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Enhancement of Nitrogen Retention in Cow Manure Composting with Biochar: An Investigation into Migration and Regulation Mechanisms

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Abstract: Context: Biochar can affect the storage and forms of nitrogen; thus, it may also play a role in altering the nitrogen cycle during the fermentation process of cow dung into organic fertilizer. Objective: To elucidate the mechanism and process of nitrogen transformation during the composting of cow manure with biochar, a comparative experiment was conducted. Method: This study investigates the use of biochar as a medium to enhance nitrogen storage during the aerobic composting of cow manure. The effectiveness was verified through a rapid composting experiment. Result and Conclusions: The results demonstrated that adding 5% biochar to the compost pile increased the total nitrogen content in manure by 12%. Specifically, the pyrrolic nitrogen in the composted cow manure increased from 38% to 44%, and the carbon-nitrogen ratio improved from 35% to 37%. Analysis of surface functional groups indicated that the C=O and C=C bonds in biochar played a key role in modifying nitrogen storage. Microbial analysis showed that biochar could significantly enhance the regional competitiveness of microorganisms, such as *Cellvibrio*, thereby boosting the expression of functional genes involved in the nitrification process, including *amoABC*, hao, and *nxrAB*. Therefore, adding 5% biochar not only enhances nitrogen storage in organic fertilizer but also changes the microbial population structure. Significance: This study carries substantial implications for the application of Biochar in the field, as well as for the development of microbial fertilizers based on cow manure.

Keywords: biochar; compost; nitrogen cycle; microbial community; manure

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1. Introduction

In modern dairy farm intensive production, cows are often overfed to maximize individual milk production [1], resulting in cow manure containing a large amount of unused nutrients [2]. These nutrients can cause environmental problems, such as the greenhouse effect, soil degradation, and water eutrophication, if not utilized rationally [3]. Therefore, the efficient utilization of nutrients in cow manure is essential for sustainable agricultural development. The primary method for utilizing the nutrients in manure is fertilization, which includes direct field application, composting, vermicomposting, and organic fertilizer production [4]. Among these, composting is the most conventional method [5]. This process involves adjusting the carbon-to-nitrogen ratio of the cow manure pile by adding specific auxiliary materials and introducing microbial agents for high-temperature fermentation to produce compost. Returning this compost to the field provides nutrients to crops and enhances the soil environment, thereby achieving nutrient recycling and utilization in cow manure. During the composting process, nitrogen is the nutrient with the highest content and best fertilizer efficiency, but it is also the most easily lost element [6,7]; thus, efficient utilization of nitrogen is the key to composting.

The most common method for enhancing nitrogen recycling involves optimizing composting process parameters and microbial population structures to improve nitrogen recovery rates. For instance, Liu et al. [8] adjusted the carbon-nitrogen-phosphorus-sulfur ratio in cow manure compost to regulate microbial nutrient mineralization and enhance nitrogen utilization. Qiao et al. [9] demonstrated that crop absorption of nitrogen in manure is influenced by the composition of the compost pile. Similarly, Duan et al. [10] showed that crop nitrogen absorption from manure is affected by the compost pile's composition. Duan et al. (Duan et al., 2020) also added exogenous bacterial agents (*Bacillus subtilis*) at varying levels during cow manure composting and found that these microbial agents could influence the composting process, potentially regulating soil microorganisms and affecting nitrogen uptake by plant roots. However, the efficiency of nitrogen cycle improvement through these methods primarily depends on the interaction between microorganisms and plant roots, without significantly increasing the nitrogen content in the compost products. Consequently, the ability to improve nitrogen cycle efficiency is limited. This limitation arises from the nutrient carrier shortage, as the original cow manure pile hardly preserves nitrogen in a fixed structure [11]. Therefore, enhancing nitrogen storage to increase overall nitrogen recycling and utilization efficiency has long been a challenging issue in cow manure utilization and has garnered widespread attention from practitioners.

Nitrogen typically exists in forms such as NH_4^+, NO_2^-, NO_3^-, and organic nitrogen. Organic nitrogen is converted into inorganic forms, and researchers have demonstrated that inorganic nitrogen can be absorbed by adsorbents such as attapulgite [12], ion exchange membrane [13], and biochar [14]. Among these, biochar has been shown to act as a nutrient adsorption slow-release agent. For instance, Morris et al. [15] found that biochar could temporarily adsorb nitrates and ammonium and then release them slowly. Similarly, Han et al. [16] discovered that biochar has a similar function for ammonia, with effects lasting several weeks. This phenomenon was also observed by Wang et al. [17], Saarela et al. [18], and Cai et al. [19]. Additionally, ammonia and nitrate nitrogen are mutually transformed via nitrifying and denitrifying bacteria during composting. This process is complex and can be influenced by the stock of nitrogen in different forms. Moreover, several studies have corroborated that the interconversion of various nitrogen forms significantly affects certain crops, such as corn [20], beans [21], and beet [22]. Thus, biochar can destabilize the nitrogen reserve, subsequently modifying the nitrogen translocation process and enhancing storage capacity and duration, ultimately fostering crop growth.

Based on the aforementioned considerations, we hypothesize that the addition of biochar may alter the nitrogen cycling pathway due to its adsorption characteristics. Consequently, this research initially involved the collection of cow manure from a large cowshed. Biochar was then added to the manure to enhance the nitrogen content in the solid matter. Subsequently, composting experiments were conducted on the manure, both with and without the addition of biochar. The nitrogen forms were identified, and to further elucidate the nitrogen transformation mechanisms, the microbial community was investigated.

2. Materials and Methods

2.1. Experimental Design

The comprehensive experimental flowchart is shown in Figure 1. Biochar was incorporated into raw cow manure (CM) and subsequently underwent a solid–liquid separation process. Using the resultant solid cow manure, blended with straw to adjust the carbon-to-nitrogen ratio, we conducted composting experiments.

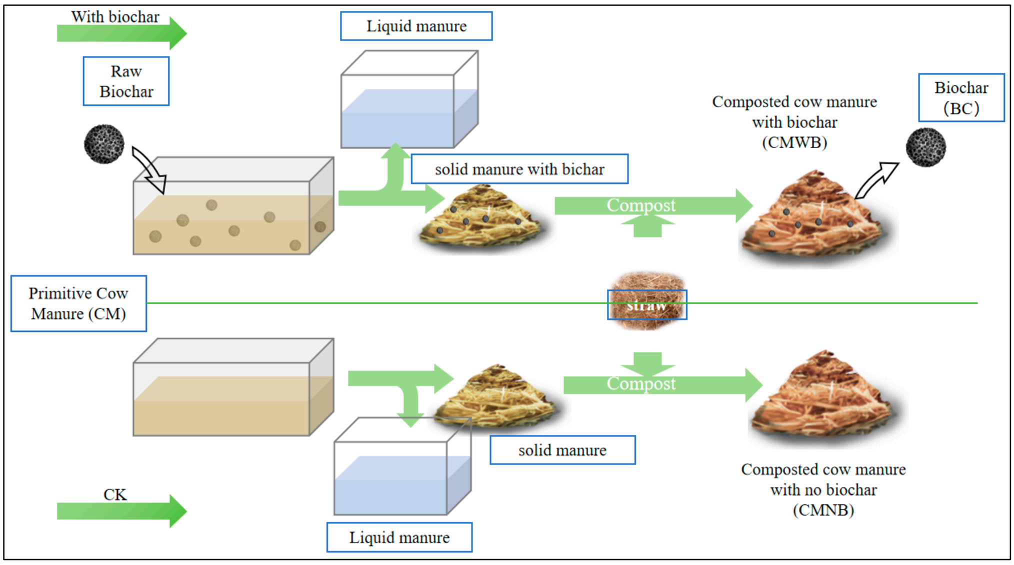

Figure 1. The overall experiment flowchart.

2.2. Materials and Compost Process

The raw materials, including primitive corn straw (through natural drying) and biochar (poplar wood shavings, 400 °C for 40 min), as well as the composting experimental site, were provided by the dairy farm of Huarui Agriculture Co., Ltd. (Qingdao, China) (100°40′20″ E, 38°43′33″ N). Each sample was measured 3 times, and the average data and standard deviation of properties are shown in Table 1.

Table 1. The detailed properties of the raw materials.

Materials	**Total Nitrogen (%)**	**Organic Matter (%)**	**Organic Carbon (%)**	**Moisture (%)**	**Carbon Nitrogen Ratio**
CM Straw	3.44 ± 1.42 0.48 ± 0.06	60.25 ± 10.15 79.25 ± 0.90	34.95 ± 5.89 45.97 ± 0.52	85.00 ± 5.94 5.00 ± 0.28	10.16 ± 4.15 95.77 ± 8.13
	Ash (%)	Volatile matter (%)	Fixed carbon (%)	Moisture (%)	Specific surface area (m^3/g)
Biochar	10.16 ± 2.00	29.86 ± 2.97	57.03 ± 4.90	2.95 ± 90.13	273.10 ± 70.02

Note: Total nitrogen was measured by UV spectroscopic, and the detailed operation process can be found in the supporting materials. Organic matter (%) was measured using the thermostatically analysis method. Organic carbon (%) was calculated based on the organic matter content. Moisture, ash, and volatile matter content were obtained by industrial analysis using a muffle furnace. The specific surface area of the biochar (m^3/g) was measured using an automated surface area and pore size analyzer (NOVA4200e, Quantachrome, Boynton Beach, FL, USA).

The compost experiment was conducted indoors, the average temperature ranged from 5 °C to 10 °C. Air humidity ranged from 40% to ~50%. Four steps had been carried for the compost experiment. (1) Adding 1% biochar to the primitive manure, followed by thorough mixing, and then separating the solid and liquid using a separator. (2) Mixing the solid manure and straw, in a ratio of 2.6:1, to adjust the carbon nitrogen ratio to around 25% and the moisture content to around 56%. (3) Stacking the mixture into piles of about 0.2 m^3, with each pile weighing 110 kg, (4) starting the composting experiment, and flipping the piles regularly.

The whole compost period lasted for 18 days, and during the this period, the temperature of the compost pile was continually measured. Pile-turning was carried out every day to mix the materials evenly, and after 6 days, the pile was turned every 3 days. After composting, the samples of cow manure with no biochar (CMNB) and cow manure with biochar (CMWB) were collected using a five-point sampling method at different positions, namely the bottom, middle, and top of the pile. The Biochar (BC) in CMWB was then extracted for further analysis.

2.3. Determination Indicators and Assay Method

2.3.1. Nitrogen Content Detection

The total nitrogen content of the samples from piles before and after composting was measured by the Kjeldahl method (the operation process is listed in the supporting materials). The same method was also applied to the liquid manure. Available nitrogen, including nitrate nitrogen and ammonium nitrogen, was detected by spectrophotometry and the colorimetric method, respectively. All the operation steps are listed in the supporting material. The samples were measured three times and average was then taken. The experimental date was recorded and visualized by Microsoft Excel.

For further mechanism analysis, the solid samples were analyzed using both X-ray Photoelectron Spectroscopy (XPS, Thermo Scientific Escalab 250Xi, Waltham, MA, USA) and Fourier Transform Infra-Red (FTIR, Bruker TENSOR II, Billerica, MA, USA) to detect surface nitrogen forms and functional groups. The result of FTIR was statistically analyzed using Ominic8.2, and all the results of this part were visualized using Origin8.1.

2.3.2. High-Throughput Sequencing of Microbial Community

To illustrate the variation in microbial species in cow manure influenced by biochar, DNA from CM, CMWB, CMNB, and BC samples was extracted using a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc., New York, NY, USA) in accordance with the manufacturer's instructions. The DNA quality, including integrity, purity, and concentration, was evaluated via a 1% agarose gel and a Nanodrop spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE, USA). The 16S rDNA V3–V4 bacterial regions of the extracted genomic DNA were amplified using the forward primer 5′-CCTAYGGGRBGCASCAG-3′ and the reverse primer 5′-GGACTACNNGGGTATCTAAT-3′. PCR reactions were performed in triplicate, and the triplicate PCR products were combined and purified using an ENZA Gel Extraction Kit (Omega Bio-Tek Inc., Norcross, GA, USA). Subsequently, the amplification products were subjected to Illumina MiSeq high-throughput sequencing and analysis. Sequencing and bioinformatic services were provided by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China).

3. Results and Discussion

3.1. Nitrogen Element Flow

The results showed that the addition of biochar resulted in more total nitrogen being stored in the solid manure compared to the liquid manure. Overall, the nitrogen content (including both solid and liquid) after compost was 1% higher than the control, with solid manure showing a 1.4% increase and liquid manure showing a 0.2% increase (Figure 2a). This phenomenon indicates that 5% biochar can reduce nitrogen loss by 1.2% compared to the control during compost.

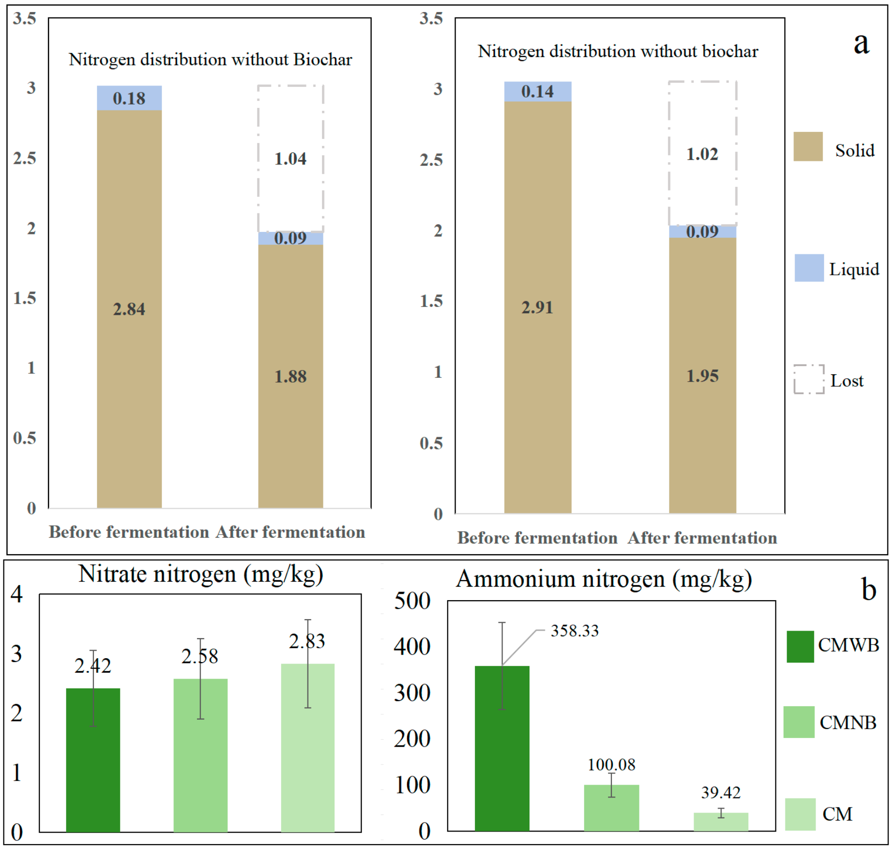

Figure 2. (**a**) Nitrogen distribution with and without biochar. (**b**) Nitrate N and ammonium nitrogen content in CMWB and CMNB.

The mechanism of nitrogen preservation by biochar may include ion exchange, chemical bond adsorption, and physical storage; these processes might alter the original nitrogen conversion pathways [23]. For instance, biochar can inhibit the mineralization of organic nitrogen and promote the polymerization of NH_4^+ with both the carbon skeleton and active groups on the surface of biochar [24,25]. Furthermore, ammonia nitrogen may react with carboxyl groups, forming ammonium salts or amide compounds. Therefore, to elucidate this process, the ammonium and nitrate nitrogen contents were quantitatively tested.

Mathematically, biochar increased the nitrate nitrogen content of the pile from 2.58 mg/kg to 2.83 mg/kg and reduced the ammonium nitrogen content from 100.08 mg/kg to 39.42 mg/kg (Figure 2b) in CMWB compared to CMNB. Consequently, biochar can influence the balance of ammonium nitrogen and nitrate nitrogen through both chemical and microbiological mechanisms. To better understand the chemical and microbiological effects of biochar on manure compost, we analyzed the changes in nitrogen chemical forms and microbial communities in the following chapters.

3.2. Nitrogen Form Varition

To gain deeper insights into the chemical composition and morphology of the elements in the samples, XPS analysis was conducted. The wide-scan XPS spectra of the CW, CMWB, and CMNB exhibit similar profiles (Figure 3a–c). Peaks corresponding to O1s, N1s, and C1s were observed, with the binding energies of oxygen and carbon being significantly higher than that of nitrogen. This is attributed to the fact that the residues of forage post-digestion and absorption by cows are primarily composed of cellulose and hemicellulose, which consist mainly of carbon and oxygen, with relatively low nitrogen content.

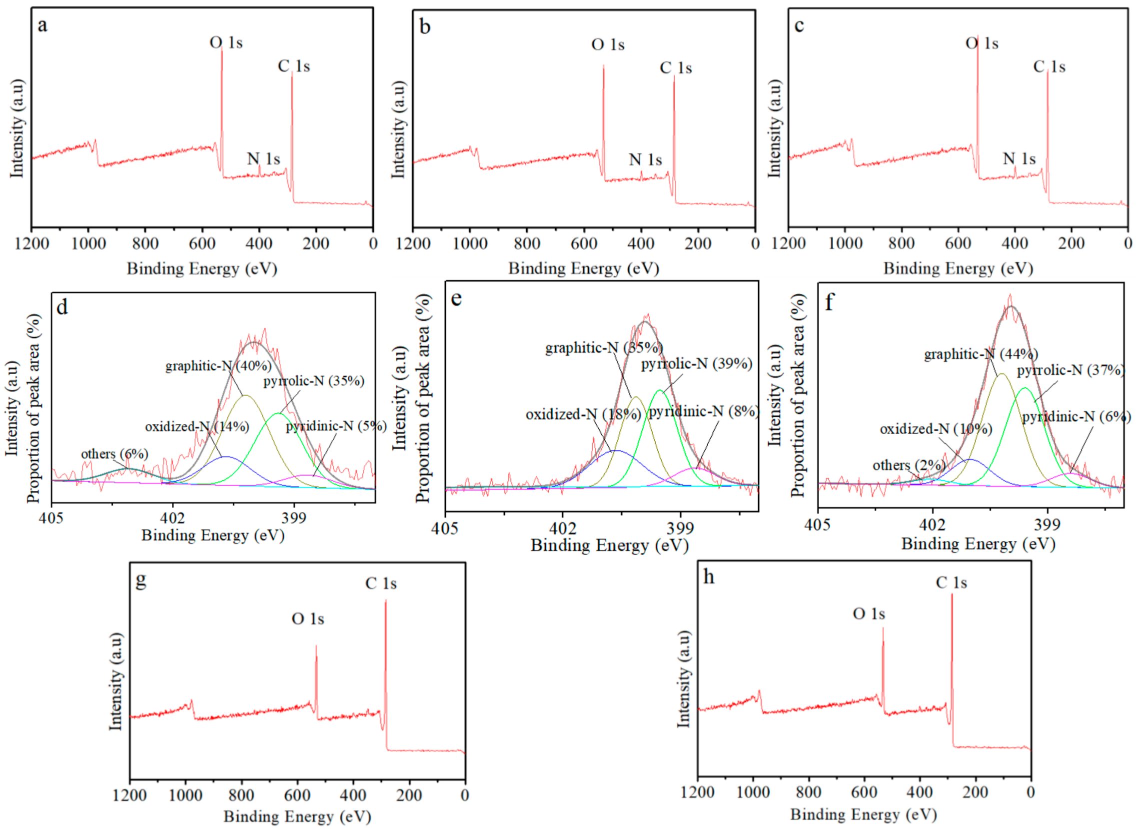

Figure 3. (**a**) Wide-scan XPS spectra of CW. (**b**) Wide-scan XPS spectra of CMWB. (**c**) Wide-scan XPS spectra of CMNB. (**d**) The deconvoluted N1s spectra of the XPS results of CW. (**e**) The deconvoluted N1s spectra of the XPS results of CMWB. (**f**) The deconvoluted N1s spectra of the XPS results of CMNB. (**g**) Wide-scan XPS spectra of primitive biochar. (**h**) Wide-scan XPS spectra of primitive BC.

The deconvoluted N1s spectra of the XPS results (Figure 3d–f) of raw cow manure exhibit four peaks between 398 and 402 eV. These peaks correspond to various forms of organic nitrogen: pyridine-N (398.7 ± 0.4 eV), pyrrole-N (400.3 ± 0.3 eV), carbon-N (401.2 ± 0.3 eV), and oxide-N (402.8 ± 0.5 eV) [26]. We analyzed the content of these nitrogen forms in each sample based on the area of each peak. In solid cow manure (Figure 3d), carbon-N and pyrrole-N constitute the largest portions, accounting for 40% and 35%, respectively. This pattern is similar to composted cow manure with biochar (Figure 3f), where carbon-N and pyrrole-N also have the highest proportions, at 44% and 37%, respectively. In contrast, composted cow manure without biochar shows a different distribution, with pyrrole-N (38%) being more prevalent than carbon-N (35%).

Notably, during the peak fitting process for both CM and CMWB, a fifth peak was identified, which was absent in CMNB. This suggests that biochar may influence nitrogen retention in cow manure. However, this retention might be due to biochar's adsorption properties or suboptimal composting results. To investigate further, we analyzed the XPS wide scan of the original biochar (Figure 3g) and the biochar post-composting (Figure 3h). The results showed no significant N1s peak, indicating that composting does not increase the nitrogen content of biochar, thus suggesting that nitrogen retention is primarily a physical adsorption process.

The functional groups of the samples are closely associated with their specific chemical properties, with types of functional groups referenced from previous studies. FTIR spectra of precipitation with manure samples, including CM, CMWB, and CMNB, showed no significant changes (Figure 4). This is primarily due to the main components in cow manure, such as forage fiber, remaining largely unaltered, resulting in unchanged surface functional groups. Biochar, regardless of whether it was composted, displayed a decrease at 1400 cm^{-1} and an increase at 1600 cm^{-1} compared to the manure samples. This could be attributed to the carbonization process, during which O and H in C-O and COO-CH_3 are removed, forming C=C bonds [27–29].

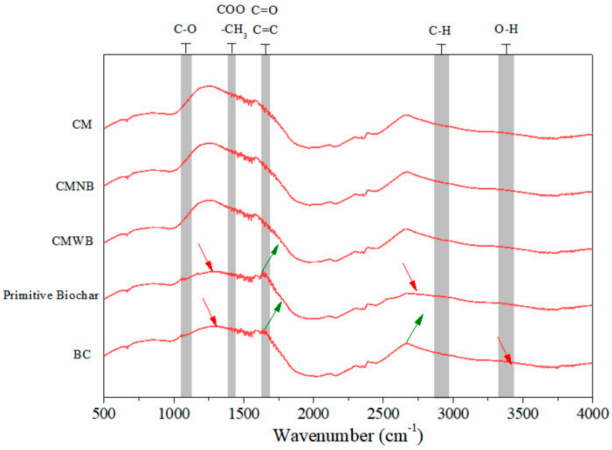

Figure 4. FTIR spectra result of CM, CMWB, CMNV, primitive biochar, and BC.

The infrared absorption of biochar before and after composting exhibited distinct trends at 2900 cm^{-1} and 3400 cm^{-1}. The primitive biochar had lower absorbance at 2900 cm^{-1}, indicating less C-H stretching, likely due to the release of H^+ from biomass during biochar preparation. Notably, the absorbance for C-H stretching on the surface of biochar increased after composting, possibly due to the absorption of other organic matter, such as amino acids, glucose, and organic polymers from cell walls. Biochar also showed lower absorbance at 3400 cm^{-1}, corresponding to O-H stretching. This may be because functional groups, such as alcohols, phenols, and carboxylic acids, which contain hydroxyl and carboxyl groups, undergo decomposition and subsequently combine with free H^+ [28].

Furthermore, biochar demonstrated reduced absorbance at 3400 cm^{-1}, corresponding to O-H bonds. This reduction may be attributed to the decomposition of functional groups, such as alcohols, phenols, and carboxylic acids, followed by their interaction with free H^+ ions [29].

3.3. Changes in Microbial Communities

Different forms of nitrogen in the soil affect microbial activities and may alter the microbial community structure. A genus-level species composition analysis was performed (Figure 5a), and the species composition of the top 20 dominant bacteria is presented below. The results showed that the dominant types (the three bacteria with the highest proportions) in CM are *Firmicutes* (including *Fundicoccus* and *Atopostipes*) and *Actinobacteria* (mainly *Jeotgalibaca*), accounting for 39% in CM. *Firmicutes* are microorganisms related to the host's digestive tract, which may play an important role in maintaining gut health and the digestive process, explaining their continued prevalence in CM. In this study, we did not introduce microbial agents, such as *Bacillus* subtilis, to adjust the microbial community structure, thus the microorganisms in the pile are in a state of natural competition. There are no obvious dominant strains, with *Cellvibrio*, *Blautia_A_141781*, *Agathobacter_164117*, *Faecalibacterium*, and *Faecalibacillus* accounting for 4%, 6%, 5%, 4%, and 4%, respectively.

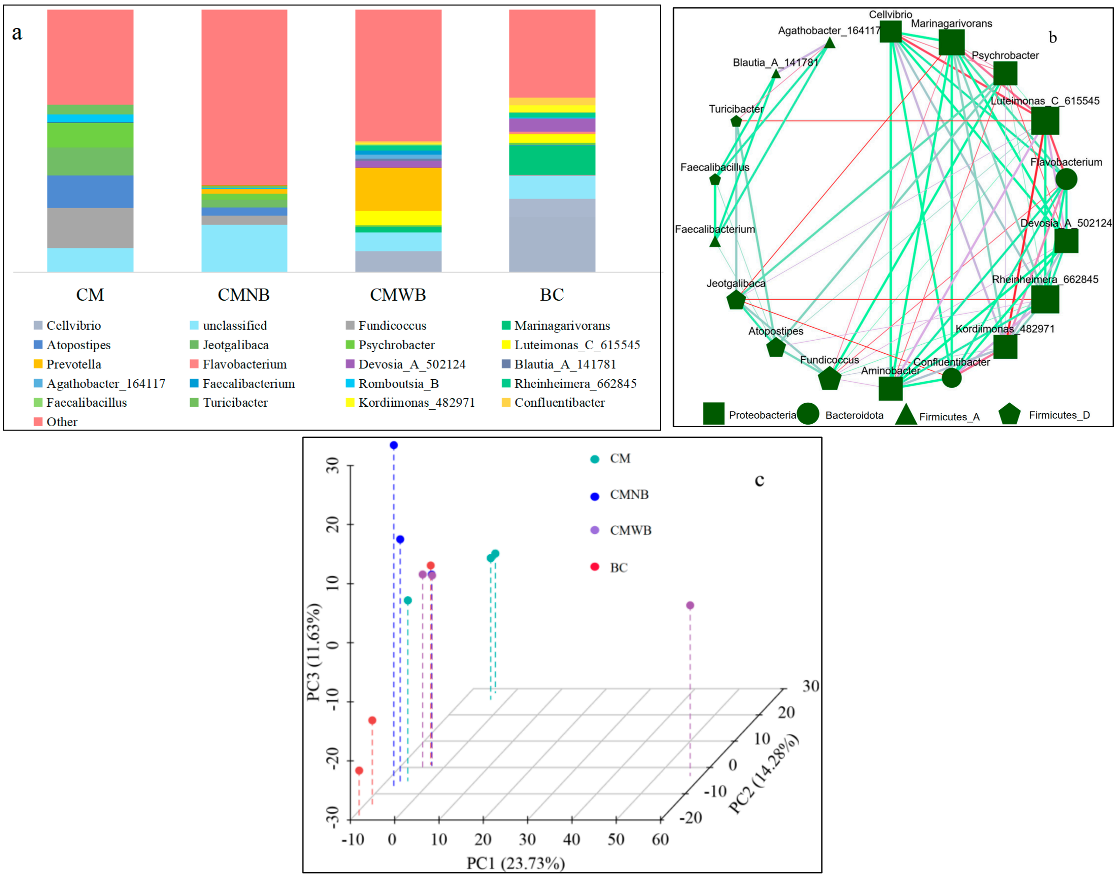

Figure 5. (**a**) Species composition analysis of CM, CMNB, CMWB, and BC. (**b**) Correlation analysis of the various microorganisms in CM, CMNB, CMWB, and BC. (**c**) Principal component analysis of the microorganisms in CM, CMNB, CMWB, and BC.

In comparison, the presence of biomass charcoal likely accounts for the significant differences in microbial community structure between CMWB and CMNB. In CMWB, the dominant microbial communities consist of *Cellvibrio* (8%), *Luteimonas_C_615545* (5%), *Prevotella* (8%), and *Flavobacterium* (8%). *Prevotella*, a member of the *Bacteroidetes* phylum, is commonly found in the intestines of animals where it participates in cellulose degradation and energy metabolism, and thus can also be present in CM. *Cellvibrio* and *Flavobacterium* are Gram-negative bacteria typically isolated from water and exhibit very low levels in both CM and CMNB. Given that the initial moisture content in CMNB and CMWB raw materials is identical, we speculate that biochar may enhance the competitiveness of these bacteria, a situation also observed with *Luteimonas_C_615545*.

To elucidate the relationships among various microbial species, we conducted a correlation analysis on the samples. As illustrated in Figure 5b, the dots represent differential microorganisms, with the dot size proportional to the degree of correlation. The red lines indicate positive correlations between two types of microorganisms, while the blue lines indicate negative correlations. The thickness of the lines represents the magnitude of the correlation in absolute terms. The results indicate that *Proteobacteria* play a significant central role in microbial networks, potentially crucial for maintaining the stability and functionality of microbial communities. Notably, *Cellvibrio* and *Bacteroidota* (indicated by circular dots) exhibited a significant negative correlation. Consequently, with the presence of biochar, *Cellvibrio* has established higher competitiveness within the microbial community.

To clarify the differences and similarities in microbial community composition among various samples, we utilized principal component analysis (PCA, Figure 5c). The results indicated that the variation ratio of PC1 is 24%, significantly higher than that of PC2 and

PC3. In this dimension, CMWB exhibited the greatest variability, while other treatments showed almost no significant variation. CM and CMNB demonstrated noticeable variability in the dimensions of PC2 and PC3, respectively, and BC showed variability in both PC2 and PC3. Overall, there is diversity among the samples of each treatment. Regarding the degree of diversity, the microbial community composition of CM and CMNB was relatively lower, whereas CMWB and BC exhibited higher variations. We speculate that these higher variations might be due to the incorporation of biochar, which could enhance the competitive capabilities of functional bacteria.

The change in microbial structure directly affects the carbon and nitrogen cycles of the entire reactor. Therefore, the C and N cycles were investigated according to DNA sequencing of the samples. The relative abundances of the pathways involved in the nitrogen cycle are shown in Figure 6. The pie chart indicates the relative abundance of each pathway in each metagenomic sample, and the size of the pie charts represents the total relative abundance of each pathway.

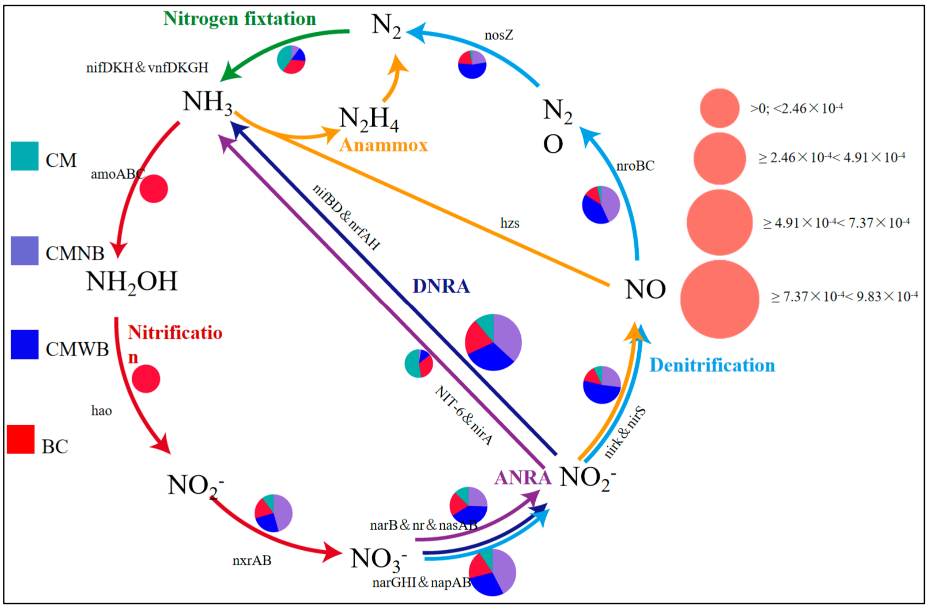

Figure 6. The relative abundances of the pathways involved in the nitrogen cycle of CM, CMNB, CMWB, and BC.

It was observed that the functional microbes involved in N-cycling varied with cow manure compost. Three functional genes for nitrification, namely *amoABC*, hao, and *nxrAB*; five functional genes for assimilatory nitrate reduction (*ANRA*), namely *narB*, *nasAB*, *NIT-6*, and *nirA*; four genes for dissimilatory nitrate reduction to ammonium (*DNRA*), namely *narGHI*, *napAB*, *nifBD*, and *nrfAH*; one functional gene for anammox, which is *hzs*; six functional genes for denitrification, namely *nirK*, *nirS*, *norBC*, and *nosZ*; and two functional genes for nitrogen fixation, namely *nifDKH* and *vnfDKGH*, were identified.

Among them, the *amoABC* genes that dominate nitrification are primarily found in BC, indicating that these nitrifying bacteria rely on the habitat provided by biochar for reproduction. It is also possible that the nitrifying bacteria are fully expressed, with their detection abundance increased due to external factors regulated by the microenvironment provided by biochar. The genes *nifBD* and *nrfAH*, which dominate DNRA, are expressed abundantly in CMNB and CMWB. These two genes encode part of the nitrogenase complex responsible for reducing atmospheric nitrogen (N_2) to ammonia (NH_3), serving as the core enzyme in biological nitrogen fixation. The expression of these two genes is relatively low in CM and BC, suggesting that microorganisms containing these genes proliferate significantly under natural competitive conditions, whereas they are suppressed in biochar. A similar situation occurs with other functional genes throughout the denitrification process.

Furthermore, the genes *NIT-6* and *nirA*, which dominate ANRA, as well as *nifDKH* and *vnfDKGH*, which dominate nitrogen fixation, show high abundance in CM and BC but lower abundance in CMNB and CMWB. This may be due to the fact that, during the composting process in this experiment, the turning of the compost heap allows certain aerobic bacteria to gain a competitive advantage, while the porous structure of biochar can reduce the competition of these aerobic bacteria against native bacteria, allowing bacteria containing these genes (such as *Archaea* and *Planctomycetes*) to form local advantages and maintain their gene abundance. A more likely explanation is that the porous structure and surface functional groups of biochar promote the substantial release of nitrogenase by certain bacteria that can produce extracellular enzymes containing these functional genes (e.g., *Bacillus*), resulting in a higher abundance of their functional genes in the detection results.

Overall, biochar can significantly enhance the competitiveness of certain bacteria and the expression of specific functional genes. The presence of the *NIT-6* and *nirA* genes may provide further evidence that some bacteria can expand their reproductive range using the substrates provided by biochar.

4. Discussion

4.1. Changes in Nitrogen Content and Chemical Structure

Our data showed that 5% biochar could reduce nitrogen loss during the process of cow manure composting. During composting, the mechanisms of nitrogen preservation include ion exchange, chemical bond adsorption, and physical institutional storage. These processes might alter the original nitrogen conversion pathway [30,31]. For example, NH_4^+ could be adsorbed through chemical reactions with the active groups on the surface of biochar. As a Lewis acid, ammonia nitrogen reacts with carboxyl groups to form ammonium salts or amide compounds [32]. Compared with nitrate nitrogen, ammonium nitrogen is more prone to volatilization loss [33]. Therefore, the addition of biochar provides more opportunities for ammonium retention in the pile during composting.

However, the results of this research indicate that the addition of biochar to manure increases the production of nitrate nitrogen. It is known that C=O bonds can form stable adsorption complexes with NH_4^+ through electrostatic attraction and hydrogen bonding, which enhances the adsorption of ammonium nitrogen [34,35]. In addition to C=O bonds (including carbonyl, carboxyl et al.), C=C bonds can also interact with nitrate ions in aqueous solutions to form ionic bonds, leading to their enrichment in the solution [34]. Our research shows that the levels of C=C and C=O on the surface of biochar are higher than those in solid substances in cow manure. Therefore, adding biochar during the composting process simultaneously strengthens the retention of both ammonium nitrogen and nitrate nitrogen, with a more pronounced effect on nitrate nitrogen.

The changes in organic nitrogen during composting are relatively complex. In this study, XRD results did not show a significant N1 peak on the surface of biochar, indicating that biochar does not directly adsorb organic nitrogen. However, the sub-peak results of organic nitrogen in CMNB and CMWB show some differences, suggesting that biochar's adsorption of inorganic nitrogen can alter the distribution of organic nitrogen species. These changes represent a complex and dynamic process influenced by various factors. In the early stages of composting, organic nitrogen content may decrease due to intensified microbial activity, which decomposes organic nitrogen into inorganic nutrients for microbial use [36,37]. As composting progresses, particularly in the mid-to-late stages, the organic nitrogen content increases mainly because microorganisms assimilate inorganic nitrogen under suitable conditions, converting it back into organic nitrogen and fixing it [36]. In this process, biochar may temporarily store or delay the supply of inorganic nutrients, leading to differences in the decomposition process of organic nitrogen. This could explain why the proportion of pyridine nitrogen and pyrrole nitrogen is lower in compost piles mixed with biochar after composting.

4.2. Changes in Microbial Communities and the Process of Nitrogen Metabolism

The transformation of nitrogen forms during composting is primarily driven by microbial activity. This study demonstrates that the addition of biochar enhances the competitive capabilities of certain bacteria, such as *Cellvibrio*, during the composting process of cow dung. Research [38,39] indicates that *Cellvibrio* is a thermophilic bacterium capable of degrading cellulose and polysaccharides, making it an essential microorganism adapted to environmental conditions. The relative abundance of *Cellvibrio* increases as the composting process matures [40]. Furthermore, *Cellvibrio*'s ability to degrade insoluble organic matter, such as cellulose in organic fertilizers [41], is crucial for the effective utilization of organic fertilizers. Consequently, *Cellvibrio* can be considered an indicator microorganism for manure maturity. Therefore, in this study, the significantly higher amount of *Cellvibrio* in BC compared to other treatments suggests that biochar in CMWB provides a favorable habitat for *Cellvibrio*, thereby locally enhancing the decomposition of cow manure.

Furthermore, the nitrogen cycling pathway is determined by the gene expression of various microbial communities [42,43]. In this study, the *amoABC* genes, which are key to nitrification, were predominantly found in BC, suggesting that nitrifying bacteria thrive in the habitat provided by biochar [44,45]. It is also possible that these bacteria exhibit increased gene expression and detection abundance due to external factors regulated within the biochar microenvironment [46]. The *nifBD* and *nrfAH* genes, crucial for DNRA, were abundantly expressed in CMNB and CMWB. These genes encode components of the nitrogenase complex responsible for converting atmospheric nitrogen (N2) to ammonia (NH3), a core enzyme in biological nitrogen fixation [47]. Their expression was relatively low in CM and BC, indicating that microorganisms with these genes proliferate more under natural competitive conditions but are suppressed in biochar. Similar patterns were observed for other functional genes involved in the denitrification process. Additionally, genes such as *NIT-6* and *nirA*, important for ANRA, as well as *nifDKH* and *vnfDKGH*, critical for nitrogen fixation, exhibited higher abundance in CM and BC but lower in CMNB and CMWB. This could be because the composting process, which includes turning the compost heap, favors certain aerobic bacteria, while the porous structure of biochar reduces competition, allowing native bacteria to maintain gene abundance. A more plausible explanation is that the porous structure and surface functional groups of biochar facilitate the significant release of nitrogenase by bacteria capable of producing extracellular enzymes with these functional genes (e.g., *Bacillus*), leading to higher detection abundances [48].

Overall, biochar can significantly enhance the competitiveness of certain bacteria and the expression capacity of specific functional genes. Additionally, the distribution of the *NIT-6* and *nirA* genes in the results might further indicate that some bacteria expand their breeding range relying on the substrate provided by biochar.

4.3. Comprehensive Effect of Biochar on Nitrogen Cycle

The incorporation of biomass carbon has significantly influenced the nitrogen cycling process during the composting of cow manure. The porous structure of biomass carbon creates an environment conducive to microbial growth, allowing specific microorganisms, such as *Cellvibrio*, to gain a competitive advantage. This advantage promotes the increased expression of functional genes involved in nitrification. The finding that biochar can enhance microbial activity has been corroborated by other studies. For instance, Li et al. [49] observed a greater increase in total microbial diversity with the addition of biochar in sandy soil. Different from this study, Sun et al. [50] found that biochar supported the population of *B. mucilaginosus*, a microbial species distinct from those in this study. This discrepancy suggests that the biochar's effect on promoting microorganism growth is selective and potentially contingent on a range of factors, such as the overall environment, the type of indigenous microorganisms, and the characteristics of the biochar.

Additionally, compared to cow dung, biomass carbon contains more carboxyl and carbonyl groups, which have a strong adsorption affinity for ammonium ions. Consequently, this results in higher retention of ammonium around the biomass carbon, further enhancing the expression of genes such as *amoABC*, hao, and *nxrAB*, which are strongly associated with nitrification, ultimately increasing the nitrate nitrogen content. The research by Gupta et al. [51] yielded similar results; however, the findings of Llovet et al. [52] and Hamada et al. [53] were contrary. They suggested that biochar reduces the nitrate nitrogen stock, possibly due to the different microbial species promoted by biochar in their cases, leading to significant variations in nitrogen storage outcomes.

5. Conclusions

In conclusion, 5% biochar assisted in establishing a separate breeding space for microorganisms in cow manure with enriched total nitrogen, which lead to the total N improving by 12% compared with composted cow manure with no biochar. At the same time, under the synergistic effect of the adsorption of biochar and microbial transformation, more nitrate nitrogen (9% higher) was preserved in cow manure, and less ammonium nitrogen (61% lower) remained. From the perspective of microbial communities and functional genes, the space offered by biochar is conducive to the reproduction and growth of certain microorganisms, such as *Cellvibrio*, *Marinagarivorans*, and *Devosia_A_502124*, while inhibiting others. This phenomenon changed the distribution of nitrogen metabolism-related genes, strengthening the nitrification process.

However, this result is not entirely positive, as more nitrate nitrogen also means more nitrogen oxides are released, which may lead to an increase in greenhouse gas emissions. The change in nitrogen forms due to biochar depends on the microbes it carries, so we believe that by utilizing this, it is possible to make biochar a specialized carrier for certain functional probiotics. This can be applied in organic fertilizers to produce microbial fertilizers, which will be the focus of our next research step.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15020265/s1.

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