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Organic Materials with High C/N Ratio: More Beneficial to Soil Improvement and Soil Health

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Abstract

In order to figure out the effect of organic fertilizers with different carbon-nitrogen (C/N) ratios on the soil improvement and the healthy cultivation, the pot experiment method was used to study effects on the physical and chemical properties and the bacterial community structure of sandy loam soil using five treatments of chemical fertilizer application with the C/N ratios of 15 (CN15), 20 (CN20), 25 (CN25), 30 (CN30) and the control (CK) respectively. Results show that the organic materials with different C/N ratios significantly improve the soil porosity and water content, which all show a linear change rule with the C/N ratio. It can also significantly increase the soil total carbon, total nitrogen, soil C/N ratio, soil microbial biomass carbon, microbial biomass nitrogen and microbial biomass C/N ratio. Among them, CN30 significantly increases the soil total carbon and C/N ratio, which are 5.34%-24.13% and 8.87%-30.15% respectively compared with other treatments. It can be also found that the dominant flora (at the phylum level) of each treatment are *Actinobacteria*, *Proteobacteria* and *Chlorobi*. The CN30 treatment presents the most obvious improvement in the diversity and richness of the soil bacterial community and is more conducive to the growth and reproduction of *Proteobacteria* and *Firmicutes.* The correlation analysis shows that C_{total}/N_{total} and C_{mic}/N_{mic} are the most important environmental factors affecting the soil physical and chemical properties and their correlation with the bacterial communities. The higher C/N ratio of organic materials results in a more significant improvement of the soil physical and chemical properties. This study provides a new theoretical basis for the soil health cultivation technology.

1 Introduction

Green and sustainable development is a necessary condition for realizing the modern agriculture and the use of organic materials is one of the key measures. Adding organic materials to soil has been demonstrated in numerous studies to lower the soil bulk density, enhance the soil porosity, increase the soil carbon pool, and increase the soil health (Chen et al. 2020; Yang et al. 2022; Yu et al. 2020; Jernigan et al. 2020; Zhou et al. 2021). According to Zhu et al., the effectiveness of nutrients in organic materials, such as nitrogen, phosphorous, and potassium, which are degraded and used by microbes or released into the soil, is strongly associated with the carbon/nitrogen (C/N) ratio of organic fertilizers (Zhu et al. 2021). Trinsoutrot et al. also indicated that the C/N ratio is the main factor affecting the nitrogen release of organic materials (Trinsoutrot et al. 2000). Soil carbon and nitrogen pools are the most important components of soil, and more than 90% of nitrogen exists in the form of organic matter (Yan et al. 2022). The coupling relationship between the soil carbon and the nitrogen pools directly or indirectly determines the soil quality and health (e.g. the stability of soil structure, soil fertility, etc.), and is inextricably linked to the crop yield quality (Ren et al. 2021). Herein, the exogenous addition of organic materials is the most direct and effective way to adjust the coupling relationship between the soil carbon and the nitrogen.

Soil microorganisms play significant roles in the mineralization of the soil organic matter and the cycle of elements such as carbon and nitrogen, as well as promoting the soil nutrient transformation and supply (Pf et al. 2021). The use of organic materials has been proven in several studies to drastically alter the makeup of microbial communities (Zhao et al. 2020; Guo et al. 2019). Cleveland et al. found that the changes in the availability of carbon and nitrogen substrates for plants and animals in ecosystems contribute to a rapid alteration of the structure and composition of microbial communities, which is directly related to the ratio of carbon and nitrogen elements in the environment (Cleveland et al. 2007). On a global scale, the soil microbial C:N:P is 60:7:1 while the soil C:N:P is 186:13:1. Meanwhile, the carbon use efficiency based on the soil microorganisms is mostly 40–60%. Therefore, it is generally believed that the exogenous substrates with a C/N ratio of 15–25 are more conducive to the development of soil microorganisms (Li et al. 2018; Manzoni et al. 2012). Zhao et al. found that the addition of organic materials might

evidently improve the stored carbon and nitrogen in the soil and the soil microbial biomass carbon, stimulate the growth of soil microorganisms, and promote the activity of soil microorganisms (Zhao et al. 2020). The addition of exogenous organic materials, such as straw (Li et al. 2021) and farmyard manure (Pei et al. 2020), could directly deliver carbon into the soil and indirectly improve the soil by the higher plant biomass such as crop residues, litter, and roots.

Previous studies mostly focused on the impacts of organic compounds (e.g. straw returning (Li et al. 2021), manure (Pei et al. 2020), and biochar (Ma et al. 2020), etc.) on the soil carbon and nitrogen parameters and the microbial community structure. However, there are few studies reporting the effects of the C/N ratio of the combined organic materials on the physical and chemical properties of sandy loam, especially the bacterial community structure. In this paper, by characterizing the carbon and nitrogen parameters of three materials, four different C/N ratio treatments were quantitatively assessed. Moreover, the impacts of material C/N ratio on the soil basic physical and chemical properties and the bacteria community structure were investigated, providing a theoretical reference for the resource utilization of agricultural and forestry waste and the cultivation of soil health. Based on this, the scientific hypothesis of this study is that the C/N ratio of organic materials has a significant correlation with the soil physicochemical properties and rhizosphere bacterial community structure.

2 Materials And Methods

2.1 Experimental materials

The test was carried out from May to September 2020. The test soil was sandy loam soil acquired from the test field's 0–20 cm soil layer at the Modern Tobacco Agricultural Science and Technology Park of Henan Agricultural University's Xuchang Campus (34°13'N, 113°80'E). Air-dried soil samples were sieved via a 2-cm sieve.The basic physicochemical properties of the test soil are as follows:pH 7.14, soil organic matter 11.54 mg/kg, alkali hydrolyzed nitrogen 74.54 mg/kg, available phosphorus 11.32 mg/kg, available potassium126.35mg/kg. The organic materials involved in the test were: biochar, vermicompost and barley straw. The test biochar was peanut shell biochar, which was carbonisation at 400 ℃ under low oxygen conditions. The percentages of total carbon and total nitrogen of biochar vermicompost and barley straw were: 9.5%, 0.88%; 37.3%,1.45%,47.3%,1.25%. Their carbon to nitrogen ratios were: 37.7, 10.8, and 25.7. Then according to a certain mass ratio, they were evenly mixed into different treatments with carbon to nitrogen ratio of 15, 20, 25 and 30. The above test materials were provided by Henan Biochar Engineering Technology Center. Cultivated crop was flue-cured tobacco.

2.2 Pot culture test design

The dimension of pots was measured:39.5×40×28 cm (outer diameter, height,bottom diameter). Each pot held 25kg of soil, 5g of pure nitrogen, and a nitrogen to phosphorus(P_2O5) to potassium (K_2O) ratio of 1:1.5:3. The treatments were designated as CK (conventional fertilisation), CN15 (CK + 100g of materials combination with C/N ratio of 15/pot), CN20 (CK + 100g of materials combination with C/N ratio of 20/pot), CN25 (CK + 100g of materials combination with C/N ratio of 25/pot), CN30 (CK + 100g of materials combination with a C/N ratio of 30/pot). Before transplanting, different materials, fertilisers and soil samples were mixed evenly in according to test design above.Under natural outdoor circumstances, the potted plants were planted on the ridge at a row spacing of 120 cm × 60 cm, and transplanting was completed on May 17, 2020. Following that, drip irrigation was used to augment natural water and reduce soil compaction.

2.3 Soil measurement and analysis

Soil bulk density and water content were measured 100 days after tobacco transplanting. Soil was obtained from the rhizosphere soil and sealed in zip-lock containers for each sample collection. The soil samples were stripped of plant debris and roots. The pH of the soil, as well as its TC, TN, Cmic, and Nmic, were then measured after sieving. DNA extraction, sequencing, and PCR were used to examine the gene of the microbial community in the rhizosphere soil. With the following modifications, the acquired gene sequences were subjected to mass screening and grouping into operational categorization units (OTUs). After grouping OTUs with 97 percent similarity, OTUs having a total abundance of less than 60 were removed using abundance filters. In addition, the OTU counts inside the subsample (n = 5) were averaged throughout the graph to get the samples, which were then rounded to integers.

Total carbon and nitrogen are calculated using Dumas combustion and the Elementar vario MAX CN (an analyzer built in Germany by Shanghai Haichao Optoelectronics Technology Co, Ltd. in Shanghai, China). (Chen et al. 2013). The oxygen and carbon dioxide flow rates were both 125 mL per minute. In 0.01M CaCl2, the pH was measured (1:5). The moisture content of the soil is dried in an oven. The ring knife method was used to determine the bulk density of soil. Porosity was estimated using the following formula:

soil porosity (%) = (1 − bulk density/specific gravity) × 100 (Eq. 1)

Soil microbial biomass carbon (Cmic) and Nitrogen (Cmic) were obtained using the flow analyzer method and chloroform fumigation direct extraction method and calculated (Chen et al. 2013) as :

soil microbial biomass carbon(Cmic)=(fumigated soil organic carbon − unfumigated soil organic carbon)/0.45 (Eq. 2)

soil microbial biomass nitrogen (Nmic)=(fumigated soil microbial biomass nitrogen − unfumigated soil microbial biomass nitrogen)/0.25 (Eq. 3)

2.4 The data analysis

The data was analyzed using the SPSS 22.0 software, and the least significant difference method was used for analysis of variance.The DPS7.0 software was used to heat map analysis. The principal coordinates analysis(PCoA) was utilized to examine changes in the makeup of the microbial community. The ratio of community composition (bar) approach was used to observe the distribution of community composition over numerous samples. Sample stratification analysis was performed to compare microbial community composition and evaluate differences across microbial communities, and redundant analysis (RDA) was utilized to analyze the influence of environmental variables on the variations in bacterial community between samples. The "vegan" package of R software was used to visualize the PCoA, bar, LefSe, and hierarchy analyses (version 3.3).

3 Results And Analysis

3.1 Variation in the soil physicochemical properties among treatments

It can be seen from Fig. 1a that the soil pH values of CN20 and CN30 increase by 0.42% and 2.11% respectively compared with the CK treatment but without a significant difference. The soil bulk density decreases with the increase of material C/N ratio. Compared with CK, the soil bulk density of CN20, CN25 and CN30 decreases significantly, with a decrease rate of 5.08%, 6.11% and 8.47% respectively (Fig. 1b). Contrary to the soil bulk density, the soil porosity increases regularly with the increase of material C/N ratio and CN20, CN25 and CN30 are

significantly different from the CK treatment (Fig. 1c). Except for CN15, the other C/N ratio materials all increase the soil moisture content (5.11%-12.58%) and CN25 and CN30 present significant differences compared with CK (Fig. 1d).

The materials with different C/N ratios substantially enhance the total amount of soil carbon, nitrogen, and C/N ratio, with CN30 showing the maximum effects compared with other three treatments (Fig. 1e, f, and g). Moreover, it can be seen from Figs. 1h to 1j that soil C_{mic}, soil N_{mic} and C_{mic}/N_{mic} ratio are significantly promoted with CN30 showing the largest increase in soil C_{mic} and C_{mic}/N_{mic} ratio up to 11.29% and 7.88% respectively compared with CK.

3.2 Correlation analysis of the soil physical and chemical parameters

Based on the results of 3.1, it can be known that the C/N ratio of organic materials influences on the soil physical and chemical properties to some extent and thus affects the substantial physical and chemical parameters. In this sense, the correlation between these parameters was explored using the person correlation analysis (Table 1). Results show that the soil bulk density and pH have a negative relationship but without a significant difference. However, it is to be noted that the soil C/N ratio and pH present a substantial positive association (R = 0.744) and an extremely negative correlation between the soil C/N ratio and the bulk density is observed (R=-0.783). Moreover, an extremely positive correlation is observed comparing the soil C/N ratio with the soil porosity, the water content, the microbial biomass carbon and the microbial biomass C/N ratio. There are significant positive correlations between the soil total carbon and total nitrogen, and the microbial biomass carbon and microbial biomass nitrogen.

Table 1
Pearson's correlation analysis between soil physical and chemical properties

Parameter	pH	Bulk density	Porosity	Water content	C_{total}	N_{total}	C/N	C_{mic}	N_{mic}
Bulk density	-0.224								
Porosity	0.314	-0.925**							
Water content	0.177	-0.816**	0.942						
C_{total}	0.573	-0.672*	0.875**	0.884**					
N_{total}	0.453	-0.323	0.121	0.527	0.830*				
C/N	0.744*	-0.783**	0.767**	0.784**	0.967**	-0.914**			
C_{mic}	0.672*	-0.282	0.667*	0.684*	0.763*	0.714**	0.857**		
N_{mic}	0.576*	-0.340	0.545*	0.584	0.774*	0.610*	-0.747**	0.643*	
C_{mic}/N_{mic}	0.845**	-0.451	0.510*	0.441	0.680*	-0.614*	0.664*	0.893**	-0.913**

Remark; *Significantly correlated at the 0.05 level, **significantly correlated at the 0.01 level.

3.3 Correlation analysis of the soil physical and chemical parameters

OTU (Operational Taxonomic Units) is a uniform marker artificially assigned to a taxonomic unit (strain, genus, species, group, etc.) in phylogenetic or population genetics studies to facilitate analysis. All sequences can be divided into OTUs according to different levels of similarity, and in this study, the OTUs at 97% similarity level were analyzed for bioinformatic statistics.The total number of OTUs detected in this test sample after the uniformed sampling is 13516. As shown in Fig. 2, the number of OTUs shared by all samples is 3017 and the OTUs of each treatment are 295(CK), 332(CN15), 240(CN20), 212(CN25) and 208(CN30) respectively. Among them, the OTUs of CN15 is the highest, accounting for 2.45% of total OTUs. Compared with the CK treatment, the numbers of OTUs for CN15, CN25, and CN30 all increase to different degrees, with the increase rates being 6.58%, 0.28%, and 0.53% respectively. In general, except for the CN20 treatment, the organic materials with different C/N ratios all increase the number of OTUs in the samples.

3.4 Effects of organic materials with different C/N ratios on the bacterial alpha diversity

It can be seen from Table 2 that the Simpson indexes of organic material treatments with different C/N ratios are all higher than that of CK treatment, although there is no significant difference between them.. The Shannon index follows a sequence of CN30 > CN25 > CN20 > CN15 and the four treatments all outperform the CK by a wide margin. As for the Chao index, the CN30 treatment shows the highest numerical value and the value is significantly higher than the rest of the treatments. Although there is no significant difference in terms of the ACE index between the different treatments of C/N ratios, CN25 shows the most obvious increase regarding the ACE index, which is 5.45% higher than that of the CK treatment.

The Bray-curtis distance method was used to perform PCoA analyses of the soil bacterial populations for different treatments. It can be seen from Fig. 3 that the interpretation degree of the sample composition difference in the first axis is 77.35%, and it is 7.66% in the second axis. The CK processing is mainly concentrated in the 1st and 2nd quadrants, CN15 is in the 1st and 3rd quadrants, CN20 is in the 1st quadrant, CN25 is in the 3rd quadrant, and CN30 is in the 4th quadrant, indicating the compositions of soil species are significantly different after the addition of materials with different C/N ratios. Moreover, the difference in the material composition between CK and CN30 treatments is particularly obvious.

Table 2
α diversity of soil bacterial community

Treatment	Shannon index	Simpson index	Chao index	ACE index
CK	5.5835 ± 0.21b	0.9973 ± 0.05a	4906.41 ± 255.47b	3720.83 ± 0.034b
CN15	6.4653 ± 0.23a	0.9981 ± 0.03a	4929.47 ± 354.25b	3883.21 ± 0.044a
CN20	6.5195 ± 0.21a	0.9991 ± 0.09a	4987.62 ± 231.47b	3885.23 ± 0.03a
CN25	6.5341 ± 0.22a	0.9993 ± 0.03a	5075.40 ± 231.33b	3911.21 ± 0.019a
CN30	6.6388 ± 0.19a	0.9984 ± 0.04a	5328.14 ± 297.14a	3898.05 ± 0.029a
Remark:Within each column, means followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$				

3.5 Effects of organic materials with different C/N ratios on the bacterial community structure

Figure 4 illustrates that the dominant floras at the level of phylum for each treatment are *Actinobacteria*, *Proteobacteria* and *Chlorobi*. For *Actinobacteria*, the relative abundance relationship is CN30 > CN25 > CN15 > CN20 > CK with CN30 increasing by 5.24%-6.47% and significantly higher than the other treatments. Compared with CK, CN20 and CN25 treatments show a relative decreased abundance of *Proteobacteria* by 0.89% and 0.31% respectively. CN30 shows the lowest relative abundance of *Chlorobi* (7.14%) while CN20 shows the highest (11.12%). Compared with the CN15 treatment, CN20 increases by 2.11% and CN30 decreases by 1.87% compared with the CK treatment. The relative abundance of *Acidobacteria* shows a trend of first decreasing and then increasing with CN20 showing the highest relative abundance (12.45%).

The heatmap can use the color changes to reflect the difference information of data between the treatments and it can intuitively represent the size of data values with the defined color shades. As shown in Fig. 5, the samples are classified and aggregated according to the similarity of abundance. It is found that the CK treatment significantly inhibits the abundance of *Bdellovibrionota* and *Nitrospirta*. CN30 is more conducive to the growth and reproduction of *Proteobacteria* and *Firmicutes*, but exhibits a substantial inhibitory effect on the Methylomirabilota's growth and reproduction.

3.6 Soil bacterial community LEfSe analysis

To explore the relationship between the specific related bacterial taxa with the different C/N ratios, the differences in the enrichment of bacterial communities across treatments at different taxonomic levels (phylum-genus) were compared using linear discriminant analysis (LDA) effect size (LEfSe). As shown in Fig. 6, there are 30 bacterial clades statistically significantly different with an LDA score of 2 or higher. The enrichment degree of 15 bacterial taxa such as *Themolepholla*, *Solirubrobacterales*, *Gaiellaceae*, *Galella*, *Gammaproteobacteria* in the CN15 treatment is significantly higher than that in other treatments. *Anaerolineae* is significantly enriched in the CK treatment. The enrichment of 11 bacterial groups in the CN30 treatment such as *Firmicutes*, *Bacilli*, *Bacteroidota*, *Bacteroidia*, etc. is significantly higher than that in other treatments. *Plantomycetota* is significantly enriched in the CN25 treatment and *Chloroflexi* is significantly enriched in the CN20 treatment.

3.7 Correlation between the soil dominant flora and the environmental factors

In the association study of the soil bacterial dominating flora and the environmental parameters (Fig. 7), pH and NB1-j flora abundance show a strong negative link. The soil bulk density is significantly positively correlated with Dependentiae, RCP2-54, *Nitrospiota*, *Bacteroidota* and *Myxococcota*. However, the relationship between the porosity and the dominant flora is opposite to that of bulk density. *Patescibacteria* shows a significant positive relationship with C_{total}, N_{total}, N_{mic} and a stronger positive correlation with C/N, Cmic, C_{mic}/N_{mic}. N_{total} shows a very significant positive correlation with *Planctomycetota* but a significant negative correlation with *Bacteroidota* and *Myxococcota.* In addition, C/N, C_{mic}/N_{mic} and NB1-j all show a significant negative correlation. It can be seen that the main environmental factors affecting the abundance of bacteria in different treatments are bulk density, porosity, C/N and C_{mic}/N_{mic}.

3.8 Effects of organic materials with different C/N ratios on the agronomic traits of tobacco plants

As shown in Table 3, the treatments with different C/N ratios increase the plant height, leaf length, leaf width and stem circumference of tobacco plants to different degrees. Except for the leaf width, the other three parameters are significantly different from CK. Between CN15-CN30, there is no discernible variation in plant height but the increase

compared with CK follows a sequence of CN30 > CN20 > CN25 > CN15. The leaf length and the stem circumference in CN30 are higher than the other treatments while the leaf width of CN25 is substantially higher than that of the rest treatments, with an increase of 16.05%, 7.64%, 5.79%, 9.34% and 12.92%, respectively.

Table 3
Effects of material carbon nitrogen ratio on agronomic characters of tobacco plants

Trentments	Plant height	Leaf length	Leaf width	Stem girth
CK	110.56 ± 2.23b	66.03 ± 1.15c	30.33 ± 0.87b	9.50 ± 0.44b
CN15	118.23 ± 1.99ab	70.66 ± 1.35bc	32.70 ± 0.54b	10.63 ± 0.68ab
CN20	124.4 ± 1.98a	70.06 ± 1.53bc	33.16 ± 1.12b	10.21 ± 0.49ab
CN25	123.36 ± 2.03a	72.10 ± 0.85b	35.20 ± 0.87a	11.06 ± 0.33ab
CN30	129.36 ± 1.67a	81.66 ± 1.88a	31.17 ± 0.85b	11.53 ± 0.57a
Remark:Within each column, means followed by different letters are significantly different according to Tukey's test at $p \leq 0.05$				

4 Discussion

4.1. Effects of C/N ratio of organic materials on the soil physicochemical properties

The treatments of organic materials with different C/N ratios significantly affect the bulk density, porosity and water content of sandy loam and the three groups of physical property values all show the linear changes with the C/N ratio. The increases of the C/N ratios enhance the soil porosity and water content, whereas the decreases of C/N ratios lower the bulk density. This might be attributed to the fact that the different organic materials show different effects on the soil improvement by their own structural characteristics, or different C/N ratios have different effects on the soil microbial activity (Birk et al. 2008).

The composition of organic materials determines the rate of nutrient decomposition and nutrient utilization (Li and Lin 2021). The soil bulk density and porosity can be significantly improved by the treatments with different C/N ratios. When the soil bulk density decreases and the porosity increases, the soil aeration and permeability increase, accelerating the metabolism and reproduction rate of soil microorganisms. With the suitable soil temperature and humidity conditions, the activities of most microorganisms are enhanced, thereby improving the rate and efficiency of soil nitrogen mineralization and explaining the reason why organic materials increase the total nitrogen and the microbial biomass nitrogen content in the soil (Wang et al. 2020). Previous studies show that the nitrogen available to crops and microorganisms depends on the C/N ratio of organic materials and the low ratio (C/N < 20) leads to a net nitrogen mineralization which increases the content of mineral nitrogen in soil (Hadas et al. 2004). In contrast, the organic compounds with a high C/N ratio result in the net nitrogen fixation (Moritsuka et al. 2004). This is consistent with the findings of this study that CN30 lowers the soil total nitrogen content and CN25 and CN30 decrease the soil microbial biomass nitrogen compared with CN20. However, further analyses of the changes in different nitrogen components are required to clarify the effects of organic materials with different C/N ratios on the nitrogen mineralization and fixation. In blends, the breakdown of low-nitrogen nutrients is stimulated by the high-nitrogen nutrients (Chapman and Koch 2007). In this study, the organic materials with high C/N ratios tend to release or retain more nitrogen, while Schwendener et al. label the litter with a low C/N ratio with ^{15}N, indicating that nitrogen

is transferred from the litter with a low C/N ratio to the litter with a high C/N ratio (Schwendener et al. 2005). Similar results are also reported by Taylor et al. that the amount of carbon in the soil is inversely proportional to the amount of nitrate nitrogen in the soil and the higher carbon content leads to a stronger ability for the soil to keep nitrogen, reduce the nitrogen flowing into the water, and clean the the water (Taylor and Townsend 2010). In terms of the relationship between carbon and nitrogen elements, it can be seen from the Person correlation analysis (Table 1) that C/N and C_{mic}/N_{mic} are the environmental factors most close to other physical and chemical parameters. In general, the relationship between the soil physicochemical properties and organic material with different C/N ratios is aligned with "Experimental Hypothesis 1" in this study, suggesting that the C/N ratio has a substantial positive link with the impact of improving the soil physical and chemical characteristics.

4.2 Effects of organic material with different C/N ratios on the soil bacterial community structure

Soil microorganisms are important factors in the soil ecosystems, affecting the crop root metabolic activities, the soil structure formation, and the fertility transformation. Previous studies of investigating the exogenous organic materials on the soil bacterial community structure were mostly focusing on the addition of a single material while limited studies reported the mixed organic materials with different C/N ratios. For example, the addition of biochar and wheat straw to the soil bacterial community may have an influence on the structure and diversity of the community and increase the diversity and richness of the bacterial community (Li et al. 2020; Cmh et al. 2020). The addition of exogenous organic materials can promote the growth of some bacteria and also inhibit some other bacteria's growthm, which results in a shift in the bacterial community structure of the soil (Niu et al. 2021). From the diversity index table (Table 2), the CN20 treatment improved the variety and richness of the soil bacterial populations to the greatest extent. The top 3 dominant species at the phylum level for each treatment are *Actinobacteria*, *Proteobacteria*, and *Chlorobi* (Fig. 4), among which, the relative abundance of *Actinobacteria* in the CN20 treatment is substantially higher than that in the other treatments. In addition, CN20 is more conducive to the growth and reproduction of *Proteobacteria* and *Firmicutes* while it shows a considerable inhibited effect on the growth and reproduction of *Methylomirabilota* (Fig. 5). The C/N ratio gradient used in this study was from 15 to30 and the results demonstrated that the C/N ratio of organic materials influencing on the soil bacterial community is not in a simple linear relationship, but also restricted by soil types, material compositions and other environmental factors (Marschner et al. 2001). *Actinomycetes* are the largest group of bacteria at the bacterial phylum level and crucial to the breakdown of organic materials and the production of humus. (Sun et al. 2014). At the same time, it allows a range of medicines to protect the soil and plant roots from the harmful microbes (Wang et al. 2017). The majority of *Proteobacteria* can use benzoic acid, salicylic acid and other substances as the sole carbon source to degrade the soil toxic substances and inhibit the growth of plant pathogens (Wang et al. 2020). *Chloroflexi*, named for its green pigment, exhibits the ability to breakdown cellulose and is strongly positively linked with the water retention capacity and the above-ground biomass (Podosokorskaya et al. 2013).

4.3 The relationship between the soil bacterial community structure and the physicochemical properties

Changes in any environmental condition may alter the organization of the soil microbial community to some extent (Rillig et al. 2021). Physicochemical qualities of soil, for instance, pH, water content, organic matter, and soil nutrients, are all important factors affecting the soil microbial communities (Lin et al. 2021; Fahey et al. 2020). The heat map of the correlation between soil dominant flora and environmental factors (Fig. 6) shows that the environmental factors affecting the abundance of dominant bacterial flora in different treatments are mainly the soil bulk density, porosity, C/N and C_{mic}/N_{mic}, which was identical to Huang et al.'s findings (Huang et al. 2012). The C/N

in soil is relatively stable. So when the exogenous organic materials enter the soil, it causes a strong competition between crops and microorganisms for the effective carbon and nitrogen sources. From the perspective of study, this competition level is not only affected by the C/N ratio of the material itself, but also significantly affected by the changes in the soil physicochemical properties after the exogenous additives applied to the soil. The impact of soil pH on the bacterial population is found to be insignificant in this work. This might be mainly due to the fact that different treatments do not cause significant differences in soil pH, thus leading to an invisible influence on the bacterial population in the soil.

4.4 The mechanism of organic material with different C/N ratios influencing on the soil physicochemical properties and bacterial community

Various studies indicated that the addition of exogenous high-carbon materials benefits the soil physicochemical properties and microbial community structure and the improvement effect varies from the materials (Li et al. 2021; Pei et al. 2020; Ma et al. 2020). In this sense, the influencing mechanism of the C/N ratio of the material is still unclear. In this study, materials with different C/N ratios improve the soil physical environment such as bulk density, porosity and water content and the C/N ratio is frequently favorably connected with this beneficial impact. The microorganism reproduction is significantly influenced by the improvements in physical and chemical features and based on the results, the CN30 treatment shows the most obvious improvement effects on the related parameters of bacterial community structure. It might be since the CN20-CN30 treatments present no significant effect on the soil PH which significantly affected bacterial community structure. So, the ability of other physicochemical properties affecting the bacterial community is particularly prominent. Meanwhile, the growth and reproduction ability of microorganisms are affected by the physical properties of the soil. Moreover, the energy and nutrients required for the growth and reproduction of microorganisms are more directly related to the soil C/N ratio, especially the microbial biomass C/N ratio. In this work, the CN30 treatment presents the most significant increase in the soil microbial biomass C/N ratio, suggesting that the carbon, nitrogen and nitrogen sources available to microorganisms in this treatment are the most abundant. In summary, the stoichiometric ratio of carbon and nitrogen in organic materials influences on the change of the soil bacterial community structure as well as their physical and chemical properties, with which are the most dramatically changed by CN30.

5 Conclusion

Various C/N ratios in the organic compounds improve the soil physicochemical properties and the improvement is enhanced with the increase of C/N ratio. The most important parameters influencing on the soil physicochemical qualities and correlated with the bacterial populations are C/N and C_{mic}/N_{mic}. The CN30 treatment shows the most obvious effect on the improvement of bacterial diversity index and beneficial flora. In the future,, further studies can be performed using the different material combinations and the long-term positioning to verify and clarify the effect of organic materials with different C/N ratios on the soil physicochemical properties, the microbial community structure and its mechanism.

Declarations

Declaration of competing interest

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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Figures

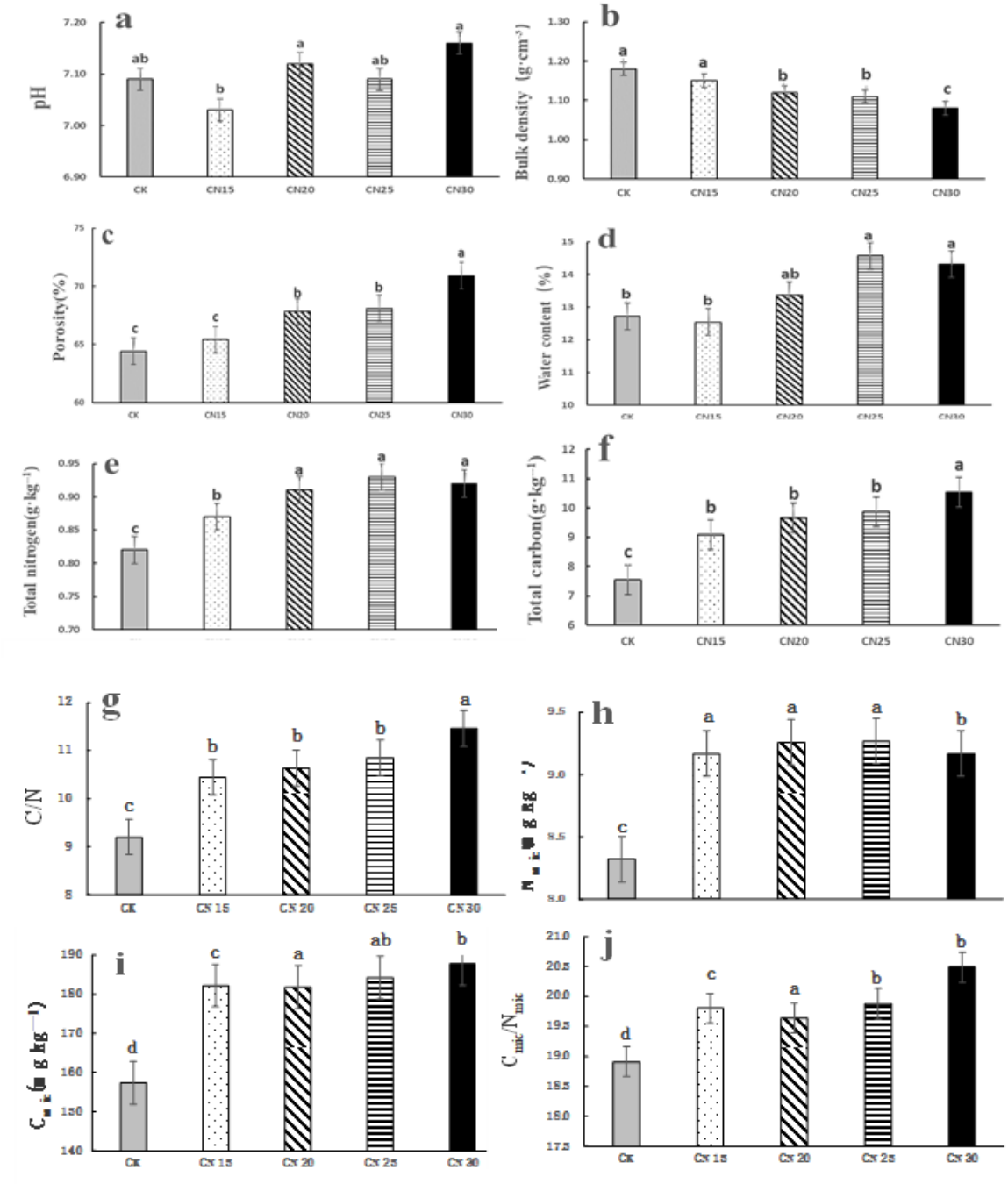

Figure 1

Effects of carbon nitrogen ratio on soil physical and chemical properties.

Remark:The different letters above the bars indicate significant differences ($p < 0.05$) among different treatments Cmic, Nmic indicate soil microbial biomass carbon, soil microbial biomass nitrogen, soil carbon content respectively.

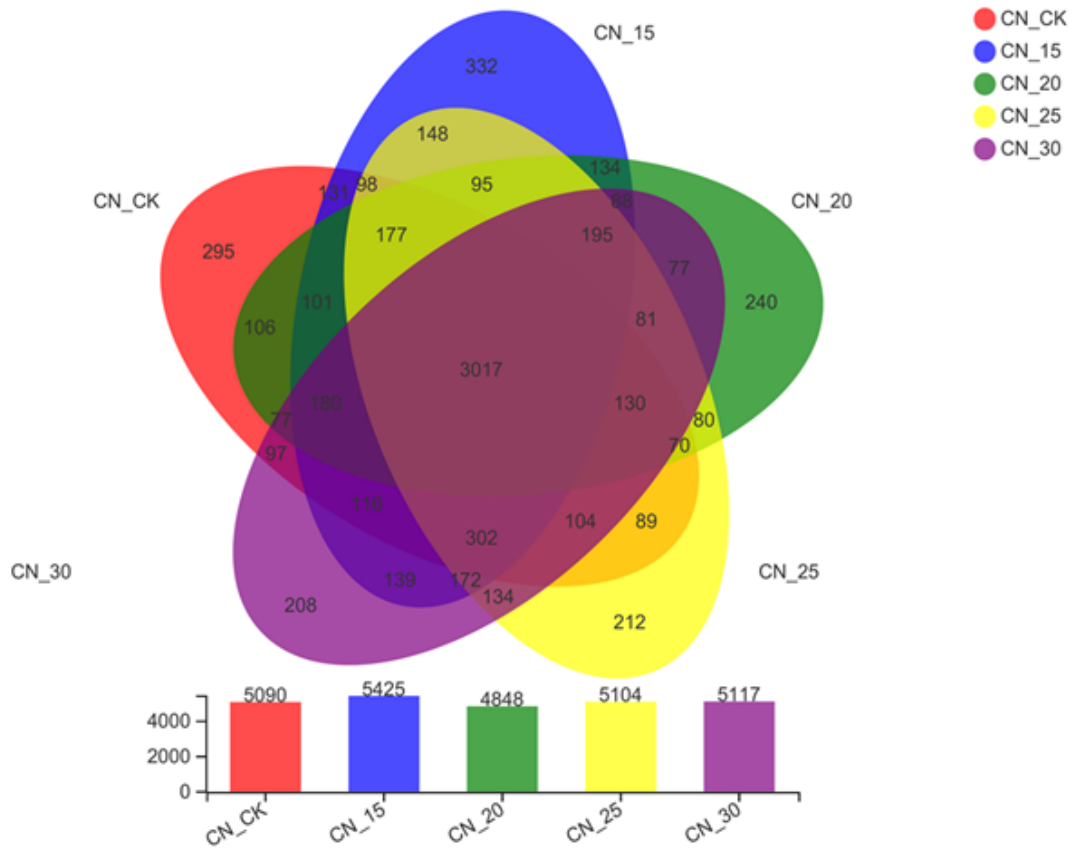

Figure 2

Venn graph analysis of the core OTUs treated with differernt C/N ratio organic materials.

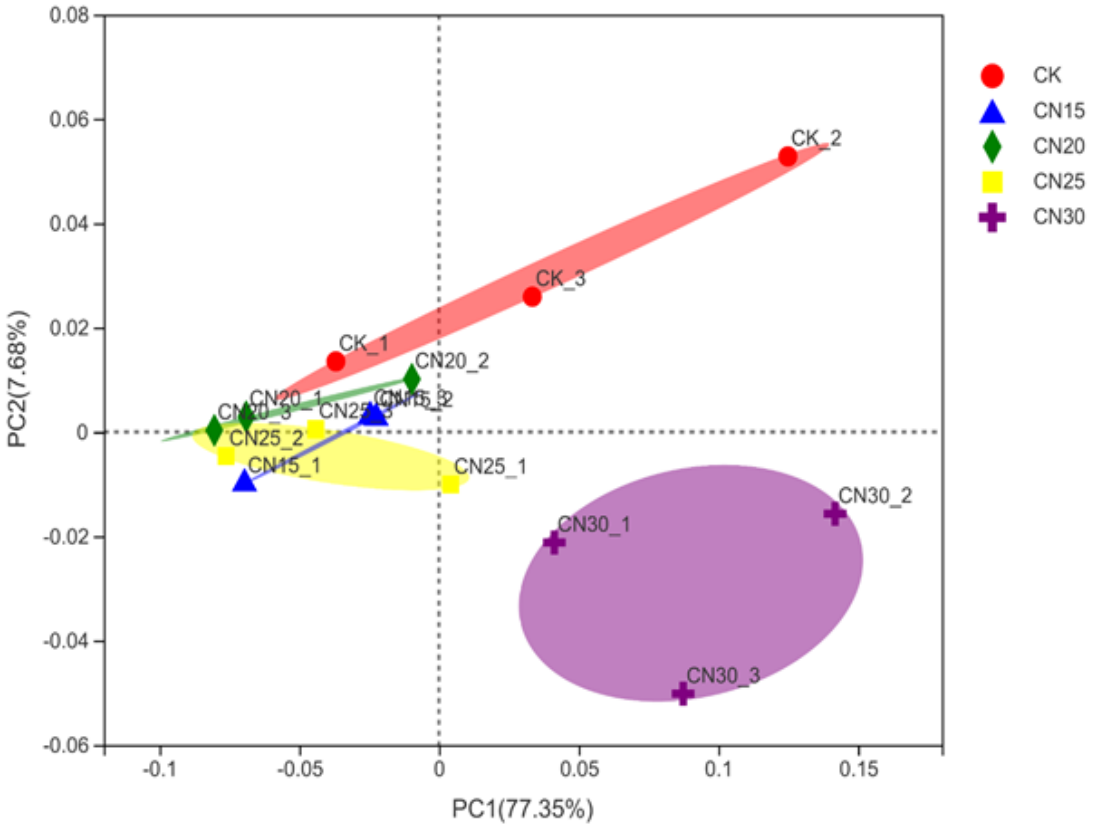

Figure 3

Principal co-ordinates analysis (PcoA) of soil bacterial communities in different treatments

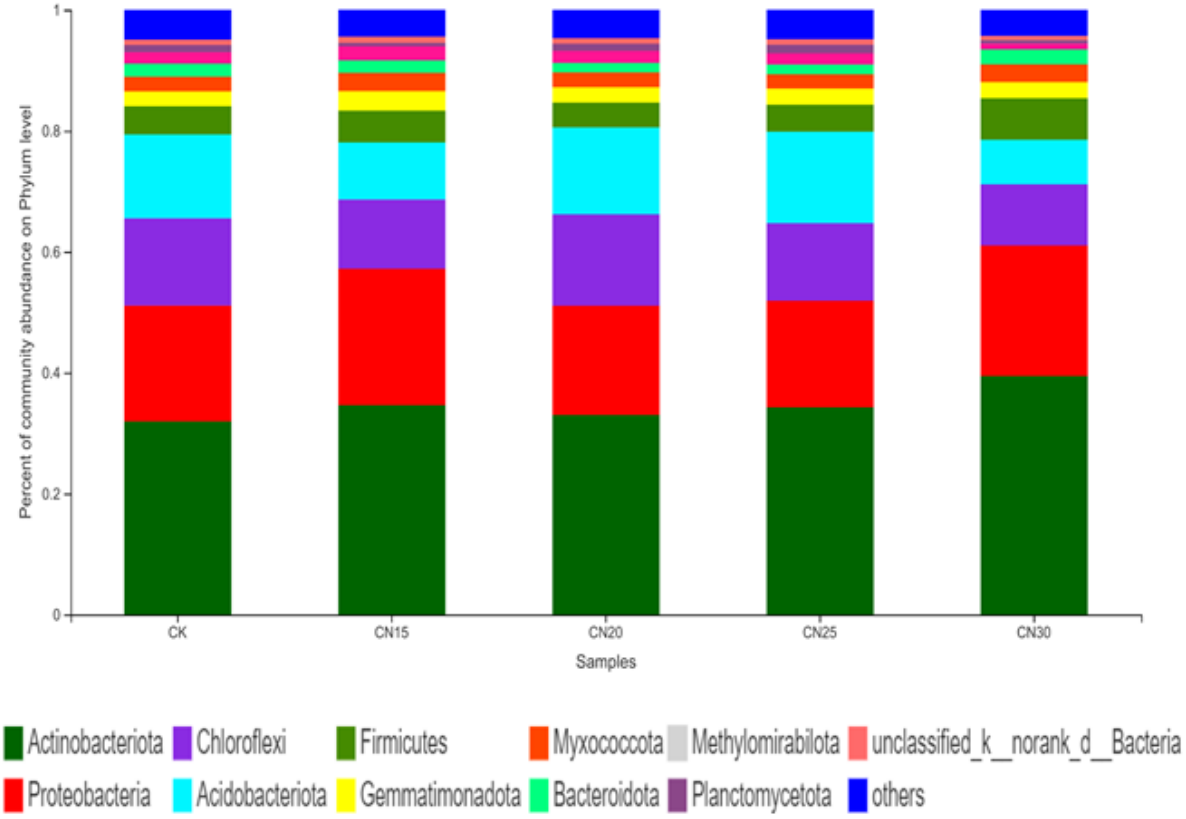

Figure 4

Composition of bacterial community in rhizosphere soil at phylum level

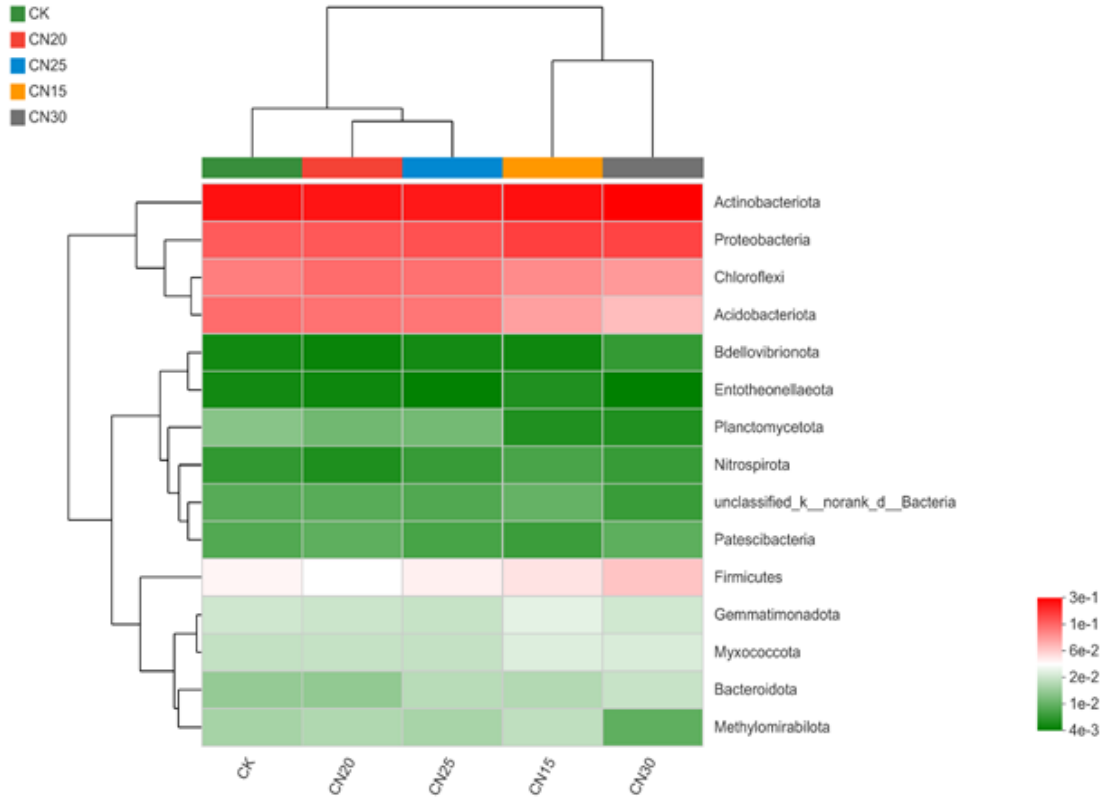

Figure 5

Heatmap map of the top 15 species with the highest abundance at the phylum level

Figure 6

LEfSe analysis of bacterial community in rhizosphere soil

Figure 7

Correlation heat map of the top thirty phyla and soil properties

Remark: The value of P<0.05 is marked with “*”, * 0.01<P≤0.05, ** 0.001<P≤0.01, and *** P≤0.001.

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