# Predicting the humification degree of multiple organic solid waste during composting using a designated bacterial community

**Hong-Yu Yang**
Northeast Agricultural University
**Shu-Bo Zhang**
Northeast Agricultural University
**Han-Han Meng**
Northeast Agricultural University
**Yue Zhao** ( zhaoyue@neau.edu.cn )
Northeast Agricultural University
**Zi-Min Wei** ( weizimin@neau.edu.cn )
Northeast Agricultural University
**Guang-Ren Zheng**
Northeast Agricultural University
**Xue Wang**

**Research Article**

**Keywords:** Bacterial community, Biological indicator, Humification degree

**Posted Date:** March 16th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-328051/v2

# Abstract

Microbes are the drivers for disposing of organic solid waste (OSW) during composting. Moreover, humus is a multipurpose biological macromolecule synthesized by microorganisms during composting. Here, we investigated the humification degree (HD), and the humus content was assessed in terms of the bacterial community. Microbial sequencing and bioinformatics approaches were combined to analyze the biological characteristics of 105 composting samples. The bacterial communities were useful indicators for making predictions and even correctly determined the categories of OSWs with 94% accuracy. Furthermore, the bacterial genera screened were designated as a bacterial code. The bacterial codes can also provide a better prediction of HD. Our results suggested that the bacteria code is a reliable biological method to assess HD effectively. Bacterial codes can be used as ecological and biological indicators to evaluate the quality of composting of different materials.


# Introduction

Composting is a significant measure to achieve organic solid waste (OSW) resources, reduction, and stabilization(Wei et al., 2022). Microbial metabolism plays a major role in the high-efficiency, rapid humification(Chen et al., 2015; Liu et al., 2021; Lu et al., 2013). Humic substances are ubiquitous natural compounds arising from biological degradation of waste during composting(Khoshnevisan et al., 2021; Lu et al., 2013). The quantity of humus and the humification degree (HD) affect the stability of the composting and determine the quality of the final product(Senesi & Plaza, 2007). Therefore, the HD and humus content are essential indicators for evaluating the quality of humification products.

The HD is a comprehensive indicator of the final quality of compost products and a key evaluation indicator to influence the safe application of compost products. Various nonbiological indicators for the traditional evaluation of the HD include physical indicators, chemical indicators, and spectroscopy indicators(Ikeya et al., 2019; Medina et al., 2020). However, physical and chemical indicators are limited in measuring the HD, and spectral analysis methods cannot provide comprehensive information, so they are not enough to quickly and accurately determine the stability of organic matter(Medina et al., 2020). Therefore, it is crucial to find a biological index to efficiently evaluate humification products. In addition, previous studies have explored the response relationship between microorganisms and HD in composting(Gong, 2007; Wu et al., 2020a). Most of them reveal the response mechanism of only a single material rather than predicting and evaluating the HD for different composting materials based on microbial community data(Wu et al., 2020a; Zhu et al., 2019a).

With the development of sequencing technology, high-throughput sequencing technology has been applied more widely in composting research(Van Rossum et al., 2020). A mushrooming number of statistical methods based on bacterial communities have emerged, especially those related to machine learning(Ye et al., 2020). Random forest has been verified as a better machine learning model to determine the responses of bacteria to environmental factors and offers a straightforward and well-documented approach for creating predictive tools(Kim et al., 2020). Importantly, there is ample evidence

that bacterial communities directly or indirectly respond to changes in the environment(Awasthi et al., 2020; Yuan et al., 2020). The bacterial data did not always classify a site as outside of target ranges when the chemical data indicated that the chemistry changes did not always affect the biological communities in the same way.

This study investigates the bioindicator ability of bacteria during composting by high-throughput sequencing for five kinds of OSW (chicken manure (CM), cow dung (CD), sewage sludge (SS), garden waste (GW), and rice straw (RS)). Bacterial diversity analysis suggests that there are similarities in bacterial communities during composting, which we call conserved bacteria. Conserved bacteria and the key divergent bacteria obtained by statistical screening constitute microbe indicators that can characterize the composting of different OSWs. Random forest method to identify significant relationships between the bacterial community and the types of OSW in composting. In addition, the humification indicators were predicted. Our study establishes bacterial codes that have a better performance in predicting humification and are proposed as bioindicators to evaluate the HD.

# Materials And Methods

*2.1. Humification samples.*

A total of 105 humus samples of five OSWs were obtained from the Laboratory of Environmental Microbiology of Northeast Agricultural University, China, in this study. CM and CD samples were collected from the College of Animal Science, Northeast Agricultural University, and SS was from the Harbin sewage treatment plant; the SS samples were dried and processed into grain sizes ranging from 10 mm-20 mm. The RS samples were taken from Xiangyang Farm, and the GW samples were taken from the mixed forest of Northeast Agricultural University and cut into fragments of approximately 20 mm. These materials were aerobically fermented in a separate reactor(Zhu et al., 2020). For sufficient humification, the initial moisture content of the solid organic waste was maintained in the range of 60–65%, the C/N ratio was 25:1, and the reactor ventilation rate was 0.5 L/min(Cui et al., 2017). The piles were turned over at each sampling time to ensure a steady supply of oxygen. Some samples were frozen at -20°C for microbiological analysis, while others were air-dried for the determination of humic acids (HA), fulvic acids (FA), organic matter (OM), and total organic carbon (TOC). TOC was estimated using the potassium dichromate volumetric method.

*2.2. Humus analysis.*

Dry samples (10 g) were evenly mingled in a mixed solution of 0.1 M $Na_4P_2O_7 \cdot 10H_2O$ and NaOH at a ratio of 1:10 (w/v). The mixture was shaken at room temperature for 24 h and centrifuged at 10,000 rpm at 4°C for 20 minutes. Therefore, the obtained supernatant was filtered through a 0.45 μm Millipore membrane, and the limpid liquid obtained was a humus solution. The pH of the solution was adjusted to 1 by 6 M HCl, and it was incubated at 4°C for 12 h. The solution was centrifuged at 10,000 RPM for 10

min. The FA solution was obtained by purifying the supernatant with XAD-7 exchange resin. After washing with 0.1 mol/L HCl solution three times, the precipitate was dissolved in 0.05 mol/L NaHCO3 to obtain HA solution. All samples were analyzed in triplicate, and the mean was calculated to reduce the error. A Shimadzu TOC-Vcph analyzer was applied to detect TOC, HA, and FA(Wu et al., 2020b). The humification indices were calculated according to the following formulas(Baffi et al., 2007; Ren et al., 2021):

Humification Index(HI)= $C_{HA}$/TOC×100;

Degree of Polymerization (DP)=$C_{HA}/C_{FA}$.

*2.3. High-throughput sequencing of 16S rRNA.*

Sample DNA was extracted with a DNA kit (Omega Biotek, Inc.). A NanoDrop Spectrophotometer (Gene Co. Ltd, Shanghai, China) was used to evaluate DNA quantity and quality. To investigate the bacterial structure of various organic solid wastes, the 16S primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) were used to amplify the V3-V4 regions of the bacterial 16S rRNA gene. The samples were finally sequenced on the Illumina HiSeq platform (Novogene Bioinformatics Technology Inc., Beijing, China)(Chen et al., 2022).

*2.4. Network analysis.*

To determine the relationship between the bacteria of different solid organic wastes, network analysis was used to describe the underlying co-occurrences among bacteria(Cardona et al., 2016; Rottjers & Faust, 2018). Network analysis was performed at the genus level, and the results are shown at the phylum level. Data filtering was performed before network construction with the 'Hmisc' package of R. The genera whose relative abundance sums were less than 0.005 were removed to reduce the computational complexity and ensure the accuracy of the calculation, avoiding the calculation error caused by a null value(Barberan et al., 2012). Spearman's correlation was used to analyze all pairwise associations(Awasthi et al., 2021b; Wu et al., 2022).

To obtain the genus with a higher correlation coefficient, the genus with a lower Spearman coefficient ($r$<0.8) was eliminated. The less-significant correlations were removed by R software, and only significant correlations ($P$<0.05) were retained for the downstream procedure(Soffer et al., 2015). The Benjamini-Hochberg multiple test was used to correct the $P$-values and adjust the false discovery rates (FDRs), and the chance of false rejection of the null hypothesis was not more than 0.05(Faust et al., 2012). The correlation results were imported into Gephi (0.9.2) and visualized with Fruchterman-Reingold algorithms. The network density, clustering coefficient, and other topological parameters of the network and the degree of each node in the network were calculated by Gephi. The clustering coefficient was used

to reflect the degree of embedding between a node and its neighbors, that is, the degree of clustering between nodes. The network density represents the degree of aggregation and the degree of connection tightness of nodes. The degree was used to search the key hubs in the network(Louca et al., 2016; Sporns et al., 2007). A higher degree for a node indicates that there are more nodes connected to it in the network. When the node changes, it will affect more nodes, making the network structure more likely to change, and highly connected hubs are also more likely to become key hubs in the network.

### *2.5. Function prediction for bacteria.*

Functional annotation of prokaryotic taxa (FAPROTAX) was used to evaluate bacterial communities' potential functions and divide OTUs into one or more functional groups that contain currently culturable bacteria based on existing literature(Liang et al., 2020). FAPROTAX contains more than 7600 functional annotations from more than 80 functional groups (e.g., xylanolysis, nitrification, methanol oxidation). Variations in specific strains within non-cultivable species were ignored; for example, the cases where horizontal gene transfer led to the differentiation between strains. This may be closely related to the degradation of different carbon compounds, which is why some carbon metabolism pathways were not divided (similarly for fermentation)(Louca et al., 2016). However, other functions were more conserved, such as those associated with nitrification, denitrification, or sulfate respiration(Ma et al., 2020). The method was run by matching the annotation information of the OTUs based on 16S rRNA sequencing with the information database through Python and outputting the predicted function (http://www.zoology.ubc.ca/louca/FAPROTAX/lib/php/index.php?p=Instructions).

### *2.6. Prediction of humification indices.*

A random forest method was used to predict the humification indices in composting and evaluate the of different organic solid wastes. Random forests have been confirmed by previous studies to be superior to other modeling methods when applied to environmental bacterial communities(Smith et al., 2015). All the samples contained a total of 988 genera after rarefaction. To reduce the number of explanatory variables entering each random forest model, the two key categories of genera (CB and KDB) provided by multiple methods in this study that best represented various samples were finally screened. The random forest method was performed by the 'randomForest' package with default parameters.(Hermans et al., 2020) Linear regression models were used to evaluate the accuracy of random forest prediction of the maturity of the material. The closer $R^2$ is to 1, the smaller the deviations of the predicted values. The closer the slope of the equation is to 1, the closer the predicted value is to the actual value.

### *2.7. Statistics analysis.*

All statistical analyses and data visualizations were performed in the R platform (4.0.2). Canoco 5 was employed to perform NMDS analysis based on OTU data of different samples, and they were visualized by OriginPro 2019. The alpha diversity of different materials in the aerobic composting process was visualized by the 'vioplot' package in R. Kruskal-Wallis tests were used to determine whether there were differences in alpha diversity among multiple materials(Coelho et al., 2021). Permutation multivariate analysis of variance (PERMANOVA) was calculated by the 'vegan' package of R software. PERMANOVA was used to determine differences in bacterial communities among different organic solid wastes.(Guo et al., 2019) Bray-Curtis distance was utilized by PERMANOVA to calculate the differences in bacterial communities, whether it was at the overall level or pairwise comparisons. Linear discriminant analysis effect size (LEfSe) (Zhou et al., 2022) was used to determine the biomarkers (according to the biosystematics, they are divided into 6 categories from the kingdom level to the genus level) of five different organic solid wastes, that is, the significant divergent bacteria between a material and the others, which was based on $P<0.05$ and $LDA>2.0$(Hermans et al., 2020; Segata et al., 2011).

# Results

*3.1. Analysis of bacterial diversity.*

This study indicated that the diversity and community structure of bacteria were significantly different for various OSWs (see supplementary files). However, NMDS analysis also showed that regardless of the plane, the projections of the bacterial communities had certain overlaps (see supplementary files). Therefore, composting products could be evaluated, both divergent bacteria and conserved bacteria should be considered during the composting of all OSWs.

*3.2. Bacterial communities diversity and conserved bacteria.*

We compared the richness and diversity of bacteria from different OSWs during composting. Four kinds of alpha diversity were analyzed with the Kruskal-Wallis test (see supplementary files). The Shannon index and Simpson index of bacteria were significantly different between these OSWs. The alpha diversity of RS significantly differed from those of CM and SS, and the alpha diversity in CD and SS was very notably different. ACE and Chao1 index analysis results showed that the richness of bacteria in CD was significantly different. In contrast, the alpha diversity of bacteria in GW was relatively similar to those of other OSWs.

Nonmetric multidimensional scaling (NMDS) showed that the differences in SS were smaller than those in other OSWs, whereas the differences in CD were larger. There were significant differences ($p<0.001$) in the bacterial communities between OSWs (see supplementary files). An effect on the microbial community structure during composting was also illustrated due to OSW heterogeneity. Moreover, the bacterial community structure had more significant differences between SS and the other OSWs, with $R^2$ values of 0.244-0.387. In contrast, the community structure was relatively similar between GW and

other OSWs. Some similarities can still be revealed in various OSWs where projections overlapped in NMDS (see supplementary files).

To explore the conserved bacteria in all OSWs, OTUs were classified into 988 genera. The phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria were found (Fig. 1 a, c, e, g, i) with high relative abundances in all OSWs. The samples of all OSWs were classified similarly at the phylum level. However, the relative abundances at the phylum level varied greatly.

In the composting of both RS and CD, the relative abundance of dominant genera (except Others) was approximately 20%. In the composting of SS, the diversity of bacteria was lower. Moreover, the relative abundances of microorganisms in the same sample changed little, and the organisms were relatively stable at the genus level (Fig. 1, b, d, f, h, j). Ten genera of bacteria were present in 70% of the samples with high relative abundances (Fig. 1). These bacteria were named as “conserved bacteria” (CB).

*3.3 Divergent bacteria.*

To identify the divergent bacteria, some bacterial genera were selected with LEfSe statistical analysis (see supplementary files). We summarized the bacteria belonging to the different groups in various OSWs (see supplementary files). Too many biomarkers obtained from the LEfSe analysis were not conducive to subsequent research. Therefore, we conducted a further classification for divergent bacteria to identify the critical divergent bacteria in various OSWs.

*3.4 Divergent hub bacteria.*

To determine which genera significantly interact with other bacteria, we constructed five networks for bacterial communities for different OSWs (Fig. 3). Overall, positive correlations were more significant than negative correlations among all OSWs (Table 1). The network density of CD was 0.169, and the CD network had a higher number of nodes and edges (184 and 2852, respectively) than the networks of other materials, indicating that the bacterial communities of CD were the most complex and structurally stable. The node number of SS was only 47, with fewer members at the phylum level than at the other levels. Nevertheless, its network density was 0.101 and close to that of GW (0.105) while being larger than those of RS and CM. These results indicate that the SS bacterial community composition was simpler than others. However, the bacterial community was more tightly linked, which was conserved with the NMDS analysis. Although the number of RS nodes was 99, the lowest network density was only 0.054, implying that the RS bacterial community was looser.

The hub bacteria were selected based on a top 10% ranking at the genus level for various OSWs. Hub bacteria may have enormous impacts on bacterial community composition. For example, *Haliangium* was ranked in the top 10% of genera in CD and was considered one of the hub bacteria in the CD bacterial community. The hub bacteria were combined with LEfSe analysis to simplify the divergent hub bacteria (DHB). Moreover, the DHB differed significantly between the various OSWs, suggesting that they may influence bacterial community structure differences.

*3.5 Divergent functional bacteria*

FAPROTAX was applied to predict the functions of bacterial communities. We found that the bacterial communities in every OSW covered roughly the same functions with 30 basic functions. Nevertheless, there were also slight differences between OSWs. Bacterial communities in GW covered more functions, followed by those in RS, CD, CM, and SS (see supplementary files). We observed that the composting mainly involved three major functional groups: C metabolism (including lignin decomposition, cellulose decomposition, etc.), N metabolism (including nitrification, denitrification, etc.), and fermentation. Therefore, the FAPROTAX results are simplified into these three aspects. (see supplementary files).

Our results indicated that the bacterial community also demonstrated functional diversity (Fig. 5a). CM and RS emphasized fermentation more than carbon and nitrogen metabolism. In contrast, the bacterial community of CD invested more in carbon and nitrogen metabolism capacity. For SS, carbon metabolism capacity was stronger than composting and nitrogen metabolism, and nitrogen metabolism was weaker. Moreover, GW was more balanced overall, with carbon and nitrogen metabolism roughly the same, and fermentation was slightly more dominant than carbon and nitrogen metabolism.

The results of FAPROTAX analysis were combined with the results of LEfSe analysis to screen out the divergent functional bacteria (DFB) for each OSW. These genera may contribute to the significant differences among OSW bacterial community functions.

*3.6 Constructing bacterial codes*

First, divergent functional bacteria and divergent hub bacteria were combined as key divergent bacteria (KDB). Then, KDB and CB were combined from bacterial codes for various OSWs. The bacterial codes contain the CB that are stably present during composting, the divergent hub bacteria that occupy critical positions in the community structure, and the divergent functional bacteria that are functionally different for different OSWs. That is, we grouped all bacteria capable of characterizing the overall bacterial profile of composting. *Random forest prediction*

After obtaining the bacterial codes of various OSWs in this experiment, the random forest model was applied to verify the feasibility of using the bacterial codes. All microbial OTUs at the genus level were input into the random forest model (Fig. 5). The results showed that the bacterial communities could correctly determine the categories of OSWs with more than 94% accuracy. In addition, the bacterial community could be used to predict and assess composting with a random forest model.

# Discussion

Determining the HD of products has been a major concern for researchers in composting(Guimaraes et al., 2013; Kawasaki et al., 2008; Zhao et al., 2017). In general, researchers use fundamental physicochemical indicators to determine the HD of composting products(de Gannes et al., 2013). Hence, the introduction of biological indicators is indispensable for the evaluation of the HD of fermented

products. Here, bacterial codes are developed for bacterial communities in different materials. Bacterial codes can evaluate the HD of different materials more accurately in this study. Moreover, a random forest model was used to predict the quality of the composting products. Collectively, the whole bacterial code recognition and prediction system can be used as a biological tool to evaluate the quality of fermentation of different materials and control fermentation cycling. Moreover, this system can also be used for other biological processes related to microbial reactions. For example, it can evaluate the hazard level of pollutants in soil or water and assess the risk of intestinal microbial diseases.

We introduce the microbial community as an unknown parameter to investigate the HD. To fully characterize the microbial community during composting, the functions and combinations of different microbes in OSWs from various sources were summarized by a multidimensional analysis. We suppose that attention should be given to the similarities of bacterial communities in experiments. Conserved bacteria are a specific group of bacteria in composting. Although their abundances differ, bacteria preserved under different nutrients and physical and chemical factors may have better characteristics under specific composting conditions. Common microbes may also form the most stable community in response to environmental perturbations. The predominance of aerobic thermophiles and thermophilic bacteria (e.g., *Truepera*) is probably due to the overall warming during aerobic fermentation(Ki et al., 2018; Krishnan et al., 2017). Moreover, *Truepera*, *Bacillus*, and *Enterococcus* are associated with the decomposition of substances such as cellulose(Hemati et al., 2021; Kong et al., 2020). Additionally, *Devosia* and *Flavobacterium* may be involved in functions related to nitrogen metabolism during aerobic fermentation(Zhang et al., 2020). This means that these genera are involved in the aerobic fermentation of most samples. Therefore, to predict the HD of samples more accurately, attention must be paid to these bacteria. They have great potential in marking the aerobic fermentation and humification quality of different OSWs.

Additionally, divergent bacterial populations can be classified into multiple categories from multiple perspectives. First, core microbial taxa can be determined based on network analysis parameters (such as degree, betweenness, and centrality)(Zhou et al., 2011). Core groups of microbes might be the dominant microbes when present in higher abundances, or they might be the prominent conductors in a microbial network(Zhu et al., 2019b). Conductors can direct minor microbes to carry out a series of life activities. These conductors may not be abundant, but the metabolites they secrete are the nutritional basis for the life activities of other microbes(Fisher & Mehta, 2014). Second, researchers select specific microbes to study metabolic pathways and microbial functions, such as N and C metabolism(Dijkstra et al., 2008). Microbes are classified according to their differences in function to investigate the metabolic potential of different taxonomic groups on specific substrates or specific life activities(Qu et al., 2020). At present, microflora, microfluidics, and flow cytometry can be used to classify or separate microbes with different functions(Kou et al., 2016; Maurice et al., 2013). Third, we aimed to better describe and predict the evolution of the whole structure and function of the community. Researchers are now making a variety of predictions using mathematical models and statistical methods. Data from random forests show that bacterial communities are instrumental in classifying and predicting HD parameters. Researchers compared the predicted values of the random forest time series of bacteria codes with the

actual values of the decay index, as shown below (fig. 7). Except for the predicted value of FA of bacterial codes being different from the actual value, the predicted values of decay values were close to the actual value, which indicates the feasibility of characterizing the decay degree by bacterial codes.

Experiments verified the bacterial community as a biological indicator to better evaluate the quality of composting. However, many experiments data are needed to prove this hypothesis in practical applications. First, the raw materials for composting were collected around Harbin, so the results may be limited by region. Additionally, the types of materials selected are limited, and many follow-up experiments are needed to verify the experimental results. In the future, the universality of this method can be verified by multipoint and multimaterial joint tests. The results verified by multiple experiments can be used to build accurate mathematical models, and a humic acid process evaluation database can be constructed. Second, since the composition of the microbial community was characterized by only 16s v3-v4 amplicon sequencing(Awasthi et al., 2021a), only the relationship between the bacterial community composition and the HD was considered during the fermentation process. Additionally, the relationships between fungi, archaea, or protozoa and HD was not discussed. Furthermore, with modern life science and technology development, multivariate analysis methods, such as quantitative proteomic analysis, metabolomics analysis, and microfluidic technology, can be applied in this study. Additionally, the quality of composting products is limited by only the humic acid concentration. External factors, such as different materials and environmental factors, can also affect the degree of humification. It is worth noting that microbes can be characteristic of these external factors. That is, the use of biological indicators to characterize environmental factors has unique advantages over traditional methods. Experiment does more than prove that composting can be characterized by biological means. Bacterial codes can also be used as ecological and biological indicators to evaluate the quality of composting among different materials.

# Conclusions

The composting is the main method to degrade OSWs, increased the monitoring of the HD during composting is essential to ensure the quality and fertility of the products after degradation. Due to microbes are the engine of composting, given the importance of bacterial communities to ensure harmless of products after composting, it is time that monitoring efforts better account for changes by biological method, rather than relying on abiotic changes to determine the quality of composting. We evaluated bacterial communities of 5 types of OSWs during composting and the HD of host materials using 105 samples. The research analyzed the bacterial community from multiple dimensions and constructed bacterial code that can better predict HD

. A greater use of the bacterial communities as indicators in degradation of OSWs will not only improve our ability to manage composting but also further provides new insights on the use of microbe to

evaluate the content of various substances, and the microenvironmental during the degradation process of OSWs.

# Abbreviations

OSW: organic solid waste; HD: humification degree; SS: sewage sludge; CD: cow dung; CM: chicken manure; GW: garden waste; RS: rice straw.

# Declarations


### Acknowledgements

We wish to thank Hao Jingkun for providing chicken manure, Zhu Longji for providing cow dung, Wang Liqin for providing garden waste, Shi Mingzi for providing sewage sludge, and Wu Di for providing rice straw.

### Funding

This work was financially supported by the National Natural Science Foundation of China (No. 51878132) and National Key Research and Development Project, No. 2019YFC1906403.


### E-Supplementary data

All raw sequences derived from this experiment were submitted into the Short Read Archive of NCBI and can be found under the BioProject accession number PRJNA710204.

# References


Awasthi, M.K., Duan, Y., Liu, T., Awasthi, S.K., Zhang, Z. 2020. Relevance of biochar to influence the bacterial succession during pig manure composting. *Bioresour Technol*, **304**, 122962.

Awasthi, S.K., Duan, Y., Liu, T., Zhang, Z., Pandey, A., Varjani, S., Awasthi, M.K., Taherzadeh, M.J. 2021a. Can biochar regulate the fate of heavy metals (Cu and Zn) resistant bacteria community during the poultry manure composting? *J Hazard Mater*, **406**, 124593.

Awasthi, S.K., Duan, Y.M., Liu, T., Zhou, Y.W., Qin, S.Y., Liu, H.M., Varjani, S., Awasthi, M.K., Zhang, Z.Q., Pandey, A., Taherzadeh, M.J. 2021b. Sequential presence of heavy metal resistant fungal communities influenced by biochar amendment in the poultry manure composting process. *Journal of Cleaner Production*, **291**.

Baffi, C., Dell'Abate, M.T., Nassisi, A., Silva, S., Benedetti, A., Genevini, P.L., Adani, F. 2007. Determination of biological stability in compost: A comparison of methodologies. *Soil Biology & Biochemistry*, **39**(6), 1284-1293.

Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N. 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. *Isme Journal*, **6**(2), 343-351.

Cardona, C., Weisenhorn, P., Henry, C., Gilbert, J.A. 2016. Network-based metabolic analysis and microbial community modeling. *Curr Opin Microbiol*, **31**, 124-131.

Chen, M., Xu, P., Zeng, G.M., Yang, C.P., Huang, D.L., Zhang, J.C. 2015. Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. *Biotechnol Adv*, **33**(6), 745-755.

Chen, X., Du, Z., Guo, T., Wu, J., Wang, B., Wei, Z., Jia, L., Kang, K. 2022. Effects of heavy metals stress on chicken manures composting via the perspective of microbial community feedback. *Environ Pollut*, **294**, 118624.

Coelho, F.F., Giasson, E., Campos, A.R., Tiecher, T., Costa, J.J.F., Coblinski, J.A. 2021. Digital soil class mapping in Brazil: a systematic review. *Sci Agr*, **78**(5).

Cui, H.Y., Zhao, Y., Chen, Y.N., Zhang, X., Wang, X.Q., Lu, Q., Jia, L.M., Wei, Z.M. 2017. Assessment of phytotoxicity grade during composting based on EEM/PARAFAC combined with projection pursuit regression. *J Hazard Mater*, **326**, 10-17.

de Gannes, V., Eudoxie, G., Hickey, W.J. 2013. Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing. *Bioresource Technology*, **133**, 573-580.

Dijkstra, P., LaViolette, C.M., Coyle, J.S., Doucett, R.R., Schwartz, E., Hart, S.C., Hungate, B.A. 2008. N-15 enrichment as an integrator of the effects of C and N on microbial metabolism and ecosystem function. *Ecol Lett*, **11**(4), 389-397.

Faust, K., Sathirapongsasuti, J.F., Izard, J., Segata, N., Gevers, D., Raes, J., Huttenhower, C. 2012. Microbial co-occurrence relationships in the human microbiome. *PLoS Comput Biol*, **8**(7), e1002606.

Fisher, C.K., Mehta, P. 2014. Identifying Keystone Species in the Human Gut Microbiome from Metagenomic Timeseries Using Sparse Linear Regression. *Plos One*, **9**(7).

Gong, C.M. 2007. Microbial safety control of compost material with cow dung by heat treatment. *J Environ Sci-China*, **19**(8), 1014-1019.

Guimaraes, D.V., Gonzaga, M.I.S., da Silva, T.O., da Silva, T.L., Dias, N.D., Matias, M.I.S. 2013. Soil organic matter pools and carbon fractions in soil under different land uses. *Soil & Tillage Research*, **126**, 177-182.

Guo, Q.X., Yan, L.J., Korpelainen, H., Niinemets, U., Li, C.Y. 2019. Plant-plant interactions and N fertilization shape soil bacterial and fungal communities. *Soil Biology & Biochemistry*, **128**, 127-138.

Hemati, A., Aliasgharzad, N., Khakvar, R., Khoshmanzar, E., Asgari Lajayer, B., van Hullebusch, E.D. 2021. Role of lignin and thermophilic lignocellulolytic bacteria in the evolution of humification indices and enzymatic activities during compost production. *Waste Manag*, **119**, 122-134.

Hermans, S.M., Buckley, H.L., Case, B.S., Curran-Cournane, F., Taylor, M., Lear, G. 2020. Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome*, **8**(1).

Ikeya, K., Maie, N., Han, X.Z., Wang, G.H., Watanabe, A. 2019. Comparison of carbon skeletal structures in black humic acids from different soil origins. *Soil Sci Plant Nutr*, **65**(2), 109-113.

Kawasaki, S., Maie, N., Watanabe, A. 2008. Composition of humic acids with respect to the degree of humification in cultivated soils with and without manure application as assessed by fractional precipitation. *Soil Sci Plant Nutr*, **54**(1), 57-61.

Khoshnevisan, B., Duan, N., Tsapekos, P., Awasthi, M.K., Liu, Z.D., Mohammadi, A., Angelidaki, I., Tsang, D.C., Zhang, Z.Q., Pan, J.T., Ma, L., Aghbashlo, M., Tabatabaei, M., Liu, H.B. 2021. A critical review on livestock manure biorefinery technologies: Sustainability, challenges, and future perspectives. *Renewable & Sustainable Energy Reviews*, **135**.

Ki, B.M., Kim, Y.M., Jeon, J.M., Ryu, H.W., Cho, K.S. 2018. Characterization of odor emissions and microbial community structure during degradation of pig carcasses using the soil burial-composting method. *Waste Manage*, **77**, 30-42.

Kim, H., Lee, K.K., Jeon, J., Harris, W.A., Lee, Y.H. 2020. Domestication of Oryza species eco-evolutionarily shapes bacterial and fungal communities in rice seed. *Microbiome*, **8**(1), 20.

Kong, Z.J., Wang, X.Q., Wang, M.M., Chai, L.F., Wang, X.S., Liu, D.Y., Shen, Q.R. 2020. Bacterial ecosystem functioning in organic matter biodegradation of different composting at the thermophilic phase. *Bioresource Technology*, **317**.

Kou, S.Z., Cheng, D.H., Sun, F., Hsing, I.M. 2016. Microfluidics and microbial engineering. *Lab Chip*, **16**(3), 432-446.

Krishnan, Y., Bong, C.P.C., Azman, N.F., Zakaria, Z., Othman, N., Abdullah, N., Ho, C.S., Lee, C.T., Hansen, S.B., Hara, H. 2017. Co-composting of palm empty fruit bunch and palm oil mill effluent: Microbial diversity and potential mitigation of greenhouse gas emission. *J Clean Prod*, **146**, 94-100.

Liang, S.C., Deng, J.J., Jiang, Y., Wu, S.J., Zhou, Y.B., Zhu, W.X. 2020. Functional Distribution of Bacterial Community under Different Land Use Patterns Based on FaProTax Function Prediction. *Pol J Environ Stud*, **29**(2), 1245-1261.

Liu, T., Kumar Awasthi, M., Jiao, M., Kumar Awasthi, S., Qin, S., Zhou, Y., Liu, H., Li, J., Zhang, Z. 2021. Changes of fungal diversity in fine coal gasification slag amendment pig manure composting. *Bioresour Technol*, **325**, 124703.

Louca, S., Parfrey, L.W., Doebeli, M. 2016. Decoupling function and taxonomy in the global ocean microbiome. *Science*, **353**(6305), 1272-1277.

Lu, L.H., Zeng, G.M., Fan, C.Z., Ren, X.J., Wang, C., Zhao, Q.R., Zhang, J.C., Chen, M., Chen, A.W., Jiang, M. 2013. Characterization of a laccase-like multicopper oxidase from newly isolated Streptomyces sp C1 in agricultural waste compost and enzymatic decolorization of azo dyes. *Biochem Eng J*, **72**, 70-76.

Ma, J., Liu, H., Zhang, C., Ding, K., Chen, R., Liu, S. 2020. Joint response of chemistry and functional microbial community to oxygenation of the reductive confined aquifer. *Science of the Total Environment*, **720**.

Maurice, C.F., Haiser, H.J., Turnbaugh, P.J. 2013. Xenobiotics Shape the Physiology and Gene Expression of the Active Human Gut Microbiome. *Cell*, **152**(1-2), 39-50.

Medina, J., Monreal, C.M., Antilen, M., Calabi-Floody, M., Velasco-Molina, M., Meier, S., Borie, F., Cornejo, P., Knicker, H. 2020. Influence of inorganic additives on wheat straw composting: Characterization and structural composition of organic matter derived from the process. *J Environ Manage*, **260**.

Qu, Z.L., Liu, B., Ma, Y., Sun, H. 2020. Differences in bacterial community structure and potential functions among Eucalyptus plantations with different ages and species of trees. *Applied Soil Ecology*, **149**.

Ren, X., Wang, Q., Chen, X., Zhang, Y., Sun, Y., Li, R., Li, J., Zhang, Z. 2021. Elucidating the optimum added dosage of Diatomite during co-composting of pig manure and sawdust: Carbon dynamics and microbial community. *Sci Total Environ*, **777**, 146058.

Rottjers, L., Faust, K. 2018. From hairballs to hypotheses-biological insights from microbial networks. *FEMS Microbiol Rev*, **42**(6), 761-780.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biol*, **12**(6).

Senesi, N., Plaza, C. 2007. Role of humification processes in recycling organic wastes of various nature and sources as soil amendments. *Clean-Soil Air Water*, **35**(1), 26-41.

Smith, M.B., Rocha, A.M., Smillie, C.S., Olesen, S.W., Paradis, C., Wu, L.Y., Campbell, J.H., Fortney, J.L., Mehlhorn, T.L., Lowe, K.A., Earles, J.E., Phillips, J., Techtmann, S.M., Joyner, D.C., Elias, D.A., Bailey, K.L., Hurt, R.A., Preheim, S.P., Sanders, M.C., Yang, J., Mueller, M.A., Brooks, S., Watson, D.B., Zhang, P., He, Z.L., Dubinsky, E.A., Adams, P.D., Arkin, A.P., Fields, M.W., Zhou, J.Z., Alm, E.J., Hazen, T.C. 2015. Natural Bacterial Communities Serve as Quantitative Geochemical Biosensors. *Mbio*, **6**(3).

Soffer, N., Zaneveld, J., Vega Thurber, R. 2015. Phage-bacteria network analysis and its implication for the understanding of coral disease. *Environ Microbiol*, **17**(4), 1203-18.

Sporns, O., Honey, C.J., Kotter, R. 2007. Identification and classification of hubs in brain networks. *Plos One*, **2**(10), e1049.

Van Rossum, T., Ferretti, P., Maistrenko, O.M., Bork, P. 2020. Diversity within species: interpreting strains in microbiomes. *Nat Rev Microbiol*, **18**(9), 491-506.

Wei, Z., Ahmed Mohamed, T., Zhao, L., Zhu, Z., Zhao, Y., Wu, J. 2022. Microhabitat drive microbial anabolism to promote carbon sequestration during composting. *Bioresour Technol*, **346**, 126577.

Wu, D., Qu, F., Li, D., Zhao, Y., Li, X., Niu, S., Zhao, M., Qi, H., Wei, Z., Song, C. 2022. Effect of Fenton pretreatment and bacterial inoculation on cellulose-degrading genes and fungal communities during rice straw composting. *Sci Total Environ*, **806**(Pt 4), 151376.

Wu, D., Wei, Z., Gao, X., Wu, J., Chen, X., Zhao, Y., Jia, L., Wen, D. 2020a. Reconstruction of core microbes based on producing lignocellulolytic enzymes causing by bacterial inoculation during rice straw composting. *Bioresour Technol*, **315**, 123849.

Wu, J.Q., Wei, Z.M., Zhu, Z.C., Zhao, Y., Jia, L.M., Lv, P. 2020b. Humus formation driven by ammonia-oxidizing bacteria during mixed materials composting. *Bioresource Technology*, **311**.

Ye, L., Mei, R., Liu, W.T., Ren, H., Zhang, X.X. 2020. Machine learning-aided analyses of thousands of draft genomes reveal specific features of activated sludge processes. *Microbiome*, **8**(1), 16.

Yuan, J., Wen, T., Zhang, H., Zhao, M., Penton, C.R., Thomashow, L.S., Shen, Q. 2020. Predicting disease occurrence with high accuracy based on soil macroecological patterns of Fusarium wilt. *ISME J*, **14**(12), 2936-2950.

Zhang, W.M., Yu, C.X., Wang, X.J., Hai, L. 2020. Increased abundance of nitrogen transforming bacteria by higher C/N ratio reduces the total losses of N and C in chicken manure and corn stover mix composting. *Bioresource Technology*, **297**.

Zhao, Y., Wei, Y.Q., Zhang, Y., Wen, X., Xi, B.D., Zhao, X.Y., Zhang, X., Wei, Z.M. 2017. Roles of composts in soil based on the assessment of humification degree of fulvic acids. *Ecol Indic*, **72**, 473-480.

Zhou, J.Z., Deng, Y., Luo, F., He, Z.L., Yang, Y.F. 2011. Phylogenetic Molecular Ecological Network of Soil Microbial Communities in Response to Elevated CO2. *Mbio*, **2**(4).

Zhou, Y., Sun, Y., Liu, J., Ren, X., Zhang, Z., Wang, Q. 2022. Effects of microplastics on humification and fungal community during cow manure composting. *Sci Total Environ*, **803**, 150029.

Zhu, L., Zhao, Y., Zhang, W., Zhou, H., Chen, X., Li, Y., Wei, D., Wei, Z. 2019a. Roles of bacterial community in the transformation of organic nitrogen toward enhanced bioavailability during composting with different wastes. *Bioresour Technol*, **285**, 121326.

Zhu, L.J., Wei, Z.M., Yang, T.X., Zhao, X.Y., Dang, Q.L., Chen, X.M., Wu, J.Q., Zhao, Y. 2020. Core microorganisms promote the transformation of DOM fractions with different molecular weights to improve the stability during composting. *Bioresource Technology*, **299**.

Zhu, L.J., Zhao, Y., Zhang, W.S., Zhou, H.X., Chen, X.M., Li, Y.J., Wei, D., Wei, Z.M. 2019b. Roles of bacterial community in the transformation of organic nitrogen toward enhanced bioavailability during composting with different wastes. *Bioresource Technology*, **285**.

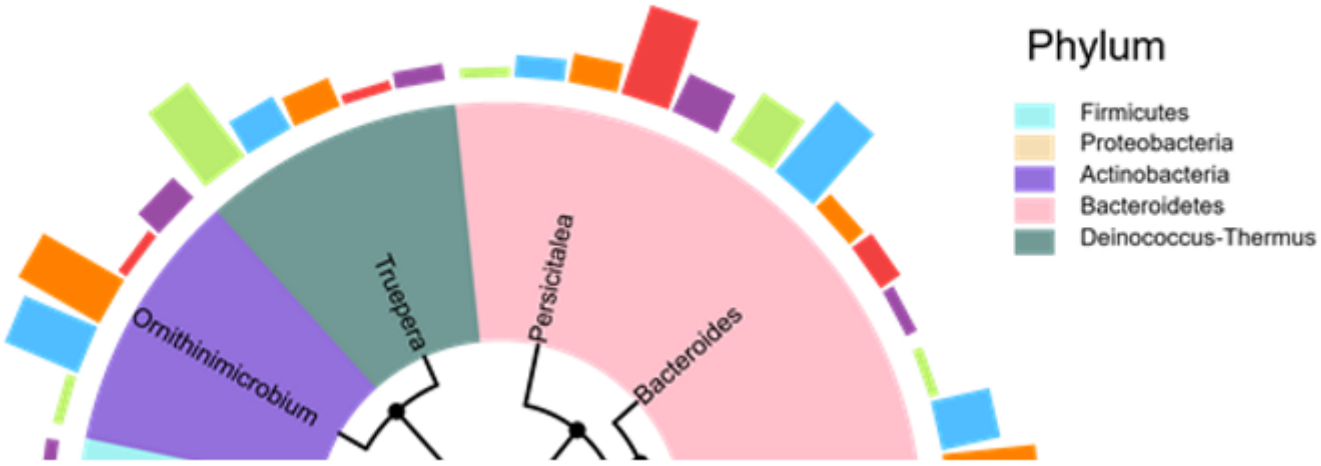
# Tables

Table 1 is in the supplementary files section.

# Figures

### Figure 1

**The relative abundance of bacteria in the compost sample.** a, c, e, g, i are the relative abundance of bacteria at the phylum level; b, d, f, h, j are the relative abundance of bacteria at the genus level. a, c, e, g, and i use different colors to mark the bacteria with the total OTU of five different raw materials aerobic high-temperature composting in the top 15 at the door level, and the remaining bacteria are marked with light gray; b, d, f, h, j It represents the top ten bacteria in the sum of OTUs at the genus level among five different raw materials, and the size of the bubbles indicates the relative abundance of bacteria.



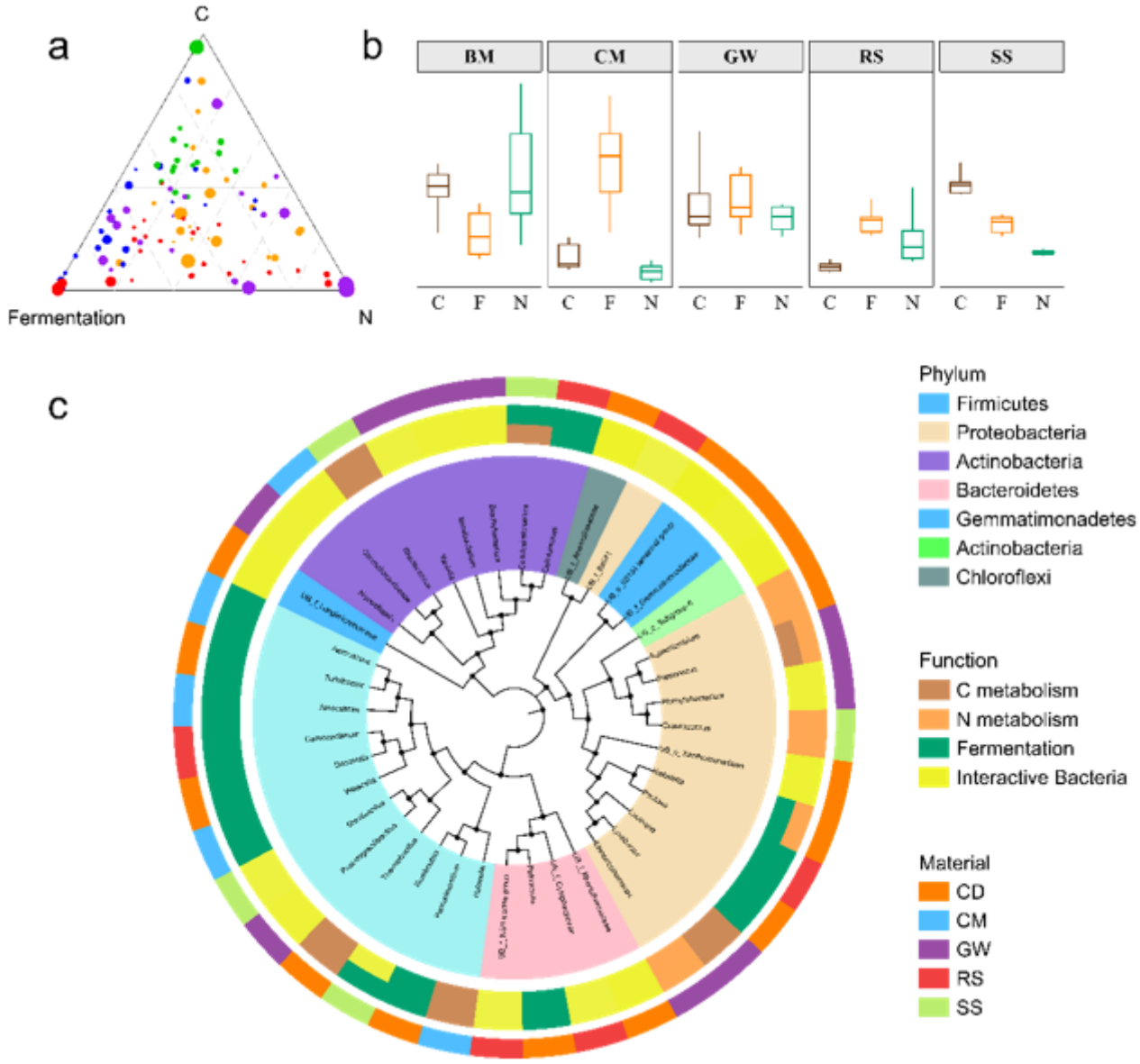

## Figure 2

Phylogenetic tree of conserved bacteria. According to OTU abundance analysis, conserved bacteria were selected; they existed in no fewer than 70% of the samples of each composting material treatment. The histogram shows the proportion of each bacterial genus in the various organic solid wastes after abundance normalization. The background color of the phylogenetic tree indicates the phyla to which bacteria belong.

## Figure 3

Network constructed based on different organic solid wastes (including chicken manure (CM), cow dung (CD), garden waste (GW), rice straw (RS), and sewage sludge (SS)) and the co-occurrences between

bacterial taxa at the genus level. The node size symbolizes the degree of each bacterium, and the nodes filled in various colors are phyla.




## Figure 4

FAPROTAX functional prediction results and a phylogenetic tree of essential differential microorganisms. **a** Ternary plots based on FAPROTAX functional prediction results. Different colors represent different organic solid waste samples, and node sizes indicate the relative abundances of functional groups in the samples. **b** Box plots of function prediction results, with box colors representing different functional groups (C metabolism, N metabolism, and fermentation). **c** Functional prediction, network analysis, and LEfSe analysis, with three circles outside the phylogenetic tree. The outermost circle represents the material to which the bacterial genus was associated with, and the two inner circles indicate the function of the bacterial genus or whether it is a crucial node microorganism. The color range of the evolutionary tree represents gate-level taxa.

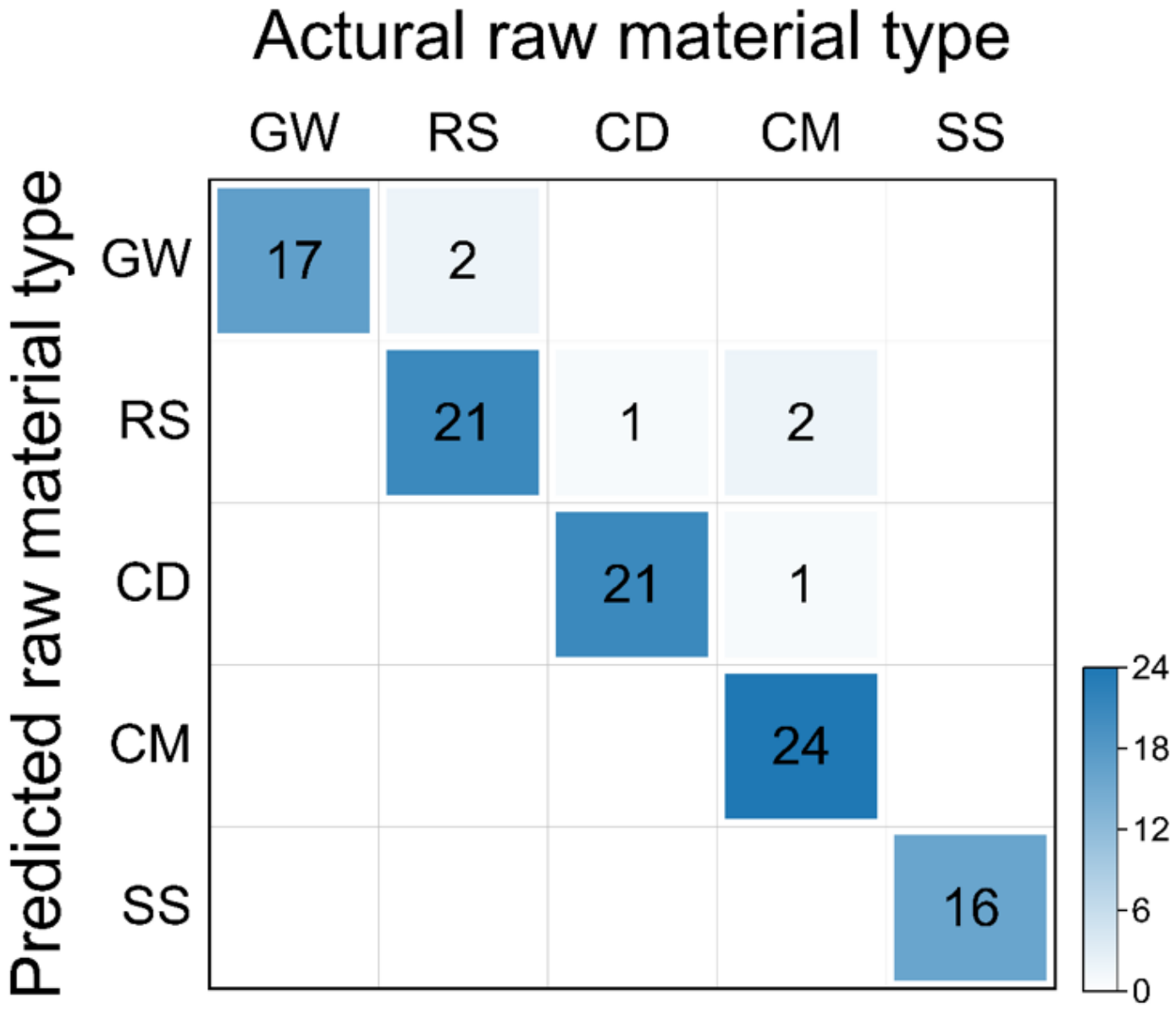



**Figure 5**

Based on the bacterial data, it is predicted by random forest classification. There are 105 samples in total, 99 of which are correct prediction values.

The bacterial codes were input into the random forest model to predict some fundamental humification indicators (Fig. 6). Furthermore, standard regression curves were plotted based on the prediction results. When the slope was closer to 1, the prediction results were more accurate. Among the four model scenarios that were fit with the linear regression analysis, the predicted humification indicator slopes were between 0.46 and 0.78, and the prediction result is relatively assuring. Except for the CM samples, the accuracies of the HA predictions were similar, and the slopes were all approximately 0.7 (Fig. 6a). The slopes were all greater than 0.6, and the slope value of CD was even 0.78, which was the highest value

among all FA prediction results (Fig. 6b). The prediction of the HD (Fig. 6c) was suitable for CD. In contrast, the CM humification index prediction had the minimum of all the predicted slope values (Fig. 6d). The accuracy of the model HD prediction using microbes varied across genera between the five OSW (see supplementary files). However, all the results suggested that bacterial codes can respond to the HD of composting from the prediction results. It is also indicted that the potential ability of bacteria codes to predict the composting HD.

**Figure 6**

Random forest predictions using four humification indicators during composting. **a-d** represent the stochastic forest predictions of four humification indicators (HA, FA, HI, and DP) during composting, respectively. The purple dots represent garden waste, the red dots represent rice straw, the orange dots represent cow manure, the blue dots represent chicken manure, and the green dots represent sludge. The gray dashed line represents the best-predicted case. The adjusted $R^2$ and slope values are indicated in the figure.

**Figure 7**

**Compared the predicted value with the actual value of the humification indexs.**

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx