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The effect of bacteria inoculation on the lignocellulose degradation and the microbial properties during cow dung composting

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Research Article

Keywords: Compost, Lignocellulose degradation, Inoculation, Transcriptome

Posted Date: April 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1541181/v1

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Abstract

The goal of this work was to explore the potential effects of bacteria inoculation on the lignocellulose degradation and the underlying microbial mechanism during cow dung composting. The results suggested that bacteria inoculation better-accelerated temperature rose and increased thermophilic phase. Compared to the control (without inoculation, CK), the cellulose, hemicellulose, and lignin degradation rates of treated group (EG) increased from 53.3% to 70.0%, 50.2% to 61.3% and 46.4% to 60.0%, respectively. The microbial community structure and diversity were obviously changed by inoculation. Moreover, inoculation contributed to modulate the key compost microbial functional populations linking to the degradation of lignocelluloses by stamp analysis. Correlation matrix analysis indicated that the expression of bacterial lingo-cellulolytic enzymes were closely related to the key microbial functional populations. Overall, the results confirmed the importance of bacteria inoculation and have important implication for promoting the efficiency and quality of cow dung compost.

Introduction

Nowadays, with the increasing demand for poultry and its products, the number of livestock farms and the amount of poultry manure are also increasing (Ma and Wang, 2015). How to accelerate the harmless, reduction, and resource utilization of poultry manure has become an urgent and important problem to the development of animal husbandry. Compost, as an economical and environmentally-friendly technology, has been widely used in the treatment of organic waste in agriculture and animal husbandry (Hossain et al. 2016). Large numbers of studies have been conducted and found that adding the key functional microorganisms to the compost is beneficial to extend the high-temperature duration, reduce the nutrient loss, expedite the decomposition of the compost, and promote the maturity of materials (Hachicha et al. 2012; Yang et al. 2017; Zhao et al. 2020). With sustainable development becoming the future trend, efficient and stable microbial agents also have a broad application prospect of poultry manure treatment.

The main organic components of poultry manure are cellulose, hemicellulose, protein, fat, and lignin and these substances differ in biodegradability during the composting fermentation process (He et al. 2012). Carbohydrates and crude fat are easily-degraded organic matter in compost, while cellulose, and hemicellulose are relatively difficult degraded organics and lignin is basically non-degraded (Zoghlami et al. 2019). Hence the degradation of hemicellulose, cellulose and lignin is an important problem in compost fermentation process (Gabhane et al. 2012).

To solve the above problems, much research has focused on how to utilize the high-temperature microorganisms which can efficiently degrade cellulose and hemicellulose, and lignin over the past few decades. For example, inoculation with the *Phanerochaete* and *Chrysosporium* was reported to improve physical and chemical parameters and increase substrate utilization rate (Liu et al. 2020). Inoculation with the *Actinomycetes* can promote the cellulose degradation and accelerate the composting process (Zhao et al. 2017). Inoculation with the *Bacillus thuringiensis* to obtain bio-pesticide properties and add value to home compost (Ballardo et al. 2020). These findings implied that applications of the key microbial inoculants are critical to compost and which is helpful to achieve sustained benefits on composting. However, many factors frequently limited the microbial inoculation to exert their beneficial effects, e.g., variations of environmental conditions, compost materials, method of the inoculant, inoculation time, and compost method (Lu et al. 2021; Yang and Doña-Grimaldi et al. 2019; Wei et al. 2018; Zhao et al. 2016). In the context of animal husbandry industry's rapid development, screening efficient and stable compost compound microbial agents, and achieving the diversity of microbial inoculation possess a vital act on improving compost quality and efficiency.

Although microbial inoculation has been popularized as a strategy to improve compost quality, its efficacy in published literature still couldn’t reach a consensus. Moreover, the mechanism of different microbial inoculation and their effects is also still unclear. The aim of the present study was to (i) compare the changes of physicochemical parameters and lignocellulose degradation in the treated group (EG) and the control (CK); (ii) assess the effect of microbial inoculation on the microbial community succession and the significant changes in functional community; (iii) analyze the potential links

between lignocellulosic-metabolizing enzymes and the specific microbial groups during compost. The information generated will get insight into the role and the adjustability of the micro-biome of composts, allowing a more specifically control of the composting process and develop more efficient and stable microbial agents.

Materials And Methods

Materials and inoculum

Cow dung compost was conducted at a poultry farm located in Xishan, Xinjiang Uygur Autonomous Region, China (43°81'N, 87°57'E) in August 2018. Air-driedreed straw as the major carbon substrate was shredded into pieces less than 5 cm long and mixed with the cow dung (1:3; v/v). Two treatment groups were built as follows: cattle manure + wheat straw (control, CK) and cattle manure + wheat straw + compound microbial inoculant (treated, EG), respectively. The treatment group with three replications was used. Each pile was approximately 1.0 m high, 0.8 m wide and 1.2 m long with a moisture content of 60% in outdoor. Compost was performed over 70 days and turned on days 11 and 27 by forklift. The physicochemical properties of initial substrates are shown in Table **1**.

Three different test strains were used in compound microbial inoculant (Co-inoculation, 1:1:1 v/v; OD_{600}, 0.6): (i) *Bacillus* sp. Q3, (ii) *Geobacillus* sp. NM6, (iii) *Bacillus* sp. ND, which were separated from compost samples based on the activity of cellulases, amylase, lipases, and protease in our previous study. They are all thermophilic bacteria, and cultured in NF9 liquid medium (Sucrose, 20.0 g/L; Peptone, 2.0 g/L; Corn syrup, 5.0 ml/L; Na_2PO_4, 1.0 g/L), and temperature range for growth are 40-73 °C. The microbial inoculation has been popularized and used at many places in Shaya with a good compost effect.

Sample collection and physicochemical parameter analysis

Before turning over the compost pile, samples were simultaneously collected from the upper, central, and lower layer of treatment groups on days 0 (initial phase), 11 (mesophilic phase), 27 (mesophilic phase), and 48 (thermophilic phase). To obtain homogenized samples, the sub-samples were well blended together as a representative sample of each group at each time point. All representative samples (approximately 1.0 kg/per sample) were divided into two servings: one was stored at −80 ◦C for the bacterial and fungal DNA extraction and another was air-dried for the determination of physical-chemical environmental factors. By using the portable thermometer, the average temperature (T) was finally obtained by measuring the surface, core, and bottom of groups. Electrical conductivity (EC) and pH were measured after shaking equilibration at a wet weight of the representative sample to distilled water ratio of 1:10. The oven-dried samples were further heated at 550 ℃ for the determination of organic matter content (OM) (Gajalakshmi et al. 2008). Furthermore, the germination index (GI) and the contents of hemicellulose, cellulose, and lignin were determined according to previous description (Ouyang et al. 2014; Van Soest et al. 2019).

DNA extraction and high-throughput sequencing

According to the manufacturer's protocols, the FastDNA Spin Kit for Soil (MP Biomedical, France) was used to extract the Total DNA from 24 compost samples. The diversity analysis of the bacterial and fungal community was performed by using the Illumina MiSeq platform by Allwegene Tech Co., Ltd (Beijing, China). For bacteria, the V3-V4 region of the 16S rRNA gene was amplified by the primers 338F and 806R (Li et al. 2020). For fungi, ITS1 and ITS4 primers were used to amplify the ITS region (Grades et al. 1993). The QIIME (1.9.1) was used to filter the quality of raw sequence data. Afterward, the clean sequences were assigned to operational taxonomic units (OTUs, 97.0% similarity) by using UPARSE (Edgar et al. 2013) of the SILVA database (for bacterial 16S rRNA gene) and UNITE database (for fungal ITS rDNA) for the sequence reference set. The 16S rRNA and ITS genes sequences obtained in this study have been deposited in the NCBI Sequence Read Archive under the accession number PRJNA772265 and PRJNA772727, respectively.

RNA Extraction and Transcriptome analysis

Transcriptome sequencing and analysis were performed in Allwegene Tech Co., Ltd (Beijing, China). Samples were collected from the EG on days 0 (initial phase) and 48 (thermophilic phase) with three biological replicates for RNA extraction. After the library preparations and Illumina sequencing, paired-end reads were generated. The PandaSeq was used to join the paired-end sequences (Masella et al. 2012) and low-quality reads were filtered out by using the Trimmomatic (Bolger et al. 2014). Subsequently, sequencing data analysis and DEG estimation were performed, including quality control and comparative analysis. Quality control of sequencing data was carried out by fasta QC, and filtered reads were considered for further analysis. Finally, transcription factors (TFs) were used for annotation and pathway analysis, including GO enrichment and KEGG pathway enrichment. The expression of each unigene was estimated by fragments per kilobase of transcript per million fragments mapped (FPKM) reads values. Genes with a threshold of fold change (FC) >2.0 (or <0.5) and a P-value <0.01 were set as the threshold for significantly differentially expressed genes (DEGs). The transcriptomic raw sequencing data were submitted in NCBI under BioProject ID PRJNA772758.

Statistical analyses

The prism GraphPad version 8.0 was also used for tabulating and processing related data. The difference in microbial community composition of two different treatments were evaluated with principal coordinate analysis (PCA) and permutational multivariate analysis of variance based on both unweighted and weighted UniFrac distances by using QIIME 2.0 (Estaki et al. 2020). Furthermore, the distribution of bacterial and fungal communities (phylum level) was demonstrated by Circos diagrams (https://www.bioincloud.tech). The correlation matrix was analyzed by using the software RStudio.

Results

Changes in physicochemical parameters during composting

During the mesophilic phase, temperature of piles rose quickly and steadily, and EG was slightly higher than the CK (Fig. 1a). After turning over piles (27 d), the temperature level increased rapidly again and subsequently entered into the thermophilic stage (>50℃). Though the temperature trends of two treatment groups were similar, temperature in the EG was significantly higher compared with the CK. EG reached the highest temperatures on day 35 at 67.3 °C and kept above 50 ◦C for 27 days, by contrast, CK reached the highest temperature on day 44 at 53.4 ◦C and kept above 50 ◦C for 8 days. Subsequently, the temperature of two treatment groups dropped on days 43 and 45, respectively, and entered the cooling stage. Results indicated that EG probably possessed more available functional microorganism led to more organic waste was metabolized and released more energy. Appropriate high temperature and keep a longer thermophilic phase is killing not more pathogens and weed seeds, but also making compost product safer.

The pH values of two treatment groups showed both a decrease first and then increase as compost time proceed (Fig.1b). The main point for pH decreased may be due to the microorganisms rapidly decomposed large quantity of easily degradable organic matter that caused organic acids produced (Gajalakshmi et al. 2008) and consumed simultaneously a part of nitrate-nitrogen (Guardia et al. 2009). The pH values of two treatment groups decreased after entering the thermophilic phase, and which was significantly lower in the EG compared to the CK. It was no surprise that on the one hand high temperatures generally cause the NH^{4+}-N to volatilize to NH_3, on the other hand, the thermophilic microorganisms worked industriously to decompose organic matter and produce small molecule acids. During the cooling-off period, undegraded organic matter, such as proteins, was generally further decomposed and produced NH^{4+}-N. Consequently, the pH values of two treatment groups the period increased slightly, and which was similar to previous studies (Meng et al. 2018a, b).

The EC values of two treatment groups were all more than 4.0 mS/cm in the whole composting process (Fig. S1), and seemed to be related with the local characteristic of the soil environment (Bi et al. 2020). The problem should give rise to people's attention because a high soluble salt content will inhibit the growth of crops. Moreover, the content of available nitrogen in the EG was higher than the CK since microbial inoculation might reduce the volatilization of ammonia and increase the available nitrogen content (Fig. S1).

Evolution of lingo-cellulosic fractions during composting

The activity of lignocellulosic decomposition depends on the species and abundance of lignocellulose-degrading microorganisms present in the mixture. The cellulose, hemicellulose, and lignin contents of two treatment groups gradually decreased with the composting proceed (Fig. 1d-f). It was obvious that the EG produced the significantly higher ($P < 0.05$) cellulose, hemicellulose, and lignin degradation rates. Specifically, the cellulose, hemicellulose, and lignin degradation rate of the EG increased from 53.3% to 70.0%, 50.2% to 61.3%, and 46.4% and 58.9% respectively in nearly finished composting (60 d) compared to the CK.

The degradation of cellulose and lignin occurred a lot 35.2% to 32.9.0% and 25.1% and 33.9%, respectively) in two treatment groups during the thermophilic phase, which was consistent with previous reports (Mei et al. 2020; Zhu et al. 2021) and Xiao et al. (2009) also found that cellulose degradation was stronger during high temperature period. EG generated the higher temperature and kept a longer thermophilic period, which was helpful for improving the decomposition efficiency of cellulose and lignin. In addition, cellulose was tightly cross-linked with lignin in the lignocellulose matrix, so the internal lignin components will be more exposed when cellulose was significantly removed. Therefore, it was more effectively promoted the lignocellulolytic enzymes secreted by microorganisms to play their role in the EG.

Seed germination index (GI) analysis

Seed germination index (GI) was used for quickly and efficiently assessing the compost maturity and has been widely accepted by people (Hussain et al. 2018). Composts with a GI value > 80.0% were considered to be mature according to Bernal et al. (2009). GI was analyzed with aqueous extracts of fresh samples using Chinese cabbage. The GI of the EG and the CK enhanced gradually along with composting process and eventually increased from 33.6% and 35.1% at day 0 to 90.7% and 83.0% at day 60, respectively (Fig. 1c). Results showed that addition of microbial inoculation had a significant impact on GI of the final compost product, the final products of EG was mature and non-phytotoxic, which in line with the national normative requirement (Luo et al. 2017).

Changes of bacterial and fungal community diversity during composting

After quality filter and potential chimeras were removed, a total of 3,799,998 high-quality bacterial and 2,111,338 fungal sequences were generated respectively for 48 samples across two treatment groups. Regarding the β-diversity, Principal Component Analysis (PCA) revealed a significant effect of the microbial inoculation on bacterial and fungal community, the difference became more obvious with compost time (Fig. 2a and c). The α-diversity was obtained for Shannon's diversity indexes, Chao1, and observed OTUs. The Shannon's diversity indexes were in general different between compost samples in the EG and the CK (Fig. 2b and d). Compared with bacteria, the Shannon's diversity index of fungi has risen notably on day 48. For bacteria and fungi, it could observe that EG samples showed significantly higher values for all the diversity indices as compared to the CK samples (Fig. S2). Moreover, although the α-diversity of the EG was relatively more stable from initial to mesophilic period (27 d), which changed markedly in both the EG and the CK in the thermophilic period. Venn diagram exhibited that EG and CK samples possessed 1214 (8.1%) and 933 (6.0%) bacterial OTUs shared by four different compost stages, respectively (Fig. S3). Meanwhile, they possessed 64 (4.4%) and 52 (3.5%) fungal OTUs shared by four compost stages, respectively. Results suggested that EG could slightly enhance the core bacterial OTUs, but not the core fungal OTUs.

At the phyla level, the abundances of the top nine phyla represented 95~97% of the total bacterial communities, including Proteobacter*ia* (21.8-52.4%), *Bacteroidetes* (14.5-39.0%), *Firmicutes* (4.0-37.3%), and *Actinobacteria* (4.2-9.6%) and the remainder belonged to the phyla *Spirochaetae*, *Chloroflexi*, *Gemmatimonadetes*, *Planctomycetes* and *Fibrobacteres* (Fig. 3a). Because of the copiotrophic strategies of the *Proteobacteria* and *Bacteroidetes*, they usually showed the rapid growth response to resource availability (Fierer et al. 2007). Thus, *Proteobacteria* and *Bacteroidetes* increased first and then decreased during composting. By contrast, *Firmicutes* increased markedly in the thermophilic phase, which was able to secrete various extracellular thermostable enzymes and degrade some macromolecular substrates (protein, pectin, and cellulose, etc.). The addition of microbial inoculation had not a significant impact on the abundances of *Actinobacteria*, but *Bacteroidetes* on day 27. On day 48, *Firmicutes* and *Gemmatimonadetes* were more abundant in the EG than the CK. The previous findings indicated that they are important bacteria for anaerobic fermentation, decomposing organic matter to hydrogen or acetic acid (Cardinali-Rezende et al. 2012), which also matched well with the levels of cellulose and lignin degradation in the compost process.

Ascomycota was the most well-represented fungal division during the composting process, composing more than 87% of the fungal species in the thermophilic stage, the remainder (10.0-20.0%) belonged to the phyla *Basidiomycota*, *Glomeromycota*, and *Mortierellomycota* (1.9-29.6%, 0.1-2.6%, and 0.3-2.7%, respectively) (Fig. 3b). The *Ascomycota* and *Glomeromycota* of two treatment groups were a few difference on days 11 and 27, while the *Basidiomycota* exhibited a great difference on days 11 and 48. At a finer taxonomic level, the abundance of 13 bacterial and 7 fungal taxa (family level) showed significant changes in different compost periods, among which *Trichocomaceae*, *Glomeraceae*, *Anaerolineaceae*, *Rhodothermaceae*, *Limnochordaceae*, and *Marinilabiaceae* were most representative (Fig. S4).

Specific differences of microbiome during composting

The observed differences in *α*-and *β*-diversity between the EG and the CK led us to explore more in-depth the differences in taxonomic identity and the abundance of bacterial and fungal taxa. For bacteria, *Moheibacter*, *Halocella*, *Marinobacter*, *Petrimonas*, and *Actinotalea* were consistently enriched in the EG, while *Halomonas* was more abundant in the CK on days 11 and 27 (mesophilic phase) (Welch's *t* test, $P < 0.05$, FDR-corrected, Fig. 4a and b). On 48 (thermophilic phase), *Methylocaldum*, *Marinobacter*, *VadinBC27_wastewater_sludge*, *Caldicoprobacter*, *Turicibacter*, and *Hydrogenispora* were more abundant in the EG, while *Halomonas* and *Galbibacter* were more abundant in the CK (Welch's *t* test, $P < 0.05$, FDR-corrected, Fig. 4c). It was noteworthy that the genera exclusive for the EG and the CK were *Marinobacter* and *Halomonas* respectively from day 11 to day 48.

The *Marinobacter* genus is mostly facultative aerobic heterotrophic and halotolerant bacteria and the main factor favoring *Marinobacter* abundance was hydrocarbon amendment (Bonin et al. 2015). The genus *Halomonas* is characteristically halophilic or halotolerant with denitrification function, and can secrete various metabolites (polyhydroxyalkanoates, PHA) with the basic carbon source (Kim et al. 2013). The genera *Petrimonas*, *Actinotalea*, and *Halocella* were mesophilic and able to utilize die-hard substances such as cellulose. By contrast, *Hydrogenispora*, *Caldicoprobacter*, and *VadinBC27_wastewater_sludge* were thermophilic biomass-degrading bacteria and could utilize complex organic compounds (chitin, xylan, and lignin) (Ungkulpasvich et al. 2020; Mhiri et al. 2020; Wang et al. 2021). In terms of metabolic characteristics, they might aid in the degradation of the big molecular substances and refractory organic compounds, and their abundance were strongly modulated by pile environment and positively or negatively affected by temperature. Further experimentation is still needed to decipher the impact of these "enriched" microbes for efficiency and quality of cow dung compost. Moreover, *Methylocaldum* was obviously enriched (48 d; 0.11% and 3.87%, respectively) in the EG compared to the CK and it could utilize methane, which possibly was contributed to reduce methane emissions during composting (Takeuchi et al. 2014).

For fungi, the genera *Pseudallescheria*, *Melanocarpus*, *Chaetomium*, *Coprinellus*, and *Penicillium* showed a higher abundance in the EG than in the CK, while *Scopulariopsis* was significantly more abundant in the CK on day 11 (Welch's

test, P <0.05, FDR-corrected, Fig. 5a). In the EG, *Chaetomium* and *Penicillium* were enriched, however, *Microascus* was enriched in the CK on day 27 (Welch's t test, P <0.05, FDR-corrected, Fig. 5b). On day 48, *Gamsia*, *Melanocarpus*, *Chaetomium*, and *Penicillium* were significantly more abundant in the EG, while *Chrysosporium*, *Scopulariopsis*, and *Acremonium* were enriched in the CK (Welch's t test, P <0.05, FDR-corrected, Fig. 5c). The *Chaetomium* and *Penicillium* are moderate thermophilic and known for its cellulose-degrading capabilities and *Melanocarpus* is very diverse and cosmopolite fungi and play important roles as decomposers of organic materials (Linkies et al. 2021; Dyer et al. 2014; Feng et al. 2021). They were associated with their ability to decompose complex carbohydrates, thereby contributing to carbon cycling in cow dung compost. *Microascus*, *Acremonium*, and *Scopulariopsis* were more enriched in the CK, which could cause widespread infection was revealed by the researcher. Taken together, the comparative results verified that microbial inoculation contributed to modulate the abundance of specific functional groups and reduce the bacterial pathogens in cow dung compost. The genus level of microbial community was shown in Fig. S5 in detail.

Transcriptional analysis

The transcriptomic analysis of bacterial community in the EG (0 d and 48 d) was generated to investigate genes encoding CAZymes related to the decomposition of cow dung lignocellulose. From six samples (three biological replicates for each period), a total of 2.2 billion cleaned reads (32.2 Gb) were got after filtering and each sample contains approximately 4.1–6.7 Gb (Table. S1). The error rate of transcribe data was 2.4-2.1% and the Q20 and Q30 values exceeded 97.6% and 92.9%, respectively, and reached the basic requirements of gene discovery. A non-redundant transcript cluster was got, including 997,517 unique genes with an average length of 925 bp and an N50 of 1,176 bp, an N90 of 414 bp, respectively. Results indicated that a total of 118,611 genes were up-regulated and 186,660 genes were down-regulated.

Enrichment

230,188 DEGs were carried out an enrichment analysis of GO functions and KEGG pathways. GO function analysis showed that 366, 316, and 423 categories were enriched in biological process (BP), molecular function (MF), and cellular component (CC), respectively. The top 20 GO enrichment circle and GO summary graphs of the DEGs were presented in Fig. 5a and b. Among the top 20 enriched GO entries, the membrane related to cell components possessed higher rich factors. Fifteen items were enriched in BP, among them GO: 0051179 (localization), GO: 0006810 (transport), and GO: 0051234 (establishment of localization) were enriched in more genes (194, 182, and 182, respectively), and the down-regulated genes were also in a higher degree enriched. Lastly, four entries were enriched in MF, among them the P-values of GO: 0015075 (transporter activity), GO: 0022857 (transmembrane transporter activity), and GO: 0016874 (ligase activity) were higher. To further understand the growth status of the microbial community, the DEGs were mapped to the KEGG database. In the top 20 enriched pathways, DEGs mapped to the ribosome (ko03010) occupy the largest proportion, with "RNA degradation (ko03018)" and "Longevity regulating pathway–worm (ko04212)" ranking second and third (Fig. 6c). Combining the analysis results of the GO functions and the KEGG pathway, it was obvious that these pathways involved more in translation, localization, membrane, and biological processes.

There were altogether 39,907 CAZyme-encoding genes were detected in different families, with 18,462 potentially involved in lignocellulose degradation enzymes were found from the auxiliary activities (AA), glycoside hydrolases (GH), and carbohydrate esterases (CE) families. Among them, 2 AAs belonged to the lignin-degrading Enzymes; 14 GHs families belonged to the cellulose-degrading enzymes and 7 CBMs families accessory proteins related to cellulose degradation; 11 GHs and 11 CEs families belonged to the hemicellulose-degrading enzyme system, and 7 CBMs families assisted the catalytic function of the hemicellulase system; 14 GHs families belonged to the cello-oligosaccharides degrading enzyme system and 3 CBMs families belonged to the cello-oligosaccharides degradation enzymes. Using the cluster analysis, we confirmed that the expression levels of lingo-cellulosic enzymes were significantly higher in the thermophilic period compared with the initial period (Fig. S6). These results implied that the inducing mechanism supporting the high

expression level of lignocellulose should exist, and it might be intimately connected with the regulation of the microbial inoculation on the resident microbes in cow dung compost.

Relationship among CAZY family genes and microbial community

The correlation matrix among CAZY (Carbohydrate-Active enzymes database) family genes and microbial population in the EG was explored. The complex interactions among bacterial species were performed (Fig. 7). *Halomonas* and *Marinobacter* were exclusively represented in the CK and the EG, respectively. *Marinobacter* exhibited the significantly ($p<0.05$) positive correlation with *VadinBC27_wastewater_sludge*, *Hydrogenispora*, *Longispora*, *Treponema*, *Methylocaldum*, *Moheibacter*, *Limnochorda*, *Caldicoprobacter*, and *Tepidimicrobium*, with only two significantly ($p<0.05$) negative connection identified as *Halomonas* and *Turicibacter*. By contrast, *Halomonas* was just the opposite, we found that it showed a significantly ($p<0.05$) negative correlation with *Halocella*, *Marinobacter*, *Limnochorda*, *Turicibacter*, *Caldicoprobacter*, *Tepidimicrobium*, and *Methylocaldum*, with only a significantly ($p<0.05$) positive connection identified as *Turicibacter*. In the correlation matrix, we observed positive correlations between bacterial populations, which suggested niche overlap, as well as negative correlations, suggesting competition or amensalism (Faust et al. 2012). The significantly enriched bacterial populations in the EG was in general positively correlated, forming well-differentiated clusters. These significantly enriched bacterial populations consisted mainly of resident functional microbes that involved in degradation of complex organic matters.

Fig. 7 showed that the expression levels of cellulose, hemicellulase, and oligosaccharidase genes were significantly ($p<0.05$) related to *Hydrogenispora*, *VadinBC27_wastewater_sludge*, *Halomonas*, and *Methylocaldum*. Fig. 8 showed that the expression levels of cellulose, hemicellulase, and oligosaccharidase genes were significantly ($p<0.05$) related to *Chaetomium*, *Melanoleuca*, *Pseudallescheria*, *Penicillium*, *Gamsia*, *Pseudogymnoascus*, *Vishniacozyma*, and *Aspergillus*. These functional microorganisms were more abundant in the EG, which possessed the important feature of microbes associated with degradation of lignocellulose besides. They might be members of the core functional microbiome and most likely better adapted and responded to compost environment in the EG, such as temperature. The diversity and abundance of these microorganisms in EG, as well as their diversity in metabolic traits, makes them potentially important functional microbes in the compost material transformation.

Conclusions

The bacteria inoculation was able to effectively extend the thermophilic phase and enhance lignocellulose decomposition because the key microbial functional populations were regulated and controlled. The microbial community diversity and structure were obviously changed by inoculation, and key microbial functional populations was more enriched in the EG. In addition, a strong correlation between the abundance of specific functional populations and the expression level of lignocellulose-degrading enzymes existed. This study has an important implication for resource utilization of livestock manure seeking higher efficiency and quality of compost.

Declarations

Authors' contributions Writing-original draft: LYZ; Writing-reviewing: XPY and YQX; Investigation: XWW and JPD; Formal analysis: ZFW, HTZ, YYL; Validation: Funding acquisition: LF.

Funding This study was supported by National Natural Science Foundation of China (51908255), Regional Collaborative Innovation Special Project of Xinjiang Uygur Autonomous Region (Nos.2020E0204), Forestry Development Subsidy Fund Project of Xinjiang Uygur Autonomous Region (Nos. XJLYKJ-2021-15).

Data availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare no competing interests.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Tables

Table 1 Characteristics of raw materials used in this study.

Parameters	pH	C/N (%)	TOC (%)	OM (%)	Moisture (%)	Carbon Source	Weight (kg)
Treated	9.2	30.0	28.3	50.1	60.0	Straw	1000.0
Control	9.2	30.0	28.3	50.1	60.0	Straw	1000.0

Abbreviation: OM, organic matters; TOC, total organic carbon (TOC=OM/1.724); C/N, the ratio of total organic carbon to total kjedahl nitrogen.

Figures

Fig. 1

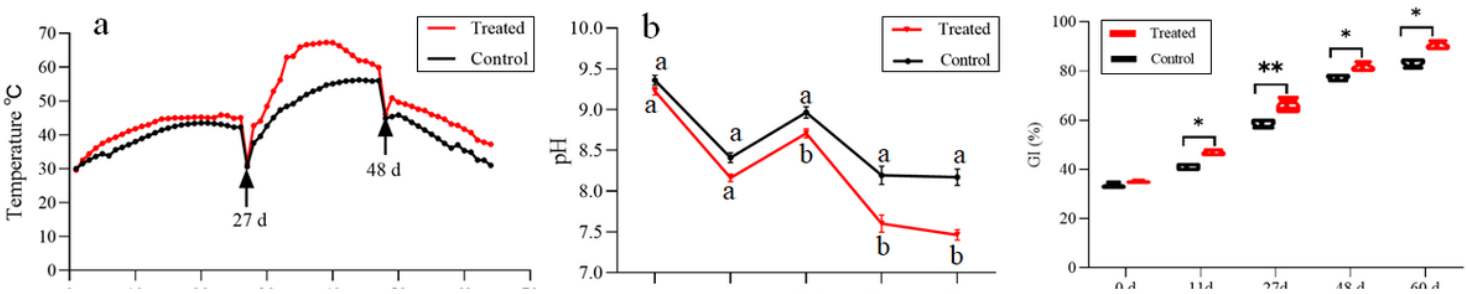

Figure 1

Changes in physicochemical parameters during the composting process of treated and control groups. (a)Temperature, (b) pH, (c) GI, (d) Lignin degradation rate, (e) Cellulose degradation rate, (f). Hemicellulose degradation rate. All data are the mean of three replicates and error bars indicate standard deviations. The same letter (a, b) are not significantly different at $p < 0.05$ level.

Fig. 2

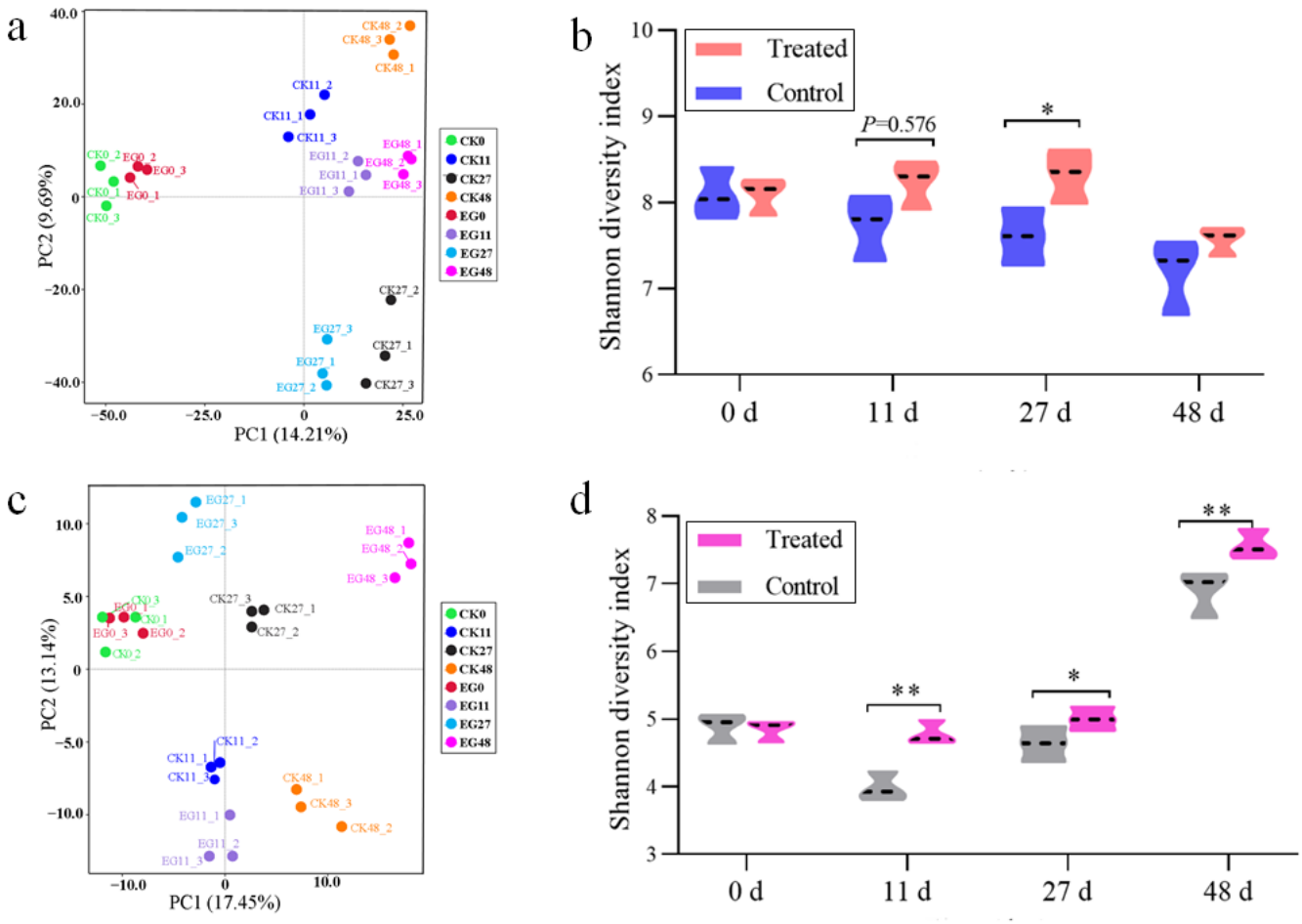

Figure 2

Successions of the microbial community during the composting process of treated and control groups. Principal component analysis (PCA) and Alpha diversity based on Shannon indexes of bacterial (a) (b) and fungal (c) (d) community composition in treated and control groups (n= 3 for each group). Boxes are vertically bounded by the 1st and 3rd numerical value and center line ismedian.

Fig. 3

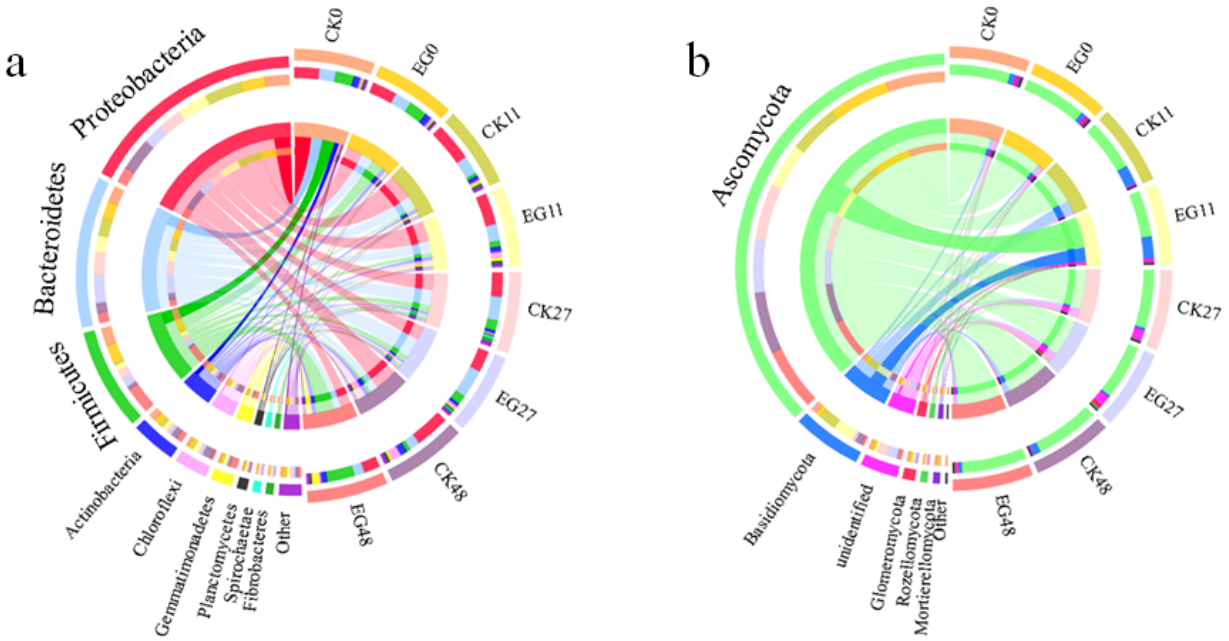

Figure 3

Changes of bacterial (a) and fungal (b) community composition in treated and control groups at the phylum level during the composting process.

Fig. 4

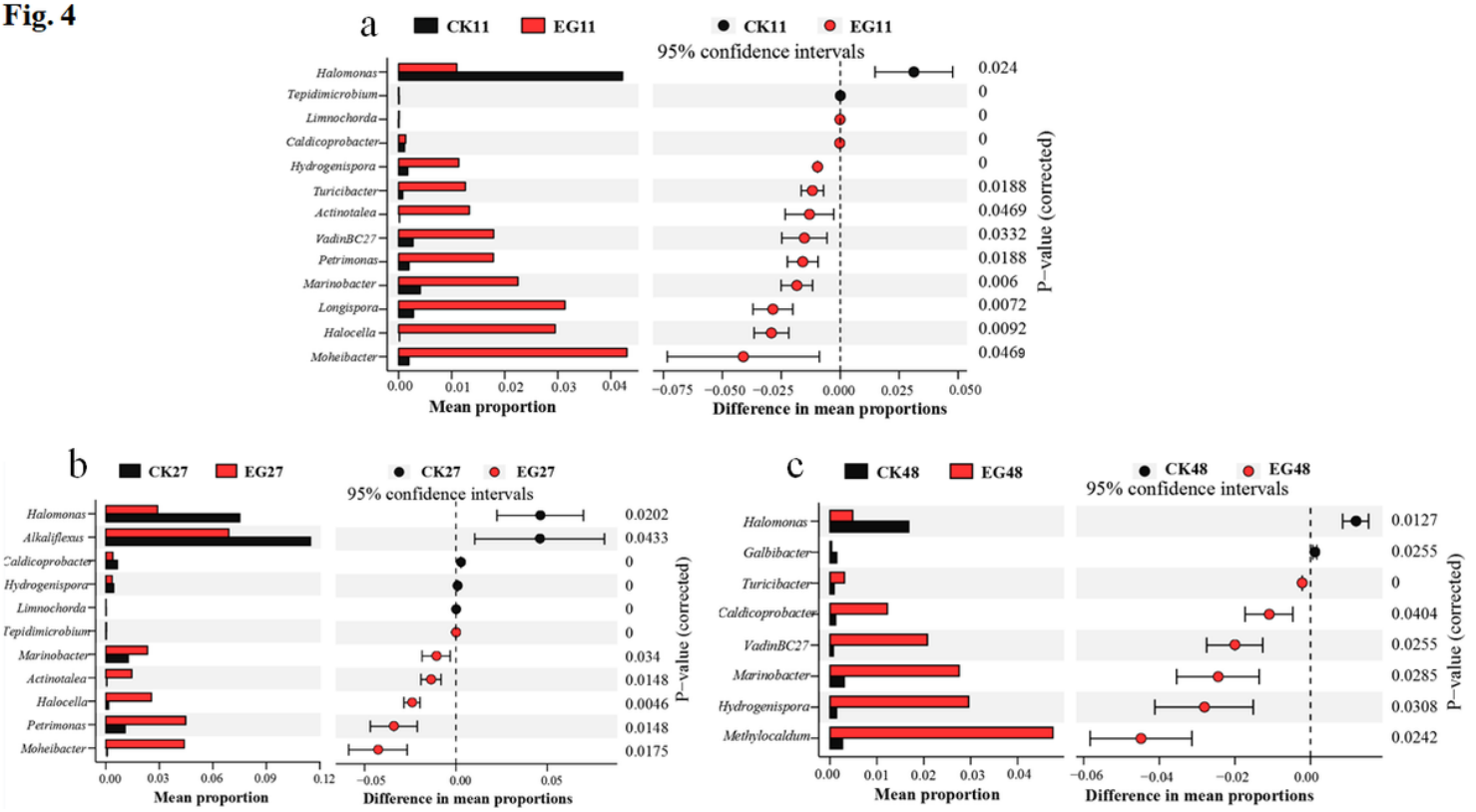

Figure 4

The significantly different (p< 0.05) groups of bacterial community (genus level). Corrected P-values were calculated using the FDR-corrected. The different colours overrepresented in the community have a positive or negative difference between proportions.

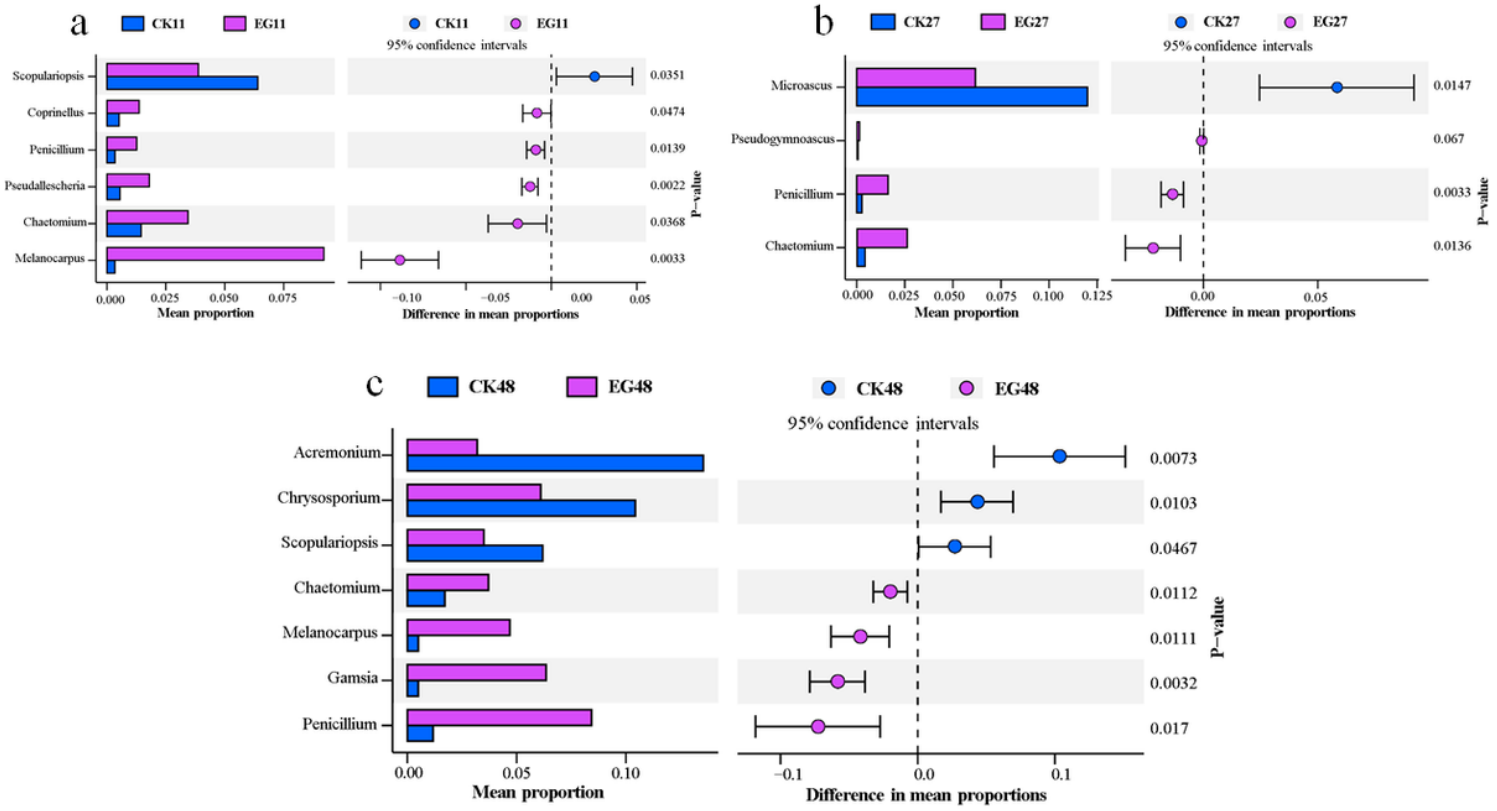

Figure 5

The significantly different (p< 0.05) groups of fungal community (genus level). Corrected P-values were calculated using the FDR-corrected. The different colours overrepresented in the community have a positive or negative difference between proportions.

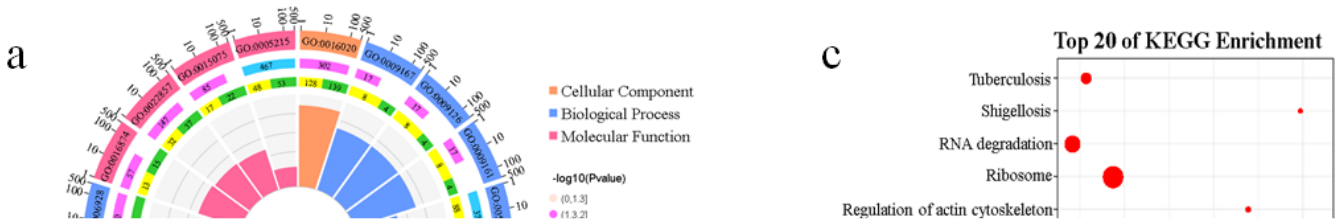

Figure 6

GO and KEGG analysis of DEGs for metabolism pathways of bacterial community in treated group. Top 20 GO enrichment circle (a) and GO summary graphs (b). KEGG enrichment analysis (c). There are four circles from outside to inside in (a). The first lap indicates top 20 GO term and the number of the genes corresponds to the outer lap. The second lap indicates the number of genes in genome background and P values for enrichment of the upregulated genes for the specified biological process. The third lap indicates the ratio of the upregulated genes (yellow purple) and downregulated genes (green purple). The fourth lap indicates the enrichment factor of each GO term.

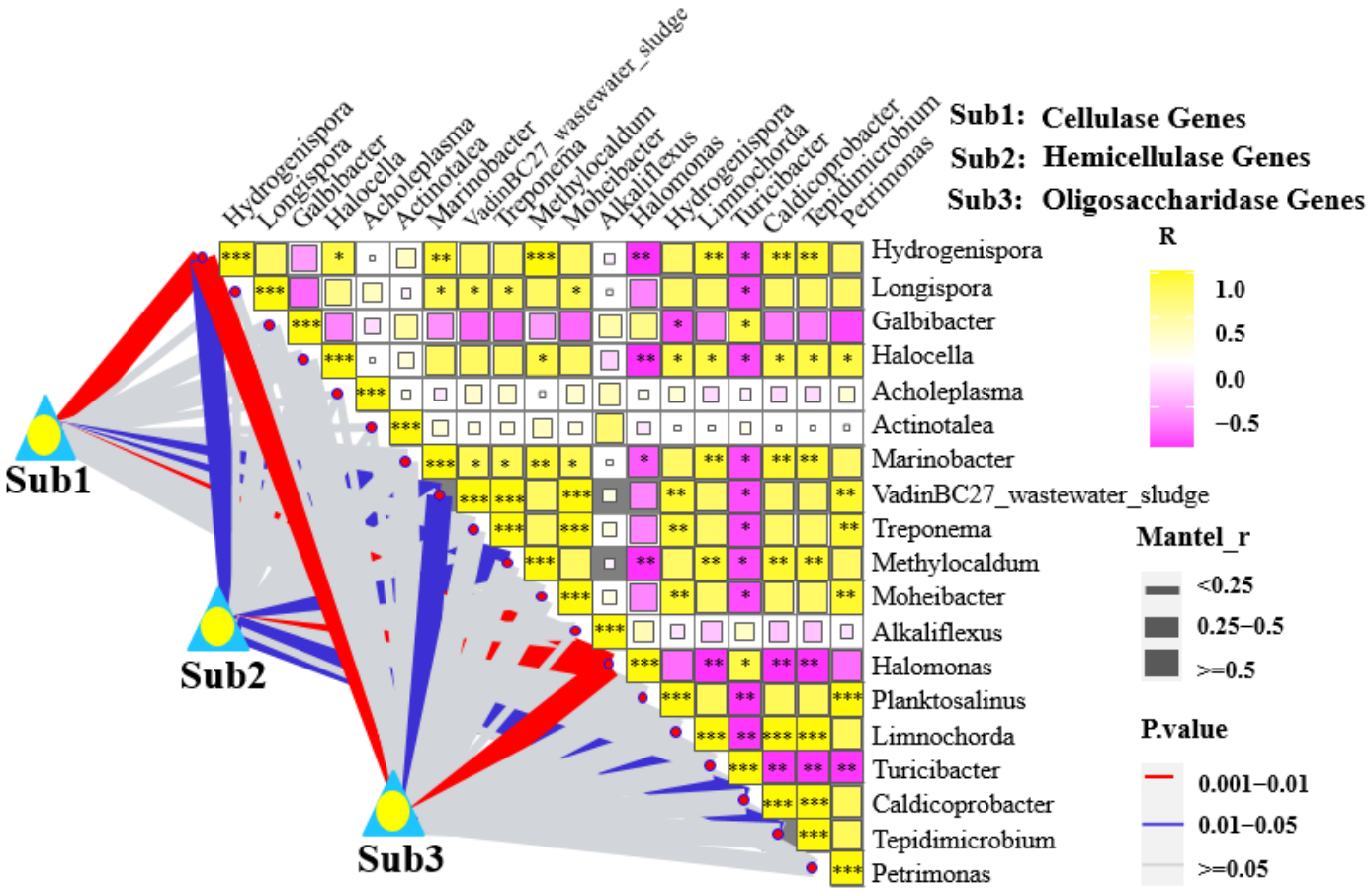

Figure 7

Pairwise comparisons of the bacterial community (genus level) with a color gradient denoting Spearman's correlation coefficients. Lignocelluloses degrading enzyme genes were related to each by mantel test.

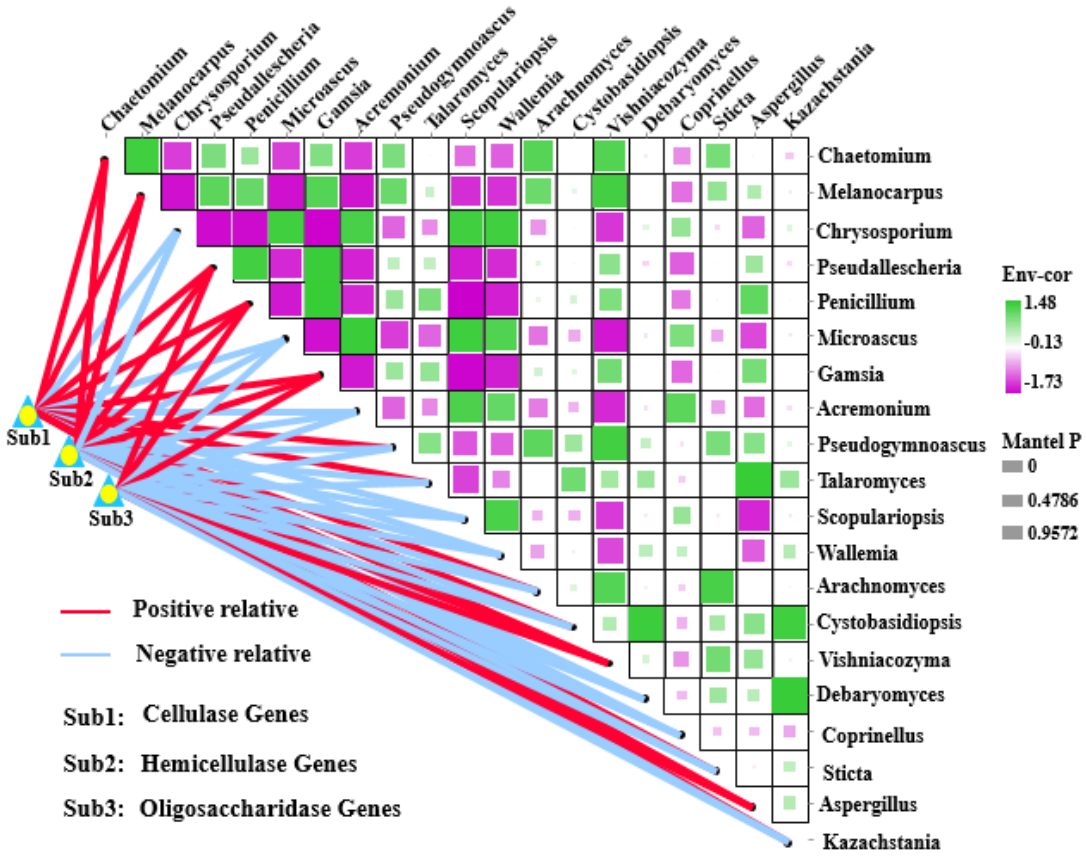

Figure 8

Figure legend not available with this version.

Supplementary Files

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