Metagenome analysis reveals potential microbial functions in topsoil of wheat–maize rotation system with five-year application of fertilizers

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Abstract: Fertilization mode affects soil quality and ecological health. The effects of four fertilization regimens on lignocellulose content, readily degradable carbohydrate decomposition, and potential microbial functions in the topsoil of a wheat–maize rotation system between 2012 and 2017 were investigated. The fertilization regimens of control (control NFNB), high chemical fertilizer (HCF), high biochar plus low chemical fertilizer (HBLCF), and biochar-based fertilizer (BBF) were compared on soil fundamental properties, microbial structure, and potential function in soil carbohydrate degradation based on metagenome analysis. The diversity of carbohydrate-active enzyme genes in the topsoil microbial consortia in the four trials was primarily distributed within the ten ecologically most dominant phyla. Application of BBF was associated with the lowest decline in total nitrogen and P_2O_5 (2012-2017: 6.5% and 28.1%, respectively) and the most effective carbohydrate decomposition (2015-2017: 67.0% for cellulose and 59.9% for readily degradable carbohydrate). Carbohydrate transport and metabolism accounted for 6.0% of reads assigned functional classification under the BBF regimen. These findings reveal the ecologically functional diversity of topsoil microorganisms and suggest BBF application as a promising strategy for sustainable agriculture and beneficial to soil health.

Keywords: biochar-based fertilizer, carbohydrate decomposition, genetic function, microbial community, topsoil
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1 Introduction

Modern agricultural practices are characterized by the overuse of chemical fertilizers to enhance yields[1-3]. The excess use of chemical fertilizers has resulted in a significant decline in soil health and soil microbial diversity and has led to serious water and soil pollution[4,5]. An increasing number of studies on biomass recycling suggest that the use of biomass fertilizer can enhance soil quality and mitigate effects of climate change[6,7]. Biochar, a carbon-rich solid material obtained from biomass through pyrolysis[8,9], has gained substantial attention because of its beneficial properties for agriculture, such as water retention, improvement of nutrient availability, promotion of microorganism growth, and sequestration of atmospheric carbon dioxide[10,12]. However, in general, pure biochar does not provide sufficient nutrients or readily degradable organic matter[13,14]. Compared with chemical fertilizers and biochar, manure compost is effective in increasing degradable organic matter and soil microbial activities[1]; however, the effects are often short-lived because of the high decomposition rate of manure compost[15]. Therefore, the incorporation of manure compost into biochar to produce biochar-based fertilizer (BBF) is considered a promising approach to developing a sustainable nutrient source.

Organic and inorganic fertilizers can significantly affect soil microorganisms[13]. Soil microbial communities are responsive to the addition of biochar or compost, which increase microbial abundance and activities by introducing microorganisms and providing an environment with ample aeration, water, and nutrients[16,17]. Given that the residence time of biochar in the soil is expected to be hundreds to thousands of years, the changes in microbial community structure and functions might persist for a long time[18], and thus, the long-term consequences of applying BBF for soil microbial communities in fields need to be considered. Nevertheless, the soil microbiological diversity after long-term application of BBF remains largely unknown.

The adaption of the soil microbial structure and function to the

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soil environment is considered essential for sustainable agricultural production[19]. Soil microbial biomass, activity, and community structure are critical for soil quality and health, which influence plant growth by affecting the nutrient turnover and disease incidence[20]. Microorganisms in soils play an important role in the decomposition of soil organic matter[21]. Carbohydrate is regarded one of the key indicators of the quality of soil organic matter owing to its roles in energy supply, nutrient cycling, and aggregate formation in the soil ecology[22,23]. Carbohydrate is more sensitive to external factors than the total soil organic matter. As such, the interaction between soil microorganisms and carbohydrate plays key roles in the soil microenvironment, and understanding how soil microorganisms respond to BBF application may help strengthen agricultural ecosystem health.

Previous studies have reported soil microbial properties in soils under different fertilization practices; however, few attempts have been made to compare the long-term effects of high chemical fertilizer (as used in local farming practice), biochar plus chemical fertilizer, and biochar-based fertilizer (BBF) on soil microbial properties, including microbial structure and function, especially, on the basis of metagenome analysis. Therefore, the objectives of this study were: (i) to analyze the 5-year effects of control (NFNB), high chemical fertilizer (HCF), high biochar plus low chemical fertilizer (HBLCF), and BBF on soil properties, and (ii) to identify the microbial composition and potential function in soil carbohydrate degradation.

2 Materials and methods

2.1 Site description

This study was conducted at Xingdong Agricultural Station in Shiye Town, Dantu District, Jiangsu Province in eastern China (32°12′34.39″N, 119°19′27.08″E). The region has a subtropical monsoon climate, with a mean annual rainfall of 1088.3 mm and an average annual temperature of 15.6 ℃.

2.2 Materials

2.2.1 Soil

The soil is a typical yellow brown soil (Hapludalf) with silty clay loam texture. Baseline soil chemical properties at the start of the experiment in 2012 were 28.27 soil organic matter g/kg, 1.95 g TN/kg, 18.43 mg P_2O_5/kg, and 198.99 mg K_2O/kg, and the water content was 26.5%.

2.2.2 Chemical fertilizer

The synthetic mineral fertilizer (Stanley, Linshu, China) used contained 15% N, 15% P_2O_5, and 15% K_2O. The urea fertilizer (Stanley, Linshu, China) had a TN content ≥46.4%. The chemical fertilizers were commercially purchased.

2.2.3 Biochar

The biochar was made from maize straw using a carbonization furnace designed by the Chinese Academy of Agricultural Engineering at a pyrolysis temperature of 650 ℃ for 35 min. The water content of the biochar was 6.1%, the fixed carbon content was 12.9%, and the total content of N, P_2O_5, and K_2O was 6.3%.

2.2.4 Biochar-based fertilizer

The BBF consisted of organic fertilizer, the synthetic mineral fertilizer described in Section 2.2.2, and the biochar described in Section 2.2.3. The organic fertilizer was compost produced from pig manure, and it was mixed with the synthetic mineral fertilizer at a mass ratio of 3:1 to form the basic fertilizer. The basic fertilizer was mixed with biochar at a mass ratio of 2:3 to form the BBF. The water content of the BBF was 10%, the organic matter content was 62.6%, and the total content of N, P_2O_5, and K_2O was 11.8%.

2.3 Fertilization treatments

The experiment consisted of 16 plots that were allocated four fertilization treatments with four replicates arranged according to a randomized complete block design. Each plot was 21 m^2 (3.5 m × 6 m), and the plots were separated by plastic boards inserted into the soil to a depth of approximately 40 cm to prevent the flow of water and fertilizer between the plots. The four fertilization treatments were as follows: a control (NFNB) without addition of any fertilizer or biochar; a standard treatment (HCF) reflecting the local farming practice with heavy chemical fertilization (for wheat, 450.0 kg synthetic mineral fertilizer per hm^2 and 225.0 kg urea fertilizer per hm^2, and for maize, 450.0 kg synthetic mineral fertilizer per hm^2 and 450.0 kg urea fertilizer per hm^2); a combined treatment (HBLCF) with high biochar addition (30.0 ton biochar per hm^2) and low chemical fertilizer addition (270.0 kg synthetic fertilizer per hm^2); and a BBF treatment (BBF) with BBF addition alone (10.5 t/hm^2). HCF plots were fertilized each year from 2012 to 2017, plots under HBLCF and BBF treatments were fertilized in both 2012 and 2015. Fertilization was applied to the soil surface and harrowed thoroughly to a depth of approximately 20 cm. A double cropping system of summer maize–winter wheat (planted in June and October, respectively) was applied from June 2012 to June 2017.

2.4 Sampling

Soil samples were collected in October, from 2012 to 2017. Each sample was a mixture of more than 5 individual soil cores collected at a depth of 5-20 cm around the roots. Visible roots, plant residues, and stones were removed from the soil samples. Soil samples were finely ground, sieved, and then were stored at −80 ℃ until analysis.

2.5 Soil chemical properties

TN, P_2O_5, K_2O, soil organic matter, and moisture content were measured after each sample was collected. The content of total Kjeldahl nitrogen was measured based on the fertilizer standard method in China (NY 525-2011), and P_2O_5, K_2O, and soil organic matter were determined following procedures for soil analysis reported by Wang et al.[24] and Yang et al.[25] Soil moisture content was measured gravimetrically. Carbohydrate composition was determined just using soil samples collected in 2015, 2016 and 2017 under the four treatments, due to the poor storage conditions of soil samples in 2012, 2013 and 2014. The content of total carbohydrates was determined by hydrolyzing them to the reducing sugar, and then measuring the absorption peak using the spectrophotometer.3,5-dinitrosalicylic acid method described by Teixeira et al.[26] Lignin, cellulose, and hemicellulose were measured by analyzing the absorption peaks using the spectrophotometer according to the procedure for fibre content analysis reported in Jung et al.[27] The content of readily degradable carbohydrate was the difference between the total carbohydrate content, the cellulose and hemicellulose contents. One-way analysis of variance (ANOVA) in SPSS 24.0 (IBM Corp., Armonk, USA) was conducted for statistical analysis.

2.6 DNA extraction

Soil samples collected from fields under the four treatments before harvesting of the maize in 2017 were subjected to sequencing analysis. DNA extraction from soil sample for metagenomics was conducted. Then the purity was quantified by

NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the quality was examined.

2.7 Metagenome sequencing

DNA was fragmented to an average size of approximately 300 bp using a Covaris M220 (Gene Company Limited, China) for paired-end library construction. The paired-end library was prepared using a TruSeq DNA Sample Prep Kit (Illumina, San Diego, CA, USA). Adapters containing the full complement of sequencing primer hybridization sites were ligated to the blunt-end fragments. Paired-end sequencing was conducted on an Illumina HiSeq 4000 platform (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China), using a HiSeq 3000/4000 PE Cluster Kit and HiSeq 3000/4000 SBS Kit according to the manufacturer's instructions (www.illumina.com). All the raw metagenomics datasets have been deposited into NCBI Sequence Read Achieve database (Accession Nnumber SRP139498). The raw reads were trimmed, and the filtered reads were assembled. Then the gene prediction and catalogue were conducted as described by Cai et al.[28]

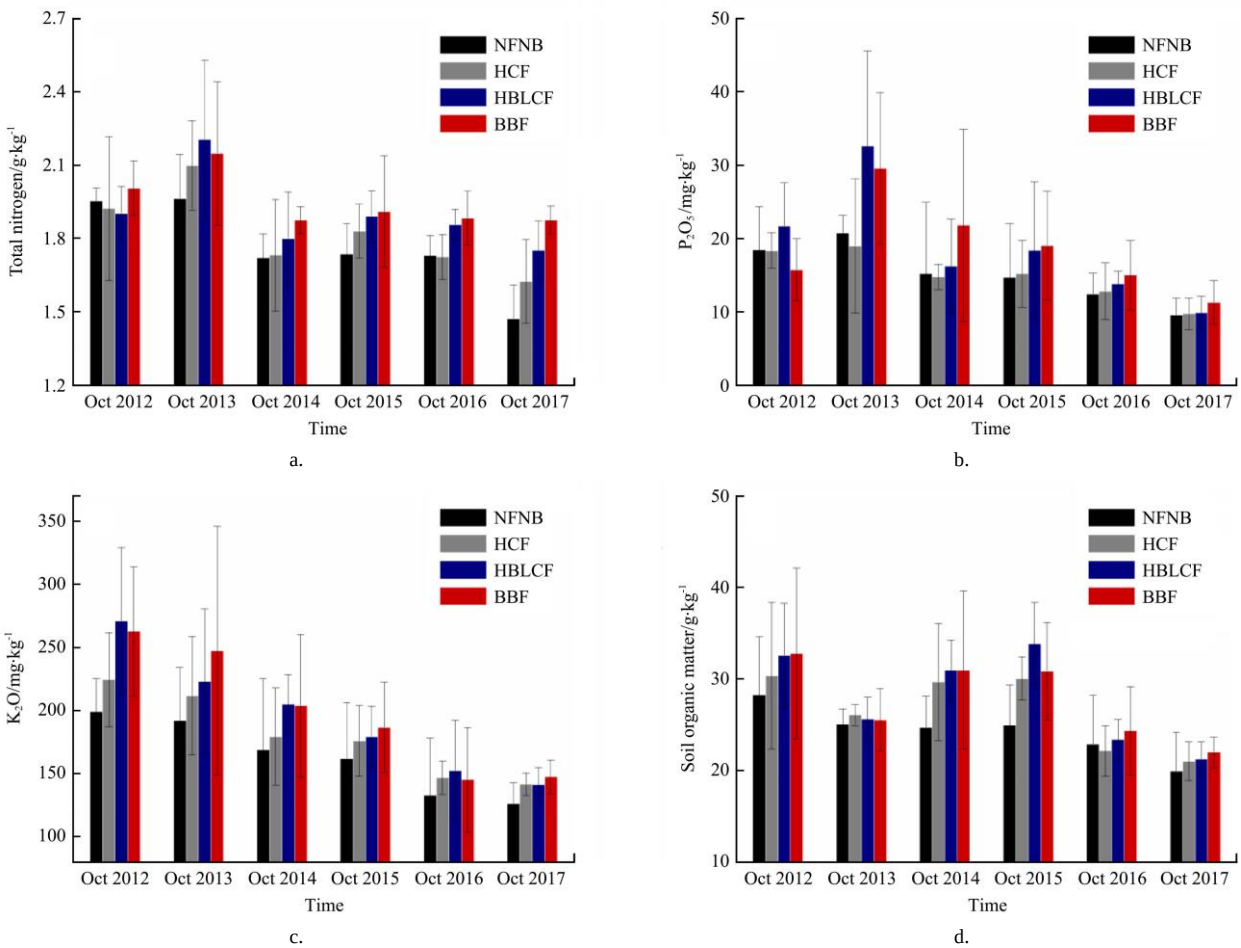
2.8 COG, KEGG, and CAZyme annotations

Genes were searched against the database of evolutionary genealogy of genes: non-supervised Orthologous Groups (eggNOG) to annotate the clusters of orthologous groups of proteins (COG)[24], searched against the database of the Kyoto Encyclopedia of Genes and Genomes (KEGG) to illuminate the degradation pathways, and were annotated against the carbohydrate-active enzyme (CAZyme) database to inform the enzyme families including: glycoside hydrolases (GHs), glycosyl transferases (GTs), carbohydrate esterases (CEs), carbohydrate binding modules (CBMs), auxiliary activities (AAs), and polysaccharide lyases (PLs)[29]. COG functional classifications of the four treatments were compared using Bray–Curtis distances, followed by principal coordinate analysis (PCoA).

3 Results

3.1 Soil chemical properties

From 2012 to 2017, the nutrient contents declined overall. For TN and P_2O_5, the percent decline was the lowest under the BBF treatment; while for K_2O and soil organic matter, the percent decline was the lowest under NFNB treatment (Figure 1). Under NFNB, HCF, HBLCF, and BBF, the decreases in TN were 24.6%, 15.5%, 7.9%, and 6.5%, respectively; the decreases in P_2O_5 content were 48.0%, 46.7%, 54.2%, and 28.1%, respectively; the decreases in K_2O content were 36.6%, 36.8%, 47.9%, and 43.9%, respectively; and the decreases in soil organic matter content were 29.4%, 30.7%, 34.8%, and 32.9%, respectively.



Note: The four regimens were as follows: a control without the addition of any fertilizer or biochar (NFNB); a standard treatment reflecting the local farming practice with high chemical fertilizer addition (HCF); a combined treatment with high biochar addition and low chemical fertilizer addition (HBLCF); and a treatment with biochar-based fertilizer alone (BBF).

Figure 1 Soil chemical properties under the four fertilization regimens

In 2017, the nutrient contents under BBF treatment were all higher than those under other treatments; however, only TN was significantly higher under BBF in 2017 (1.88 g/kg) than under the other treatments ($p<0.05$), while the differences for P_2O_5, K_2O, and soil organic matter were not significant. Taken together, the percent declines and final contents of nutrients after five-year

cultivation indicated that BBF performed the best in terms of nutrient conservation.

3.2 Ariations in lignocellulose and readily degradable carbohydrate

From 2015 to 2017, the contents of lignin, cellulose, and readily degradable carbohydrate decreased significantly (p<0.05) in all regimens, while the hemicellulose content showed variation. Under BBF, HBLCF, HCF, and NFNB, lignin decreased by 53.0%, 57.1%, 57.0%, and 41.5%, respectively; cellulose decreased by 67.0%, 37.2%, 56.3%, and 51.1%, respectively; and readily degradable carbohydrate decreased by 59.9%, 57.2%, 45.7%, and 44.1%, respectively. The hemicellulose content increased by 7.8% under BBF and by 10.9% under HCF, while it decreased by 7.1% under HBLCF and by 20.1% under NFNB. In 2016, the hemicellulose content increased under HCF, HBLCF, and BBF, but not under NFNB. This may be due to the biodegradation of biochar and BBF after the fertilization in 2015 or hemicellulose biosynthesis occurring in the soil microenvironment (Figure 2). Accordingly, the topsoil microenvironment under BBF showed better carbohydrate degradation ability.

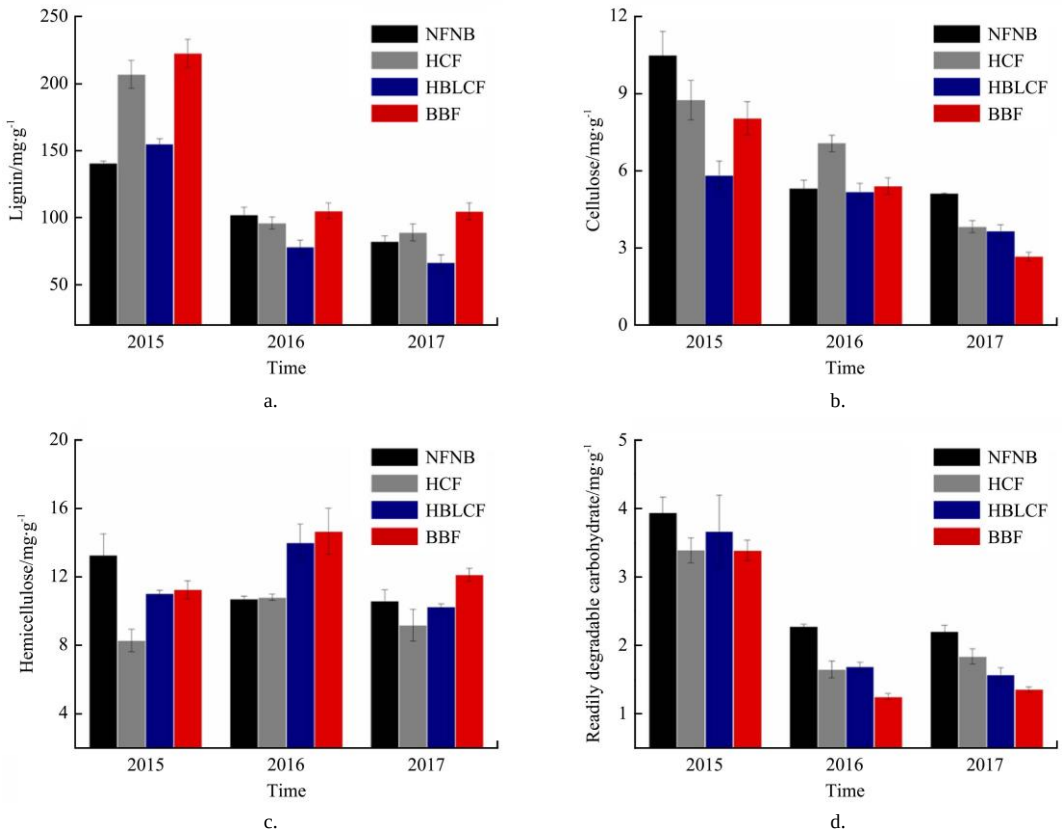


Figure 2 Soil contents of lignocellulose and readily degradable carbohydrate under the four regimens in 2015, 2016, and 2017

3.3 Genome data

Metagenome sequencing of the microorganisms in topsoil samples under treatments BBF, HBLCF, HCF, and NFNB yielded 104 525 886, 103 344 656, 103 550 946, and 96 914 240 raw reads, respectively, which accounted for 15.8 Gb, 15.6 Gb, 15.6 Gb, and 14.6 Gb, respectively. The percentages of clean reads in raw reads were 98.8%, 98.8%, 98.3%, and 98.6%, respectively. The numbers of contigs were 806240, 825450, 808750, and 740556. The total open reading frames (ORFs) of four samples were 9 944 824, and the average length was 405.29 bp.

3.4 Microbial community

The most abundant phyla in the domains of bacteria, archaea, and eukaryotes are listed in Figure 3. As for bacteria, Proteobacteria, Actinobacteria, and Acidobacteria were the three most abundant phyla, which together accounted for >70% of all phyla in the soil samples. The relative abundances of Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes, Firmicutes, Bacteroidetes, Nitrospirae under BBF and HCF were higher than those under HBLCF and NFNB, while Actinobacteria under HBLCF and NFNB were higher than those under BBF and HCF. As for archaea, Thaumarchaeota showed the highest abundance, and it was also among the ten most abundant total phyla under the four regimens. Soil under BBF treatment was low in archaea, while NFNB-treated soil had a higher abundance of this domain. Regarding eukaryotes, the relative abundances were all <0.05%. In this domain, the abundant phyla were mainly from the kingdoms of metazoa, fungi, and viridiplantae. The existence of metazoa may be due to animal activities, and the three most abundant fungal phyla were Ascomycota, Basidiomycota, and Chytridiomycota.

3.5 COG and CAZyme annotations reveal relationships between enzyme genes and microorganisms

In eggNOG, for BBF, HBLCF, HCF, and NFNB samples, 1 169 456, 1 233 468, 1 178 836, and 1 164 420 reads were assigned to carbohydrate transport and metabolism, respectively. In BBF-treated soils, the functions of energy production and conversion, and carbohydrate transport and metabolism accounted for 7.9% and 6.0%, respectively.

For BBF, HBLCF, HCF, and NFNB samples, 679906, 710332, 688644, and 668514 reads were annotated to CAZyme-encoding genes, respectively.

In all four trials, the CAZyme-encoding genes were predominantly derived from the phyla of Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Firmicutes, and Thaumarchaeota (Figure 4). Among these, Thaumarchaeota are

archaea and all other phyla are bacteria. Supplementary Figure S1 indicated that the CAZyme-encoding genes showed similar overall distributions under the four treatments, with GHs and GTs, which are critical for the cleavage of polymeric substrates[30], being the most abundant. Supplementary Figure S1 shows the proportion of CAZyme-encoding genes under the four treatments.

In the BBF-treated soil, the read abundances of GHs, GTs, CEs, CBMs, AAs, and PLs were 33.1%, 31.1%, 18.3%, 9.2%, 5.3%, and 3.0%, respectively. Figure 5 presents the relationship between the functional bacteria and the CAZyme-encoding genes. The functional bacteria were also ecologically dominant. Most of the CAZyme-encoding genes under BBF were derived from unclassified Acidobacteria, followed by *Nocardioides* (phylum Actinobacteria), *Solirubrobacter* (phylum Actinobacteria), *Streptomyces* (phylum Actinobacteria), and unclassified Betaproteobacteria (phylum Proteobacteria). Though *Phycicoccus* (phylum Actinobacteria) was ranked the 19th in relative abundance in BBF samples, it was still among the ten genera the most enriched with CAZyme-encoding genes.

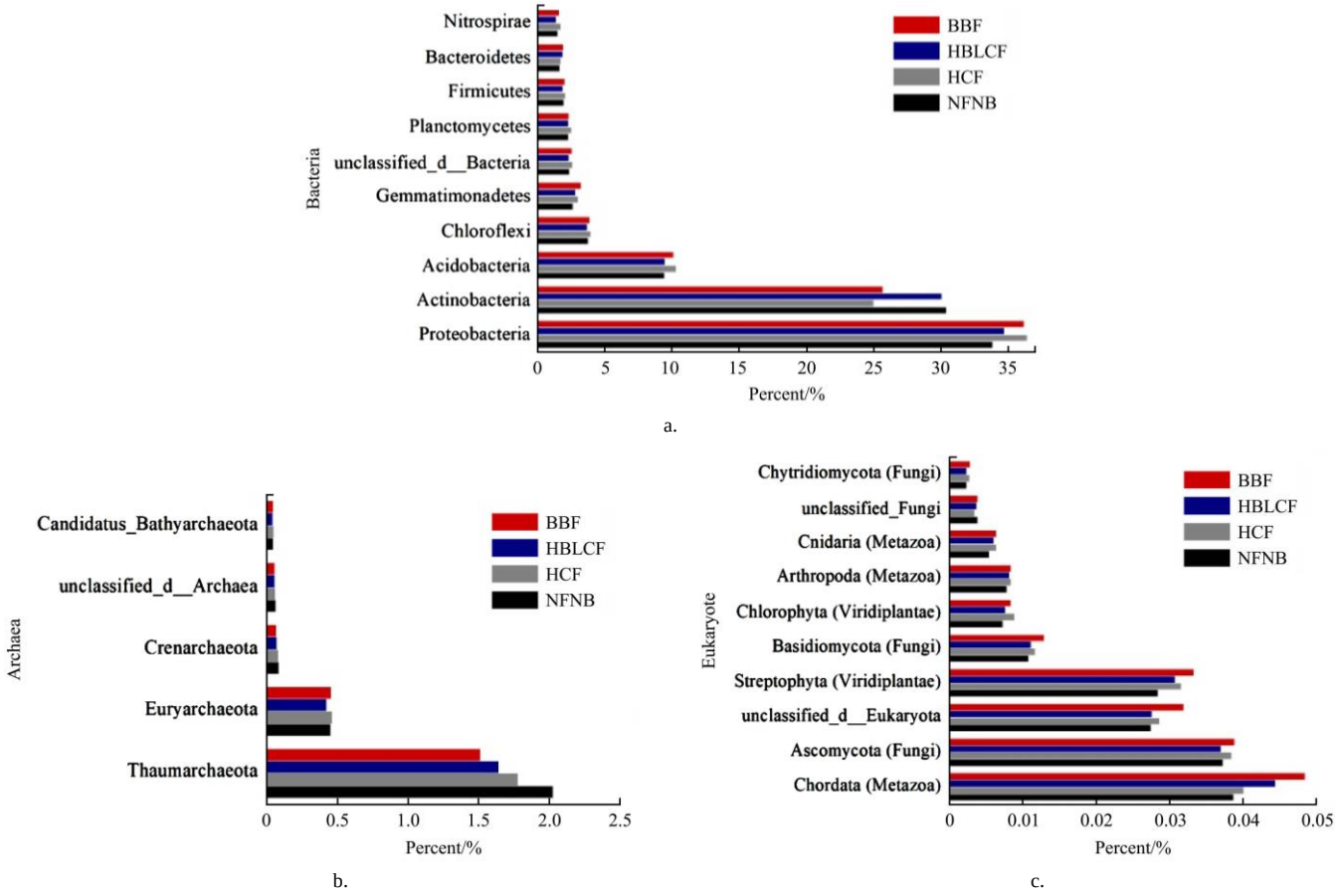


Figure 3 Soil microbial community distribution under the four regimens at the phylum level in the domains bacteria (a), archaea (b), and eukaryote (c), based on metagenome sequencing

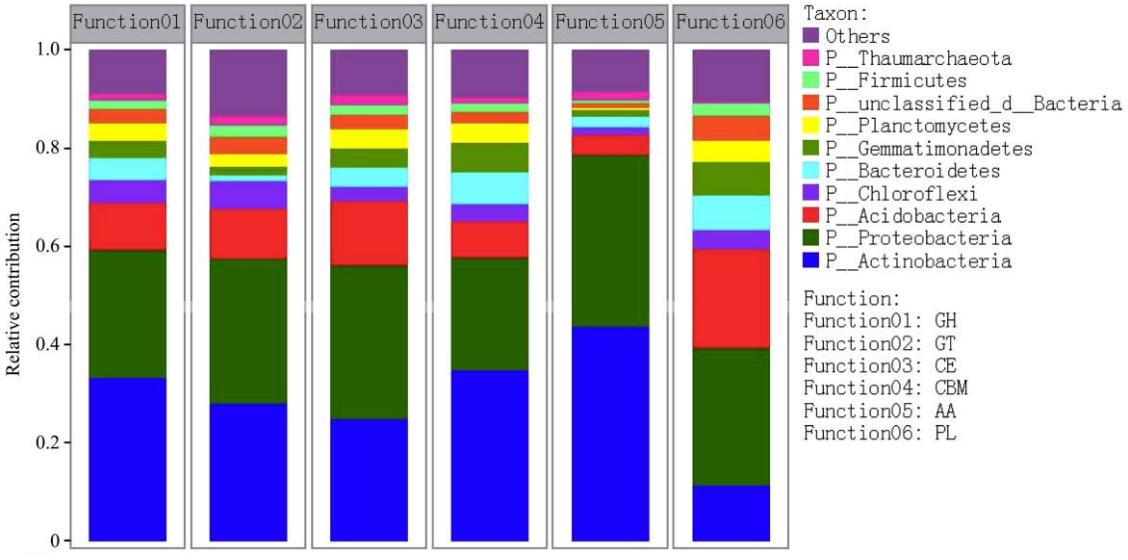


Figure 4 Contributions of carbohydrate-active enzyme (CAZyme) genes of functional phyla under the four regimens

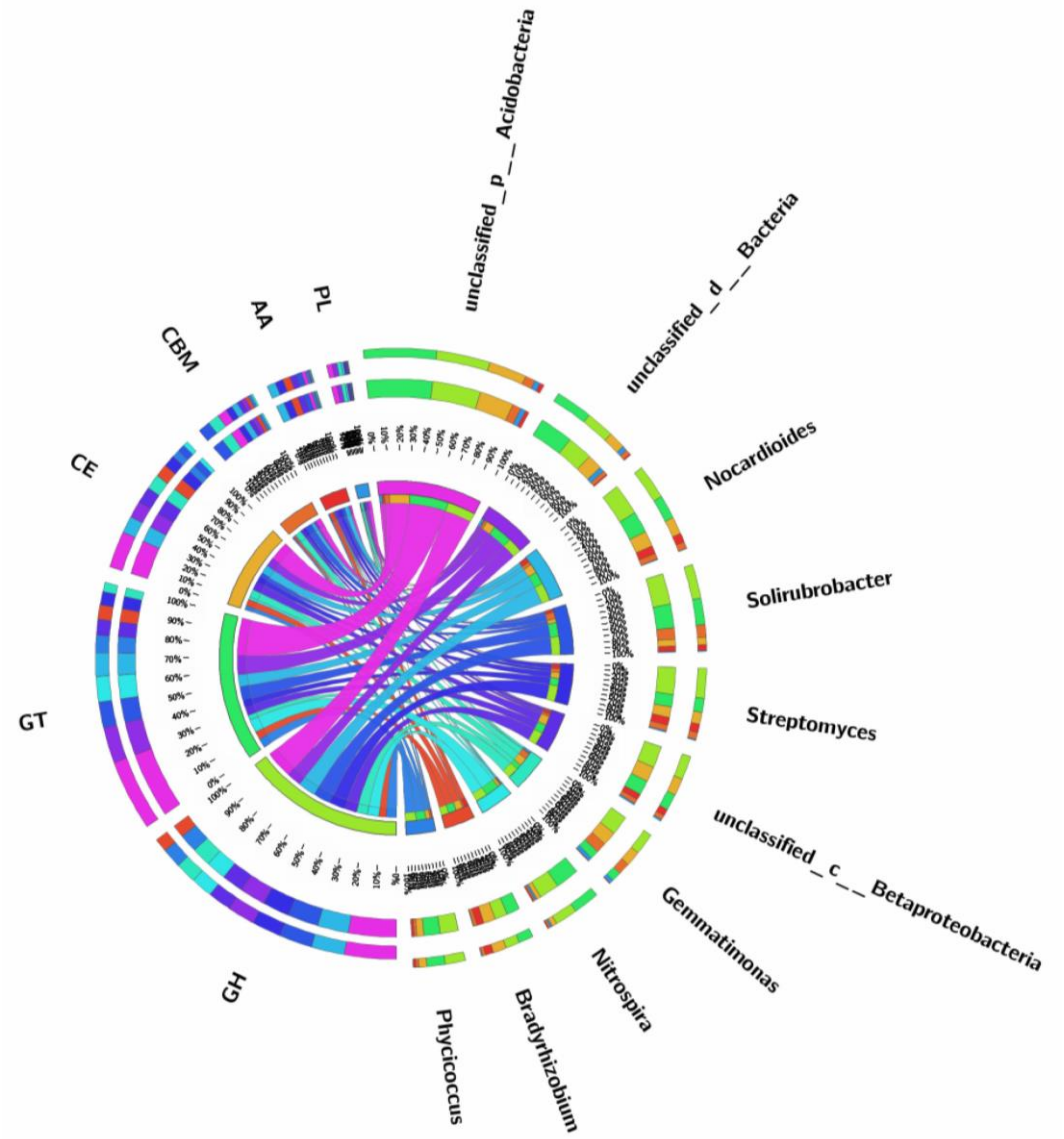


Figure 5 Relationship between the functional bacteria and the CAZyme-encoding genes

3.6 KEGG annotation elucidates pathways involved in carbohydrate metabolism

To illustrate the metabolic pathways critical to carbohydrate metabolism, related genes identified in BBF-treated soil samples were annotated to KEGG pathways. Glucose is converted into pyruvate through glycolysis, which will generate free energy. In BBF-treated soil, a variety of related enzyme-encoding genes were annotated to glycolysis (ko00010, 299334 reads). The extracellular D-Glucose was metabolized to α-D-Glucose 6-phosphate under the action of phosphotransferases (EC 2.7.1.199), and then metabolized to D-Glucose 1-phosphate, *β*-D-Glucose 6-phosphate and *β*-D-Fructose 6-phosphate under the action of enzymes of phosphoglucomutase (EC 5.4.2.2), glucose-6-phosphate isomerase (EC 5.3.1.9), and glucose-6-phosphate 1-epimerase (EC 5.1.3.15). *α*-D-Glucose 6-phosphate connected with the pentose phosphate pathway, and D-Glucose 1-phosphate connected with the starch and sucrose metabolism. *β*-D-Fructose 6-phosphate was gradually metabolized into the phosphoenolpyruvate, and then transferred into the pyruvate by pyruvate kinase (EC 2.7.1.40), connected with the pyruvate metabolism and the citrate cycle. The pyruvate was metabolized into L-lactate by L-lactate dehydrogenase (EC 1.1.1.27). L-lactate connected with the propanoate metabolism.

The plentiful types of enzyme-encoding genes annotated to amino sugar and nucleotide sugar metabolism in BBF soil (ko00520, 211582 reads) facilitate the metabolism of polysaccharides such as hemicellulose and chitin, and monosaccharides such as fructose, mannose, glucose, and galactose. Chitin could be metabolized to acetyl glucosamine or to D-fructose 6-phosphate, which is connected to the glycolysis. Fructose was phosphortransferred into *β*-D-Fructose 6-phosphate, then entering the glycolysis. L-Arabinose was metabolized from arabinan by *α*-L-arabinofuranosidase (EC 3.2.1.55). 1,4-*β*-D-xylan could be hydrolyzed to D-xylose by *β*-xylosidase (EC 3.2.1.37), which connected with the pentose and glucuronate interconversions. Glucose could be metabolized into D-Glucose 1-phosphate, which is connected with the acarbose and validamycin biosynthesis. It could be sequentially metabolized into uridine diphosphate glucose, and connected with the ascorbate and aldarate metabolism. The *α*-D-Galactose could be finally metabolized into UDP-*α*-D-galactofuranose.

The genes annotated to starch and sucrose metabolism under BBF application (ko00500, 196744 reads) indicated that extensive metabolism of these carbohydrates might occur in this soil. The cellulose could be metabolized into cellodextrin by endoglucanase (EC 3.2.1.4), then into cellobiose by *β*-glucosidase (EC 3.2.1.21)

and cellulose 1,4-β-cellobiosidase (EC 3.2.1.91); the cellulose could also be metabolized into cellobiose by cellulose 1,4-β-cellobiosidase (EC 3.2.1.91). Cellobiose would be transferred into D-Glucose or D-Glucose 1-phosphate by cellobiose phosphorylase (EC 2.4.1.20). For starch metabolism, the resistant amylose could be metabolized to sucrose by amylosucrase (EC 2.4.1.4), and then degraded into fructose and glucose. Amylose could also be metabolized into maltose by isoamylase (EC 3.2.1.68) and α-amylase (EC 3.2.1.1). Finally, starch could degrade into glucose by maltase-glucoamylase (EC 3.2.1.3), α-amylase (EC 3.2.1.1), and sucrase-isomaltase (EC 3.2.1.10). The sucrose could be hydrolyzed into D-Fructose and D-Glucose under the action of maltase-glucoamylase (EC 3.2.1.20) and β-fructofuranosidase (EC 3.2.1.26). Supplementary data Figures S2-S4 show the pathways involved in carbohydrate metabolism.

4 Discussion

Under all four regimens, the percent declines and final contents of nutrients after five-year cultivation indicated that BBF performed the best in terms of nutrient conservation and carbohydrate degradation ability. The most abundant bacterial phyla of Proteobacteria, Actinobacteria, and Acidobacteria are capable of degrading complex carbohydrates such as cellulose and hemicellulose[31,32]. Ammonia-oxidizing Thaumarchaeota and methane-producing Euryarchaeota, which play important roles in carbon and nitrogen cycling, were the dominant Archaea in all experimental fields[33].

Under BBF treatment, 1026 NOGs were related in the classification of carbohydrate transport and metabolism. The most abundant NOGs are discussed below.

Dehydrogenase, including COG4993 (48354 reads) and COG2133 (35928 reads), was the most abundant involved in carbohydrate transport and metabolism. Dehydrogenase is considered to be an indicator of overall soil microbial activity and is one of the most commonly used biological indicators of the ecological wellbeing of soil environments[34]. The major facilitator superfamily COG0477 (38256 reads), selectively transports a wide spectrum of substrates across biomembranes and plays a pivotal role in multiple physiological processes[35,36]. COG0366 (25032 reads) is an amylase-encoding gene that is related to the degradation of cellulose, hemicellulose, and starch[37,38]. COG1109 (18724 reads) catalyzes the glucosamine-6-phosphate be metabolized to the glucosamine-1-phosphate[39]. Cellobiose phosphorylase COG3459 (19800 reads) and glycosyltransferase COG1472 (17984 reads) are involved in the degradation of xylan and pectin[40]. The distribution of NOGs reveals the potentially microbial functions in topsoil ecosystem amended with BBF for carbohydrate degradation, uptake, and transport.

There are several implications according to the genetic annotation. The most functional microbes for carbohydrates degradation were ecologically dominant, that the CAZyme-encoding genes were predominantly from the phyla of Acidobacteria, Actinobacteria, and Proteobacteria. Besides, the genetic annotation verified the microbial capabilities of *Nocardioides*, *Solirubrobacter*, and *Streptomyces* in lignocellulose-degrading, indicating they are rich in genes encoding GHs and GTs. Furthermore, the distributions of CAZyme families were similar under the four regimens; however, the responsible microbial communities were different and were associated with the various treatments; as for dominant carbohydrate-active microorganisms (Acidobacteria, Proteobacteria, Firmicutes, and Bacteroidetes), these were the most abundant under BBF application.

5 Conclusions

Lignin, cellulose, and readily degradable carbohydrate significantly degraded in all regimens. Amendment of the soil with BBF resulted in better nutrient conservation and carbohydrate decomposition when compared to the other treatments. The diversity of CAZyme genes in the topsoil microorganisms from four fertilization regimens were primarily distributed within Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Firmicutes, and Thaumarchaeota, which are also among the 10 ecologically most dominant phyla. The gene annotations based on the metagenome sequencing revealed that carbohydrate transport and metabolism accounted for 6.0% of the COG functional classification under BBF application, and illustrated the microbial potential in the fibre and carbohydrates degradation in the topsoil microenvironment.

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