# β-glucosidase genes differentially expressed during composting

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**Research**

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# Abstract

Background: Cellulose degradation by cellulase is brought about by complex communities of interacting microorganism, which significantly to the cycling of carbon on a global scale. β-Glucosidase is the rate-limiting enzyme of degradation of cellulose. Thus, analysis of expression of genes involved in cellulose degradation and regulation of β-glucosidase gene expression in composting is beneficial to a better understanding of cellulose degradation mechanism. According to our previous researches, we present the hypothesis that “microbial functional communities differentially regulate the expression of glucose-tolerant β-glucosidase and glucose sensitive β-glucosidase (up or down regulation) to adapt to the changes in cellulose degradation.”

Results: Here, the functional microbial community structure and function change in association with cellulose degradation during the process of natural and inoculated composts was investigated by metatranscriptome and DNA clone library. Compared with inoculated compost, cellulose degradation was obviously inhibited during natural composting. Especially, the cooling phase of natural compost exhibited carbon catabolite repression (CCR) effect due to high concentration of glucose and cellobiose. The expression of genes encoding endoglucanase and exoglucanase were significantly down-regulation, while the CCR has no effect on β-glucosidase genes expression levels. But functional microbial community composition changed significantly, the composition of glucose-tolerant β-glucosidase increased.

Conclusions: These results indicated that microbial functional communities differentially regulate the expression of glucose tolerant β-glucosidase (up regulation) and non-glucose tolerant β-glucosidase (down regulation) under CCR. This work provides a frame work to predict how functional microbial communities will respond to cellulose degradation conditions changes.


# Background

Composting is a widely used way to degradation of biodegradation components by microbial communities. Microbes play the vital role in this process. Generally, the function of the microbial community was closely related to the community structure. A thorough knowledge on the succession of bacteria community is necessary for effective management of composting [1]. Cellulose can be used as a carbon source during composting, microbes produce cellulases to sustain their growth. Cellulase is a multi-enzyme complex of three different enzymes; exoglucanase [EC 3.2.1.74 and EC 3.2.1.91], endoglucanase [EC 3.2.1.4] and beta-glucosidase (BGL) [EC 3.2.1.21] which acts synergistically for complete hydrolysis of cellulose. β-glucosidase have a pivotal role in this enzymatic system, β-glucosidase completes the final step of hydrolysis by converting the cellobiose (an intermediate product of cellulose hydrolysis) to glucose [2]. And this reaction is considered to be the rate limiting step in the enzymatic hydrolysis of cellulose to glucose [2]. Berlemont and Martini [3] reported that β-glucosidase genes are present in nearly all bacterial phyla, which was confirmed in Pathan’s research [4]. These facts demonstrate that a wide phylogenetic diversity of microorganisms likely participate in the last step of

enzymatic cellulose hydrolysis. Therefore, in order to achieve high conversion efficiency for cellulose, understanding the β-glucosidase-producing microbial communities are significantly important. Several sets of degenerate primer have been designed to analyze β-glucosidase genes diversity from different environmental niches [4–9].

β-glucosidases (β-D-glucoside glucohydrolase, EC 3.2.1.21) are ubiquitous enzymes found in archaea, eubacteria, to eukaryotes [10]. Regarding their evolutionary and structural properties, they can be classified into one of five glycoside hydrolase families (GHs):1, 3, 5, 9 and 30 [11, 12]. The vast majority of β-glucosidases (BGLs) characterized so far belong to GH1 [12, 13]. High concentration of glucose inhibits enzyme activity of β-glucosidase. However, there are a number of β-glucosidases that are tolerant to glucose inhibition. So far, many glucose resistant enzymes have been isolated from bacteria, archaea and metagenome [14]. For example, rBgl4 from Taloromyces fuiculosus [15], BGL from Termoanaerobacterium aotearoense P8G3#4 [16], G1mgNtBG1 from Nasutitermesta kasagoensis [17] and BGL6 and Ks5A7 from the metagenomic library [18, 19]. All the glucose-tolerant β-glucosidases discovered to date are members of the GH1 and GH3 families [20]. GH1 family show better glucose tolerance [21, 22]. Comparative analysis of the 3D structures of GH1 β-glucosidases reveal the $(\beta/\alpha)_8$ TIM-barrel architecture that is typical of this enzyme family [23]. De Giuseppe et al. found a molecular mechanism of glucose tolerance. It suggested that the bulky side chains W168 and L173 in the Bglhi cause a narrowing of the + 2 aglycone site, restricting access of saccharides to the active site [21, 24]

However, “when, why and how” the functional microorganisms encoding and expressing glucose-tolerant β-glucosidase in natural environment have not yet been elucidated. According to our previous researches, we present the hypothesis that “microbial functional communities differentially regulate the expression of glucose-tolerant β-glucosidase and glucose sensitive β-glucosidase (up or down regulation) to adapt to the changes in cellulose degradation.” In this study, the function and succession of cellulolytic microbial community in the thermophilic and cooling phase during natural and inoculated composting were investigated by metatranscriptome and DNA clone library. And focus on the function and regulation of β-glucosidase-producing microbial community and individual in cooling phase of natural compost in order to provide theoretical basis for verifying this hypothesis. Answers to these questions are critical for understanding how microbial populations interact with substrates and products to drive fundamental ecological processes of cellulose degradation.

# Results

### Metatranscriptome reveals the transcription of genes involved in cellulose degradation

To examine the transcriptional responses of the cellulose degrading microbial community in natural compost and inoculated compost, RNA-seq metatranscriptomic analysis were performed on four samples of different phases, including thermophilic phase (TN, day 22 for natural compost; TI, day 22 for inoculated compost) and cooling phase (CN, day 46 for natural compost; CI, day 31 for inoculated compost). On average, 45

million raw sequence reads were obtained from the metatranscriptome of each compost, and a total of 27.17Gbp of high-quality sequences were obtained after removing the adapters and quality filtering. The Q30 base percentages of each sample were above 98%. (Additional file 1: Table S1). A total of 707980 unigenes were identified after de novo assembling using Trinity and cluztered with CD-HIT, the average length of these unigenes was 425bp. The average GC content was 49.07% (Additional file 1: Table S2). Rarefaction analysis showed clear asymptotes for four communities, suggesting that there was sufficient sequence coverage to detect most expressed genes (Additional file 2: Figure S1).

Phylogenetic classification of total gene expression and expression of the cellulolytic metatranscriptome were revealed. At the phylum level, total gene expression in all samples was predominantly by Proteobacteria, Bacteroidetes, Firmicutes and Spirochctes (Fig.1a). Notably, Firmicutes, Proteobacteria, Bacteroidetes and Actinobacteria were also reported as dominant phyla in other lignocellulosic composts [25,26]. Expression of the cellulolytic metatranscriptome was similarly dominated by Proteobacteria and Bacteroidetes in all communities (Fig.1b). As observed previously, proteobacteria dominates oxygenated habitats [27] and Bacteroidetes are known for their contribution to the largest reservoir of CAZymes in various environments [27,28]. For these communities, Actinobacteria, Firmicutes and Ascomycota expressed a similar yet minor fraction of the total cellulolytic metatranscriptome (Fig.1b). This result is similar with Berlemont et al. [29] study. The contribution of Proteobacteria phylum in thermophilic community was higher than cooling community in total metatranscriptome of natural compost, this phenomenon also observed in cellulolytic metatranscriptome of natural compost and inoculated compost (Fig.1). In total cellulolytic metatranscriptome, a considerable proportion of the unigenes encoding cellulolytic enzymes remained unclassified even at the phylum level (Fig.1b), which might be attributed to the relatively short gene sequences or suggest the existence of novel and uncharacterized cellulolytic microbes in the compost.

The transcription of genes involved in degradation of cellulose (such as endoglucanase and exoglucanase) and cellobiose (such as cellobiose-specific ABC family transporter and β-glucosidase) were detected in metatranscriptome (Fig. 2). In the natural compost, the transcription of endoglucanases genes and exoglucanases genes in thermophilic phase were higher than cooling phase (Fig. 2), this result was consisted with the result of CMCase activity (Additional file 1: Table S5), the up-regulated level of CMCase activity observed in thermophilic phase was significantly higher compared with cooling phase. In inoculated compost, the samples in thermophilic phase show higher transcription of β-glucosidases genes than cooling phase (Fig. 2), and the β-glucosidases activity of thermophilic phase also show higher than cooling phase (Additional file 1: Table S5). Interesting, in natural compost, β-glucosidases activity was significant higher in thermophilic phase than cooling phase, but the transcript level of β-glucosidase in thermophilic phase is similar with cooling phase of natural compost. These results indicated that the expression of β-glucosidase

genes seems more resistant to high concentration glucose than endoglucanase and exoglucanase. The change trend of expression of β-glucosidase genes is contradicted with that of β-glucosidase activity, a possible reason for this phenomenon was speculated to be from the accumulation of cellobiose and glucose, and thus, changed β-glucosidase-producing microbial community composition and produced different kinds of β-glucosidases. The genes encode ABC family cellobiose-specific transporters were also detected in metatranscriptome. In the inoculated compost, the genes of CI sample encode cellobiose-specific ABC family homologs of CebE, CebF, CebG and MsiK [30] are higher than TI sample. Interestingly, on the day 31 of inoculated compost, glucose was not detected in microbial metabolism, but cellobiose was detected in microbial metabolism (Additional file 1: Table S5). These results suggested that, the product glucose of cellulolytic degradation was not enough to support microbial utilization. In the no glucose condition, a part of microorganisms metabolizing cellobiose intracellularly using transporters and intracellular β-glucosidases.

Lignocellulolytic enzymes are regulated by the combinations of many transcription factors, ClrB, XlnR, CreA and AmyR transcription factors as critical dose-dependent regulators of cellulase expression [31]. ClrB and XlnR had additive effects on positively regulating the cellulose and hemicellulose gene expressions, the down-regulation of expression of ClrB and XlnR occurred in cooling phase of natural compost (Table 1). CreA is carbon catabolite repressors, played a negative role in the degradation of plant cell wall polymers [32], the up-regulation of expression of CreA occurred in cooling phase of natural compost. Content of glucose and cellobiose kept at a high level in the natural compost during cooling phase, meanwhile, both CMCase activity and β-glucosidase activity decreased in cooling phase of natural compost. These results indicated that significantly carbon catabolite repression (CCR) was observed in the cooling phase of natural compost. Interestingly, the AmyR as a fungal repressor, the transcription of AmyR in the thermophilic phase is higher than cooling phase in natural compost, this up-regulation of AmyR result in the up-regulation of expression of endoglucanase gene of fungi (Table 1).

**Table 1** The expression of transcription factors in the both composts

| | | Expression of TN | Expression of CN | Expression of TI | Expression of CI | source |
|---|---|---|---|---|---|---|
| activator | clrb | 17.41 | 10.11 | 1.46 | 2.21 | Bacteria |
| | xlnR | 17.82 | 3.05 | 12.17 | 2.79 | Fungi |
| repressor | creA | 5.13 | 33.82 | 31.58 | 14.56 | Bacteria/Fungi |
| | AmyR | 69.81 | 3.47 | 9.99 | 42.35 | Fungi |

The transcript pools differed significantly between thermophilic phase and cooling phase in natural compost. The sample in cooling phase of natural compost (CN) were marked by an increase in the use of simple compounds (starch and trehalose) while the share of CAZymes targeting recalcitrant plant biomass (cellulose and lignin) decreased (Fig. 3). These results indicated that the degradation of complex compounds, such as cellulose and lignin, were inhibited due to carbon catabolite repression (CCR), while biomass may need to be maintained at the cost of simple C compounds. Higher transcription of CAZymes targeting fungal and bacterial cell wall components, such as selected glucans and peptidoglycan, was observed in thermophilic phase compared to cooling phase in the natural and inoculated compost (Fig. 3), indicating higher turnover and growth rates during thermophilic composting phase.

## Characterization of GH1 family β-glucosidase-producing microbial communities and β-glucosidase gene quantification

Analysis of the community structure of family 1 β-glucosidase-producing bacteria at the levels of phylum by construct DNA library. Proteobacteria and Actinobacteria were predominant groups in both composts (Fig. 4). The contribution of Proteobacteria phylum in thermophilic community was higher than cooling community in both composts, this result is consistent with the expression of β-glucosidase in metatranscriptome (Fig. 3). The diversity of functional microbes was higher in inoculated compost than natural compost, as also seen in Liu et al. studies [33]. The abundant of Actinobacteria increased with the composting process of natural compost while this phenomenon is not seen in inoculated compost, we speculate that this is closely related to the accumulation of glucose and cellobiose in cooling phase of natural compost.

All the glucose-tolerant β-glucosidases discovered to date are members of the GH1 and GH3 families [20]. Compared with GH3 family β-glucosidase, the sequence of GH1 family β-glucosidase gene was more conservative and thorough characteristics. In particular, it had the highly conserved residues Trp168 and Leu173, that closely related to the tolerance of the enzyme to high glucose concentrations [21], making it easier to predict the characters of gene and enzyme activity. The designed degenerate primer can amplify a gene sequence of about 1100 to 1200 bp in length including the characteristic sequence. A total of 457 β-glucosidase genes were obtained from DNA library. We observed Actinobacteria and Proteobacteria contributed most β-glucosidases sequences of the GH1 family, this result consistent with Pathan et al. [4] and Zang et al. study [34] which found these two phyla account for the majority of β-glucosidases sequences of the GH1 family. We also observed that the lower diversity in the cooling phase of natural compost than that of inoculated compost, indicating a strong CCR selection of the functional microbial communities. The abundances and expression of the eighteen individual community members (Additional file 2: Figure S2) were measured by quantifying from DNA and RNA pools. Expression of most

β-glucosidase genes were inhibited by CCR. In the cooling phase of natural compost (CCR period), the translational efficiency of six β-glucosidase genes showed decreased, such as the gene GH1B-b2-20, GH1B-13-2-6, GH1B-b4-37, GH1B-b4-26, GH1B-13-12 and GH1B-10-4-50 showed decreased by 107.37%, 22.50%, 13.10%, 12.01%, 9.29% and 7.53% respectively (Fig. 5). However, the translational efficiency of five β-glucosidase genes showed obviously increased. Such as the gene GH1B-3-55, GH1B-b4-18, GH1B-12-33, GH1B-13-55 and GH1B-14-21 showed increased by 27.67%, 15.14%, 22.24%, 22.30% and 20.23% respectively (Fig. 5). The Trp168 and Leu173 glucose-tolerant conservative residues [21] were observed in the GH1B-3-55, GH1B-b4-18, GH1B-12-33 and GH1B-13-55. In order to explore the sites and mechanism by which glucose affects the actives of β-glucosidases, interactions between the ligand and the enzymes were simulated by molecular docking [35]. Base on some β-glucosidases shaing > 40% sequence identity with other β-glucosidases in Protein Data Bank (PDB) are available, the structures of the fourteen β-glucosidases were successfully simulated with the SWISS-MODEL program. The templates and the global quality estimation score GMQE of the models as shown in (Additional file 1: Table S7). GH1B-13-55 β-glucosidase were successfully simulated by molecular docking, the result showed that glucose preferentially binds to outside sites at the middle of the channel, where the bound glucose does not hinder substrate binding to the active site, therefore leading to glucose tolerant (Fig. 6a). Interestingly, the GH1B-14-21 gene sequences has no Trp 168 and Leu173 glucose-tolerant conservative residues observed, but the result of molecular docking is same with GH1B-13-55 which has characteristic features of glucose-tolerant (Fig. 6b).

# Discussion

Functional microbial community plays an important role in cellulosic biomass degradation. The study of functional microorganism how to regulated and involved in cellulose degradation is significantly to the cycling of carbon on a global scale. Increasing glucose concentration would lead to carbon catabolite repression (CCR) which has a great impact on the decomposition of cellulose by functional microorganism. Clarifies functional microorganism how to respond under the inhibited conditions, which provide benefit reference for study of biomass energy utilization and efficient decomposition of cellulose. In this study, expression of genes related to the degradation of cellulose was investigated in natural and inoculated composting by metatranscriptomic and DNA library analyses. These results showed that inoculating agents caused differences between the two composts, including degradation efficiencies of cellulose, functional microbial activity and cellulolytic microbial communities. Compared with inoculated compost, the carbon catabolite repression (CCR) occurred when glucose and cellobiose contents accumulated in the cooling phase of natural compost.

The dominant microbial community of cellulolytic enzymes genes expression is similar to total genes expression, including Protobacteria and Bacteroidetes. Functional group of microorganisms and dominating taxa occurred succession during cellulosic composts, Protobacteria were even more

dominant during thermophilic phase of natural and inoculated compost, the relative abundant of Protobacteria decreased in cooling phase of natural and inoculated compost. In natural compost, the abundant of phyla Actinobacteria and Firmicutes were higher in cooling phase than thermophilic phase. However, in inoculated compost, the abundant of phyla Bacteroidetes and Ascomycota were higher in cooling phase than thermophilic phase. We speculated the reason for this difference is the different conditions of cellulose degradation in natural compost and inoculated composts (compared with inoculated compost, there is more residue of degradable material in the cooling phase of natural compost, and CCR occurred in this phase).

Compared with inoculated compost, cellulose degradation was obviously inhibited during natural composting, which can reflect in the functional of microbial community involved in cellulose degradation. Cellulase formation apparently occurred because of consistent respective regulators. In the report of Li et al. [31], the transcriptional-regulatory network as a “seesaw model” in which the coordinated regulation of cellulolytic genes is established by counteracting activators and repressors. In the natural compost of this study, the expression of activators (ClrB and xlnR) are higher in thermophilic phase, while the expression repressors (creA) is significantly higher in the cooling phase (Table 1). Meanwhile, in natural compost, both CMCase activity and β-glucosidase activity decreased during the cooling phase (31d- 46d) of composting, the transcription of endoglucanase genes and exoglucanase genes showed similar trends with the change of the CMCase activity, and the contents of glucose and cellobiose accumulated in cooling phase with the maximum values (Additional file 1: Table S5). These results of this study further confirmed that significantly carbon catabolite repression (CCR) was observed in the cooling phase of natural compost.

β-glucosidase gene expression is also subject to carbon catabolite repression (CCR), which functions when a favorable carbon source, such as glucose, is present. Most BGLs are commonly inhibited by glucose [36]. As expected, in natural compost, β-glucosidase activity significantly decreased due to carbon catabolite repression (CCR) during the cooling phase. However, the transcription level of β-glucosidase genes in this phase was not decline compared with thermophilic phase. It is noteworthy that this phenomenon is not seen in inoculated compost. This could be explained by functional microbial communities differentially regulated the expression of β-glucosidase genes to adapt to CCR. These results can be confirmed in other studies, it had been found that the differently expression of β-glucosidase in some cellulolytic microbes such as Clostridium T.reesei, A.oryzae, Clostridium thermocellum, aspergillus.terreus. The differential expression between cel3a and cel1a regulated in response to glucose concentration was found in T.reesei, the expression of cel3a was higher when glucose was present in excess than when glucose was depleted from the medium [37]. In the presence of glucose, the other β-glucosidase genes had decreased expression in the Δxyr1 mutant compared to parental strain excepted for cel3a [38]. Interestingly, the T.reesei strains TRB1 and SEU-7 displayed a better resistance to carbon catabolite repression for higher expression of cel3a [39, 40]. A. oryzae produced two distinct extracellular β-glucosidases, the major form BGL was highly inhibited by glucose and the minor form HGT-BG was highly glucose tolerant. The expression levels of both were significantly changed on various carbon sources media. It’s worth noting that β-glucosidase exhibited the lowest level

of hydrolyzing activity on lactose medium, but high ratio of HGT-BG produced by A. oryzaethe [41]. In our previous research work, at sufficiently high glucose concentration, the functional microbial community in compost was altered, up-regulation of glucose tolerant β-glucosidase genes and down-regulation of non-glucose tolerant β-glucosidase may contribute to maintaining β-glucosidase activity despite the high glucose content [9].

In this study, based on the observation that Trp 168 and Leu173 were conserved in glucose-tolerant GH1 enzymes using Giuseppe's method [21]. The translational efficiency of glucose-tolerant GH1B-3-55, GH1B-b4-18, GH1B-12-33 and GH1B-13-55 genes encoding glucose-tolerant β-glucosidases were obviously increased in natural compost from day 31 to day 46 with the increase of concentration of glucose (Fig. 5). It is noteworthy that the GH1B-14-21 gene has no Trp 168 and Leu173, but the translational efficiency of the GH1B-14-21 gene was also obviously increased in natural compost from day 31 to day 46 (Fig. 5). The studies on Bgl1A and Bgl1B by Yang et al. [35] proposed that glucose products can inhibit the enzymatic activity of β-glucosidases by competing with substrate to bind directly to the active site. In this study, the β-glucosidase structure modeling and ligands docking results of GH1B-14-21 were similar with GH1B-13-55, In GH1B-14-21 and GH1B-13-55, glucose preferentially binds to outside sites of the channel, where the bound glucose does not hinder substrate binding to the active site (Fig. 6), therefore leading to glucose tolerant. However, the translational efficiency of most non-glucose tolerant β-glucosidase genes was significantly inhibited, such as GH1B-b2-20, GH1B-13-2-6, GH1B-b4-37, GH1B-b4-26, GH1B-13-12 and GH1B-10-4-50 (Fig. 5). This finding was consistent with the work of Pérezpons who reported that β-glucosidase activity of Streptomyces sp. QM-B814 was inhibited in the presence of glucose [42]. The response of others β-glucosidase gene expression to CCR exhibited middle state behavior. These results suggested that at sufficiently high glucose concentrations, the transcriptional efficiency of glucose-tolerant β-glucosidase genes are higher than that of non-glucose-tolerant β-glucosidase genes. That confirms the hypothesis that "microbial functional communities differentially regulate the expression of glucose tolerant β-glucosidases and non-glucose tolerant β-glucosidases (up or down regulation) to adapt to the changes in cellulose degradation."

## Conclusion

The cellulolytic microbial community was closely related to the condition of cellulose degradation. In the cooling phase of natural compost (CCR period), β-glucosidase-producing microbial communities differentially regulate the expression of glucose tolerant β-glucosidases and non-glucose tolerant β-glucosidases. These results provide evidence to our hypothesis.

## Methods

## Samples collection and physicochemical properties analysis

The aerobic composting samples collected at College of Resources and Environmental Sciences of Northeast Agricultural University in China. The details of the process of compost and the physico-chemical properties of straw-cattle manure compost are described by zang [9]. We have collected four time-series samples from natural compost and inoculated compost (day 12, 22, 31 and 46). Samples were immediately frozen in liquid nitrogen and stored at − 80 °C until analysis.

# Metranscriptome assembly and annotation

RNA was extracted using the RNA PowerSoil Total RNA Isolation Kit (MoBio Laboratories), the quality of the RNA and removal of potential contaminating DNA were detected by 1% agarose gels. RNA concentration was confirmed using the Qubit® 3.0 Flurometer (Thermo Fisher Scientific, MA, USA);RNA integrity and concentration were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

A total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (#E7530L, NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample.

Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads for eucaryote (For prokaryote, rRNA was removed from total RNA using Ribo-Zero rRNA Removal Kit to purify mRNA). Mixed with the fragmentation buffer, the mRNA is fragmented into short fragments. First strand cDNA was synthesized using random hexamer primer and RNaseH. Second strand cDNA synthesis was subsequently performed using buffer, dNTPs, DNA polymerase I and RNase H. The library fragments were purified and resolved with EB buffer, then terminal repair, A-tailing and adapter added were implemented. After size selecting and retrieving by AMPure XP beads, the products were used as the index PCR templates.

RNA concentration of library was measured using Qubit® RNA Assay Kit in Qubit® 3.0 to preliminary quantify and then dilute to 1 ng/μl. Insert size was assessed using the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA), and qualified insert size was accurate quantification using StepOnePlus™ Real-Time PCR System(Thermo Fisher Scientific,MA，USA)(Library valid concentration＞10 nM).

The library could be sequenced using Illumina HiSeqTM X TEN or others sequencer when necessary.

# DNA extraction and cDNA preparation

Total DNA was extracted from samples by a Hipure Stool DNA Kit (Magen,China), total RNA was extracted from samples using a RNA PowerSoil total RNA isolation kit (MoBio Laboratories, Inc.). cDNA was generated from total RNA using a PrimeScriptTM RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Bio, Inc.) according to the manufacturer's instructions. Random hexamers and the RT

Primer Mix were used as primers during strand synthesis. RT Primer mix contain oligo (dT) primer and random 6-mers.

# Construction of DNA library

DNA library targeting β-glucosidase gene of all simples were constructed to analyze cellulolytic communities in each sample. Family 1 β-glucosidase genes of DNA were amplified using the primer pairs GH1F3–GH1R16 (Additional file 1: Table S3). PCR reactions were conducted in triplicate 50μL volumes containing 12.5 μL 2 × Taq Master Mix(novoprotein), 0.2 μmol/L (each) primer, 0.5μL of DNA templates and 11.6μL ddH2O, amplification was conducted with an initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 10 s, annealing for 34 s at 50.0 °C, and 72 °C for 30 s; and a final elongation at 72 °C for 5 min. Pooled PCR products were visualized on a 1% agarose gel, then purified using the Gel Extraction kit (Omega). The purified products were ligated to pMD™18-T Vector (Takara, USA) and transformed into E.coli DH5α Competent Cells (Takara,USA) by heat shock. The recombinant plasmids were placed in solid Luria-Bertani (LB) medium containing X-gal, IPTG, and ampicillin (AMP+) and cultured 12 h at 37 °C. Meanwhile, white single colonies were inoculated in liquid LB medium containing AMP + and cultured 12 h at 37 °C. After the bacterial liquid was used as PCR template to amplify the target gene, the amplified products were detected by agarose gel electrophoresis. Positive clones containing the gene fragment were sent to Beijing Huada Gene Company (Beijing, PR China) for sequencing.

# Real-time Quantitantive PCR of β-glucosidase genes

CFD-3120 real-time PCR system (Bio-Rad) was used to conduct qPCR, DNA and RNA were used as the template for quantify the β-glucosidase gene of GH1 family, and the fluorescent dye SYBR Green was used to quantitative analysis of the functional gene. Each reaction was conducted in a 20μL system including 10μL of NovoStart ®SYBR qPCR SuperMix Plus (2×, novoprotein, China); The primer for qPCR in this study are listed in (Additional file 1 :Table S4) and amplification was conducted with 95 °C for 60 s, 40 cycles of amplification (95 °C for 20 s, 60 °C for 60 s). Melting curve analysis was used to confirm the product specificity. All assays were repeated three times and no signal was observed in negative controls.

# Homology modeling and protein-ligand docking

The 3D structure models of β-glucosidase were constructed using the homology modeling by SWISS-MODEL. The structures were visualized using the visualization tool PyMOL (https://www.pymol.org/) Autodock 4.0[36] was employed for docking to discover the potential glucose binding sites in the substrate channel, the chair conformations of glucose and pNPG were downloaded from PDB as a part of 2O9T (a β-glucosidase from Bacillus polymyxa with glucose) and 3AI0 (a β-glucosidase from termite Neotermes koshunensis with pNPG) respectively. We set the enzyme active site as center of the grid box, and the search was performed in a retangular box with dimensions 40 Å×40 Å × 40 Å. The ligand and the receptor molecules with the lowest binding energy were considered to have the best docking conformations .The files were transformed to. pdb form by PyMOL. The docking results were visualized by PyMOL.

# Statistical Analysis

Date was analyzed using the program SPSS 13.0 for windows. One-way ANOVA with repeated measures was used to test differences in the measured parameters during composting, and post-hoc Tukey test was used to further investigate the differences ($p < 0.05$).

# Abbreviations

GH
Glycoside hydrolases;

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and material

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

### Competing interests

The authors declare that they have no competing interests.


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### Author contributions

Z.X., L.H., C.X. and L.S. performed the majority of experiments and statistical analyses with the help of L.J., G.L., S.E. and M.B. L.H., Z.X. and M.B. designed the study. L.H. and M.B. drafted the manuscript.


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Not applicable.


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# Figures

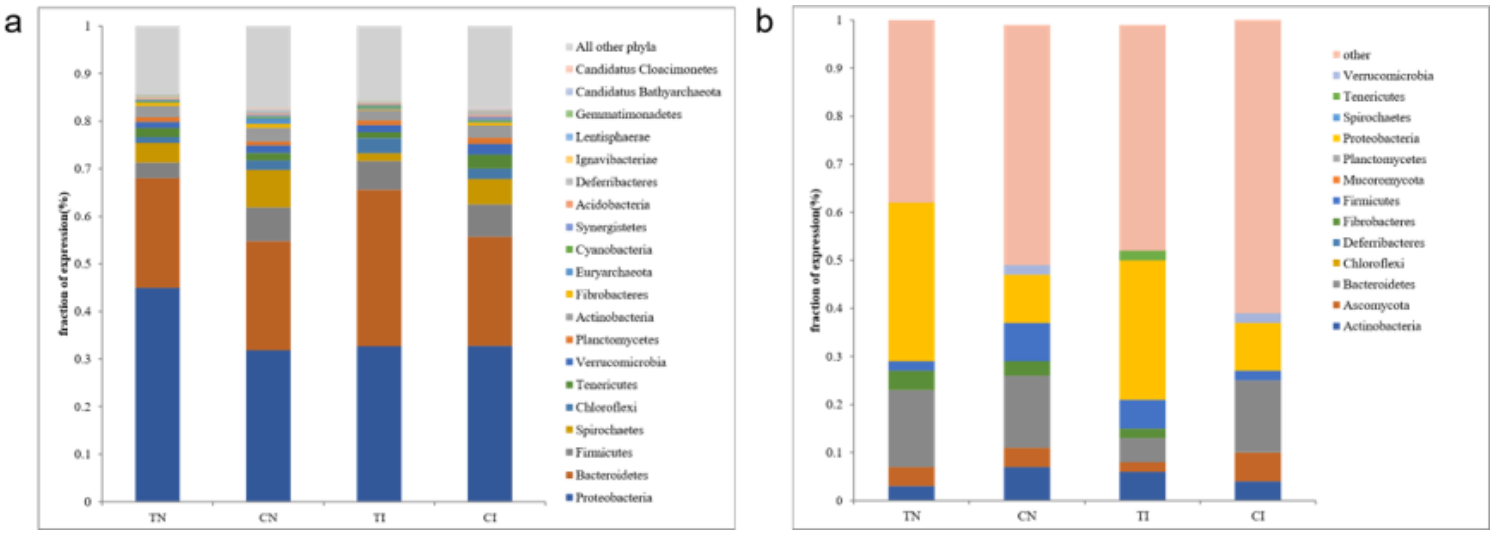
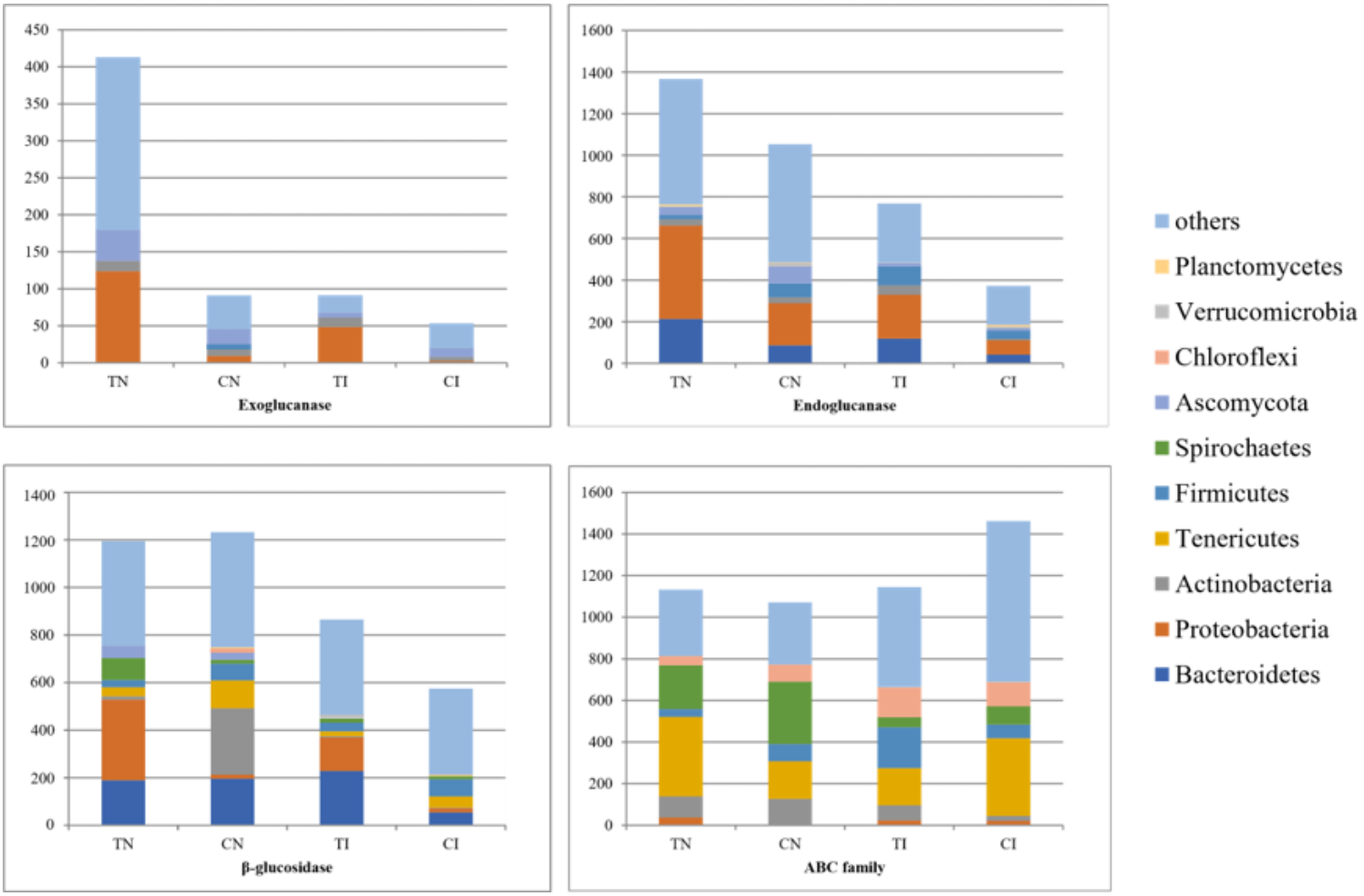


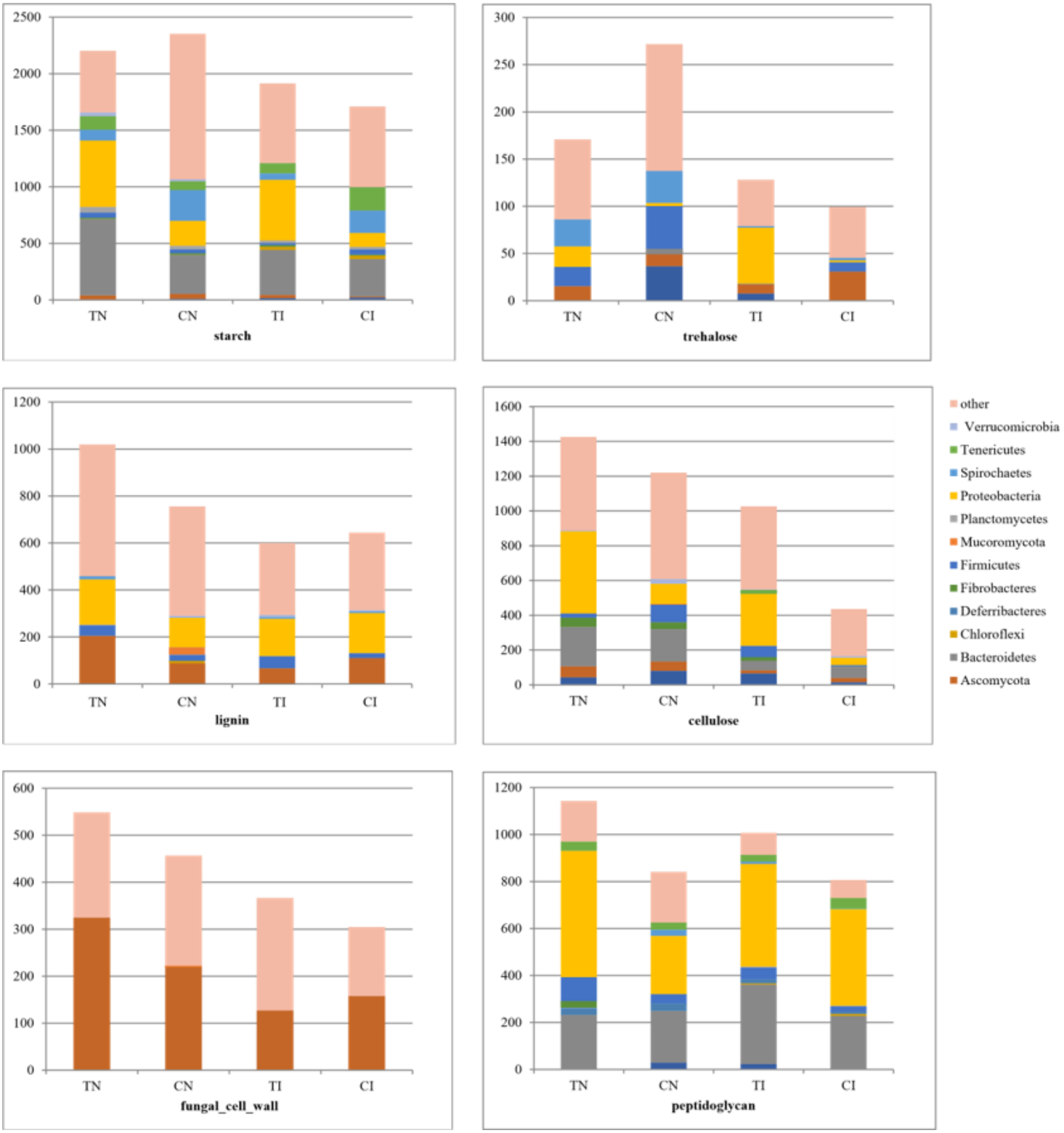

**Figure 1**

Total gene expression and expression of GH families relevant to cellulose deconstruction by phylum in thermophilic stage and cooling stage of natural and innoculated composts. a Total gene expression relevant to GH families by phylum in thermophilic stage and cooling stage of natural and inoculated composts. b Expression of GH families relevant to cellulose deconstruction by phylum in thermophilic stage and cooling stage of natural and inoculated composts. Abbreviations: TN thermophilic stage in natural compost, CN cooling stage in natural compost, TI thermophilic stage in inoculated compost, CI cooling phase in inoculated compost.



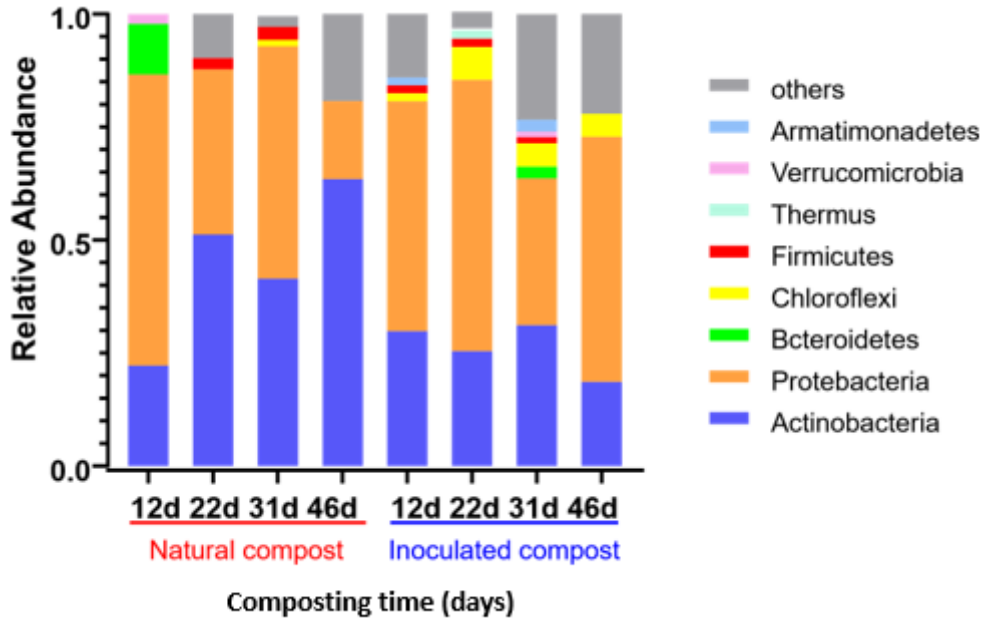

## Figure 2

Transcription of cellulolytic enzymes and ABC family transport. Numbers indicate the share of reads in the total metatranscriptome in ppm (reads per one million reads). Abbreviations: TN thermophilic stage in natural compost, CN cooling stage in natural compost, TI thermophilic stage in inoculated compost, CI cooling phase in inoculated compost.



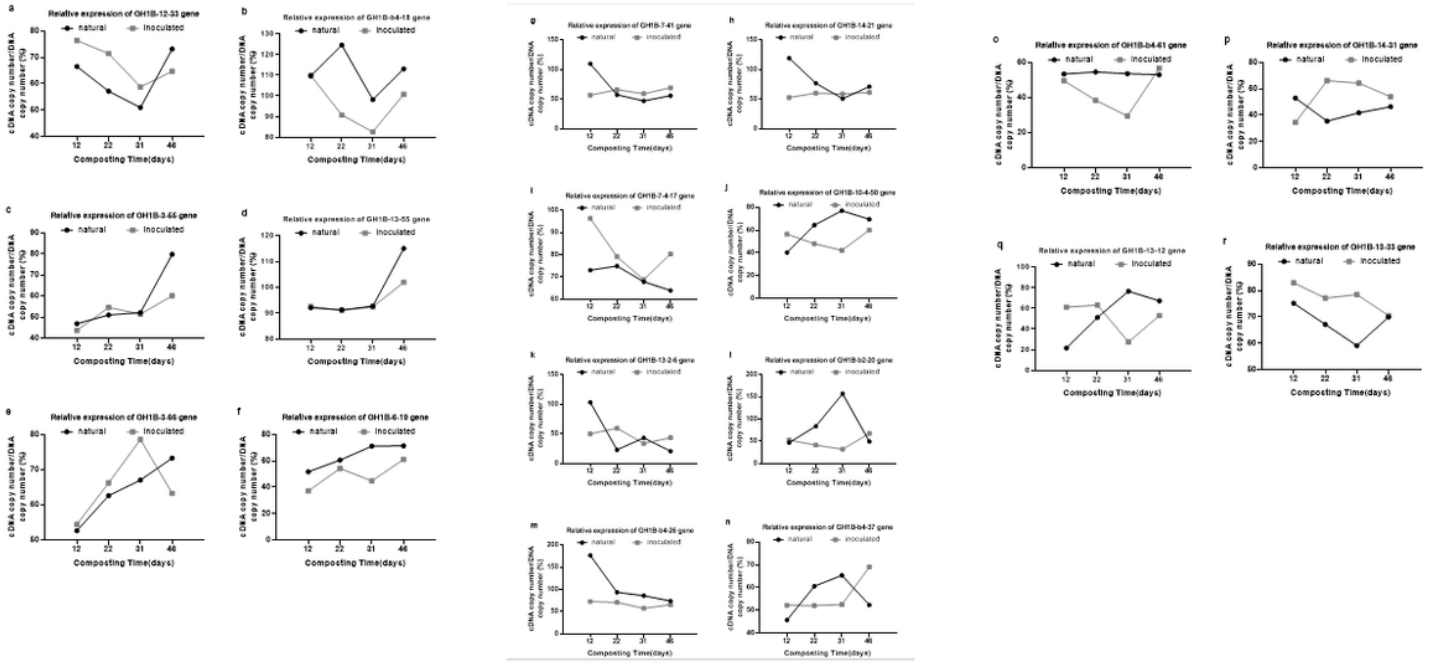

## Figure 3

Transcription of functional groups in composts. Numbers indicate the share of reads in the total metatranscriptome in ppm (reads per one million reads). Abbreviations: TN thermophilic stage in natural compost, CN cooling stage in natural compost, TI thermophilic stage in inoculated compost, CI cooling phase in inoculated compost.




## Figure 4

Taxonomic assignment of β-glucosidase genes from GH1 family during two composting. Colors of stack bars indicate taxonomic affiliation of genes.



## Figure 5

Differences in the relative expression of family 1 β-glucosidase genes from bacteria (GH1) in the natural composting and the inoculated composting.

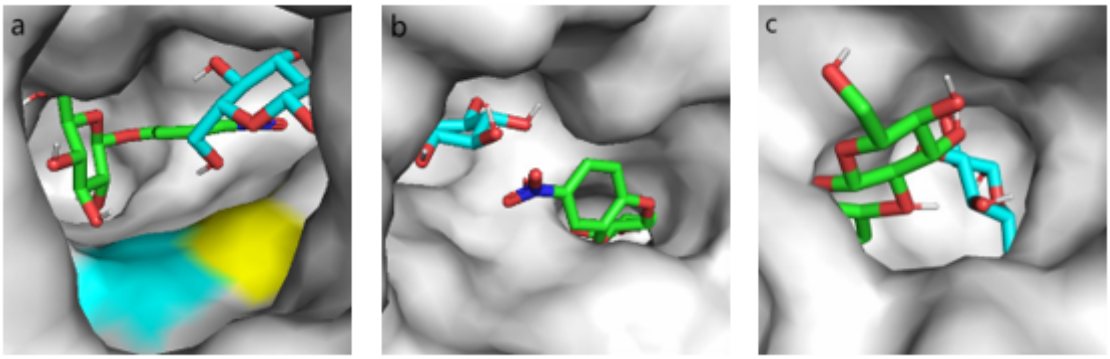



**Figure 6**

Structures of glucose and pNPG docked on β-glucosidases. Glucose and pNPG are shown with thick stick, with oxygen and hydrogen colored in red and white respectively, carbon colored in cyan and green in glucose and pNPG respectively, and nitrogen colored in blue in pNPG. a The structural basis of glucose tolerant for GH1B-13-55, cyan and yellow, Trp 168(W) and Leu173(L) amino acid residues located at the active site entrance. b The structural basis of glucose tolerant for GH1B-14-21, glucose bound to the substrate channel wall at middle, but pNPG bound at the channel bottom. c The structural basis of non-glucose-tolerant for GH1B-7-41, pNPG bound to the substrate channel wall at middle, but the glucose bound at the channel bottom. The binding was simulated by molecular docking and the images were made with PyMOL.

## Supplementary Files

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

- SupplementaryTable.xlsx
- SupplementaryFigure.docx