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Metagenomics reveals N-induced changes in carbon-degrading genes and microbial communities of tea (*Camellia sinensis* L.) plantation soil under long-term fertilization

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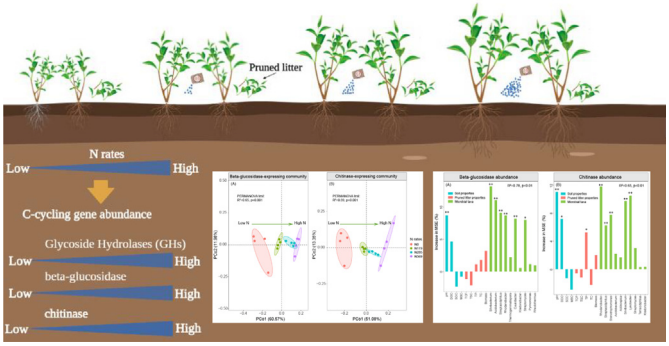
HIGHLIGHTS

- N fertilization significantly affected C-cycling genes.
- N fertilization increased most SOC-degrading microbial abundance.
- Beta-glucosidase and chitinase-expressing communities showed a significant variation under different N rates.
- Similar dominant microbial phyla performed different SOC-degrading associated functions.
- DOC and pH of soil and biomass and polyphenols of pruned litter largely contributed to SOC degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil organic carbon (SOC) is an important C pool of the global ecosystem and is affected by various agricultural practices including fertilization. Excessive nitrogen (N) application is an important field management measure in tea plantation systems. However, the mechanism underlying the impact of N fertilization on SOC, especially the microscopic mechanism remain unclear. The present study explored the effects of N fertilization on C-cycling genes, SOC-degrading enzymes and microbes expressing these enzymes by using a metagenomic approach in a tea plantation under long-term fertilization with different N rates. Results showed that N application significantly changed the abundance of C-cycling genes, SOC-degrading enzymes, especially those associated with labile and recalcitrant C degradation. In addition, the beta-glucosidase and chitinase-expressing microbial communities showed a significant difference under different N rates. At the phylum level, microbial taxa involved in C degradation were highly similar and abundant, while at the genus level, only specific taxa performed labile and recalcitrant C degradation; these SOC-degrading microbes were significantly enriched under N application. Redundancy analysis (RDA) revealed that the soil and pruned litter properties greatly influenced the SOC-degrading communities; pH and DOC of the soil and biomass and total polyphenol (TP) of the pruned litter exerted significant effects. Additionally, the random forest (RF) algorithm revealed that soil pH and dominant taxa efficiently predicted the beta-glucosidase abundance, while soil pH and DOC, pruned litter TP, and the highly abundant microbial taxa efficiently predicted chitinase abundance. Our

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study indicated that long-term N fertilization exerted a significant positive effect on SOC-degrading enzymes and microbes expressing these enzymes, resulting in potential impact on soil C storage in a perennial tea plantation ecosystem.

Abbreviations

Soil properties

DOC	dissolved organic carbon
MBC	microbial biomass carbon
SOC	soil organic carbon

Pruned litter properties

TC	total carbon
TCF	total crude fiber
TP	total polyphenols
TSC	total soluble carbohydrates
Biomass	the biomass of pruned tea litter

C-cycling gene groups

GHs	glycoside hydrolases
GTS	glycosyl transferases
CEs	carbohydrate esterases
AAs	auxiliary activities
PLs	polysaccharide lyases
CBMs	carbohydrate-binding modules

1. Introduction

Soil organic carbon (SOC) is an important indicator of soil fertility and plays a critical role in maintaining soil quality (Lal, 2004; Yang et al., 2020). Even slight changes in SOC influence carbon dioxide (CO_2) emissions, potentially exacerbating global climate change (Lal, 2018). In addition, anthropogenic activities, such as fertilization, considerably impact soil C pools (Álvaro-Fuentes et al., 2012). Therefore, understanding how agricultural management practices influence SOC dynamics is significant for improving soil fertility and predicting climate change. Multiple factors, such as exogenous C input, affect soil C dynamic, probably due to the impact on SOC-degrading microbes (Chowdhury et al., 2021). As the major C source, exogenous C input not only provides food and energy for soil microbes (Preece and Peñuelas, 2020) but also potentially influence (positive and negative priming effect) native soil organic C (Prommer et al., 2020), leading to disrupting the SOC balance. In addition, meteorological conditions, such as mean annual temperature (MAT) and mean annual precipitation (MAP), also affect soil C balance (Jian et al., 2020) due to the effect on the microbes involved in soil C cycling.

Microorganisms are vital in mediating biogeochemical cycles, especially the functional microorganisms, could perform some specific processes, such as C-, N-, P- and S-cycling nutrient cycles (Wang et al., 2018). In the process of terrestrial C cycling, beta-glucosidase and chitinase were the key enzymes performing the labile and recalcitrant C degradation. These enzymes were generally produced by specific microbes, and exerted a significant impact on SOC dynamics by the catabolic and anabolic processes, especially the SOC degradation. Evidence shows that agricultural management measures, such as N fertilization, influence soil native C, exerting a positive, negative, or even null effect on total SOC accumulation (Fang et al., 2018; Zhang et al., 2021). Previous studies mainly focused on soil C composition and enzyme activities (Jian et al., 2016; Chen et al., 2018; Wang et al., 2018). However, the responses of the microbial functional traits, such as C-cycling groups, at the genetic level to N addition have attracted little attention; moreover, the results on the response of microbial functional traits to N rates are inconsistent. For example, Jing et al. (2021) revealed that labile C-degrading genes were significantly stimulated only under low N addition. Wei et al. (2018) reported abundant C-cycling genes at a 450 kg ha^{-1} N rate in agricultural ecosystems, whereas Eisenlord et al. (2013) observed that 3 g N m^{-2} y^{-1} led to a significant decrease in C-cycling genes at sites with low ambient N deposition in the hardwood forests of North America. These differences between studies were related to the initial N status of the soil, which differs among the ecosystems, and different N rates (Guo et al., 2017). In summary, the mechanism underlying the effect of N fertilization on SOC, such as C-cycling genes, SOC-degrading enzymes and the microbes expressing these enzymes, remains unclear. Therefore, understanding the response of SOC-degradation enzymes and associated functional communities to field management strategies, especially N fertilization, is crucial for predicting soil C loss in agricultural systems.

Tea (*Camellia sinensis* L.) plantation is widely distributed in the tropical and subtropical acidic regions with soil pH < 4.5 (Ruan et al., 2010). High N fertilizer rates and periodic pruning for obtaining desirable yields and high leaf amino acid content are the major features of these tea plantations. According to our previous investigation, nearly 500 kg of pure N per hectare is applied to plantations across the main tea-producing areas of China each year (Ni et al., 2019). Moreover, the harvested area of tea plantation in 2020 of China has reached 3,365,697 ha (2020 FAO, https://www.fao.org/home/en). All these factors have the potential relationship with soil C loss. Therefore, soil C sequestration is essential for maintaining soil fertility and coping with global climate change, which demands understanding how N fertilization impacts soil C cycling, especially SOC degradation, in tea plantation systems.

Previous studies on SOC dynamics in tea plantations primarily focused on the changes in total organic C and its driving factors. For example, Sun et al. (2020) identified soil N and oxalic acid extractable iron as the important environmental variables leading to SOC changes in the tea plantations of Xishuangbanna, Yunnan. Besides, land use is an important factor affecting C storage. Ma et al. (2022) recently showed that the conversion of secondary forests to tea plantations reduced the content of soil microbial necromass C, which was not conducive to SOC accumulation. Moreover, N fertilization in a tea plantation system maintains and improves soil fertility, including SOC accumulation. However, the mechanism underlying the impact of N fertilization on SOC dynamics, the microscopic mechanism, such as the SOC degrading enzymes and microbes expressing these enzymes in tea plantation soil remains unclear.

Therefore, the present study explored the effects of N application on C-cycling genes, SOC-degrading enzymes and microbes expressing these enzymes in a tea plantation under long-term fertilization with different N rates. The study assessed the relationship between soil and pruned litter properties, C-cycling gens, SOC-degrading enzymes, and microbes expressing these enzymes. The objectives of this study were to (i) explore the response of soil C-cycling genes, SOC-degrading enzymes and associated microbes to long-term N fertilization, (ii) identify the driving factors affecting beta-glucosidase and chitinase-expressing microbial communities, and (iii) predict the factors driving the abundance of beta-glucosidase and chitinase associated with labile and recalcitrant C degradation. The study's findings will deepen our understanding of the influence of long-term N fertilization on C-cycling genes, SOC-degrading enzymes and microbes expressing SOC-degrading enzymes in a tea plantation. The study will provide

a new perspective for improving soil fertility and predicting C storage in a perennial teaplantation ecosystem.

2. Materials and methods

2.1. Site description

The long-term experiment was initiated in 2005 at Hangzhou, Zhejiang, China (120°05′E, 30°10′N, elevation 60 m). The area is characterized by a typical monsoon climate with a mean annual temperature (MAT) of 17.0 °C and precipitation (MAP) of 1533 mm. The soil of this region has been classified as an Alisol based on the WRB soil classification system (IUSS Working Group WRB, 2015). The soil had an initial pH (H_2O) of 4.16, organic C of 7.83 g kg^{-1}, total N of 1.39 g kg^{-1}, available phosphorus of 25 mg kg^{-1}, and available potassium of 76 mg kg^{-1}. The domestic tea clone Longjing-43 was cultivated in this region in single rows (150 cm between rows and 33 cm between plants) at a density of 60,000 plants ha^{-1}.

2.2. Experimental design

The experiment was implemented in a randomized design with four N fertilizer rates (N0, N119, N285, and N569; 0, 119, 285, and 569 kg N ha^{-1} yr^{-1}, respectively); four replicates were maintained per treatment, with a plot area of 24 m^2 per replicate, creating 16 plots. The N fertilizer urea (46 % pure N) was applied four times a year in early February (30 %), late May (20 %), early July (20 %), and late October (30 %) based on the nutrient demand of tea plants. Phosphorus (superphosphate) and potassium (sulfate of potash) fertilizers were applied in late October as the base fertilizer for all treatment plots based on soil testing. All fertilizers were manually incorporated into temporarily plowed furrows (15 cm width and 15 depth) and covered with soil. Pruning practices were carried out in late April and July, and the pruned litter was returned entirely to the tea plantation.

2.3. Sampling of soil and pruned tea litter

In May 2017, after the spring tea harvest, surface soils from a depth of 0–20 cm were collected using a pre-cleaned stainless steel hand sampler (inner diameter 5 cm). Samples collected from 16 randomly selected locations in a plot were pooled to obtain each replicate sample. Large stones, plant residues, and visible roots were removed manually, and the soil sample was passed through a 2 mm sieve and subsampled into two parts. One part was stored at −80 °C for molecular analysis; the second part was immediately used to determine the dissolved organic carbon (DOC) and microbial biomass carbon (MBC) and then air-dried to determine the remaining soil properties, including pH. Meanwhile, pruned litter was collected immediately after pruning; pruner litter of length 1 m was selected to estimate biomass for each hectare. Meanwhile, for chemical analysis, pruned litter, including twigs and leaves, was collected from each plot, oven-dried, and ground. We analyzed the soil and pruned litter properties following the methods described in our previous study (Yang et al., 2019).

2.4. DNA extraction, library construction, and metagenomic sequencing

DNA was extracted from the soil sample using the MoBio PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's protocol. The concentration and quality of the extracted DNA were assessed with a TBS-380 Fluorometer (TurnerBioSystems, USA) and NanoDrop 2000 (Thermo Fisher Scientific, USA). The quality of DNA was verified on a 1 % agarose gel. High-quality DNA samples were then sent to the Majorbio Biomedical Technology Co., Ltd. (Shanghai, China) for shotgun metagenomics sequencing.

The DNA was fragmented to approximately 300 bp with a Covaris M220 ultrasonicator (Gene Company Limited, China), and the paired-end library was constructed with the TruSeq™ DNA Sample Prep Kit (Illumina, San Diego, CA, USA). The blunt-end DNA fragments were ligated with adapters containing the complete complementary sequencing primer hybridization sites. Finally, paired-end sequencing was performed using HiSeq 3000/4000 PE Cluster Kit and HiSeq 3000/4000 SBS Kit on an Illumina HiSeq4000 platform (Illumina Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) as per the manufacturer's instructions (www.illumina.com). The metagenome sequences have been deposited in the NCBI database under the accession number PRJNA559847.

2.5. Data processing

The clean reads were generated from the raw reads in fastq format by removing the adapter sequences and low-quality bases (length < 50 bp or quality value < 20 or having N bases). SOAPdenovo, a De Bruijn graph-based assembler, was used to assemble the short reads. Further, we checked for K-mers with ⅓–⅔ the read length in each sample. Coverage scaffolds with a length >500 bp were retained for subsequent statistical tests, and the quantity and quality of scaffolds generated by each assembly were evaluated. The best K-mer, which yielded the minimum scaffold number and the maximum N50 and N90 values, was selected. Finally, the scaffolds with a length > 500 bp were extracted, broken into contigs without gaps, and used for further gene prediction and annotation.

2.6. Gene prediction, taxonomy, and functional annotation

The contigs' open reading frames (ORFs) and annotations were predicted with MetaGene Annotator (http://metagene.cb.k.u-tokyo.ac.jp/). The predicted ORFs with length > 100 bp were retained and translated to amino acid sequences via NCBI ORF finder, followed by annotation using BLASTP (BLAST Version 2.2.28+, http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI-NR database, including SwissProt. The optimized e-value threshold was set as $1e^{-5}$ for BLAST alignment.

All sequences from the gene sets with a 95 % sequence identity (90 % coverage) were clustered to obtain the non-redundant gene catalog using the CD-HIT program (http://www.bioinformatics.org/cd-hit/). Reads after quality control were mapped to the representative genes with 95 % identity using SOAPaligner, and gene abundance in each sample was evaluated. Representative sequences of the non-redundant gene catalog were annotated based on the NCBI-NR database using the BLASTP alternative in Diamond (Version 0.8.35, http://www.diamondsearch.org/index.php), with an e-value cutoff of $1e^{-5}$. Alpha diversity of beta-glucosidase and chitinase-expressing microbial communities were calculated to determine the community richness and diversity using the Mothur software. The carbohydrate-active enzymes (CAZy) annotation was conducted using hmmscan (http://hmmer.janelia.org/search/hmmscan) against the database (http://www.cazy.org/) with an e-value cutoff of $1e^{-5}$. The Kyoto Encyclopedia of Genes and Genomes (KEGG) (Version 94.2, http://www.genome.jp/keeg/) annotation was conducted using Diamond against the database with an e-value cutoff of $1e^{-5}$. This study analyzed sequencing data using Majorbio Cloud (www.majorbio.com), a comprehensive bioinformatics platform (Ren et al., 2022).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) combined with Duncan's post hoc test was used to compare the C-cycling genes, microbial diversity, and community composition under four N rates. Nonlinear regression described the relationship between the N rates and the C-cycling genes, functional gene diversity, and microbial diversity. Principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity was performed to explore the similarities in the microbial communities carrying the C-cycling genes and the beta-glucosidase and chitinase-expressing microbial communities, and the permutational multivariate analysis of variance (PERMANOVA) to assess the effects of different N rates on C-cycling genes and beta-glucosidase and chitinase-expressing microbial communities.

Redundancy analysis (RDA) explored the correlation between the beta-glucosidase and chitinase-expressing microbial communities and the soil and pruned litter properties. Variance partition analysis (VPA) dissected the relative contributions of soil and pruned litter properties to the changes in soil beta-glucosidase and chitinase-expressing microbial communities. PERMANOVA test identified the individual variables affecting the beta-glucosidase and chitinase-expressing microbial communities. Finally, a random forest (RF) algorithm was used to determine the most important variables associated with soil, pruned litter, and specific microbial taxa to predict the abundance of beta-glucosidase and chitinase.

All statistical analyses were conducted in R (Version 4.0.1). We applied the 'stats' package for one-way ANOVA, Pearson correlation analysis, and simple regression analysis, the 'vegan' package for PCoA, RDA, and PERMANOVA, and the 'randomforest' package for RF.

3. Results

3.1. Soil C-cycling functional genes

Based on the CAZyme database, 196C-cycling genes were identified with a frequency >75 % and a significant response ($p < 0.05$) of these genes to N application. PCoA showed that the C-cycling genes were significantly different under various N rates ($R^2 = 0.77$, $p < 0.001$; Fig. S1). The N application significantly changed the diversity of the C-cycling genes; however, the richness and diversity indices of these C-cycling genes showed opposite trends (Fig. S2). The Chao1 index of C-cycling genes decreased with increasing N rates, while the Shannon index decreased. In addition, the quadratic regression explained the relationship between the diversity indices of the C-cycling genes and the N rates (Fig. S2).

The C-cycling genes were grouped into six categories, including glycoside hydrolases (GHs), glycosyl transferases (GTs), carbohydrate esterases (CEs), auxiliary activities (AAs), polysaccharide lyases (PLs), and carbohydrate-binding modules (CBMs), based on their specific functions; these six genes groups were detected in the following decreasing order of relative abundance in: GHs > GTs > CEs > AAs > CBMs > PLs (Fig. 1). Detailed analysis revealed that N application significantly changed the relative abundance of the six functional gene categories; the abundance of most genes decreased significantly with increasing N rates, while only that of GHs and AAs increased. Meanwhile, the quadratic regression described the relationship between N rates and the C-cycling genes ($R^2 = 0.63$–0.76, $p < 0.01$), except for CBMs and PLs because of less correlation with N rates.

Subsequent correlation analysis to explore the association between soil and pruned litter properties and C-cycling gene abundance and diversity indices revealed strong correlations of these C-cycling genes, except for PLs, with soil pH and DOC (Fig. S3); these genes negatively correlated with soil pH but positively with DOC. The GHs, GTs, CEs, AAs, and CBMs positively correlated with the biomass of pruned litter; however, the PLs showed no significant correlation with the pruned litter properties. Among the diversity indices, the Chao1 index of C-cycling genes positively

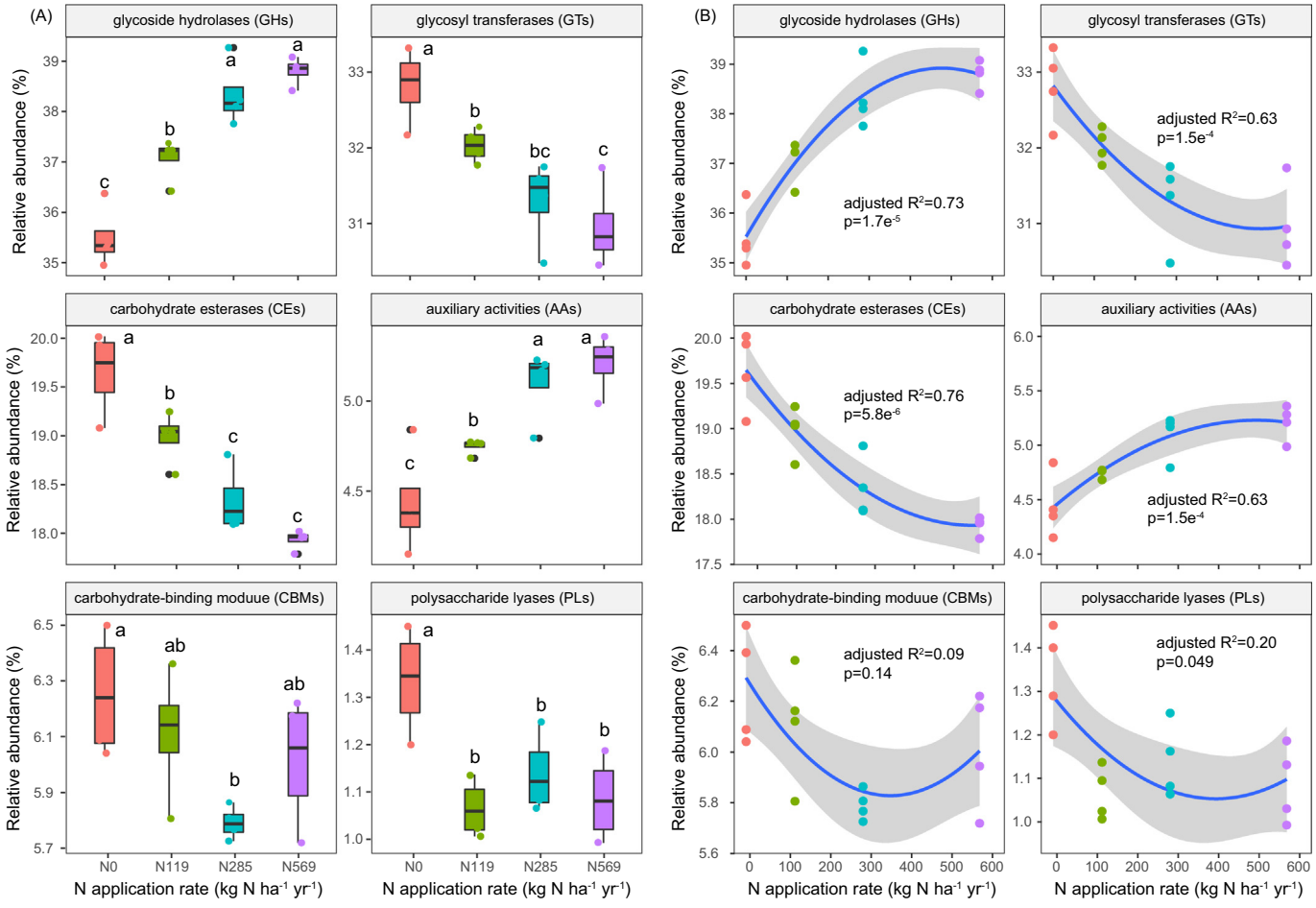

Fig. 1. Changes in the relative abundance of the microbial genes encoding glycoside hydrolases (GHs), glycosyltransferases (GTs), carbohydrate esterases (CEs), auxiliary activities (AAs), carbohydrate-binding modules (CBMs), and polysaccharide lyases (PLs) in response to long-term N fertilization. Box plots (A, left panel) show the ANOVA results of the effect of N application rates on the six groups of C-cycling genes; trend lines (B, right panel) show the regression curves indicating the relationships between the six groups of C-cycling genes and the N rates, and gray shading represents 95 % confidence intervals. Lowercase letters above boxes indicate significant differences ($p < 0.05$) in the C-cycling gene groups among the different N application rates.

correlated with soil pH and TP, while the Shannon index negatively correlated with soil pH but positively with DOC. The diversity indices showed no significant correlation with the other pruned litter properties (Fig. S3).

3.2. Functional genes involved in SOC degradation

Among the six types of C-cycling genes, GHs demonstrated the highest relative abundance (37.43 %; Fig. 1A). We identified 71 genes encoding GHs, which perform SOC degradation; however, only the top 20 genes were listed in our study. Detailed analysis revealed that N application significantly increased the abundance of GH genes (Fig. 2). Additionally, the GH genes significantly correlated with soil pH and DOC (Fig. S4); the GHs negatively correlated with soil pH but positively with DOC. However, no significant correlation was detected between the GHs and other soil properties. Among the pruned litter properties, biomass positively correlated with most genes of the GH family, while TP negatively correlated with a few genes. The GHs showed no significant correlation with any other pruned litter property.

3.3. Diversity and composition of microbial communities involved in SOC degradation

Carbon degradation is the main process leading to soil C loss. The present study focused on two key genes of the GH family, GH3 and GH18, encoding beta-glucosidase and chitinase, respectively, which degrade labile and recalcitrant C (Fig. 2). The absolute abundance of both beta-glucosidase and chitinase significantly increased with increasing N rates (Fig. 2). Meanwhile, the relative abundances of beta-glucosidase and chitinase correlated considerably with pH and DOC of soil and biomass and TP of pruned litter but not with any other soil and pruned litter properties (Fig. S5).

The PCoA results showed a significant different of the beta-glucosidase and chitinase-expressing microbial communities with increasing N rates. The community structures of microbes (at the genus level) carrying the beta-glucosidase and chitinase coding genes differed significantly among the N levels (PERMANOVA test: $R^2 = 0.65$, $p < 0.001$ and $R^2 = 0.59$, $p < 0.001$; Fig. 3). In the plot, the first and second axes explained 72.55 % and 64.43 % of the total variance in beta-glucosidase (Fig. 3A) and chitinase expressing microbial communities (Fig. 3B). Soil samples under low N (N0 and N119) and high N treatments (N285 and N569) were clearly separated along the first axis. Further analysis showed no difference in the Chao1 index of beta-glucosidase and chitinase-expressing microbes under different N levels. Meanwhile, N application significantly influenced the Shannon index; the Shannon index of beta-glucosidase and chitinase-expressing microbes decreased substantially with increasing N rates (Fig. S6). Likewise, a quadratic regression fitted only the Shannon index with the different N rates.

Taxonomic annotation identified that similar taxa at the phylum level expressed beta-glucosidase and chitinase (Fig. 4A, B). The dominant phyla carrying these genes were Acidobacteria (34.92 % vs. 36.09 %), Proteobacteria (27.71 % vs. 25.48 %), Actinobacteria (19.37 % vs. 20.61 %), Chloroflexi (5.68 % vs 4.26 %), and Bacteroidetes (4.78 % vs. 3.13 %), which together accounted for 92.46 %–89.57 % of the microbes. Moreover, N application significantly affected the beta-glucosidase-expressing microbial taxa at the phylum level (Fig. S7A); the abundance of

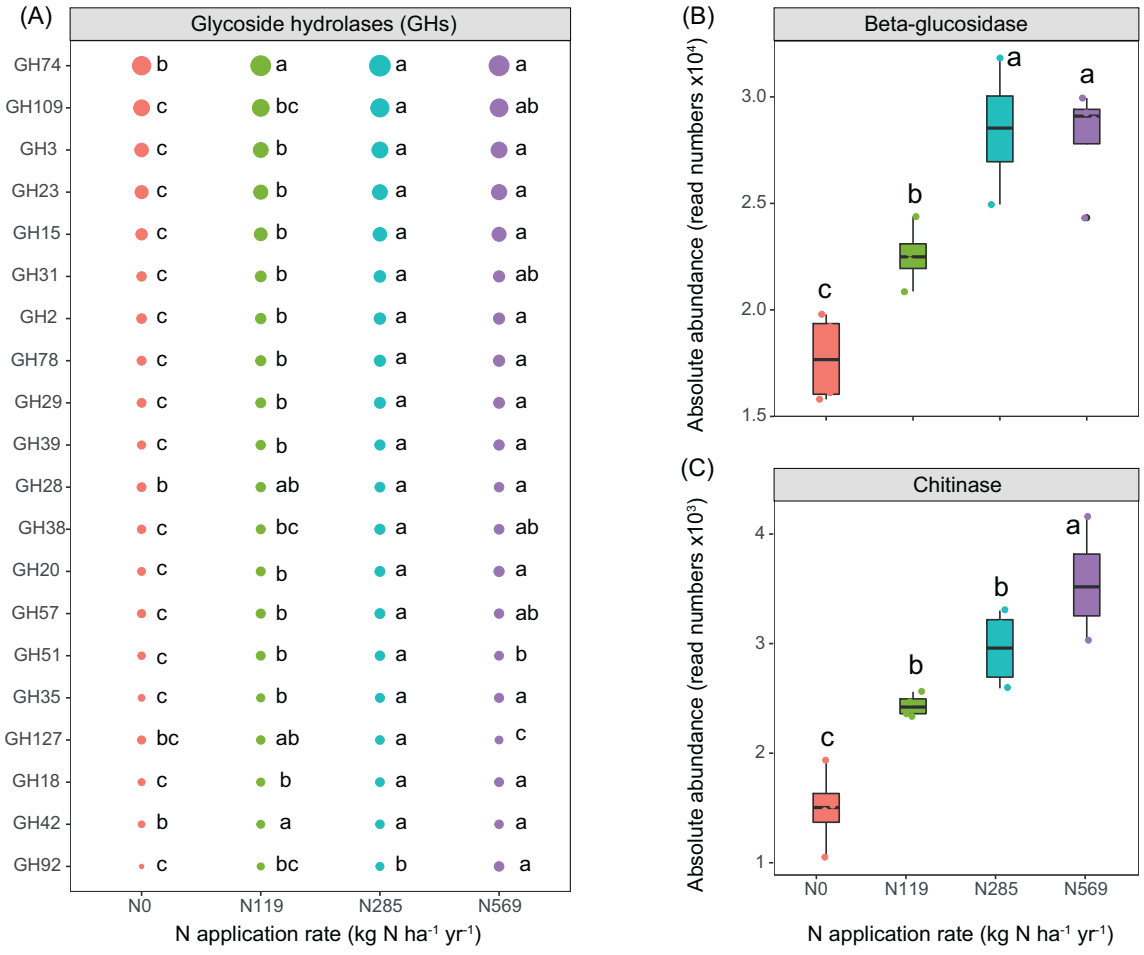

Fig. 2. Changes in the abundance of C-cycling glycoside hydrolase genes (GHs) involved in organic C degradation in the tea plantation soils under different N application rates. The bubble plots show the GH family's absolute abundance in terms of reads per sample (A). The 20 most abundant GHs are shown. Box plots show the ANOVA results of the effect of N application rates on the abundances of beta-glucosidase (B) and chitinase (C) genes. Lowercase letters on the right of the bubbles and above the bars indicate significant differences among the N rates (One-way ANOVA, $p < 0.05$).

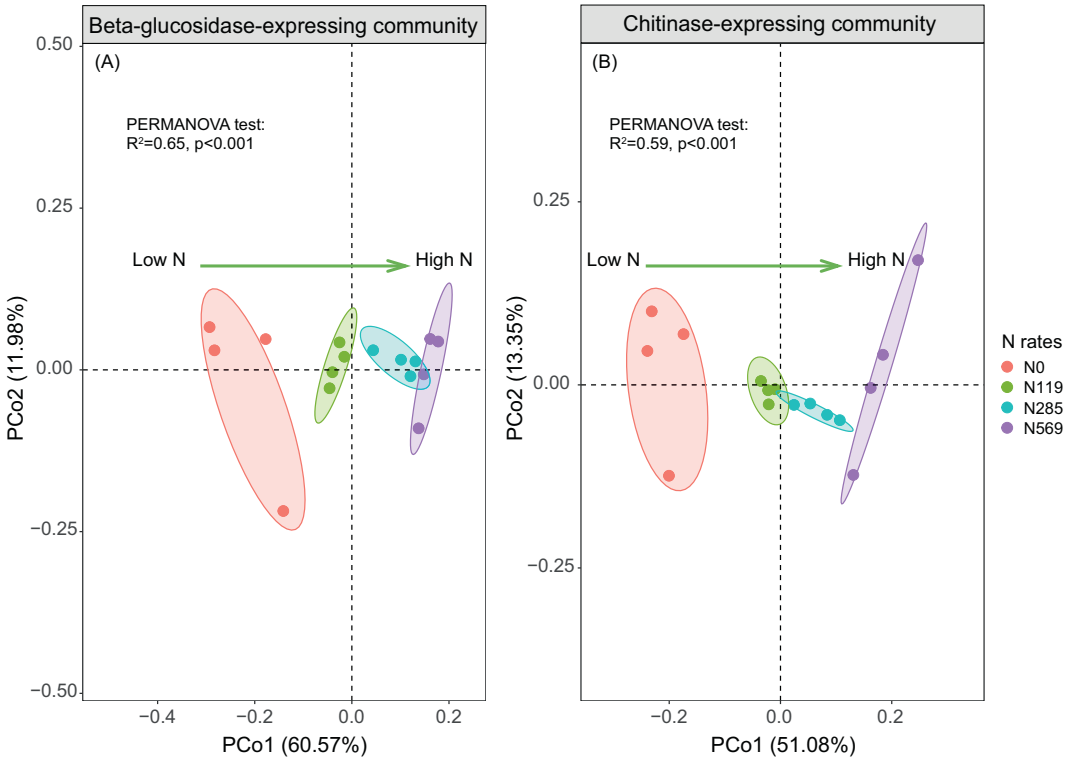

Fig. 3. Principal coordinate analysis (PCoA) of beta-glucosidase (A, left panel) and chitinase-expressing (B, right panel) community composition at the genus level based on Bray-Curtis distances. The variations explained by PCoA 1 and PCoA 2 are shown as percent values along the axes. A permutational multivariate analysis of variance (PERMANOVA) was used to determine the significant effect of N rates on the functional community structure.

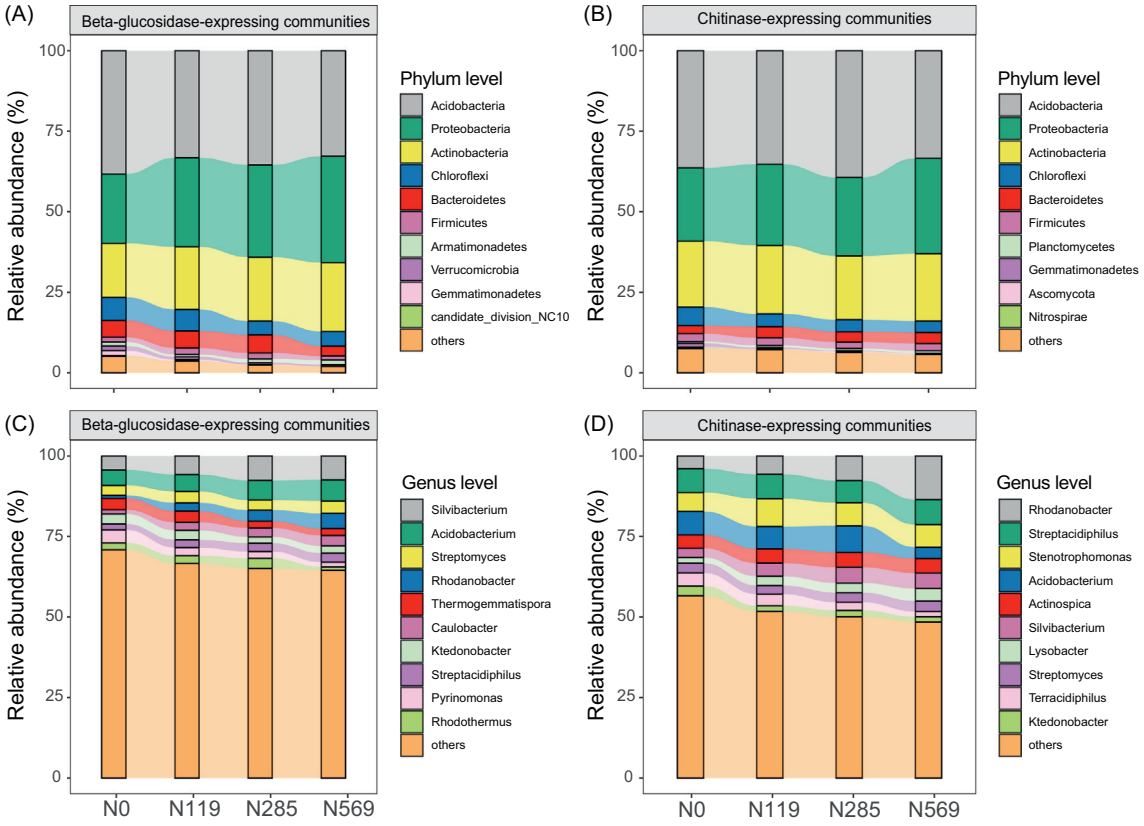

Fig. 4. The relative abundance of the top 10 microbial taxa expressing beta-glucosidase (A, C; left panel) and chitinase (B, D; right panel) at the phylum (A, B; top panel) and genus (C, D; bottom panel) levels in the tea plantation soils under four long-term N application rates.

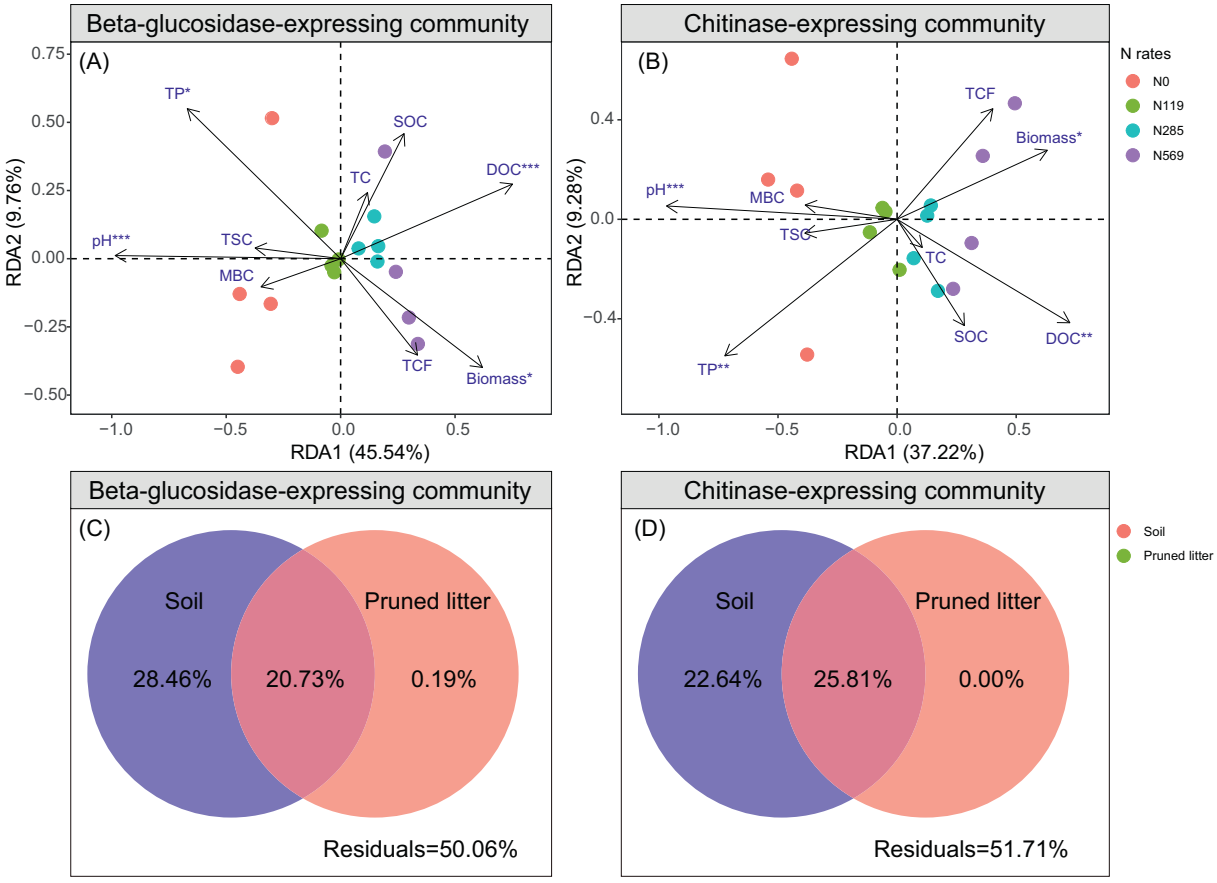

Fig. 5. Redundancy analysis (RDA) and variance partitioning analysis (VPA) show the relationship between beta-glucosidase (A, left panel) and chitinase-expressing (B, right panel) community composition and the soil and pruned litter properties. A permutational multivariate analysis of variance (PERMANOVA) was used to test the effect of environmental factor on the functional microbial communities; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. For VPA, soil and pruned litter properties were significant variables influencing the beta-glucosidase and chitinase-expressing microbial community in the RDA plots.

seven phyla increased with increasing N rates. However, N application did not influence the abundance of Chloroflexi, Armatimonadetes, and candidate_division_NC10. Similar to the beta-glucosidase-expressing microbial communities, the abundance of the eight chitinase-expressing microbial taxa increased significantly with increasing N rates; however, Chloroflexi and Nitrospirae did not respond to N application (Fig. S7B).

Subsequent taxonomic annotation of microbes associated with beta-glucosidase and chitinase-expressing microbial communities at the genus level identified 25 beta-glucosidase-expressing genera with a relative abundance >1 %. The top five taxa identified in the decreasing order of relative abundance were *Silvibacterium*, *Acidobacterium*, *Streptomyces*, *Rhodanobacter*, and *Thermogemmatispora* (Fig. 4C). Among the top ten taxa, *Thermogemmatispora* and *Ktedonobacter* showed no significant difference among the N application rates, while the other eight displayed an increase in abundance with increasing N rates (Fig. S7C). A total of 13 genera with relative abundance >1 % were identified as associated with chitinase. The top five taxa in the decreasing order were *Rhodanobacter*, *Streptacidiphilus*, *Stenotrophomonas*, *Acidobacterium*, and *Ktedonobacter* (Fig. 4D). Except for *Ktedonobacter*, the other nine taxa increased with increasing N rates (Fig. S7D).

3.4. Relationship between microbial communities involved in SOC degradation and soil and pruned litter properties

The present study used RDA to assess the effects of soil and pruned litter properties on the beta-glucosidase and chitinase-expressing communities. The first two axes from RDA explained 55.30 % and 46.50 % of the total variation in the beta-glucosidase and chitinase-expressing microbial communities, respectively (Fig. 5A, B). PERMANOVA test revealed that soil pH ($R^2 = 0.56$, $p = 0.001$ and $R^2 = 0.52$, $p = 0.001$) and DOC ($R^2 = 0.34$, $p = 0.001$ and $R^2 = 0.31$, $p = 0.002$) significantly influenced beta-glucosidase and chitinase-expressing microbial communities (Table 1). Among the pruned litter properties, only biomass ($R^2 = 0.24$, $p = 0.016$ and $R^2 = 0.27$, $p = 0.003$) and TP ($R^2 = 0.22$, $p = 0.019$ and $R^2 = 0.21$, $p = 0.030$) significantly affected the beta-glucosidase and chitinase-expressing communities (Table 1). Further, VPA was used to dissect the relative contributions of the soil and pruned litter properties to the soil beta-glucosidase and chitinase-expressing microbial communities. Results showed that soil pH, DOC, biomass, and TP significantly affected the

Table 1
Results of permutational multivariate analysis of variance (PERMANOVA).

Beta-glucosidase-expressing community			Chitinase-expressing community		
	R^2	*p*-Value		R^2	*p*-Value
Soil properties					
pH	**0.56**	**0.001**	pH	**0.52**	**0.001**
DOC	**0.34**	**0.001**	DOC	**0.31**	**0.002**
SOC	0.08	0.269	SOC	0.08	0.260
MBC	0.07	0.329	MBC	0.09	0.236
Pruned litter properties					
TCF	0.07	0.306	TCF	0.08	0.219
TSC	0.09	0.251	TSC	0.09	0.209
TP	**0.24**	**0.016**	TP	**0.27**	**0.003**
TC	0.04	0.616	TC	0.04	0.659
Biomass	**0.22**	**0.019**	Biomass	**0.21**	**0.030**

Bold text indicates statistical significance as tested by PERMANOVA.

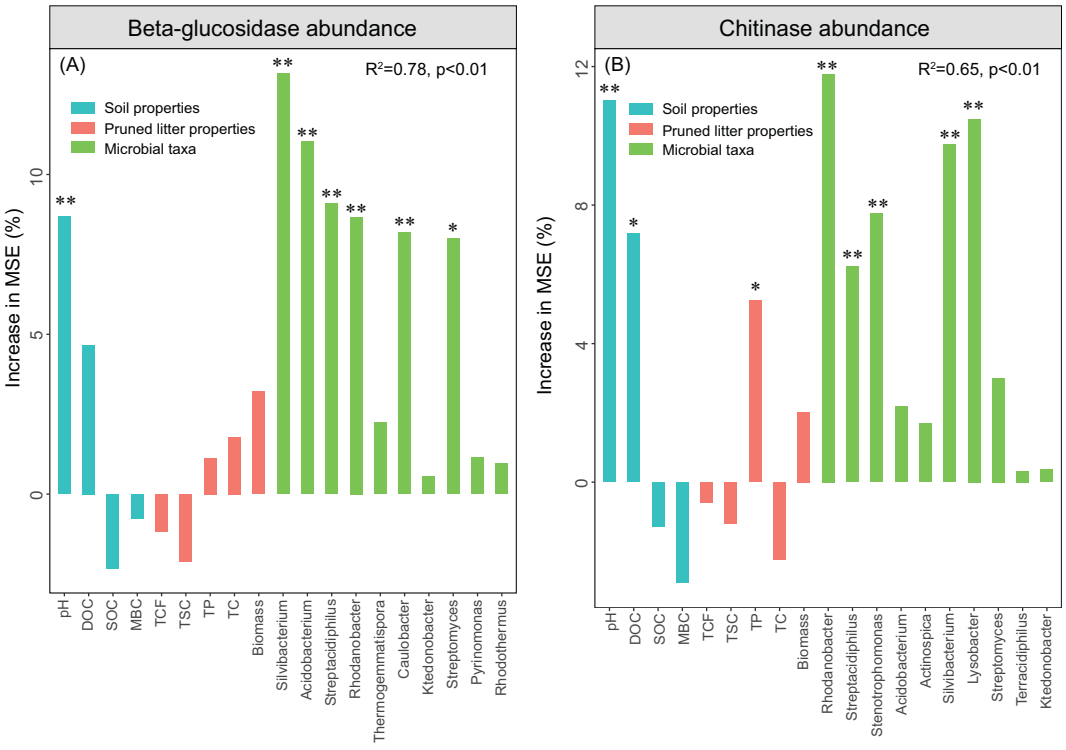

Fig. 6. Random forest regression model shows the key factors influencing the abundance of beta-glucosidase (A) and chitinase (B) genes. MSE stands for "mean square error", and R^2 refers to the model's goodness of fit. The star above the bars indicates that the factors had significant effects on the abundance of beta-glucosidase and chitinase; $*p < 0.05$, $**p < 0.01$.

functional microbial community structure; soil properties (pH and DOC) largely explained variations in the beta-glucosidase (28.46 %) and chitinase (22.64 %) expressing microbial communities while pruned litter properties (TP and biomass) accounted for a very low variation rate (<1 %) (Fig. 5C, D). These observations suggest that the soil and pruned litter properties significantly affected (20.73 % and 25.81 % variations, respectively) the beta-glucosidase and chitinase-expressing microbial communities.

Finally, the Pearson correlation showed that only soil pH and DOC significantly correlated with the beta-glucosidase and chitinase expressing microbial taxa; however, these two properties displayed opposite correlations (Fig. S8). Soil pH was significantly associated with seven phyla and seven genera of microbes expressing beta-glucosidase and eight phyla and seven genera of microbes expressing chitinase, while DOC correlated considerably with five phyla and six genera of microbes expressing beta-glucosidase and four phyla and three genera of microbes expressing chitinase. Among the pruned litter properties, biomass was significantly associated with three phyla and five genera beta-glucosidase expressing taxa and four phyla and three genera chitinase expressing taxa, while TP was significantly associated with two phyla and five genera of beta-glucosidase expressing taxa and four phyla and three genera of chitinase expressing taxa. Other soil and pruned litter properties showed weaker correlations with beta-glucosidase and chitinase-expressing microbial taxa.

3.5. Driving factors for predicting the abundance of beta-glucosidase and chitinase

The present study used an RF model to screen the main soil and pruned litter properties and the key microbial taxa affecting the abundance of beta-glucosidase and chitinase (Fig. 6). Both beta-glucosidase and chitinase modeling demonstrated good predictive efficiencies ($R^2 = 0.78$, $p < 0.01$ and $R^2 = 0.65$, $p < 0.01$). In the beta-glucosidase model, only soil pH was identified as the key variable among the soil properties that significantly affected beta-glucosidase abundance. Meanwhile, six out of ten taxa at the genus level, namely *Silvibacterium*, *Acidobacterium*, *Streptomyces*, *Rhodanobacter*, *Caulobacter*, and *Streptacidiphilus*, were identified as key microbial taxa. However, no pruned litter property influenced the abundance of beta-glucosidase. In the chitinase model, soil pH and DOC and pruned litter TP content predicted the abundance of chitinase with high accuracy (Fig. 6); the model also identified five microbial taxa, which significantly influenced chitinase abundance.

4. Discussion

4.1. Effect of long-term N fertilization on organic C-decomposing genes

In our study, long-term N fertilization significantly affected soil C-cycling genes in the tea plantation (Figs. 1, 2; Fig. S1). The Chao1 index of C-cycling functional genes was decreased with increasing N rates, while the Shannon index was increased (Fig. S2), indicating an increase in microbial diversity related to C degradation in soil under N application (Cheng et al., 2017). Previously, Trivedi et al. (2016) also reported that N addition significantly changed the composition of functional genes; the richness and diversity of C-degrading genes, such as *amyA*, *glucoamylase*, *ara*, *xylanase*, *cellobiase*, and *endochitinase*, decreased with N addition. However, the present study found an increase in the relative abundance of organic C-degrading genes (GHs) with increasing N rates (Fig. 1), consistent with a previous meta-analysis that showed increased activities of C-cycling enzymes β-1,4-glucosidase (BG) and β-D-cellobiosidase (CBH) with N application (Jian et al., 2016). These findings indicate that N application is not conducive to SOC accumulation. Similarly, Luo et al. (2019) also found that N application accelerated SOC loss in the grassland ecosystem of the Qinghai-Tibet Plateau due to a significant increase in the activities of the C-degrading enzymes. Moreover, the present study found a substantial decrease in the relative abundance of organic C-synthesizing genes (e.g., GTs) under N application (Fig. 1), consistent with our previous report on decreased SOC content under high N application (Yang et al., 2019), probably due to the positive priming effect of pruned litter also

(Yang et al., 2022). Thus, the finding suggested that exogenous C input exerted a positive priming effect, resulting in less SOC accumulation.

Detailed analysis showed that the relative abundance of the top 20 SOC-degrading genes increased significantly with increasing N rates (Fig. 2A), indicating a positive association between these genes and N input (Jian et al., 2016). Among the SOC-degrading genes, beta-glucosidase and chitinase are the key enzymes that specifically degrade the labile and recalcitrant C (Dai et al., 2021). Analysis based on the KEGG database found that the activity of beta-glucosidase was ten times higher than that of chitinase (Fig. 2B, C), suggesting labile C as the dominant C component in tea plantation soil (Yang et al., 2019). This observation is consistent with our recent results on relatively low C storage in tea plantation soil under long-term fertilization even after returning pruned litter (Yang et al., 2022). Large quantities of pruned litter returned to tea plantation soil contain labile C, which is degraded by soil microorganisms (Yang et al., 2022). Moreover, the abundance of beta-glucosidase and chitinase increased significantly with increasing N rates (Fig. 2B and C), indicating that N application promoted the decomposition of labile and recalcitrant C and accelerated the SOC loss, consistent with the decrease in SOC under high N conditions (Yang et al., 2019). These findings indicate that pruned tea litter incorporation results in less SOC accumulation, especially under excessive N application, in the tea plantation system.

4.2. Effect of long-term N fertilization on organic C-decomposing microbial communities

Microbes are important drivers of biogeochemical cycles (Rousk and Bengtson, 2014). This study focused on beta-glucosidase and chitinase-expressing microbial communities associated with labile and recalcitrant C degradation (Figs. 3 and 4). In the tea plantation soils, long-term N application significantly changed the community compositions of beta-glucosidase and chitinase-expressing microbes, especially under low N (N0 and N119) and high N (N285 and N569) rates (Fig. 3). Besides, the glucosidase and chitinase-expressing communities showed a significant different under different N rates (Fig. 3). Under high N application, the diversity of beta-glucosidase and chitinase-expressing microbes significantly decreased (Fig. S2). The microbial variation observed in this study is consistent with the response of the microbial community to N application in other ecosystems, such as grassland, forests, and farmlands (Ramirez et al., 2010; Wu et al., 2013; Zeng et al., 2016), which occurred probably due to the changes in soil N availability under different N rates. According to the oligotrophic-copiotrophic theory (Fierer et al., 2007), specific microbes respond differently to soil N availability. In the present study, the N-induced decrease in the functional microbial diversity was probably due to oligotrophic communities (Zeng et al., 2016).

At the phylum level, most beta-glucosidase and chitinase-expressing microbes were abundant and similar (Fig. 4A, B), indicating that these dominant microbial taxa may belong to habitat generalist taxa (Crump et al., 2012) that perform most ecological functions (Lladó et al., 2016). Accordingly, the dominant phyla Acidobacteria, Proteobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes, and Gemmatimonadetes may be the generalist taxa. This finding is consistent with the previous studies that showed that the five most abundant phyla were all generalists and accounted for over three-quarters of the total microbial abundance (Wu et al., 2018). Among the beta-glucosidase-expressing microbial communities, Armatimonadetes and Verrucomicrobia were the specific microbial taxa responsible for decomposing labile C, while among the chitinase-expressing microbial communities, Planctomycetes, Ascomycota, and Nitrospira were the specific ones responsible for decomposing recalcitrant C. These specific microbes could be habitat specialists, generally less abundant (Crump et al., 2012). These observations suggest that both habitat generalists and specialist taxa perform ecological functions. In other words, both abundant and rare microbial communities perform critical ecological functions (Huber et al., 2007).

Meanwhile, at the genus level, the microbial taxa degrading labile and recalcitrant C differed largely, indicating that most ecological functions were performed by specific taxa at higher taxonomic levels (Louca et al., 2018). *Silvibacterium*, *Acidobacterium*, *Streptomyces*, and *Ktedonobacter* were the specific microbial genera associated with labile and recalcitrant C degradation (Fig. 4C, D). Moreover, the relative abundance of the microbes expressing beta-glucosidase and chitinases differed significantly at the genus level; microbial taxa with high abundance performed labile C degradation, while those with low abundance were responsible for recalcitrant C degradation (Fig. 4C, D). These results suggest that microorganisms perform specific ecological functions highly depending on the differences in the substrate (Louca et al., 2018). However, it should be noted that functional microbial taxa, especially at higher taxonomic levels (i.e., species levels), should be further analyzed since a single strain can perform various ecological functions (Vorholt et al., 2017). Additionally, we found a strong positive relationship between the functional taxa and the N rates (Fig. S7); these functional taxa belonged to copiotrophic taxa. Thus, the results indicate that N application improved the growth and reproduction of functional taxa and promoted SOC degradation, decreasing SOC content in the tea plantation (Yang et al., 2019).

4.3. Factors driving organic C decomposition-associated functional genes and microbial communities in tea soils under N application

Various biotic and abiotic factors are crucial in structuring functional microbial communities. RDA results of the present study indicated that soil pH is an important factor affecting the beta-glucosidase and chitinase-expressing microbial communities in tea plantation soil (Fig. 5). This observation is consistent with Dai et al. (2021), who reported that soil pH is a crucial factor regulating soil C-cycling, including C-cycling genes and associated functional communities. Besides, evidence shows that soil pH is a key factor regulating microbial growth; microbes grow in a neutral environment; therefore, acidic and alkali conditions inhibit their growth and reproduction (Beales, 2004). In the present study, organic C-degrading genes and microbial communities negatively correlated with soil pH, consistent with Dai et al. (2021) findings, indicating that relatively low pH may benefit SOC degradation.

In addition, soil DOC significantly affected C-degrading genes and beta-glucosidase and chitinase-expressing microbial communities (Fig. 5). As an unstable form of C, DOC is easily broken down and utilized by microbes (Liu et al., 2019). During decomposition, DOC is a direct Source of energy for bacterial growth and activity, and its availability affects the transformation of nutrients, including N, P, and S cycling (Roesch et al., 2007). DOC is widely recognized as a source of energy and nutrients for soil microorganisms (Kaiser and Kalbitz, 2012). Several studies have shown that DOC impacts soil biological properties more than other physicochemical properties (Li et al., 2015). Moreover, consistent with Zhong et al. (2020), DOC and lignin accounted for a higher rate of microbial community variation during straw degradation under different N application rates.

In addition to soil properties, pruned litter properties also significantly affected SOC-degrading enzymes and beta-glucosidase and chitinase-expressing microbial communities (Fig. 5). Plant biomass is the primary source of energy for microbes, and it provides high-quality substrates, such as SOC, thereby increasing the population of soil microbial communities (Yang et al., 2021). Chen et al. (2020) found a positive relationship between soil bacterial diversity and plant biomass. Besides, polyphenols significantly affect beta-glucosidase and chitinase-expressing microbial communities (Fig. 5), similar to the effect on soil microbial communities (Yang et al., 2019). Studies have demonstrated that polyphenols, as a C source, greatly influence various soil processes, such as nitrification (Tang et al., 2021). There are multiple reports on the effects of polyphenols on soil C-cycling. For example, polyphenols stimulate litter decomposition rate, influence soil humus formation (Stevenson, 1994) and nutrient release patterns (Nannipieri and Badalucco, 2003), and directly or indirectly alter the microbial community. Polyphenols also control other macronutrients, such as phosphorus and sulfur, and micronutrients, such as iron, copper, zinc, and manganese (Cesco et al., 2012). Polyphenols positively or negatively affect soil N content, depending on the specific phenolic compound

and the microbial activity (Hättenschwiler and Vitousek, 2000). Thus, these earlier reports and the present findings indicate plant properties should also be considered while exploring the drivers of microbial communities.

5. Conclusions

In this study, long-term N application significantly changed beta-glucosidase and chitinase-expressing communities in tea plantations. N fertilization significantly improved the abundance of C-cycling genes, especially expressing the beta-glucosidase and chitinase. Similar dominant microbial taxa at the phylum level and distinct taxa at the genus level degraded the labile and recalcitrant C of the tea plantation soil. Further random forest models indicated that pH and DOC of the soil, polyphenol content of pruned litter, and the dominant taxa performing ecological functions were important variables for predicting the abundance of beta-glucosidase and chitinase in the soil. Thus, our results concluded that N application significantly enhanced the SOC degradation ability of the tea plantation soils, reflected by the functional genes and microbial taxa, which was not conducive to SOC accumulation. These findings provide a basis for exploring the effects of agricultural management measures on soil C transformation from the perspective of genes-enzymes-microbes.

CRediT authorship contribution statement

Xiangde Yang: Experimental design, Investigation, Data curation, Writing-original draft. Jianyun Ruan and Lifeng Ma: Supervision, Funding acquisition, Writing-review & editing. Kang Ni: Investigation, Data curation, Revised. Yuanzhi Shi, Xiaoyun Yi, Lingfei Ji, Sirou Wei, Yanyan Jiang and Yongli Zhang: Data analysis and Discussion. Yanjiang Cai, Qingxu Ma and Sheng Tang: Review and Discussion.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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