Runing title：Intercropping increase the tobacco biomass

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Tobacco/garlic intercropping system improve chemical properties by changing C,N cycling

and plant degradation pathway in rhizosphere soil, thus increase the tobacco plant biomass

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**Abstract****:**As the mechanism of the microbe-soil-tobacco interaction remains unclear and the contribution of tobacco plant growth is still difficult to predict, the chemical propertie and microbes of soil in tobacco/garlic intercropping system, the relevance of the soil chemical properties and the genes involved in C, N cycling and plant degradation (organic matter turnover) were studied by metagenome sequencing. The results showed that the intercropping treatment (T)significantly enhanced the content of organic matter(OM) ,the available nitrogen (AN) , the available phosphorus(AP) ,the available potassium content(AK), microbe number and the microbial biomass nitrogen,as well as the activity of urease , phosphatase ,invertase compared to monocropping treatment (CK), Especially the content of OM ,AN,AP,AK increased significantly by 29.46%,19.75%, 10.37%,17.42% in rhizosphere of T treatment than CK treatment. The content of polyphenol oxidase activity and microbial biomass carbon significantly decreased in T treatment with by 22.61% and 9.03% relative to CK treatment. Metagenomic analysis showed that the relative abundances of genes related to C cycling (ACA,sdhA,sdhB, sucD, mdh) , N cycling (glnA)and plant degradation (bglX) were higher in the T treatment than the CK treatment. Compared to the CK treatmen, the relative abundance of ACA, sdhA, sdhB, sucD,mdh,glnA and bglX were espectively 26.06%, 39.37%, 48.27%, 32.44%, 57.55%,14.28% and 2.39% higher in the T treatment. The intercropping system changed the chemical properties as well as the abundance of microbes, and subsequently regulate genes involved in C, N cycling and plant degradation, these improved the soil environment and leaded to the increase of tobacco plant biomass.

****Keywords:****Flue-cured tobacco, Garlic, Intercropping, Chemical properties, C and N cycling, Organic matter, Metagenome sequencing

**Introduction**

Flue-cured tobacco is an important agro-economic crop worldwide, but led to the long-term monoculture because the increasing demand for yield and limited arable land(Li et al.,2022;Fu et al.,2018;Chen et al.,2016).However, long-term monoculture may affect the diversity and abundance of microbial communities in tobacco-growing soil, thereby reducing soil quality, ultimately hindering the growth and development of tobacco plants, and leading to the decline of tobacco yield and quality(Gong et al.,2018;Ma et al.,2021;Wang et al.,2016).

Intercropping had effects on microbial communities and chemical properties in soil ([Cao et al.,2017](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-6)). The interactions among microbes, nutrients and enzymes in intercropping systems leads to an increase or decrease in microbe quantity and enzyme activity, contributing to the improvement of the soil micro-ecological environment ([Zhou et al.,2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-76)),these interactions also affect plant productivity directly or indirectly. [Verma et al. (2014)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-56) reported that the higher organic C input obtained through the decomposition of plant residues in the intercropping system helps to increase microbial activity and thus promote plant growth.In addition, intercropping can efficiently utilize light and nutrients and increase yields ([Li et al.,2010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-26);[Quan et al.,2013](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-42)).So, intercropping makes full use of soil nutrients and land resources and increases economic benefits, which contributes to the development of efficient and sustainable production of agriculture.

The microecological environment plays an important role in the growth of intercropping crops and the development of sustainable agriculture.Although the impact of tobacco/corn intercropping on soil nutrients, soil enzyme activity and bacterial population has been investigated in several studies (Zhou et al.,2015b), little is known about how tobacco/garlic intercropping affects the microecological environment, especially the interaction mechanism of microbes-nutrients-enzymes involved in C / N cycling and organic matter degradation in intercropping systems. In this study, our objective was to investigate the nutrients, root zone soil microbes and enzyme activity under tobacco/garlic intercropping conditions, including analysis the content of N, P,K and organic matter, microbe quantity and soil metagenomic sequencing. Through metagenomic sequencing, we can not only obtain the characteristic information of all the microbial communities in the sample but also perform the analysis of genes and metabolic pathways ([Zhang et al., 2019b](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-71)).

**1Materials and Methods**

### 1.1 Experimental site and plant materials

The experiments were performed at the Qixingguan Demonstration Base (27°03′67″N, 104°51′23″E), Bijie city, Guizhou Province, China. The field site was previously used for monocropping flue-cured tobacco. The tested soil was clayey soil , in which organic matter (OM), available nitrogen (AN), available phosphorus (AP) and available potassium content (AK)were 19.32 g/kg, 79.29 mg/kg, 21.28mg/kg and108.19 mg/kg, respectively. The pH value was 5.7. The tobacco variety “Yunyan 87” and the garlic variety “Guizhou white garlic” were provided by the China Tobacco Guizhou Industrial Co Ltd Bijie Company.

**1.2 Experimental design**

On April, 2022, tobacco and garlic were planted simultaneously in the field. On June and July, 2022,garlic and tobacco were harvested respectively. Monocropping tobacco (CK) was the control, and the tobacco/ garlic intercropping system was the treatment(T). For CK, tobacco was planted with a row spacing of 1.2 m and the plant spacing is 0.5 meters; For T, the garlic is planted below the tobacco, at a distance of 0.1 meters from the tobacco. The same approach was taken in 2023.

### **1.3** Soil sampling

After the flue-cured tobacco is harvested On July 30th,2023,ten plants of tobacco per treatment were uprooted. The soil from both bulk soil and soil attached to the plant roots was collected, mixed and separated into two sealed virus-free bags . One bag (about 50 g) was stored in a refrigerator at -80 °C and used to extract soil DNA and for metagenome sequencing. The One bag (about 400 g) was dried naturally, ground and sieved and used for the determination of the nutrient content and the soil enzyme activity.

### **1.4** Soil physicochemical property analysis

The available N, available P, available K and organic matter contents were measured by the alkaline hydrolysis diffusion method , sodium bicarbonate extraction method , ammonium acetate extractionmethod and the potassium dichromate titrimetric method , respectively(Zhao et al., 2020).

Urease activity, sucrase activity, phosphatase activity, and polyphenol oxidase were determined by sodium phenolate sodium hypochlorite colorimetric method, 3, 5-

dinitrosalicylic acid colorimetric method, sodium phenyl phosphate colorimetric method and substrate induction method, respectively([Jia et al., 2019b](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-71)).

### **1.5** Genomics DNA extraction and **Metagenomic** sequencing

Biome DNA was extracted from each treated Soil sample using the NucleoSpin Soil Kit (machery-nagel, Germany) and checked for quality on 1% agarose gel. Covaris M220 (Gene Company Limited, China) software was used to construct a library of the extracted DNA and qualified by QC. The qualified libraries were sequenced on the MGISEQ-2000 platform (BGI-Shenzhen, China) ([Zhu et al., 2020](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-77); [Yang et al., 2020](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-65)).

### **1.6** Statistical analysis

MEGAHIT and MetaGeneMark soft was used to assemble the original sequence and gene prediction, gene catalog was constructed, and abundance information in each sample was obtained(Zhu et al., 2010).To obtain functional information, the sequences were aligned against the KEGG, eggNOG,CAZy,COG and CARD database by DIAMOND ([Buchfink et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-5)) . Data of metabolic pathways and relative abundances of genes analysis were analyzed as previously described in [Zheng et al. (2019)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-75). Based on the abundance profiles, the features with significantly differential abundances across groups were determined using Wilcoxon’s rank sum test ([Matsouaka et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-36)).

All of the sequence data have been deposited in the NCBI database under accession number SAMN35711959, SAMN35711960, SAMN35711961, SAMN35711962.

**2 Results**

### 2.1 Effects of tobacco**/**garlic intercropping on rhizosphere soil ****of tobacco****

### 2.1.1 Effects of intercropping on the chemical properties

Compared with the CK, a significant increasing trend of OM,AN,AP,AK was found in T, which were increasing by 29.46% in OM content ([Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)A), 19.75%in AN content ([Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)B), 10.37% in APcontent([Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)C), 17.42% in AK content([Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)D).

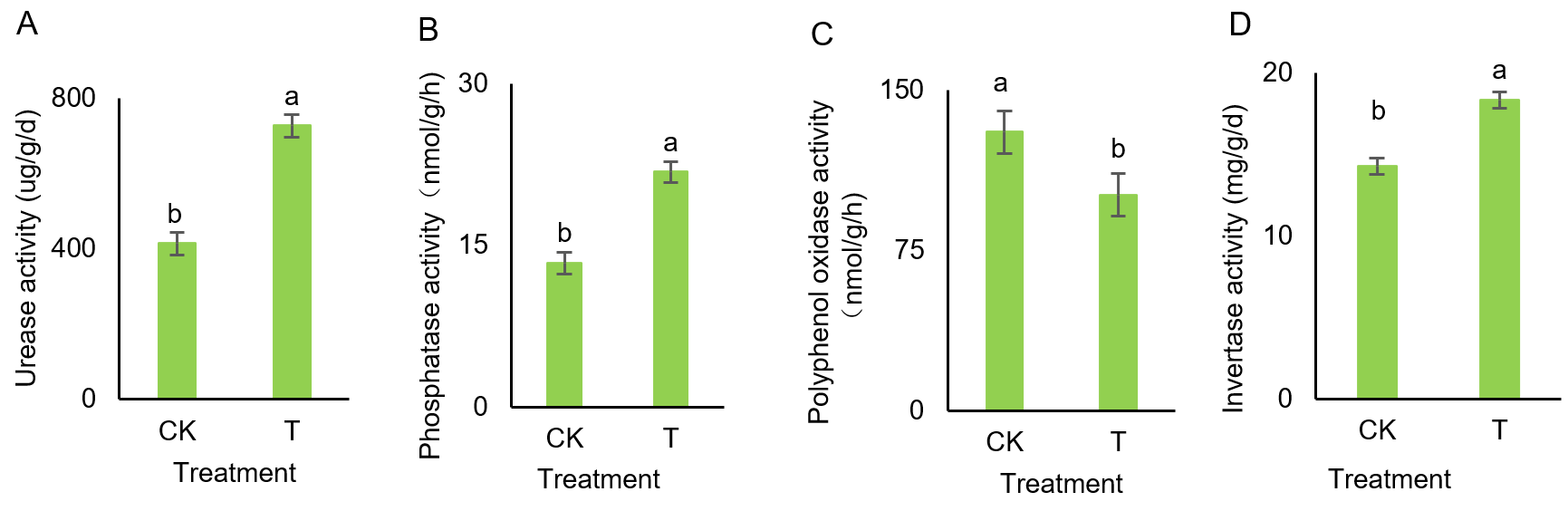
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### [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) **Basic soil chemical properties of CK and T treatment in** rhizosphere **soil of tobacco**

Note: A,Organic matter content; B,Available nitrogen content; C,Available phosphorus content; D,Available potassium content. Different lowercase letters in the same column indicate significant differences(p<0.05), the same as below.

### 2.1.2 Effects of intercropping on the enzyme activity

A comparison of enzymes in rhizosphere soil is shown in [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table). The activity of urease, phosphatase and invertase in T, with percentage increases of 75.62%, 63.06% and 28.54%, respectively,was significantly higher than in CK. Compared to CK, the polyphenol oxidase activity showed a decrease in T with percentage decrease of 22.61% .

[Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) **Major enzyme activities of CK and T treatment in** rhizosphere **soil of tobacco**

### 2.2 Effects of tobacco**/**garlic intercropping on microbe of rhizosphere soil

### 2.2.1 Effect sof intercropping on the ****microbial quantity****

Intercropping affected the diversity of soil microbes in rhizosphere soils ([Table1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)). Compared with the CK, there was a significantly higher number of bacteria and fungi in the rhizosphere soils of T, of which the number increased by 11.32% and 49.56% ; the biomass nitrogen content increased significantly by 28.43% in T; the biomass carbon content decreased significantly in intercropping treatments with decreased by 9.03% in T.

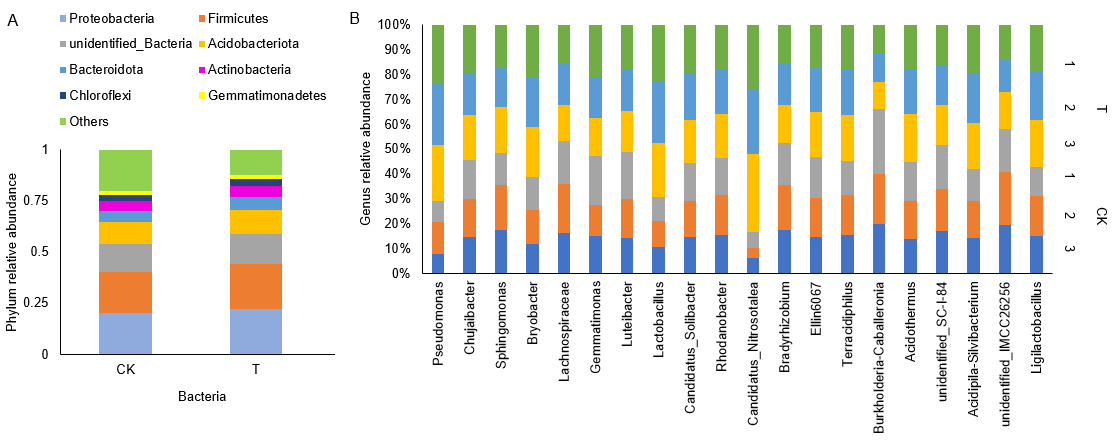
### Table 1 **Microbial quantity of CK and T in rhizosphere soil of tobacco**

| **Treatment** | Bacteria  (103·g−1) | Fungi  (103·g−1) | Microbial biomass nitrogen (mg·kg−1) | Microbial biomass carbon (mg·kg−1) |
| --- | --- | --- | --- | --- |
| CK | 3.18± 0.26b | 1.15± 0.43b | 45.44 ± 9.38b | 489.69 ± 5.65a |
| T | 3.54± 0.12a | 1.72± 0.17a | 58.36 ± 5.69a | 445.50 ± 4.45b |

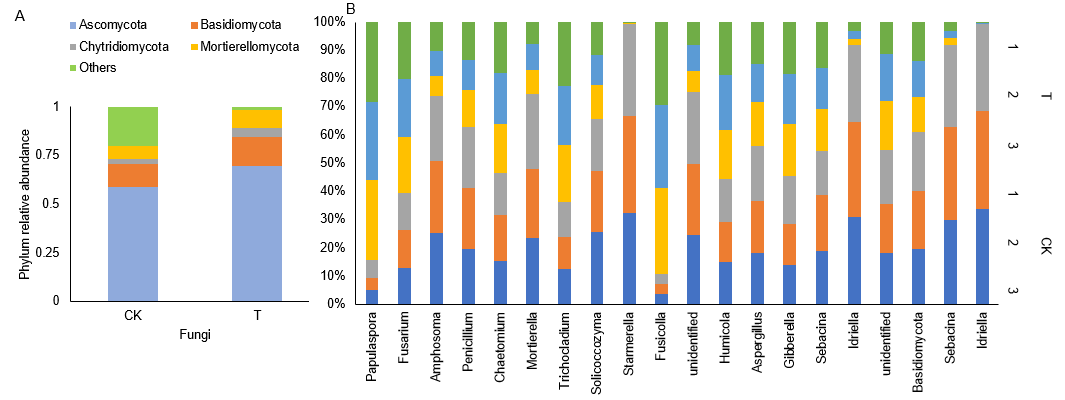
### 2.2.2 Effects of intercropping on the ****microbial**** abundance

In the top 100 abundance at the genus level, the dominant bacterial at the phylum level are *Proteobacteria*, *Firmicutes*,*unidentified\_Bacteroidota*, *Acidobacteriota*, *Bacteroidota*, *Actinobacteriota*, *Gemmatimonadetes*, *Chloroflexi* ([Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [3](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)A) and dominant fungal communities at the phylum level are *Mortierellomycota, Ascomycota*, *Basidiomycota*, *Chytridiomycota*( [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [4](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)A) in the two treatments , but the microbial abundance at genus level was significantly different between the two treatments( [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [3](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)B and [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [4](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)B) .

The operational taxonomic unit (OTU) richness and gene copy of microbial community in T treatment were much higher than that of CK treatment (Fig. 5).

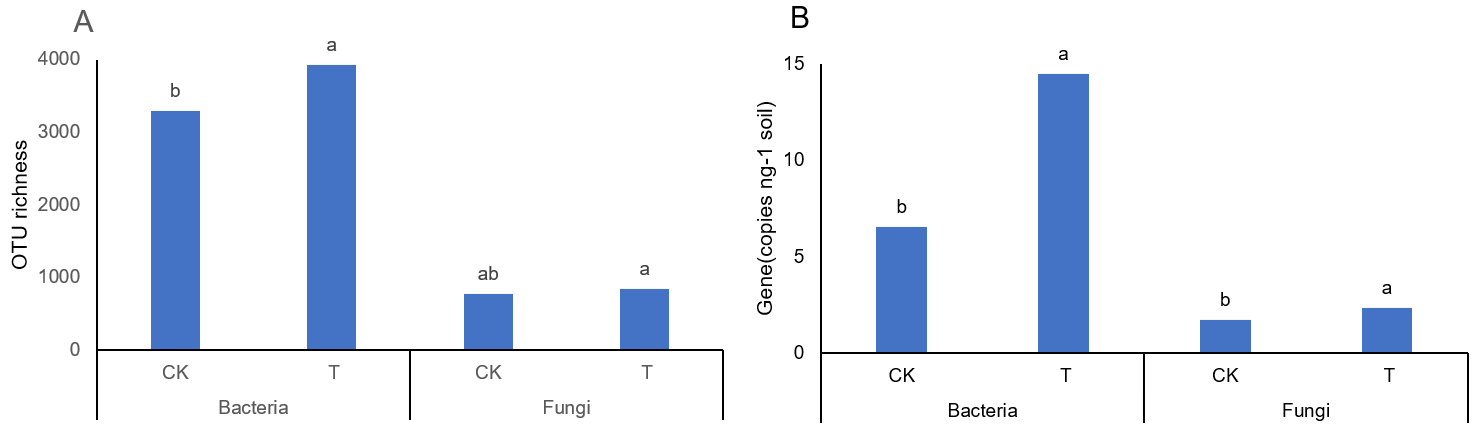


### [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [3](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) Bacterial communities **of CK and T treatment in** rhizosphere **soil of tobacco**

Note:A and B is respectively bacterial community composition at the phylum and genus levels. Genera of all samples with greater than 0.5% abundance are listed. 

### [Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [4](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) Fungal communities **of CK and T treatment in** rhizosphere **soil of tobacco**

Note:A and B is respectively fungal community composition at the phylum and genus levels. Genera of all samples with greater than 0.5% abundance are listed.



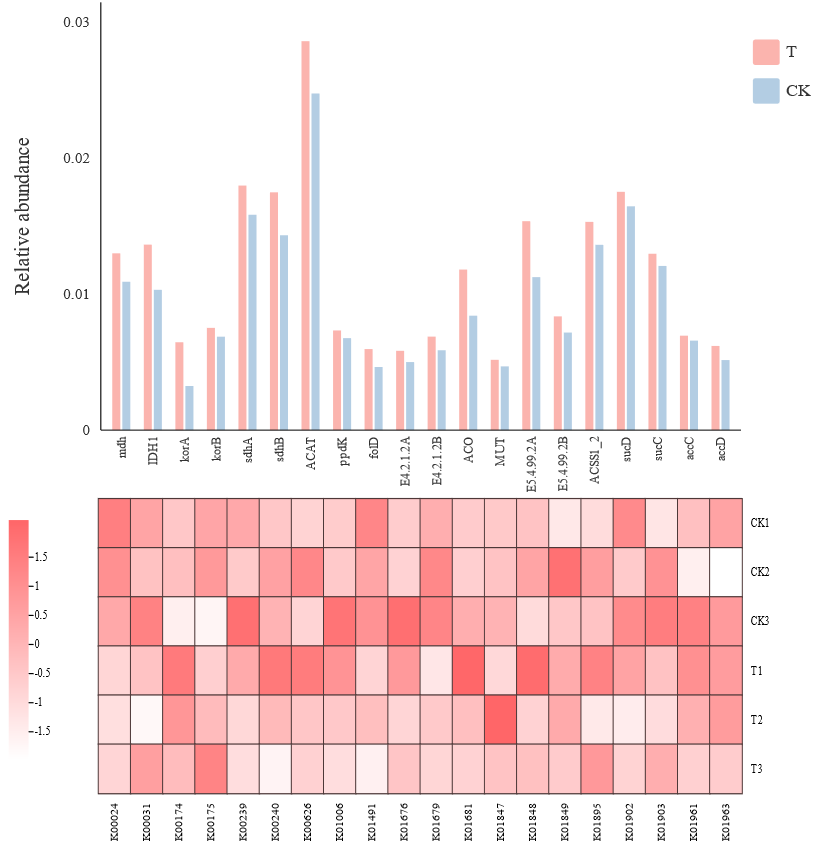
[Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [5](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) OTU richness and gene of bacterial and fungal communities **in** rhizosphere **soil of tobacco**

Note:A and B is respectively OTU richness and gene copy.

### 2.3 **Effects of tobacco/garlic intercropping on** gene abundance involved in C cycling, N cycling and plant degradation of rhizosphere soil ****of tobacco****

**2.3.1** **Effects of intercropping on major genes abundance of C cycling**

By comparison with the carbon cycling pathway, among the top twenty major genes in abundance, ACAT(K00626), sucD(K01902),sdhA(K00239), sdhB(K00240), ACSS1\_2 (K01895) ,E5.4.99.2A(K01848)were higher, with intercropping treatments increasing by 15.54%,6.47%,13.55% ,22.01%,12.40% nd 36.54%, respectively, compared with controls, which indicated that intercropping could change the abundance of major functional genes of carbon cycling (Fig. 6).



[Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [6](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) **Relative abundances of genes related to C cycling in** rhizosphere **soil of tobacco**

The results of correlation analysis between microbial of genus level and major gene in C cycling pathway showed (Figure 7) that microbial (*Rhodolanes*,*Cytobacillus*,

*Egicoccus*,*Microlunatus*,*Planococcus*) were significantly associated with more than five genes (p<0.01),which showed they were the marker microbial communities of soil microbial carbon cycling pathway in intercropping; genes (accD,sucD,korB, MUT, sohB, AOC,E4.2.1.2B) were significantly associated with more than four microbial (p<0.01),

which showed they were the marker genes of soil microbial carbon cycling pathway in intercropping.

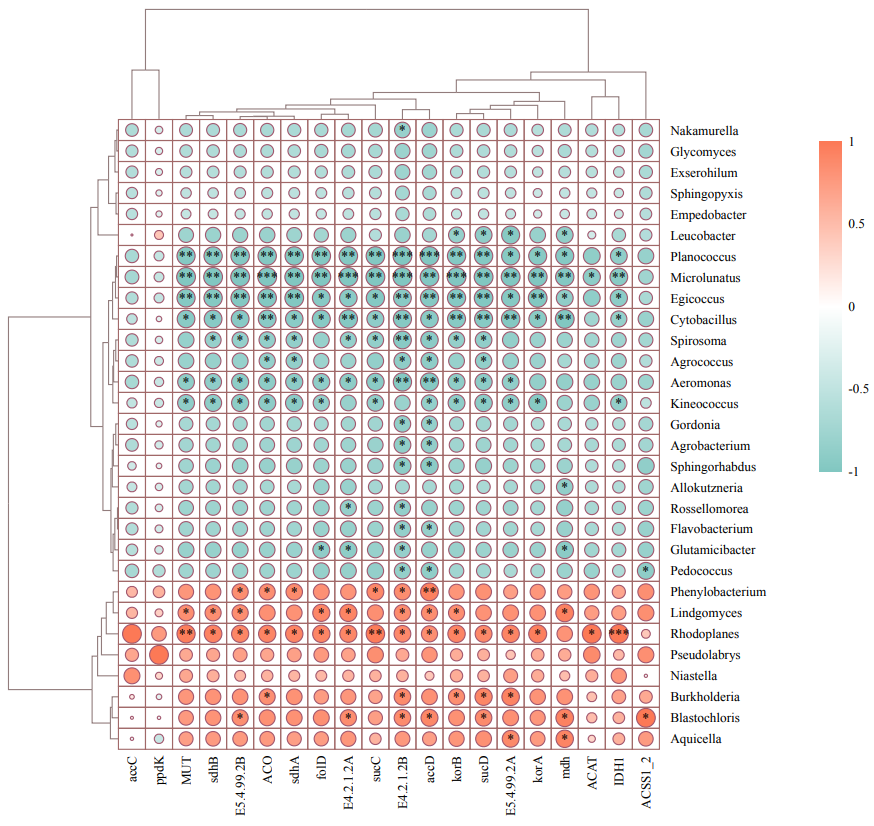


Figure 7 The correlation between microbial of genus level and major gene in C cycling pathway

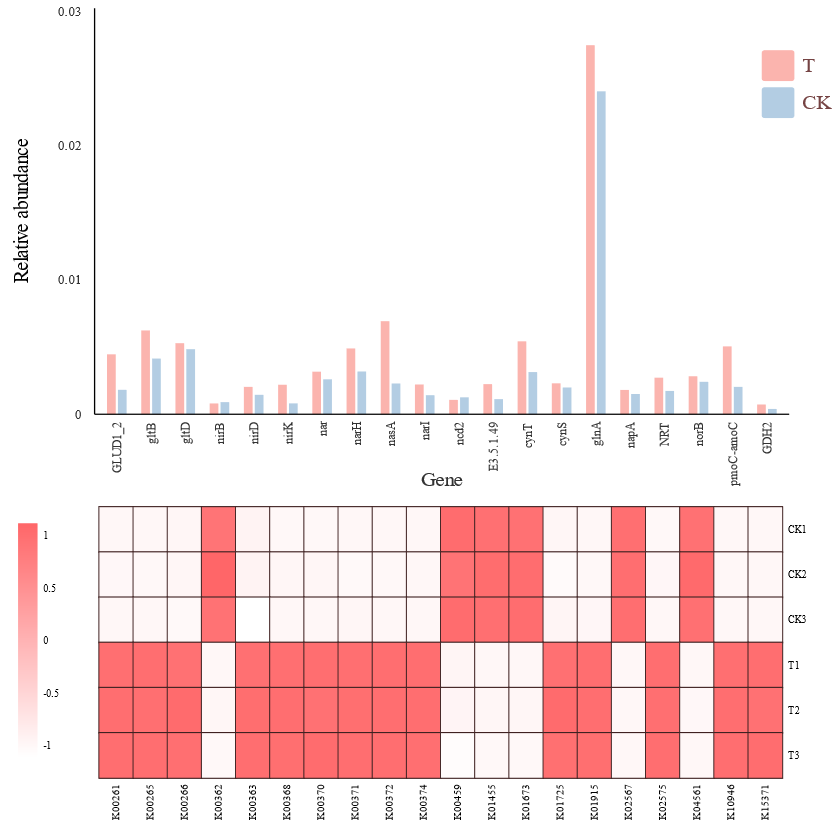
**2.3.2 Effects of intercropping on major genes abundance** **of N cycling**

By comparison with the nitrogen cycling pathway, among the top twenty major genes in abundance, glnA(K01915) was the gene with the highest relative abundance among the top 20 major functional genes abundance, and the intertreatment increased by 14.28% compared with the control (Figure 8).

The results of correlation analysis between microbial of genus level and major gene in

nitrogen cycling pathway showed (Figure 9) that microbial (*Egicoccus*,*Microlunatus*,

*Isosphaera*)were significantly associated with more than five genes (p<0.01),which showed they were the marker microbial communities of soil microbial N cycling pathway in intercropping; genes (nbd2) were significantly associated with more than four microbial (p<0.01),which showedthey were the marker genes of soil microbial nitrogen cycling pathway in intercropping.



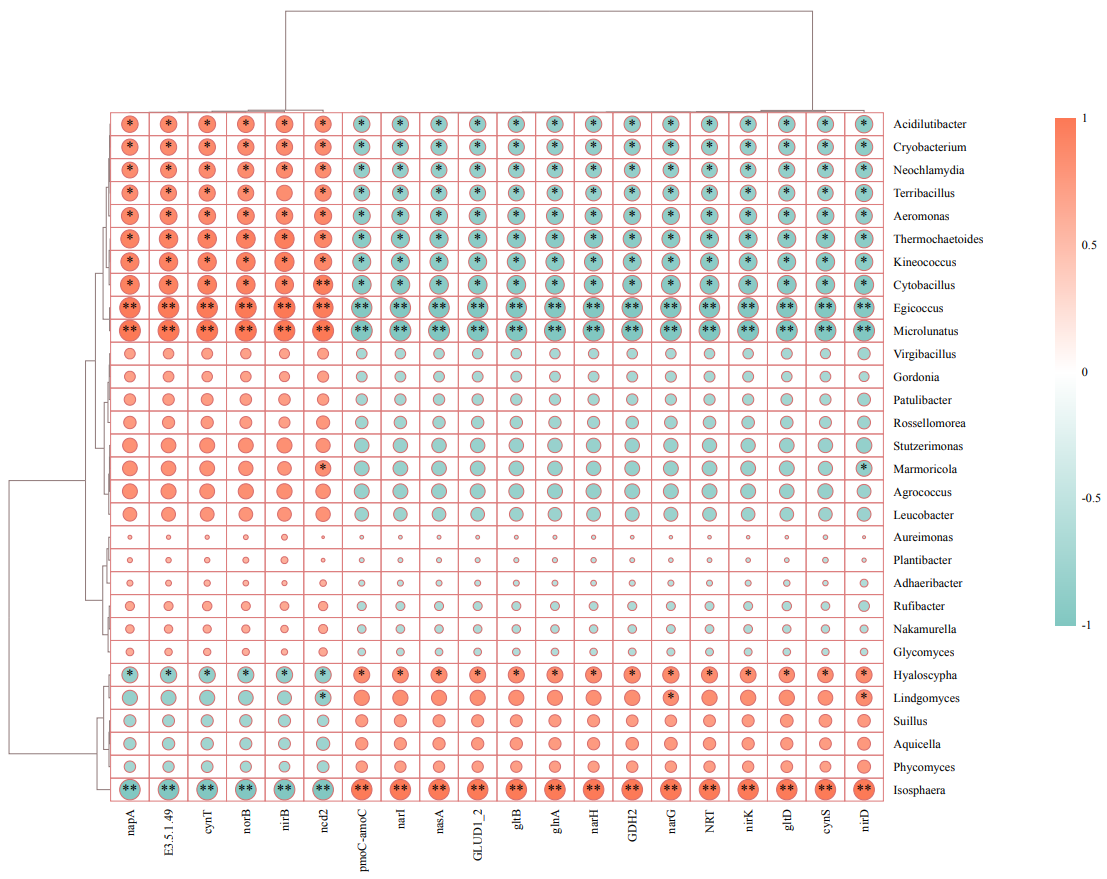
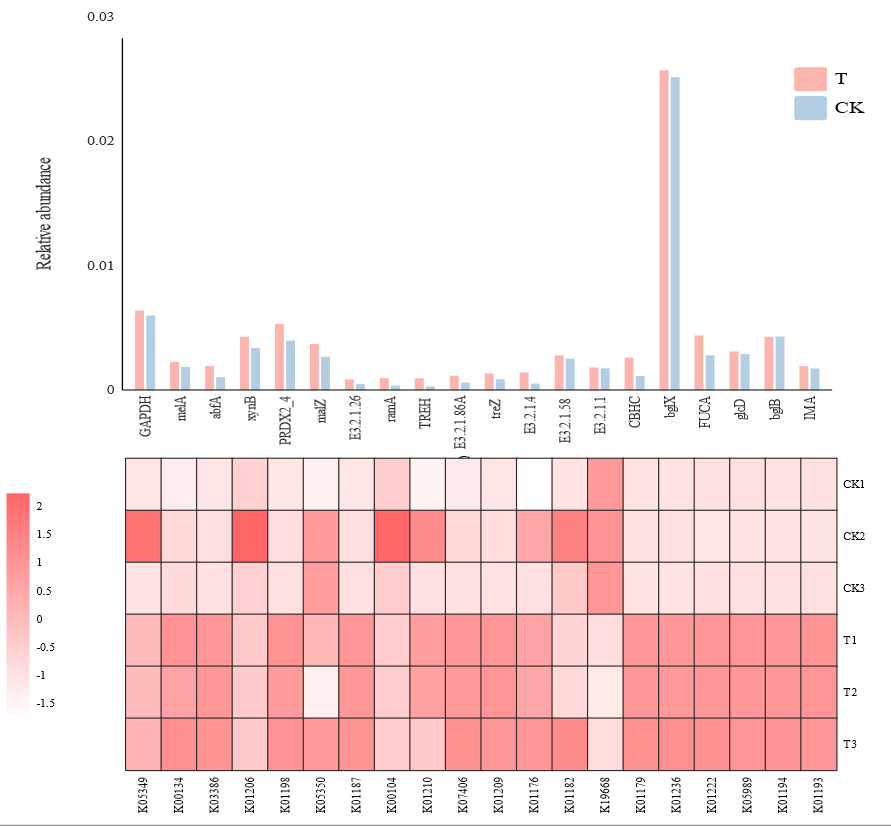
[Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [8](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table) **Relative abundances of genes related to N cycling ofin** rhizosphere **soil of tobacco**

Figure 9 The correlation between microbial of genus level and major gene in N cycling pathway

**2.3.3 Effects of intercropping on major genes abundance of plant degradation pathway**

By comparison with the plant degradation pathway, among the top twenty major genes in abundance,bglX(K05349) was the gene with the highest relative abundance , and the intertreatment increased by 2.39% compared with the control (Fig.10).

[Figure](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-3/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) [1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table/table-1/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/table)0 **Relative abundances of genes related to** plant degradation pathway **oftreatment in** rhizosphere **soil of tobacco**

The results of correlation analysis between microbial of genus level and major gene in plant degradation pathway showed (Fig.11) that (*Isosphaera*)were significantly associated with more than three genes(p<0.01),which showed they were the marker microbial communities of plant degradation pathway in intercropping; genes (E3.2.1.1,) were significantly associated with more than four microbial(p<0.01),which showedthey were the marker genes of soil microbial plant degradation pathway in intercropping.

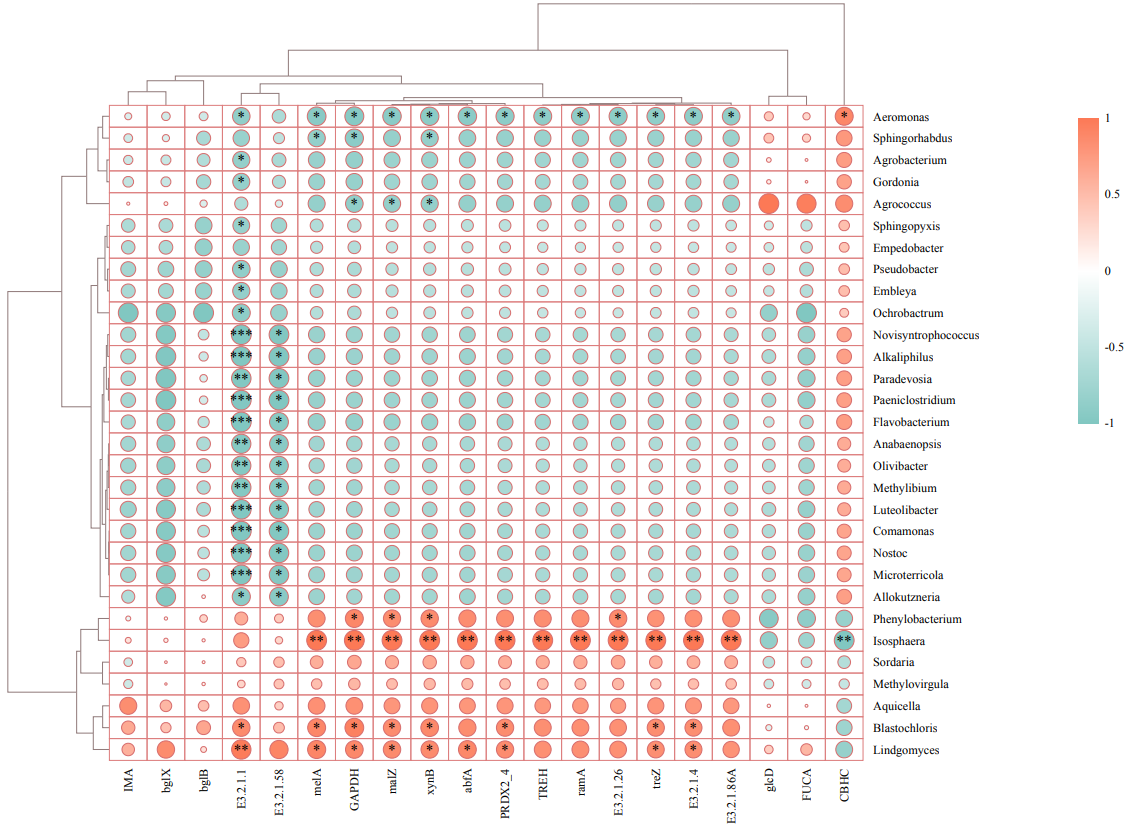


Figure 11 The correlation between microbial of genus level and major gene in plant degradation pathway

**2.4 Effects of tobacco/garlic intercropping on biomass of flue-cured tobacco**

From 30 to 90 days after the plant transplanting, the roots and stems biomass of tobacco plants in the T treatments were higher than of the CK treatment,but the difference are not significant.However, from 60 to 90 days after the plant transplanting, the leaves and total biomass of the T treatments was significant higher than that of the CK treatment (p<0.05)(Fig. 12).

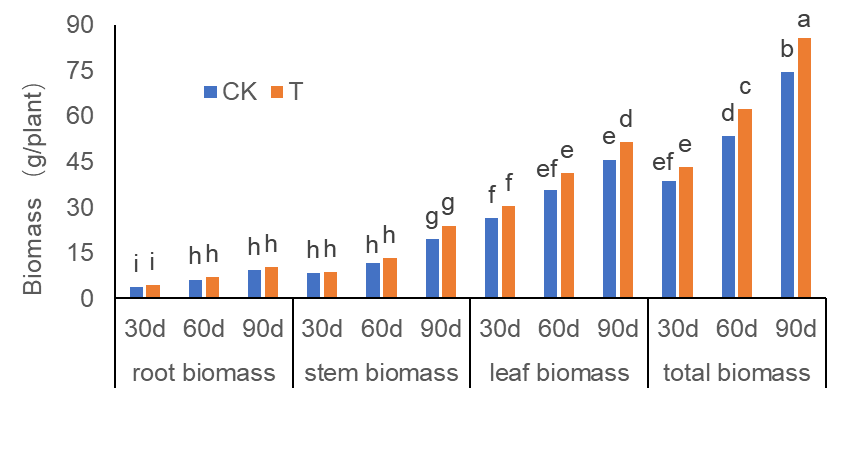


Figure 12 Effects on the roots, stems and leaves biomass of plant in tobacco intercropping

Note: A,root biomass; B,Stem biomass; C, leaf biomass; D, total biomass.

**2.5 Correlation analysis**

### **2.5.1 Correlation analysis between** major **genes and soil** chemical properties

In C cycling pathway,the OM, AN, AP, AK,UE,ACP, INVof soil chemical properties were positively correlated with all the genes while PPO was negatively correlated with all the genes. The correlation of soil chemical properties INV and E5.4.99.2B gene, soil chemical properties OM and sdh gene, soil chemical properties UE and the genes of ACO, ppdK,sucC,E5.4.99.2B,soil chemical properties AN and the genes of foID,E4.1.1.2A,soil chemical properties ACP and the genes of korB, korA,sdhB were significantly (p<0.001) (Fig. 13).

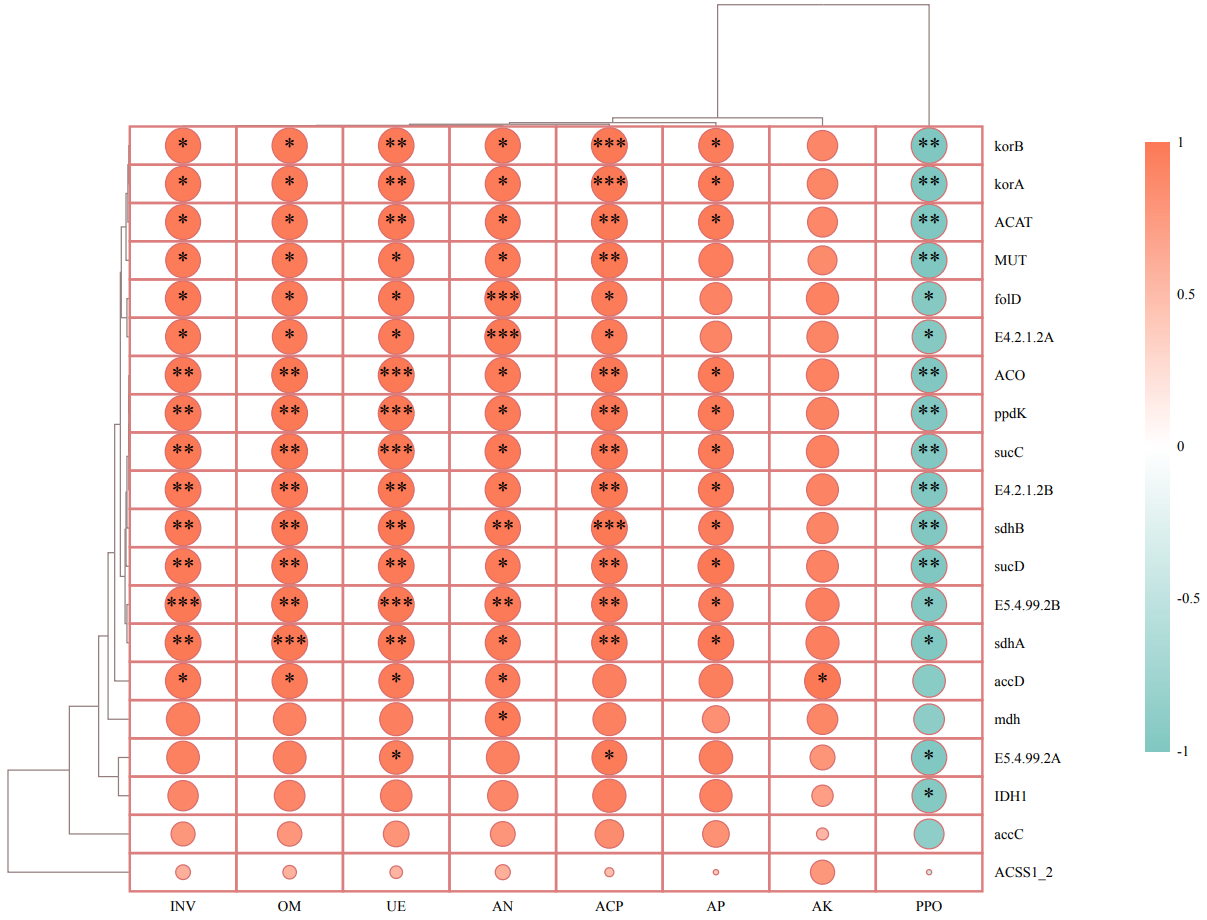


Figure 13 Correlation analysis between major geneand chemical properties of C cycling pathway

in the rhizosphere soil of tobacco plant

Note: OM, AN, AP, AK, UE, PPO,ACP,INV and represent respectively organic matter, available nitrogen, available phosphorus, available potassium, urease, peroxidase,polyphenol oxidase, acid phosphatase ands ucrase ,the same as below.

In N cycling pathway, the OM, AN, AP, AK,UE,ACP, INV were positively correlated with the genes of GDH2, NRT, gltB, narG, glnA,cynS, narI, narH, GLUD1\_2, nirK, pmoC-amoC, nasA,gltD,nirD while were negatively correlated with the genes of norB, napA, E3.5.1.49, cynT,nirB,ncd2. PPO is the opposite. The correlation of four chemical properties index(ACP,OM,UE,INV) and all genes were significantly (p<0.01)(Fig. 14).

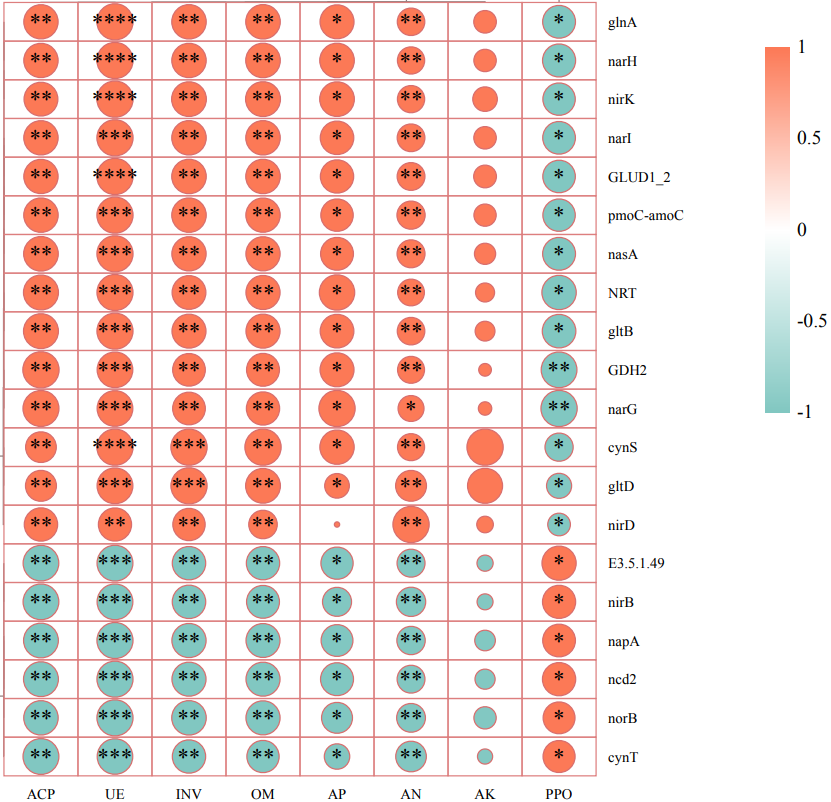


Figure 14 Correlation analysis between major geneand chemical properties of N cycling pathway

in the rhizosphere soil of tobacco plant

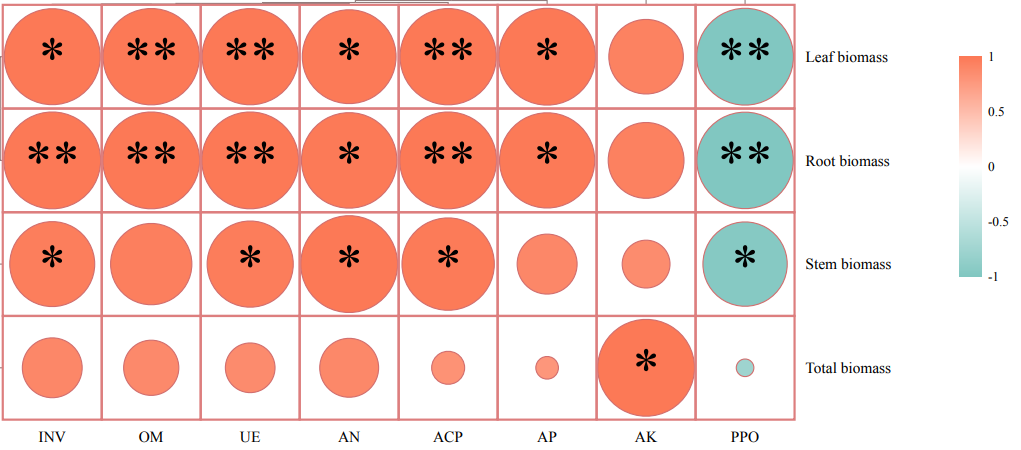
In plant degradation pathway,the OM, AN, AP, AK,UE,ACP, INVwere negatively correlated with the genes of IMA,bjlB,CBHC,while were positively correlated with other genes. PPO is the opposite (Fig. 15).

### **2.5.2 Correlation analysis** **between soil** chemical properties **and tobacco plant biomass**

The biomass of tobacco plants were positively correlated with OM, AN, AP, AK, UE, ACP and INV while negatively correlated with PPO (Fig. 16)..Among them,the leaf biomass of plant and OM,UE,ACP,PPO reached very significant level(p<0.001),the root biomass of plant and INV,OM,UE,ACP,PPO reached very significant level(p<0.001).

### **1704793684490**

Figure 15 Correlation analysis between major geneand chemical properties of plant degradation pathway in the rhizosphere soil of tobacco plant



### Figure 16 Correlation analysis between soil chemical properties and tobacco plant biomass

### 3 Discussion

### 3.1Tobacco**/**garlic intercropping system changed the chemical properties of rhizosphere soil of **tobacco plant**

Previous studies have shown that intercropping systems have an important impact on the content of various soil nutrients ([Liu et al., 2019a](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-33); [Wang et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-58)),the content of available nitrogen and available phosphorus increased in cassava/peanut intercropping ([Li et al., 2012](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-29)),the content of the available potassium and available phosphorus increased in legume/tomato intercropping ([Dai et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-14)),while the content of available nitrogen decreased in milk vetch/rape intercropping ([Zhou et al., 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-76)), which indicated that soil nutrients varied greatly in rhizosphere soil due to the different intercropped crops.Our study indicated that the content of organic matter ,the available nitrogen ,the available phosphorus,the available potassium in rhizosphere soil increased([Fig. 1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure)).

In this study , the activity of urease, phosphatase and invertase increased significantly in intercropping treatments compared to the monocropping treatment([Fig. 2](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure)). The same results was also observed in maize/peanut and maize/ soybean ([Zhang et al., 2012a](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-69)) intercropping systems.

### 3.2 Improved rhizosphere soil chemical properties were related to the microbial community in tobacco**/**garlicintercropping systems

Relative to the CK, the content of bacteria,fungi and biomass nitrogen increased significantly while the content of biomass carbon decreased in the intercropping treatments([Table 1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure)),which indicated that intercropping had an important impact on the structure of microbe communities.

Intercropping significantly affected the diversity of microbe and increased the number of bacteria and fungi(Chen et al., 2019). Many studies have shown that the number of microbe, such as bacteria and fungi, increased significantly in intercropping systems ( [Solanki et al., 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-51);[Solanki et al., 2018](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-50);[Solanki et al., 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-49); [Chen et al., 2018a](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-7)). However, in the intercropping system of Chinese milk vetch and rape, the content of soil microbial communities decreased ([Zhou et al., 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-76)).These differences demonstrated that microbe quantity and activity were correlated with the species of intercropped crops, soil conditions and other impact factors.

### 3.3 Metagenomic data analysis

### Previous reports indicated that many members of *Proteobacteria* and *Bacteroidetes* of the soil bacteria community were closely associated with the carbon (C)and nitrogen (N) cycles (Leff et al., 2015), while the *Ascomycota* of the soil fungal community can rapidly metabolize organic substrates of rhizosphere soil (Bastida et al., 2013).As a result, the intercropping systems might increase soil nutrient accumulation and nutrient utilization efficiency by promoting microbial growth that is closely associated with N fixation or other C-N processes. In this study, the abundance of *Proteobacteria* of bacteria and *Ascomycota* of fungiin the intercropping treatment was significantly higher than the monocropping treatment (Fig.3 and Fig.4), indicating the improved soil microbial conditions after intercropping.

### In addition, we analysised intercropping crops for metagenomic sequencing. The gene abundance of such as ACAT(K00626), sdhA(K00239), sdhB(K00240), sucD (K01902), ACSS1\_2 (K01895) ,E5.4.99.2A(K01848)involved in C cycling, glnA(K01915) involved in N cycling,and bglX (K05349) involved in plant degradation pathway in the intercropping treatments were generally higher than that of monocropping treatments([Fig. 6](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure),[Fig.8](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) and [Fig.1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure)0),and there are respectively different marker microbial communities in the C,N cycling and plant degradation pathway([Fig.7](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure),[Fig.9](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure) and [Fig.1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-2/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure)1). These results suggested that intercropping may affect the structure and quantities of microbial communities by mediating carbon and nitrogen cycling.Similar results appear in the former study ( Pardon et al., 2017).

### **3.4 Effects of intercropping on tobacco growth**

### In this study, intercropping can substantially increase the biomassof tobacco plant (Fig.12) . The growth improvement results of tobacco plant are consistent with previous research on A. carmichaeli-rice intercropping systems (Ren et al., 2018). These results revealed that intercropping can induce changes in soil microbial community structure, such as the increase in *Acidobacteria* abundance of bacteria can better degrade plant residue polymers and enhance photosynthesis, thereby increasing soil nutrients and plant biomass; while the increase of plant residue polymers also promoted the increase of *Ascomycota* abundance of fungi,and the fungi can rapidly metabolize organic substrates of rhizosphere soil (Bastida et al., 2013).

As gene analysis showed, related genes involved in C cycling, N cycling and organic matter turnover vary significantly between intercropping and monocropping treatments ([Fig. 1](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure/fig-4/" \t "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/figure)3-15), contributing to changes in metabolic pathways and more portions of the soil environment. Act as decomposers in ecosystem, microbes play an important role in anabolism and catabolism. When microbes increased, the activities of genes related to metabolism increased, subsequently leading to more active N/P cycling and organic matter turnover. The synthesis and degradation of carbohydrates is the basis of the growth and fruit maturation of plants and the basic substances essential to the life of microbes. This process contributed to the growth of plants and finally reflected in biomass of crops.

### Therefore, we concluded that the increased microbial abundance of tobacco intercropping system could accelerate the soil C cycling, N cycling and organic matter turnover, which in turn may have been beneficial to maintaining high soil fertility and growth of plant.

### **3.5 Soil-microbe-plant interaction**

The content of chemical properties and enzyme activity in rhizosphere soil is closely correlated with soil microbe communities and their biological activities ([Solanki et al., 2019](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-49) ;[Wang et al., 2015](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-58)). The abundance of bacteria significantly affect invertase while the abundance of fungi affects urease and acid phosphatase ([Hu et al., 2002](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7894120/" \l "ref-21)). Microbial activity connected to metabolic processes results in changes in enzymes and chemical properties of soil , which supports the growth of microbial communities(Zhang et al., 2021b). According to the results of study, the abundance of microbe in T treatment significantly increased relative to CK treatment , and increase of microbe caused increase of activities of genes, which contributed to higher level of organic matter turnover and enhanced metabolism in rhizosphere soil.

### A diverse microbial community provides more nutrients for plants to absorb and less competition from microorganisms. Increased microbial diversity may promote the accumulation of available N and OM in the soil, all of which could increase overall plant productivity (Banerjee et al., 2018; Delgado-Baquerizo et al., 2020). Higher tobacco biomass and soil fertility of intercropping may depend on the regulating ability of belowground interactions, since soils with high diversity also have higher plant productivity (Lan et al., 2023) and decomposition ability in organic matters (Liu et al., 2020b).

In tobacco**/**garlic intercropping system, we consider it influenced the abundances of genes involved in various metabolisms and improved the soil environment of rhizosphere soil by mediating the chemical properties and microbes , finally increase the nutrients in rhizosphere soil which is beneficial to the growth and development of tobacco.

**4 Conclusions**

Tobacco**/**garlic intercropping significantly affects rhizosphere soil physicochemical properties, enzyme activities and microbial community quantities. Metagenomic analysis suggested that the relative abundances of genes related to C cycling (ACA,sdhA,sdhB, sucD,ACSS1\_2 ,E5.4.99.2A) , N cycling (glnA)and organic matter degradation (bglX) were higher in the soil of intercropping treatments. The intercropping system changed chemical properties by regulating genes involved in C cycling, N cycling and organic matter turnover and then improved the soil environment. The results of this study showed that nutrients, enzymes and microbes of soil work together and reach a dynamic balance responsible for the positive effects on the growth of tabocco plants.

**Author contributions**

Wencheng Wu and Qingyu Guo performed the statistical analysis, the preliminary manuscript was composed by Jun Zhong. Zhenbao Luo, Caibin Li,Heqing Cai and Yi He contributed to conception and design of the study. Wencheng Wu and Qingyu Guo are the co-author, Jun Zhong and Yi He are the co-corresponding author. All authors contributed to manuscript revision, read, and approved the submitted version.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships.

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