

Improved soil fertility and microbial diversity by microbial organic fertilizers in tobacco continuously cropping area



Hui Guo[1]; Huijuan Yang[1]; Junwei Sun[2]; Yuehui Wei[1]; Hongzhi Shi[1]
[1] College of Tobacco Science, Henan Agricultural University, Zhengzhou, 540002, China
[2] Yunnan Tobacco Company, Dali State Company, Dali ,671000,China

Introduction

Soil is the cornerstone on which microorganisms, animals and plants depend, and it plays a vital role, and soil microorganisms are crucial in the decomposition of organic matter and circulating nutrients, and are essential in maintaining the soil system. Microbial bacterial fertilizer has a significant effect on the improvement of soil microbial biomass and enzyme activity in facility agriculture, and it also has a good promotion effect on the continuous cropping obstacles of flue-cured tobacco and other crops.

In this experiment, the application of different microbial bacterial fertilizers was used to analyze and compare the effects of different fertilization methods. The effects of microbial bacterial fertilizers on soil microbes in Dali, Yunnan were studied to provide a basis for the scientific application of microbial bacterial fertilizers.

Materials and Methods

- ◆ Tobacco Varieties:HD
- ◆ Sample:Take a sample of rhizosphere soil
- ◆ Community: Conventional fertilization CK; Conventional fertilization + Xiangyun Guansheng organic fertilizer T1 (50kg/ha); Conventional fertilization + Midu Guofa organic fertilizer T2 (50kg/ha); Conventional fertilization + solid microbial fertilizer provided by Fujian Sanju (Gengduobang) T3 (50kg/ha) conventional fertilization + liquid microbial fertilizer provided by Fujian Sanju (Gengduobang) T4 (500ml/ha gold irrigation liquid ratio With fixed root water)
- ◆ Sequencing technology: Illumina Miseq high-throughput

Table 1. Technical indexes and manufacturers of 4 fertilizers

Serial number	Fertilizer	Microbial bacteria	Effective viable count	Factory
T1	Xiangyun Guansheng organic fertilizer	Bacillus amyloliquefaciens	≥0.2billion/g	Xiangyun Guansheng Fertilizer Industry Co. Ltd
T2	Midu Guofa organic fertilizer	\	\	Dali Guofa Pixionghe Agricultural Technology Co., Ltd.
T3	Fujian Sanju solid bacteria fertilizer	Bacillus amyloliquefaciens, Arthrobacter nicotianae,Jelly-like bacillus	≥2.0billion/g	Fujian Sanju Biological Technology Co., Ltd.
T4	Fujian Sanju liquid bacteria fertilizer	Jelly-like bacillus	≥2.0billion/g	Fujian Sanju Biological Technology Co., Ltd.

Table 2. Bioinformatics analysis process

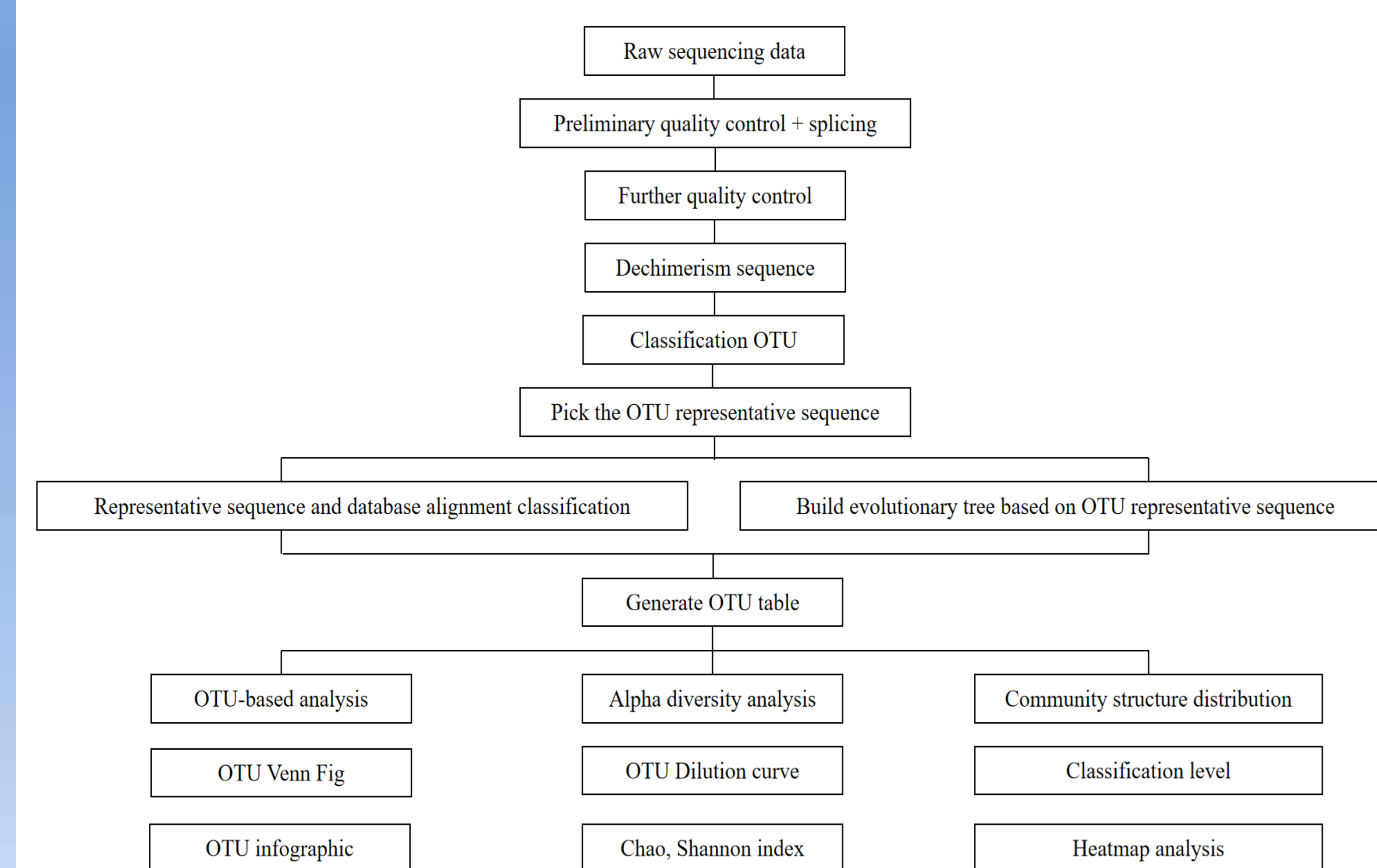


Table 3. Sample DNA test results

sample_ID	concentration (ng/μL)	A260/280	A260/230	volume(μL)	Total (μg)	result
CK 5.15	24±8.83	1.89±0.07	1.3±0.12	55±0	1.32±0.49	A
T1 5.15	26.68±8.37	1.91±0.06	1.34±0.13	55±0	1.47±0.46	A
T2 5.15	28.8±5.64	1.93±0.06	1.39±0.09	55±0	1.59±0.31	A
T3 5.15	25.58±9.45	1.93±0.06	1.36±0.19	55±0	1.51±0.42	A
T4 5.15	23.55±3.92	1.98±0.05	1.3±0.05	55±0	1.3±0.22	A
CK 5.30	27.05±7.89	1.87±0.06	1.65±0.38	60±0	1.62±0.47	A
T1 5.30	34.3±13.13	1.87±0.09	1.65±0.56	60±0	2.06±0.79	A
T2 5.30	31.75±7.39	1.96±0.06	1.96±0.33	60±0	1.91±0.44	A
T3 5.30	32.03±11.52	1.9±0.07	2±0.09	60±0	1.92±0.69	A
T4 5.30	30.58±12.02	1.89±0.09	1.45±0.47	60±0	1.83±0.72	A

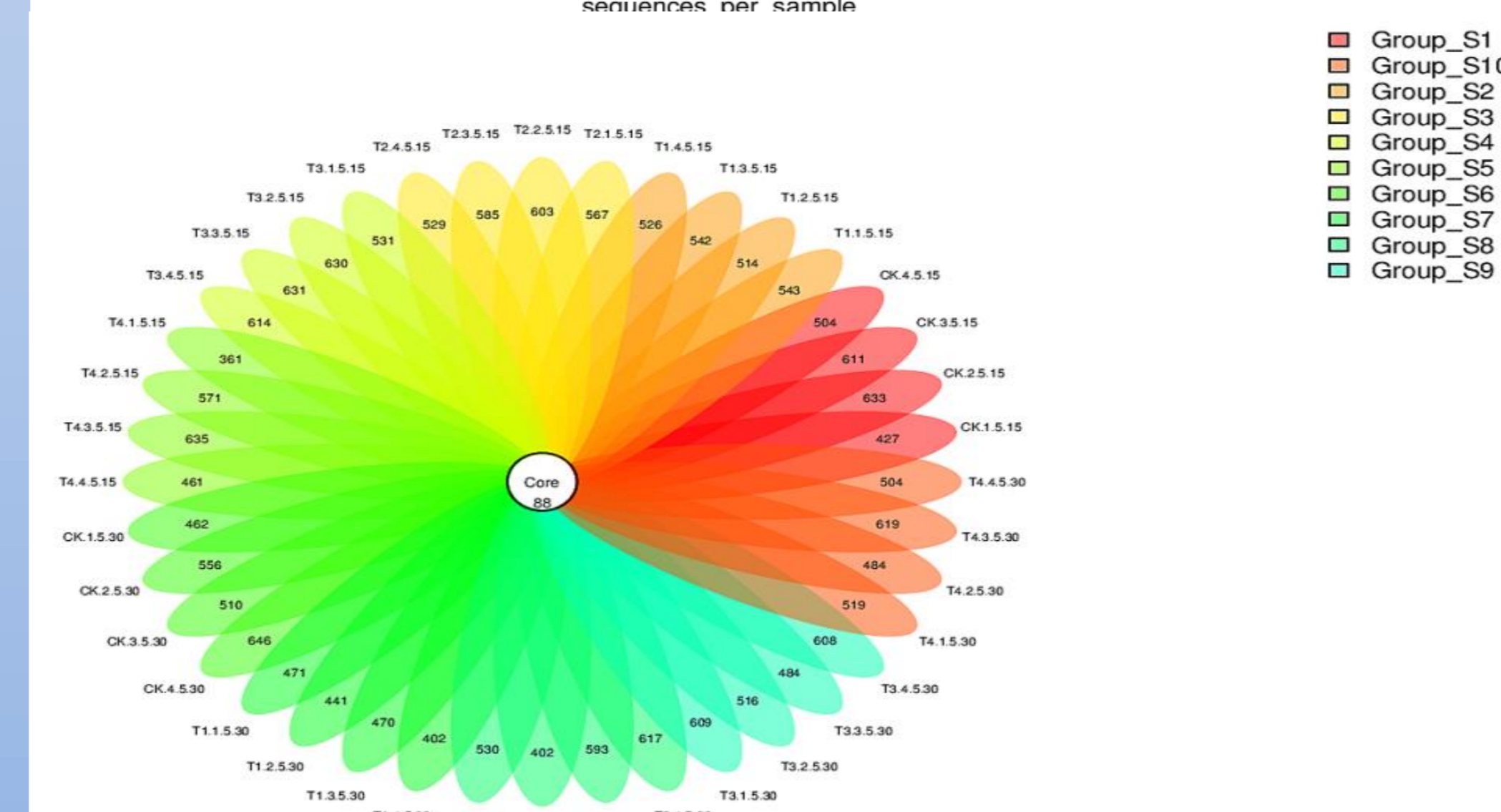
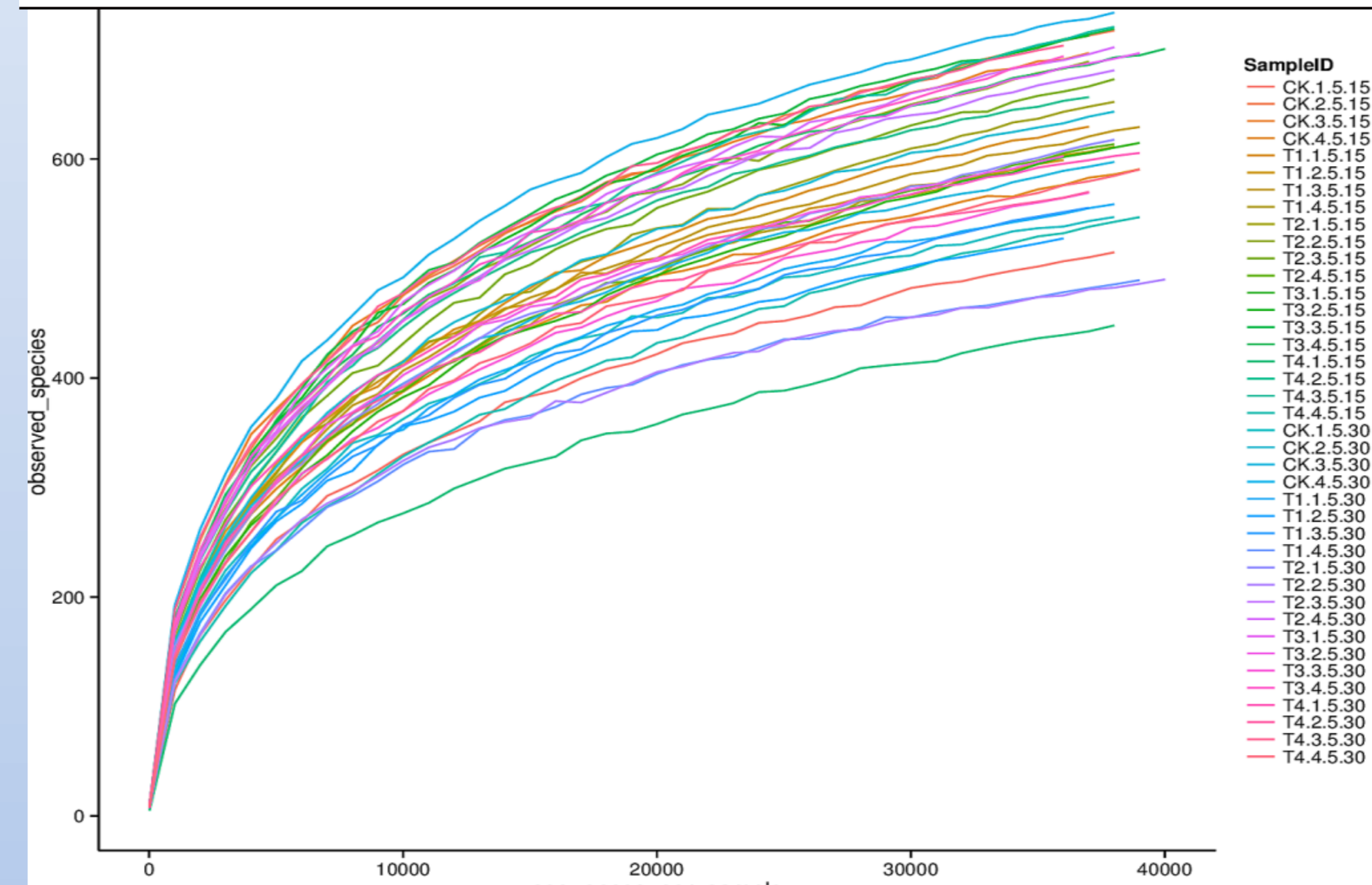


Table 4. Quantity of OTUs in rhizosphere soil

sample_ID	clean_tags	valid_tags	valid minLength	valid meanLength	valid maxLength	OTU_counts	Total_OFs	valid_percent
CK.5.15	40309±801.21a	38384±865.4a	208±10a	263.24±2.69ab	455.75±11.98a	631.7596.09ab	2143	0.96±0.01a
T1.5.15	39456.5±1142.74a	37998.751061.16a	202.75±0.5a	259.8±2.39a	461.75±2.5a	619.2513.89ab	2143	0.96±0.01a
T2.5.15	39716±641.93a	38191.25±531.9a	202.75±0.5a	262.59±4ab	464±2a	659±31.62b	2143	0.96±0a
T3.5.15	40536.251253.29a	39002.751254.86a	203±0a	260.72±3.66ab	462.25±0.5a	689.5±47.64b	2143	0.96±0.01a
T4.5.15	40013.5±768.03a	38456.5±736.13a	212.5±10.97a	271.76±18.13ab	460.5±4.36a	595±120.98ab	2143	0.96±0.01a
CK.5.30	39655.75±260.39a	38346.75±337.39a	202.75±0.5a	261.28±6.89b	458.25±4.65a	631.5±78.37ab	2143	0.96±0.01a
T1.5.30	39702.5±1226.9a	37870.5±1161.81a	202.75±0.5a	267.61±3.85ab	459.25±7.5a	534±32.47a	2143	0.96±0.01a
T2.5.30	40723.75±855.15a	38727.25±928.57a	202.75±0.5a	269.13±1.59ab	460.25±5.5a	623.5±96.27ab	2143	0.96±0a
T3.5.30	39253.751341.04a	37417±1260.79a	211.5±10.12a	269.95±6.07ab	462.75±0.5a	642.2563.99ab	2143	0.96±0.01a
T4.5.30	40023.251430.36a	38319±1315.08a	211.5±10.12a	262.3±1.1ab	463.75±2.22a	619.5±60.07ab	2143	0.96±0a

Table 5. Analysis of alpha diversity of tobacco soil

Samples	chao1	goods_coverage	observed_species	shannon	simpson
CK.5.15	788.95±113.08ab	0.9954±0.0005ab	621.35±95.82ab	5.64±0.92a	0.92±0.06a
T1.5.15	763.33±32.34ab	0.9955±0.0005ab	611.33±11.37ab	5.92±0.12a	0.96±0a
T2.5.15	827.52±28.37b	0.9949±0.0001a	648.45±34.24b	5.94±0.52a	0.95±0.03a
T3.5.15	871.01±39.63b	0.9948±0.0003a	674.28±51.16b	6.17±0.13a	0.96±0a
T4.5.15	751±121.46ab	0.9953±0.0009ab	583.88±121.19ab	5.23±1.11a	0.89±0.12a
CK.5.30	764.63±74.5ab	0.9956±0.0003ab	622.4±78.13ab	5.96±0.45a	0.96±0.02a
T1.5.30	659.53±60.57a	0.996±0.0005b	526.95±34.05a	5.44±0.37a	0.94±0.03a
T2.5.30	777.77±126.54ab	0.9954±0.0008ab	612.03±96.78ab	5.86±0.33a	0.96±0.01a
T3.5.30	836.42±111.69b	0.9949±0.0007a	635.85±62.04ab	5.97±0.56a	0.95±0.03a
T4.5.30	751.97±67b	0.9957±0.0006ab	610.1±64.18ab	6.06±0.52a	0.96±0.02a

The tested variety was Honghua Dajinyuan. The soil type in this area is red loam, the field row spacing is 120 cm, the plant spacing is 55 cm, and the experiment is set up with 5 treatments, 3 repetitions, a total of 15 plots, randomly arranged, each plot area is 0.3 mu.

Fig 1. The similar level of 16S rDNA of tobacco soil bacteria was a dilution curve of 97%

Fig 2. Venn diagram of OTU composition of bacterial species in tobacco soil

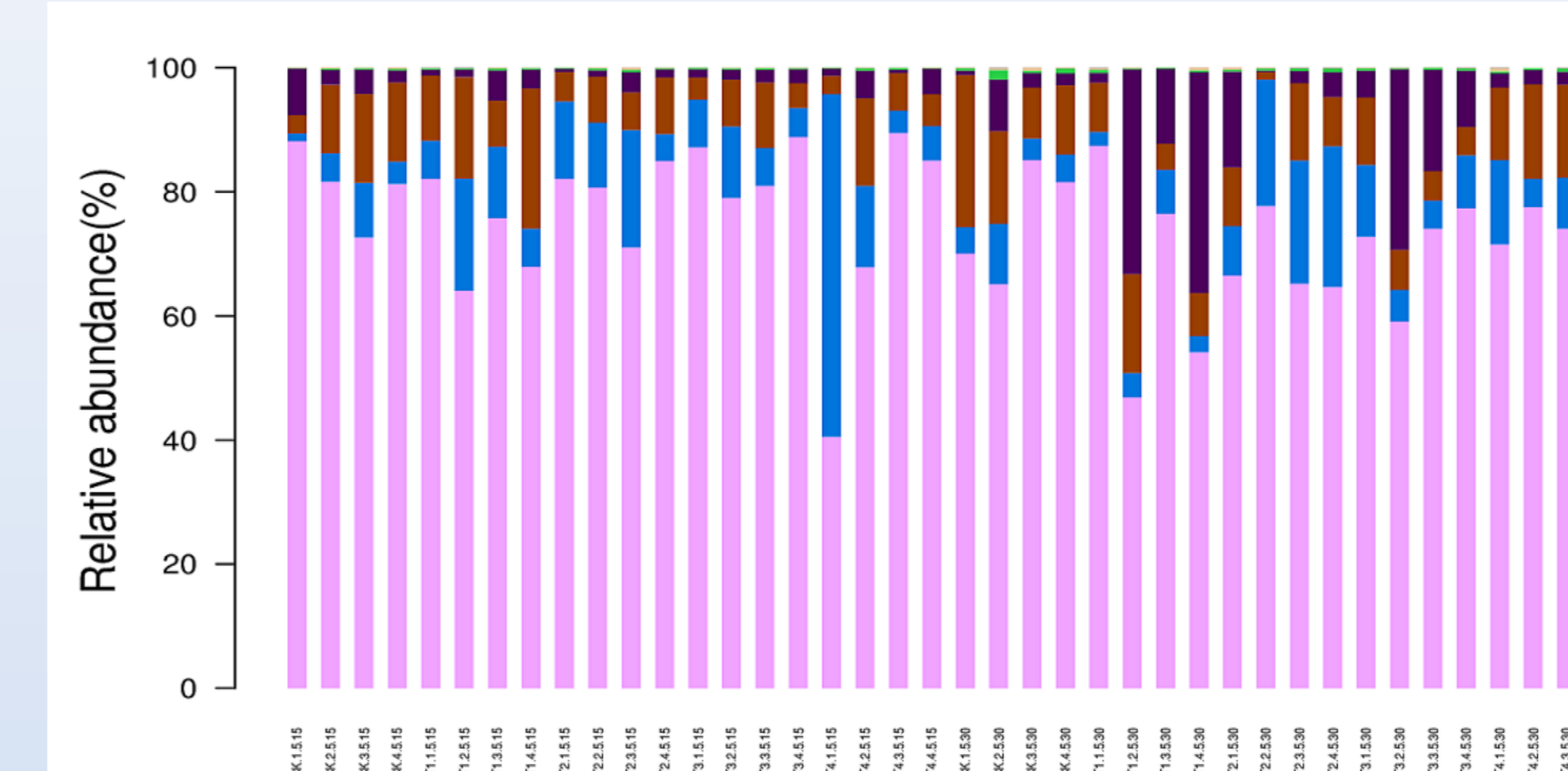


Fig 3. Relative abundance of samples in different test groups at gate level

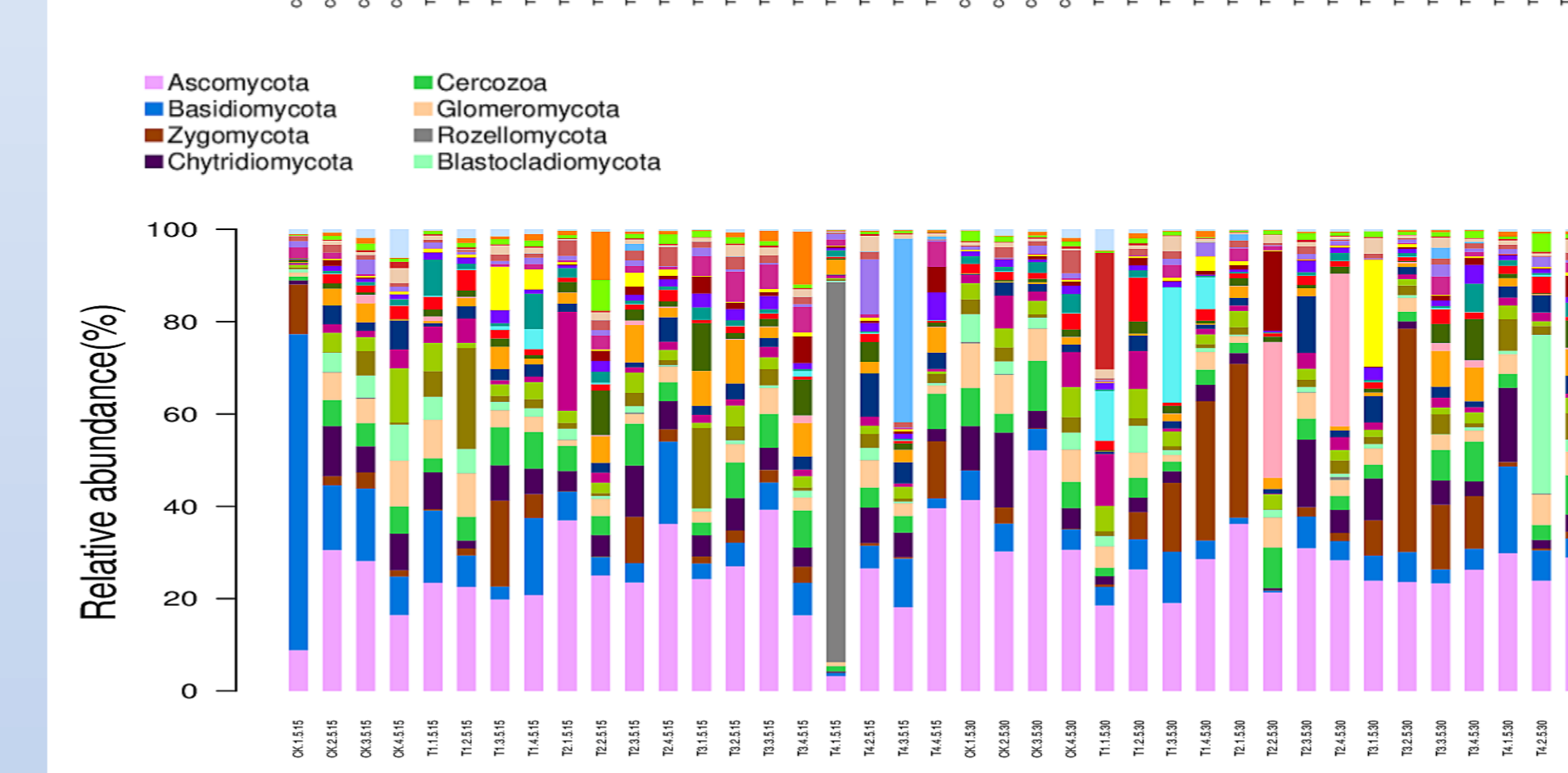


Fig 4. Relative abundance of samples in different test groups at genus level

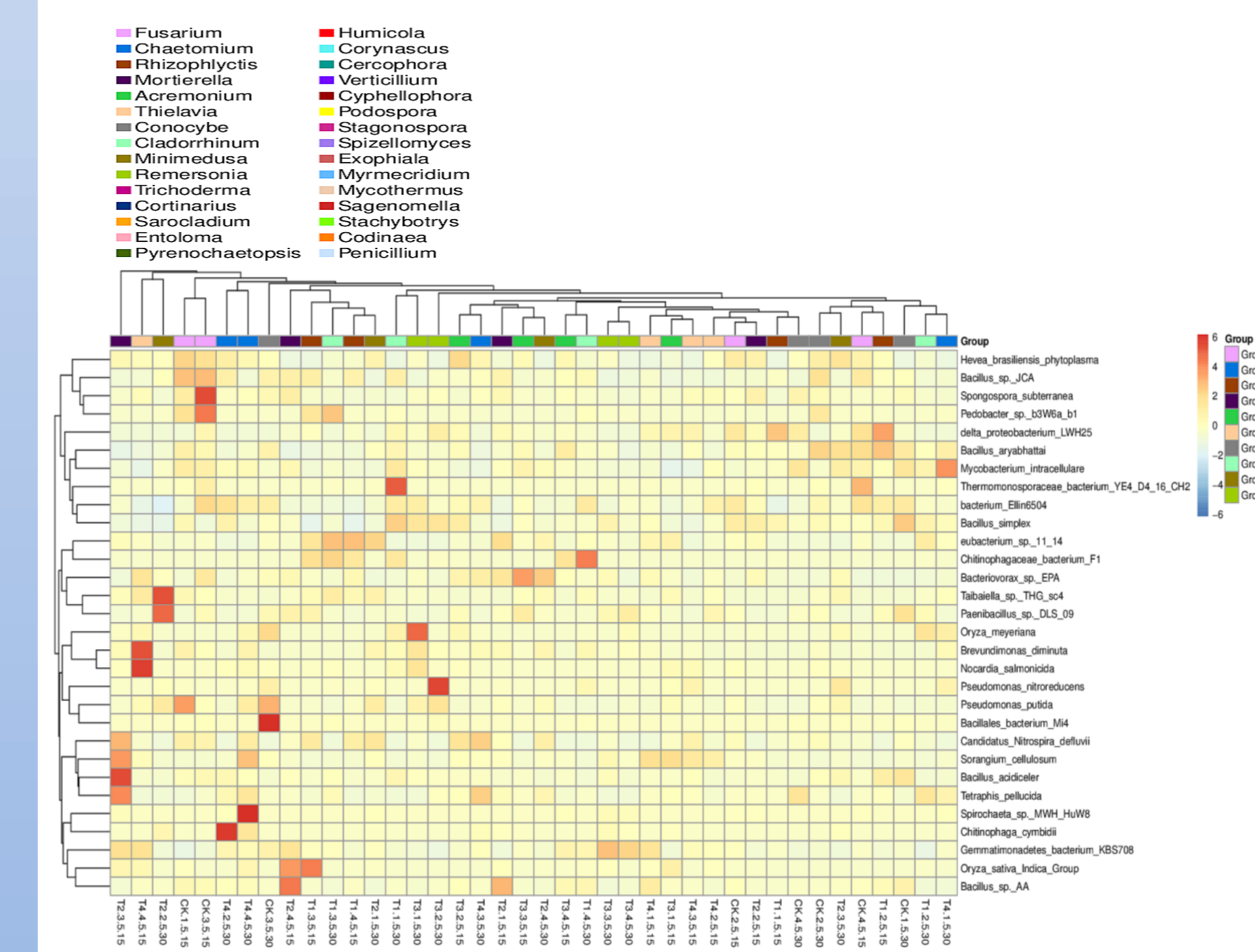


Fig 5. Heatmap analysis of species abundance

Results and Discussion

- ◆ The amount of valid sequence data obtained after sequencing allegations was distributed between 37417-390002.75 reads, the average length was distributed between 259.8-271.7625bp, and the numbers of operation classification units (OTUs) in each sample were distributed between 455.75-642.25.
- ◆ The Wayne diagram shows that totally 2143 OTUs were obtained, with a total of 88 OTUs.
- ◆ Alpha diversity analysis showed that the richness microbial community in the soil treated with microbial fertilizer gradually increased according to the ACE index and Chao index, while no obvious changes were observed in the other treatments. This increasing in the microbial community and diversity is more obvious according to the Shannon index and Simpson index. The diversity of microbial species in all the soil samples were increased.
- ◆ Community structure analysis showed that the soil bacteria types in the sampled soil were categorized in 16 phyla, 44 classes, 45 orders, 45 families, 45 genera, and Ascomycota, Basidiomycota, and Zygomycota were in higher abundance.
- ◆ The colony heat map showed that the all the organic fertilization treatments increased the bacterial community composition in the soil compared with the control which was inorganic fertilizer applied. The treated samples also had more bacterial diversities than that in control.

Conclusion

Application of microbial bacterial fertilizer can improve the diversity of tobacco planting soil community structure in Dali, Yunnan and improve the soil environment.