Effects of different fertilization methods on soil microbial community and diversity and crop yield in a sweet potato field

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Research Article

Keywords: fertilization methods, functional flora, Illumina paired-end sequencing, microbial community, soil microorganisms, sweet potato

Posted Date: August 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1869341/v1

Abstract

o Aims Different fertilizers such as microbial fertilizer, vegetable cake, mushroom residue, quicklime, soil conditioner, organic fertilizer, and compound fertilizer were used, and their effects on soil microbial community and sweet potato yield were studied. Thirty different treatments were set up, and the agronomic traits, quality, and characteristics of sweet potato were determined. The effects of these treatments on soil pH, nutrient indicators, and microbial community structure were also investigated.

o Methods Combining field agronomic character investigation, soil nutrient and other indicators determination and bioinformatics analysis of soil microorganisms.

o Results Differences among the indicators under different planting modes. Seaweed microbial fertilizer and quicklime + biomass charcoal appropriately increased the growth of sweet potato stems and leaves. Zeolite and sheep manure organic fertilizer appropriately increased the yield of sweet potato. In the case of combined application of organic fertilizer and compound fertilizer, when the proportion of organic fertilizer was high, the stems and leaves of sweet potato grew vigorously, and when the proportion of compound fertilizer was high, the yield of sweet potato was high.

o Conclusions Although both soil nutrient content and organic matter content equally affected sweet potato yield, there were constraints and balances between them, and a higher value of either of them did not provide a better yield. The relative abundance of Proteobacteria in soil microorganisms was positively correlated with sweet potato yield. Firmicutes was also associated with yield, no positive correlation was found. Latescibacteria, Ignavibacteriae, and RBG-1 (Zixibacteria) were probably associated with elevated soil pH.

Introduction

Strategies used in food production systems highlight the importance of sustainability. Crop production systems depend on healthy and fertile soil. Soil ecosystems are complex habitats that harbor diverse communities of bacteria and fungi, which form the soil microbiome. Fertilization is one of the main strategies to ensure sustainable food production. As early as the 19th century, under the pressure of rapid increase in global population, the trend of application of fertilizers for crop production began to urgently meet the increased human demand for food. Long-term fertilization significantly changes crop yield (Wang et al.,2011), soil physicochemical properties (Liang et al.,2011), and microbial activity(Ma et al.,2010) and has a profound impact on soil quality and sustainable use(Ge et al.,2010). However, in recent years, with the deterioration of global environment, to increase crop yield and gain maximum benefits, farmers have begun to use several chemical substances such as fertilizers and pesticides, which has led to a decline in soil quality and fertilizer utilization rate each year (Sradnick et al., 2013). Severe loss of soil quality, thinning of the black soil layer, decreased nutrient content, soil acidification, decreased soil microbial diversity (Nautiyal et al.,2010), frequent outbreaks of soil-borne diseases (Sradnick et al., 2013; Tamm et al., 2001), and a series of other issues(Wei et al., 2008; Zhou et al., 2015) hinder the sustainable development of agriculture(Tamm et al., 2001). According to the relevant data(Tao et al., 2014), short-term application of organic fertilizers will not significantly increase crop yield. Previous surveys have shown that the use of organic fertilizers in China has exhibited a significant downward trend in the past 50 years. From the 1960s to the 1980s, organic fertilizers accounted for the total fertilizers used for crop production. However, by the beginning of the 21st century, the application of organic fertilizers accounted for only 10–30% of the total fertilizer application(Zhao et al., 2016). Based on the current situation of an increase in chemical fertilizer application and a decrease in organic fertilizer application each year, China has proposed fertilization guidance strategies such as combined application of organic and inorganic fertilizers (Ding et al.,2017) and restoring the use of straw from the field for fertilization (Shen et al., 2001).

The degradation of land resources and the deterioration of agro-ecological environment in world agriculture have forced researchers to focus more on the study of ecosystem changes, especially soil ecology (Wang et al., 2002; Zhou et al., 2002). Soil microorganisms are an extremely important and active part of the soil ecosystem. They are an important medium in

the flow of soil organic matter, nutrients, and energy and function as a power source of biochemical elements. They also play a leading role in crop productivity (He et al., 2013). Different fertilizer types and planting patterns have different effects on soil fertility, soil microbial biomass, soil microbial diversity, and microbial community structure in soil (Wang, 2014). Therefore, soil microbial parametersare used to evaluate soil quality. The study of these parameters is gaining increasing attention, and these parameters could serve as an early warning and sensitive indicator of soil ecosystem changes (Li et al., 2005; Zhao et al., 2013). Fertilization is a direct and practical method to improve soil fertility, and it can significantly improve soil quality and crop yield; therefore, the appropriate application of fertilizers has been the focus of investigation of most researchers since many years (Basak et al., 2013; Wang et al., 2013). At present, the effect of fertilization on soil biological properties is a hot topic of research; however, researchers are no longer interested in studies with only a single parameter to explore the effect of fertilization on soil properties, but they desire to conduct a comprehensive research study involving multiple perspectives (Stepien et al., 2014). Therefore, soil microorganisms, as a health-sensitive indicator for monitoring, evaluating, and reflecting the changes in soil fertility, will certainly receive increasing attention of researchers in coming years. To promote the sustainable development of agriculture in China and to enable sustainable utilization of soil, it is necessary to strengthen the ecological balance, increase research on biological and ecological characteristics of soil microorganisms, and use the obtained results to monitor, evaluate, and provide early warning signs of changes in soil fertility.

Soil microbial communities are highly susceptible to anthropogenic factors, and long-term continuous cropping of the same crop has been reported to increase the likelihood of occurrence of soil plant pathogens in annual crops and reduce crop yield(Cao et al.,2011;Feng et al.,2003;Lu et al.,2013). Plant diseases are usually caused by soil microorganisms (Bailey & Lazarovits,2003). However, some microorganisms have a positive influence on plant growth (Tkacz &Poole, 2015). Soil microbial diversity is also considered as an important indicator of soil conditions (Bruggen & Semenov, 2000). Therefore, it is critical to study the community and diversity of soil microorganisms to overcome the obstacles related to continuous cropping.

Recently, several studies have been conducted on the effect of long-term fertilization on corn, rice, and other crops (Jin, 2008; Wang, 2014; Wu, 2018; Xu, 2017); however, there are relatively few studies on sweet potato. Therefore, the present study considered sweet potato as the research object and examined the effect of different fertilization treatments on its yield. The combination of Illumina paired-end (PE) sequencing technology and modern molecular biology techniques such as real-time PCR was used to determine the effects of different fertilization conditions on the agronomic traits of sweet potato fields as well as to examine the structural characteristics and composition of soil microbial communities and to understand their evolution process. The formulation of appropriate fertilization strategies and the development of a good soil ecological environment could provide a theoretical basis for improving soil ecology and could also have practical significance.

Materials And Methods

Experiment materials

Tested sweet potato variety (line): “ZH1042” is a sweet potato line independently selected by Hangzhou Academy of Agricultural Sciences; it is currently registered with the name “Hangshu No. 2.” This strain has red skin and white heart and has a high dry rate. According to Jiangsu Xuzhou Sweet Potato Research Center(China Xuzhou 34.277988°N, 117.296692°E), this variety shows the following characteristics: dry rate, 36.90%;starch rate,74.80%;protein content,3.89%;soluble sugar content,6.64%;reducing sugar content,1.23%;and β-carotene content,0.78mg/100g.Table 1 shows detailed soil physical and chemical properties of the site at the start of the experiment.

Table 1
Fundamental characteristics of the tested soil

Soil depth(cm)	pH	organic matter(g/kg)	total nitrogen(g/kg)	Available phosphorus(mg/kg)	fast-acting potassium(mg/kg)
0–20	4.11	8.44	0.55	15.10	346.5

Experimental design and treatments

The study materials were cut potato seedlings sown on May 15, 2020 and harvested on September 4, 2020. A single-row planting was followed, with each variety having 3 plots; each plot was 5 m long and 4.2 m wide, and the plot area was 21m^2. Thirty different treatments were performed, and the specific treatments are shown in Table 2. Among these treatments, the “LYX” series was designed to study the effect of different organic fertilizer types on the cultivated land; the “FZW” series was designed to study the effect of soil acid reduction and slowing down of restoration techniques; and the ‘T’ series was designed to determine the appropriate ratio of carbon-based fertilizers, organic fertilizers, and compound fertilizers. All treatments were repeated three times, and the results were calculated as the average of three replicates.

The following botanical characteristics of sweet potato were measured and recorded on the spot during harvest: vine weight per plant, potato weight, commercial potato weight, number of tubers, number of commercial potato in a plot, yield of a plot, weight of the aboveground part of a plot, and weight of commercial potato in a plot. Soil samples from 5 sampling points were uniformly and randomly collected from each plot by the plum-blossom method. The sampling tool was a stainless steel soil drill. The sampling depth was 0–20 cm in the plough layer. pH, organic matter, urease, microbial carbon, microbial nitrogen, and bacterial, fungal, and functional microorganism abundance and activity were measured for all soil samples.

Table 2
Details of test treatments and doses

Number	Treatment	Kg/667m^2	Community usage
LYX-1	Soil-Tibetan Gold Microbial Fertilizer	550	17.3
LYX-2	Carbon energy microbial inoculants	1000	31.5
LYX-3	Pure sheep manure organic fertilizer	750	23.6
LYX-4	commercial organic fertilizer	1000	31.5
LYX-5	vegetable cake	500	15.7
LYX-6	Mushroom residue after decomposing	2000	63.0
LYX-7	Compound Microbial Fertilizer	650	20.5
LYX-8	Foliar fertilizer	-	Dilute 600 times
LYX-9	Soybean meal microbial fertilizer	650	20.5
LYX-10	Protein Microbial Fertilizer	650	20.5
LYX-11	Seaweed Microbial Fertilizer	900	28.4
LYX-12	Organic Soil Conditioner	600	18.9
FZW-1	Conventional fertilization (commercial organic fertilizer + compound fertilizer)	500 + 25	15.7 + 0.8
FZW-2	quicklime	200	6.3
FZW-3	Biochar	400	12.6
FZW-4	Zeolite	300	9.4
FZW-5	immature mushroom residue	600	18.9
FZW-6	Potassium humate	200	6.3
FZW-7	1/2 quicklime + 1/2 biomass charcoal	100 + 200	3.1 + 6.3
FZW-8	1/2 quicklime + 1/2 zeolite	100 + 150	3.1 + 4.7
FZW-9	1/2 quicklime + 1/2 potassium humate	100 + 100	3.1 + 3.1
FZW-10	1/2 quicklime + 1/2 mushroom residue	100 + 300	3.1 + 9.4
A	Compound fertilizer	50	1.6
B	commercial organic fertilizer	800	25.2
C	Charcoal based fertilizer	800	25.2
D	Organic fertilizer + compound fertilizer	800 + 25	25.2 + 0.8
E	Carbon-based fertilizer + compound fertilizer	800 + 25	25.2 + 0.8
F	Organic fertilizer + compound fertilizer	400 + 50	12.6 + 1.6
G	Carbon-based fertilizer + compound fertilizer	400 + 50	12.6 + 1.6
CK	CK	0	0

Collection of soil samples for analysis

Soil samples were collected before sowing and after harvest, and the basic soil properties were analyzed in a laboratory. Soil was sampled at three points in each plot and then mixed well to form a composite sample. Soil pH was measured by the NY/T 1377–2007 method; soil organic matter was measured by the NY/T 1121.6–2006 method; urease was determined by the sodium phenate-sodium hypochlorite colorimetric method; and microbial carbon and microbial nitrogen were measured by the chloroform fumigation method. The alkali-hydrolysable nitrogen content in soil was determined by the alkaline hydrolysis diffusion method; available phosphorus was extracted by the $NaHCO_3$ method, and its content was determined by the molybdenum blue colorimetric method; and available potassium was extracted by NH_3COONH_4 and measured by a flame photometer. The samples were placed in an ice box and stored at − 80°C for DNA extraction.

Sweet potato quality determination

The following methods were used to analyze the quality of sweet potato: dry matter: GB 5009.3–2016 first method; starch,GB5009.9-2016 second method; soluble sugar, NY/T 1278–2007 method; reducing sugar,GB5009.7-2016 first method; crude protein,GB5009.5-2016 first method; β-carotene, GB5009.83-2016method.

Illumina PE sequencing

PCR amplification

Genomic DNA was extracted from the sample, and the extracted genomic DNA was detected by 1% agarose gel electrophoresis. Specific primers with barcodes were synthesized and TransStart FastPfu DNA Polymerase (China Beijing TransGen Biotech TransGen AP221-02) and ABI GeneAmp® (Model 9700)(China Shagnhai Epbais Applied Biosystems Trading (Shanghai) Co., Ltd.) were used for PCR amplification. Each sample was replicated three times. The PCR products of the same sample were mixed and detected by 2% agarose gel electrophoresis. The AxyPrep DNA gel recovery kit (AXYGEN) was used to cut the gel to recover the PCR products, and the recovered products were eluted with Tris_HCl.

Fluorescence quantification

By referring to the preliminary quantitative results of electrophoresis, the PCR products were detected and quantified with the QuantiFluor™-ST blue fluorescence quantitative system (Promega) and then mixed in corresponding proportions according to the sequencing volume requirements of each sample.

Illumina PE library construction

The following steps were used for library construction: Connect the“Y” shaped connector, and then use magnetic bead screening to remove linker self-ligating fragments. Next, enrich the library templates by PCR amplification, and finally, perform denaturation with NaOH to yield single-stranded DNA fragments.

Illumina PE sequencing

The following steps were used for PE sequencing: one end of the DNA fragment complementary to the primer base was fixed on the chip; the other end was randomly complementary to another nearby primer and was also fixed to form a “bridge”. PCR amplification was performed to generate DNA clusters. DNA amplicons were linearized into single strands. The modified DNA polymerase and dNTPs with 4 fluorescent labels were added, and only one base per cycle was synthesized. The surface of the reaction plate was scanned with a laser to read the nucleotide species polymerized in the first round of reaction of each template sequence. The “fluorophore” and “termination group” were chemically cut to restore the viscosity of the 3′-end, and polymerization was continued for the second nucleotide. The fluorescent signals were counted in each round to know the sequence of the template DNA fragment.

Bioinformatics Analysis

The PE reads obtained by Illumina PE sequencing were first spliced according to the overlap relationship, and the sequence quality was simultaneously controlled and filtered. After differentiating the samples, operational taxonomic unit (OTU) cluster analysis and species taxonomy analysis were performed. Based on OTU, diversity index analysis was performed. The results of OTU cluster analysis were used to analyze the diversity index of OTUs and to detect the sequencing depth; by using taxonomic information, statistical analysis of community structure was performed at each taxonomic level. On the basis of the above analysis, a series of in-depth statistical and visual analyses of community structure and phylogeny were performed.

Sweet potato yield

The weight of vines per plant, weight of potato per plant, weight of commercial potato per plant, number of tubers per plant, and number of commercial potatoes per plant were randomly selected from 10 adjacent plants in each plot and measured, and the average value was considered. Commercial potato was defined according to the following criteria: appearance is free of pests and diseases, size is relatively uniform, and the prepared potato chips can be directly sold in the market. Plot yield was calculated as the total weight of sweet potato per plot at harvest.

Results

Comparison of agronomic characteristics of sweet potato

The vine weight per plant, potato weight per plant, commercial potato weight per plant, number of tubers per plant, number of commercial potatoes per plant, plot yield, plot vine weight, plot commercial potato weight, and other indicators were measured under different treatments. The following results were obtained: in the “LYX” series, the yield of plots and commercial potato yields were the highest under the LYX-3 treatment(17.89 kg and 14.07 kg, respectively); the aboveground part of sweet potato grew most vigorously under the LYX-11 treatment; and the yield in terms of number of potatoes was the largest under the LYX-12 treatment(4.93). In the “FZW” series, the yield of the FZW-4 plot and commercial potato plot was the highest(24.97 kg and 23.57 kg, respectively); the sweet potato aerial part grew most vigorously under the FZW-7 treatment; and the number of tubers per plant was the highest under the FZW-5 treatment(4.80 pcs). In the “T” series, the yield of the sweet potato plot and the commercial potato plot under treatment F were the highest(27.58 kg and 25.82 kg, respectively);the aboveground part of sweet potato grew most vigorously under treatment D, and the number of tubers per plant was the highest (Table 3).

Table 3
Comparison of field agronomic characteristics of sweet potato under different treatments

Treatment	Single vine weight(kg)	Single sweet potato weight(kg)	Commercial sweet potato weight per plant(kg)	Number of tubers per plant (pieces)	Number of commercial sweet potatoes per plant (pieces)	Plot yield (kg)	Community rattan weight (kg)	Community commercial sweet potato weight (kg)
LYX-1	0.32	0.21	0.19	3.80	2.67	15.33	31.24	12.62
LYX-2	0.27	0.22	0.19	4.20	2.80	12.06	31.58	9.65
LYX-3	0.21	0.24	0.22	4.77	3.87	17.89	19.47	14.07
LYX-4	0.18	0.23	0.21	4.90	3.53	16.85	15.14	12.87
LYX-5	0.28	0.23	0.20	4.43	3.07	15.55	25.74	12.71
LYX-6	0.19	0.24	0.22	4.80	3.53	16.25	21.89	13.16
LYX-7	0.22	0.22	0.18	4.77	3.60	14.67	15.06	11.85
LYX-8	0.19	0.20	0.17	4.80	3.33	14.07	15.51	9.93
LYX-9	0.21	0.24	0.21	4.77	3.43	15.59	17.23	11.80
LYX-10	0.15	0.18	0.16	4.20	2.80	13.77	12.73	10.33
LYX-11	0.37	0.22	0.19	4.07	2.53	13.83	**32.53**	11.36
LYX-12	0.20	0.26	0.23	4.93	3.57	16.39	21.07	13.81
FZW-1	0.22	0.26	0.24	3.73	2.50	15.98	8.90	13.97
FZW-2	0.19	0.24	0.21	3.43	2.10	23.24	15.33	20.44
FZW-3	0.16	0.26	0.22	4.33	2.70	18.14	14.40	14.25
FZW-4	0.14	0.45	0.41	4.50	3.30	24.97	16.29	23.57
FZW-5	0.14	0.27	0.23	4.80	3.03	22.87	13.84	20.17
FZW-6	0.17	0.37	0.33	3.83	2.63	19.78	11.86	18.57
FZW-7	0.26	0.18	0.15	3.80	2.27	13.84	21.03	10.09
FZW-8	0.12	0.29	0.25	4.27	2.77	24.52	13.70	22.06
FZW-9	0.19	0.29	0.25	4.33	3.07	21.70	17.45	19.62
FZW-10	0.13	0.29	0.20	4.00	2.23	14.21	8.91	10.77
A	0.31	0.30	0.25	4.47	3.03	15.76	21.79	14.45
B	0.25	0.30	0.25	4.07	2.78	22.28	17.95	21.16
C	0.25	0.44	0.39	4.37	2.90	22.02	14.94	20.75
D	0.31	0.34	0.31	4.80	4.32	25.65	24.89	24.02
E	0.27	0.34	0.28	4.77	4.13	22.55	19.51	20.59
F	0.18	0.24	0.20	3.10	2.07	**27.58**	21.29	**25.82**
G	0.22	0.33	0.28	4.07	2.54	23.74	17.33	21.76

Treatment	Single vine weight(kg)	Single sweet potato weight(kg)	Commercial sweet potato weight per plant(kg)	Number of tubers per plant (pieces)	Number of commercial sweet potatoes per plant (pieces)	Plot yield (kg)	Community rattan weight (kg)	Community commercial sweet potato weight (kg)
CK	0.18	0.29	0.24	4.03	2.62	20.57	14.94	19.44

Determination of soil pH value and nutrient indices under different treatments

The soil pH value, organic matter content, urease, microbial carbon, and microbial nitrogen indices were measured under different treatments. The results showed that the soil organic matter content and microbial carbon content of the “LYX” series were the highest under the LYX-2 treatment(19.2 g/kg and 481 mg/kg , respectively); pH and urease were the highest under the LYX-6 treatment(5.84 and 0.69 mg/±24 h ammonia nitrogen, respectively); and soil microbial nitrogen content was the highest under the LYX-12 treatment(97.4 mg/kg). In the “FZW” series, the urease content was the highest under the FZW-1 treatment(0.76 mg/±24 h ammonia nitrogen); the microbial nitrogen content was the highest under the FZW-6 treatment(66.5 mg/kg); the soil pH value was the highest under the FZW-9 treatment (6.40); and the soil organic matter content and microbial carbon content were the highest under the FZW-10 treatment(14.3 g/kg and 359 mg/kg, respectively). In the “T” series, the contents of soil organic matter, microbial carbon, and microbial nitrogen were the highest under treatment B(24.8 g/kg, 619 mg/kg, and 79.7 mg/kg, respectively);the highest urease content was under treatment E(0.68 mg/±24 h ammonia);and the soil pH value was the highest under treatment F(5.70) (Table 4).

Table 4
Determination of soil pH value and nutrient indices under different treatments

Treatment	PH	Organic matter (g/kg)	Urine enzyme (mg/±24 hours ammonia nitrogen)	Microbial carbon (mg/kg)	Microbial nitrogen (mg/kg)
LYX-1	4.92	13.9	0.60	349	90.5
LYX-2	4.80	19.2	0.67	481	97.3
LYX-3	5.40	16.1	0.64	402	92.2
LYX-4	5.62	15.8	0.66	396	71.6
LYX-5	5.08	16.2	0.60	405	39.9
LYX-6	5.84	12.8	0.69	320	56.5
LYX-7	4.91	13.4	0.55	335	76.1
LYX-8	4.94	16.1	0.57	403	78.5
LYX-9	5.24	10.5	0.62	263	81.0
LYX-10	5.18	12.7	0.61	318	73.7
LYX-11	4.75	16.6	0.58	414	91.5
LYX-12	5.70	14.7	0.68	368	97.4
FZW-1	**6.32**	11.3	**0.76**	333	59.0
FZW-2	5.80	8.5	0.68	252	60.6
FZW-3	5.45	10.0	0.65	300	62.7
FZW-4	5.41	9.9	0.64	297	64.9
FZW-5	5.38	10.2	0.63	305	63.2
FZW-6	5.50	8.6	0.65	264	66.5
FZW-7	6.08	9.2	0.72	280	57.8
FZW-8	5.80	9.4	0.68	286	62.4
FZW-9	6.40	8.7	0.75	268	64.3
FZW-10	5.90	14.3	0.69	359	53.2
A	4.79	13.6	0.65	339	62.6
B	5.66	**24.8**	0.64	**619**	79.7
C	5.56	19.1	0.62	477	77.3
D	5.41	15.4	0.62	385	61.1
E	5.23	19.1	0.68	477	74.4
F	5.70	18.6	0.63	464	78.5
G	5.10	19.9	0.63	498	64.5
CK	5.16	7.9	0.61	324	77.9

Differences in sweet potato quality

After harvesting, the contents of dry matter, starch, soluble sugar, reducing sugar, crude protein, and β-carotene in sweet potato were determined under different treatments (Table 5). It was found that the reducing sugar and β-carotene contents of LYX-1 in the “LYX” series were the highest at 1.8g/100g and 158μg/100g. In the “LYX” series, the LYX-2 treatment showed the highest crude protein content(2.88g/100g); the LYX-7 treatment showed the highest dry matter content(38.7g/100g); and the LYX-11 treatment showed the lowest sweet potato starch content and the highest soluble sugar content (36.1g/100g and 3.58%, respectively). In the “FZW” series, the content of soluble sugar and reducing sugar was the highest under the FZW-4 treatment(5.83% and 4.9g/100g, respectively); the content of β-carotene was the highest under the FZW-8 treatment(81.2μg/100g; and the dry matter, starch, and crude protein contents were the highest under the FZW-9 treatment(24.6 g/100 g, 19.2 g/100 g, and 1.05 g/100 g, respectively). In the “T” series, the dry matter and β-carotene contents of sweet potato were the highest under treatment B(37.1 g/100g and 123 μg/100g, respectively); the content of starch was the highest under treatment E(32.5 g/100g);the content of crude protein was the highest under treatment F (2.11g/100g); and the highest content of soluble sugar and reducing sugar was observed under treatment G(4.12% and 3.3g/100g, respectively).

Table 5
Difference analysis of sweet potato quality under different treatments

Treatment	dry matter (g/100g)	Starch (g/100g)	soluble sugar (%)	reducing sugar (g/100g)	crude protein (g/100g)	β-carotene(μg/100g)
LYX-1	36.2	31.0	3.17	1.8	2.51	158
LYX-2	37.4	35.8	3.06	1.5	2.88	89.6
LYX-3	34.7	30.1	3.14	1.6	1.23	73.4
LYX-4	37.5	30.7	2.95	1.1	1.23	122
LYX-5	37.4	33.2	3.14	1.4	1.74	118
LYX-6	36.2	32.4	3.13	1.8	1.22	60.7
LYX-7	38.7	34.2	1.92	1.0	1.73	96
LYX-8	37.6	34.1	2.69	1.2	1.42	128
LYX-9	36.9	36.0	2.31	1.5	1.35	112
LYX-10	37.8	34.3	3.14	1.3	1.69	88.4
LYX-11	37.6	36.1	3.58	1.5	3.2	97.4
LYX-12	36.4	32.4	2.71	1.7	1.7	148
FZW-1	32.8	27.3	4.83	3.8	0.92	55.9
FZW-2	33.8	26.4	4.76	3.9	0.94	59.5
FZW-3	32.8	27.8	5.02	3.9	0.97	45.5
FZW-4	30.3	24.4	5.83	4.9	0.77	64.9
FZW-5	33.6	27.8	4.42	3.7	1.10	41.2
FZW-6	33.3	27.7	4.23	2.9	1.21	41.1
FZW-7	32.4	25.9	4.17	3.7	0.97	53.1
FZW-8	33.5	24.9	4.94	3.3	0.94	81.2
FZW-9	34.6	29.2	4.61	3.4	1.05	73.3
FZW-10	31.4	26.4	4.55	4.4	0.81	65.0
A	36.5	30.8	3.02	1.8	1.75	116.0
B	37.1	29.7	2.17	2.7	1.63	123.0
C	35.3	30.4	3.25	2.5	1.22	76.0
D	34.6	31.6	3.69	2.9	0.74	87.0
E	33.7	32.5	4.07	3.1	1.55	66.5
F	32.2	30.3	4.11	2.8	2.11	81.3
G	34.1	29.8	4.12	3.3	1.97	72.1
CK	33.4	32.1	1.87	1.1	0.69	40.8

Alpha diversity of soil microorganisms

Rareness curves were constructed for the total number of species, and the relative abundance contained in the corresponding sequences for each species was determined, along with the microbial richness and diversity of the samples. As shown in Fig. 1, the soil microbial richness levels under different fertilization treatments increased gradually with the increase in sample sequencing depth; moreover, with the increase in sample sequencing depth, the differences among the different fertilization treatments gradually increased. Analysis of the rarefaction curves for each treatment confirmed that the number of OTUs in the microbial community increased significantly with the increase in the sequence depth; the number of new OTUs decreased with increasing sequence depth, and the sequence depth varied across the treatments. Therefore, the arrangement order of different soil microorganisms could better reflect the type and quantity of microbial communities. The rarefaction curves of the soil samples showed that the number of OTUs was the highest for the LYX-12 treatment, followed by treatment C and treatment B; however, the sequence depth for treatment B was less deep than that for treatment C. The least amount of OTUs were observed for the LYX-1 treatment, followed by LYX-5 and FZW-3 treatments.

Relative abundances and community compositions of soil microbial phyla

According to the OTU classification, the soil species and relative abundances in the samples were statistically analyzed at the phylum level (Fig. 2). The microbial species in all soil samples covered 43 phyla, including 8 major microbial communities in the rhizosphere soil. These included Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Bacteroidetes, Planctomycetes, Gemmatimonadetes, and Firmicutes. The abundant Proteobacteria and Actinobacteria accounted for the highest proportions(28.1% and 22.3%, respectively). The proportions of Chloroflexi, Acidobacteria, Bacteroidetes, Lanctomycetes, Gemmatimonadetes, and Firmicutes were 15.8%, 12.7%, 6.5%, 6.2%, 2.6%, and 1.9%, respectively. The proportion of other 36 bacterial phyla were extremely low, with a total of only 3.9%.

The composition and abundance of soil microbial phyla varied greatly under different fertilization treatments. The highest content of Proteobacteria in soil was observed for LYX-8(33.7%), followed by FZW-8, and the lowest content was observed for FZW-7(21.2%). The highest content of Actinobacteria was observed for LYX-1(34.3%), followed by LYX-10(30.6%), and the lowest content was observed for LYX-8 (11.3%). The highest content of Chloroflexi in soil was noted for FZW-7(22.8%), followed by LYX-4(21.6%), and the lowest content was observed for FZW-8(10.0%). The highest content of Acidobacteria in soil was observed for FZW-10(21.4%), followed by treatment G(21.3%), and the lowest content was noted for LYX-1(3.3%). Regarding the proportion of the main bacterial phyla, the rhizosphere microbial communities of sweet potato varied widely after different fertilizer treatments, and the proportions were also different when the communities were the same.

Cluster analysis of soil microorganisms under different treatments revealed that FZW-1 and FZW-9 belonged to one class; FZW-3, FZW-2, FZW-10, FZW-5, FZW-4, FZW-7, FZW-8,and FZW-6 were in one class; LYX-1 was a single class; LYX-8 was a single class; and LYX-7, LYX-4, E, D, CK, B, G, A, F, C, LYX-11, LYX-5, LYX-2, LYX-9, LYX-3, LYX-12, LYX-6, and LYX-10 were in one class (Fig. 3).

Soil microbial community heatmap

The species and relative abundances of soil microorganisms in the samples were analyzed by phylum-level and genus-level heat map analysis (Figs. 4 and 5). The abundance of genus decreased significantly.The abundance of Nitrospirae decreased the most under the LYX-5 treatment; the abundance of Latescibacteria increased significantly under LYX-12 and FZW-9 treatments, and the abundance was higher in FZW-9 than in LYX-12; and the abundance of Ignavibacteriae and RBG-1 (Zixibacteria) decreased significantly under the FZW-9 treatment. The results of genus-level heat map analysis showed that the abundances of Mesoflavibacter, Nitrosomonadaceae_uncultured, Donghicola, and Roseiflexus decreased significantly under the LYX-1 treatment, with Donghicola showing the most decrease. The abundances of Cytophagaceae_uncultured in LYX-7 and LYX-8 treatments showed a decreasing trend to varying degrees. The abundances

of Mizugakiibacter, JG30a − KF − 32_norank, and Elev − 1554_norank decreased significantly under the FZW-1 treatment, with Mizugakiibacter showing the most decrease.The abundance of Mizugakiibacter decreased significantly under FZW-4, FZW-5,FZW-9, and FZW-10 treatments.The abundances of Mizugakiibacter, JG37 − AG − 4_norank, Actinospica, HSB OF53 − F07_norank, JG30a − KF − 32_norank, ODP1230B8.23_norank, FCPS473_norank, Acetobacteraceae_uncultured, Granulicella, Thermosporotrichaceae_uncultured, and Elev − 1554_norank decreased to varying degrees.

Soil microbial community Venn

A Venn map (Fig. 6) was constructed for soil microorganisms under different treatments. The figure that 168 sequences of 30 samples were divided into the same OTU. From LYX-1 to CK, there were 22, 30, 42, 36 16, 31, 30, 40, 37,31, 25, 86, 27, 15, 20, 17, 29, 18, 19, 25, 183, 13, 15, 24, 49, 16, 21, 28, 13 and 13 special OUT, among which FZW-9 has the largest number of special OUTs;the total number of OUT is 1474, 1498, 2239, 2159, 1484, 2214, 1771, 1547,2142, 1889, 1600, 2386, 1898, 1564, 1516, 1824, 1802, 1716, 1834, 1543, 1876,1711, 1696, 2306, 2307, 2124, 2029, 2284, 1781 and 1692, and LYX-12 has the most total OUTs (Fig. 6).

Differences in soil microbial community structure

Linear discriminant analysis Effect Size (LEfSe) analysis (LDA values shown in Fig. 7) was performed to determine significant differences in taxon abundance and to validate communities or species that had significantly different effects on sweet potato growth under different fertilizer treatments (Fig. 8). Specifically, c_Betaproteobacteria, o_Nitrosomonadales, f_Nitrosomonadaceae, and c_JG37_AG_4 were the four dominant biomarkers of soil microorganisms with the maximum LDA values for samples under FZW treatment (Fig. 7). Similarly, P_Actinobacteria, c_Actinobacteria, o_Solirubrobacterales, and c_Alphaproteobacteria were the four dominant biomarkers of soil microorganisms with the maximum LDA values for samples under LYX treatment (Fig. 7). Moreover, p_Acidobacteria, o_Acidobacteriales, c_Acidobacteria, and f_Acidobacteriaceae_Subgroup_1 were the four dominant biomarkers of soil microorganisms with the maximum LDA values for samples under T treatment (Fig. 7). Additionally, c_Subgroup_2, o_Acidimicrobiales, c_Acidimicrobiia, o_Gaiellales, c_JG37_AG_4, f_Nitrosomonadaceae, o_Nitrosomonadales, and c_Betaproteobacteria were primarily changes for soil microorganisms under FZW treatment (Fig. 8). f_Actinospicaceae, o_Catenulisporales, o_Corynebacteriales, f_Geodermatophilaceae, f_Microbacteriaceae, o_Micrococcales, f_Pseudonocardiaceae, o_Pseudonocardiales, f_Streptomycetaceae, o_Streptomycetales, c_Actinobacteria, f_Elev_16S_1332, f_YNPFFP1, o_Solirubrobacterales, f_FCPS473, f_JG30a_KF_32, o_Bacillales, c_Bacilli, f_Planctomycetaceae, o_Planctomycetales, c_Planctomycetacia, f_Bradyrhizobiaceae, f_Hyphomicrobiaceae, o_Rhizobiales, and c_Alphaproteobacteria were primarily changes for soil microorganisms under LYX treatment (Fig. 8). f_Acidobacteriaceae_Subgroup_1, o_Acidobacteriales, c_Acidobacteria, f_Solibacteraceae_Subgroup_3, o_Solibacterales, c_Solibacteres, f_Frankiaceae, o_JG30_KF_AS9, c_Thermomicrobia, f_ODP1230B8_23, o_Halanaerobiales, c_Clostridia, f_Gemmatimonadaceae, o_Gemmatimonadales, c_Gemmatimonadetes, f_Acetobacteraceae, and o_Rhodospirillales were primarily changes for soil microorganisms under T treatment (Fig. 8).

Discussion

The present study is the first to examine differences in the agronomic traits, yield, and quality of sweet potato and soil microbiome structure after the application of different types of fertilizers. Our study found that the three fertilization modes of pure sheep manure organic fertilizer, zeolite, and 1/2 organic fertilizer + compound fertilizer could increase the yield of sweet potato, in the following order: organic fertilizer + compound fertilizer > zeolite > pure sheep manure organic fertilizer.Seaweed microbial fertilizer, 1/2 quicklime + 1/2 biomass charcoal, and organic fertilizer + 1/2 compound fertilizer were the three fertilization modes beneficial to the growth of sweet potato stems and leaves,with the following order: seaweed microbial fertilizer > organic fertilizer + 1/2 compound fertilizer > 1/2 quicklime + 1/2 biomass charcoal.Organic soil conditioner, fungal residue, and organic fertilizer + 1/2 compound fertilizer were the three fertilization modes that increased the number of sweet potatoes per plant, in the following order: organic soil conditioner > fungal residue = organic fertilizer + 1/2 compound fertilizer.

The analysis of soil nutrient indicators revealed that the soil pH value increased the most under the following three fertilization modes: fungal residue, 1/2 quicklime + 1/2 potassium fulvic acid, and 1/2 organic fertilizer + compound fertilizer, in the following order: 1/2 quicklime + 1/2 potassium humate > fungal residue > 1/2 organic fertilizer + compound fertilizer.Carbon energy microbial inoculant, 1/2 quicklime + 1/2 fungal residue, and commercial organic fertilizer were the three fertilization modesthat led to the highest soil organic matter content, in the following order: commercial organic fertilizer > carbonenergy microbial inoculant > 1/2 quicklime + 1/2 fungal residue.

Soil urease content was the highest under the following three fertilization modes: fungal residue, conventional fertilization, and carbon-based fertilizer + 1/2 compound fertilizer, in the following order: conventional fertilization > fungal residue > carbon-based fertilizer + 1/2 compound fertilizer.Carbon energy microbial bacterial fertilizer, 1/2 quicklime + 1/2 bacterial residue, and commercial organic fertilizer were the three fertilization modesthat resulted in the highest soil microbial carbon content, in the following order: commercial organic fertilizer > carbon energy microbial bacterial fertilizer > 1/2 quicklime + 1/2 bacterial residue.Soil microbial nitrogen content was the highest under the three fertilization modes: organic source soil conditioner, potassium fulvic acid, and commercial organic fertilizer, with the following order: organic source soil conditioner > commercial organic fertilizer > potassium fulvic acid.

The analysis of sweet potato quality showed that the dry matter content of sweet potato was the highest under the following three fertilization modes: compound microbial bacterial fertilizer, 1/2 quicklime + 1/2 potassium fulvic acid, and commercial organic fertilizer, in the following order: compound microbial bacterial fertilizer > commercial organic fertilizer > 1/2 quicklime + 1/2 potassium fulvic acid.Seaweed microbial bacterial fertilizer, 1/2 quicklime + 1/2 potassium fulvic acid, and carbon-based fertilizer + 1/2 compound fertilizer were the three fertilization modesthat yielded the highest sweet potato starch content, in the following order: seaweed microbial bacterial fertilizer > charcoal-based fertilizer + 1/2 compound fertilizer > 1/2 quicklime + 1/2 potassium fulvic acid.

Seaweed microbial fertilizer, zeolite, and 1/2 carbon-based fertilizer + compound fertilizer were the three fertilization modes that provided the highest sweet potato soluble sugar content, in the following order: zeolite > 1/2 carbon-based fertilizer + compound fertilizer > seaweed microbial fertilizer. Soil Tibetan gold microbial fertilizer, zeolite, and 1/2 carbon-based fertilizer + compound fertilizer were the three fertilization modes for yielding the highest sweet potato reducing sugar content, in the following order: zeolite > 1/2 carbon-based fertilizer + compound fertilizer > SoilTibetan gold microbial fertilizer.

Carbon-energy microbial agent, 1/2 quicklime + 1/2 potassium fulvicate, and 1/2 organic fertilizer + compound fertilizer were the three fertilization modesthat showed the highest sweet potato crude protein content, in the following order: carbon microbial agent > 1/2 organic fertilizer + compound fertilizer > 1/2 quicklime + 1/2 potassium fulvic acid.The highest β-carotene content in sweet potato was observed for soil Tibetan gold microbial bacterial fertilizer, 1/2 quicklime + 1/2 zeolite, and commercial organic fertilizer,in the following order: soil Tibetan gold microbial fertilizer > commercial organic fertilizer > 1/2 quicklime + 1/2 zeolite.

Previous research has shown that the combined application of chemical fertilizers with pig manure can effectively maintain the balance of soil nutrients, and by increasing soil total nitrogen and available nutrients, a better yield increase of wheat can be achieved in the yellow fluvo-aquic soil area (Wei et al., 2017a). Compared to the application of straw, long-term pig manure compost combined with chemical fertilizers has the best effect on increasing wheat yield by improving soil nitrogen content(Yu et al.,2012). In addition, the combined application of organic and inorganic fertilizers not only compensates for the lack of soil nutrients caused by single application of chemical fertilizers, but it also solves the problem of low levels of soil organic matter(Yuan et al.,2017). The content of soil organic matter is one of the important factors that restrict crop yield. The results of the present study showed that compared to no fertilization, fertilization significantly increased soil organic matter content. In this study, in the comparison of the application of different organic fertilizers, the combined application of 1/2 organic fertilizer + compound fertilizer had a significant effect on improving the yield of fresh

potato, which was the same as that observed in a previous study (Wei et al., 2017b). Previous research has shown that long-term combined application of organic and inorganic fertilizers can significantly increase soil organic carbon content, and crop yield can only be improved after soil organic carbon reaches a certain level. Therefore, crop yield is jointly affected by soil nutrient content and soil organic matter content(Zhang et al.,2019). In the present study, soil pH, organic matter content, microbial carbon, microbial nitrogen, and other indicators were measured, and it was found that the organic matter content of the treatments with high yield was relatively high, but not the highest among all treatments. There were constraints and balances between them, and a higher value of either of them did not provide a better yield.

Soil microorganisms are important promoters of ecosystem functions and have significant effects on root metabolic activities, soil structure formation, and fertility transformation(Jiang et al.,2010). Some studies(Li et al.,2017) have shown that the more complex the soil microbial community structure and the higher the species richness, the higher is the stability of the soil ecosystem. Crop roots and their exudates provide soil microorganisms with a variety of carbon sources and play an important role in the interaction between crops and soil microorganisms(Bais et al.,2006). Previous studies(Liu et al., 2014; Song et al.,2007;Venter et al.,2016; Zhang et al.,2017) have shown that soil nutrient changes have a greater impact on the structure and functional diversity of soil microbial communities. In the present study, compared to the control, 1/2 organic fertilizer + compound fertilizer, zeolite, and pure sheep manure organic fertilizer treatment had the highest sweet potato yield, and the relative abundances of Proteobacteria in soil microorganisms under these treatments were 33.06%, 32.39%, and 27.96%, respectively, which were higher than those of the control (27.56%);this finding indicated that the relative abundance of Proteobacteria is positively correlated with sweet potato yield. Previous research (Wang Guanghua et al., 2016; Zhang Yanhong et al., 2016) reported that Firmicutes can effectively degrade soil residues and participate in the metabolic synthesis of carbon and nitrogen compounds, which is more conducive to the degradation of soil organic matter and formation of humus and promotes the increase in soil fertility and cellulase activity. Increasing the application of an organic fertilizer or a bio-organic fertilizer can significantly improve the relative abundance of soil Firmicutes.The relative abundance of walnut soil Proteobacteria and Firmicutes under chemical fertilizer + organic fertilizer + bio-organic fertilizer treatment was the highest and significantly higher than that achieved by CK and single application of a chemical fertilizer (Zhang et al.,2021);this finding is consistent with the results of Zhang et al.(2020). However, our study found that the relative abundance of Firmicutes was 2.41% in the control, 1.84% under 1/2 organic fertilizer + compound fertilizer treatment, and 0.75% under zeolite treatment. The relative abundance of Firmicutes under organic fertilizer treatment was 2.32%, which was inconsistent with the results of Zhang Qian and Zhang Meng. It is speculated that the difference among the main communities of soil microorganisms may be caused by different crops. Soil microorganisms are an important part of soil and participate in most of the material transformation and energy exchange processes in the soil. They play an important role in the process of nutrient cycling, organic matter decomposition, and inhibition of soil-borne pathogens in the soil rhizosphere ecosystem, and they also reflect changes in the soil environmentby acting as important indicators (Hao et al., 2010; Li et al., 2012). Crops can affect the rhizosphere microbial community by producing root exudates, and microbial functional diversity has a greater impact on the stability of soil micro-ecosystems and soil fertility(Huang et al., 2015; Yang et al., 2014).

After 1/2 quicklime + 1/2 potassium humate treatment, the abundance of Latescibacteria in the soil increased significantly, and the abundance of Ignavibacteriae and RBG-1 (Zixibacteria) decreased significantly, which may be related to the increase in soil pH. The abundance of Mizugakiibacter decreased significantly under the FZW-4 treatment, which may be related to yield. Under the FZW-9 treatment, the abundance of Mizugakiibacter, JG37 − AG − 4_norank, Actinospica, HSB OF53 − F07_norank, JG30a − KF − 32_norank, ODP1230B8.23_norank, FCPS473_norank, Acetobacteraceae_uncultured, Granulicella, Thermosporotrichaceae_uncultured, and Elev − 1554_norank decreased to varying degrees, which may be related to the increase in soil pH and the increase in dry matter and starch content of sweet potato.

Conclusions

Our research shows significant differences in sweet potato agronomic traits and quality, soil physicochemical properties, and microbial communities under different planting modes. Seaweed microbial fertilizer and quicklime + biomass char can appropriately improve the growth of sweet potato stems and leaves. Zeolite and sheep manure organic fertilizer can appropriately increase the yield of sweet potato. The combination of organic fertilizer + 1/2 compound fertilizer can promote the growth of sweet potato stems and leaves, while the combination of 1/2 organic fertilizer + compound fertilizer can promote the increase in sweet potato yield. Although both soil nutrient content and organic matter content equally affect the yield of sweet potato, there were constraints and balances between them, and a higher value of either of them did not provide a better yield.The relative abundance of Proteobacteria in soil microorganisms was positively correlated with sweet potato yield;the abundance of Firmicutes was also associated with yield, but no positive correlation was observed. The abundance of Latescibacteria, Ignavibacteriae, and RBG-1 (Zixibacteria) may be associated with elevated soil pH.

Declarations

ACKNOWLEDGMENTS

This research was funded by the Key Research and Development Program of Zhejiang Province (2019C02035), We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

References

1. Bailey, K. L. & Lazarovits, G. (2003). Suppressing soil-borne diseases with residue management and organic amendments. Soil and Tillage Research, 72, 169–180. https://doi.org/10.1016/S0167-1987(03)00086-2.
2. Bais H P，Weir T L，Perry L G，et a1．The role of root exudates in rhizosphere intercmions with plants and other organisms．Annual Review ofPlant Biology，2006，57：233．
3. Basak B.B., Biswas D.R., Pal S. Soil biochemical properties and grain quality as affected by organic manures and mineral fertilizers in soil under maize-wheat rotation. Agrochimica, 2013,57(1):49-66.
4. Bruggen, A. H. C. V. & Semenov, A. M. (2000). In search of biological indicators for soil health and disease suppression. Applied Soil Ecology, 15, 13–24. https://doi.org/10.1016/S0929-1393(00)00068-8.
5. Cao, Y., Zhang, Z. H., Ling, N., Yuan, Y. J., Zheng, X. Y., Shen, B. &Shen, Q. R. (2011). Bacillus subtilis SQR 9 can control Fusarium wilt in cucumber by colonizing plant roots. Biology and Fertility of Soils, 47, 495–506. https://doi.org/10.1007/s00374-011-0556-2.
6. Ding J.L, Jiang X, Ma M.C, et al. (2017). Effects of long-term combined application of organic and inorganic fertilizers on fungal community structure in black soil in Northeast China . Journal of Plant Nutrition and Fertilizers, 23(04): 914-923.
7. Feng, Y., Motta, A. C., Reeves, D. W., Burmester, C. H., Santen, E. V.& Osborne, J. A. (2003). Soil microbial communities under con- ventional-till and no-till continuous cotton systems. Soil Biology and Biochemistry, 35, 1693–1703. https://doi.org/10.1016/j.soilb io.2003.08.016.
8. Ge G F, Li Z J, Fan F L, et al. Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers.[J]. Plant & Soil, 2010, 326(1-2):31.
9. Hao W Y，Ren L X，Ran W，et a1．Allelopathic effects of root exudates from watermelon and rice plants on *Fusariumoxysporum*f. sp. *Niveum*. Plant and Soil，2010，336：485-497．
10. He J. Z, Li J, Zheng Y.M. (2013). Reflections on the relationship between microbial diversity and stability in soil ecosystems. Biodiversity, 21(4):1-1.
11. Huang Y Q, Han X R, Yang J F, et al.,(2015). Effects of peanut root exudates on soil microbiological characteristics and community functional diversity,Journal of Shenyang Agricultural University, 46(1)：48-54．

12. Jiang J, Song M H.(2010).The role of plants and soil microbes in regulating nutrient cycling in ecosystems.Chinese Journal of Plant Ecology, 34(8)：979－988．
13. Jin Z.J. (2008). Effects of tillage and long-term fertilization on soil microbial community structure and activity in paddy fields. Nanjing: Nanjing Agricultural University.
14. Li P，Ma L，Feng Y L，et a1．Diversity and chemotaxis of soil bacteria with antifungal activity against *Fusarium* wilt of banana. Journal of Industrial Microbiology，2012，39：1495-1505．
15. Li X J, Xu T L, Chen B D,et al.,(2017).Arbuscular mycorrhizal fungi diversity and community structure in desert and grassland ecosystems.Journal of Ecology, 36(10)：2734-2743．
16. Li X.Y, Zhao B.Q, Li X.H, et al.(2005). Effects of different fertilization regimes on soil microorganisms and their relationship with soil fertility[J]. Chinese Agricultural Sciences, 38(8): 1591-1599.
17. Liang B, Yang X Y, He X H, Zhou J B. Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. Biology and Fertility of Soils, 2011, 47(2): 121-128.
18. Liu W，Jiang L，Hu S J，et a1．Decoupling of soil microbes and plants with increasing anthropogenic nitrogen inputs in a temperate steppe，Soil Biology and Biochemistry，2014，72(6)：116-122．
19. Lu, L. H., Yin, S. X., Liu, X., Zhang, W. M., Gu, T. Y., Shen, Q. R.& Qiu, H. Z. (2013). Fungal networks in yield-invigorating and debilitating soils induced by prolonged potato monoculture. Soil Biology and Biochemistry, 65, 186–194. https://doi.org/10.1016/j. soilbio.2013.05.025.
20. Ma N.N, Li T.L, Wu C.C, Zhang E.P. (2010) Effects of long-term fertilization on soil enzyme activities and soil physicochemical properties in facility vegetable fields. Chinese Journal of Applied Ecology, 21 (7): 1766-1771.
21. Nautiyal C.S.,Chauhan P. S.,Bhatia C.R. Changes in soil physic-chemical properties and microbial functional diversity due to 14 years of conversion of grassland to organic agriculture in semi-arid agroecosystem. Soil and tillage research, 2010,109(2):55-60.
22. Shen Q R, Tan J F, Qian X Q. An introduction to soil and fertilizer. Beijing: Higher Education Press, 2001: 4–6, 270–271.
23. Song M H，Xu X L，Hu Q w，et a1．Interactions of plant species mediated plant competition for inorganic nitrogen with soil Microorganisms in an alpine meadow，Plant and Soil，2007，297(1/2)：127-137．
24. Sradnick A., Murugan R.,Oltmanns M.,Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer. Applied soil ecology, 2013,63:23-28.
25. Stepien W.,Gorska E.B., Pietkiewicz S. et al. Long-term mineral fertilization impact on chemical and microbiological properties of soil and Miscanthus x giganteus yield. Plant soil and environment, 2014, 60(3):117-122.
26. Tamm L. Organic agriculture: development and state of the art. Journal of environmental monitoring, 2001, 3(6):92-96.
27. Tao L, Chu G.X, Liu T, et al.(2014) Effects of replacing some chemical fertilizers with organic fertilizers on soil microbial counts and enzyme activities on long-term continuous cropping cotton field yield . Chinese Journal of Ecology, 34(21):6137–6146.
28. Tkacz, A. & Poole, P. (2015). Role of root microbiota in plant productivity. Journal of Experimental Botany, 66, 2167–2175. https://doi. org/10.1093/jxb/erv157.
29. VenterZS，JacobsK，HawkinsH，The impact of crop rotation on soil microbial diversity，A meta-analysis，Pedobiologia，2016， 59(4)，215-223．
30. Wang F, Lin C, Li Q.H, et al. (2011). Effects of different long-term fertilization treatments on rice grain quality traits and soil fertility factors in southern Huangdi fields. Journal of Plant Nutrition and Fertilizers. 17(2): 283-290.
31. Wang G H, Liu J J, Yu Z H, et al., (2016).Advances in Bacterial Ecology of Agroacidobacteria, biotechnology bulletin, 26(2): 14-20.

32. Wang L.L, Dong M, Zhang Q, (2013). et al. Effects of organic fertilizers with different carbon to nitrogen ratios on soil microbial biomass in organic agriculture. Chinese Journal of Ecological Agriculture, 21(9):1073-1077.
33. Wang X, Chen T, Liang R.l, et al.(2002). Effects of sludge land use on crops and soil. Chinese Journal of Applied Ecology, 13(2):163-166.
34. Wang Y.(2014). Effects of long-term fertilization on soil microorganisms in greenhouse and field. Beijing: China Agricultural University.
35. Wei D, Yang Q, Zhang J Z, et al. Bacterial communities structure and diversity in a black soil as affected by long-term fertilization.Pedosphere, 2008, 18(5): 582–592.
36. Wei M., Zhang A J., Zhu G YP., et al., (2017a).Effects of different long-term fertilization on winter wheat yield and soil nutrients in yellow fluvo-aquic soil region, Journal of Plant Nutrition and Fertilizers, 23(2):304-312.
37. Wei M., Zhang A J., Zhu G YP., et al., (2017b).Study on yield stability and quality characteristics of sweet potato under long-term fertilization,Northwest Agricultural Journal, 26(4):588-595.
38. Wu J.N.(2018). Effects of different long-term fertilization on microbial diversity and functional flora of maize rhizosphere. Jilin: Jilin Agricultural University.
39. Xu B.L. (2017). Effects of long-term fertilization and temperature on nitrifying microbial and total bacterial communities in acidic dryland soil.. Nanjing: Nanjing Normal University.
40. Yang Z X, Tang L, Zheng Y, et al,(2014).Effects of different varieties of wheat and broad bean intercropping on fusarium wilt, root exudates and functional diversity of rhizosphere microbial communities, 20(3)：570-579
41. Yu J, Jiang Y, Xu C M, et al.,(2012) Effects of different organic matter and its combination of compost and chemical fertilizer on wheat growth and nitrogen uptake,Journal of Plant Nutrition and Fertilizers, 18(6):1293-1302.
42. Yuan M M, Wu G, Hu R, et al.,(2017). Effects of straw returning and fertilizer application on soil organic carbon composition and yield of rice oil rotation, Journal of Plant Nutrition and Fertilizers, 23(1):27-35.
43. Zhang M, Liu Y L, Wei Q Q, et al.,2020.Effects of short-term application of distiller's grain biochar on nitrogen availability and bacterial community structure diversity in Guizhou yellow soil,Environmental Science, 16(10): 253-327.
44. Zhang Q.(2021). Effects of different fertilization treatments on soil biological characteristics and walnut yield and quality,Xianyang, Northwest Agriculture and Forestry University.
45. Zhang X，Zhang R，Gao J，et a1，Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and enrich beneficial bacteria，Soil Biology and Biochemistry，2017，104，208-217，
46. Zhang X Z, Gao H J, Peng C, et al, (2019).Variation characteristics of organic carbon, total nitrogen and maize yield stability in long-term organic fertilization black soil,Journal of Plant Nutrition and Fertilizers, 25(9):1473-1481.
47. Zhang Y H, Dou S, Dong S S, et al., (2016). Effects of deep straw returning and combined application of chemical fertilizers on soil humus composition and humic acid structure.Soil Journal, 53(03): 694-702.
48. Zhao J, Ni T, Li J, et al. Effects of organic-inorganic compound fertilizer with reduced chemical fertilizer application on crop yields, soil biological activity and bacterial community structure in a rice–wheat cropping system. Applied Soil Ecology, 2016, 99:1–12.
49. Zhao T, Jiang Y.L , Yan H, et al. (2013). Effects of different slope aspects on soil microbial biomass and soluble organic carbon in loess hilly area[J].Environmental Science, 34(8):3223-3230.
50. Zhou J, Guan D, Zhou B, et al. Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. Soil Biology and Biochemistry, 2015, 90: 42–51.
51. Zhou Q.X, Sun S.J. (2002). Research and Development Trend of Applied Ecology. Journal of Applied Ecology, 13(7):879-884.

Figures

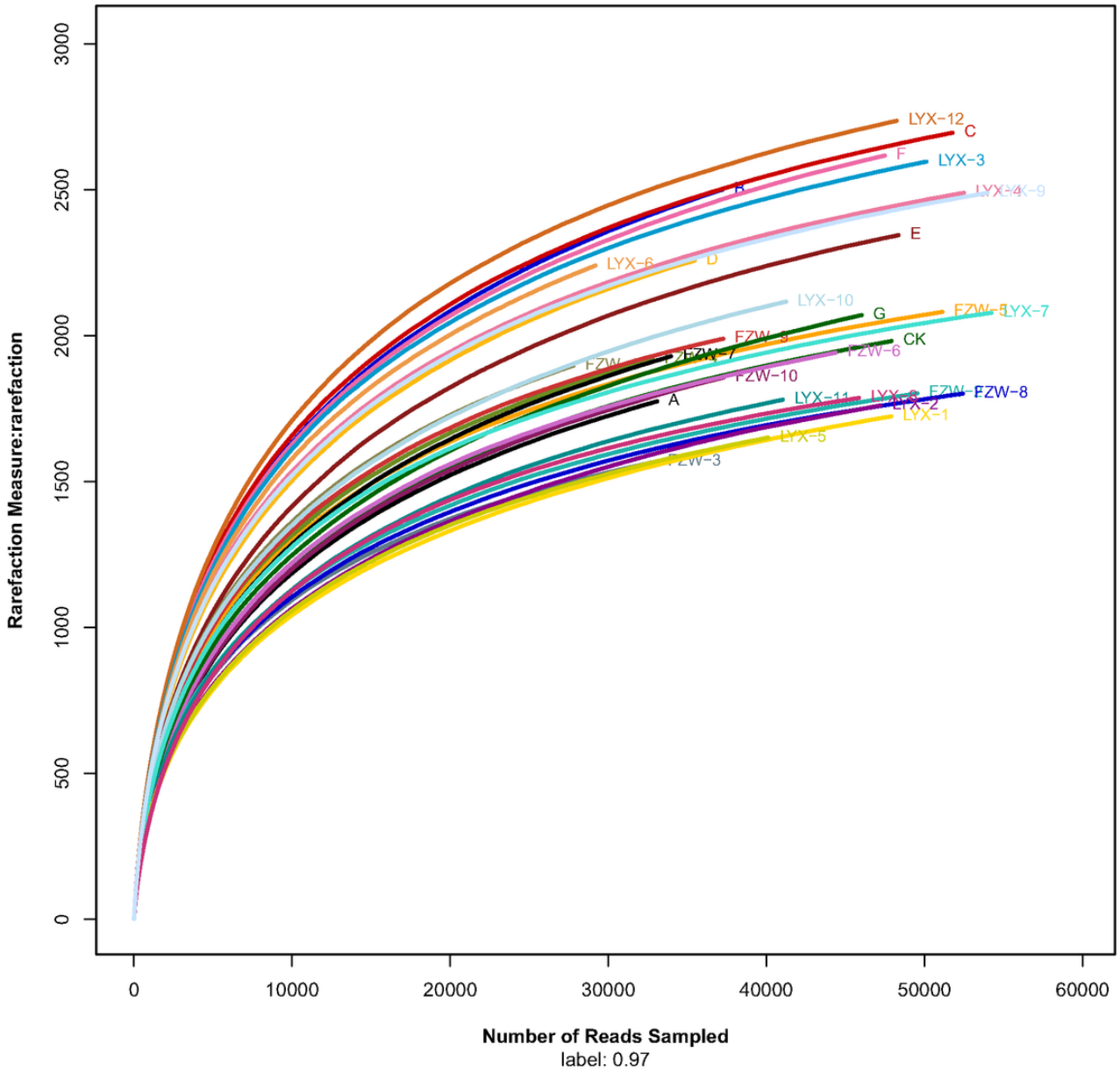

Figure 1

Rarefaction curves of soil microorganisms.

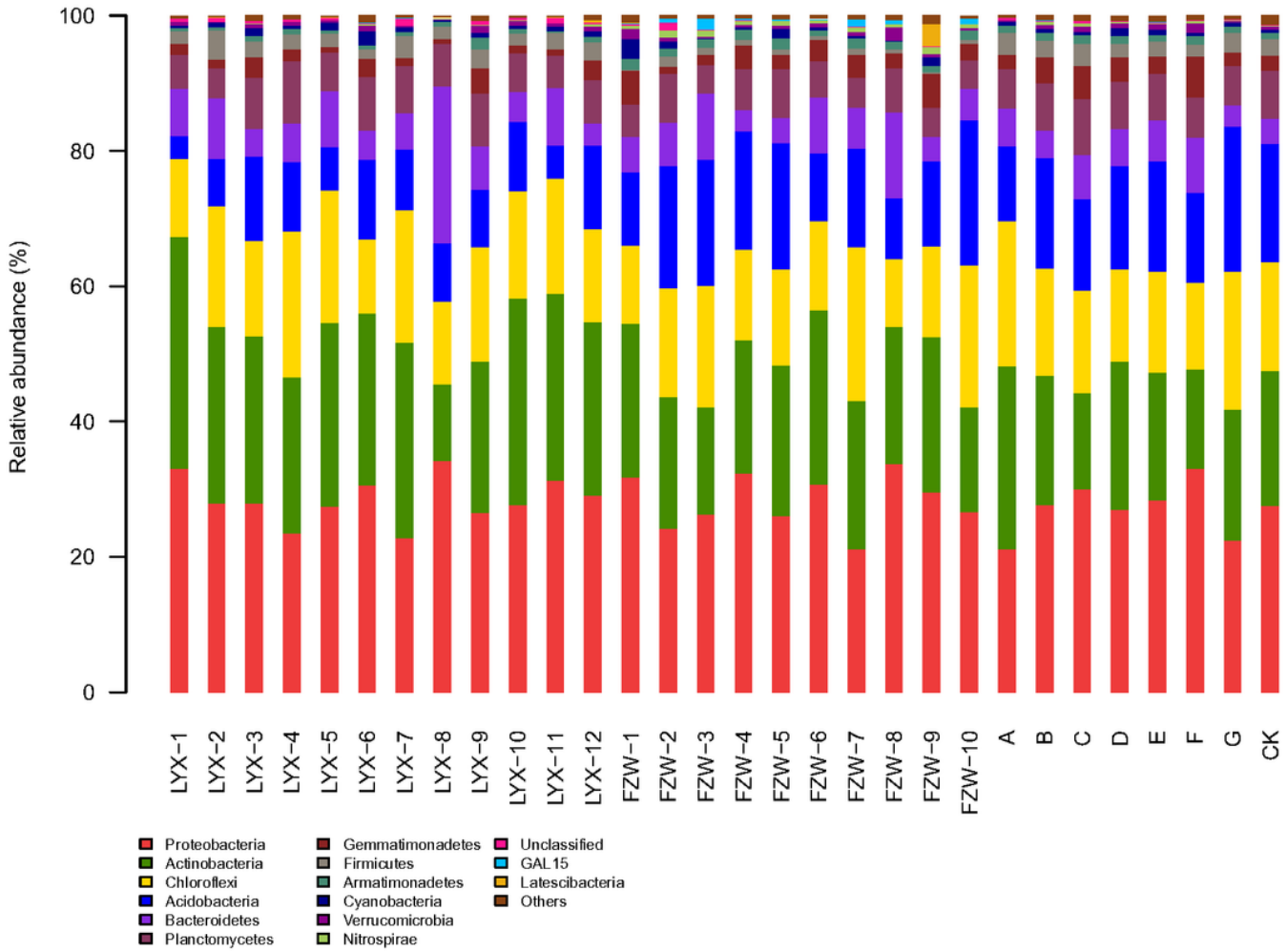

Figure 2

Relative abundances and community compositions of soil microbial phyla.

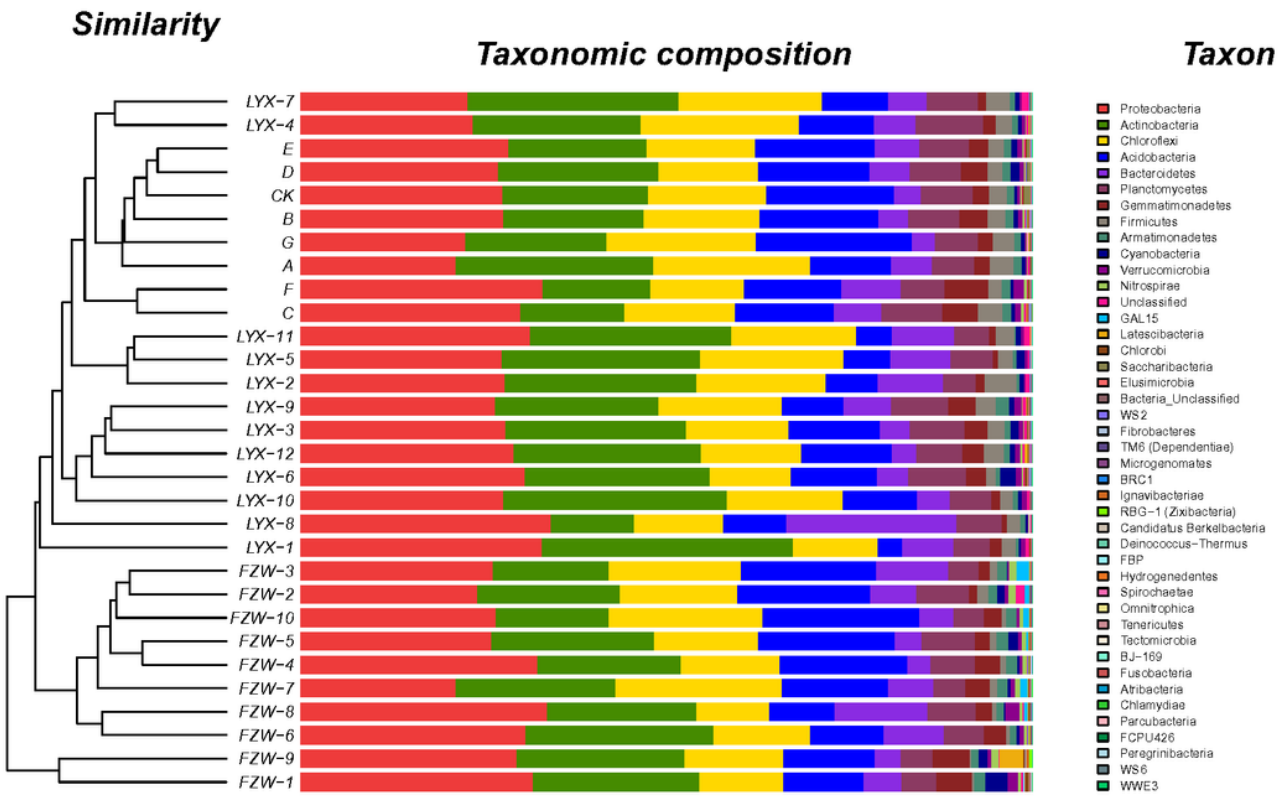

Figure 3

Microbial community barplot with cluster tree.

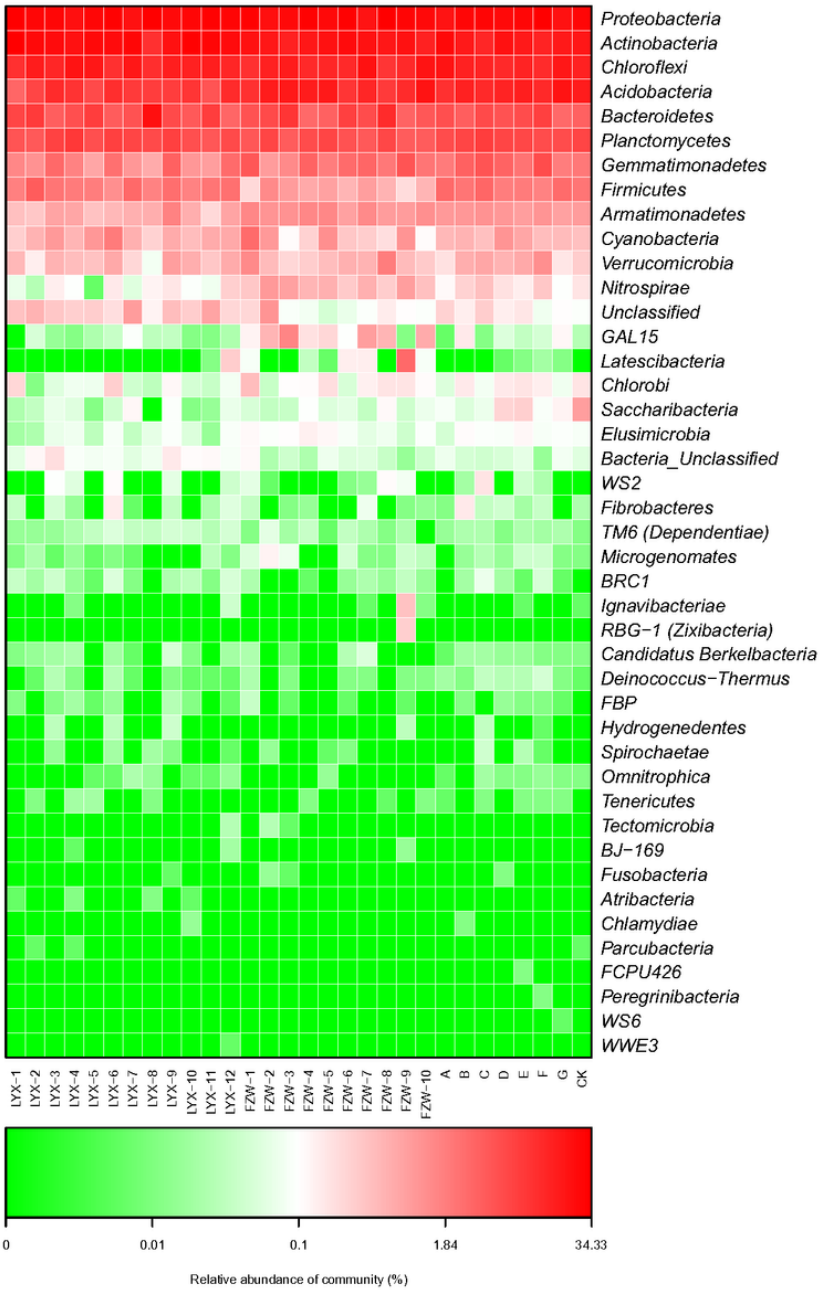

Figure 4

Microbial community heatmap analysis at the phylum level.

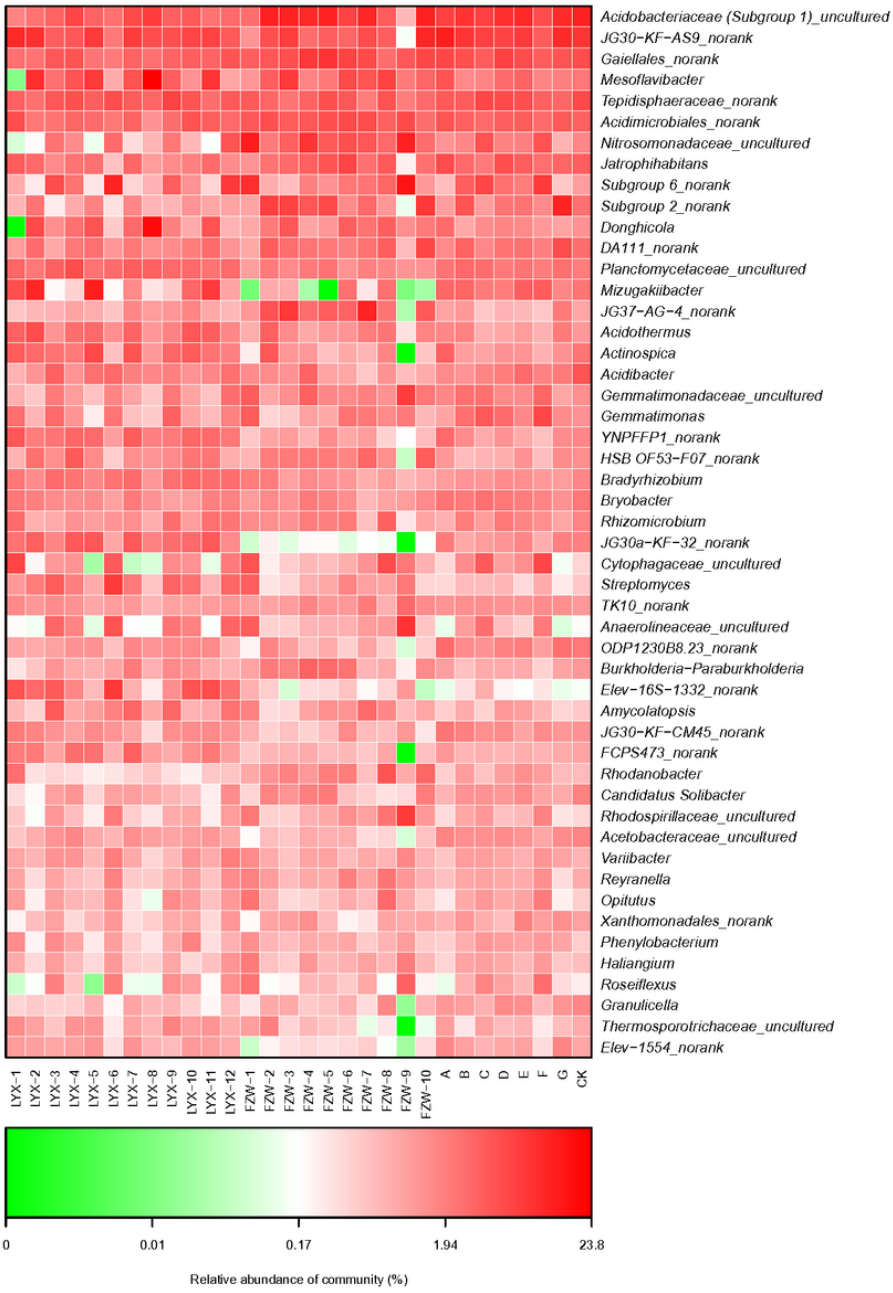

Figure 5

Microbial community heatmap analysis at the genuslevel.

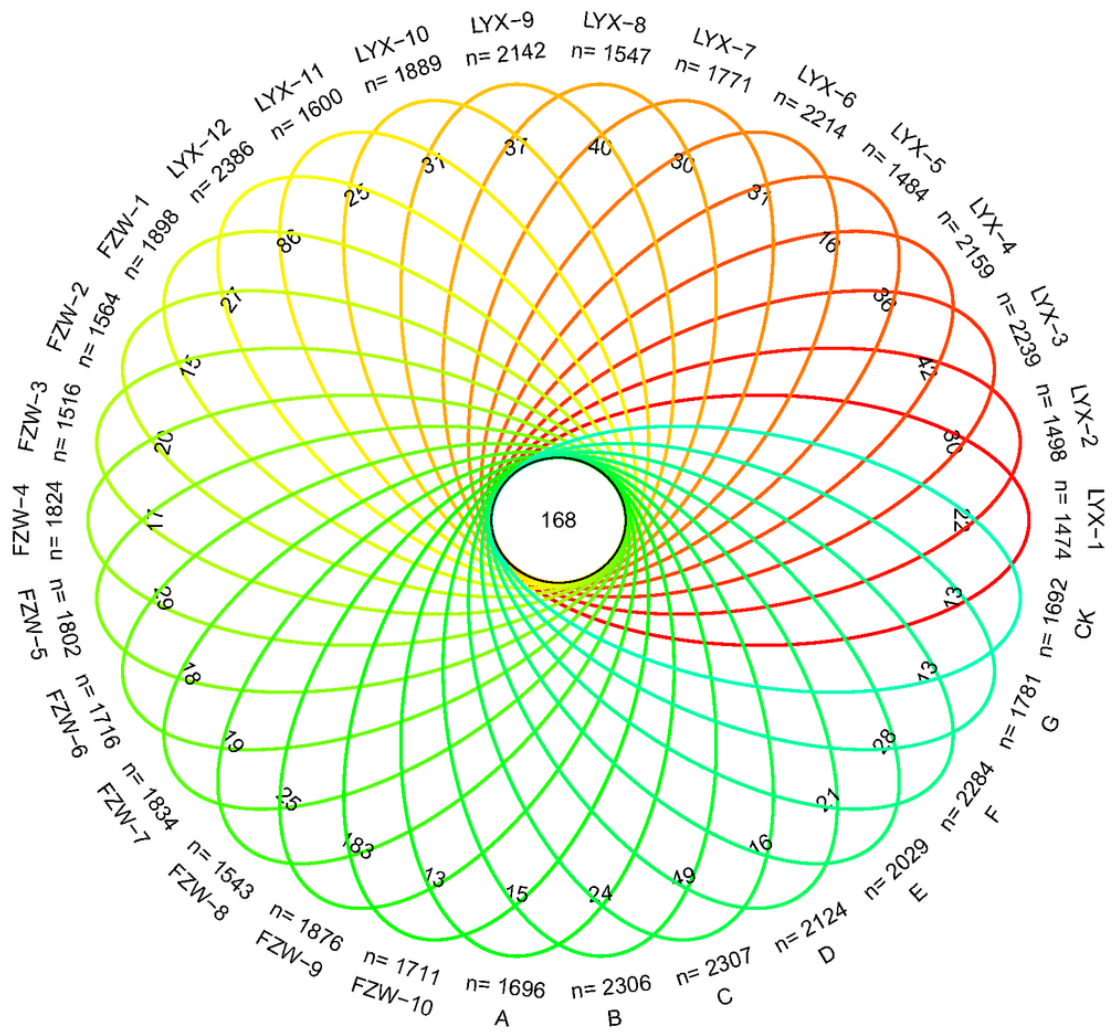

Figure 6

Venn map for the soil microbial community under different treatments.

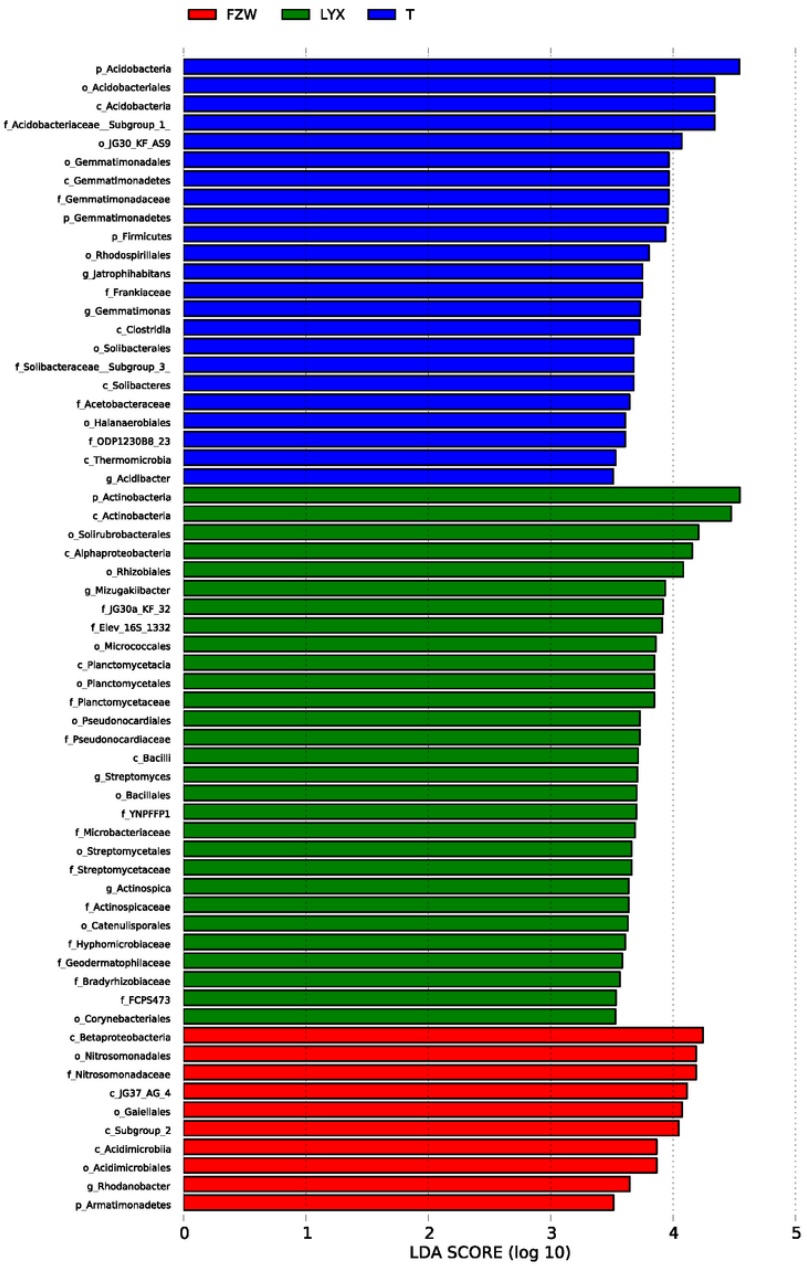

Figure 7

LDA scores for soil microorganisms at the phylum (p), class (c), order (o), family (f), genus (g), and species (s) levels. Only taxa with mean abundances >1% were considered to be significant.

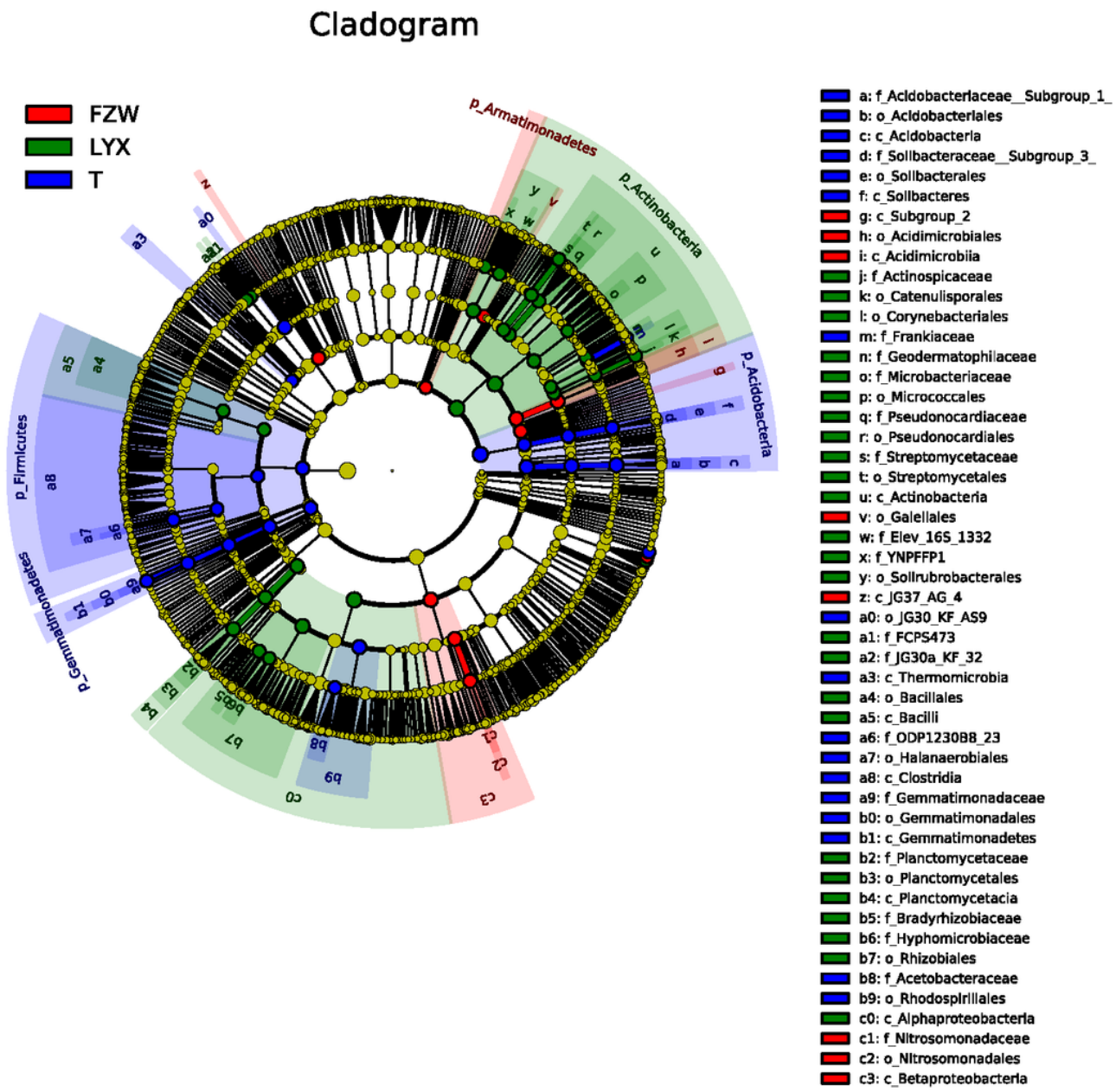

Figure 8

The LEfSe method was used to identify the significantly different abundant taxa of soil microorganisms. The taxa with significantly different abundances among the treatments are represented by colored dots, and from the center outward, they represent the kingdom, phylum, class, order, family, genus, and species levels. The colored shadows represent the trends of the significantly differed taxa. Each colored dot has an effect size LDA score, as shown in Figure 7.