Changes of Eukaryotic Microorganism Structures in Soil during Lettuce-spinach Rotation

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Abstract: The purpose of this study is to analyze and compare eukaryotic microorganisms in soil samples by rotation of lettuce and spinach. High-throughput sequencing technology was used to analyze the eukaryotes present in soil samples before and after crop planting and the effect of soil sucrase activity on eukaryotic communities, in order to provide data that will aid in alleviating the problems resulting from the continuous cropping of lettuce. The results showed that eukaryotic microorganism, such as *Heterodera*, *Pseudallescheria* and *Podosphaera species*, were inhibited by rotation of lettuce with spinach and had a certain effect on the recovery of soil quality. Rotation of lettuce with spinach could increase lettuce production and soil sucrase activity by 31.4% and 9.5%, respectively. It could improve the diversity of eukaryotic microbial community in soil.

1 INTRODUCTION

Soil microbial diversity is important for sustainable agricultural development, because microorganisms can participate in several biochemical processes that promote agricultural production, including plant nutrient recycling, soil structure maintenance and agricultural chemical degradation (Lanzén 2013). Soil microorganisms are mainly composed of bacteria, fungi, actinomycetes and some algae. Among them, soil eukaryotes play an important role in maintaining soil nutrients and biogeochemical cycles. There are many factors affecting soil microbial community, including soil characteristics, environmental conditions, crop management strategies and other factors, among which plant species and tillage are important factors affecting soil microbial community (Larkin, 2003). Soil enzyme activity is also an important indicator of microbial and biochemical processes, usually involving the decomposition and synthesis of soil organic matter, nutrient cycling and availability, soil fertility and quality, and its determination can be used to quantify and monitor changes in microbial community structure and activity, and soil Dynamic response index of organic matter to human disturbance (Raiesi, 2018). Researchers such as Lawton and Li found that the overall composition of species and microbial

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communities and planting methods with certain functional characteristics could change the structure and diversity of soil microbial communities and affected ecosystem functions (Lawton, 1994, Li, Liu, 2017).

Lettuce is one of the most popular raw leafy vegetables in China. So far, there have been few reports on the interaction of eukaryotic microorganisms in the soil during lettuce cultivation and with different planting methods. In soil microbiology research, soil quality indicators have been obtained by measuring changes in microbial activity and community structure over time. In this paper, the soil collection of the rotation of lettuce and spinach was used to analyze the changes in soil eukaryotic microbial community diversity under this planting method, in order to provide some theoretical basis for alleviating the obstacle of continuous cropping of lettuce.

2 MATERIALS AND METHODS

2.1 Site Description

The experiments were conducted in a plastic greenhouse in an experimental demonstration base (east longitude 116.14°, north latitude 40.19°) in Beijing, China. Prior to the experiments, mung beans were grown within the greenhouse for a long period of time. The annual average temperature in this area was 12.6 ℃, and the annual precipitation was 680.6 mm.

2.2 Experimental Design

The experimental setup the lettuce-spinach rotation group (S). The sample naming format was used S - planting year – cultivation number - 1 (before planting) / 2 (after planting) - soil depth. The adjacent lands were protected by two furrows that were 1.2 m wide and 6.5 m in length. The experiments were conducted between September 2016 and June 2017. Due to low temperatures during the winter, the experimental field used for the continuous cropping of lettuce was filled in, and no crops were planted during this period. Other treatments were consistent throughout the planting period. Randomly selected 20 × 20 cm area and the yield of lettuce in this area was weighed by weighing method. Each field soil sample was collected using a five-point sampling method, during which four samples were collected at each of four corners, and one sample was collected from the center of the field; samples were collected before and after crop cultivation at sampling depths of 0-10 cm and 10-20 cm. After the removal of residual leaves and roots, the soil samples were placed into aseptic sampling bags. The samples from each of the five points were combined and divided into two parts: one part was used for the experiments, while the other was stored at -40 ℃ for follow-up experiments. A description of each of the soil samples is given in Table 1.

Table 1: Description of soil samples.

Sample	Collection date	Depth (cm)	State of crop growth	Cultivation time
S.16.1.1.10	2016.09.09	0-10	Before cultivation	1st
S.16.1.1.20	2016.09.09	10-20	Before cultivation	1st
S.16.1.2.10	2016.10.20	0-10	Harvest	1st
S.16.1.2.20	2016.10.20	10-20	Harvest	1st
S.16.2.1.10	2016.10.27	0-10	Before cultivation	2nd
S.16.2.1.20	2016.10.27	10-20	Before cultivation	2nd
S.17.2.2.10	2017.03.10	0-10	Harvest	2nd
S.17.2.2.20	2017.03.10	10-20	Harvest	2nd
S.17.3.1.10	2017.03.21	0-10	Before cultivation	3rd
S.17.3.1.20	2017.03.21	10-20	Before cultivation	3rd
S.17.3.2.10	2017.04.27	0-10	Harvest	3rd
S.17.3.2.20	2017.04.27	10-20	Harvest	3rd
S.17.4.1.10	2017.05.23	0-10	Before cultivation	4th
S.17.4.1.20	2017.05.23	10-20	Before cultivation	4th
S.17.4.2.10	2017.06.20	0-10	Harvest	4th
S.17.4.2.20	2017.06.20	10-20	Harvest	4th

2.3 DNA Extraction and PCR Amplification

According to the manufacturer's instructions, the Mag-Bind® Universal Metagenomics Kit was used to extract DNA from 1.0 g of soil samples. The extracted DNA was measured by agarose gel electrophoresis (0.8%), and the DNA was quantified by an ultraviolet spectrophotometer. The extracted DNA was stored at -80°C for analysis. The V4-V5 region within the 18S rRNA gene was amplified from each sample using general eukaryotic primers TAReuk454F WD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAReu kREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Logares, R 2016) according to previously published protocols. PCR amplification was conducted using the Q5 high fidelity DNA polymerase (NEB, UK); Strictly control the number of amplification cycles to ensure that the number of cycles was as few as possible, and the amplification conditions of each batch of samples were consistent. The high-throughput sequencing of the 18S rRNA gene was conducted using the Illumina MiSeq PE300 platform at the Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China). The read sequences were deposited into the NCBI Sequence Read Archive under accession numbers SRP155301 and SRP154689.

2.4 Sequence Analysis

In order to integrate the original double-end sequencing data into our analysis, the sliding window method was used to individually screen double-end sequences in FASTQ format. The FLASH software (v1.2.7; http://ccb.jhu.edu/ software/FLASH/) was used to pair double-ended sequences through primary quality screening of overlapping bases. The sequencing results were analyzed using the QIME software (v1.8.0; http://qiime.org/). Sequences that met the following criteria were filtered out: (1) length<150 bp; (2) contained fuzzy bases; (3) number of mismatched bases in 5'-end primers > 1; (4) number of consecutive identical bases > 8. Chimeric sequences were verified and removed using USEARCH (v5.2.236; http://www.drive5.com/usearch/). The QIIME and UCLUST softwares were used to divide the operational taxonomic units (OTU) at 97% similarity; The most abundant sequence in each OTU was selected as the representative sequence of the OTU. Then, according to the number of sequences corresponding to each OTU in each sample, a matrix file containing the abundance of OTU in each sample was constructed. For each OTU representative sequence, used the default parameters in the QIIME software to compare the representative sequence with the template sequence in the Silva database to obtain the classification information corresponding to each OTU (Release 115; http://www.arb-silva.de).

3 RESUITS

3.1 Difference in Lettuce Yield and Soil Sucrase Activity

Lettuce grown in rotation with spinach yields were 4.78 kg/m2 and 6.28 kg/m2. The average yield of the two rotations is 5.53 kg/m2. The output of lettuce increased by 31.4% after rotation, significantly. In the process of lettuce rotation, the activity of invertase fluctuates greatly, and the overall level remained at 15.25 U/g. Sucrose activity increased when lettuce was harvested compared to pre-planting activity. The soil invertase activity in rotation treatment increased by 9.5% on average compared with that before planting. (Fig. 1). In the crop rotation soil samples, *Ascomycota*, *Chlorophyta*, Nematoda, Streptophyta, Apicomplexa, *Chytridiomycota*, *Arthropoda* and *Eustigmatophyceae* accounted for a large proportion of the eukaryotes phyla present during cultivation. In addition, *Eustigmatophyceae* appeared during lettuce cultivation, and *Bacillariophyta* was found in all of the 0-10 cm soil samples (Fig. 2).

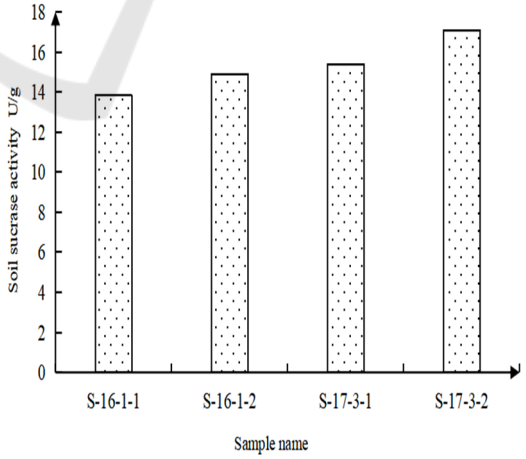

Figure 1: Soil sucrase activity in rotation cropping soil samples.

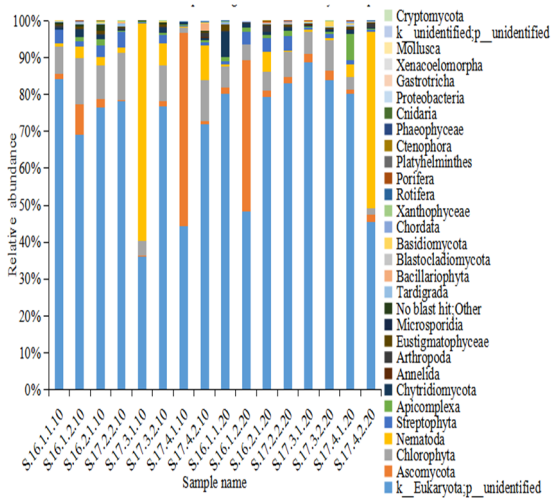

Figure 2: Relative abundance of the eukaryotes phyla presents in the crop rotation soil samples. Relative abundances are reported as the percentage of the total eukaryotes sequences.

3.2 Soil Eukaryotes Diversity and Community Structure during Crop Rotation

In the 0-10 cm soil samples, the diversity indices showed a rising trend during the cultivation of lettuce, with increases observed in the Chao1 (13.46~65.04%), ACE (14.05~63.79%), Simpson (2.90~50.35%) and Shannon (18.55~108.24%) indices. The diversity indices, including Chao1 (-12.14%), ACE (-12.21%), Simpson (-2.17%), and Shannon (-13.19%), decreased during the first cultivation of spinach. During the second spinach planting, the diversity indices, including Chao1 (36.95%), ACE (36.85%), Simpson (9.86%) and Shannon (32.35%), were increased. In the 10-20 cm soil samples, the diversity indices during lettuce cultivation were increased (Chao1, 6.48~11.89%; ACE, 6.35~12.97%; Simpson, -3.5~ -3.11%; Shannon, -10.70~ -9.52%. During the spinach planting period, all of the indices, including Chao1 (17.55~24.31%), ACE (16.75~22.56%), Simpson (-8.70~3.88%) and Shannon (-25.12~21.55%), were increased (Table 2).

Table 2: The proportion of the shared and unique OTUs in the harvest and cultivation soil samples from each cultivation period.

Samples	Chao1	ACE	Simpson	Shannon	Goods_coverage	Evenness
S.16.1.1.10	900.4000	880.5166	0.9421	5.5672	0.9978	0.5788
S.16.1.2.10	1021.5543	1004.2072	0.9694	6.6001	0.9980	0.6694
S.16.2.1.10	1068.1728	1085.3210	0.9722	6.8789	0.9986	0.6860
S.17.2.2.10	938.4615	952.7641	0.9511	5.9713	0.9991	0.6055
S.17.3.1.10	670.1648	681.4558	0.6239	2.8731	0.9981	0.3126
S.17.3.2.10	1106.0067	1116.1486	0.9381	5.9829	0.9977	0.5978
S.17.4.1.10	820.0132	821.0723	0.8845	5.2434	1.0000	0.5417
S.17.4.2.10	1123.0038	1123.6392	0.9718	6.9342	1.0000	0.6843
S.16.1.1.20	998.0000	998.0000	0.9645	6.9054	1.0000	0.6931
S.16.1.2.20	1116.6429	1127.4693	0.9307	6.2483	0.9994	0.6173
S.16.2.1.20	831.0000	843.8775	0.9412	5.9878	0.9983	0.6239
S.17.2.2.20	1033.0339	1034.2674	0.9777	7.2781	0.9999	0.7269
S.17.3.1.20	939.9874	958.8076	0.9654	6.6445	0.9987	0.6752
S.17.3.2.20	1000.9198	1019.7156	0.9354	5.9335	0.9991	0.5961
S.17.4.1.20	878.6081	882.2762	0.9696	7.0500	0.9993	0.7227
S.17.4.2.20	1032.8430	1030.0534	0.8852	5.2790	0.9977	0.5347

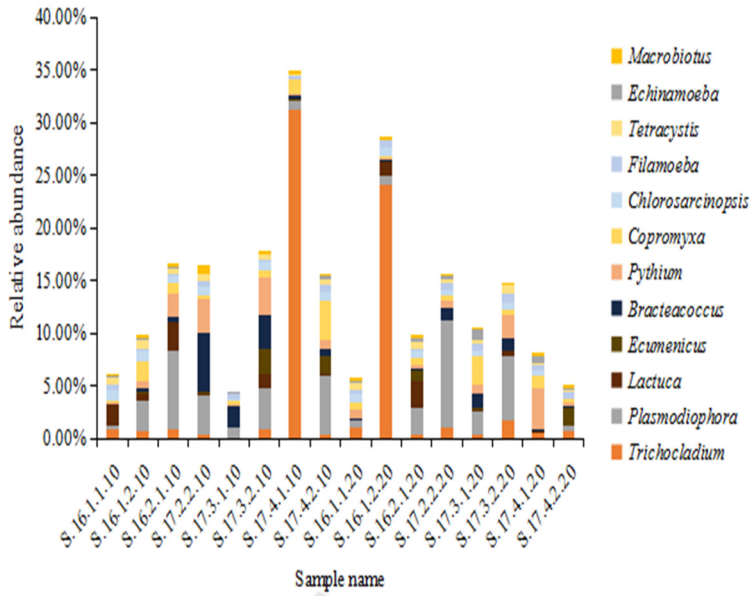

Figure 3: Relative abundance of different eukaryotic genera in the crop rotation soil samples

During crop rotation, *Ecumenicus*, *Bracteacoccus*, *Pythium*, *Chlorosarcinopsis*, *Tetracystis* and *Macrobiotus* species showed an upward trend in the 0-10 cm soil samples obtained during the cultivation of lettuce and spinach. In the 10-20 cm soil samples, *Trichocladium*, *Plasmodiophora* and *Filamoeba* species showed an increasing trend during the rotation process, while *Copromyxa* and *Echinamoeba* species showed a downward trend. There were also a number of eukaryotic genera that demonstrated opposing trends during the cultivation of lettuce and spinach, such as *Lactuca*, whose species increased in number during lettuce cultivation but decreased during the spinach planting period in the 10-20 cm soil samples (Fig. 3).

4 DISCUSSION

Sucrase is an important enzyme to characterize the biological activity intensity of soil, which is positively correlated with the content of humus, organic matter and clay, the content of N and P, the number of microorganisms and the respiration intensity (Wang, 2005). In this experiment, the activity of sucrase and the yield of lettuce both increased 9.5% and 31.4%, respectively, after rotation. *Nematoda* were the most dominant faunal group in this experiment, which is highly diverse, ranked third in terms of richness during these experiments. In the soil samples obtained during crop rotation, the changes in *Nematoda* were different due to the different crops that were planted. Among them, *Heterodera* species showed an opposing trend during the process of lettuce and spinach cultivation.

In this experiment, changes in the genus of some fungi were also different, these differences in eukaryotic community compositions might be the result of the different crops. Some studies have found that members of *Ascomycota* are the main soil fungal decomposers (Ma, 2013). Interestingly, in the crop rotation soil samples, this phylum was increased during lettuce cultivation and decreased during spinach cultivation, due to different planting methods. After the lettuce-spinach rotation the relative abundance of genus such as *Bacillus*, *Pseudomonas*, *Sphingomonas*, *Nitrospira* and *Zephyr* in soil is higher than the crop rotation. But the the relative abundance of *Acidocaldarius* was going low. Research findings after rotation, the proportion of bacteria in the soil, such as *Acidbacteria*, *Firmicutes* and *Fusarium*, is less than that of crop rotation soil. And *Proteobacteria* and *Bacillus* constitute the dominant flora in the soil in rotation (Pieterse, 2014). *Pseudomonas* and its secondary metabolites, including the antibiotic 2,4-diacetylphloroglucinol (DAPG), protect plants directly by inhibiting plant pathogens by inducing plant systemic resistance (Zhang, 2018).

5 CONCLUSIONS

The results showed that crop rotation of spinach and lettuce could effectively control increased in *Heterodera*, *Pseudallescheria* and *Podosphaera* species. However, some eukaryotes species, such as those of *Fusarium* and *Plasmodiophora*, did not decrease during crop rotation. Further experiments were needed to identify the specific components of spinach root exudates that have inhibitory effects on eukaryotes. In addition, rotation with spinach improved soil sucrase activity and lettuce yield.

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