Changes of Eukaryotes Microorganism Structures in Soil during Continuous Cropping of Lettuce

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Abstract: This study aimed to analyse eukaryotes in soil during the continuous cropping of lettuce. High-throughput sequencing technology was used to analyze the eukaryotes present in soil samples before and after crop planting, in order to provide data that will aid in alleviating the problems resulting from the continuous cropping of lettuce. The results showed that Trichocladium, Chlorosarcinopsis, Hindakia, Zea, Diploscapter and Tylenchorhynchus species were increased during continuous cropping. It indicates that the continuous cropping of lettuce affected the eukaryotic microbial community.

1 INTRODUCTION

Soil microbes consist mainly of bacteria, fungi, actinomycetes and some algae, all of which play important roles in the ecological environment and constitute the core of the soil ecosystem in terms of maintaining soil quality and health (Vessey 2003). Because of the rapid response to environmental changes, microorganisms is regarded as an effective biological indicator to assess soil conditions and land management success (Chen 2012). Soil eukaryotes play important roles in the maintenance of soil nutrients and in biogeochemical cycles.

However, soil microbiology studies, at present, mainly assess microbial biomass, the soil respiration rate, and other quantitative aspects. Analysis of soil microbial biomass may only reflect the influence of some functionally specialized microorganisms (Tang 2007). The microbial respiration rate can be regarded as an index of the total number of active soil microorganisms. Neither of these parameters are able to measure changes in the composition of the microflora. To obtain complete knowledge of changes in soil quality in terms of the microorganisms in soil at the genus or species level, the determination of soil microbial diversity and microbial structure should be combined. The measurement of changes in microbial activity and community structure over time has been considered to be a better indicator of soil quality. Lawton and other researchers have proposed that, in addition to the richness of species, the presence of species with certain functional properties and the overall composition of the microbial community can affect ecosystem function (Lawton 1994). During research into the use of continuous planting and rotation in the farming of eggplants, Li found that continuous planting changed the structure and diversity of the microbial community in soil (Li 2017).

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The purpose of this study was to investigate the impact of the continuous cropping of lettuce. The eukaryotes community structure was examined using high-throughput sequencing technology.

2 MATERIALS AND METHODS

2.1 Site Description

The test was carried out in a plastic greenhouse at a test demonstration base in Beijing (116.14°E longitude, 40.19°N latitude). Before the experiment, mung beans were grown in the greenhouse for a long time. The annual average temperature was 12.6℃, and the annual precipitation was 680.6 mm.

2.2 Experimental Design

The adjacent land was protected by two ditches 1.2 m wide and 6.5 m long. These experiments were conducted between September 2016 and June 2017. Due to the low temperature in winter, the experimental field for continuous lettuce cultivation was landfilled and no crops were planted during this period. Other treatments were consistent throughout the planting period. An area of 20 × 20 cm was randomly selected, and the lettuce production in this area was weighed by a weighing method. The field soil samples were collected by the five-point method, four samples were collected at the four corners, and one sample was collected at the center of the field; samples were collected at a sampling depth of 0-10 cm and 10-20 cm before and after crop planting. After removing the residual leaves and roots, putted the soil sample in a sterile sampling bag. Combined the five-point samples and divided them into two parts: one part was used for experiments, and the other part was stored at -40 ℃ for subsequent experiments. Table 1 gives the description of each soil sample. The lettuce continuous cropping group (N) naming format used was N - planting year - cultivation number - 1 (before planting) / 2 (after planting) - soil depth.

Table 1: Description of soil samples.

Sample	Collection date	Depth (cm)	State of crop growth	Cultivation time
N.16.1.1.10	2016.09.09	0-10	Before cultivation	1st
N.16.1.1.20	2016.09.09	10-20	Before cultivation	1st
N.16.1.2.10	2016.10.20	0-10	Harvest	1st
N.16.1.2.20	2016.10.20	10-20	Harvest	1st
N.17.2.1.10	2017.03.10	0-10	Before cultivation	2nd
N.17.2.1.20	2017.03.10	10-20	Before cultivation	2nd
N.17.2.2.10	2017.03.21	0-10	Mid-cultivation	2nd
N.17.2.2.20	2017.03.21	10-20	Mid-cultivation	2nd
N.17.2.3.10	2017.04.27	0-10	Harvest	2nd
N.17.2.3.20	2017.04.27	10-20	Harvest	2nd
N.17.3.1.10	2017.05.23	0-10	Before cultivation	3rd
N.17.3.1.20	2017.05.23	10-20	Before cultivation	3rd
N.17.3.2.10	2017.06.20	0-10	Harvest	3rd
N.17.3.2.20	2017.06.20	10-20	Harvest	3rd

2.3 DNA Extraction and PCR Amplification

DNA was extracted from 1.0 g of soil sample using the Mag-Bind® Universal Metagenomics Kit according to the manufacturer's instructions. The quality of the extracted DNA was determined using agarose gel electrophoresis (0.8%), and the DNA was quantified using a UV spectrophotometer. The extracted DNA was stored at -80℃ prior to 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 2016) according to previously published protocols. PCR amplification was conducted using the Q5 high fidelity DNA polymerase (NEB, UK); the number of amplification cycles was strictly controlled to ensure that the least number of cycles were used as

possible and the amplification conditions used for 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

To integrate the original double-ended sequencing data into our analysis, a sliding window method was used to individually screen the double-end sequences in FASTQ format. The FLASH software (v1.2.7; http://ccb.jhu.edu/ software/FLASH/) was used to pair the double-ended sequences via a primary quality screen of the 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, the matrix file containing the OTU abundances in each sample was constructed. For each OTU representative sequence, the default parameters were used in the QIIME software to obtain the taxonomic information corresponding to each OTU by comparing the representative sequence to the template sequence in the Silva database (Release 115; http://www.arb-silva.de).

3 RESULTS

3.1 Lettuce Yield

The yields of continuous cropping lettuce were 4.88 kg/m^2, 5.54 kg/m^2 and 5.29 kg/m^2 respectively.

3.2 Soil Eukaryotes Diversity and Community Structure during Continuous Cropping

After the DNA sequences obtained from the soil samples were trimmed and filtered for quality and chimeric reads, pyrosequencing was conducted. These experiments resulted in a total of 2 995 049 sequence reads that were obtained from eukaryotes 18S rRNA in 30 soil samples. The sequences that had a similarity of greater than 97 % were classified as belonging to the same OTU. The description of the indices that were used, including ACE, Chao1, Shannon, Simpson, goods-coverage and Simpson- evenness, are shown in Table 2. During the first cultivation period, the Chao1 (78%), ACE (83%), Simpson (2%), and Shannon (2%) indices for the 0-10 cm harvest soil samples were increased compared with those for the samples obtained before cultivation, and the same trend was observed for the 10-20 cm soil samples, for which the Chao1, ACE, Simpson and Shannon indices were increased by 4%, 2%, 2% and 4%, respectively. During the second cultivation period, the Chao1 (-1%) and ACE (-2%) indices were decreased for the 0-10 cm soil samples, in contrast with the Simpson (1%), and Shannon (1%) indices, which were increased. For the 10-20 cm soil samples, all of the diversity indices, including Chao1 (-34%), ACE (-32%), Simpson (-23%), and Shannon (-40%), were decreased. The same phenomenon was observed during the third cultivation period; however, compared to the first period, all of the diversity indices were increased. For the 0-10 cm samples, the increases were as follows: Chao1 (113%), ACE (115%), Simpson (13%) and Shannon (63%); for the 10-20 cm samples, the increases were: Chao1 (14%), ACE (17%), Simpson (17%) and Shannon (62%). The good-coverage index was between 99.7%-100%, indicating that the sequencing depth was sufficient to cover all of the species present in the sample (Table 2).

In contrast, there were 414 shared OTUs (7.21%) found in all of the soil samples, and there were 436 unique OTUs (45+37+48+92+214; 7.59%) (Fig.1), accounting for 3.6%, 2.4%, 3.1%, 6.0%, and 16.6% of the total number of OTUs in each sample, respectively. The overall trend in the proportion of unique OTUs in the soil samples was observed to be one of gradual increase, which indicates that the planting of lettuce affected the eukaryotic microbial community. The proportion of unique OTUs increased during the first cultivation period but declined during the second and third cultivation periods. During each cultivation period, the

proportion of shared OTUs in the harvest and cultivation soil samples decreased as the cultivation frequency increased (Table 3).

At the genus level, there were significant differences in the species present in soil at different depths. In the 0-10 cm soil samples, *Mortierella* and *Lactuca* species decreased over time during the three periods of lettuce planting. In contrast, *Trichocladium*, *Chlorosarcinopsis*, *Diploscapter*, *Hindakia*, *Tylenchorhynchus* and *Zea* species increased over the same period of time. In addition, some eukaryotes were affected by the idle period; *Chlamydomonas*, *Copromyxa*, *Pterygota*, *Desmochloris*, *Heterococcus* and *Rubus* species showed a rise-fall-rise trend, while *Plasmodiophora* and *Alogomyces* species presented a fall-rise-fall trend. In the 10-20 cm soil samples, *Fusarium*, *Trichophaeopsis*, *Pseudallescheria* and *Orbicula* species increased during continuous cropping, while *Mortierella*, *Eocercomonas*, *Pythium*, *Chlorosarcinopsis*, *Tetracystis*, *Macrobiotus*, and *Gallus* species demonstrated a rise-fall-rise trend. Additionally, *Stachyamoeba* species showed a fall-rise-fall trend (Fig. 2).

Table 2. Eukaryotes diversity within the continuous cropping soil samples.

Samples	Chao1	ACE	Simpson	Shannon	Goods_coverage	Evenness
N.16.1.1.10	517.0000	517.0000	0.8408	4.4452	1.0000	0.4931
N.16.1.2.10	919.5301	945.0977	0.8612	4.5395	0.9977	0.4662
N.17.2.1.10	1202.7557	1220.3624	0.9064	5.6456	0.9974	0.5571
N.17.2.2.10	1103.1321	1115.4176	0.8984	5.4381	0.9975	0.5444
N.17.2.3.10	1188.3860	1199.2055	0.9190	5.6780	0.9976	0.5610
N.17.3.1.10	621.0000	621.3391	0.8673	4.4233	1.0000	0.4767
N.17.3.2.10	1320.7807	1337.8595	0.9766	7.2261	0.9978	0.7010
N.16.1.1.20	1031.8201	1043.0437	0.8846	5.5050	0.9982	0.5538
N.16.1.2.20	1068.5283	1063.3434	0.9066	5.7253	0.9983	0.5734
N.17.2.1.20	1207.3285	1204.0233	0.9023	5.7121	0.9978	0.5633
N.17.2.2.20	1239.6391	1229.8505	0.8946	5.6729	0.9976	0.5584
N.17.2.3.20	798.2095	823.3581	0.6946	3.4453	0.9979	0.3616
N.17.3.1.20	1070.6346	1035.2957	0.8375	4.5212	0.9972	0.4597
N.17.3.2.20	1220.2435	1213.2584	0.9769	7.3027	0.9981	0.7178

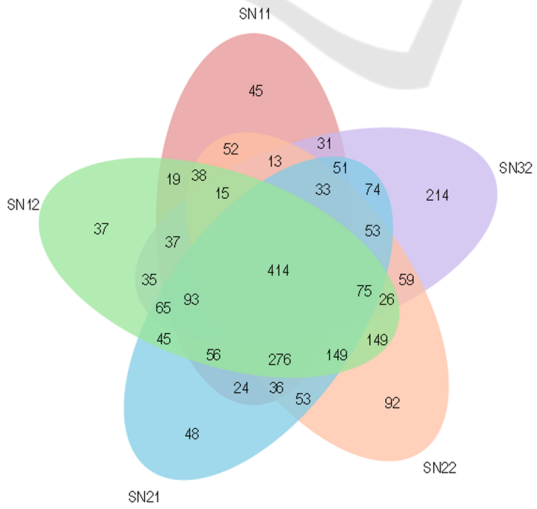

Figure 1: Venn diagram showing the shared eukaryotes OTUs (at a distance of 0.03) in the continuous cropping soil samples. SN11, before the first planting; SN12, after the first planting; SN21, before the second planting; SN22, after the second planting; SN32, after the third planting.

Table 3: The proportion of shared and unique OTUs in the harvest and cultivation soil samples from each cultivation period.

	Unique OTUs in cultivation soil samples (%)	Unique OTUs in harvest soil samples (%)	Shared OTUs (%)
1st	15.7	32.0	52.3
2nd	22.9	22.3	54.8
3rd	39.6	28.1	32.3

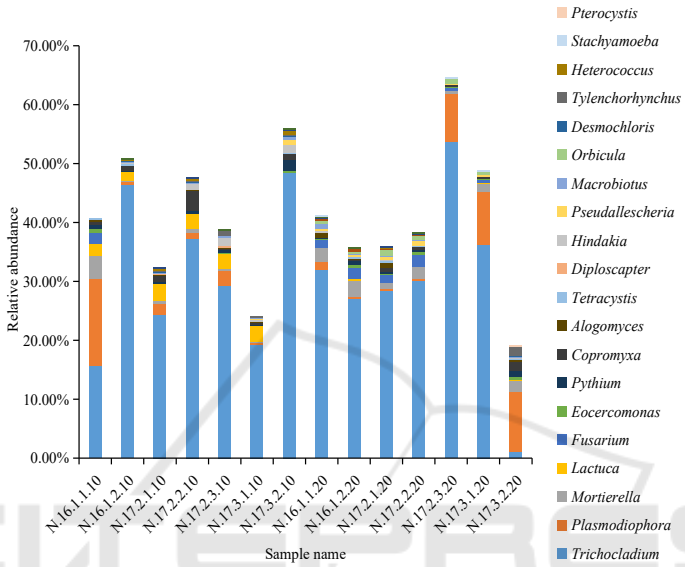

Figure 2: Relative abundance of different eukaryotic genera in the continuous cropping soil samples.

4 DISCUSSION

The main purpose of this study was to analyze the changes in eukaryotes communities in soil during the continuous cropping of lettuce via the high-throughput sequencing of microbes in soil samples.

Nematoda are the most dominant faunal group in this experiment, which is highly diverse, ranked third in terms of richness during these experiments. There are approximately 100,000 - 1 million nematodes worldwide, which account for 80% of the total number of animals (Parkinson 2004). *Nematoda* are divided into three types of species, namely, plant-feeding nematodes, bacterial-feeding nematodes, and fungal-feeding nematodes; some of these species cause economic losses during plant cultivation (Mcsorley 2016). During the continuous cropping process, the abundance of *Nematoda* in the soil was gradually increased as the planting times were increased. *Prismatolais* and *Ceratoplectus* were two bacterial nematode genera that appeared during the third planting. Studies have shown that nematodes that feed on bacteria have a great potential to function as predators of soil bacteria (Xiao 2014). When nematodes reach a certain abundance, their feeding reduces the number and activity of bacteria, which results in a reduction in nitrogen mineralization due to the consumption of fixed and nutritive components (Mao 2005). Compared with the early stages of planting, the number of nematodes that feed on fungi in the soil was increased; *Aphelenchoides* species were increased in the 0-10 cm soil, samples, while the percentage of *Aphelenchus* and *Tylencholaimus* species were increased in the 10-20 cm soil samples. This may be related to the increase in the amount of eukaryotes in the soil that resulted from the increase in planting times.

5 CONCLUSION

Using Illumina MiSeq to sequence eukaryotes 18S rRNA, changes in eukaryotes community structures occurring during the continuous cropping of lettuce

were observed. The results showed that *Trichocladium*, *Chlorosarcin- opsis*, *Hindakia, Zea, Diploscapter* and *Tylenchorhynchus* species were increased during continuous cropping.

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