

## Article

# Dynamics of a Soil Fungal Community in a Three-Year Green Garlic/Cucumber Crop Rotation System in Northwest China

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**Abstract:** A decreased soil biological environment in terms of an unstable microbial population and diversity index is primarily caused by intensive continuous cropping systems. In this study, a crop rotation system was established to evaluate the soil fungal diversity using a pyrosequencing-based analysis during three successive growing seasons (2013, 2014, and 2015) under a field pot investigation. We found that overall green garlic/cucumber rotational effects increased cucumber productivity under these growing practices. Analysis of fungal communities in rhizosphere soils by high-throughput pyrosequencing showed that the estimated treatment effects were more obvious during the three-year trials, and OTU richness was much higher than the control in cucumber-planted soils. The relative frequencies of fungal diversity showed variable responses before and after rotation practices, and mainly  $\alpha$ -diversity of fungi increased in garlic planted soil and again decreased after cucumber planted soil in each year. The phylogenetic classification illustrated that the fungal communities were dominated by the taxa *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, and *Zygomycota* across all samples. Particularly, the relative abundance of the taxon *Ascomycota* was largely and predominantly enriched with the increasing number of garlic bulbs during the three years. Moreover, the majority of abundant taxa positively correlated with available P and K contents, while being highly negatively correlated with soil pH, EC, and SOC. These results indicate that garlic-cucumber-based crop rotations induce fungal richness and diversity and promote the sustainability of agricultural ecosystems, thus enhancing crop growth and production.

**Keywords:** *Allium sativum*; *Cucumis sativus*; sustainable agriculture; fungal diversity

## 1. Introduction

Cucumber (*Cucumis sativus* L.) is an important cash vegetable crop widely cultivated in continuous cropping under protected conditions in China. Commercial greenhouse cucumber production has greatly expanded in the northwest region due to the maximum economic return and high yield, and continuous cropping has emerged as a popular cultivation trend among farmers [1]. However, a decline in cucumber productivity and soil microbiology degradation has become more severe even though it is renowned for having a short turnover. This cropping system under intensive crop production regimes is largely associated with several detrimental factors via the consumption of excessive inputs and agrochemicals. These harmful conditions caused by continuous mono-cropping include the deterioration of soil quality indexes, increased soil-borne pathogens, and decreased crop yield and plant biomass, all of which further exacerbate soil sickness problems and autotoxicity. Moreover, functional diversity in microbial populations is also significantly restricted by continuous cropping obstacles, which is unacceptable for the sustainable production of vegetable crops [2–4]. Therefore,

innovative approaches could prevent these problems in order to meet the sustainable food production target. Meanwhile, the maintenance of microbial diversity and composition is also a very important consideration in this situation. Subsequently, recent research assessing the effects of ecology-based cropping systems has gained more attention by demonstrating long-term positive plant-soil feedback where production is simultaneously environmentally friendly [5,6]. Such practices, including organic crop fertilization, cover crops, multi-cropping, and biological pest control, are well-known agricultural practices that have been widely used to maintain soil microbial ecology in China [7–11].

The optimization of ecological services for better crop management, utilizing multiple cropping systems, is a good choice for growing a variety of crops in sequence while providing several ecosystem functions in the same space. In the multi-cropping system, crop rotation is the most efficient and alternative way to boost crop yield and reduce the incidence and severity of soil-related factors [12,13]. There is increasing evidence that crop rotation could be a possible substitute for chemical fertilizers and pesticides by regulating several plant nutrition functions, such as increased nutrient uptake [14–16] and improved mineral use efficiency [17] and nutrient leaching recovery [18], as reported in conventional or organic and low-input systems.

Crop rotation has been utilized as a soil ecological conservation practice that triggers the belowground microbial community, creating overall good soil geometry and a healthy soil profile, which can indirectly influence cropping productivity and environmental sustainability [14,15,19]. A number of rotating crops with deep root systems or shallow-rooted vegetables during fallow periods are often employed for soil biodiversity purposes because they supply vegetative residues and food sources, reduce the buildup and carryover of soil-borne disease organisms and insect pests (breaks disease and pest cycles), and can help create favorable growing conditions for healthy, well-developed crop root systems. Additional plant diversity resulting from frequent rotation can stimulate a greater variety of soil microbial activity/diversity, enhance carbon and nutrient cycling, and promote root health [12,20].

In addition, studies have demonstrated that the impact of crop rotation in response to various cultural treatments strongly affected the soil configurations and had a variable influence on the ecological microclimate. Soil microbial diversity is highly correlated with some soil parameters, such as organic matter, soil microbial biomass, and enzymatic activities [21–23].

Soil microorganisms comprising fungi, bacteria, actinomycetes, protozoa, and nematodes are major soil entities that perform numerous activities, including nutrient cycling [24], ecosystem functioning [25], soil structure formation, nitrogen fixation [26,27], organic matter decomposition [28], and toxin removal [29,30]. Research on microbial communities in the soils of continuous cropping systems has mainly focused on bacteria [2,31,32]. A wealth of bacterial groups has been shown to contribute to disease suppression through antibiosis, plant growth promotion, or systemic induced resistance [33–35]. However, several studies have suggested that fungi are one of the key constituents of a microbial population and can influence the soil structure stability and fertility and perform a wide range of critical ecological functions, including decomposition, parasitism, pathogenesis, and symbiosis. In addition, soil with a higher disease suppressive potential has been found to exhibit higher fungal diversity, which plays a very important role in improving plant health [36]. Despite their high biodiversity and their ecological and economical roles, soil fungal genetic and functional diversity are still poorly studied relative to soil bacterial communities in intensive vegetable production systems in China.

Adopting crop rotation to enhance microbial diversity may require the use of specific crop combinations that are expected to have a greater effect on soil microbial diversity. Garlic (*Allium sativum* L.) has effectively been incorporated into crop rotation systems as a companion crop due to its allelopathic and antimicrobial effects [37–40]. It has been reported that crop rotation with garlic can prevent insect attacks and plant invasions [41–43]; moreover, garlic root exudate was shown to significantly inhibit vegetable pathogens [37]. Furthermore, crop rotation with garlic can alleviate problems associated with the continuous cropping of some vegetables under protected

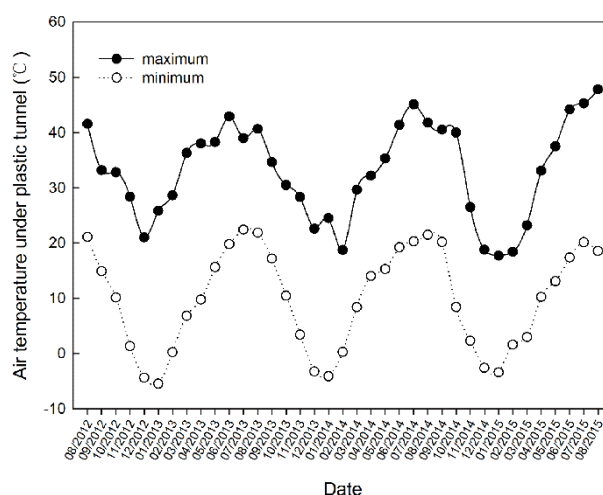
cultivation [38,39,44]. Unfortunately, no scientific reports have studied the variation of soil microbial diversity in a garlic rotation system by using modern biological tools. Therefore, it is important to explore the invisible plant-microbe-soil interactions in the garlic rotation system.

In this study, we employed a high-throughput pyrosequencing method to reveal the soil indigenous fungal diversity and community structure in a three-year green garlic/cucumber crop rotation system relative to mono-cropping in a pot experiment in China. We compared cucumber mono-cropping with to a three-year green garlic/cucumber crop rotation system to pursue the following objectives: (1) to determine the yield increase of cucumber; (2) to determine the dynamics of the soil fungal community; and (3) to evaluate the correlation between the richest fungal communities and soil physico-chemical features

## 2. Materials and Methods

### 2.1. Site Description and Experimental Design

A field pot-based experiment was conducted from August 2012 to July 2015 in a plastic tunnel at the Horticultural Experimental Station (34°17' N, 108°04' E), College of Horticulture, Northwest A&F University, Yangling, Shaanxi Province, China. The temperature in the plastic tunnel was recorded during this experiment (Figure 1). Fresh soil that had not been planted with garlic or cucumber within the previous ten years was utilized. The soil of the experimental site is classified as having a sandy loam texture with the following physicochemical characteristics: electrical conductivity (1:5 water) 138.5  $\mu\text{S}\cdot\text{cm}^{-1}$ , pH (1:2.5 water) 7.96, organic matter 18.176  $\text{g}\cdot\text{kg}^{-1}$ , total nitrogen (N) 1.759  $\text{g}\cdot\text{kg}^{-1}$ , total phosphorus (P) 1.004  $\text{g}\cdot\text{kg}^{-1}$ , total potassium (K) 16.632  $\text{g}\cdot\text{kg}^{-1}$ , available N 60.67  $\text{mg}\cdot\text{kg}^{-1}$ , available P 63.72  $\text{mg}\cdot\text{kg}^{-1}$ , and available K 305.333  $\text{mg}\cdot\text{kg}^{-1}$ ; the invertase, urease, catalase, and alkaline phosphatase activities were 14.29 glucose  $\text{mg}\cdot\text{g}^{-1}$ , 1.864  $\text{NH}_3\text{-N mg}\cdot\text{g}^{-1}$ , 11.18 0.01 N  $\text{KMnO}_4 \text{ mL}\cdot\text{g}^{-1}$ , and 0.515  $\text{P}_2\text{O}_5 \text{ mg}\cdot\text{g}^{-1}$ , respectively.



**Figure 1.** Monthly maximum and minimum air temperatures in the plastic tunnel in the three experimental years from August 2012 to August 2015.

At the trial site, the crop management practice of a garlic-cucumber rotation was practiced during three years of experimentation, and the garlic-focused rotations consisted of six treatments with garlic bulbs, which were evaluated in each growing season. The garlic rotation was considered a sustainable rotation versus cucumber continuous mono-cropping (termed 'conventional' cropping system, as this was common commercial practice in the region). The sequence of the different numbers of garlic bulbs according to treatment was as follows: 5, 10, 15, 20, and 25 garlic bulbs (cucumber/green garlic ratios of 2:5, 2:10, 2:15, 2:20, and 2:25, presented as T<sub>5</sub>, T<sub>10</sub>, T<sub>15</sub>, T<sub>20</sub>, and T<sub>25</sub>, respectively). All the pots were

organized in a randomized block design with five replications (one pot per replication), and each plastic pot (38 cm diameter  $\times$  33 cm height) received fifteen kilograms of soil. The cucumber cultivar “Bo-Nai 13B” and the garlic cultivar “G064” were provided by the horticultural experimental station.

Garlic bulbs of a uniform size (approximately 5 cm diameter, 4 cm height, 45 g in weight) were chosen and planted in pots on 15 August 2012 and grew until 13 March 2013. Green sprouts were harvested three times during the growth period when the sprouts were approximately 30 cm in height. Next, two cucumber seedlings were planted in the same pots on 15 March and grew until 15 July 2013. The distance between cucumber plants in each pot was 25 cm, and the distance between pots were 50 and 30 cm (rows and pots). The experiment was repeated under the same growing conditions for the next two years (2014 and 2015), and all the field management practices were carefully performed and kept the same throughout the study period.

Every year, before garlic bulb planting and cucumber seedling transplantation, the uniform soil in each pot was manually tilled, and 30 g of organic fertilizer (“Peng-Di-Xin” (manure substitute, Zhengzhou, China), containing 30% organic matter, 4% N + P<sub>2</sub>O + K<sub>2</sub>O, 20% humic acid, 2% trace elements, and 5% organic sylvite), and 30 g of compound fertilizer (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O at 18-18-18) was added per pot as base fertilizer according to local farming conventions. During the reproductive growth stage of cucumber, 1 L of Hoagland’s nutrient solution was applied to each pot every fifteen days from 15 April until the 10 July to provide enough nutrition for the cucumber plants. For garlic nutrition, they were only irrigated with water as required at the growing stage. No other farm management techniques were performed on the garlic plants.

## 2.2. Soil Sampling, Soil Characteristics Analysis, and Yield Records

Soil was sampled from the plow layer (0–20 cm depth) of each treatment. In the first phase, the soil was sampled after uprooting garlic bulbs on 15 March, and in the second phase, the soil was sampled after cucumber uprooting from 2013 to 2015. Subsequently, all of the sub-samples taken from a single pot were thoroughly mixed to form a composite sample (a total of six sample points during three years. Treatments details are available in Table 1. The rhizosphere soil samples collected from each treatment were packed in an icebox and immediately transferred to the laboratory. The soil was sieved through a 1 mm size mesh and thoroughly homogenized; one portion was air-dried for soil properties analysis, and the other part was stored at  $-80^{\circ}\text{C}$  for DNA extraction for soil microbial analysis. Soil characteristics analysis and the yield record of green garlic and cucumber were determined according to a previously described method [45].

## 2.3. DNA Extraction, PCR Amplification, and Pyrosequencing

Soil DNA was extracted from 0.5 g mixed soil samples using the soil DNA kit (OMEGA, Bio-Tek, Norcross, GA, USA) according to the manufacturer’s protocols. Total DNA concentration was quantified through the Nanodrop 2000C Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). To amplify the fungal fragments of the appropriate size for pyrosequencing, the fungal primer pair ITS1 5'-barcode-TCCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' was employed [46]. PCR amplifications were performed in triplicate in a 20  $\mu\text{L}$  mixture containing 4  $\mu\text{L}$  of 5 $\times$  FastPfu Buffer, 2  $\mu\text{L}$  of 2.5 mM dNTPs, 0.8  $\mu\text{L}$  of each primer (5  $\mu\text{M}$ ), 0.4  $\mu\text{L}$  of FastPfu Polymerase, and 10 ng of template DNA; the volume was brought up to 25  $\mu\text{L}$  with double-distilled water. The PCR conditions used for the amplification of the ITS rRNA genes were as follows: 95  $^{\circ}\text{C}$  for 3 min, 35 cycles of 95  $^{\circ}\text{C}$  for 30 s for denaturation, 55  $^{\circ}\text{C}$  for 30 s for annealing, 72  $^{\circ}\text{C}$  for 1 min for extension, and 72  $^{\circ}\text{C}$  for 10 min. For each sample, 20 parallel PCRs (including two negative control reactions) were performed, and the presence of PCR products was determined by analyzing 2  $\mu\text{L}$  of the product on a 1.5% agarose gel. Next, all PCR products of the same sample were pooled, and the PCR products of approximately 400 bp were purified using a PCR Purification Kit (Axygen Bio, Union City, CA, USA) before sending the samples to Majorbio Biotech Co., Ltd. (Shanghai, China) for sequencing.

**Table 1.** Sample description and yield of cucumber and green garlic.

Sampling Date	Sample	Crop	Treatment	Yield (g Plant <sup>-1</sup> )
15 March 2013	DS1_1	green garlic	0 bulb	0
	DS1_2	green garlic	5 bulbs	29.50 ± 1.30a
	DS1_3	green garlic	10 bulbs	24.45 ± 2.10b
	DS1_4	green garlic	15 bulbs	21.37 ± 0.88c
	DS1_5	green garlic	20 bulbs	20.23 ± 1.21c
	DS1_6	green garlic	25 bulbs	18.68 ± 1.55c
15 July 2013	HG1_1	cucumber	2 cucumber plants	1199.96 ± 29.52d
	HG1_2	cucumber	2 cucumber plants	1223.14 ± 18.49d
	HG1_3	cucumber	2 cucumber plants	1475.10 ± 32.37c
	HG1_4	cucumber	2 cucumber plants	1660.05 ± 42.34b
	HG1_5	cucumber	2 cucumber plants	1952.00 ± 21.95a
	HG1_6	cucumber	2 cucumber plants	1984.14 ± 25.99a
15 March 2014	DS2_1	green garlic	0 bulb	0
	DS2_2	green garlic	5 bulbs	21.60 ± 2.10a
	DS2_3	green garlic	10 bulbs	17.97 ± 1.20b
	DS2_4	green garlic	15 bulbs	15.58 ± 0.51c
	DS2_5	green garlic	20 bulbs	14.72 ± 0.33c
	DS2_6	green garlic	25 bulbs	13.29 ± 1.10c
15 July 2014	HG2_1	cucumber	2 cucumber plants	1009.07 ± 33.66d
	HG2_2	cucumber	2 cucumber plants	1092.22 ± 14.58c
	HG2_3	cucumber	2 cucumber plants	1123.82 ± 22.49bc
	HG2_4	cucumber	2 cucumber plants	1150.87 ± 28.57bc
	HG2_5	cucumber	2 cucumber plants	1362.96 ± 12.96a
	HG2_6	cucumber	2 cucumber plants	1176.12 ± 23.12b
15 March 2015	DS3_1	green garlic	0 bulb	0
	DS3_2	green garlic	5 bulbs	18.40 ± 0.25a
	DS3_3	green garlic	10 bulbs	15.17 ± 1.03b
	DS3_4	green garlic	15 bulbs	13.42 ± 0.85c
	DS3_5	green garlic	20 bulbs	12.47 ± 2.10c
	DS3_6	green garlic	25 bulbs	13.8 ± 1.66c
15 July 2015	HG3_1	cucumber	2 cucumber plants	963.51 ± 15.35d
	HG3_2	cucumber	2 cucumber plants	1086.47 ± 40.03c
	HG3_3	cucumber	2 cucumber plants	1099.77 ± 32.19c
	HG3_4	cucumber	2 cucumber plants	1141.46 ± 44.23c
	HG3_5	cucumber	2 cucumber plants	1448.15 ± 25.46a
	HG3_6	cucumber	2 cucumber plants	1313.07 ± 25.42b

Different letters indicate significant differences at the  $p < 0.05$  level,  $n = 5$ . DS, garlic-planted soil. HG, cucumber-planted soil.

Sequence analysis was performed using Mothur software. In brief, sequence reads belonging to the samples were extracted from the data obtained from the 454 FLX sequencer based on tags with no ambiguous base pairs. The primers were removed, and the sequences were trimmed to remove low-quality sequences. Next, all sequences were aligned against the reference sequence database (Silva database). The sequencing data were used to measure the relative abundance of operational taxonomic units (OTUs) at a dissimilarity of 0.03 and to calculate the diversity index of soil samples. These data were then correlated with several measurable properties of soil to reveal any relationships.

#### 2.4. Statistical Analysis

The experiment was performed using a randomized complete block design (RCBD). An OTU-based analysis was performed to calculate the richness, diversity, and coverage at a dissimilarity of 0.03. Principal component analysis (PCA) of the sites and redundancy analysis (RDA) were carried out using CANOCO for Windows. Richness indexes, the Chao estimator, and the abundance-based

coverage estimator (ACE) were calculated to estimate the number of observed OTUs. The diversity within each individual sample was estimated using the nonparametric Shannon's diversity index. Spearman's rank correlations between abundant taxa and soil properties during three years were calculated by using IBM SPSS Statistics 20. Soil properties were determined five times for each sampling point during three years, with values used as the mean for each treatment.

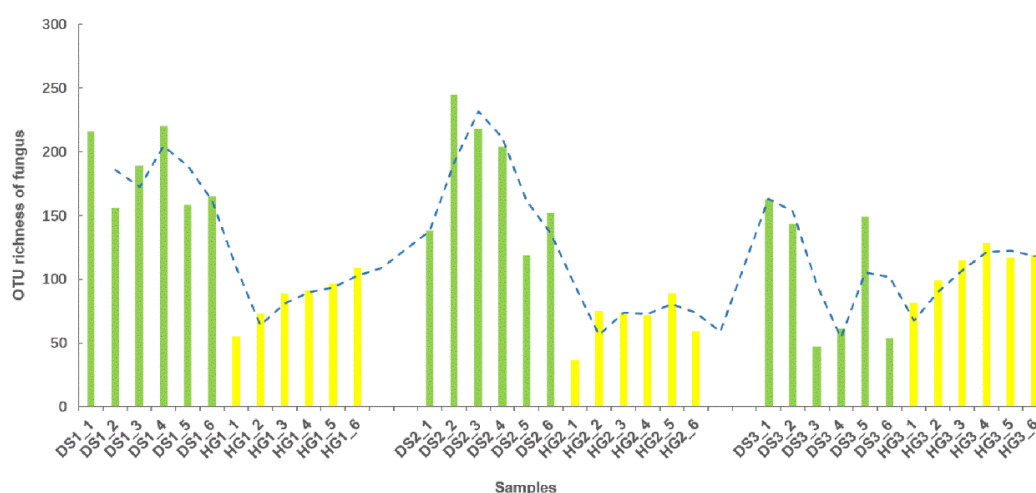
### 3. Results

#### 3.1. Effects of a Three-Year Green Garlic/Cucumber Crop Rotation System on Yields

The green garlic and cucumber yields obtained during the three-year (from 2012 to 2015) crop rotation system are presented in Table 1. The yields of green garlic and cucumber showed a generally decreasing trend during the three years, as continuous cropping obstacles severely affected yields in 2014 and 2015, which were dramatically lower compared to yields in 2013. However, compared to cucumber mono-cropping, cucumber yields in the five treatments increased with the number of garlic bulbs planted by 1.9%, 22.9%, 38.3%, 62.7%, and 65.4%, respectively, in 2013; similarly, the same increasing trend was observed in 2014 and 2015. In contrast, the yields of green garlic decreased along with the number of garlic bulbs planted in each year.

#### 3.2. Effects of a Three-Year Green Garlic/Cucumber Crop Rotation System on Fungal Diversity in the Soil

After equalizing the sampling effort, 94,680 sequences were retained for an analysis from the three years sampled. These high-quality sequences were clustered into 4234 OTUs at 97% sequence similarity, with 37,245 OTUs per sample. Fungal richness of the different treatments in different years were calculated based on the 2630 sequences. As shown in Figure 2, a remarkable difference was observed for the OTU richness of fungi between the garlic- and cucumber-planted soils; in each year, the OTU richness of fungi species increased after garlic was planted and decreased after cucumber was planted. Beyond that, the OTU number in the cucumber-planted soil varied with the number of garlic bulbs during the three-year cultivation when compared to the mono-cropping system, and generally, the number of fungal OTUs was enhanced.

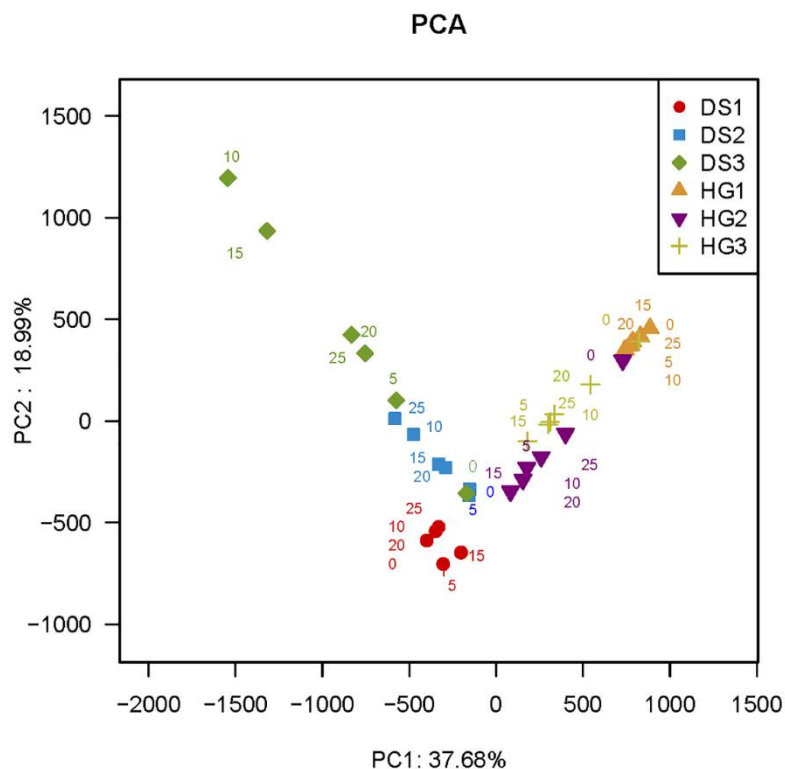


**Figure 2.** Estimated number of observed OTUs of fungi in individual samples from the cucumber/garlic cropping system for three years. OTUs, operational taxonomic units. DS, garlic-planted soil. HG, cucumber-planted soil.

Concomitantly, the PCA of the fungal community clearly showed variations between green garlic- and cucumber-planted soils (Figure 3). The first two components explained 56.67% of the total fungal community variation among the individual samples during the three years. The fungal community



compositions shifted greatly between the two species along the different directions of the first principal component axis. In addition, the samples of the same species (green garlic and cucumber) in the three different years are also ungrouped very well and shifted along the second principal component axis. However, the samples from cucumber mono-cropping in the two different growing seasons during the three years were grouped into two groups (DS1-1, DS2-1, DS3-1 and HG1-1, HG2-1, HG3-1), and the remaining samples of treatments were grouped by year.

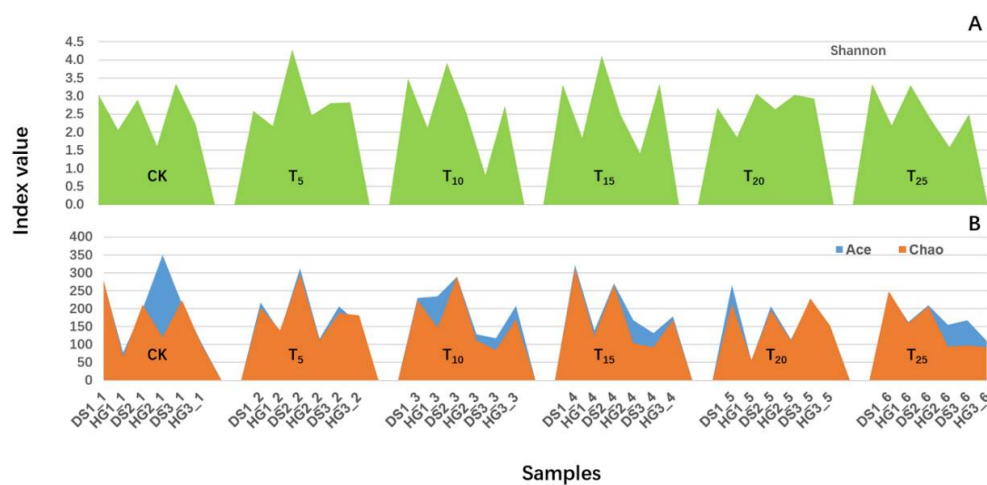


**Figure 3.** Principal component analysis (PCA) of fungal communities based on OTUs at a distance of 3% for individual samples from the cucumber/garlic cropping system for three years. The first two components are 37.68% and 18.99%.

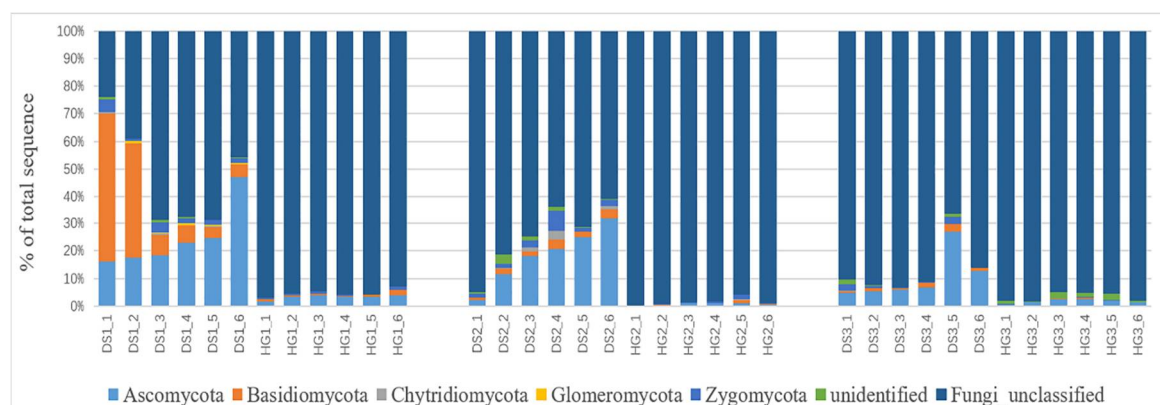
Fungal  $\alpha$ -diversity of the different treatments for the two crop seasons in each year was calculated based on a total of 2630 high quality sequences belonging to particular samples. During the three years, the relative frequencies of fungal diversity showed variable responses before and after rotation practices; mainly, the diversity of fungi increased after garlic planting, and it decreased after cucumber planting each year. However, no significant differences for the fungal  $\alpha$ -diversity indices were found between the mono-cropping system (CK) and the cucumber/garlic (treatments T<sub>5</sub>, T<sub>10</sub>, T<sub>15</sub>, T<sub>20</sub>, and T<sub>25</sub>) cropping system during the three year's experiment (Figure 4).

The dominant taxa across all samples were *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Glomeromycota*, and *Zygomycota*, except for the unclassified and unidentified. The highest relative abundance taxon was *Ascomycota* under the garlic rotation scheme during the entire experimental period, and its variation is shown in Figure 5, which showed a similar trend to the OTU richness of fungi. The treatment effect for *Ascomycota* abundance was more obvious, especially in garlic-planted soil niches during the three years compared to the control, which increased with the increasing number of garlic bulbs added to this crop rotation system. The relative abundance of *Basidiomycota* decreased greatly in the garlic-planted treatments compared to the control during the first year, but there was no significant difference between the treatments and control in the next two years. Nevertheless, the

relative abundance in these five dominate taxa decreased dramatically to an extremely low level at the end of the experimental period.



**Figure 4.**  $\alpha$ -diversity of individual samples from the cucumber/garlic cropping system. Shannon (A), nonparametric Shannon diversity index. ACE, abundance-based coverage estimator (B).



**Figure 5.** Phylogenetic classification of the relative abundance of the dominant fungal taxa in individual samples from the cucumber/garlic cropping system for three years. Relative abundance is based on the proportional frequencies that could be classified at the phylum level.

### 3.3. The Relationships between Microorganism Taxa and Soil Features in the Three-Year Green Garlic/Cucumber Crop Rotation System

To evaluate the relationships between some of the soil edaphic properties and the relative abundance of the fungal taxa, Spearman's rank correlation coefficient was used in this study. All the major groups of fungi exhibited different correlation patterns with the soil properties (Table 2). We found that all of the abundant taxa were negatively correlated with soil pH, EC, and SOC, but were generally positively correlated with soil available nutrients P (except for *Glomeromycota*) and K. For available N, the abundance of *Ascomycota* and *Glomeromycota* were negatively correlated, but *Basidiomycota*, *Chytridiomycota*, and *Zygomycota* were positively correlated.

The RDA (redundancy analysis) biplot clearly showed the relationship between soil characteristics and the fungal community structure (Figure 6). The effects of soil properties on the fungal community are shown by the direction and length of the vectors. The arrows for the process parameters point in the direction of maximum change for a particular variable across the diagram, and their length is proportional to the rate of change in this direction. Long arrows are more closely related to the soil

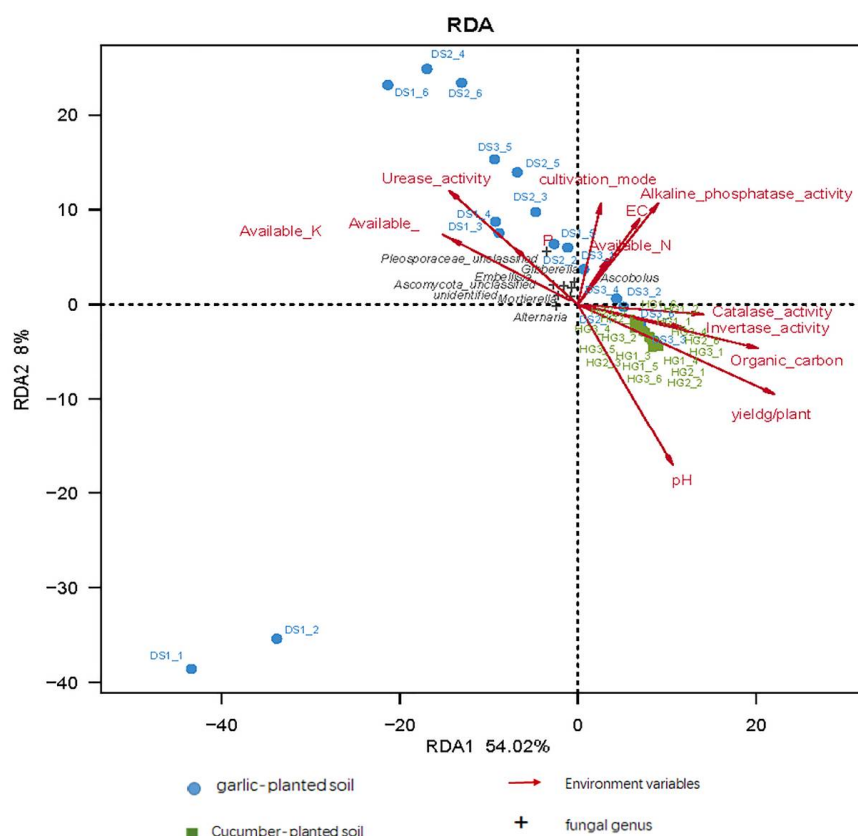


biological pattern variations. In this experiment, RDA analysis was performed to determine the variable effects of environmental factors (soil physicochemical properties, cultivation mode, and seasons) on the abundant genus. The first two axes of RDA explained 54.02% and 8% of the total variation in the data. The fungal communities of garlic-planted soil were grouped separately from the fungal communities of cucumber-planted soil along both axes; the abundant genus of garlic-planted soils related more to higher contents of soil available nutrients, activities of urease and alkaline phosphatase, and to the different cultivation modes. Meanwhile, the fungal communities of cucumber-planted soils formed a separate group associated with a higher pH, organic carbon content, activities of invertase and catalase, and higher yields. Most of the main abundant genera in this system differed within garlic-planted soils, which were *Ascobolus*, *Ascomycota\_unclassified*, *Alternaria*, *Embellisia*, *Gibberella*, *Mortierella*, and *Pleiosporaceae\_unclassified*.

**Table 2.** Spearman's rank correlations ( $r$ ) between the abundant taxa and soil properties.

	pH	EC	Available N	Available P	Available K	SOIL Organic Carbon
<i>Ascomycota</i>	<b>−0.583 **</b>	−0.003	−0.106	0.091	<b>0.455 **</b>	<b>−0.471 **</b>
<i>Basidiomycota</i>	<b>−0.498 **</b>	<b>−0.355 *</b>	0.040	0.274	<b>0.394 *</b>	<b>−0.667 **</b>
<i>Chytridiomycota</i>	−0.246	−0.243	0.217	<b>0.574 **</b>	<b>0.342 *</b>	<b>−0.379 *</b>
<i>Glomeromycota</i>	−0.204	<b>−0.383 *</b>	−0.304	−0.061	0.143	<b>−0.446 **</b>
<i>Zygomycota</i>	<b>−0.483 **</b>	−0.251	0.132	<b>0.397 *</b>	<b>0.463 **</b>	<b>−0.619 **</b>

Values in bold indicate statistical significance at \*  $p < 0.05$  and \*\*  $p < 0.01$ .



**Figure 6.** Redundancy analysis (RDA) of abundant taxa and the environmental variable eigenvectors for individual samples during three years.

#### 4. Discussion

The changes in soil structural properties were induced by alternative agricultural management practices, and soil microbes made a significant contribution to organic matter decomposition, nutrient cycling, and functions related to soil health, quality, and ecosystem stability [47–49]. Among different soil microbial diversities, soil fungal communities are an essential and functional component of microorganisms; in emphasizing its prominence, we taxonomically classified the soil fungal community structure as influenced by the inclusion of a crop-specific sustainable cropping system examined through high-throughput sequencing technology. This is a popular and effective method to analyze specific microbial groups at the molecular level [50]. The significance of crop rotation for the development of agro-ecological sustainability has been well documented in terms of soil structure maintenance, soil fertility improvement, and total soil microbial diversity richness [51–53]. Thus, it is important to explore the effects of the garlic/cucumber crop rotation system on microbial diversity or richness under long-term field conditions.

##### *4.1. Effects of a Three-Year Green Garlic/Cucumber Crop Rotation System on Yield of Cucumber Compared to Cucumber Mono-Cropping*

Soil toxicity due to negative plant-soil feedback can play a crucial role in plant biomass and yield loss [54,55]. In this study, the yields of green garlic and cucumber showed a declining trend (2013 > 2014 > 2015) over time through the three cropping years, indicating that continuous cropping obstacles emerged in both the cucumber mono-cropping system and the green garlic/cucumber crop rotation system. The decline in cucumber yield could be related to the activity of cucumber root exudates that cause autotoxicity and its co-effects that disturb soil ecological indicators and consequently imbalance soil microbial biomass [3,56–58]. In addition, the difference in cucumber yield with respect to the amount of incorporated garlic bulbs could be due to allelopathic interference phenomenon in the vicinity of cucumber soil. Because certain assumptions could be derived due to the diverse allelopathic activity of garlic species and degree of inhibition or promotion greatly depended on their concentration and different plant organs (bulbs, root exudates, garlic extract, garlic residues) possess different allelopathic potential in a dose dependent manner [37–40].

However, this study also revealed that cucumber-garlic rotation prevents cucumber yield losses due to mono-cropping. It indicated that the green garlic/cucumber cropping system is an efficient cropping system for sustainable cucumber production and can alleviate soil sickness to some extent. The results were supported by previous findings that multi-cropping with garlic had a stimulatory effect on the growth and yield of eggplant, tomato, and cucumber [39,44,59]. However, the yields of green garlic decreased with the increasing number of green garlic bulbs planted in the crop rotation system, which confirmed the guideline that yields of garlic linearly respond to plant density in that yield decreases with the increase in plant density [60,61].

##### *4.2. The Dynamics of a Three-Year Green Garlic/Cucumber Crop Rotation System on the Fungal Community and Fungal Diversity in Soil Compared to Cucumber Mono-Cropping*

Crop production is affected by soil biological processes, including enzyme activities and soil fertility, which are influenced by microbial activities. Soil fungal diversity is considered very critical to the long-term sustainability of soil ecosystems, which is usually reduced by agricultural management [62,63]. Recently, studies have demonstrated that greater biodiversity can enhance the ecosystem and the stability of microbial functions [31]. Hence, it is important to maintain and restore fungal biodiversity in sustainable agricultural systems. In the present study, the dynamics of a three-year green garlic/cucumber crop rotation system on the fungal community and fungal diversity in the soil compared to cucumber mono-cropping were evaluated under a greenhouse plastic tunnel. During the three-year cultivation, the fungi OTU richness of cucumber-planted soil generally increased with the increasing number of garlic bulbs planted. In addition, the OTU richness indexes of fungi significantly increased after the growing period of green garlic, and a dramatic

temporally declining tendency was observed in the community composition after cucumber was planted in 2013 and 2014 (Figure 2). It can be explained by the fact that environmental variables have clear effects on fungal interaction, especially the temperature at the end of the green garlic growing period (15 March), which is more suitable for fungal growth [64]. Our findings showed that the fungal communities were well grouped according to the cropping systems, not only in the alpha diversity, but also in the community structure. The principal component analysis (PCA) of the fungal community, as shown in Figure 3, also elucidated that significant variations occurred between the green garlic- and cucumber-planted soils among the individual samples during the whole experiment duration, as well as between the treatments with green garlic and cucumber mono-cropping. Agricultural soil management practices and cropping systems can directly affect the fungal community composition, as reported by numerous authors [49,65–68]. Continuous cropping under a protected cultivation system is becoming an unrealistic approach, which considerably affects the biomass and activity of soil microorganisms unless the cropping pattern is modified [44,69,70]. The population and diversity of selective fungal communities are linked to soil sickness and are associated with continuous obstacles of cucumber cultivation [71]. Our results from this investigation were consistent with previous studies showing that the specific microbiota composition changed within different microenvironments in the soil matrix [72]. However, the degree of variation in the taxonomic community composition and fungal richness as influenced by different crop rotation systems in protected vegetable cultivation conditions are poorly understood. Only a few studies have been reported in this context; for example, Yao et al. evaluated the diversity of the soil microbial communities in continuous cucumber and in cucumber-wheat and cucumber-tomato rotation under long-term plastic-greenhouse cultivation [73]. The observed parameters, the coefficients of DNA sequence similarity, OTU richness, and the Shannon–Weaver index indicated that soils under both types of rotation attained a higher fungal diversity compared to mono-cropping soils. Moreover, they also indicated that continuous cultivation and the proper cropping system were largely associated with the proliferation of microbiota species diversity. The continuous cropping of watermelon production followed by soil amendments in combination with garlic rotation can ameliorate the negative effects of continuous cropping obstacles and enhance the belowground microbe population [74]. A higher level of fungal diversity in the rhizosphere of garlic-planted soil was greatly influenced by the garlic rotation system, which renovated the soil functional properties, created a higher qualitative and quantitative distribution of organic matter content, and increased nutrient uptake levels; moreover, its efficacy was linearly associated with the number of garlic bulbs [38,75]. Organic matter reshaped the soil edaphic properties in micro aggregates that delivered relatively unique microenvironments for fungal growth [76]. Meanwhile, it could be assumed that garlic has strong allelopathic activity on both crop productivity and soil biology. It has been reported that its promotional or inhibitory action is usually dependent on host species biogeography, micro-climatic factors, and the frequency of intensive growing practices [77]. Surprisingly, in our findings, the garlic crop rotation played a beneficial role in cucumber-planted soil structural transformation and fungal propagation. It is interesting to know that the possible alteration in the soil biological dynamics was obviously affected by the different agricultural practices. In the present work, not only the fungal diversity, but also the relative abundance of some important fungal taxa classified by the pyrosequencing approach, were affected by garlic-cucumber cropping management. Subsequently, *Ascomycota* was identified as the most dominant fungal taxon among them in the cultivation-dependent soil samples. This taxon had a greater geographical distribution in the diversified soil profile and usually showed a tendency to tolerate maximum temperature [77]. The higher richness of this genus was previously reported by [78], which achieved soil health and soil eco-stability targets by meeting ecological goals under an intensive potato cropping system. In addition, the majority of the fungal community species altered by different species belonged to our recognized genus, and *Ascomycetes* were much more frequent [79].

#### 4.3. The Relationships between Microorganism Taxa and Soil Features in the Three-Year Green Garlic/Cucumber Crop Rotation System

Crop rotation is an excellent practice for protected vegetable production; this cultivation system effectively improves soil physicochemical characteristics, regulates soil fertility status, and increases crop production by changing the soil micro-environment [14,80]. An increasing number of studies demonstrate that environmental factors shape the fungal community and structure [21,80]. In the present study, we found that the proportions of abundant taxa were highly negatively correlated with soil pH, EC, and SOC. Our results were consistent with some other studies that have demonstrated that soil pH was a very strong factor in structuring microbial communities [31,81]. In contrast, the proportions of abundant taxa were positively correlated with soil available P and K (Table 2). The results were also confirmed by the redundancy analysis (RDA) of the abundant taxa and the environmental variables for individual samples during the three years (Figure 6). Our results are consistent with reports that the physicochemical soil microenvironment has a very strong impact on soil microbial community and structure [31,82,83]. Until now, this interaction between environmental variables and the fungal community has proved to be beneficial to cucumber plants.

## 5. Conclusions

We conclude that allelopathic crops incorporated into systemic rotational sequences could be a suitable option for short duration cash crops under intensive greenhouse production. The altered soil micro-environment was improved by crop rotation with a different number of garlic bulbs; consequently, the yields of cucumber in the green garlic/cucumber crop rotation system were enhanced compared to cucumber mono-cropping. From these findings, we have suggested that crop rotation with green garlic is an economic, sustainable, and diversified cropping system that alleviates continuous cropping obstacles and can accelerate cucumber crop productivity by improving the soil microbiology environment. Furthermore, this study may provide a greater opportunity to explore the sequential changes in soil microbial biomass composition with garlic rotation for some other crops utilizing modern analytical techniques. Additional research is also needed to assess the fungal network in yield-invigorating soils induced by a crop rotation with garlic.

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