



Article Effects of the Continuous Cropping of Amomum villosum on Rhizosphere Soil Physicochemical Properties, Enzyme Activities, and Microbial Communities

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Abstract: *Amomum villosum*, which is an important perennial medicinal plant, easily suffers from continuous cropping obstacles in the plantation. The aim of this study is to find an effective method to solve the problem of *A. villosum* continuous cropping. In this study, we analyzed four fields in which *A. villosum* was continuously cropped and a fallow field to reveal the effects of continuous cropping on the rhizosphere soil physicochemical properties, enzyme activities, and bacterial and fungal communities. Most of the soil nutrient contents gradually increased as the number of years of continuous cropping increased, whereas the soil pH decreased slightly. The soil urease and acid phosphatase activities tended to increase as the length of the continuous cropping period increased, which may have accelerated the conversion of soil substances. Furthermore, the alpha diversity of the bacterial and fungal communities decreased as the duration of the continuous cropping period increased. Additionally, the redundancy analysis revealed that bacterial and fungal community structures at the phylum level were the most correlated with pH value and catalase activity, respectively. This study may be useful for promoting the continuous cropping and sustainable development of *A. villosum*.

Keywords: perennial medicinal plant; successive cropping obstacle; next-generation sequencing; soil bacterial community; soil fungal community

1. Introduction

Amomum villosum Lour., which belongs to the family Zingiberaceae, is a perennial medicinal plant [1]. The dried ripe fruits of *A. villosum* are highly valued as one of four southern medicines in traditional Chinese medicine [2]. Because the fruits may be useful for resolving dampness, promoting appetite, improving spleen functions, stopping diarrhea, regulating vital energy, and preventing miscarriages, they have been used for medicinal purposes for more than 1300 years [3,4]. Furthermore, they have been included as ingredients in condiments and health-promoting food products because of their flavor [5]. According to an earlier phytochemical analysis, dried ripe *A. villosum* fruits contain large amounts of volatile components, including bornyl acetate, camphor, limonene, and borneol, as well as flavonoids, polysaccharides, and inorganic matter [6].

In China, *A. villosum* is cultivated or grows naturally in shady and humid mountainous regions in Fujian, Guangdong, Guangxi, and Yunnan provinces [7]. The large-scale production of high-quality *A. villosum* is heavily dependent on the cultivation method, with the continuous cropping of *A. villosum* becoming increasingly common [1]. However, long-term continuous cropping frequently leads to disease outbreaks and decreased productivity [8–10]. The continuous cropping of *A. villosum* may lead to substantial decreases in yield, with stem wilt, leaf spot, and fruit rot diseases identified as major biotic stresses with detrimental effects on *A. villosum* yields [11].



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Many external factors, including soil physicochemical properties (such as organic matter, nitrogen, phosphorus, and potassium contents, etc.), changes in soil enzymatic activities, and shifts in the soil microbial population, influence the crop yield and quality in continuous cropping systems [12,13]. Soil enzymes, which are one of the most active components of soil ecosystems, promote soil metabolic processes and are highly responsive to changes induced by natural and human factors [14,15]. These enzymes are highly catalytic proteins that affect soil development, fertility, and evolution [16]. In an earlier study, soil enzyme activities were modulated by the continuous cropping of three medicinal plants (Coptis chinensis, Polygonum multiflorum, and Fritillaria thunbergii) [17]. Microorganisms are also an important part of the soil ecosystem because they are the main promoters of soil chemical processes, thereby influencing soil fertility and sustainable development [18]. Soil microorganisms affect soil fertility and plant growth in many ways. In turn, the soil nutrient status as well as the plant biomass and health status affect the soil microbial community structure and function [19]. Research on soil microbial ecology has indicated that long-term continuous cropping can substantially alter the structure and diversity of the rhizospheric microbial community [20–23].

Next-generation sequencing platforms, such as Illumina MiSeq, are increasingly being used to analyze the structure, diversity, and composition of soil bacterial and fungal communities in continuous cropping systems. Previous studies involving high-throughput sequencing technology revealed changes in the rhizospheric bacterial community caused by continuous cropping [8,21,24,25]. Similarly, Illumina MiSeq sequencing or 454 pyrosequencing analyses detected significant changes in the diversity and composition of rhizospheric fungal populations induced by continuous cropping [9,24–26].

Thus, this study aims to provide information on how the continuous cropping of *A. villosum* affects the rhizosphere soil physicochemical properties, enzyme activities, and microbial communities. In the present study, the diversity and composition of rhizospheric bacterial and fungal communities were analyzed in four fields in which *A. villosum* was grown in a continuous cropping system for 3–6 years as well as in a fallow field. Specifically, the NovaSeq PE250 platform was used to sequence the internal transcribed spacer (ITS) and 16S rRNA gene sequences to thoroughly examine the biodiversity of bacterial and fungal communities. Furthermore, the soil physicochemical properties and enzyme activities were determined and correlations with the bacterial and fungal communities of the *A. villosum* rhizosphere were revealed. The present study generated baseline data on the effects of continuous cropping of *A. villosum* on rhizosphere soil physicochemical properties, enzyme activities, and microbial communities, and will serve as a theoretical basis for sustainable management along with improvement in yield and quality of *A. villosum*.

2. Materials and Methods

2.1. Field Experiments and Soil Sampling

The *A. villosum* rhizosphere soil samples analyzed in this study were collected in Jiangcheng county, Pu'er city, Yunnan province (latitude 22°34′48″ N, longitude 101°52′48″ E; 776 m above sea level), which is located in a subtropical humid monsoon climatic zone with an annual mean temperature of 18.7 °C and an annual mean precipitation of 2283 mm. Sandy and clay soils are the main soil types in this region. Organic and compound fertilizers should be applied once to the fields where *A. villosum* plants are cultivated between June and September every year. The organic and compound fertilizers are applied 1000 kg and 5000 kg per hectare, respectively, and nitrogen, phosphorus, and potassium contents accounted for 15% of the compound fertilizer content, respectively. The rhizosphere soil samples were obtained from fields in which *A. villosum* was continuously cropped for 3 years (AV3), 4 years (AV4), 5 years (AV5), or 6 years (AV6). Samples collected from a fallow field lacking cultivated plants for at least 6 years served as the control (CK). For each field, four samples were collected from the rhizosphere of 15 randomly selected *A. villosum* plants. Whole *A. villosum* plants were removed from the soil, after which the rhizosphere soil was collected by shaking the roots vigorously to separate the soil from the roots. For

the fallow field, five core samples were collected from the topsoil layer (approximately 15 cm depth). All soil samples were transferred to sterile centrifuge tubes and transported to the laboratory in ice boxes. Each soil sample was passed through a 2 mm sieve in the laboratory to remove debris and other stony material before being homogenized. Finally, 10 g soil samples were added to sterilized tubes and stored at -80 °C for the subsequent DNA extraction. Other soil samples were used to analyze soil enzyme activities and physicochemical properties. Each experimental treatment (AV3, AV4, AV5, AV6, and CK) was analyzed using three biological replicates.

2.2. Analysis of Soil Physicochemical Properties

The soil's physicochemical properties were determined based on the method described by Bao et al. [27]. Water was added to the soil samples at a 2.5:1 ratio, after which the resulting solutions were shaken (200 rpm for 10 min) using the TS-2 shaker (Qi Lin Bei Er, Shanghai, China). The solutions were left undisturbed for 30 min before measuring the soil pH using the FE28 Standard pH meter (Mettler-Toledo, Columbus, OH, USA). The organic matter content was determined using the high-temperature external thermal potassium dichromate oxidation capacity method. The total nitrogen content was measured according to the Kjeldahl digestion method, whereas the total phosphorus content was determined using the sodium hydroxide melting molybdenum antimony anti-colorimetric method. The protocol used to determine the total potassium content involved hydrofluoric acid digestion and flame photometric measurements. The available nitrogen content was measured via the alkali hydrolyzed diffusion method. The available phosphorus content was determined using the hydrochloric acid-sulfuric acid double acid extraction colorimetric method. The available potassium content was estimated on the basis of a neutral ammonium acetate extraction and flame photometric measurements. The samples for each experimental treatment were analyzed using three biological replicates and two technical replicates.

2.3. Analysis of Soil Enzyme Activities

Fresh soil is dried in the shade under ventilated conditions, and the dry soil samples were used for the analysis of soil enzyme activities, and soil enzyme activities were determined based on the method described by Guan et al. [28]. The soil urease activity was determined using the sodium phenol colorimetric method, whereas the soil phosphatase activity was measured according to the disodium phosphate colorimetric method. The soil catalase activity was determined according to a titration involving potassium permanganate. The soil sucrase activity was determined using the 3,5-dinitrosalicylic acid colorimetric method. The samples for each experimental treatment were analyzed using three biological replicates and two technical replicates.

2.4. DNA Extraction and MiSeq Sequencing

Genomic DNA was extracted from 0.5 g soil samples (dry weight) using the E.Z.N.A Soil Kit (Omega Bio-Tek, Norcross, GA, USA). The concentration and purity of the DNA were determined using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The quality of the DNA was assessed by 1% agarose gel electrophoresis. The total DNA was eluted in 50 μ L Elution buffer and stored at -80 °C for the subsequent PCR analysis.

The V3-V4 regions of the bacterial 16S rRNA genes were amplified by PCR using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAA TCC-3') [29]. The ITS2 regions of the fungal small-subunit rRNA genes were amplified by PCR using primers ITS1FI2 (5'-GTGARTCATCGAATCTTTG-3') and ITS2 (5'-TCCTCCG CTTATTGATATGC-3'). The 5' ends of the primers were tagged with specific barcodes for each sample and universal tags for sequencing. The PCR conditions for amplifying the V3–V4 and ITS fragments were as follows: 98 °C for 30 s; 32 cycles of 98 °C for 10 s, 54 °C for 30 s, and 72 °C for 45 s; and 72 °C for 10 min. The PCR amplification was performed in a solution comprising 25 ng template DNA, 12.5 μ L PCR Premix, 2.5 μ L each primer, and

PCR-grade water for a final volume of 25 µL. The PCR products were analyzed using the QuantiFluor[™]-ST Blue Fluorescence Quantification system (Promega) and by 2% agarose gel electrophoresis. Amplicon pools were prepared for sequencing. The size and quantity of the amplicon libraries were measured using the Agilent 2100 Bioanalyzer (Agilent, USA) and the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on the Illumina NovaSeq PE250 platform according to the manufacturer-recommended protocol.

2.5. Bioinformatic Analyses

Paired-end reads were assigned to samples according to their unique barcodes and then truncated by cleaving the barcode and primer sequence. Additionally, the paired-end reads were merged using FLASH (v1.2.8) (http://ccb.jhu.edu/software/FLASH/; accessed on 1 August 2022). The raw reads were filtered for quality to obtain high-quality clean reads using fqtrim (v0.94) (http://ccb.jhu.edu/software/fqtrim/; accessed on 1 August 2022). Chimeric sequences were filtered using the Vsearch software (v2.3.4) (https://github.com/torognes/vsearch; accessed on 1 August 2022). After a dereplication step using DADA2 (https://qiime2.org/; accessed on 1 August 2022), we obtained feature tables and feature sequences. The SILVA classifier (release 138; https://www.arbsilva.de/documentation/release138/; accessed on 1 August 2022) was then used to normalize the feature abundances according to the relative abundances of each sample. Alpha diversity and beta diversity were calculated using QIIME2 [30] and visualized in graphs, which were drawn using the R package. Sequences were annotated on the basis of the SILVA database. Other diagrams were drawn using the R package (v3.5.2).

2.6. Statistical Analyses

Statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) 18.0 software and the R vegan package. Alpha diversity was used to assess microbial community richness and evenness and the sequencing depth for the bacterial and fungal communities of each sample. Specifically, alpha diversity was mainly reflected by the Chao1, Observed_OTUs, Goods_coverage, Shannon, Simpson, and Pielou_e indices. Chao1 and Observed_OTUs were used to estimate the number of bacteria and fungi in a community. Goods_coverage refers to microbial coverage, whereas the Shannon and Simpson indices represent diversity. The Pielou_e index reflects the uniformity of the community. Alpha diversity and beta diversity were calculated by randomly normalizing the data for the same sequences. Beta diversity refers to the differences in bacteria and fungi among different environmental communities. Beta diversity was estimated by calculating the distance matrix between environmental samples and determining the differences between samples through a principal coordinate analysis (PCoA). Correlations between the operational taxonomic unit (OTU) data and soil physicochemical properties and enzyme activities were determined on the basis of a redundancy analysis (RDA), which was conducted using the vegan package of R.

3. Results

3.1. Soil Physicochemical Properties

The continuous cropping of *A. villosum* resulted in an initial increase in the soil pH, which differed significantly among all five fields (p < 0.05) (Table 1). However, the soil pH was significantly lower for the AV4, AV5, and AV6 fields than for the CK field. The soil organic matter content also varied among fields. Accordingly, the soil organic matter content increased as the number of years of continuous cropping increased.

Samples	pН	OM (g/kg ⁻¹)	TN (g/kg ⁻¹)	AN (g/kg ⁻¹)	TP (g/kg ⁻¹)	AP (g/kg ⁻¹)	TK (g/kg ⁻¹)	AK (g/kg ⁻¹)
AV3	$4.42\pm0.12a$	$23.50\pm1.10a$	$0.70\pm0.01a$	$107.51\pm0.55a$	$0.70\pm0.07a$	$4.30\pm0.29a$	$4.42\pm0.06a$	$84.02\pm2.18a$
AV4	$4.25\pm0.01b$	$23.50\pm0.42a$	$0.95\pm0.03b$	$142.48\pm3.13b$	$0.78\pm0.05b$	$4.17\pm0.43b$	$4.25\pm0.11b$	$81.96\pm3.99a$
AV5	$4.18\pm0.03\mathrm{c}$	$32.33 \pm 1.77 \mathrm{b}$	$1.35\pm0.05c$	$177.82\pm0.68c$	$0.89\pm0.07c$	$3.65 \pm 0.16c$	$3.98 \pm 0.12c$	$79.62 \pm 4.56a$
AV6	$4.10\pm0.02d$	$33.43 \pm 1.06 \text{b}$	$1.58\pm0.11d$	$341.62 \pm 10.72d$	$0.94\pm0.05d$	$3.60 \pm 0.13c$	$3.94 \pm 0.25c$	$78.98 \pm 3.38a$
CK	$4.36\pm0.06e$	$32.70 \pm 1.72b$	$0.57\pm0.01\mathrm{e}$	$72.38 \pm 1.58 e$	$0.63 \pm 0.07 \mathrm{e}$	$3.59 \pm 0.11c$	$4.02\pm0.27c$	$60.87 \pm 4.54b$

Table 1. Soil chemical properties among the examined fields.

OM, TN, AN, TP, AP, TK, and AK represent organic matter, total nitrogen, available nitrogen, total phosphorus, available phosphorus, total potassium, and available potassium, respectively; AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field. Different letters in columns indicate significant differences (p < 0.05, n = 6).

The total nitrogen and total phosphorus contents were lowest for the fallow field (CK) and increased significantly (p < 0.05) from AV3 to AV6 (Table 1). In contrast, the total potassium content decreased as the number of years of continuous cropping increased, although there were no significant differences among the AV5, AV6, and CK fields.

The changes in the soil's available nitrogen, phosphorus, and potassium contents are presented in Table 1. The available nitrogen content increased significantly from AV3 to AV6. Conversely, the available phosphorus content tended to decrease from AV3 to AV6, but was lowest for the CK field. The available potassium content tended to decrease as the number of years of continuous cropping increased, but this change was not significant.

3.2. Enzyme Activities

The soil urease activity was lowest for the CK field and it increased slightly in response to the continuous cropping (Table 2). The soil acid phosphatase activity differed significantly among the five fields (p < 0.05). More specifically, it was lowest in the CK field and increased as the duration of the continuous cropping increased. The soil catalase and sucrase activities fluctuated over the experimental period, with the highest levels in the AV5 field, followed by the AV3 field, and the lowest level in the CK field.

Samples	Urease Activities (mg/d/g)	Acid Phosphatase Activities (µmol/d/g)	Catalase Activities (µmol/d/g)	Sucrase Activities (U/g)
AV3	$0.42\pm0.03a$	$4.36\pm0.22a$	$53.27 \pm 1.80 \mathrm{a}$	$235.21 \pm 16.00 \text{ab}$
AV4	$0.47\pm0.02 \mathrm{ab}$	$4.52\pm0.45b$	$52.24\pm0.73b$	$221.63\pm10.83a$
AV5	$0.50\pm0.02b$	$4.84\pm0.14\mathrm{c}$	$54.42\pm0.67\mathrm{c}$	$252.89\pm6.29\mathrm{b}$
AV6	$0.52\pm0.02b$	$5.12\pm0.32d$	$52.70\pm0.46d$	$210.14\pm9.79a$
CK	$0.40\pm0.02a$	$3.94\pm0.19e$	$49.97\pm0.67b$	$187.83\pm7.31c$

Table 2. Soil enzyme activities among the examined fields.

AV3, AVAV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field. Different letters in columns indicate significant differences (p < 0.05, n = 6).

3.3. Description of the Bacterial and Fungal Communities

A total of 9610 bacterial OTUs were obtained (Figure 1A). Additionally, 2786, 2699, 2382, 2222, and 3370 bacterial OTUs were obtained for the AV3, AV4, AV5, AV6, and CK fields, respectively, with 204 OTUs common to all five fields. A total of 4555 fungal OTUs were obtained (Figure 1B). Moreover, 789, 710, 773, 1032, and 2277 fungal OTUs were obtained for the AV3, AV4, AV5, AV6, and CK fields, respectively, with 21 shared OTUs among the five fields.

The V3-V4 regions of the bacterial 16S rRNA genes in the soil samples from five fields were sequenced and classified (including no-rank and unclassified). A total of 3 kingdoms, 38 phyla, 119 classes, 247 orders, 397 families, 751 genera, and 943 species were detected in the 15 analyzed soil samples.



Figure 1. Venn diagram of the bacterial and fungal OTUs for the *A. villosum* rhizosphere soil samples and the fallow field soil sample. (**A**) Venn diagram of the bacterial OTUs; (**B**) Venn diagram of the fungal OTUs; AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field.

At the bacterial phylum level (Figure 2A,B), the dominant phyla in all samples were *Proteobacteria* (29.57%), *Acidobacteria* (25.80%), *Chloroflexi* (7.22%), *Planctomycetota* (6.22%), and *Actinobacteriota* (6.13%). As the number of years of continuous cropping increased, the relative abundances of *Proteobacteria* and *Acidobacteria* gradually increased and decreased, respectively, whereas the relative abundances of *Chloroflexi*, *Planctomycetota*, and *Actinobacteriota* fluctuated.



Figure 2. Cont.

С

AV5

A V 6

СК

A V 4

AV3

anctomycetes

0

0.20 0.15 0.10 0.05 0.00

Bray-Curtis distance

D

Alphaproteobacteria



Genus



Figure 2. Cont.



Figure 2. Bacterial composition and community structure in *A. villosum* rhizosphere soils and a fallow field. (**A**) Bar plot of the bacterial community at the phylum level. (**B**) Circos plot of the top five bacterial communities at the phylum level. (**C**) Bar plot of the bacterial community at the class level. (**D**) Circos plot of the top five bacterial communities at the genus level. (**C**) Circos plot of the top five bacterial communities at the genus level. (**D**) Circos plot of the top five bacterial communities at the class level. (**E**) Bar plot of the bacterial community at the genus level. (**F**) Circos plot of the top five bacterial communities at the genus level. The relative abundance in each sample was calculated on the basis of the percentage of the total effective bacterial sequences, which were classified using the SILVA databank. Phyla, classes, and genera representing less than 1% of the total composition in both libraries were classified as 'other.' AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field.

At the bacterial class level (Figure 2C,D), the dominant classes in all samples were *Acidobacteriae* (21.07%), *Alphaproteobacteria* (16.66%), *Gammaproteobacteria* (11.18%), *Verrucomicrobiae* (5.97%), and *Planctomycetes* (5.36%). The relative abundance of *Acidobacteriae* tended to gradually decrease as the number of years of continuous cropping increased. The relative abundances of *Alphaproteobacteria* and *Gammaproteobacteria* initially increased and then decreased as the number of years of continuous cropping increased. In contrast, the relative abundance of *Verrucomicrobiae* initially decreased and then increased. The relative abundance of *Planctomycetes* fluctuated in response to the continuous cropping. Relative abundances (%) of bacteria at the phylum, class, and genus level were showed in Supplementary Tables S1–S3, respectively.

At the bacterial genus level (Figure 2E,F), *Subgroup_2_unclassified* (8.89%), *Elsterales_unclassified* (5.59%), *Acidobacteriales_unclassified* (5.41%), *Gemmataceae_unclassified* (4.09%), and *Acidothermus* (3.71%) were the top five genera in all rhizosphere soils. As the duration of the continuous cropping increased, the relative abundances of *Subgroup_2_unclassified* and *Acidobacteriales_unclassified* increased and decreased. The relative abundances of *Elsterales_unclassified* and *Acidothermus* increased at first and then decreased as the number of years of continuous cropping increased, whereas the opposite changes were observed for the relative abundance of *Gemmataceae_unclassified* (i.e., U-shaped curve).

The ITS1-1 F regions of the fungal genes in the 15 analyzed soil samples were sequenced and classified (including no-rank and unclassified), after which the OTUs were divided into 6 phyla, 26 classes, 75 orders, 165 families, 343 genera, and 557 species. Relative abundances (%) of fungi at the phylum, class, and genus level were showed in Supplementary Tables S4–S6, respectively.

At the phylum taxonomic level, the soil fungal kingdom consisted of the following six fungal phyla (Figure 3A,B): *Ascomycota* (41.93%), *Basidiomycota* (39.62%), *Fungi_unclassified* (10.38%), *Zygomycota* (6.71%), *Glomeromycota* (1.25%), and *Chytridiomycota* (0.12%). Thus, *Ascomycota* and *Basidiomycota* were the two most dominant phyla in the fungal community.

The relative abundance of *Ascomycota* initially decreased and then increased as the number of years of continuous cropping increased, which was in contrast to the fluctuations in the relative abundance of *Basidiomycota*.



Figure 3. Cont.



Figure 3. Fungal composition and community structure in *A. villosum* rhizosphere soils and a fallow field. (**A**) Bar plot of the fungal community at the phylum level. (**B**) Circos plot of the top five fungal communities at the phylum level. (**C**) Bar plot of the fungal community at the class level. (**D**) Circos plot of the top five fungal communities at the class level. (**E**) Bar plot of the fungal community at the genus level. (**F**) Circos plot of the top five fungal communities at the genus level. (**F**) Circos plot of the top five fungal communities at the genus level. The relative abundance in each sample was calculated on the basis of the percentage of the total effective fungal sequences, which were classified using the SILVA databank. Phyla, classes, and genera representing less than 1% of the total composition in both libraries were classified as 'other.' AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field.

The dominant classes in the *A. villosum* and fallow fields were *Agaricomycetes* (30.03%), *Ascomycota_unclassified* (26.80%), *Sordariomycetes* (13.15%), *Fungi_unclassified* (11.25%), and *Mucoromycotina_incertae_sedis* (6.23%) (Figure 3C,D). The relative abundances of *Agaricomycetes*, *Sordariomycetes*, and *Fungi_unclassified* increased and decreased as the duration of the continuous cropping increased. The changes in the relative abundance of *Ascomycota_unclassified* as the number of years of continuous cropping increased showed a U-shaped curve, whereas the relative abundance of *Mucoromycotina_incertae_sedis* initially increased and then decreased in response to the continuous cropping.

The five most dominant genera in the continuously cropped and fallow fields were *Ascomycota_unclassified* (26.80%), *Agaricomycetes_unclassified* (15.47%), *Fungi_unclassified* (11.25%), *Agaricales_unclassified* (6.26%), and *Mortierella* (6.21%) (Figure 3E,F). The relative abundance of *Verrucomicrobiae* decreased at first and then increased with the increase in the number of years of continuous cropping, whereas the opposite changes were observed for the relative abundance of *Mortierella*. As the length of the continuous cropping period increased, the relative abundances of *Agaricomycetes_unclassified* and *Fungi_unclassified*

increased and decreased. The relative abundance of *Agaricales_unclassified* exhibited a gradual increasing trend as the number of years of continuous cropping increased.

3.4. Microbial Diversity

3.4.1. Alpha Diversity Indices of the Bacterial and Fungal Communities

Alpha diversity indices were used to reveal the bacterial community diversity in the samples (Table 3). The richness (Observed_OTUs and Chao1) of the bacterial communities was highest for the CK field and decreased steadily as the duration of the continuous cropping increased. However, there were almost no differences in the Shannon and Simpson indices of the bacterial communities between the continuously cropped fields and the fallow field. The bacterial coverage rate (Goods_coverage) indicated that the sequencing results for all samples reflected the actual bacterial communities in the samples. The Pielou_e index (evenness) of the bacterial communities was relatively high in all samples, with no major differences among fields.

Table 3. Alpha diversity indices of the bacterial community in *A. villosum* rhizosphere soils and a fallow field.

Samples	Observed_OTUs	Shannon	Simpson	Chao1	Goods_coverage	Pielou_e
AV3	$1524.67 \pm 78.02a$	$9.55\pm0.13a$	$1.00\pm0.00a$	$1532.47 \pm 77.92a$	$1.00\pm0.00a$	$0.90\pm0.01a$
AV4	$1460.00 \pm 60.00 b$	$9.59\pm0.09a$	$1.00\pm0.00 \mathrm{a}$	$1465.07 \pm 58.46 \mathrm{b}$	1.00 ± 0.00 a	$0.91\pm0.00b$
AV5	$1448.50 \pm 94.50b$	$9.45\pm0.10a$	$1.00\pm0.00a$	$1459.23 \pm 87.73b$	1.00 ± 0.00 a	$0.90\pm0.00a$
AV6	$1428.33 \pm 19.33b$	$9.46\pm0.07a$	$1.00\pm0.00a$	$1439.18 \pm 25.85b$	1.00 ± 0.00 a	$0.90\pm0.01a$
СК	$1681.00 \pm 39.00c$	$9.91\pm0.05b$	$1.00\pm0.00 \mathrm{a}$	$1686.32 \pm 37.63c$	$1.00\pm0.00 a$	$0.92\pm0.00c$

AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field. Different letters in columns indicate significant differences (p < 0.05, n = 3).

Alpha diversity indices were also used to reveal the fungal community diversity in the samples (Table 4). The richness (Observed_OTUs and Chao1) of the fungal communities was highest in the AV3 field, but it clearly decreased as the length of the continuous cropping period increased. Additionally, the Shannon and Simpson indices of the fungal communities decreased significantly as the number of years of continuous cropping increased. Similar to the bacterial communities, the coverage rate (Goods_coverage) of the fungal communities demonstrated that the sequencing results for all samples accurately reflected the fungal communities in the samples. The Pielou_e index (evenness) of the fungal communities in all samples decreased in response to the continuous cropping.

Table 4. Alpha diversity indices of the fungal community in *A. villosum* rhizosphere soils and a fallow field.

Samples	Observed_OTUs	Shannon	Simpson	Chao1	Goods_coverage	Pielou_e
AV3	$969.67 \pm 3.03a$	$6.98\pm0.63a$	$0.96\pm0.02a$	$971.03 \pm 7.85a$	$1.00\pm0.00a$	$0.71\pm0.03a$
AV4	$576.00 \pm 0.00b$	$6.06\pm0.06\mathrm{b}$	$0.95\pm0.00a$	$577.04 \pm 0.62b$	$1.00\pm0.00a$	$0.66 \pm 0.01 \mathrm{ab}$
AV5	$439.50 \pm 6.50 b$	$4.95\pm0.16c$	$0.87 \pm 0.02b$	$439.62\pm6.47b$	$1.00\pm0.00a$	$0.56 \pm 0.02b$
AV6	$329.33\pm5.79b$	$3.35\pm0.85d$	$0.75\pm0.07c$	$329.98\pm5.26b$	$1.00\pm0.00a$	$0.40\pm0.02c$

AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field. Different letters in columns indicate significant differences (p < 0.05, n = 3).

3.4.2. Beta Diversity Indices of the Bacterial and Fungal Communities

The distributions of the bacterial communities in the five examined fields along the first two PCoA axes are presented in Figure 4A. On the basis of the first principal coordinate, the bacterial communities in the AV3-AV6 fields were grouped separately from that of the fallow field. On the basis of the second principal coordinate, the rhizospheric bacterial communities of the AV3 and AV4 fields were generally grouped separately from the corresponding communities of the AV5 and AV6 fields.



Figure 4. Principal coordinate analysis (PCoA) of the bacterial and fungal communities. (**A**) Results of the PCoA of the bacterial communities. (**B**) Results of the PCoA of the fungal communities. AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field.

The rhizospheric fungal communities in the AV3–AV6 fields were clustered separately from the fungal community in the fallow field according to the first principal coordinate (Figure 4B). The fungal community in the AV3 rhizosphere soil was distinguished from those of the AV4, AV5, and AV6 rhizosphere soil samples according to the second principal coordinate.

3.5. Effects of Environmental Factors on Bacterial and Fungal Communities

The RDA of the data for the top ten bacterial phyla and the soil environmental factors revealed clear variations in the bacterial community structures in the AV3–AV6 fields and the fallow field (Figure 5A). The rank-order of the effects of the soil physicochemical properties and enzyme activities on the bacterial community structure was as follows: pH > UA > TN > AN > TP > PA > AK > OM > CA > TK > AP. The soil pH was the top environmental factor influencing the bacterial community composition at the phylum level. The soil pH was positively correlated with *Chloroflexi*.



Figure 5. Correlations among microbial phyla, soil physicochemical properties, and soil enzyme activities. **(A)** Results of the redundancy analysis (RDA) of the dominant bacterial phyla, soil physicochemical properties, and soil enzyme activities. **(B)** Results of the RDA of the dominant fungal phyla, soil physicochemical properties, and soil enzyme activities. AV3, AV4, AV5, and AV6 represent the rhizosphere soil samples from the fields in which *A. villosum* was continuously cropped for 3, 4, 5, and 6 years, respectively, whereas CK represents the soil sample from the fallow field. OM: organic matter; TN: total nitrogen; AN: available nitrogen; TP: total phosphorus; AP: available phosphorus; TK: total potassium; AK: available potassium; UA: urease activity; PA: acid phosphatase activity; CA: catalase activity; and SA: sucrase activity.

The RDA of the data for all six fungal phyla and the soil environmental factors detected obvious changes in the fungal community structure in the AV3-AV6 fields and the fallow field (Figure 5B). The rank-order of the effects of the soil physicochemical properties and enzyme activities on the fungal phyla was as follows: CA > PA > AK > TP > UA > OM > TN > TK > pH > AN > AP. The soil CA was the top environmental factor affecting the fungal community composition at the phylum level. A negative correlation was detected between CA and *Basidiomycota*. The RDA values of each variable with explanation percentage and associated P value at the bacterial and fungal phylum levels were presented in Supplementary Table S7 and Table S8, respectively.

4. Discussion

Analyzing the soil physicochemical properties and enzyme activities during the continuous cropping of different plants is important for characterizing the effects of continuous cropping on the rhizosphere soil fertility. In this study, the continuous cropping of A. villosum affected the soil pH, resulting in the gradual acidification of the soil, which is in accordance with the findings of other studies on Codonopsis tangshen [10], P. multiflorum [17], Cannabis sativa [31], and Piper nigrum [32]. The overuse of chemical fertilizers and pesticides has various effects on soil pH. The chemical fertilizers are rich in N, P, and K, which are highly water soluble and change pH. The pesticides are known to remain in soil for longer periods, form transformation products having toxic and harmful effects, and are retained in the soil. This retention is directly proportional to the soil pH. Therefore, chemical fertilizers (N, P, and K) and pesticides could cause the acidification of soil by lowering its pH [31–35]. In the present study, as the number of years of continuous cropping increased, the total nitrogen, available nitrogen, and total phosphorus contents gradually increased, meanwhile, the soil pH gradually decreased. In addition, no pesticide was used in these experimental sites because A. villosum was organically cultivated. Consequently, the total nitrogen, available nitrogen, and total phosphorus may be the main factors leading to soil acidification in this study. In a previous study, the excessive application of nitrogen fertilizers led to soil acidification, although the application of nitrogen fertilizers can satisfy the demand for available nitrogen by A. villosum to some extent [36]. Besides, plant species also differ greatly in their capacity to change rhizospheric soil properties such as pH. Some species such as chickpea are capable of acidifying their rhizosphere to a greater extent than others such as white lupin and field pea, due to greater excess uptake of cations over anions [37]. However, the long-term influences of A. villosum plant root exudates or residues on rhizospheric soil pH could not be determined in the range of this study, and we will carry out relevant research in subsequent experiments.

In the current study, as the number of years of continuous cropping increased, the soil organic matter, total nitrogen, available nitrogen, and total phosphorus contents gradually increased, which was in contrast to the gradual decrease in the available phosphorus, total potassium, and available potassium contents, which might be related to inappropriate fertilizer management practices. These results were consistent with the results of earlier research on *C. chinensis* [8,9] and *P. multiflorum* [17], even though the experiments conducted in these studies differed in terms of fertilizer management. Inorganic or chemical fertilizers are often misused in continuous cropping systems, which usually results in detrimental changes to soil physicochemical properties.

The soil urease and acid phosphatase activities in the present study tended to increase as the length of the continuous cropping period increased, possibly reflecting the conversion of soil substances. Similar results were obtained by Alami et al. [17] during a study on *Zea mays*. In our study, the increase in the soil urease activity was consistent with the upward trend in the soil organic matter and total nitrogen contents as the number of years of continuous cropping increased. The activity of soil urease, which can convert urea to ammonia, was positively correlated with the soil organic matter and total nitrogen contents [38–40]. In addition, the changes in the acid phosphatase activity and the soil pH had the opposite trends as the duration of the continuous cropping increased. Similarly, a previous investigation confirmed

14 of 18

that the soil acid phosphatase activity is negatively correlated with the soil pH, with an increase in soil acidity leading to increased enzymatic activity [41].

Proteobacteria and Acidobacteria were the top two bacterial phyla in this study (relative abundance exceeding 25%). Proteobacteria is the largest bacterial phylum, which comprises many nitrogen-fixing bacteria [42]. Actinomycetes from the phylum Acidobacteria are mostly saprophytes and are widely distributed in soil [43]. They mainly promote the degradation of plant debris in the soil, while also contributing to the nitrogen cycle under natural conditions [39]. These results are consistent with those of other studies that revealed that Proteobacteria and Acidobacteria are the most common bacterial phyla in different agricultural systems or soil types [8,10,17]. Proteobacteria could decompose organic matter and promote the growth of sweet potato and sugarcane [36,44]. In the present study, the relative abundances of Proteobacteria increased gradually as the number of years of continuous cropping increased, and meanwhile, the fruits of A. villosum also presented a gradual increase in production (unpublished). Acidobacteria usually exists in nutrient-poor and highly acidic soil environments and also promotes plant growth through degrading complex and stubborn carbon sources [45]. In our study, the relative abundances of Acidobacteria decreased gradually from AV3 to AV6, although rhizospheric soil acidized gradually and was more suitable for the growth of Acidobacteria. Therefore, Acidobacteria was not one of the factors that increases A. villosum production in this study.

Ascomycota and Basidiomycota were the top two fungal phyla in the present study (relative abundance exceeding 35%). During the vegetative phase, processes related to saprophylaxis, parasitism, and symbiosis are active in ascomycetes from Ascomycota, which enables these fungi to decompose plant residues [46]. Species belonging to *Basidiomycota*, which is one of the largest fungal phyla, coexist with plants to form mycorrhizae, which are beneficial for crop cultivation [47]. However, some basidiomycetes can cause plant diseases, many of which are responsible for severe economic losses [47]. Our findings are in accordance with the results of previous studies that identified Ascomycota and Basidiomycota as the two most abundant phyla in C. chinensis and Panax ginseng grown in continuous cropping systems [9,41]. Some fungi belonging to Ascomycota are classified as plant growth-promoting fungi, and directly promote plant growth, but some other are harmful to plants and can cause many diseases [48]. Fungi of the Basidiomycota represent major pathogen lineages and mushroom-forming species [49]. In the present study, the relative abundance of *Ascomycota* initially decreased and then increased as the number of years of continuous cropping increased, which was in contrast to the fluctuations in the relative abundance of *Basidiomycota*. However, the variation tendencies in the relative abundance of Ascomycota and Basidiomycota were inconsistent with the growth trend of A. villosum production as the length of the continuous cropping period increased. Therefore, Ascomycota and Basidiomycota were not the factors that increased A. villosum production in this study.

Soil microorganisms, which promote the decomposition of soil organic matter and the transformation of soil nutrients, vary in terms of their types and quantities in different soil environments [50]. Bacteria and fungi, two important groups of microbes in the soil ecosystem, have vital effects on soil functions and plant health [51]. In our study, the continuous cropping of *A. villosum* significantly influenced the composition, diversity, and structure of the soil bacterial and fungal communities. More specifically, compared with the corresponding indices for the CK field, the Observed_OTUs and Chao1 indices (richness) for the bacterial communities were lower for the AV3-AV6 fields. Additionally, the richness of the bacterial communities steadily decreased as the number of years of continuous cropping of *Glycine max* [1], *C. chinensis* [8], and *Z. mays* [17] over multiple years. In terms of the fungal communities examined in our study, the richness (Observed_OTUs and Chao1) and diversity (Shannon and Simpson) indices decreased significantly as the length of the continuous cropping period increased. Similarly, Alami et al. [9] observed that the richness

and diversity indices for fields in which *C. chinensis* was continuously cropped tended to decrease over time. The suppressive effects of continuous cropping on soil bacterial and fungal populations may be due to the toxic effects of long-term continuous cropping, including the accumulation of antimicrobial compounds [52,53].

Due to the high economic and medicinal value of perennial medicinal plants, the area of planting is significantly increasing, and continuous monoculture systems are widely used, leading to outbreaks of soil-borne diseases and a reduction in yield and quality (antioxidant and anti-inflammatory effects) through several pathways [54]. The monocultures are usually accompanied by unstable soil microbial communities, with increased abundances of pathogens and reduced beneficial microorganisms, resulting in outbreaks of soil-borne diseases [55]. Finally, yield reductions from monocultures are related to autotoxic substances in rhizosphere soil [56]. In contrast, in the present study, a gradual increase was found in *A. villosum* production and soil-borne diseases were not apparent as the number of years of continuous cropping increased, although the diversity of the soil bacterial and fungal communities decreased gradually.

Our PCoA results suggested that the continuous cropping of *A. villosum* substantially altered the soil bacterial and fungal community structures. Specifically, the bacterial and fungal communities in the CK soil samples were clustered together and clearly separated from the corresponding communities in the AV3-AV6 soil samples. Moreover, the distance separating the CK soil samples from the *A. villosum* rhizosphere samples was greater than the distance between the different *A. villosum* rhizosphere samples (AV3-AV6). This result was consistent with the related findings of studies on the continuous cropping of annual and perennial plants, including *Saccharum officinarum* [57], *Panax notoginseng* [58], *Coffea arabica* [59], and *Arachis hypogaea* [60]. Thus, soil bacterial and fungal communities appear to be affected by continuous cropping systems.

The correlation between the diversity of soil microbial communities and soil environmental factors may provide fundamental insights useful for addressing problems associated with continuous cropping [32]. In the current study, the RDA results demonstrated that pH and CA were the most important soil environmental factors affecting the soil bacterial and fungal community compositions. Similar results were reported for C. sativa [31] and S. officinarum [57], indicating that the soil microbial community composition and structure are correlated with pH in continuous cropping systems. In an earlier study on the consequences of continuous cropping, the soil urease activity was revealed to have the strongest effect on the soil microbial community among the analyzed factors [17]. Therefore, the changes in soil physicochemical properties and enzyme activities caused by long-term continuous cropping may be the main factor modulating bacterial and fungal communities and may also be one of the main problems associated with the continuous cropping of A. villosum. Besides, changes in bacterial and fungal community structures in response to the continuous cropping of A. villosum may also be under the long-term influences of A. villosum plant root exudates or residues, which could not be determined in the range of this study.

5. Conclusions

The long-term continuous cropping of *A. villosum* resulted in distinct changes to soil physicochemical properties and enzyme activities, which differed between the *A. villosum* rhizosphere soil samples and the fallow field soil samples. The soil organic matter, total nitrogen, available nitrogen, and total phosphorus contents gradually increased as the length of the continuous cropping period increased, whereas the soil pH, available phosphorus, total potassium, and available potassium content tended to decrease slightly. Continuous cropping promoted soil urease and acid phosphatase activities, and these two enzymes might have accelerated the conversion of soil substances. The continuous cropping of *A. villosum* markedly changed the bacterial and fungal community composition, structure, and diversity. Moreover, the bacterial and fungal communities were more diverse in the fallow field than in the fields in which *A. villosum* was continuously cropped. Besides, RDA

analyses showed that the pH value has the greatest impact on the bacterial community at the phylum taxonomic level, and the soil pH was positively correlated with *Chloroflexi*. RDA analyses also revealed that catalase activity had the greatest effect on the fungal community at the fungal genus level, and catalase activity was negatively correlated with *Basidiomycota*. These observations provide the basis for future research on sustainable agricultural production and for increasing soil bacterial and fungal activities as well as *A. villosum* yield and quality in continuous cropping systems in China.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/agronomy12102548/s1, Table S1. Relative abundances (%) of bacteria at the phylum level; Table S2. Relative abundances (%) of bacteria at the class level; Table S3. Relative abundances (%) of bacteria at the genus level; Table S4. Relative abundances (%) of fungi at the phylum level; Table S5. Relative abundances (%) of fungi at the class level; Table S6. Relative abundances (%) of fungi at the genus level; Table S7. The RDA value of each variable with explanation percentage and associated *P* value at the bacterial phylum level; Table S8. The RDA value of each variable with explanation percentage and associated *P* value at the fungal phylum level.

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References

- 1. Cui, Y.; Chen, X.; Nie, L.; Sun, W.; Hu, H.; Lin, Y.; Li, H.; Zheng, X.; Song, J.; Yao, H. Comparison and phylogenetic analysis of chloroplast genomes of three medicinal and edible *Amonum* species. *Int. J. Mol. Sci.* **2019**, *20*, 4040. [CrossRef] [PubMed]
- Doh, E.J.; Kim, J.-H.; Lee, G. Identification and monitoring of *Amomifructus* and its adulterants based on DNA barcoding analysis and designed DNA markers. *Molecules* 2019, 24, 4193. [CrossRef] [PubMed]
- 3. Kim, H.-L.; Lee, S.-K.; Min, D.-E.; Choi, B.-K.; Lee, D.-R. Anti-obesity effects of a mixture of *Atractylodes macrocephala* and *Amomum villosum* extracts on 3T3-L1 adipocytes and high-fat diet-induced obesity in mice. *Molecules* **2022**, *27*, 906. [CrossRef]
- Zhou, Q.; Li, X.; Wang, X.; Shi, D.; Zhang, S.; Yin, Y.; Zhang, H.; Liu, B.; Song, N.; Zhang, Y. Vanillic acid as a promising xanthine oxidase inhibitor: Extraction from *Amomum villosum* Lour and biocompatibility improvement via extract nanoemulsion. *Foods* 2022, 11, 968. [CrossRef]
- Cho, J.-H.; Lee, J.-S.; Kim, H.-G.; Lee, H.W.; Fang, Z.; Kwon, H.-H.; Kim, D.W.; Lee, C.-M.; Jeong, J.-W. Ethyl acetate fraction of *Amomum villosum* var. *xanthioides* attenuates hepatic endoplasmic reticulum stress-induced non-alcoholic steatohepatitis via improvement of antioxidant capacities. *Antioxidants* 2021, 10, 998. [CrossRef] [PubMed]
- Ao, H.; Wang, J.; Chen, L.; Li, S.; Dai, C. Comparison of volatile oil between the fruits of *Amomum villosum* Lour. and *Amomum villosum* Lour. var. *xanthioides* T. L. Wu et Senjen based on GC-MS and chemometric techniques. *Molecules* 2019, 24, 1663. [CrossRef]
- Huang, Q.; Duan, Z.; Yang, J.; Ma, X.; Zhan, R.; Xu, H.; Chen, W. SNP typing for germplasm identification of *Amomum villosum* Lour. based on DNA barcoding markers. *PLoS ONE* 2014, 9, e114940. [CrossRef]
- 8. Alami, M.M.; Xue, J.; Ma, Y.; Zhu, D.; Gong, Z.; Shu, S.; Wang, X. Structure, Diversity, and composition of bacterial communities in rhizospheric soil of *Coptis chinensis* Franch under continuously cropped fields. *Diversity* **2020**, *12*, 57. [CrossRef]

- Alami, M.M.; Xue, J.; Ma, Y.; Zhu, D.; Abbas, A.; Gong, Z.; Wang, X. Structure, function, diversity, and composition of fungal communities in rhizospheric soil of *Coptis chinensis* Franch under a successive cropping system. *Plants* 2020, 9, 244. [CrossRef]
- Zhang, M.; He, Y.; Zhou, W.; Ai, L.; Liu, H.; Chen, L.; Xie, Y. Effects of continuous cropping of *Codonopsis tangshen* on rhizospheric soil bacterial community as determined by pyrosequencing. *Diversity* 2021, 13, 317. [CrossRef]
- 11. Tang, L.; He, G.; Su, J.; Xu, H. The strategy to promote the development of industry of genuine medicinal material of *Amomum* villosum. Chin. Agric. Sci. Bull. **2012**, *28*, 94–99.
- 12. Cheng, J.; Jin, H.; Zhang, J.; Xu, Z.; Yang, X.; Liu, H.; Xu, X.; Min, D.; Lu, D.; Qin, B. Effects of allelochemicals, soil enzyme activities, and environmental factors on rhizosphere soil microbial community of *Stellera chamaejasme* L. along a growth-coverage gradient. *Microorganisms* **2022**, *10*, 158. [CrossRef]
- 13. Shi, R.; Gu, H.; He, S.; Xiong, B.; Huang, Y.; Horowitz, A.R.; He, X. Comparative metagenomic and metabolomic profiling of rhizospheres of *Panax notoginseng* grown under forest and field conditions. *Agronomy* **2021**, *11*, 2488. [CrossRef]
- Bogati, K.; Walczak, M. The impact of drought stress on soil microbial community, enzyme activities and plants. *Agronomy* 2022, 12, 189. [CrossRef]
- Muhammad, I.; Lv, J.Z.; Yang, L.; Ahmas, S.; Farooq, S.; Zeeshan, M.; Zhou, X.B. Low irrigation water minimizes the nitrate nitrogen losses without compromising the soil fertility, enzymatic activities and maize growth. *BMC Plant Biol.* 2022, 22, 159. [CrossRef]
- Bueis, T.; Turrión, M.B.; Bravo, F.; Pando, V.; Muscolo, A. Factors determining enzyme activities in soils under *Pinus halepensis* and *Pinus sylvestris* plantations in Spain: A basis for establishing sustainable forest management strategies. *Ann. For. Sci.* 2018, 75, 34. [CrossRef]
- 17. Alami, M.M.; Pang, Q.; Gong, Z.; Yang, T.; Tu, D.; Zhen, O.; Yu, W.; Alami, M.J.; Wang, X. Continuous cropping changes the composition and diversity of bacterial communities: A meta-analysis in nine different fields with different plant cultivation. *Agriculture* **2021**, *11*, 1224. [CrossRef]
- Ishida, J.K.; Bini, A.P.; Creste, S.; Sluys, M.V. Towards defining the core *Saccharum* microbiome: Input from five genotypes. *BMC Microbiol.* 2022, 22, 193. [CrossRef]
- 19. Schmidt, J.E.; Firl, A.; Hamran, H.; Imaniar, N.I.; Crow, T.M.; Forbes, S.J. Impacts of shade trees on the adjacent cacao rhizosphere in a young diversified agroforestry system. *Agronomy* **2022**, *12*, 195. [CrossRef]
- 20. Chen, J.; Huang, X.; Tong, B.; Wang, D.; Liu, J.M.; Liao, X.F.; Sun, Q.W. Effects of rhizosphere fungi on the chemical composition of fruits of the medicinal plant *Cinnamomum migao* endemic to southwestern China. *BMC Microbiol.* **2021**, *21*, 206. [CrossRef]
- 21. Zuo, J.; Zu, M.; Liu, L.; Song, X.M.; Yuan, Y.D. Composition and diversity of bacterial communities in the rhizosphere of the Chinese medicinal herb *Dendrobium*. *BMC Plant Biol*. **2021**, *21*, 127. [CrossRef] [PubMed]
- Chen, C.Y.; Zhong, C.F.; Gao, X.; Tan, C.Y.; Bai, H.; Ning, K. *Glycyrrhiza uralensis* Fisch. Root-associated microbiota: The multifaceted hubs associated with environmental factors, growth status and accumulation of secondary metabolites. *Environ. Microbiome* 2022, 17, 23. [CrossRef] [PubMed]
- 23. Gu, X.; Yang, N.; Zhao, Y.; Liu, W.H.; Li, T.F. Long-term watermelon continuous cropping leads to drastic shifts in soil bacterial and fungal community composition across gravel mulch fields. *BMC Microbiol.* **2022**, 22, 189. [CrossRef] [PubMed]
- Dong, L.; Xu, J.; Zhang, L.; Yang, J.; Liao, B.S.; Li, X.W.; Chen, S.L. High-throughput sequencing technology reveals that continuous cropping of American ginseng results in changes in the microbial community in arable soil. *Chin. Med.* 2017, 12, 18. [CrossRef] [PubMed]
- 25. Dong, L.; Bian, X.; Zhao, Y.; Yang, H.; Xu, Y.H.; Han, Y.Z.; Zhang, L.X. Rhizosphere analysis of field-grown *Panax ginseng* with different degrees of red skin provides the basis for preventing red skin syndrome. *BMC Microbiol.* **2022**, 22, 12. [CrossRef]
- Luo, Y.; Wang, Z.; He, Y.; Li, G.F.; Lv, X.H.; Zhuang, L. High-throughput sequencing analysis of the rhizosphere arbuscular mycorrhizal fungi (AMF) community composition associated with *Ferula sinkiangensis*. BMC Microbiol. 2020, 20, 335. [CrossRef]
- 27. Bao, S.D. Analysis Method of Soil and Agricultural Chemistry, 3rd ed.; China Agricultural Press: Beijing, China, 2000; pp. 25–108.
- 28. Guan, S.Y. Soil Enzyme and Its Study Method; China Agriculture Press: Beijing, China, 1986; pp. 270–285.
- Logue, J.B.; Stedmon, C.A.; Kellerman, A.M.; Nielsen, N.J.; Andersson, A.F.; Laudon, H.; Lindstrom, E.S.; Kritzberg, E.S. Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J.* 2016, 10, 533–545. [CrossRef]
- Bolyen, E.; Rideout, J.R.; Matthew, R.D.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Author correction: Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 2019, 37, 1091. [CrossRef]
- Guo, L.; Chen, X.; Li, Z.; Wang, M.; Che, Y.; Zhang, L.; Jiang, Z.; Jie, S. Effects of continuous cropping on bacterial community and diversity in rhizosphere soil of industrial hemp: A five-year experiment. *Diversity* 2022, 14, 250. [CrossRef]
- Xiong, W.; Li, Z.; Liu, H.; Xue, C.; Zhang, R.; Wu, H.; Li, R.; Shen, Q. The Effect of long-term continuous cropping of black pepper on soil bacterial communities as determined by 454 Pyrosequencing. *PLoS ONE* 2015, 10, e0136946. [CrossRef]
- Barak, P.; Jobe, B.O.; Krueger, A.R.; Peterson, L.A.; Laird, D.A. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant Soil* 1997, 197, 61–69. [CrossRef]
- 34. Ehrenfeld, J.G.; Ravit, B.; Elgersma, K. Feedback in the plant-soil system. Annu. Rev. Environ. Resour. 2005, 30, 75–115. [CrossRef]
- 35. Baweja, P.; Kumar, S.; Kumar, G. Fertilizers and Pesticides: Their Impact on Soil Health and Environment; Springer Nature: Berne, Switzerland, 2020; p. 270.

- 36. Pang, Z.; Dong, F.; Liu, Q.; Lin, W.; Hu, C.; Yuan, Z. Soil Metagenomics reveals effects of continuous sugarcane cropping on the structure and functional pathway of rhizospheric microbial community. *Front. Microbiol.* **2021**, *12*, 369. [CrossRef] [PubMed]
- Wang, X.J.; Tang, C.X.; Severi, J.; Butterly, C.R.; Baldock, J.A. Rhizosphere priming effect on soil organic carbon decomposition under plant species differing in soil acidification and root exudation. *New Phytol.* 2016, 211, 864–873. [CrossRef] [PubMed]
- Gong, J.; Hou, W.; Liu, J.; Malik, K.; Kong, X.; Wang, L.; Chen, X.; Tang, M.; Zhu, R.; Cheng, C.; et al. Effects of different land use types and soil depths on soil mineral elements, soil enzyme activity, and fungal community in karst area of southwest China. *Int. J. Environ. Res. Public Health* 2022, 19, 3120. [CrossRef] [PubMed]
- 39. Liang, C.; Liu, L.; Zhang, Z.; Ze, S.; Ji, M.; Li, Z.; Yu, J.; Yang, B.; Zhao, N. Do mixed *Pinus yunnanensis* plantations improve soil's physicochemical properties and enzyme activities? *Diversity* **2022**, *14*, 214. [CrossRef]
- Wang, F.; Cui, H.; He, F.; Liu, Q.; Zhu, Q.; Wang, W.; Liao, H.; Yao, D.; Cao, W.; Lu, P. The green manure (*Astragalus sinicus* L.) improved rice yield and quality and changed soil microbial communities of rice in the karst mountains area. *Agronomy* 2022, 12, 1851. [CrossRef]
- 41. Bian, X.; Yang, X.; Li, Q.; Sun, X. Effects of planting of two common crops, *Allium fistulosum* and *Brassica napus*, on soil properties and microbial communities of ginseng cultivation in northeast China. *BMC Microbiol.* **2022**, 22, 182. [CrossRef]
- 42. Gupta, R.S.; Mok, A. Phylogenomics and signature proteins for the alpha *Proteobacteria* and its main groups. *BMC Microbiol.* 2007, 7, 106. [CrossRef]
- 43. Jiang, C.; Sun, X.R.; Feng, J.; Zhu, S.F.; Shui, W. Metagenomic analysis reveals the different characteristics of microbial communities inside and outside the karst tiankeng. *BMC Microbiol.* **2022**, *22*, 115. [CrossRef]
- 44. Gao, Z.; Hu, Y.; Han, M.; Xu, J.; Wang, X.; Liu, L.; Tang, Z.; Jiao, W.; Jin, R.; Liu, M.; et al. Effects of continuous cropping of sweet potatoes on the bacterial community structure in rhizospheric soil. *BMC Microbiol.* **2021**, *21*, 102. [CrossRef] [PubMed]
- 45. Sikorski, J.; Baumgartner, V.; Birkhofer, K.; Boeddinghaus, R.S.; Bunk, B.; Fischer, M.; Fösel, B.U.; Friedrich, M.W.; Göker, M.; Hölzel, N.; et al. The evolution of ecological diversity in Acidobacteria. *Front. Microbiol.* **2022**, *13*, 715637. [CrossRef] [PubMed]
- 46. Peters, B.A.; Wu, J.; Hayes, R.B.; Ahn, J.Y. The oral fungal mycobiome: Characteristics and relation to periodontitis in a pilot study. *BMC Microbiol.* **2017**, *17*, 157. [CrossRef] [PubMed]
- Badotti, F.; de Oliveira, F.S.; Garcia, C.F.; Vaz, A.B.M.; Fonseca, P.L.C.; Nahum, L.A.; Oliveira, G.; Goes-Neto, A. Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of *Basidiomycota* (Fungi). *BMC Microbiol.* 2017, 17, 42. [CrossRef]
- Khalil, A.M.A.; Hassan, S.E.-D.; Alsharif, S.M.; Eid, A.M.; Ewais, E.E.-D.; Azab, E.; Gobouri, A.A.; Elkelish, A.; Fouda, A. Isolation and characterization of fungal endophytes isolated from medicinal plant ephedra pachyclada as plant growth-promoting. *Biomolecules* 2021, 11, 140. [CrossRef]
- 49. Coelho, M.A.; Bakkeren, G.; Sun, S.; Hood, M.E.; Giraud, T. Fungal sex: The Basidiomycota. Microbiol. Spectr. 2017, 10, 1128.
- 50. Marler, T.E. Soil from *Serianthes* rhizosphere influences growth and leaf nutrient content of *Serianthes* plants. *Agronomy* **2022**, 12, 1938. [CrossRef]
- 51. Li, Y.; He, X.; Yuan, H.; Lv, G. Differed growth stage dynamics of root-associated bacterial and fungal community structure associated with halophytic plant *Lycium ruthenicum*. *Microorganisms* **2022**, *10*, 1644. [CrossRef]
- 52. Zhao, J.; Wu, X.; Wu, T.; Dai, W.; Liu, H.; Yang, R. Analysis of unculturable bacterial communities in tea orchard soils based on nested PCR-DGGE. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1967–1979. [CrossRef]
- 53. Li, Y.; Li, Z.; Jiang, Y.; Weng, B.; Lin, W. Variations of rhizosphere bacterial communities in tea (*Camellia sinensis* L.) continuous cropping soil by high-throughput pyrosequencing approach. *J. Appl. Microbiol.* **2016**, *121*, 787–799. [CrossRef]
- 54. An, S.; Wei, Y.; Li, H.; Zhao, Z.; Hu, J.; Philp, J.; Ryder, M.; Toh, R.; Li, J.; Zhou, Y.; et al. Long-term monocultures of American ginseng change the rhizosphere microbiome by reducing phenolic acids in soil. *Agriculture* **2022**, *12*, 640. [CrossRef]
- Manici, L.M.; Kelderer, M.; Franke-Whittle, I.H.; Rühmer, T.; Baab, G.; Nicoletti, F.; Caputo, F.; Topp, A.; Insam, H.; Naef, A. Relationship between root-endophytic microbial communities and replant disease in specialized apple growing areas in Europe. *Appl. Soil Ecol.* 2013, 72, 207–214. [CrossRef]
- 56. Cui, J.; Zhang, E.; Zhang, X.; Wang, Q.; Liu, Q. Effects of 2,4-di-tert-butylphenol at different concentrations on soil functionality and microbial community structure in the Lanzhou lily rhizosphere. *Appl. Soil Ecol.* **2022**, *172*, 104367. [CrossRef]
- Pang, Z.; Tayyab, M.; Kong, C.; Liu, Q.; Liu, Y.; Hu, C.; Huang, J.; Weng, P.; Islam, W.; Lin, W.; et al. Continuous sugarcane planting negatively impacts soil microbial community structure, soil fertility, and sugarcane agronomic parameters. *Microorganisms* 2021, 9, 2008. [CrossRef] [PubMed]
- 58. Tan, Y.; Cui, Y.; Li, H.; Kuang, A.; Li, X.; Wei, Y.; Ji, X. Rhizospheric soil and root endogenous fungal diversity and composition in response to continuous *Panax notoginseng* cropping practices. *Microbiol. Res.* **2017**, *194*, 10–19. [CrossRef] [PubMed]
- Zhao, Q.; Xiong, W.; Xing, Y.; Sun, Y.; Lin, X.; Dong, Y. Long-term coffee monoculture alters soil chemical properties and microbial communities. *Sci. Rep.* 2018, *8*, 6116. [CrossRef] [PubMed]
- 60. Li, X.; Ding, C.; Zhang, T.; Wang, X. Soil biology & biochemistry fungal pathogen accumulation at the expense of plant-beneficial fungi as a consequence of consecutive peanut monoculturing. *Soil Biol. Biochem.* **2014**, 72, 11–18.