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Effect of Different Cultivation Patterns on *Amomum villosum* Yield and Quality Parameters, Rhizosphere Soil Properties, and Rhizosphere Soil Microbes

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Abstract: The forest–medicinal plant management system has benefited the commercial production of *Amomum villosum*. However, little is known about the influence of different forestlands on the cultivation of *A. villosum*. The present study investigated the potential differences in the *A. villosum* yield and quality parameters, rhizosphere soil properties, and rhizosphere soil microbiota between a rubber plantation (RP) and a natural secondary forest (NSF). No significant differences in yield or rhizosphere soil properties of *A. villosum* were observed between RP and NSF, although most of the *A. villosum* yield parameters, the rhizosphere soil physicochemical properties, and soil enzyme activities were higher in NSF than in RP. Furthermore, the 38 volatile components had significantly higher relative abundances in NSF than in RP. Furthermore, the alpha diversity indices for the microbiota communities in the *A. villosum* rhizosphere soil indicated that the richness of the bacterial and fungal communities was significantly higher in NSF than in RP. These findings suggest that NSF conditions may be more appropriate than RP conditions for growing *A. villosum*. The data generated in this study may be useful for increasing the production of high-quality *A. villosum* via the exploitation of natural environments.

Keywords: flavonoid; volatile components; soil physical-chemical characters; soil enzyme activities; soil microbial community

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1. Introduction

Amomum villosum Lour. is a perennial medicinal plant [1] that is widely distributed in China and in Southeast Asian countries, including Malaysia, Vietnam, Myanmar, and Laos [2]. Dried ripe *A. villosum* fruits have been used as herbal medicine for more than 1300 years to treat and stimulate the appetite, promote spleen functions, stop diarrhea, regulate vital energy, and prevent miscarriages [3–6]. In addition, *A. villosum* fruits have become the main commercialized herbal medicinal product used in clinical settings as well as in food products (e.g., tea) and as a flavor and health enhancer and veterinary drug [7].

According to phytochemical analyses, dried ripe *A. villosum* fruits include plentiful volatile substances, of which approximately 1.7–3% have attracted increasing attention because of their utility in the pharmaceutical, food, and cosmetic industries [8,9]. According to previous studies involving GC-MS analyses, *A. villosum* contains more than 60 highly aromatic volatile components [6,10], some of which can serve as a pain killer, anti-inflammatory agent, and analgesic, while also effectively eliminating phlegm [11]. Additionally, flavonoids, which are the main phenolic compounds in *A. villosum* fruits, have multiple bioactivities, such as antioxidant, cardioprotective, and anticarcinogenic

activities, and are useful for treating nontransmissible chronic diseases [6,9,12,13]. The predominant flavonoids include quercetin and isoquercetin [13].

In China, *A. villosum* is distributed in several provinces in southern China, wherein they grow under the shady and humid conditions provided by natural forests [7]. However, with the gradual exhaustion of wild *A. villosum* resources, the current availability of *A. villosum* cannot satisfy the needs of social and economic development, which is an increasing problem [2]. The production of high yields of dried ripe *A. villosum* fruits is mainly related to cultivation methods, which primarily involve field cultivation and understory cultivation [14]. The advantages of the forest medicinal plant management model include the fact it can help preserve cultivated land resources, and it is relatively cost-effective compared with traditional *A. villosum* field-cultivation methods [1]. In addition, forest resources can be used for the cultivation of medicinal plants, while also enriching the forest's ecological structure and improving soil physicochemical properties and organic matter contents, thereby increasing the economic benefits of forests [15]. For example, previous reports have indicated that, compared with field cultivation, growing medicinal plants such as *Dendrobium huoshanense* [15] and *Panax notoginseng* [16] in the forest can lead to higher yields.

Many factors, including changes in soil physical–chemical characteristics, variations in enzyme activities, and alterations in edaphon, can affect the quality and biomass of plants under different cultivation conditions [15–17]. Soil enzymes cooperatively accelerate the metabolizing processes of soil, while reacting quickly to alterations generated by natural and human factors [18,19]. Soil enzymes broadly modulate soil development with the addition of soil evolution and fertility [20]. Soil enzyme activities are affected by different plant cultivation practices [21]. Microorganisms are a significant constituent part of soil ecosystems [22,23]. Micro-ecology-based studies have demonstrated that different plant cultivation practices could dramatically changes the diversity and structure of rhizospheric soil microbial populations [16,24,25].

Second-generation sequencing platforms are increasingly being applied to resolve soil microbial communities in succession cropping modes. Prior research involving second-generation sequencing technology indicated that different cultivation practices can alter the rhizosphere soil bacteria and fungi [16,24,25]. Thus, clarifying the effects of cultivating *A. villosum* in RP and NSF on yield and quality parameters, rhizospheric soil physical-chemical characters, and rhizosphere soil microbiota is critical for optimizing the cultivation of high-quality *A. villosum*. In this study, the diversity and composition of rhizospheric soil microbial communities following the cultivation of *A. villosum* in RP and NSF were examined. Furthermore, yield and quality parameters as well as the physicochemical properties and enzyme activities of the rhizosphere soil, were also determined. This experiment is the first to study the influence of different *A. villosum* cultivation practices on yield, quality, rhizospheric soil physical–chemical characteristics, enzyme activities, and microbial communities. Thus, the study findings will not only serve as a theoretical basis for improving the sustainable management and production of high-quality *A. villosum,* but also bring enlightenment to the future development of forest medicinal plant management model.

2. Materials and Methods

2.1. Experimental Sites and Collection of Rhizosphere Samples

The experimental samples were collected on 22 August 2022 from RP and NSF in which *A. villosum* had been cultivated for 5 years. The sample sites were located in Jiangcheng county, Pu'er city, Yunnan province (latitude 22°34′48″ N, longitude 101°52′48″ E) at an altitude of 776 m. The region comprised mostly sandy and clay soil. The NSF mainly consisted of camphor and eucalyptus trees. Organic and compound fertilizers were applied once in RP, whereas fertilizers were not applied in NSF. The organic and compound fertilizer application rates were 1000 kg and 5000 kg per hectare, respectively. Organic fertilizer was obtained from chicken and cattle manures by fermentation. For compound fertilizer, the N, P, and K elements each accounted for 15%, and the other chemical elements accounted for

55%. Three biological replicates of composite rhizosphere soil samples were obtained from the roots of 15 randomly selected *A. villosum* plants in RP (RP1, RP2, and RP3) and NSF (NSF1, NSF2, and NSF3). Whole *A. villosum* plants were removed from the ground and then the rhizosphere soil samples were collected by shaking the roots vigorously to separate the soil from the roots, and then, the sifted soil particles (1 mm diameter) were collected using a 1 mm sieve. All rhizospheric soils were put into sterile centrifuge tubes and refrigerated in the lab. Each rhizospheric soil was homogenized, and then 10 g of rhizospheric soils were transferred to tubes and preserved at −80 °C for microbial sequencing analysis. The remaining rhizospheric soils were used for the analyses of physical-chemical characters and enzyme activities. The yield and quality parameters of the *A. villosum* plants cultivated in RP and NSF were also measured.

2.2. Measurement of Yield and Quality Parameters

The 15 *A. villosum* plants in each biological replicate were randomly selected from RP and NSF. Ten fruits were randomly selected from each plant for the analysis of yield and quality parameters. The longitudinal diameter, transverse diameter, and fresh weight of each fruit were measured. The fresh fruits were kept in an air-dry oven (GZX-9146 MBE, Shanghai, China) at 55 °C for 30 h, and then the longitudinal diameter, transverse diameter, and dry weight were measured. All measurements were carried out in triplicate for each experimental site, and each biological replicate contains two technical replicates. The total flavonoid content of the dried *A. villosum* seed mass samples was measured as described by An et al. [26]. All measurements were carried out in triplicate for each experimental site, and each biological replicate contains two technical replicates. The total volatile oil content of the dried *A. villosum* seed mass samples was measured using a distillation method. The dried *A. villosum* seed mass samples were crushed and 35 g ground material was soaked in deionized water for 0.5 h prior to the steam distillation. The total volatile oil content of each sample was dehydrated using anhydrous sodium sulfate. Finally, the total volatile oil content was measured by volume (mL). All measurements were carried out in triplicate for each experimental site, and each biological replicate contained two technical replicates.

2.3. Analysis of Volatile Components Using a Gas Chromatograph Coupled with a Time-of-Flight Mass Spectrometer

The volatile components in the dried *A. villosum* seed mass samples (10 ± 1 mg) were added to 2 mL EP tubes, after which 500 µL pre-cooled extract methanol/dH_2O (3:1, v/v) (Adonitol, 0.5 mg/mL stock) was added. After the solutions were centrifuged (12,000 rpm, 15 min, and 4 °C), 200 µL of supernatants were transferred to 1.5 mL tubes. After the samples were evaporated in a vacuum concentrator, 40 µL methoxyamination hydrochloride (20 mg/mL in pyridine) was added. The samples were incubated at 80 °C for 30 min and then derivatized using 60 µL BSTFA reagent (1% TMCS, v/v) at 70 °C for 1.5 h. Additionally, 5 µL FAME solution (in chloroform) was added to the quality-control sample. All samples were then analyzed using the gas chromatography and time-of-flight mass spectrometry (GC-TOF-MS) system. The GC-TOF-MS analysis was performed using an Agilent 7890 gas chromatograph coupled with a time-of-flight mass spectrometer. The system included a DB-5MS capillary column. The MS data were acquired in the full-scan mode, with an m/z range of 50–500 at a rate of 12.5 spectra per second after a solvent delay of 6.25 min. Compounds with peaks that were detected in less than half of the QC samples or with RSD > 30% in the QC samples were removed [27–29]. All measurements were performed in triplicate for each experimental site.

2.4. Analysis of Quercetin and Isoquercetin Contents by High-Performance Liquid Chromatography

The quercetin and isoquercetin contents were analyzed as described by Kalinova et al. [30]. Quercetin and isoquercetin were quantified at 220 nm using the linear calibration curves for the standards ($R^2 \geq 0.995$). All measurements were carried out in triplicate for each experimental site, and each biological replicate contained two technical replicates.

2.5. Measurement of Soil Physicochemical Properties

The rhizospheric soil physical–chemical characteristics were examined as described by our previous report [31]. All measurements were carried out in triplicate for each experimental site, and each biological replicate contained two technical replicates.

2.6. Analysis of Soil Enzyme Activities

The samples were used for the analysis of soil enzyme activities, which were determined as described by our previous report [31]. All measurements were carried out in triplicate for each experimental site, and each biological replicate contained two technical replicates.

2.7. MiSeq Sequencing and Bioinformatic Analyses

DNA extraction and MiSeq sequencing were examined as described by our previous report [31]. The libraries were analyzed through the Illumina NovaSeq platform based on the manufacturer's recommendations. The reads were divided into libraries based on the unique barcode and truncated by deleting the barcode and primer sequence. The reads were integrated by FLASH v.1.2.8. The high-quality clean reads were obtained by filtering raw reads using fqtrim v.0.94. Chimeric sequences were filtered by the Vsearch software v.2.3.4. After the dereplication step performed by DADA2, the feature tables and sequences were obtained. Using the SILVA (release 138), feature abundances were normalized by the relative abundances of each sample. Alpha and beta diversity indices were calculated by QIIME2, with graphs drawn by the R package. The BLAST online tool was applied to align sequences. The features of representative sequences were annotated using the SILVA database.

2.8. Statistical Analyses

Data of all analyses in the replications were processed for the determination as mean ± standard deviation values using the SPSS Version 18.0 (IBM Corp., Armonk, NY, USA). Alpha diversity was primarily applied to reflect the evenness and richness, as well as the sequencing depth of the microbes. It was presented through the Observed_OTUs, Chao1, Pielou-E, Shannon, Simpson, and Goods_coverage indices, which reflect richness and uniformity. Specifically, Observed_OTUs indicates the type of operational taxonomic unit (out) that can be detected in bacteria and fungi. Chao1 is applied to analyse the richness of the microbes. The Shannon and Simpson indices represent diversity, whereas Goods_coverage and Pielou-E refer to the microbial coverage and uniformity, respectively. In contrast, beta diversity was applied to reflect the microbial differences among populations. Briefly, beta diversity was presented through a principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). PCoA analyses were performed using the procedures in the NTSYS pc 2.1 software.

3. Results

3.1. Comparative Analysis of the Yield and Quality Parameters of A. villosum Cultivated in a Rubber Plantation and a Natural Secondary Forest

As shown in Table 1, no significant difference in eight yield and quality parameters of *A. villosum* cultivated for 5 years were observed between RP and NSF ($p < 0.05$). However, the longitudinal diameter and the weight of the fresh and dry *A. villosum* fruits were greater for NSF than for RP, whereas the transverse diameter of the fresh and dry fruits was greater for RP than for NSF. In addition, the total flavonoid and total volatile oil contents of the dried *A. villosum* seed mass were greater for NSF than for RP.

Table 1. Yield and quality parameters of *A. villosum* cultivated for 5 years in RP and NSF.

Parameter	RP	NSF
Longitudinal diameter of fresh *A. villosum* fruit (mm)	18.63 ± 0.19 A	19.19 ± 0.76 A
Transverse diameter of fresh *A. villosum* fruit (mm)	19.51 ± 0.35 A	18.68 ± 0.74 A
Fresh weight of *A. villosum* fruit (g)	3.70 ± 0.06 A	4.07 ± 0.42 A
Longitudinal diameter of dry *A. villosum* fruit (mm)	15.09 ± 0.12 A	16.10 ± 1.07 A
Transverse diameter of dry *A. villosum* fruit (mm)	12.86 ± 0.24 A	12.46 ± 0.80 A
Dry weight of *A. villosum* fruit (g)	0.75 ± 0.02 A	0.78 ± 0.03 A
Total flavonoid content of dried *A. villosum* seed mass (mg·100 g^{-1})	32.96 ± 1.80 A	36.86 ± 0.72 A
Total volatile oil content of dried *A. villosum* seed mass (mg·100 g^{-1})	3.17 ± 0.08 A	3.27 ± 0.21 A

RP and NSF, respectively, refer to the rubber plantation and the natural secondary forest in which *A. villosum* plants were cultivated for 5 years. Different letters within the same row are significantly different ($p < 0.05$).

3.2. Quercetin and Isoquercetin Content Differences between A. villosum Cultivated in RP and NSF

There was no significant difference in quercetin and isoquercetin content of the dried *A. villosum* seed mass between RP and NSF ($p < 0.05$), although the quercetin content of the dried *A. villosum* seed mass was almost 1.4-fold greater in NSF than in RP, and the isoquercetin content of the dried *A. villosum* seed mass was slightly greater in NSF than in RP (Table 2). The HPLC chromatograms for the quercetin and isoquercetin in the dried *A. villosum* seed masses in RP and NSF are provided in Figure S1.

Table 2. Quercetin and isoquercetin contents in *A. villosum* cultivated for 5 years in RP and NSF.

Parameter	RP	NSF
Quercetin content of dried *A. villosum* seed mass (mg·100 g^{-1})	7.13 ± 0.49 A	9.88 ± 0.47 A
Isoquercetin content of dried *A. villosum* seed mass (mg·100 g^{-1})	1.31 ± 0.08 A	1.51 ± 0.03 A

RP and NSF, respectively, refer to the rubber plantation and the natural secondary forest in which *A. villosum* plants were cultivated for 5 years. Different letters within the same row are significantly different ($p < 0.05$).

3.3. Volatile Component Differences between A. villosum Cultivated in a Rubber Plantation and A. villosum Cultivated in a Natural Secondary Forest

A GC-TOF-MS system was used to analyze the volatile components in *A. villosum* cultivated in RP and NSF for 5 years. A comparison between the mass spectra of the analytes and the mass spectra of commercial standard compounds resulted in the identification of 266 volatile components (Table S1). The data were subjected to a principal component analysis, which clearly separated the RP and NSF *A. villosum* samples in the PC1 (56.10%) and PC2 (18.50%) score plots (Figure S2). Accordingly, we used the first two principal components to examine the volatile component profiles for the *A. villosum* cultivated in RP and NSF.

On the basis of the mutual comparison, the significant differences in the volatile component compositions between the plants cultivated in RP and the plants cultivated in NSF were determined using the following criteria: variable importance in projection value > 1 and *p*-value < 0.05. Significant differences between RP and NSF were detected for 51 volatile components (Table S2). The relative abundances of 38 volatile components were significantly higher in NSF than in RP (Figure 1), whereas the relative abundances of 13 volatile components were significantly lower in NSF than in RP. The volatile components with relative contents that differed significantly between RP and NSF by a factor of 1000 were identified as raffinose, 3′-adenylic acid, phloretin, citric acid, and guanidinosuccinic acid (Table S2). The profiles of these five volatile components might be potential candidate biomarkers that are useful for differentiating between *A. villosum* plants cultivated in RP and NSF. Additionally, the comprehensive analysis revealed that the changes to KEGG metabolic pathways (e.g., pantothenate and CoA biosynthesis) in *A. villosum* plants differed significantly between RP and NSF (Figure 2).

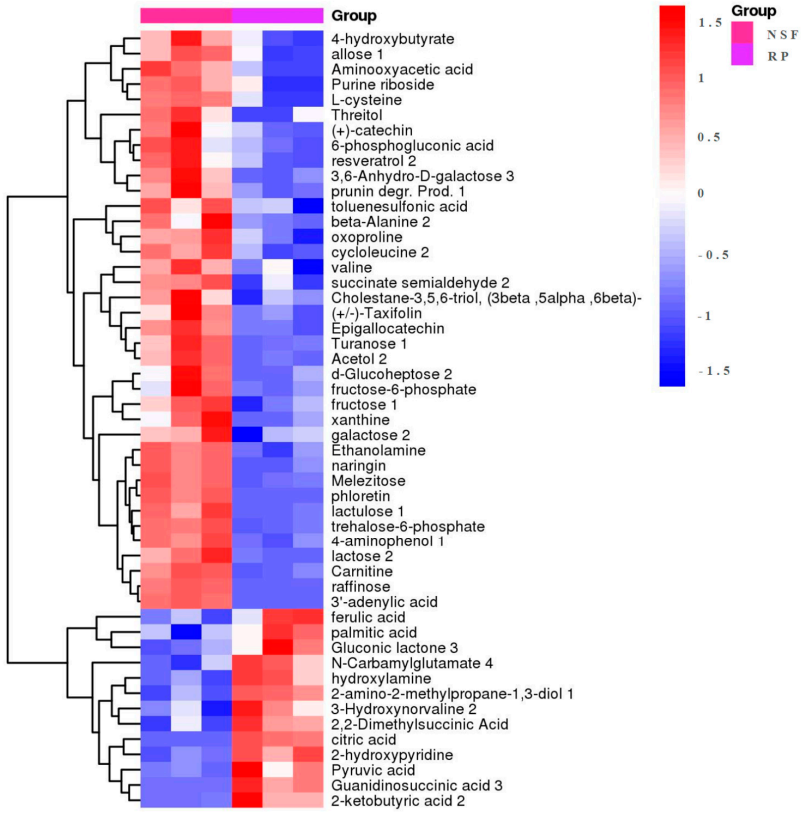

Figure 1. Heatmap of the volatile component profiles in *A. villosum* plants cultivated for 5 years in a rubber plantation (RP) and a natural secondary forest (NSF). Three replicates are shown in each row for each cultivation system.

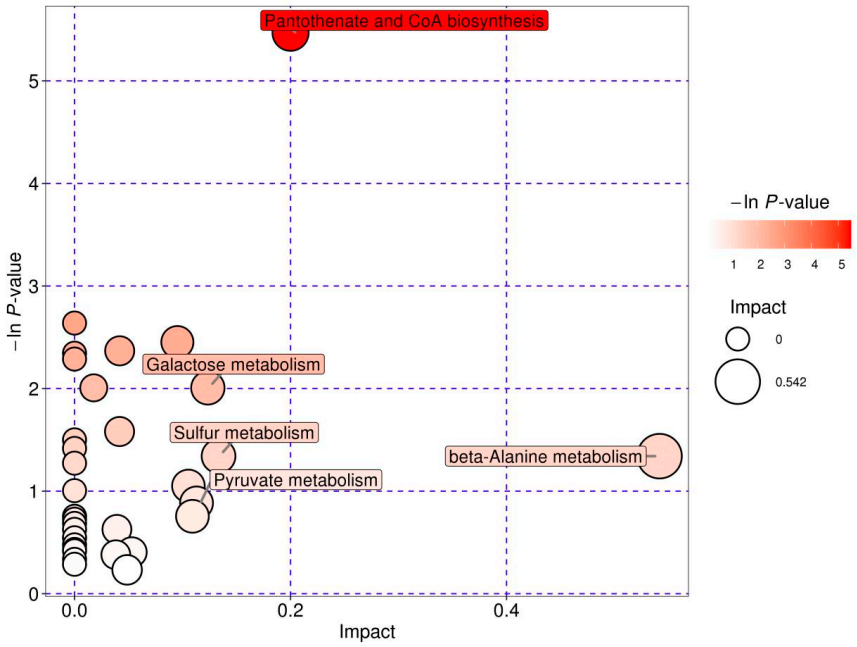

Figure 2. Overview of the differences in the metabolic pathways between *A. villosum* plants cultivated in a rubber plantation and *A. villosum* plants cultivated in a natural secondary forest for 5 years. The *y*-axis indicates the significance of the metabolic pathway enrichment. The *x*-axis indicates the effect of the pathway as determined by the Pathway Topology Analysis.

3.4. Soil Physicochemical Property Differences between RP and NSF in Which A. villosum Plants Were Cultivated

The physicochemical properties of the rhizosphere soil samples in RP and NSF are presented in Figure 3, with no significant difference in eight rhizosphere soil physicochemical properties between RP and NSF ($p < 0.05$). Among eight rhizosphere soil physicochemical properties, the pH, total phosphorus content, available phosphorus content, total potassium content, and available potassium content were lower in RP than in NSF, whereas the organic matter content, total nitrogen content, and available nitrogen content were greater in RP than in NSF.

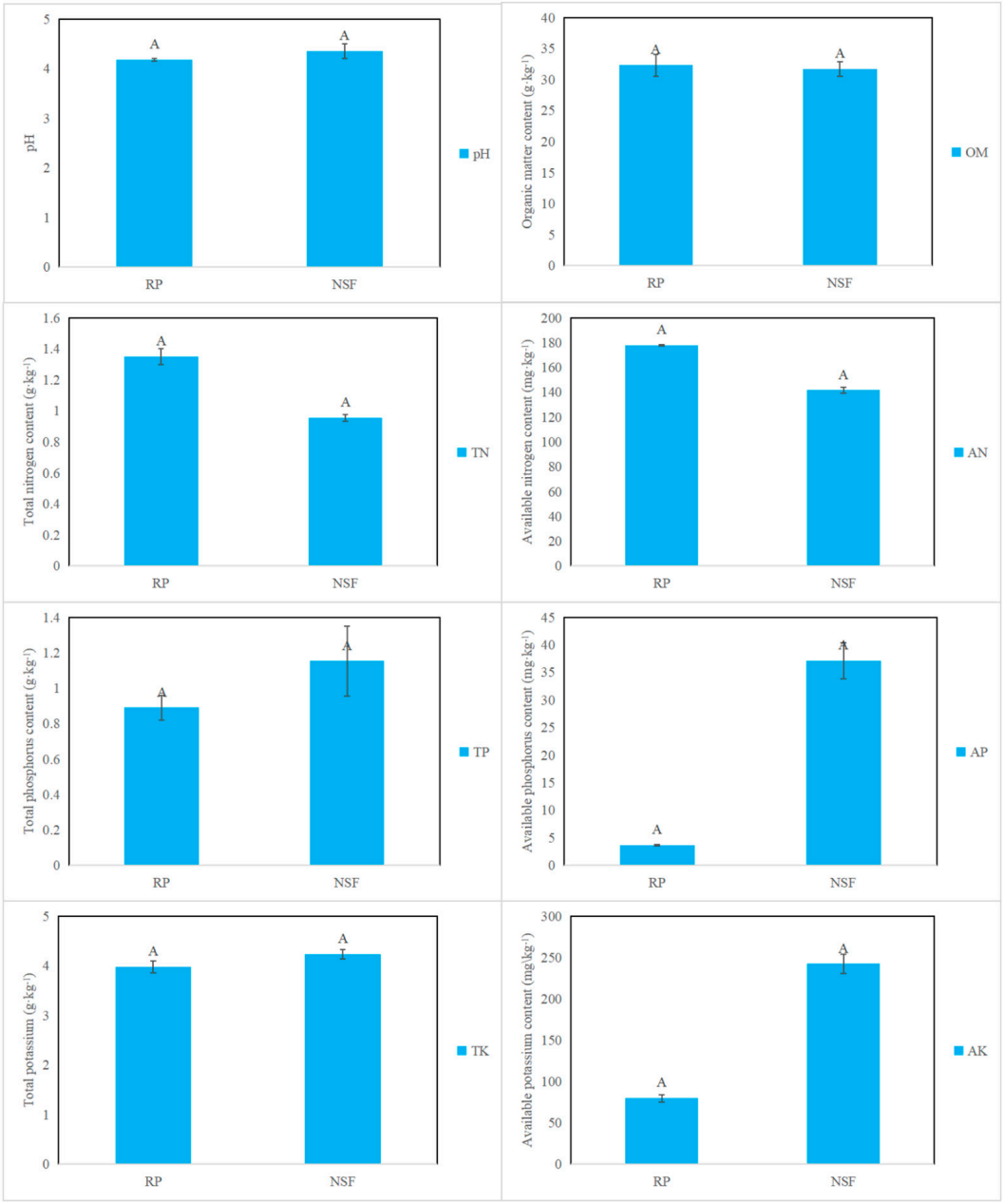

Figure 3. Physicochemical properties of the rhizosphere soil samples from a rubber plantation (RP) and a natural secondary forest (NSF) in which *A. villosum* was cultivated for 5 years. Letters within the same row are significantly different ($p < 0.05$).

3.5. Soil Enzyme Activity Differences between RP and NSF in Which A. villosum Plants Were Cultivated

The urease, acid phosphatase, and catalase activities were higher in the NSF rhizosphere soil than in the RP rhizosphere soil (Figure 4). Only the sucrase activity was higher in the RP rhizosphere soil than in the NSF rhizosphere soil, but no significant difference was found in four soil enzyme activities between RP and NSF ($p < 0.05$).

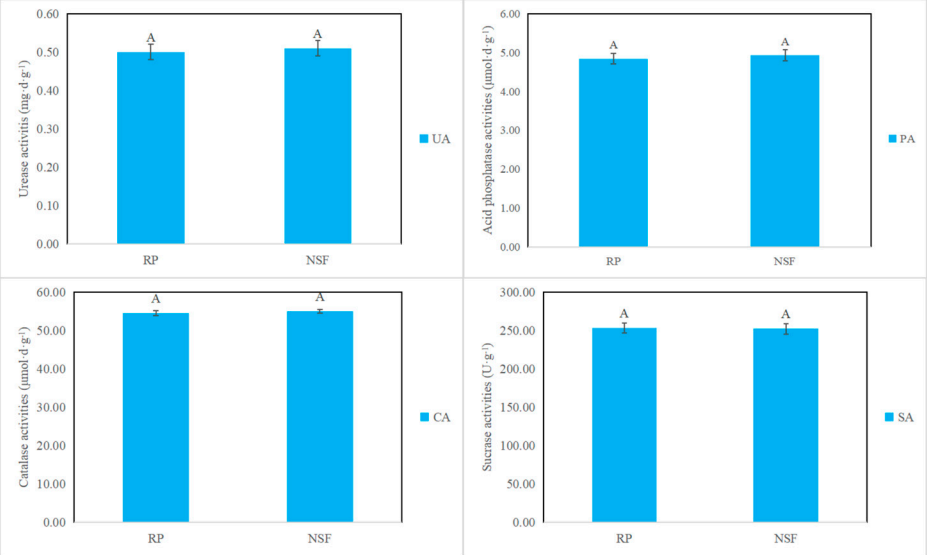

Figure 4. Enzyme activities of the rhizosphere soil samples collected from a rubber plantation (RP) and a natural secondary forest (NSF) in which *A. villosum* was cultivated for 5 years. Letters within the same row are significantly different ($p < 0.05$).

3.6. Description of the Bacterial and Fungal Communities in the Rhizosphere Soil Samples from RP and NSF in Which A. villosum Was Cultivated

A total of 4617 bacterial OTUs (97% similarity) were obtained, with 2840 and 2691 bacterial OTUs in RP and NSF, respectively. Only 924 bacterial OTUs were common to RP and NSF (i.e., 20.01% of all bacterial OTUs), reflecting the relatively large difference in the bacterial groups in the two fields (Figure 5A). Furthermore, 1827 fungal OTUs (97% similarity) were obtained, with 786 and 1286 fungal OTUs in RP and NSF, respectively. Moreover, only 245 fungal OTUs were shared by RP and NSF (i.e., 13.41% of all fungal OTUs). Accordingly, the fungal groups differed substantially between the two fields (Figure 5B).

The top 10 bacterial phyla in the rhizosphere soil samples from RP and NSF were determined, with noticeable differences observed in Planctomyccetota and firmicutes between RP and NSF ($p < 0.05$). For both RP and NSF, Proteobacteria had the highest relative abundance. The comparison between RP and NSF indicated that the relative abundances of Proteobacteria, Verrucomicrobiota, Chloroflexi, Actinobacteriota, Myxococcota, and Methylomirabilota were higher in NSF than in RP, whereas the opposite trend was observed for the other four phyla (Acidobacteriota, Planctomycetota, Firmicutes, and Bacteroidota).

The top 10 bacterial genera in the rhizosphere soil samples from RP and NSF were also determined, showing significant differences in Gemmataceae between RP and NSF (Figure 6B). The relative abundances of *Acidibacter*, *Acidobacteriota_unclassified*, *Candidatus_Koribacter*, and *Acidothermus* were higher in NSF than in RP, whereas the relative abundances of *Subgroup_2_unclassified*, *Gemmataceae_unclassified*, *Acidobacteriales_unclassified*, *Candidatus_Solibacter*, *Elsterales_unclassified*, and *Candidatus_Udaeobacter* were higher in RP than in NSF.

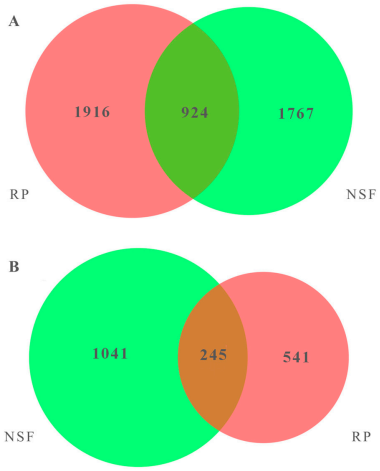

Figure 5. Venn diagrams of the bacterial and fungal OTUs in the rhizosphere soil samples in RP and NSF in which *A. villosum* was cultivated for 5 years. (**A**) Venn diagram of the bacterial OTUs. (**B**) Venn diagram of the fungal OTUs.

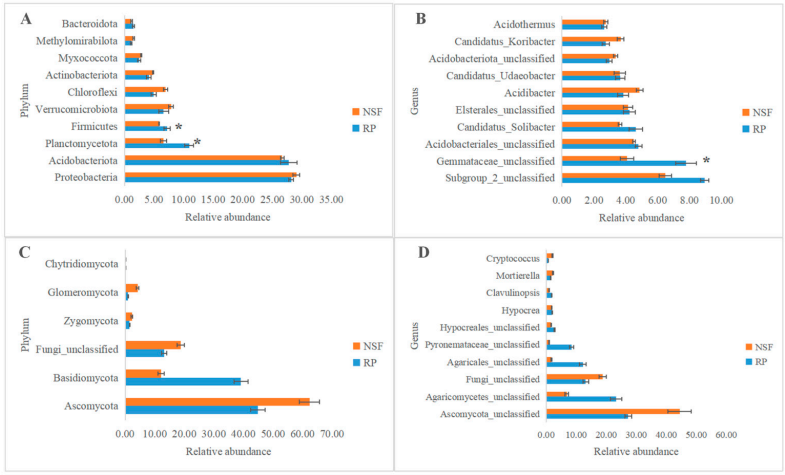

Figure 6. Bacterial community composition and structure in the rhizosphere soil samples from a rubber plantation (RP) and a natural secondary forest (NSF) in which *A. villosum* was cultivated for 5 years. (**A**) Bacterial community bar plot at the phylum level. (**B**) Bacterial community bar plot at the genus level. (**C**) Fungal community bar plot at the phylum level. (**D**) Fungal community bar plot at the genus level. The relative abundance (%) was calculated as the proportion of all effective microbial sequences in the sample using the SILVA database. Phyla and genera were classified as 'other' if they represented less than 1% of the total composition in RP and NSF. Asterisks represent significant differences ($p < 0.05$).

The identified fungi in the rhizosphere soil samples from RP and NSF were mainly from six phyla; however, they were not significantly different between RP and NSF ($p < 0.05$) (Figure 6C). Ascomycota was detected as the dominant fungal phylum in RP and NSF. The relative abundances of Ascomycota, Fungi_unclassified, Zygomycota, Glomeromycota, and Chytridiomycota were higher in NSF than in RP, whereas the relative abundance of only Basidiomycota was lower in NSF than in RP.

The top 10 fungal genera in the rhizosphere soil samples from RP and NSF are presented, with no memorable difference observed in the top 10 fungal genera between RP and NSF ($p < 0.05$) (Figure 6D). *Ascomycota_unclassified* was the primary genus in the fungal communities in RP and NSF. Furthermore, the relative abundances of *Ascomycota_unclassified*, *Fungi_unclassified*, *Mortierella*, and *Cryptococcus* were higher in NSF than in RP, but the relative abundances of the other six genera were lower in NSF than in RP.

3.7. Microbial Diversity in an RP and NSF in Which A. villosum Was Cultivated

3.7.1. Alpha Diversity Indices of the Bacterial and Fungal Communities

Alpha diversity indices (i.e., Observed_OTUs, Shannon, Simpson, Chao1, Goods_coverage, and Pielou-E) were used to reveal the diversity in the bacterial and fungal communities in RP and NSF (Figure 7). Among six alpha diversity indices, significant differences in Observed_OTUs and Chao1 were present in bacterial and fungal communities ($p < 0.05$). The Observed_OTUs and Chao1 indices, which reflected the richness of the bacterial and fungal communities, were significantly higher in NSF than in RP. The fungal community Shannon and Simpson indices (i.e., diversity) were higher in NSF than in RP. In contrast, the analysis of the bacterial communities in RP and NSF revealed that the Shannon index was only slightly higher in NSF than in RP, and there was no difference in the Simpson index. The Goods_coverage index (bacterial and fungal coverage rates) was 1 for both NSF and RP, implying that the sequencing results for all samples represented the actual situations in the samples and that the probability that a species was not detected in the samples was statistically zero. The bacterial community Pielou-E index (i.e., evenness) was essentially the same for RP and NSF, whereas the fungal community Pielou-E index was clearly higher for NSF than for RP.

Figure 7. Alpha diversity indices of the bacteria (16S) and fungi (ITS) in the rhizosphere soil samples collected from RP and NSF in which *A. villosum* was cultivated for 5 years. Different letters within the same row are significantly different ($p < 0.05$).

3.7.2. Beta Diversity Indices of the Bacterial and Fungal Communities

The differences between the bacterial communities in the rhizosphere soil samples of RP and NSF are presented in Figure 6A. The first two principal coordinates (PCoA1 and PCoA2) explained 28.48% and 26.64% of the total variance, respectively (Figure 8A). Similarly, the NMDS analysis indicated that the rhizosphere soil samples from RP and NSF were clearly separated (Figure 8C). The PCoA also revealed a clear difference between the fungal communities of RP and NSF, with PCoA1 and PCoA2 explaining 38.81% and 20.76% of the total variance, respectively (Figure 8B). Furthermore, the NMDS analysis indicated that the fungal community structures were similar for the three replicates of each site, but there were obvious differences between RP and NSF (Figure 8D).

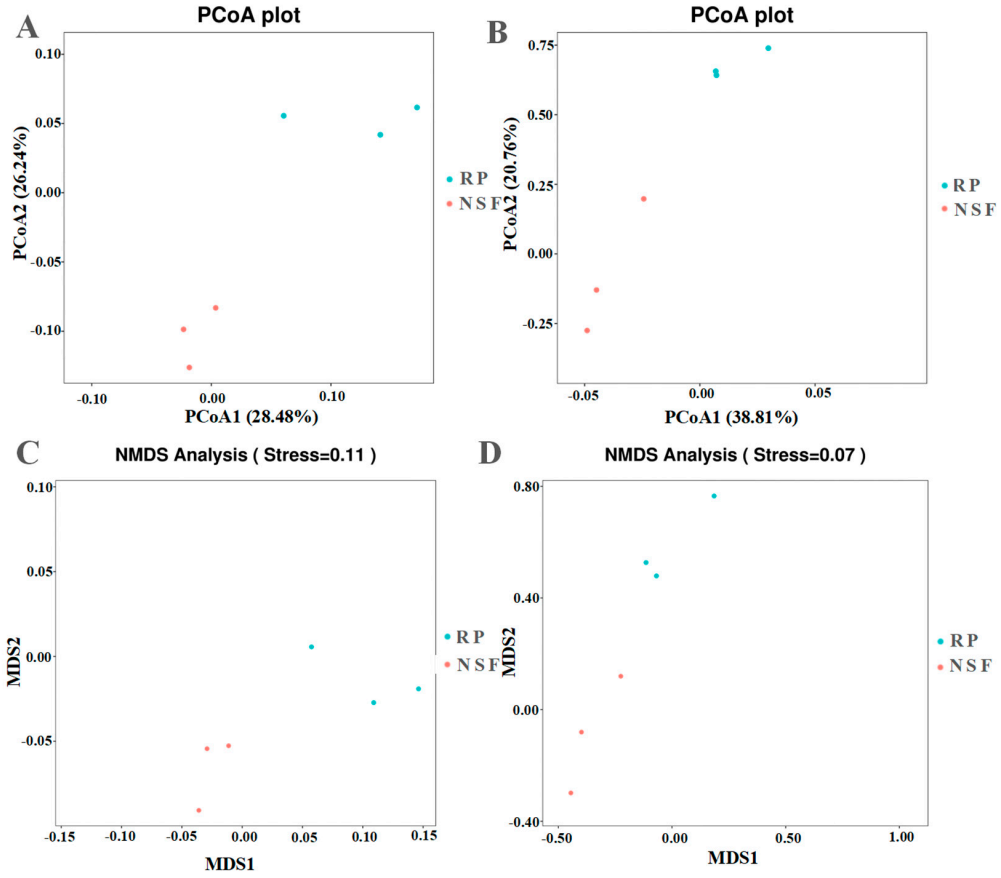

Figure 8. Principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) analysis of the bacterial and fungal communities in the rhizosphere soil samples from a rubber plantation (RP) and a natural secondary forest (NSF) in which *A. villosum* was cultivated for 5 years. (**A**) PCoA of the bacterial community. (**B**) PCoA of the fungal community. (**C**) NMDS analysis of the bacterial community. (**D**) NMDS analysis of the fungal community.

4. Discussion

The rhizosphere is the primary site for the direct interactions among the soil, plant roots, and microbial communities [16]. Rhizosphere soil properties and microbes can affect plant growth and development in various ways. For example, they can regulate plant hormone levels, affect the generation and transmission of plant signals, enhance nutrient acquisition, and increase stress resistance [25]. Our report indicated that the changes in rhizosphere soil physicochemical properties, enzyme activities, and microbial communities increase as the number of years of continuous *A. villosum* cropping increases [31]. However, there is limited available information regarding the effects of different *A. villosum* cultivation practices on yield and quality parameters, rhizosphere soil properties, and soil microbiota.

In terms of the examined rhizosphere soil properties, the organic matter, total nitrogen, and available nitrogen contents were higher in RP than in NSF. Moreover, the pH was lower in RP than in NSF. These findings may reflect inappropriate fertilizer management practices in RP, with detrimental effects on soil fertility as well as the growth and medicinal value of *A. villosum.* In contrast, because inorganic and chemical fertilizers were not applied in NSF, the soil fertility and pH were conducive to *A. villosum* fruit growth and the accumulation of medicinal substances. In our previous study on *A. villosum*, inappropriate applications of inorganic or chemical fertilizers adversely affected soil physicochemical properties [31]. Furthermore, the better soil fertility and suitable pH in NSF contribute to the production of the high-quality *A. villosum* seed mass. The present study showed that the total flavonoid, total volatile oil, quercetin, and isoquercetin contents in the dried *A. villosum* seed mass were higher for NSF than for RP, and the relative amounts of approximately 66% of the 51 volatile components detected by the GC-TOF-MS system were higher in NSF than in RP. The results of the current study were in accordance with the results of earlier research on *Coptis chinensis* [32,33] and *Polygonum multiflorum* [34], even though the experiments conducted in these studies differed in terms of fertilizer management. Inorganic or chemical fertilizers (N, P, and K) may be major drivers of soil acidification and degradation, which may lead to decreased agricultural production.

Three rhizosphere soil enzymes (urease, acid phosphatase, and catalase) were more active in NSF than in RP, although no significant differences in them were observed between RP and NSF. We speculated that the increased urease, acid phosphatase, and catalase activities in NSF increased the soil compound conversion rate, resulting in the increased availability of nutrients for *A. villosum* plants. Earlier investigations confirmed that soil enzymes, such as urease, acid phosphatase, and catalase, are critical for soil biochemical reactions and can modulate soil fertility to promote plant growth indirectly [35].

The analysis of bacterial communities identified Proteobacteria and Acidobacteria as the two predominant bacterial phyla in the RP and NSF rhizospheres, which is consistent with the findings of our earlier study on *A. villosum* [31]. In the current study, the Proteobacteria-to-Acidobacteria ratio was lower for the RP rhizosphere than for the NSF rhizosphere, indicative of a shift toward Acidobacteria over Proteobacteria in the RP rhizosphere. This ratio is reportedly a useful indicator of nutrient availability in the rhizosphere soil because Proteobacteria is common in nutrient-rich soil, whereas Acidobacteria is generally abundant in nutrient-deficient soil [36–40]. In addition, Proteobacteria species, which have a pivotal role in nutrient recycling [41,42], are prevalent in rhizosphere soils [43–45]. Therefore, the Proteobacteria-to-Acidobacteria ratio is also one of the factors that promoted the production and quality of *A. villosum* in this study. In an earlier investigation, the Proteobacteria-to-Acidobacteria ratio for the *P. notoginseng* rhizosphere was higher in the forest than in the field, which may have contributed to the observed increase in the root weight of the plants grown in the forest [16].

Ascomycota was the main phylum in the RP and NSF rhizosphere fungal communities, which is in accordance with the results of our earlier study on *A. villosum* [31]. During the vegetative growth phase, processes related to prophylaxis are active in ascomycetes from Ascomycota, thereby enabling these fungi to decompose plant residues [46]. Some fungi belonging to Ascomycota are classified as plant-growth-promoting fungi [47]. In the present study, the relative abundance of Ascomycota was higher in NSF than in RP. Therefore, Ascomycota species may contribute to the growth and accumulation of medicinal compounds in *A. villosum* fruits. Ascomycota fungi are important drivers of carbon and nitrogen cycling and modulate soil stability, plant biomass decomposition, and endophytic interactions with plants [48,49]. However, we previously determined that Ascomycota species are not the primary factor that increases *A. villosum* production with the increase in continuous cropping years [31].

Different cultivation practices can considerably affect the diversity of rhizosphere microbial communities [50]. In the current study, alpha diversity indices revealed that the richness of bacterial and fungal communities was significantly higher in NSF than in RP. In

addition, our PCoA and NMDS analysis of the bacterial and fungal communities in the RP and NSF rhizosphere soil samples suggested that different *A. villosum* cultivation practices might substantially alter the soil microorganism population structures. The microorganism populations in the NSF rhizosphere were grouped together and precisely distinguished from the respective populations in the RP rhizosphere. Similarly, a more diverse microbial community was detected in natural forests than in monoculture forest plantations in the Greater Mekong Subregion [51].

In the present study, many soil parameters, including soil fertility, plant-growth-promoting microbe contents, bacterial proportions, and the richness and diversity of the rhizosphere microbial community, were better in the NSF rhizosphere than in the monoculture RP rhizosphere. There are interactions among soil parameters and plant-growth-related parameters in different cultivation systems [52,53]. Chemical, physical, and biological (e.g., microbial) components contribute to the maintenance of soil health conducive to plant growth [54,55]. The results of the present study suggest that the healthy soil in NSF promoted *A. villosum* fruit growth and the accumulation of medicinal compounds better than the soil in the monoculture RP. Some studies confirmed that rubber farming may negatively affect soil properties [56–59]. The intensive agricultural practices in RPs are major drivers of soil degradation, which may lead to decreased crop output and quality [60,61]. Monoculture cultivation practices may be associated with yield and quality losses because of substantial changes to soil processes and properties [59,62,63].

5. Conclusions

Cultivation practices (e.g., in RP and NSF) may induce distinct changes to *A. villosum* yield and quality parameters, rhizosphere soil properties, and the rhizosphere soil microbiota. The results of yield characteristics, quality parameters, and rhizosphere soil properties implied that more high-quality *A. villosum* were produced in NSF than in RP and the fertility of the *A. villosum* rhizosphere soil was better in NSF than in RP. Moreover, compared with RP, the *A. villosum* rhizosphere soil microbes were richer in NSF. Based on the above research, the multi-crop culture can be considered more conducive to the production of high-quality *A. villosum* fruit than the monoculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9030306/s1, Table S1. Volatile composition data measured in *A. villosum* cultivated under different cultivation patterns. Table S2. Differentially accumulated volatile compositions between the *A. villosum* cultivated under rubber plantation and natural secondary forest. Figure S1. The HPLC chromatograms for the quercetin and isoquercetin in the dried *A. villosum* seed masses in RP and NSF. Figure S2. PCA scores plot for the volatile compositions in *A. villosum* cultivated rubber plantation and natural secondary forest.

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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 *Amomum* species. *Int. J. Mol. Sci.* **2019**, *20*, 4040. [CrossRef] [PubMed]
2. Wang, X.; Qin, Y.; Dai, Z. Research progress of *Amomum villosum*. *Chin. J. Ethnomed. Ethnopharm.* **2016**, *25*, 37–38.
3. 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]
4. 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]
5. 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]
6. Ai, Z.; Mowafy, S.; Liu, Y. Comparative analyses of five drying techniques on drying attributes, physicochemical aspects, and flavor components of *Amomum villosum* fruits. *LWT* **2022**, *154*, 112879. [CrossRef]
7. 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]
8. Lin, J.M.; Zheng, Y.H.; Xu, Y.C.; Xia, P.G.; Wu, Z.; Chen, F.L.; Song, L.C. Analysis of essential oil from *Amomum tsaoko* by extraction of supercritical CO_2 fluid. *Zhong Yao Cai* **2000**, *23*, 145–148. (In Chinese)
9. Cui, Q.; Wang, L.T.; Liu, J.Z.; Wang, H.M.; Guo, N.; Gu, C.B.; Fu, Y.J. Rapid extraction of *Amomum tsaoko* essential oil and determination of its chemical composition, antioxidant and antimicrobial activities. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2017**, *1*, 1061–1062. [CrossRef]
10. Deng, C.; Wang, A.; Shen, S.; Fu, D.; Chen, J.; Zhang, X. Rapid analysis of essential oil from *Fructus Amomi* by pressurized hot water extraction followed by solid-phase microextraction and gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **2005**, *38*, 326–331. [CrossRef]
11. Wu, X.S.; Li, X.G.; Xiao, F.; Zhang, Z.D.; Xu, Z.X.; Wang, H. Studies on the analgesic and anti-inflammatory effect of bornyl acetate in volatile oil from *Amomum villosum*. *Zhong Yao Cai* **2004**, *27*, 438–439. (In Chinese) [PubMed]
12. 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] [PubMed]
13. Zou, X.; Cao, C.; Zeng, Y.; Jiang, B. Determination of total phenolic content and antioxidant activities of *Fructus amomi* from various habitats. *Mod. Chin. Med.* **2018**, *20*, 811–815.
14. 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] [PubMed]
15. Wu, L.; Meng, X.; Huang, H.; Liu, Y.; Jiang, W.; Su, X.; Wang, Z.; Meng, F.; Wang, L.; Peng, D.; et al. Comparative proteome and phosphoproteome analyses reveal different molecular mechanism between stone planting under the forest and greenhouse planting of *Dendrobium huoshanense*. *Front. Plant Sci.* **2022**, *13*, 937392. [CrossRef]
16. 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]
17. Yi, S.Y.; Kang, C.Z.; Wang, W.; Song, X.W.; Xu, T.; Lu, H.B. Comparison of planting modes of *Dendrobium huoshanense* and analysis of advantages of simulated cultivation. *China J. Chin. Mater. Med.* **2021**, *46*, 1864–1868.
18. Bogati, K.; Walczak, M. The impact of drought stress on soil microbial community, enzyme activities and plants. *Agronomy* **2022**, *12*, 189. [CrossRef]
19. 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]
20. 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. Forest Sci.* **2018**, *75*, 34. [CrossRef]
21. Wang, L.; Kaur, M.; Zhang, P.; Li, J.; Xu, M. Effect of different agricultural farming practices on microbial biomass and enzyme activities of celery growing field soil. *Int. J. Environ. Res. Public Health* **2021**, *18*, 12862. [CrossRef]
22. 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]
23. 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]
24. Tong, A.Z.; Liu, W.; Liu, Q.; Xia, G.Q.; Zhu, J.Y. Diversity and composition of the *Panax ginseng* rhizosphere microbiome in various cultivation modesand ages. *BMC Microbiol.* **2021**, *21*, 18. [CrossRef] [PubMed]

25. Fang, X.; Wang, H.; Zhao, L.; Wang, M.Q.; Sun, M.Z. Diversity and structure of the rhizosphere microbial communities of wild and cultivated ginseng. *BMC Microbiol.* **2022**, *22*, 2. [CrossRef]
26. An, K.; Zhao, D.; Wang, Z.; Wu, J.; Xu, Y.; Xiao, G. Comparison of different drying methods on Chinese ginger (*Zingiber Officinale Roscoe*): Changes in volatiles, chemical profile, antioxidant properties, and microstructure. *Food Chem.* **2016**, *197*, 1292–1300. [CrossRef] [PubMed]
27. Chen, L.; Lai, Y.; Zhang, W.; Cai, J.; Hu, H.; Wang, Y.; Zhao, J.; Li, S. Comparison of volatile compounds in different parts of fresh *Amomum villosum* Lour. from different geographical areas using cryogenic grinding combined HS-SPME-GC-MS. *Chin. Med.* **2020**, *15*, 97. [CrossRef] [PubMed]
28. Kind, T.; Wohlgemuth, G.; Lee, D.Y. FiehnLib: Mass spectral and retention index libraries for metabolomics based on quadrupole and time-of-flight gas chromatography/mass spectrometry. *Anal. Chem.* **2009**, *81*, 10038–10048. [CrossRef]
29. Dunn, W.B.; Broadhurst, D.; Begley, P. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat. Protoc.* **2011**, *6*, 1060–1083. [CrossRef]
30. Kalinova, J.; Vrchotova, N. Level of catechin, myricetin, quercetin and isoquercitrin in buckwheat (*Fagopyrum esculentum* Moench), changes of their levels during vegetation and their effect on the growth of selected weeds. *J. Agric. Food Chem.* **2009**, *57*, 2719–2725. [CrossRef]
31. Wang, B.; Lu, Y.; Li, W.; He, S.; Lin, R.; Qu, P.; Chen, H.; Zhang, F.; Zhao, M.; Shi, X.; et al. Effects of the continuous cropping of *Amomum villosum* on rhizosphere soil physicochemical properties, enzyme activities, and microbial communities. *Agronomy* **2022**, *12*, 2548. [CrossRef]
32. 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]
33. 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]
34. 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]
35. Li, C.; Shi, Z.; Cai, J.; Wang, P.; Wang, F.; Ju, M.; Liu, J.; Yu, Q. Synthesis of phenylboronic acid-functionalized magnetic nanoparticles for sensitive soil enzyme assays. *Molecules* **2022**, *27*, 6883. [CrossRef]
36. Smit, E.; Leeflflang, P.; Gommans, S.; Broek, J.V.D.; van Mil, S.; Wernars, K. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl. Environ. Microbiol.* **2001**, *67*, 2284–2291. [CrossRef] [PubMed]
37. Castro, H.F.; Classen, A.; Austin, E.E.; Norby, R.; Schadt, C.W. Soil microbial community responses to multiple experimental climate change drivers. *Appl. Environ. Microbiol.* **2010**, *76*, 999–1007. [CrossRef]
38. 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]
39. 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]
40. 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]
41. Hedrich, S.; Schlömann, M.; Johnson, D.B. The iron-oxidizing proteobacteria. *Microbiology* **2011**, *157*, 1551–1564. [CrossRef]
42. Rosenzweig, N.; Tiedje, J.M.; Quensen, J.F.; Meng, Q.; Hao, J.J. Microbial communities associated with potato common scab-suppressive soil determined by pyrosequencing analyses. *Plant Dis.* **2012**, *96*, 718–725. [CrossRef] [PubMed]
43. She, S.; Niu, J.; Zhang, C.; Xiao, Y.; Chen, W.; Dai, L.; Liu, X.; Yin, H. Significant relationship between soil bacterial community structure and incidence of bacterial wilt disease under continuous cropping system. *Arch. Microbiol.* **2016**, *199*, 267–275. [CrossRef]
44. Tian, Y.; Gao, L. Bacterial diversity in the rhizosphere of cucumbers grown in soils covering a wide range of cucumber cropping histories and environmental conditions. *Microb. Ecol.* **2014**, *68*, 794–806. [CrossRef]
45. Xiong, W.; Zhao, Q.; Zhao, J.; Xun, W.; Li, R.; Zhang, R.; Wu, H.; Shen, Q. Different continuous cropping spans significantly affect microbial community membership and structure in a vanilla-grown soil as revealed by deep pyrosequencing. *Microb. Ecol.* **2014**, *70*, 209–218. [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]
47. 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] [PubMed]
48. Challacombe, J.F.; Hesse, C.N.; Bramer, L.M.; McCue, L.A.; Lipton, M.; Purvine, S.; Nicora, C.; Gallegos-Graves, L.V.; Alfaro, A.P.; Kuske, C.R. Genomes and secretomes of Ascomycota fungi reveal diverse functions in plant biomass decomposition and pathogenesis. *BMC Genom.* **2019**, *20*, 976. [CrossRef]
49. Nguyen, T.A.; Cissé, O.H.; Wong, J.Y.; Zheng, P.; Hewitt, D.; Nowrousian, M.; Stajich, J.E.; Jedd, G. Innovation and constraint leading to complex multicellularity in the Ascomycota. *Nat. Commun.* **2017**, *8*, 14444. [CrossRef]

50. 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]
51. Li, H.; Guo, J.; Karunarathna, S.C.; Ye, L.; Xu, J.; Hyde, K.D.; Mortimer, P.E. Native forests have a higher diversity of macrofungi than comparable plantation forests in the greater mekong subregion. *Forests* **2018**, *9*, 402. [CrossRef]
52. Chandra, L.R.; Gupta, S.; Pande, V.; Singh, N. Impact of forest vegetation on soil characteristics: A correlation between soil biological and physico-chemical properties. *3 Biotech* **2016**, *6*, 188. [CrossRef] [PubMed]
53. QuynhAnh, P.T.; Gomi, T.; MacDonald, L.; Mizugaki, S.; Van Khoa, P.; Furuichi, T. Linkages among land use, macronutrient levels, and soil erosion in northern Vietnam: A plot-scale study. *Geoderma* **2014**, *1*, 232–234.
54. Trivedi, P.; Delgado-Baquerizo, M.; Anderson, I.C.; Singh, B.K. Response of soil properties and microbial communities to agriculture: Implications for primary productivity and soil health indicators. *Front. Plant Sci.* **2016**, *7*, 990. [CrossRef] [PubMed]
55. Franzluebbers, A.J. Stratification of Soil Porosity and Organic Matter. In *Encyclopedia of Agrophysics*; Horabik, J., Lipiec, J., Eds.; Springer: Dordrecht, The Netherlands, 2011.
56. Singh, D.; Ferry, S.J.W.; Jeon, Y.S.; Tomlinson, K.W.; Yang, X.; Kerfahi, D.; Porazinska, D.L.; Adams, J.M. Tropical forest conversion to rubber plantation affects soil micro- & mesofaunal community & diversity. *Sci. Rep.* **2019**, *9*, 5893. [PubMed]
57. Peerawat, M.; Blaud, A.; Trap, J.; Chevallier, T.; Pascal, A.; Gay, F.; Thaler, P.; Spor, A.; Sebag, D.; Choosai, C.; et al. Rubber plantation ageing controls soil biodiversity after land conversion from cassava. *Agric. Ecosyst. Environ.* **2018**, *257*, 92–102. [CrossRef]
58. Allen, K.; Corre, M.D.; Tjoa, A.; Veldkamp, E. Soil nitrogen-cycling responses to conversion of lowland forests to oil palm and rubber plantations in sumatra, Indonesia. *PLoS ONE* **2015**, *10*, e0133325. [CrossRef] [PubMed]
59. Kotowska, M.M.; Leuschner, C.; Triadiati, T.; Meriem, S.; Hertel, D. Quantifying above- and belowground biomass carbon loss with forest conversion in tropical lowlands of sumatra (Indonesia). *Glob. Change Biol.* **2015**, *21*, 3620–3634. [CrossRef]
60. Blagodatsky, S.; Xu, J.C.; Cadisch, G. Carbon balance of rubber (*Hevea brasiliensis*) plantations: A review of uncertainties at plot, landscape and production level. *Agric. Ecosyst. Environ.* **2016**, *221*, 8–19. [CrossRef]
61. Schneider, D.; Engelhaupt, M.; Allen, K.; Kurniawan, S.; Heinemann, M.; Krashevska, V.; Nacke, H.; Wijayanti, M.; Meryandini, A.; Corre, M.D.; et al. Impact of lowland rainforest transformation on diversity and composition of soil prokaryotic communities in sumatra (Indonesia). *Front. Microbiol.* **2015**, *6*, 1339. [CrossRef]
62. Pauli, N.; Donough, C.R.; Oberthür, T.; Cock, J.H.; Verdooren, R.; Rahmadsyah, G.; Abdurrohim, R.; Indrasuara, K.; Lubis, A.; Dolong, T.; et al. Changes in soil quality indicators under oil palm plantations following application of 'best management practices' in a four-year field trial. *Agric. Ecosyst. Environ.* **2014**, *195*, 98–111. [CrossRef]
63. Velmourougane, K. Impact of organic and conventional systems of coffee farming on soil properties and culturable microbial diversity. *Scientifica* **2016**, *1*, 3604026. [CrossRef] [PubMed]