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Soil Microbial Community Structure and Physicochemical Properties in *Amomum tsaoko*-based Agroforestry Systems in the Gaoligong Mountains, Southwest China

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Abstract: *Amomum tsaoko* is cultivated in forests of tropical and subtropical regions of China, and the planting area is expanding gradually. However, little attention has been paid to the impact of *A. tsaoko* cultivation on the soil characteristics of the regions. We analyzed the effects of the *A. tsaoko*-forest agroforestry system (AFs) on the composition of soil microbial communities with increasing stand ages. We also compared the soil physicochemical properties, microbial biomass, and phospholipid fatty acid (PLFA) composition between native forest (NF) and AFs. The results showed that the level of total carbon, nitrogen, and organic matter dramatically dropped in AFs with increasing stand ages. pH affected other soil properties and showed close correlation to total carbon ($P = 0.0057$), total nitrogen ($P = 0.0146$), organic matter ($P = 0.0075$), hydrolyzable nitrogen ($P = 0.0085$), available phosphorus ($P < 0.0001$), and available potassium ($P = 0.0031$). PLFAs of bacteria ($F = 4.650$, $P = 0.037$), gram-positive bacteria ($F = 6.640$, $P = 0.015$), anaerobe ($F = 5.672$, $P = 0.022$), and total PLFA ($F = 4.349$, $P = 0.043$) were significantly affected by different treatments, with the greatest value for NF treatment, and least value for AF5. However, the microbial biomass declined during the initial 5 years of cultivation, but it reached the previous level after more than 10 years of cultivation. Our research suggests that AFs is a profitable land-use practice in the Gaoligong Mountains and that AFs showed a recovering trend of the soil nutrient condition with increasing stand ages. However, the severe loss of nitrogen in the soil of AFs requires additional nitrogen during cultivation to restore it to pre-cultivation levels.

Keywords: land-use change; agroforestry system; *Amomum tsaoko*; phospholipid fatty acid; microbial community structure; soil physicochemical properties

1. Introduction

Land resources are limited, and the area of arable land is decreasing significantly in response to construction occupation, natural disasters, ecological restoration, and agricultural structure adjustment [1]. However, in China, forest land area is increasing due to governmental policies implemented to return farmland to the forest and ecological civilization construction [2]. As far as the Nujiang river region, Northwest Yunnan, is concerned, over 98% of the area is an alpine canyon, and land with a slope of more than 25 degrees accounts for 76.6% of arable land area, and the coefficient

of cultivated land is less than 4% [3]. Furthermore, shifting cultivation has been forbidden by the government since the 1980s in this region to preserve natural resources, e.g., wild medicinal plants, threatened plants and animals, and to manage ecosystems sustainably in steeply-sloping environments. To compensate for the loss caused by the policy of converting farmland to woodland, *Amomum tsaoko*, an aromatic and medicinal plant, was introduced to Gongshan County for trial planting in the early 1990s. Subsequently, as an economic or medicinal plant with higher economic income and lower labor input, the planting area of *A. tsaoko* was expanding. It has developed into a mature planting model—the *A. tsaoko*-forest agroforestry system (AFs).

Agroforestry systems can represent a complementary system of land management, where trees and/or shrubs are grown alongside with crops and/or livestock to promote diversity and ecological sustainability while offering social, economic, and environmental benefits [4]; it has been considered as a profitable land-use practice and has been implemented worldwide for considerable periods [5]. Rational agroforestry management contributes to the improvement of soil quality and soil biodiversity [6–8]. Healthy soil supports high levels of biological diversity, activity, internal nutrient cycling, and resilience to disturbance [9]. On the contrary, the poor land-management practices, including urbanization, intensive agriculture, deforestation, and desertification are affecting belowground communities globally [10]. Soils are one of the main living places for microorganisms, in which microbes play important roles such as the cycling and transformation of soil organic matter and soil nutrients including carbon, nitrogen, phosphorus, and sulfur, and are also involved in the decomposition of organic matter [11,12]. 80–90% of the processes in soil are reactions mediated by microbes [13]; moreover, many studies have shown a correlation between microorganisms and plant root systems [14,15] and soil functionality [16,17]. Soil serves as a potential indicator for monitoring sustainable land management [9,18,19], and analyses of the physicochemical and microbial properties are the main ways to evaluate soil quality changes [6,8,17,20–27].

However, with the increased planting area of *A. tsaoko*, a series of ecological problems followed, such as soil ecosystem imbalance, decreasing biodiversity, and so on. Although many studies on agroforestry systems have shown positive results, a negative impact on soil nutrient and plant diversity in tropical rainforests was found—*A. villosum* cultivation simplifies tree stand structure, destroys sapling-seedling banks, results in the disappearance of species, and reduces plant diversity, tree biomass, litter production, and soil nutrient levels [28].

Due to the thinning of forest trees and the clearing of bush and herbs prior to *A. tsaoko* cultivation, plant diversity at these sites has visibly reduced, while underground changes in diversity or composition are harder to detect. Although the effective components [29] and genetic system [30] of *A. tsaoko* have been studied intensively, little attention has been paid to the influence of *A. tsaoko* cultivation on the environment, especially on soil properties. The objective of this study was to determine the soil physicochemical and microbial properties in AFs with increasing stand ages. We hypothesized that the negative effects of *A. tsaoko* cultivation on soil properties will gradually improve with the increase of AFs stand ages.

2. Materials and Methods

2.1. Study Area

Experimental sites located at Puladi village of (Gongshan County) and Maji village (Fugong County) of Nujiang prefecture, respectively (Figure 1), range from 1366–1715 m above sea level with a low-latitude plateau monsoon climate, and a remarkable vertical climate change with a mean annual temperature of 16.8–20.1 °C and a mean annual precipitation of 1444.3 mm. At the sites, soils are faintly acidic red, lateritic red, yellow-red, and yellow-brown earth. Four bioclimatic zones occur in this prefecture, favoring the development and diversity of plants. This area has also been classified as one of 25 global biodiversity hotspots [31]; 25.9% of the hotspot area is protected. In the 1990s, *A. tsaoko* was formally introduced and cultivated in Puladi village, which is famous as the hometown of *A. tsaoko*

in China, and it was regarded by the government as an act to increase local residents' income and enhance forest-land availability. Subsequently, *A. tsaoko* was also introduced to Maji village, which now owns the largest area of *A. tsaoko*.

This study was conducted with four replicated treatments: (1) Native forest (NF), a *A. tsaoko*-forest agroforestry system (AFs) chronosequence with stand age(s) of (2) 1 year (AF1), (3) 5–7 years (AF5), and (4) 10–12 years (AF10). Each treatment was performed with three replicates. In this region, available forest land has been prepared prior to cultivating *A. tsaoko*, including the removal of shrubs and herbs as conventional practice. Normally, after 5 years of cultivation, the mother plants of *A. tsaoko* bear fruit, and ripened fruits are harvested in October over the next few years. After the harvest, the withered old branches are cut off and placed on stubble surface around standing plants and uncovered again in March or April of the next year. It is not necessary to use any fertilizers during the whole growth period of *A. tsaoko*.

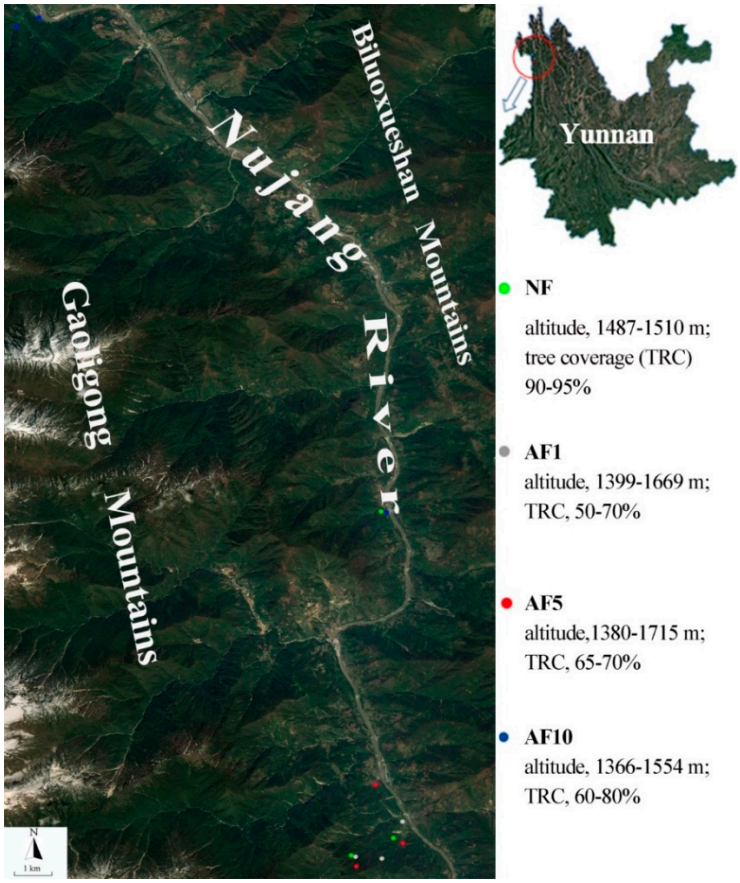

Figure 1. Study site locations (N 27°22′–27°37′, E 98°43′–98°49′).

2.2. Field Sampling and Soil Physicochemical Properties Determination

The three chronosequences (AF1, AF5, AF10) and native forest (NF) described above were studied by establishing three 20 × 20 m^2 sample plots in each type of system. The sampling plots were selected by similar environmental characteristics with an east-facing slope of 30–40 degrees as well as an equivalent intensity of agricultural management. In each sampling plot, the soil was collected by pooling five cores of 20 cm depth and by removing roots, rocks, and litter to perform one sample. Half of each soil sample was stored at 4 °C for microbe characteristics analysis, and the other half was air-dried and sieved to determine pH, hydrolyzable nitrogen (N_{hy}), available phosphorus (P_{avail}) according to Porta et al. [32], and available potassium (K_{avail}) as described by Chen et al. [27]. The total carbon (C_{total}) and organic matter (OM) were measured using a Vario MAX CN (Elementar Analysensysteme

GmhH, Hanau, Germany). Total phosphorus (P_{total}) and total potassium (K_{total}) were determined using high-resolution inductively couple plasma atomic emission spectrometry (ICP-AES), and soil moisture was determined with drying method (LY/T 1213—1999) [33].

2.3. Analysis of Microbial Biomass and Structure

Soil subsamples were prepared by leaching, separation, esterification, and extraction; the contents of the phospholipid fatty acids (PLFAs) were analyzed using gas chromatography (HP series GC, FID) with MIDI software. Soil microbial biomass was determined using the chloroform fumigation-extraction method [34,35]. Lipids were extracted from 8.0 g of dry-weight-equivalent fresh soil using a one-phase extraction mixture containing chloroform: methanol: phosphate buffer (1:2:0.8 v/v/v). The lipid extracts were then fractionated into neutral lipids, glycolipids, and phospholipids using solid-phase extraction columns by eluting with $CHCl_3$, acetone, and methanol, respectively, and then fatty acid methyl esters were recovered. Subsequently, the fatty acid methyl esters were extracted in 200 µL hexane containing 19:0 as an internal standard and analyzed using gas chromatography. A 2 µL injection with a 1:50 split, was analyzed at an oven temperature of 260 °C, a flame ionization detector temperature of 300 °C, and a pressure of 10.7 PSI at a constant flow rate of 0.4 mL min^{-1}. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE, USA). Concentrations of each PLFA were calculated based on the 19:0 internal standard concentrations. The relative abundance of individual fatty acids was shown by the proportion (mol %) of the sum of all fatty acids.

The following PLFA-marks were used: (a) Gram-positive bacteria: a15:0, i15:0, i16:0, a17:0 and i17:0 [36]; (b) Gram-negative bacteria: 16:1ω7c, 16:1 2OH, cy17:0 [36,37], i17:1ω5c, 17:1ω8c [38], i17:0 3OH [39], 18:1ω5c [40], 18:1ω7c [41] and 11Me18:1ω7c [42]; 16:1ω11c was used as *Nitrospira moscoviensis* PLFA-mark [43,44], but can be used as an indicator for Gram-negative bacteria [45]; (c) non-specific bacteria: 15:0, 16:0 and 18:0; (d) 17:0 as general bacterial PLFAs [38]; (e) the fungi: 18:2ω6, 9c/18:0 ante [46,47] and 18:1ω9c [48,49]; (f) arbuscular mycorrhizal fungi (AMF): 16:1ω5c [50]; (g) actinomycetes was identified by 10Me16:0 [41], 10Me17:0 [49,51] and 10Me18:0 [48,49,51]. The sum of (a), (b), (c), and (d) represented total bacteria PLFA. The PLFAs of 16:1ω5c, 16:1ω7c, 18:1ω7c and 18:1ω9c were chosen to identify aerobic bacteria; and a15:0, i15:0, i16:0, a17:0, i17:0, 16:1 2OH, 10Me16:0, 10Me17:0 and 10Me18:0 to identify anaerobic bacteria [52–54]. The ratio of total fungal to total bacterial PLFAs (F/B) and gram-positive to gram-negative bacteria (G+/G-) were then calculated. The sum of all of the PLFAs indicated above were calculated and used as the total PLFAs of soil microbial community.

2.4. Data Processing and Analysis

The microbial biomass was calculated as the sum of the individual PLFAs (nmol g^{-1} soil). All statistical analyses were carried out using the SPSS 16.0 software (SPSS INC, Chicago, IL), and the graphs were created using SigmaPlot 12.0 software. All data are expressed as the mean ± SD. Statistical comparisons of different treatments were made using one-way analysis of variance (one-way ANOVA) and followed by the LSD test as a post-hoc analysis. Significant differences were set at $P < 0.05$. Correlations among variables were assessed using Pearson's correlative analysis.

3. Results

3.1. Soil Properties

The physical and chemical properties of soil under *A. tsaoko* varied with the chronosequence of AFs ages (Figure 2). With increasing of AFs stand ages, soil fertility was mainly changed by the increase in the contents of available phosphorous, available potassium and total potassium, and the decrease in the contents of hydrolyzable nitrogen, total nitrogen, total carbon and organic matter.

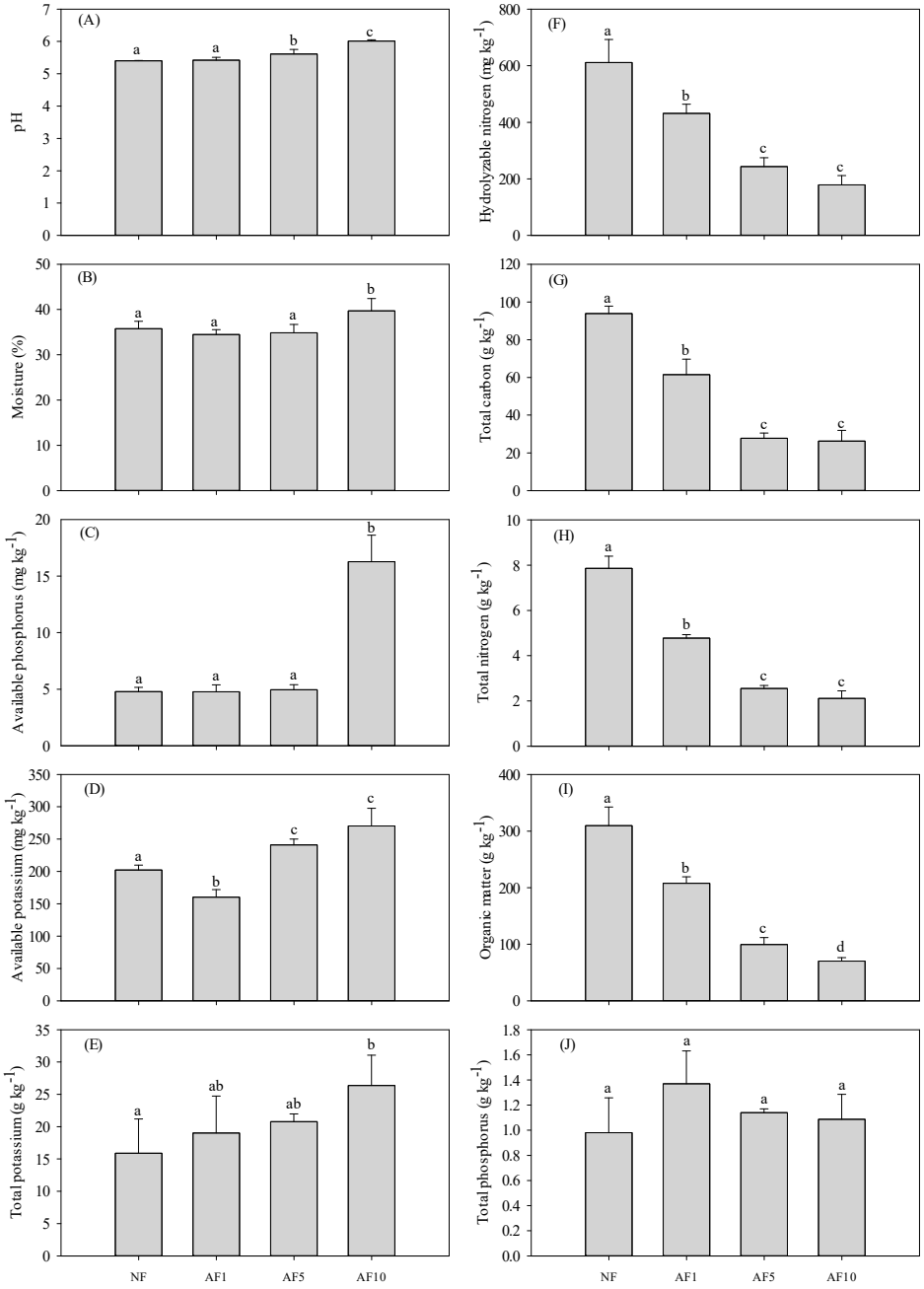

Figure 2. Soil properties in different *A. tsaoko*-forest agroforestry systems. Each bar is the mean ± SD; n = 3. Bars without the same letters are statistically significant at $P < 0.05$. (**A**) pH; (**B**) Moisture (%); (**C**) Available phosphorus (mg kg^{-1}); (**D**) Available potassium (mg kg^{-1}); (**E**) Total potassium (g kg^{-1}); (**F**) Hydrolyzable nitrogen (mg kg^{-1}); (**G**) Total carbon (g kg^{-1}); (**H**) Total nitrogen (g kg^{-1}); (**I**) Organic matter (g kg^{-1}); (**J**) Total phosphorus (g kg^{-1}).

Soil pH values ranged from 5.33–6.05, indicating that all test soils were acidic and no significant differences in pH values between NF and AF1 were observed. However, soil pH obviously increased from NF or AF1 to AF5, as well as to AF10 (Figure 2A). The soil moisture and P_{avail} values of AFs were found to be initially comparable to those of native forest (NF) at the early stages (from AF1–AF5), but they increased significantly at AF10 (Figure 2B,C). Significant variations of K_{avail} were shown in Figure 2D. The content of K_{avail} decreased significantly in AF1 when compared to NF and then increased significantly in AF5 and AF10 when compared to NF or AF1. The content of K_{total} showed an increasing trend, but there was no significant variation among NF, AF1, and AF5, nor among AF1,

AF5 and AF10. However, a significant difference was shown between NF and AF10 (0 E). A significant decrease was observed in the contents of N_{hy}, C_{total}, N_{total}, and OM from NF to AFs, and decreased significantly with increasing AFs stand ages (Figure 2F–I). However, there was no significant difference in P_{total} content among treatments (Figure 2J). In addition, pH affected other soil properties and was closely and negatively related to total carbon (P = 0.0057), total nitrogen (P = 0.0146), organic matter (P = 0.0075) and hydrolyzable nitrogen (P = 0.0085), and positively related to available phosphorus (P < 0.0001) and available potassium (P = 0.0031) (Figure 3).

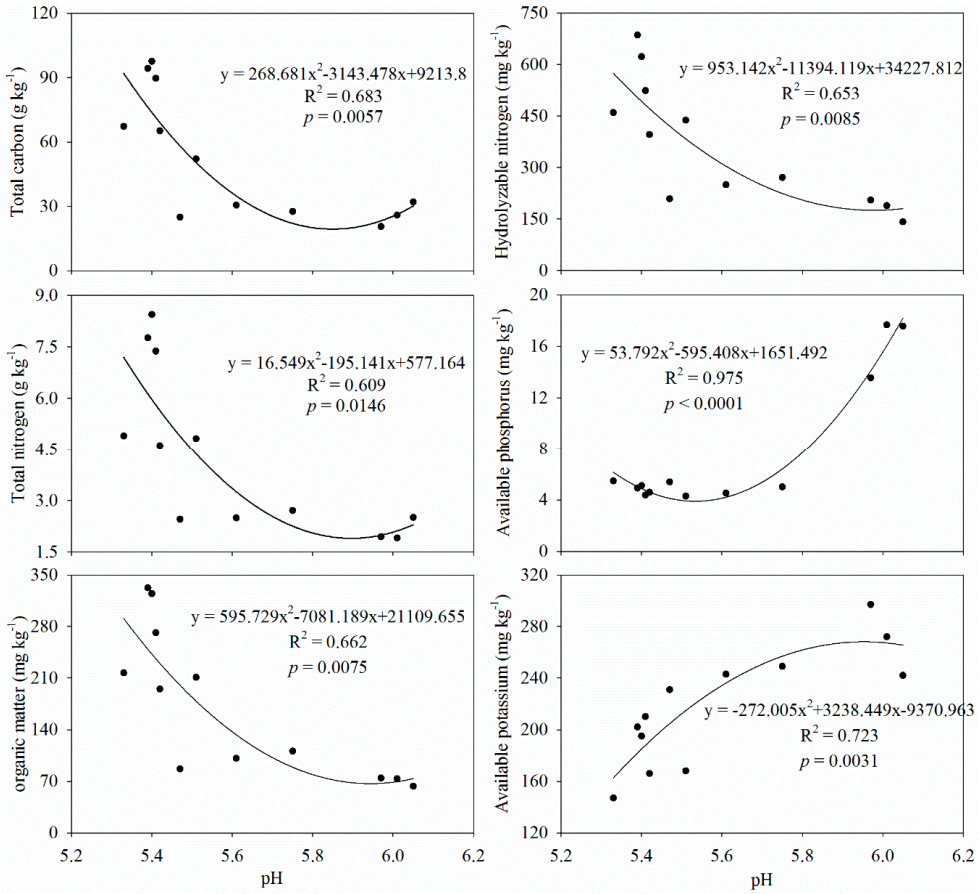

Figure 3. The correlation between pH and soil properties.

3.2. The Microbial Community Component

The number of individual PLFAs detected in soil samples ranged from 30–40, and the total amount of PLFAs ranged between 16.46 and 34.06 nmol g^{-1} soil. The total PLFAs had the highest value of 34.06 nmol g^{-1} soil in NF and decreased to 16.46–22.73 nmol g^{-1} in AFs, and it showed a significant difference between NF and AF5, but there was no significant difference between NF and AF1 or AF10, nor between NF and AFs. Among all the treatments, the proportion of bacteria was the greatest, followed by anaerobe and Gram-negative bacteria (G^-), while arbuscular mycorrhizal fungi (AMF) showed the lowest concentration. PLFAs of bacteria (F = 4.650, P = 0.037), anaerobe (F = 5.672, P = 0.022), G^+ (F = 6.640, P = 0.015) and total PLFA (F = 4.349, P = 0.043) were significantly affected by different treatments, being greatest in NF treatment, and the least in AF5. The number of bacteria in NF was significantly higher than in F5 and F10, but there was no significant difference between NF and F1 or AFs. The amounts of anaerobe and G^+ had the highest value in NF and decreased significantly in AFs, but there was no significant difference among AFs. There were no differences in the abundances of G^-, aerobe, actinomycetes, fungi, and AMF among all treatments, but the same tendency showed that the abundances decreased to the lowest point in AF5 and then increased

in AF10 (Figure 4A). The ratios of fungi to bacteria (F/B), gram-positive bacteria to gram-negative bacteria (G^+/G^-), and aerobe to anaerobe did not show any significant differences among treatments (Figure 4B). However, the different ratios showed different trends; the treatment of NF had the lowest value of F/B but highest of G^+/G^-.

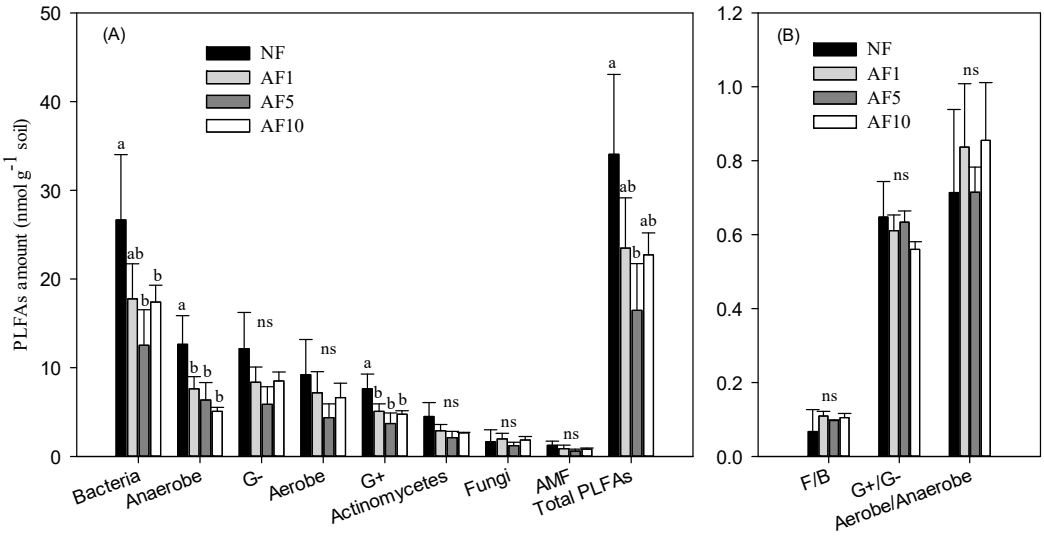

Figure 4. The influence of different treatments on phospholipid fatty acid (PLFA) patterns (**A**) and proportions (**B**) in soils. **ns** indicates no significant difference, and different letters in the same group histogram indicate significant differences ($P < 0.05$) among treatments.

The principal component analysis (PCA) showed that the first two principal components explained the total variance of 93.3%, PC1 accounted for 82.8% of the variation, while PC2 accounted for 10.5% (Figure 5A). The PCA of individual PLFAs shown for PC1 was characterized mainly by monounsaturated PLFAs (18:1ω7c, 16:1ω7c, 17:1ω8c) and iso-branching PLFA (i17:1ω5c) as well as hydroxyl PLFA (i17:0 3OH) normally correlated with gram-negative bacteria. The characteristics of PLFAs (10Me16:0, 10Me17:0, 10Me18:0) also received higher weights on PC1. The highest values along PC2 were characterized by monounsaturated PLFA (18:1ω9c), which represented fungi (Figure 5B).

3.3. Correlations Between Microbial Community and Soil Characteristics

Some strong correlations between soil chemical characteristics and PLFA concentration were observed in Table 1. In terms of PLFAs, the contents of C_{total}, N_{total}, N_{hy}, and OM were highly correlated with the concentration of total PLFA, bacteria, gram-positive bacteria, gram-negative bacteria, actinomycetes, and AMF. A significant difference was also observed between the content of P_{total} and the ratio of fungi to bacteria. However, there was no significant correlation between soil physicochemical properties and fungi, as well as G^+/G^-; similarly, there were no significant correlations between all of the PLFAs and soil pH, moisture, and K, as well as P_{avail}. Aerobe concentration is mainly related to the contents of soil N_{hy} and OM, while AMF concentration is mainly related to N_{total}, N_{hy}, and OM (Table 1). In terms of individual lipids, the contents of C_{total}, N_{total}, N_{hy}, and OM were correlated to most individual lipids, but P_{total} was only significantly correlated with 18:1ω9c, which represents fungi (Table 2).

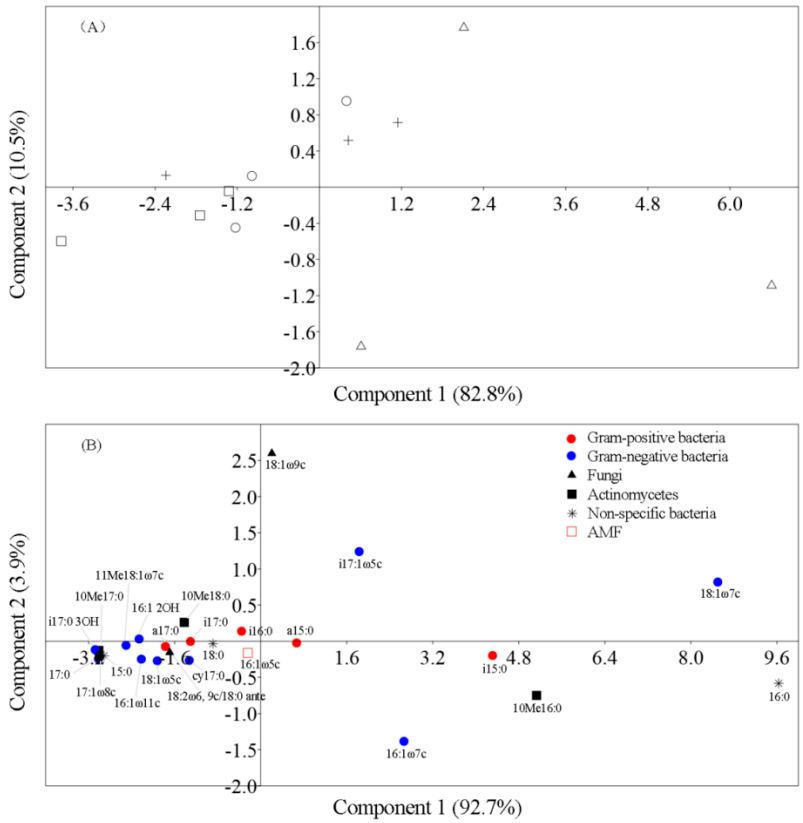

Figure 5. (**A**) Principal component analysis (PCA) of PLFAs in soils of different agroforestry system and stand ages: (Δ) Native forest (NF), (+) agroforestry system (AF)1, (□) AF5, (○) AF10. (**B**) PCA showing loading values for individual PLFAs in the soils of plots given in (**A**).

Table 1. The correlations between soil physicochemical properties and the PLFAs.

	Total PLFA	Fungi	Bacteria	G^+	G^-	F/B	G^+/G^-	Actinomycetes	Aerobe	Anaerobe	AMF
pH	−0.302	0.035	−0.304	−0.377	−0.236	0.236	−0.474	−0.346	−0.230	−0.380	−0.288
Moisture	0.111	−0.004	0.127	0.064	0.194	−0.037	−0.467	0.042	0.147	0.052	0.089
C_{total}	0.681 *	0.104	0.686 *	0.759 **	0.615 *	−0.385	0.380	0.693 *	0.533	0.750 **	0.558
N_{total}	0.714 **	0.142	0.714 **	0.783 **	0.643 *	−0.369	0.355	0.729 **	0.571	0.776 **	0.592 *
P_{total}	−0.284	0.500	−0.334	−0.358	−0.271	0.684 *	−0.260	−0.332	−0.007	−0.336	−0.322
K_{total}	−0.385	−0.171	−0.363	−0.424	−0.331	0.062	−0.284	−0.441	−0.379	−0.442	−0.375
N_{hy}	0.753 **	0.119	0.753 **	0.808 **	0.697 *	−0.380	0.269	0.776 **	0.643 *	0.810 **	0.666 *
P_{avail}	−0.086	0.168	−0.091	−0.168	−0.026	0.216	−0.551	−0.173	−0.017	−0.182	−0.070
K_{avail}	−0.278	−0.184	−0.260	−0.306	−0.216	0.011	−0.261	−0.294	−0.282	−0.316	−0.257
OM	0.734 **	0.142	0.733 **	0.793 **	0.672 *	−0.360	0.300	0.753 **	0.618 *	0.793 **	0.633 *

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

Table 2. The correlations between individual PLFAs and soil properties.

PLFA Marks	Signature of	Soil Chemical Variables				
		C_{total}	N_{total}	OM	P_{total}	N_{hy}
15:0	non-specific bacteria	0.794 **	0.818 **	0.821 **	−0.394	0.830 **
16:0	non-specific bacteria	0.705 *	0.732 **	0.741 **	−0.405	0.755 **
18:0	non-specific bacteria	0.706 *	0.742 **	0.755 **	−0.346	0.772 **
17:0	General bacteria	0.529	0.578 *	0.585 *	−0.466	0.606 *
a15:0	Gram-positive bacteria	0.584 *	0.623 *	0.662 *	−0.307	0.695 *
i15:0	Gram-positive bacteria	0.739 **	0.756 **	0.762 **	−0.405	0.775 **

Table 2. *Cont.*

PLFA Marks	Signature of	Soil Chemical Variables				
		C_{total}	N_{total}	OM	P_{total}	N_{hy}
i16:0	Gram-positive bacteria	0.828 **	0.850 **	0.807 **	−0.382	0.793 **
a17:0	Gram-positive bacteria	0.702 *	0.744 **	0.778 **	−0.251	0.803 **
i17:0	Gram-positive bacteria	0.785 **	0.783 **	0.811 **	−0.172	0.824 **
16:1 2OH	Gram-negative bacteria	0.758 **	0.708 **	0.727 **	0.122	0.716 **
16:1ω7c	Gram-negative bacteria	0.510 *	0.545	0.596 *	−0.352	0.636 *
16:1ω11c	Gram-negative bacteria	0.507	0.548	0.608 *	−0.266	0.651 *
17:1ω8c	Gram-negative bacteria	0.591 *	0.623 *	0.663 *	−0.369	0.695 *
i17:1ω5c	Gram-negative bacteria	−0.109	−0.123	−0.223	−0.535	−0.249
i17:0 3OH	Gram-negative bacteria	0.300	0.250	0.175	−0.372	0.134
cy17:0	Gram-negative bacteria	0.512	0.548	0.594 *	−0.336	0.631 *
18:1ω5c	Gram-negative bacteria	0.706 *	0.740 **	0.763 **	−0.359	0.785 **
18:1ω7c	Gram-negative bacteria	0.618 *	0.646 *	0.686 *	−0.020	0.706 *
11Me18:1ω7c	Gram-negative bacteria	0.666 *	0.679 **	0.676 *	−0.077	0.674 *
18:1ω9c	Fungi	−0.133	−0.108	−0.092	0.660 *	−0.109
18:2ω6,9c/18:0ante	Fungi	0.740 **	0.784 **	0.730 **	−0.490	0.715 **
16:1ω5c	Arbuscular mycorrhizal fungi	0.558	0.592 *	0.633 *	−0.322	0.666 *
10Me16:0	Actinomycetes	0.661 *	0.699 *	0.728 **	−0.336	0.754 **
10Me17:0	Actinomycetes	0.784 **	0.794 **	0.754 **	−0.338	0.740 **
10Me18:0	Actinomycetes	0.598 *	0.621 *	0.625 *	−0.180	0.636 *

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

4.1. AFs Effects on Soil Physicochemical Properties

Statistical data showed that AFs had great effects on soil physical and chemical properties (Figure 2). The main reason for the high value of soil available phosphorous observed in the AF10 treatment could be explained by the higher population density of *A. tsaoko*, which leads to worse ventilation during the growth stage. This can eventually result in the occurrence of disease that was prevented using the application of lime by the local farmer [55–58]. The elevated content of potassium and available phosphorus in soil may also be related to the return of the withered parts of *A. tsaoko* [32]. However, studies of the elementary composition of *A. tsaoko*, including aboveground parts, are lacking. The contents of nitrogen decreased with the chronosequence of AFs in this study, which is contrary to the results of the agroforestry coffee system (ACS) [59]. This is mostly due to the use of fertilizer in ACS; correspondingly, we did not use any fertilizer in the *A. tsaoko*-forest agroforestry system. The contents of soil nitrogen and organic matter decreased with the chronosequence of AFs mostly because of (1) more weeding and forest thinning before the fruit-bearing stages in which litter decreases and herbs largely emerge (because of less canopy and more sunlight); these vigorous herbs quickly consume more nutrients from the soil; (2) nitrogen is likely the main component of the fruits which are removed from the field every year by harvesting; this tendency is consistent with the actual output of *A. tsaoko* over the years [60,61]; and (3) nitrogen was leached easily while phosphorus and potassium were fixed in the soils; the fixed phosphorus and potassium are released by the soil with the increasing planting ages [32,62] or because the HPO_4^{2-} phosphate ions, at more acidic pH values, react with aluminum (Al) and iron (Fe) to form less soluble compounds [63] which then are released when pH reaches to 5.7–5.9. This phenomenon is related to the change of the pH value which contributes to agricultural measures, such as liming. In turn, the availability of some plant nutrients is greatly affected by soil pH [63], which is highly correlated with other physical and chemical properties of the soil (Figure 3 and Supplementary Material). However, soil, as an important natural resource, contributes to determining the way in which natural vegetation, crops, human settlements and above- or below-ground organisms are distributed on the territory; the changing of soil physiochemical properties is a complex and comprehensive process.

4.2. AFs Effects on Soil Microbial Community Structure

The change of land-use not only affects soil physicochemical properties but also affects soil microorganisms. Soil microbes play a very important role in improving soil fertility and productivity and may indirectly or directly influence plant growth. The soil microbial community is an important indicator of soil properties, which is confirmed by the same changing pattern of the soil microbial community and the soil physiochemical properties. However, several causes may exist for the changed structure and function of soil microbial communities, as well as soil physiochemical properties in *A. tsaoko*-based agroforestry systems. Our studies demonstrated that the soil microbial community structure was significantly changed from NF to AFs (Figure 4). The decrease in total PLFA from NF to AFs was mostly associated with a decrease in the bacteria, especially with gram-positive bacteria (Figure 4). This decrease in bacteria could be explained by the significant decrease physicochemical properties indicated that PLFA (Figure 2 and Table 1). Due to the loss of plant diversity caused by forest land-use conversion, the range of organic substrates entering the soil yielded insufficient levels of nutrients that are required for the sustainable growth of heterotrophic microorganisms [64,65]. Furthermore, the inherent characteristics of a plant might affect the microbial community; the fruit of *A. tsaoko* is used as a kind of spice, and the essential oil extracted from fruit, stem [66], or leaves [67] has been proven to have bacteriostasis properties [68–70].

The fluctuation of PLFAs concentrations and soil physicochemical properties might be consistent with the growth stages and field management of *A. tsaoko* for the changing of plant communities and soil resource availability [17]. Initially, after chopping the overcrowded trees and all the shrubs and herbs, the soil was severely disturbed, leading to a reduction in PLFAs content, and then reaching a higher level at AF10. The results indicated that the *A. tsaoko*-based agroforestry system contributed to the restoration of the microbial community at AF10 in which *A. tsaoko* fruit reached a higher yield. This resilient phenomenon may also be affected by abiotic and biotic factors [71,72]. Moreover, the correlation between the microbial community structure and soil physicochemical properties indicated that PLFA were highly correlated with the contents of total carbon, nitrogen, and organic matter (Tables 1 and 2), which is consistent with prior research [73]. This indicates that the inverted parabolic tendency of PLFAs content helps to restore the microbial population across increasing AFs, and it is a good signal for the sustainable utilization of land resources, especially in poor mountainous regions. Similar results suggesting that agroforestry may increase soil microbial resilience have been verified in wheat-based agroforestry systems [74]. The ratio of gram-positive to gram-negative bacteria (G^+/G^-) was used to indicate nutrient deficiencies in soils [75–78]. However, in this study, there was no significant difference in the ratio of G^+/G^-.

5. Conclusions

Prior research indicated that soil quality and biodiversity can be maintained and partially restored if managed sustainably; agroforestry systems, conversely, have the potential to enhance soil quality [6–8], which is consistent with our results. According to our research, a positive trend in the concentrations of soil phosphorus and potassium and in microbial communities after planting *A. tsaoko* for 10 years was observed. These results indicated that the *A. tsaoko*-based agroforestry system is a sustainable land-use practice, even though additional nitrogen is necessary, since a significant loss of nitrogen in the soil was observed during planting season. However, knowledge regarding the impact of *A. tsaoko* on soil microbial communities is still limited, and we could not classify the microorganism presence using the PLFA method. Hence, a high-throughput sequencing method should be implemented in the future.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/11/2/546/s1. Table S1: Correlations between soil physicochemical properties. * Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

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