


Article

Prolonging Rotation of Chinese Fir to over 25 Years Could Maintain a Better Soil Status in Subtropical China

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Abstract: Although Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is an important species for wood production in subtropical China, its serious declines in soil nutrients and timber productivity in plantations have been reported, probably caused by successive rotation and inappropriate cutting time. Although the significant effect of stand age on soil properties has been widely recognized, research on soil enzymes and microbial communities is relatively rare. In this study, assuming that short rotation period is one important reason for soil degradation, we measured soil physicochemical properties, microbial community composition, and enzyme activity in 3-, 15-, 25- and 45-year Chinese fir forests in Jiangxi province of China. Soil organic carbon (SOC) content decreased from 3-year to 25-year stands and then increased in 45-year stands. Despite the significant relationship between SOC and the abundance of total phospholipid fatty acids (PLFAs), no notable changes in the abundance of PLFAs were detected with increasing tree ages, except for the abundances of arbuscular mycorrhizal fungi (AMF) which were significantly higher in 25-year stands. However, the ratios of gram-positive to gram-negative bacteria (G+/G-) and fungi to bacteria (F/B) both decreased with increasing stand age. 45-year stands showed the highest activities of both phosphatase and β -glucosidase. Total potassium (TK) content and net N mineralization rate both had significant links with soil microbial community structure. Collectively, our study emphasized that stand age could significantly affect soil physicochemical properties and the microbial community. In general, 25-year stands showed poorer soil status compared to that of 45-year stands. Thus, the cutting age of Chinese fir should be increased to over 25 years to maintain a better soil status.

Keywords: N cycling; microbial communities; enzyme activities; PLFA; stand age

1. Introduction

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is cultivated in the south of China because of its fast growth and high yield [1,2]. It usually matures between 25 to 30 years and is harvested from 20 to 25 years to meet the growing need for timber [3,4]. However, in recent years, Chinese fir plantations have faced problems, such as reduced yield and growth rate as well as depletion of soil nutrients, which has led managers to question whether short-rotation management is sustainable [5–7] and to the increased need for an improved understanding of soil changes during the growth of Chinese fir forests.

There are many biological and physicochemical characteristics to assess soil nutritional conditions, and soil organic carbon has been shown to be important [8–10]. Furthermore, soil microorganisms and extracellular enzymes excreted by microbes are also useful indicators given their important roles in

nutrients conversion and organic matter decomposition [11]. Changes in microbial communities or enzyme activities can reflect shifts in soil fertility [12–14]. To assess soil fertility more comprehensively, soil physicochemical and microbial parameters should be synthesized in studies while in recent years, more efforts have been made towards exploring soil physicochemical properties of Chinese fir plantations [15–17].

Studies have shown relationships between tree age and soil properties of Chinese fir, but the results varied with studies. Yu et al. reported that soil organic carbon (SOC) contents generally increased from 6-year to 31-year plantations [18]; Li et al. found that soil fertility decreased from 5-year to 15-year stands and then increased in 25-year stands [19]. Most studies found that microbial biomass first decreased and then increased with tree age [20,21], but an overall increasing trend [22] or a consistently decreasing tendency [23] were also detected. Liu et al. stated that the gram-positive to gram-negative (G+/G−) bacteria ratio decreased with increasing age [24]. Enzyme activities also showed inconsistent changes with stand age in different studies [11,15]. It is noteworthy that previous studies of Chinese fir were mostly confined to the years before Chinese fir was cut down [25–27] and it remains unclear whether prolonging the cultivation period of Chinese fir could alleviate soil degradation problems.

Based on these considerations, we selected four Chinese fir forests at different ages (3 years, 15 years, 25 years, and 45 years) in Xinjiang Township, Suichuan County, Jiangxi Province. As a major production area of Chinese fir in South China, this region provides an ideal soil matrix for our research. Considering that short rotation may be one important reason for soil degradation, this study aims to (1) thoroughly investigate the impacts of tree age on soil physicochemical properties, and biomass, structure, and activity of the microbial community and (2) determine if 25 years is an appropriate cutting age for Chinese fir from the perspective of soil status. We addressed two hypotheses: (1) soil nutrients would increase in older stands; (2) soil microbial community biomass and enzyme activities would show a V-shaped trend with increasing age.

2. Materials and Methods

2.1. Study Site

The study was undertaken in Xinjiang Township (26°41′N, 114°27′E), Suichuan County, Jiangxi Province of China. This township is a main producing area of Chinese fir in Jiangxi and covers an area of 185 square kilometers, 83% of which is occupied by woods (Figure 1). Chinese fir is usually cut before 25 years in this area. The typical climate of this region is humid subtropical monsoon with a mean annual temperature of 16.5°C and a mean annual rainfall of 1421 mm. The soil type of the studied forest stands was classified as ultisols with a loamy texture [28].

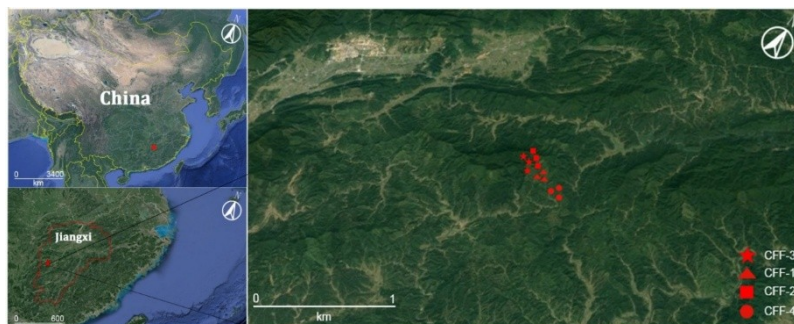


Figure 1. Location of Chinese fir plantations with varying ages in Jiangxi Province of China. CFF-3, 3-year Chinese fir forests; CFF-15, 15-year Chinese fir forests; CFF-25, 25-year Chinese fir forests; CFF-45, 45-year Chinese fir forests.

2.2. Field Sampling

In April 2018, we selected four Chinese fir plantations, namely 3 years, 15 years, 25 years, and 45 years, respectively. For each age, we selected three 30 m × 30 m plots located on three adjoining south-facing hillsides (Figure 1) with similar topographic and soil conditions (Table 1). Within each plot, we used an S-shaped sampling method to collect nine soil cores at 0 to 10 cm depths to form a composite sample for each plot. Soil samples, sealed in polyethylene bags, were transported to the laboratory within 48 h in an icebox. The fresh soil was first passed through a 2-mm sieve after the removal of plant materials, mollusks, and stones, and part of the soil samples was stored at 4 °C for phospholipid fatty acid (PLFA) analysis and the remaining were air-dried, ground and then used in physicochemical and enzymatic analysis.

Table 1. Stand and soil characteristics of Chinese fir plantations with varying ages in Jiangxi Province.

Characteristics	CFF-3	CFF-15	CFF-25	CFF-45
Stand age (year)	3	15	25	45
DBH (cm)	1.3	10.2	18.3	40.5
Canopy height (m)	1.5	8.5	11.2	25.4
Tree density (trees ha ⁻¹)	4430	3713	1325	583
Elevation (m)	315	327	322	306
Slope (°)	21	19	20	23
Aspect	South	South	South	South
pH (in water)	4.47 ± 0.06ab	4.24 ± 0.05b	4.49 ± 0.08a	4.30 ± 0.08ab
Moisture (%)	0.43 ± 0.01ab	0.40 ± 0.00b	0.40 ± 0.00b	0.45 ± 0.02a
SOC (g/kg)	37.21 ± 0.11a	33.09 ± 0.34ab	29.17 ± 0.08b	32.02 ± 0.27ab
TN (g/kg)	3.04 ± 0.04a	2.76 ± 0.02a	2.44 ± 0.02a	2.54 ± 0.02a
C/N	12.62 ± 1.48a	11.94 ± 0.55a	12.01 ± 0.55a	12.57 ± 0.15a
Net N mineralization (µg g ⁻¹ d ⁻¹)	0.61 ± 0.01b	0.58 ± 0.03bc	0.55 ± 0.01c	0.89 ± 0.01a
TK (g/kg)	8.97 ± 0.02c	16.94 ± 0.01a	17.08 ± 0.04a	15.20 ± 0.07b
TP (g/kg)	0.40 ± 0.000a	0.44 ± 0.00a	0.44 ± 0.00a	0.40 ± 0.01a

DBH, stem diameter at breast height; SOC, soil organic carbon; TN, total nitrogen; C/N, carbon to nitrogen ratio; TK, total potassium; TP, total phosphorus. Values are means followed by standard errors. Via Duncan's multiple comparison test, different letters in the same columns indicate significant difference among ages. CFF-3, 3-year Chinese fir forests; CFF-15, 15-year Chinese fir forests; CFF-25, 25-year Chinese fir forests; CFF-45, 45-year Chinese fir forests.

2.3. Soil Physicochemical Properties Analysis

Fresh soil samples were dried at 105 °C for 12 h to a constant weight, and soil moisture was measured based on the lost weight. A slurry of soil and water in a ratio of 1:2.5 was used to measure soil pH. After using 2 M hydrochloric acid to eliminate the interference of calcium carbonate, SOC and total nitrogen (TN) contents were determined by an Elemental Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Langensfeld, Germany). Net N mineralization rate was tested by comparing the difference of inorganic nitrogen concentrations (NH₄⁺-N plus NO₃⁻-N) before and after incubation for 28 days. Total potassium (TK) and total phosphorus (TP) content were measured by an iCAP7600 inductively coupled plasma atomic emission spectrometry (Thermo Fisher Scientific, Waltham, MA, USA).

2.4. Phospholipid Fatty Acid (PLFA) Analysis

The soil microbial communities were estimated by PLFA analysis. For detailed procedures, refer to Kang et al. [29]. Briefly, a mixed solution of chloroform, methanol, and citrate buffer (pH = 4.5) at a volume ratio of 1:2:0.8 was added to extract lipids from 10.00 g fresh sample. Lipid fractionation was performed by solid-phase extraction (SPE) chromatography, and phospholipids were trans esterified with a mild-alkaline methanolysis. PLFA analysis was conducted by using an Agilent 7890A-5975C Gas chromatograph-mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). PLFA biomarkers

were identified based on the retention times and mass spectra of standards and the NIST 2014 spectral database. Methyl nonadecanoate was used as an internal standard to quantify PLFAs.

A standard nomenclature is used to describe PLFAs [30]. Forty-two PLFAs were determined in all samples. Specific fatty acids were assigned to different microbial groups (Table 2). The relative abundance of each different group, the fungal-to-bacterial (F/B) ratio and the ratio of gram-positive to gram-negative (G+/G−) bacteria were also calculated [29].

Table 2. Phospholipid fatty acids (PLFA) biomarkers for calculating soil microbial biomass.

Microbial Group	PLFA Biomarkers	Reference
Saprotrophic fungi	18: 2 ω 6, 9 and 18: 1 ω 9	[31]
Gram-positive bacteria	i15: 0, a15: 0, i16: 0, i17: 0, a17: 0	[32–34]
Gram-negative bacteria	16: 1 ω 9, 16: 1 ω 7, cy17: 0, cy19: 0	[32–34]
Bacteria	i15: 0, a15: 0, i16: 0, i17: 0, a17: 0, 16: 1 ω 9, 16: 1 ω 7, cy17: 0, cy19: 0	[32–34]
Arbuscular mycorrhizal fungi	C16: 1 ω 5	[35]

2.5. Enzyme Activity Analysis

Activities of β -glucosidase, urease, and acid phosphatase were measured to determine microbial function. All three enzymes were incubated at a specific temperature with their respective substrates for a specific time before testing (Table 3). Comparing the amounts of specific products released from 1 g of dry soil after 24 h, urease activity was determined by sodium benzoate–sodium hypochlorite colorimetric method, the activity of β -glucosidase was tested by 3, 5-dinitrosalicylic acid colorimetric method, and the activity of phosphatase was detected by sodium phenyl phosphate colorimetric method.

Table 3. Substrates, incubated conditions, and products of soil enzyme activities assays.

	β -glucosidase	Urease	Phosphatase
Substrate	sucrose	urea	disodium phenyl phosphate
Incubation temperature ($^{\circ}$ C)	37	37	37
Incubation time (h)	24	24	24
Absorbance (nm)	508	578	570
Product (mg g ^{−1} 24 h ^{−1})	glucose	NH ₄ ⁺	phenol

2.6. Statistical Analysis

Statistical analyses were conducted by the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Significant differences between samples with different tree ages on soil physicochemical and biological properties were determined by one-way analysis of variance (ANOVA) coupled with Duncan's multiple comparison test. Pearson correlation analysis was used to evaluate associations between soil physicochemical and biological variables. The links between soil properties and microbial community composition were also determined based on a redundancy analysis (RDA). It was implemented in Canoco for Windows 4.5 (Biometris, Wageningen, Netherlands) and performed on the relative abundances of all PLFA biomarkers. A Monte Carlo permutation test was used to examine if soil properties could significantly explain variations in the PLFA data ($P < 0.05$). Significant properties were expressed by solid lines, while insignificant properties were denoted by dotted lines [36]. The sampling map was generated in Google Earth Pro (Google Inc., Santa Clara, CA, USA).

3. Results

3.1. Soil Physicochemical Properties

Except for TN contents (ranging from 2.44 g/kg to 3.04 g/kg) and C/N ratios (ranging from 11.94 to 12.62), soil physicochemical properties showed significant differences among ages (Table 1;

Table S1). Soil pH levels varied between 4.24 and 4.49. SOC contents were 29.17 to 37.21 g/kg and strongly correlated with TN contents ($R = 0.729$, $P < 0.01$) with the highest values in the 3-year plantations. The net N mineralization rates were highest in the 45-year plantations. Both SOC contents and net N mineralization rates decreased from 3-year to 25-year stands, then increased in 45-year stands. TK concentrations were higher in the 15-year and 25-year plantations. TP contents showed no differences among ages.

3.2. Microbial PLFA Biomass and Structure

Except for the abundances of arbuscular mycorrhizal fungi (AMF) which were significantly lower in the 45-year stands, no significant differences were found for the absolute abundances of total bacterial, fungal, G+ and G− bacterial PLFAs between tree ages (Table S1; Figure 2). The F/B ratio and the relative abundance of fungi were both highest in 3-year stands, while the proportion of bacteria was lowest there (Figure 3). The relative abundance of G+ showed no significant changes among stands. The proportion of G− increased with tree age, while the G+/G− ratio showed an opposite pattern.

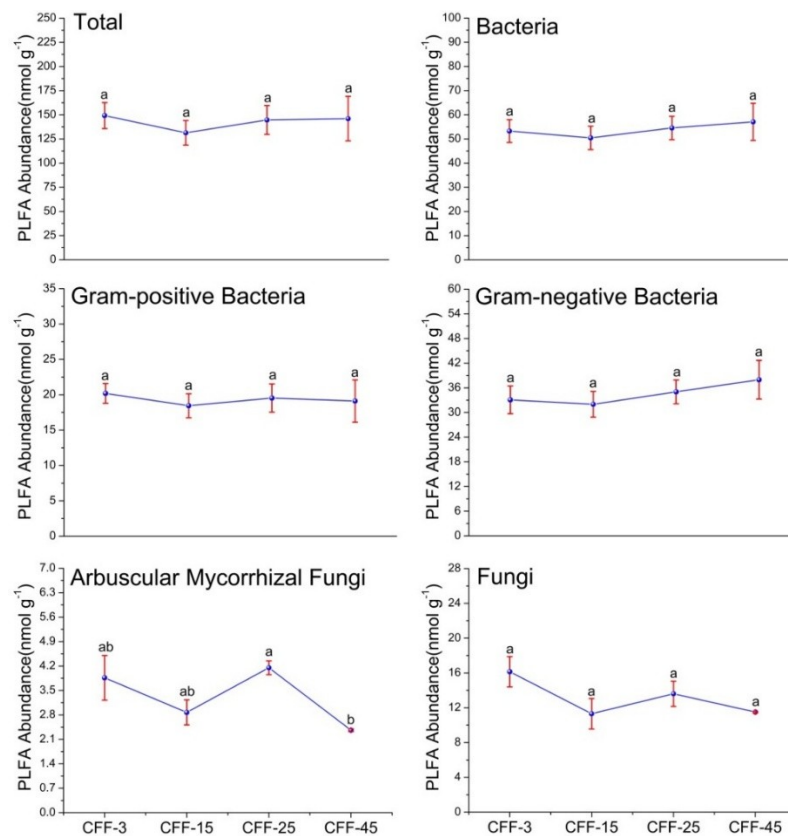


Figure 2. Phospholipid fatty acids (PLFAs) abundance of Chinese fir stands with different ages in Jiangxi Province. Values are means ($n = 3$) with a bidirectional stand error bar. Via Duncan's multiple comparison test, different letters in the same columns indicate significant difference among ages. CFF-3, 3-year Chinese fir forests; CFF-15, 15-year Chinese fir forests; CFF-25, 25-year Chinese fir forests; CFF-45, 45-year Chinese fir forests.

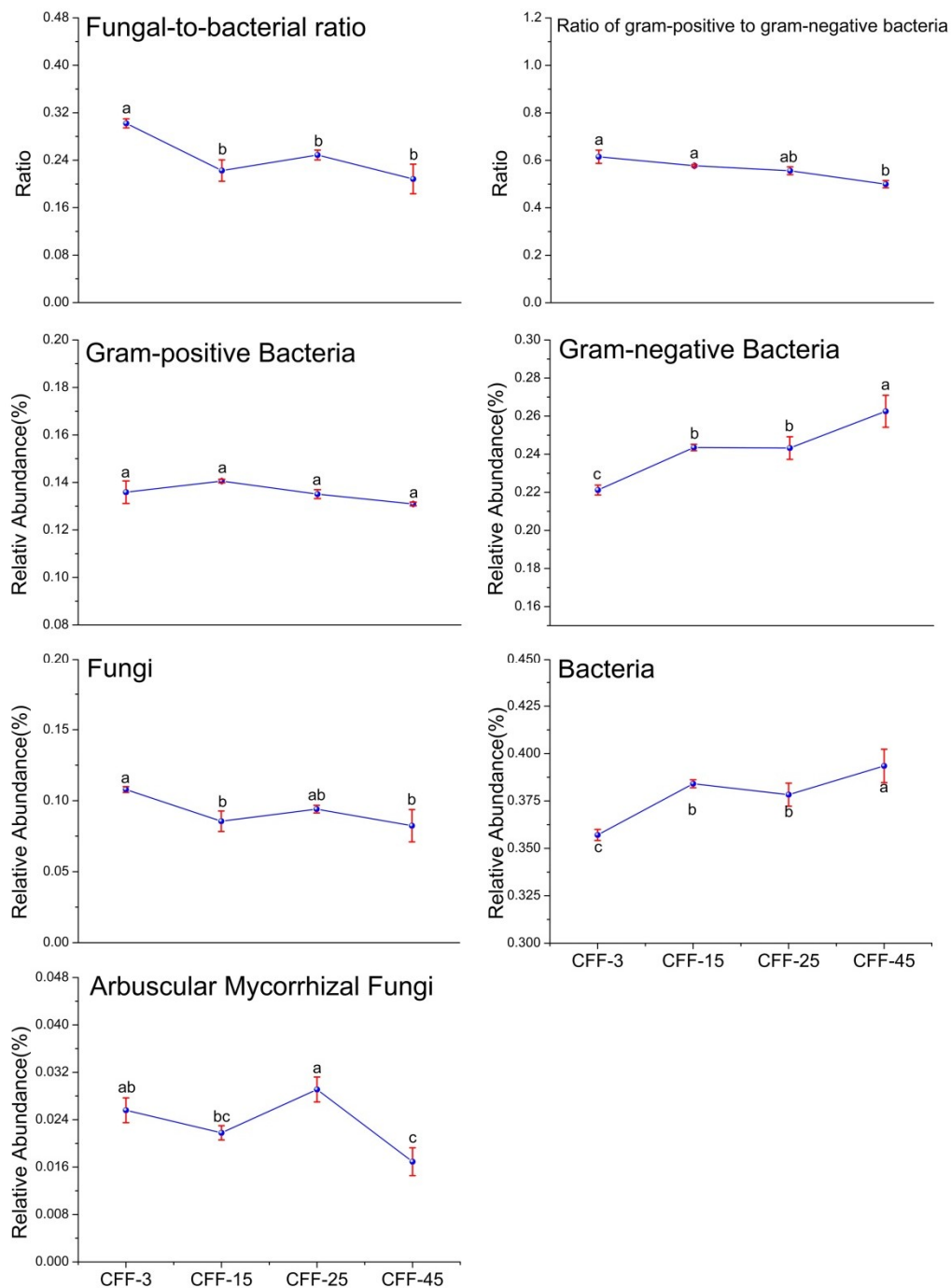


Figure 3. Relative abundance of PLFAs in Chinese fir stands with different ages in Jiangxi Province. Values are means ($n = 3$) with a bidirectional stand error bar. Via Duncan's multiple comparison test, different letters in the same columns indicate significant difference among ages. CFF-3, 3-year Chinese fir forests; CFF-15, 15-year Chinese fir forests; CFF-25, 25-year Chinese fir forests; CFF-45, 45-year Chinese fir forests.

3.3. Soil Enzyme Activity

The activities of β -glucosidase and urease did not consistently increase or decrease with tree age (Table S1; Figure 4) and differed significantly among stands. The β -glucosidase and urease activities were highest in 45-year and 15-year stands, respectively. The potential activity of phosphatase was also highest in the 45-year stands, although not significantly.

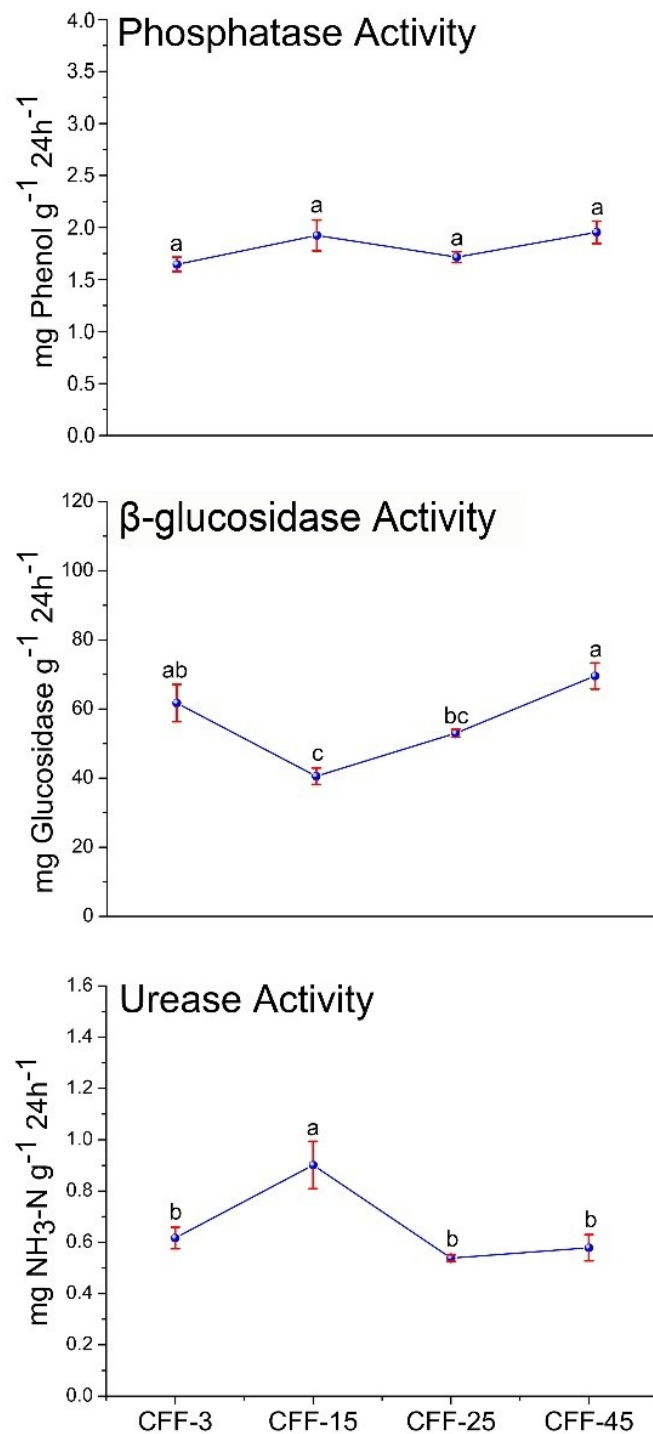


Figure 4. Soil enzyme activities under Chinese fir stands with different ages in Jiangxi Province. Values are means ($n = 3$) with a bidirectional stand error bar. Via Duncan's multiple comparison test, different letters in the same columns indicate significant difference among ages. CFF-3, 3-year Chinese fir forests; CFF-15, 15-year Chinese fir forests; CFF-25, 25-year Chinese fir forests; CFF-45, 45-year Chinese fir forests.

3.4. Relationship between Soil Physicochemical and Biological Indicators

The overall composition of the soil microbial community represented by the relative abundances of 42 PLFA biomarkers varied with tree ages (Figure 5). The 3-year (except for one stand) and 15-year stands were separated from the 25-year and 45-year stands along axis 2. Axis 1 and axis 2 explained

56.0% and 21.3% of the variation, respectively. Soil pH, SOC content, and net N mineralization rate were significantly related to microbial community structure ($P < 0.05$). SOC content and pH were negatively associated with axis 1, and the net N mineralization rate showed the opposite. The relative abundances of 18:2 ω 6, 16:1 ω 7, and 16:1 ω 5 were closely linked with pH. The relative abundances of i17:0 and cy19:0 were positively and negatively associated with SOC concentration, respectively. The RDA result based on the absolute abundances of PLFAs was not shown as it could not reveal a clear separation between age groups.

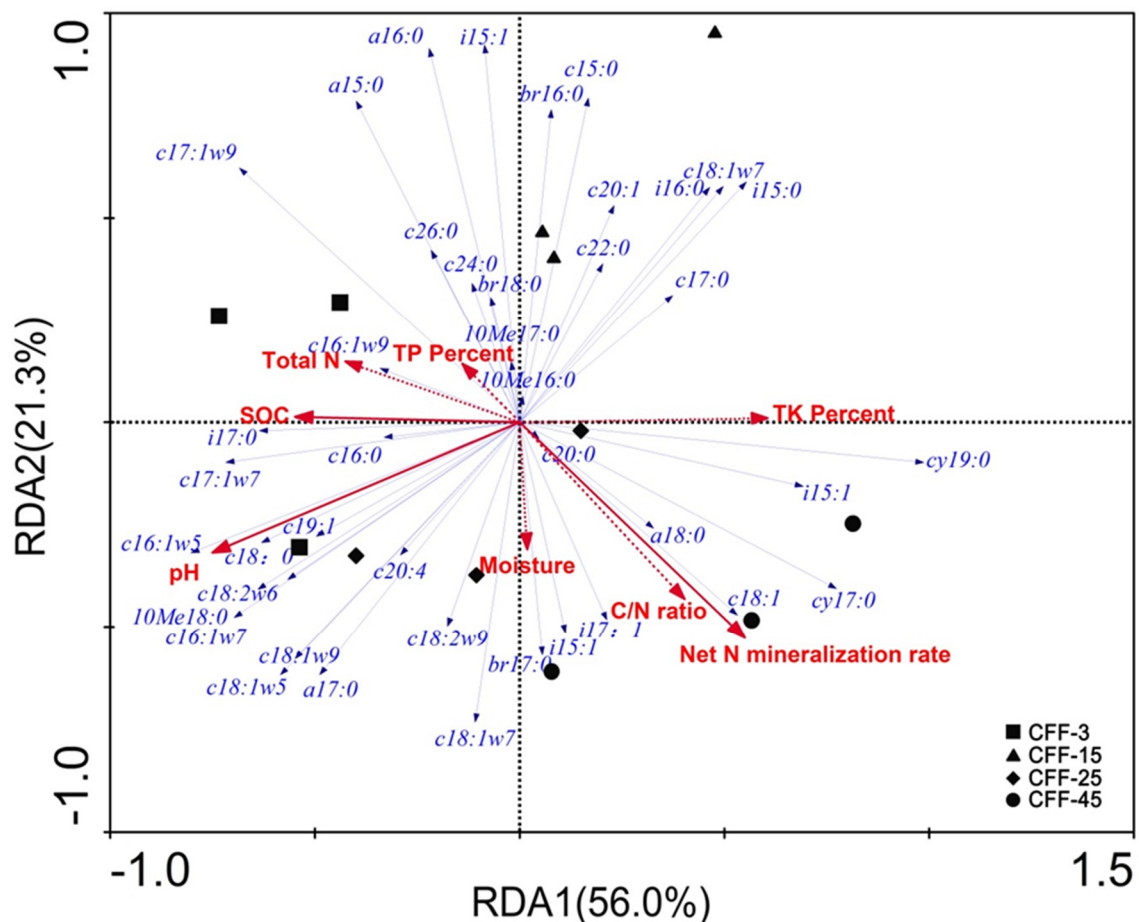


Figure 5. Redundancy analysis on the relative abundance of PLFA biomarkers and soil physicochemical properties in Jiangxi Province. Soil properties that were significantly correlated with factors were stressed (Monte Carlo permutation tests, $P < 0.05$). Vectors (indicating soil properties) of greater magnitude and forming smaller angles with an axis are more strongly correlated with that axis. CFF-3, 3-year Chinese fir forests; CFF-15, 15-year Chinese fir forests; CFF-25, 25-year Chinese fir forests; CFF-45, 45-year Chinese fir forests.

The total PLFA abundance had a positive correlation with SOC content ($R = 0.61$, $P < 0.05$; Table 4). No significant correlations between the C/N ratio and microbial parameters were detected. Both soil net N mineralization and TK content were positively related to the relative abundances of bacteria and G- bacteria. Furthermore, TK content was negatively correlated with the relative abundance of fungi and F/B ratio; soil net N mineralization had negative correlations with the relative abundance of AMF and G+/G-.

Table 4. Correlations analysis between indicators of soil microbial community, enzyme activity, and physicochemical properties in Chinese fir plantations with varying ages in Jiangxi Province.

Microbial Communities	Soil Physicochemical Properties							
	pH	Moisture	SOC	TN	C/N	TK	TP	Net N Mineralization
Total PLFAs	0.34	0.36	0.61 *	0.45	0.06	−0.22	0.169	0.07
Bacterial PLFAs	0.20	0.46	0.52	0.33	0.11	−0.05	0.219	0.26
G+ bacterial PLFAs	0.36	0.37	0.64 *	0.43	0.14	−0.19	0.312	0.01
G− bacterial PLFAs	0.10	0.48	0.44	0.26	0.08	0.04	0.158	0.38
Fungal PLFAs	0.37	−0.04	0.58	0.46	0.08	−0.58 *	0.033	−0.19
AM Fungal PLFAs	0.45	−0.42	0.24	0.12	0.12	−0.20	0.113	−0.57
F/B ratio	0.34	−0.26	0.31	0.31	0.04	−0.68 *	−0.132	−0.36
G+/G− ratio	0.52	−0.17	0.39	0.29	0.15	−0.47	0.294	−0.68 *
G+ bacteria%	0.11	−0.05	0.14	−0.12	0.35	0.10	0.639 *	−0.36
G− bacteria%	−0.58	0.21	−0.41	−0.45	0.04	0.59 *	−0.033	0.71 **
Fungi%	0.23	−0.29	0.23	0.20	0.09	−0.59 *	−1.00	−0.25
Bacteria%	−0.57	0.20	−0.39	−0.50	0.15	0.64 *	0.153	0.63 *
AM Fungi%	0.33	−0.66 *	−0.11	−0.18	0.16	−0.05	0.016	−0.72 **
β−glucosidase activity	0.18	0.68 *	0.18	0.19	−0.02	−0.41	−0.47	0.66 *
Urease activity	−0.34	−0.12	0.04	0.20	−0.26	0.11	0.223	−0.28
Phosphatase activity	−0.42	−0.16	−0.66 *	−0.51	−0.14	0.40	0.015	0.35

* $P < 0.05$, ** $P < 0.01$; G+, gram-positive; G−, gram-negative; AMF, arbuscular mycorrhizal fungi; F/B ratio, the ratio of saprotrophic fungi to bacteria; G+/G− ratio, the ratio of gram-positive bacteria to gram-negative bacteria; % represents the relative abundance of a group. Significant values are shown in bold.

4. Discussion

4.1. Effect of Stand Age on Soil Physicochemical Properties

We observed that SOC contents significantly decreased from 3-year to 25-year stands, then slightly increased in 45-year stands (Table 1). This trend was in line with the results of Wang et al. and Wei et al. who reported similar results from 7 to 49 years and from 10 to 37 years in Chinese fir plantations, respectively [21,37], but inconsistent with many studies on Chinese fir. For example, Wang et al. and Yu et al. found continuous increases of SOC concentrations from 6 to 31 years and from 18 to 49 years, respectively [11,18]; Chen et al. reported a consistent decrease in SOC contents from 2 to 40 years [38]. In our study, TN contents showed a similar trend as SOC but was not significant among stand ages. This might be attributed to ample nitrogen levels (ranging from 2.44 to 3.04 g/kg), compared with other research. For example, Wang et al., Chen et al., and Wang et al. all reported that the TN concentrations were less than 2.0 g/kg for Chinese fir from 3 to 49 years [11,21,38].

The initial reduction of SOC concentration possibly results from tree growth, as before Chinese fir trees reach maturity (usually 25–30 years), the amount of soil nutrients consumed by trees would exceed those returned to soil [17,23]. The decrease can also be explained by a decline of understory vegetation, associated with gradual canopy closure with tree growth before maturity [38,39]. Our observation that understory vegetation decreased from 3-year to 25-year stands also supported this explanation. Moreover, erosion induced by highly weathered soils, high precipitation, and steep slopes typical in the subtropical mountainous area of China, can also be a considerable avenue for SOC decline [38,40], and this effect is expected to increase with time.

The slight increase in SOC contents from 25-year to 45-year stands may still be explained by understory vegetation, as we found a regrowth of underground vegetation in 45-year stands, which was also reported by Jia et al. [41]. This phenomenon was possibly caused by canopy reopening with human disturbance (i.e., thinning) as well as natural sparsity in the 45-year stands [42]. Other studies have also observed the initial decrease and then regeneration of underground vegetation in forests [42,43]. The increase can also be linked to higher amounts of aboveground and belowground litter input in older stands [11,44]. Similar to our findings, several studies also observed relatively low levels of soil C when Chinese fir is usually cut (20–25 years) and thus suggested prolonging the rotation length to maintain soil nutrient status for successive plantings [4,26,45].

4.2. Effect of Stand Age on Microbial Communities and Enzyme Activities

Many studies have found that the microbial biomass first declined and then increased with the growth of Chinese fir [20,21]. This trend can be explained by the positive correlation between total PLFA abundance and SOC content [29,46,47], which was also observed in this study (Table 4). However, contrary to our hypothesis, microbial biomass showed no significant variations at different developmental stages (Figure 2). Mackay et al. and Smith et al. also found this phenomenon in mixed-species forests [14,48]; Yang et al. found that total PLFA abundance significantly changed only in the first 2 or 4 years in artificially planted *Sonneratia apetala* and *Sonneratia caseolaris* stands, pointing out that microbial biomass might have reached a stable state in the first few years [49]. AMF plays an important role in improving soil fertility [12,50], and Luo et al. found the highest amounts of AMF in the oldest *Picea asperata* plantations (50-year) [51]. Interestingly, AMF abundances were significantly highest in 25-year stands and then decreased in 45-year ones in our study, probably because young trees have more developed fine roots than older trees [52,53].

Although there were no notable differences in the absolute abundances of PLFA biomarkers across the four stand ages, the ratios of PLFA biomarkers significantly differed among stands (Figure 3). Consistent with the study of Yang et al. [52] and contrary to the result of Cavagnaro et al. [54], we found that the F/B ratio significantly decreased in older stands, suggesting a lower soil nutritional stress with tree growth, as bacteria tend to accumulate when soil nutrients are enough while a shift towards fungal dominance will occur when soil nutrient contents decline [55,56]. In addition, the same as the findings of Liu et al. [24], we also found decreased G+/G− ratio with increasing stand age, indicating accumulation of easily-decomposable substrates in soils [29,50]. Both ratios implied a continuous improvement of soil nutritional status over the 45 years. Thus, the rotation time should be over 25 years to relieve the soil degradation of Chinese fir. Furthermore, although the absolute abundances of PLFAs did not exhibit changes across the stands, the F/B and G+/G− ratios could still be used as sensitive parameters of microbial responses to soil fertility changes [36,57], confirming our hypothesis that the microbial communities changed with increasing stand age.

In our study, the phosphatase and β -glucosidase activities were highest in 45-year stands (Figure 4). Wang et al. also demonstrated higher enzyme activities in 49-year Chinese fir plantations [11]. Surprisingly, the highest urease activities were found in the 15-year stands, not in the 45-year ones, which was not in line with Kang et al. who reported a continuous increase of urease activity from 15 years to 35 years in dawn redwood plantations [17]. The reasons remain unknown. Our finding that insignificant changes of microbial PLFAs accompanied by notable variations in soil enzyme activities in different developmental stages was consistent with that of Yang et al. [49], possibly suggesting that not all microbes participate in nutrient metabolism.

4.3. Relationships Between Physicochemical Properties and Microbial Communities

Both the results of Pearson correlation and RDA demonstrated that net N mineralization was significantly associated with microbial communities (Table 4, Figure 5). The significant links between net nitrogen mineralization rate and microbial community structure are not surprising given that changes in soil microbial community have a close correlation with soil N cycling [58] and N mineralization in soil is a result of microbial activity [59]. However, inconsistent with Wu et al. and Wan et al. who both mentioned that soil C/N ratio was linked with soil biological indicators of Chinese fir forests [55,60], we did not observe obvious correlations between C/N ratio and biological variables. This result might be explained by the small range of C/N ratio (11.94–12.62) compared to other studies on Chinese fir [48]. For example, Zhang et al. and Wang et al. reported that the C/N ratio ranged from 8 to 11 and from 12 to 18, respectively [11,40].

5. Conclusions

In this study, soil physicochemical and biological properties varied with planting age of Chinese fir. Although no significant changes were found for the absolute abundances of PLFAs, the F/B and G+/G− ratios significantly differed across the four stand ages. We, thus, suggest that these ratios might be used as sensitive parameters of microbiome responses to soil changes with stand age. Furthermore, we found that soil chemical and biological variables were generally lower in 25-year stands compared to those in 45-year stands. Therefore, it is recommended that the rotation period should be prolonged to over 25 years, but the exact cutting age of Chinese fir needs further study to achieve a balance between ecological and economic benefits.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/8/629/s1>, Table S1. ANOVA results of the effects of stand ages.

Author Contributions: H.K. and Q.M. designed research; H.K., Q.M., J.W., and W.Y. performed research; W.Y. and Q.M. analyzed data; and Q.M., W.Y., and H.K. wrote the paper.

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Conflicts of Interest: The authors declare no conflict of interest.

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