



# Article Contrasting Effect of Thinning and Understory Removal on Soil Microbial Communities in a Subtropical Moso Bamboo Plantation

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Abstract: Thinning and understory clearance are among the two most popular forest management practices carried out to improve stand productivity in subtropical plantations. Unfortunately, studies have not fully explored the single and combination effect of thinning and understory clearance on soil microbial properties. By conducting a field manipulation experiment in a subtropical moso bamboo (Phyllostachys edulis) plantation in Southern China, we assessed the effects of thinning, understory clearance, and their combination on soil microbial phospholipid fatty acids (PLFAs) three years after treatments were first applied. We also examined the changes in soil properties after thinning and/or understory clearance. Thinning decreased soil fungal and bacterial PLFAs, and consequently soil total microbial PLFAs due to the increased soil  $NH_4^+$ -N, and  $NO_3^-N$  concentrations. Understory clearance decreased soil pH and soil water content resulting in increased soil fungal PLFAs and the ratio of soil fungal to bacterial (F:B). In addition, thinning and understory clearance caused apparent interactive effects on soil total microbial PLFAs and bacterial PLFAs, and the negative influence of thinning on soil total microbial and bacterial PLFAs were partly compensated by understory clearance. These results suggest the contrasting and interactive effect of thinning and understory clearance should be considered to assess the changes of soil microbial community and ecological processes in subtropical moso bamboo (Phyllostachys edulis) plantations in southern China.

**Keywords:** thinning; understory removal; soil microbial community; interactive effect; moso bamboo plantation

# 1. Introduction

Soil microbes play a vital role in maintaining ecosystem services and functions, including nutrient cycling [1], litter degradation [2], primary productivity [3], climate regulation [4], etc. Experiments conducted under controlled conditions suggest that soil microbial communities are generally sensitive to environmental changes, and it can be affected by forest management practices, such as fertilization [5], lime application [6], understory removal [1], thinning or clear-cut [7], and litter alternation [2], thereby influencing ecosystem functions and services through feedbacks to ecological processes. Lots of previous studies have explored mixed responses of soil microbial composition to forest management practice due to the different study region and/or forest types [1,6,8,9]. For example, Wan et al. (2019) reported that understory removal significantly decreased soil fungal PLFA and the ratio of soil fungal to bacterial in subtropical Eucalyptus plantation. While, Lei et al. (2021) explored the idea that understory clearance increased soil fungal PLFA in a subtropical Chinese fir plantation. Clearly, more experimental studies are still needed to assess the response of soil microbial community to forest management practices. In addition, management practices may be applied concurrently in forests and produce combination effects (facilitate, offset, and neutral) on soil microbes [6,10]. Accordingly, an in-depth knowing of the effect of forest artificial measures on soil microbial compositions



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). is valuable in both theoretical and practical for improving our knowledge of the mechanisms concerning soil C cycling and fertility in plantation ecosystems and enhancing the predictability sustainable forest management.

Thinning is a commonly applied plantation practice aiming to improve plantation productivity directly and indirectly [8,11]. The direct facilitate effects of thinning on plantations including reducing competition, optimizing microclimate, and increasing nutrient availability were highly focused [12–14]. However, the indirect beneficial effects driven by soil microorganisms were relatively less examined [7]. Studies related to the thinning affecting soil microbial community have provided conflicting observations. Lei et al. (2021) found that thinning increased soil fungal to bacterial ratio in a *Pinus massoniana* plantation [7]. Overby et al. (2015) explored that thinning inhibited the F:B ratio, but facilitated the abundance and diversity of arbuscular mycorrhizal fungi [13]. On the other hand, Li et al. (2019) found that thinning caused a negligible effect on soil microbial compositions [8]. Given that thinning may affect soil microbial communities, and lead to influence soil carbon cycling and nutrient availability [15,16], a better understanding of the effect of thinning on soil microbial communities would be helpful to optimize forest management practices.

Understory vegetation removal is a prevailing management practice in subtropical plantations for reducing competition with trees for water and nutrients [1,2,6]. Studies have explored the idea that understory plants can mediate soil microbial communities by maintaining the microclimate, nutrient cycling, and biodiversity in subtropical plantations [5,6]. However, the results of understory clearance affecting soil microbial compositions are usually context-dependent (depending on study region and/or forest type). Some findings have reported that understory clearance apparently altered soil microbial community [1,2], while others did not [17]. These observations suggest that the response of soil microbial composition to understory clearance needs to be further studied. Additionally, the management practice of understory removal was regularly simultaneously applied and produced interactive effects with other forest management practices, such as fertilization [10], lime application [6], litter alternation [2], and thinning [7]. For instance, Wan et al. (2021) found that understory clearance offset the stimulation effects on both soil fungal and bacterial biomass induced by litter addition in a subtropical Chinese fir plantation [2]. Nevertheless, the interactive effect between understory removal and thinning on soil microbial composition has not yet been fully explored.

Moso bamboo (*Phyllostachys edulis*), a valuable non-wood- and shoot-producing bamboo species having a fast growth rate, high productivity, short rotation time, and wide application, has been extensively planted across China and its neighboring countries [1,18]. Its planting area has exceeded 4.7 million hectares in southern China according to the ninth National Forest Resource Survey [9]. In these plantations, thinning and understory removal have increasingly been adopted to enhance timber production and the quality of moso bamboo. Therefore, the objective of the present study was to advance our knowledge of the single and combination effect of thinning and understory clearance on soil microbial compositions in a subtropical *Phyllostachys edulis* plantation. We hypothesize that: (1) thinning would stimulate soil microbial biomass and shift soil microbial community from bacterial to fungal, as thinning enhance soil carbon available for decomposition such as dead roots, which may facilitate the fungi growth as they preferentially utilize recalcitrant carbon [3]; and that (2) understory removal would offset the facilitate effects of thinning on soil microbial communities due to increase "bottom-up" limitation for microbial growth and activity [1].

#### 2. Materials and Methods

### 2.1. Field Site and Experimental Design

This study was conducted at the Dagang Experimental Forest Farm (28°37′ N, 114°56′ E) in Jiangxi Province, China (Figure 1). The climate in this region is overall subtropical, with the mean annual precipitation and air temperature are 1600 mm and 17.5 °C, respectively. The soil in this field study site is Ultisols according to the World Reference Base for soil

Resource (2006), which is developed from Quaternary Red Clay. The area of the farm is about 700 hm<sup>2</sup>, with about 470 hm<sup>2</sup> is moso bamboo plantation, with the density of about 3700 plants hm<sup>-2</sup>. The understory vegetation is dominated by *Rhanmus crenata*, *Maesa japonica* and *Callicarpa cathayana*, with some representatives of other species, notably *Ciraea cordata* Royle, *Woodwardia japonica*, and *Broussonetiapapyrifera*.



Figure 1. The geographical location of moso bamboo plantation in this experiment.

The field manipulation was conducted in September of 2015, with four plots ( $20 \text{ m} \times 20 \text{ m}$ ) located within each of four moso bamboo plantation replicates were randomly appointed to one of four treatments: CK (no treatment or control), UR (understory removal), Th (thinning), and TUR (understory clearance and thinning). In understory removal treatments (UR and TUR), both aboveground and belowground components were manually cleared by harvesting hooks prior to the experiment, and germinated plants were removed physically every month during the experimental period. In thinning treatments (Th and TUR), about 35 bamboos were removed from each plot, with the remaining bamboos were comprised by 40% bamboos aged from one to four years, 40% bamboos aged from five to six years, and 30% bamboos aged from seven to eight years, and corresponding to 25% thinning intensity. Each treatment plot was embedded with PVC (polyvinyl chloride) boards to block interference from the outside plot.

#### 2.2. Soil Sampling and Measurement

Soils were sampled in Feb, May, and Aug three years after the treatments were first applied, with any surface organic matter was cleared away before sampling. Each treatment plot was randomly sampled nine times at a depth of 0–5 cm by a soil corer with diameter at 2.5 cm. All soil samples collected within each plot combined, cleared of visible plant litter and roots, sieved (2 mm), and then portioned into two subsamples. Of these, one subsample destined for soil physico-chemical measurement was stored at 4 °C, and the other one was stored at -20 °C for phospholipid fatty acid (PLFA) measurement.

Soil pH was measured using a combination glass electrode immersed in a 1:2.5 soil-water slurry. Soil water content was determined by oven-drying for 48 h at 105 °C. Soil  $NO_3^-$ -N and  $NH_4^+$ -N were extracted and determined by a flow injection auto-analyzer (FIA, Lachat Instruments, Milwaukee, WI, USA). Soil available P (AP) was measured by the

molybdate blue method [19]. Soil organic C (SOC) was measured according to the  $H_2SO_4$ - $K_2CrO_7$  oxidation method. Soil total N (TN) and P (TP) contents were determined by a continuous-flow autoanalyzer (Auto Analyzer III, Bran + Luebbe GmbH, Cologne, Germany). The phospholipid fatty acid (PLFAs) method described by Bossio and Scow. (1998) was employed to assay soil microbial community [20]. The 19:0 internal reference concentration was used to standardized the each PLFA concentration and microbial biomass. The concentration of biomarker 18:2 $\omega$ 6,9c was appointed to indicate soil fungal biomass, and the concentrations of the following PLFAs: cy17:0, a17:0, 16:1 $\omega$ 5c, i15:0, a15:0, i17:0,14:0, 15:0, i16:0, 17:0, and 16:1 $\omega$ 7 were considered as soil bacterial biomass [2,21]. Soil microbial community was indicated by the ratio of fungal to bacterial PLFAs (F:B). Total microbial PLFAs is the sum of soil fungal and bacterial. All PLFAs were expressed as ng g<sup>-1</sup> dry soil.

#### 2.3. Statistical Analysis

In the present study, all data met the homogeneity and normality of variance according to the formal normality test. Two-way repeated measurement analysis of variance was applied to examine the effect of sampling time, treatments, and their interactions on soil microbial biomass and community, and soil physico-chemical properties throughout the experimental period. A one-way analysis of variance (ANOVA) and least significant difference tests (LSD) were used to performed differences among treatments within a given sampling time. All of the analysis and the least significant difference were performed in SPSS 18.0 (SPSS Inc., Chicago, IL, USA), and the statistical significance was determined at  $p \le 0.05$ . Relationships between soil properties and microbial compositions were determined by a redundancy analysis (RDA). The "forward selection" based on the Monte Carlo permutation (n = 499) was conducted to select the most discriminating soil property variables. RDA was performed with the CANOCO 4.5 software (Ithaca, NY, USA).

#### 3. Results

#### 3.1. Effects of Thinning and Understory Removal on Soil Physico-Chemical Properties

The statistical results in the present section are based on a two-way repeated measure ANOVAs. In addition to soil pH, sampling time produced apparent effects on soil physicochemical characters (Table 1). Thinning enhanced in soil  $NH_4^+$ -N and  $NO_3^-N$  contents, as well as TN content, but did not produce significant effects on SWC, soil pH, AP, TP, SOC, and soil C:N, C:P, N:P ratios (Figure 2, Table 1). Understory removal increased soil AP and TN contents, while caused a decline in SWC, TP, pH, and soil C:N ratio (Figure 2, Table 1). In addition, thinning and understory removal caused apparently interactive effects on soil AP and  $NO_3^-$ -N contents (Table 1).

**Table 1.** Effects of time, thinning, understory clearance, and their combination on soil properties at 0–5 cm depth.

Treatments	SWC	AP	$NH_4^+$	TN	ТР	NO <sub>3</sub> -	SOC	pН	C:N	C:P	N:P
Time	< 0.01	< 0.01	< 0.01	0.011	0.025	0.025	< 0.01	0.123	0.016	0.011	0.011
Th	0.268	0.194	0.028	< 0.01	0.067	0.035	0.107	0.185	0.189	0.508	0.081
UR	< 0.01	0.014	0.076	0.05	< 0.01	0.079	0.331	0.019	0.011	0.121	0.078
$\mathrm{Th}  imes \mathrm{UR}$	0.317	0.011	0.054	0.067	0.919	0.043	0.531	0.139	0.387	0.484	0.859

Note: Time, sampling time; Th, thinning; UR, understory removal; Th×UR, interaction effect of thinning and understory clearance. SWC (%), soil water content; AP (mg/kg), soil available phosphorus;  $NH_4^+$  (mg/kg), soil ammonium content; TN (g/kg), soil total nitrogen; TP (g/kg), soil total phosphorus;  $NO_3^-$  (mg/kg), soil nitrate content; SOC (g/kg), soil organic carbon; pH, soil pH; C:N, C:P, N:P stand for stoichiometric ratios among soil organic carbon, total nitrogen, and total phosphorus, respectively. Results are from a two-way repeated measure ANOVA, sampling time (levels: February 2018, May 2018, and August 2018), thinning (levels: thinning, not thinning), and understory removal (levels: understory removed, understory plants intact). Values in table are statistical significance.



**Figure 2.** Soil characters at 0–5 cm depth as affected by thinning, understory clearance, and their combination. (a) Soil water; (b) soil available phosphorus; (c) soil  $NH_4^+-N$ ; (d) soil total nitrogen; (e) soil total phosphorus; (f) soil  $NO_3^--N$ . Values are means  $\pm$  SE, n = 4. Statistical results are from a two-way repeated measurement of variance.

# 3.2. Effects of Thinning and Understory Removal on Soil Microbial Community

Soil total microbial biomass, fungal biomass, and bacterial biomass as indicated by total PLFAs, fungal PLFAs, and bacterial PLFAs were ranged from 923 to 5123 ng  $g^{-1}$ , from 125 to 838 ng  $g^{-1}$ , and from 786 to 4346 ng  $g^{-1}$ , respectively (Figure 3). Soil sampling time caused significantly affects soil total microbial, soil fungal, and soil bacterial PLFAs, as well as the F:B ratio (Figure 3, Table 2). During the experimental period, thinning significantly decreased soil fungal and bacterial PLFAs, consequently the total PLFAs, although the F:B ratio remain unchanged (Figure 3, Table 2). In contrast, understory clearance significantly enhanced fungal PLFAs and the F:B ratio (Figure 3, Table 2). In addition, thinning and understory clearance produced significantly combination effects on total and bacterial PLFAs (Figure 3, Table 2). Specifically, both total (Figure 3a) and bacterial PLFAs (Figure 3c)



were significantly lower in the UR treatment comparing to the CK treatment, while they were higher in the TUR treatment comparing to the Th treatment.

**Figure 3.** Soil microbial biomass and community (PLFAs profile) as affected by thinning, understory clearance, and their combination among the sampling time. (a) Soil total microbial PLFAs; (b) soil fungal PLFAs; (c) soil bacterial PLFAs; (d) the ratio of soil fungal PLFAs to bacterial PLFAs. Values are means  $\pm$  SE, n = 4. values with different lowercase letters are significantly different (p < 0.05) among treatment in each sampling event by LSD test. Values with different uppercase letters are significantly different (p < 0.05) among sampling event by LSD test.

**Table 2.** Effects of sampling time, thinning, understory clearance, and their interactions on soilmicrobial PLFAs. See Table 1 for abbreviations notes. Values in table are statistical significance.

Treatments	<b>Total PLFAs</b>	Fungal PLFAs	<b>Bacterial PLFAs</b>	F:B	
Time	< 0.01	< 0.01	< 0.01	< 0.01	
Th	< 0.01	< 0.01	< 0.01	0.157	
UR	0.875	< 0.01	0.277	< 0.01	
$\mathrm{Th} \times \mathrm{UR}$	0.022	0.288	0.02	0.015	

3.3. Relationship between Soil Microbial Community and Physico-Chemical Properties

Results from redundancy analysis indicated that soil NH<sub>4</sub><sup>+</sup>-N (p < 0.01), SWC (p < 0.05), soil NO<sub>3</sub><sup>-</sup>-N (p = 0.04), and soil pH (p = 0.05) were apparently correlated with soil PLFA profiles (Figure 4, Table S2). In specific, soil pH, SWC, and soil NO<sub>3</sub><sup>-</sup>-N were correlated negatively with biomarker 18:2 $\omega$ 6,9c, while correlated positively with biomarker i14:0, i15:0,i16:0, and cy17:0 (Figure 4). Soil NH<sub>4</sub><sup>+</sup>-N was correlated positively with biomarker cy17:0, 16:1 $\omega$ 7c, and 16:1 $\omega$ 5, while correlated negatively with biomarker i15:0, i17:0, a15:0, and a17:0 (Figure 4). The variance of soil microbial community was totally explained 92.8% by the environmental data, and the first axis contributed 84.2% and the other 8.6% variance (Figure 4).



**Figure 4.** RDA of soil microbial PLFA biomarkers. Ordination diagrams presenting species scores and environmental factor scores (vectors). See Table 1 for abbreviations notes.

### 4. Discussion

Our first hypothesis is that thinning would stimulate soil microbial biomass and shift soil microbial community from bacterial to fungal. Our results are inconsistent with the first hypothesis; thinning decreased both soil fungal and bacterial PLFAs, and consequently soil total PLFAs, although the F:B ratio remained unchanged in the present moso bamboo plantation (Table 2). This observation is in line with some other observations from *Pinus massoniana* and *Eucalyptus* plantations in subtropical China, confirming that thinning reduced soil microbial biomass and altered microbial composition in these ecosystems [7,22]. In a specific forest, thinning can affect soil microbial biomass and composition by reducing the input of substrates, changing microclimate, and nutrient availability [23–25]. Although the growth of soil microbial is generally controlled by soil carbon substrate and nutrient availability, it is well recognized that excessive inorganic nitrogen content might cause "toxicity or salt effects" on soil microbes, leading to the inhibition of the biomass of soil microbial and altering the soil microbial community [5,6,26]. Despite unchanged SWC, soil C, and P availability in the study, thinning caused a significant increase in soil  $NH_4^+$ -N and  $NO_3^-$ -N concentrations (Figure 2, Table 1) due to reduced competition [13]. Therefore, thinning decreased soil fungal and bacterial biomass, and the decreased soil fungal biomass most likely induced by the increased soil NO<sub>3</sub><sup>-</sup>-N concentration, as proved by the biomarker of  $18:2\omega 6.9c$  correlating negatively with soil NO<sub>3</sub><sup>-</sup>-N (Figure 4). In addition, the biomarkers of i15:0, i17:0, a15:0, and i17:0 prevailed the inhibited soil bacterial biomass with higher soil  $NH_4^+$ -N concentration in thinning soils, due to the fact that soil NH<sub>4</sub><sup>+</sup>-N was significantly negatively correlated with these biomarkers (Figure 4). Consistent with the findings, observations from Wu et al. (2011) and Wan et al. (2019) also explored that higher inorganic N availability suppress soil microbial biomass [6,27]. Moreover, soil fungal and bacterial differ in their resource utilization, it has been previously shown that soil bacteria generally need more N and fungal require more C for unit biomass accumulation [28,29]. Therefore, thinning had a negligible effect on the F:B ratio in the present study might attribute to the unchanged soil C:N, N:P, and C:P ratios (Table 1 and Table S1). Considering that soil microorganism is the main driver of litter decomposition, and decreased soil fungal and bacterial biomass would suppress the litter decomposition rate as previous studies revealed [1,2], our observations suggest

that thinning exerts a high degree of control on soil nutrient availability (i.e., soil inorganic N concentration), which further inhibit soil microbial biomass and, consequently, soil C cycling and forest productivity in this moso bamboo plantation.

Understory removal increased soil fungal PLFAs and the F:B ratio, although this ratio remained unchanged in soil bacterial PLFAs of this moso bamboo plantation (Figure 3, Table 2). However, most previous studies have observed negative or neutral effects on soil microbial biomass in temperate and subtropical plantations [1,2,7,29,30]. These inconsistent observations confirm the highly controls of understory vegetation on soil microbial biomass and composition, but the status is context-dependent (e.g., forest types, treatment duration, diversity, and biomass of the understory plants) [1,2,27]. As most soil fungi are moderately acidophilic, the activities of soil fungi would be stimulated and the composition of soil microbial might transfer from bacterial prevailed to fungi prevailed with the decrease of soil pH [6,31]. Therefore, understory clearance stimulated soil fungal would be partly due to the lowered soil pH, and soil pH negatively correlated with  $18:2\omega 6.9c$  in this study further supported this assumption (Figure 4). In addition, considering that SWC and soil pH are two significant factors affecting soil microbial composition, the potential explanations for the unchanged soil bacterial PLFAs are twofold and comparable to those former provided. Firstly, decreased soil water content suppressed the effective soil matrix diffusion and caused a "bottom-up" regulation for soil bacterial [7]. Secondly, some group of bacteria can be promoted in growth and activity, while other groups of bacteria can be suppressed in growth and activity under lower soil pH [31]. These findings propose that understory clearance changed soil pH and SWC, and consequently affected microbial biomass and community, and reduced litter decomposition rate as our published study reported in the present subtropical moso bamboo plantation [1].

Our second hypothesis is that understory removal would offset the facilitate effects of thinning on soil microbial communities due to increase "bottom-up" limitation for microbial growth and activity. Partly consistent with the second hypothesis, the interaction between thinning and understory clearance had an apparent effect on soil microbial PLFAs. In the present study, the UR treatment reduced soil total microbial and bacterial PLFAs, while the UR treatment enhanced soil total microbial and bacterial PLFAs under the treatment of thinning, as soil total microbial and bacterial PLFAs are apparently higher in the TUR treatment than in the Th treatment (Figure 3, Table 2), indicating that understory removal offset the suppress effects of thinning on soil microbial biomass. Indeed, the UR treatment generally decreased soil inorganic N concentration related to the CK, and we observed significantly lower soil  $NO_3^-$ -N and  $NH_4^+$ -N concentrations in the TUR treatment than in the Th treatment (Figure 2 and Table S1). Thus, thinning-induced increases in inorganic nitrogen would compensate after understory removal. Considering subtropical plantations often have been undergoing thinning and understory clearance [1,2,7], our observations propose that the interactive effect of these practice factors should be fully considered to understand and predict the changes of soil microbial community in these ecosystems.

#### 5. Conclusions

In conclusion, soil microbial biomass and composition were affected by both thinning and understory removal, as well as their combination. Thinning decreased soil fungal and bacterial biomasses, and consequently soil total microbial biomass. Understory removal enhanced soil fungal PLFAs and the F:B ratio in subtropical moso bamboo plantations. Moreover, the combination between thinning and understory removal caused a significantly effect on soil total microbial biomass and bacterial biomass, and understory removal offsets the suppressed effects of thinning on soil microbial biomass. These findings highlight that the interaction between thinning and understory removal should be considered to assess the changes in soil microbial biomass and composition. Considering the vital roles of soil microbial in driving soil C cycling and soil fertility, our findings will improve the predictability of the effect of forest management measures on ecosystem structure and function in subtropical moso bamboo plantations. **Supplementary Materials:** The following supporting information can be downloaded at: https://www. mdpi.com/article/10.3390/f13101574/s1, Table S1: Soil physic-chemical properties at 0–5 cm depth in February, May, and August in 2018 as affected by thinning, understory removal, and their interaction; Table S2: Statistical significance and variance explained by the variables selected according to the redundancy analysis (RDA). The statistical significance was determined at  $p \le 0.05$ .

**Author Contributions:** S.W. designed the experiment. Y.X., J.X., B.Z. and K.L. performed the study data collection. Y.X., J.X. and L.Z. analyzed the results. S.W. and J.L. reviewed the article. S.W. and Y.X. wrote the final article. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict interest.

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