

Article

# Long-Term Thinning Does not Significantly Affect Soil Water-Stable Aggregates and Diversity of Bacteria and Fungi in Chinese Fir (*Cunninghamia lanceolata*) Plantations in Eastern China

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Abstract: Soil structure and microbial communities are sensitive to forest disturbance. However, little is known about the long-term effects of forest thinning on water-stable aggregates (WSA), and the community composition and diversity of soil microorganisms. In this study, we investigated soil chemical properties, WSA, and communities of bacteria and fungi in conventionally managed Chinese fir plantation stands and repeatedly thinned plantation stands with medium and high tree densities 18 years after the thinning treatments. The distribution patterns of WSA fractions were similar in the three thinning treatments. The mass proportion was the highest in the macro-aggregates fraction, followed by the clay + silt fraction, and it was the lowest in the micro-aggregates fraction. The soil organic carbon (SOC) concentrations in different WSA fractions decreased with decreasing aggregate size. The WSA fractions, stability, and aggregate-associated carbon were not significantly different among the three treatments 18 years after the thinning treatments. The total nitrogen concentration of the macro-aggregates fraction was significantly higher in the stands thinned intensively than in the conventionally managed stands. The abundance of minor bacteria and fungi species was different, although no significant differences were observed in the overall bacterial and fungal composition and diversity between the three treatments. Our results indicate that, compared with the conventionally managed stands, soil WSA stability and soil microbial communities in repeatedly thinned Chinese fir stands may recover over one rotation of Chinese fir plantation and that this is accompanied by the recovery of stand growth and soil nutrition.

**Keywords:** thinning; soil water-stable aggregate; soil organic carbon; soil nitrogen; bacterial diversity; fungal diversity

## 1. Introduction

Forest management plays a key role in improving forest production and stand quality [1]. Thinning reduces tree densities and enhances growth of the remaining trees and understory vegetation [2]. Changes in stand structure strongly influence abiotic and biotic factors both aboveground and belowground within modified stands, i.e., aboveground and belowground productivity, root density, total radiation, and litterfall [3–5]. Such changes lead to modifications in soil physical, chemical, and biological properties [6,7]. Some studies have shown that thinning increases soil temperature [8] and soil bulk density [9], and decreases the carbon stocks [10], soil



nutrient contents [7], and cellulase and phenol oxidase activities [11]. Other studies have shown that thinning has a negligible [12,13] or positive effect on soil properties [6,14,15]. The discrepancy appears to relate to thinning intensity, forest ecosystem type, soil type, and time elapsed between thinning and the beginning of measurements [5]. Additionally, most studies are based on short-term experimental designs. In contrast, limited information is available on the effects of repeated thinning on soil properties, over a long period [2].

Soil aggregates, which are composed of different sized soil particles and organic and inorganic materials, are an important parameter used to evaluate soil structure. In particular, water-stable aggregates (WSA) are closely related to soil susceptibility to erosion [16], plant growth [17], and soil organic carbon (SOC) storage [18–20]. Macro-aggregates (>250  $\mu$ m) are formed by micro-aggregates (53–250 µm) bonded by root and fungal hyphae [21] and are considered more sensitive to changes in different management practices [22]. Micro-aggregates are formed by the interaction of clay particles with organic compounds and inorganic cementing materials [23], and they have less variation than macro-aggregates [24]. Physical protection of SOC by soil aggregates is considered an important mechanism to sustain carbon stability in soil, because it inhibits microbial access to SOC [25]. Previous studies have reported changes in soil aggregate fractions and their SOC content under different management practices [26–28]. However, most of these studies focused on agricultural systems, and the effects of forest management practices, such as thinning, on soil aggregate fractions and aggregate-associated carbon and nitrogen are less known. Soil compaction is a common problem associated with forest harvest operations, as it affects soil properties, especially soil structure. Page-Dumroese et al. [29] summarized the literature on effects of thinning harvests in the Western United States. They found that thinning is less likely to lead to marked soil compaction compared with that by clear-cutting, and the effects of mechanical operations on soil physical properties depend on the harvest method, operation technique, soil texture, soil condition and properties, and possibly other factors. Limited studies have reported the effects of harvest (including partial harvest, e.g., thinning) on soil aggregates [30,31]. Therefore, to evaluate the influence of a thinning operation on changes in soil structure and soil carbon and nitrogen content, it is important to study the soil aggregate fractions and aggregate-associated carbon and nitrogen following thinning.

Soil microorganisms are the main decomposers in forest systems. Fungi and bacteria account for 80%–90% of all the decomposition activity in the soil [19] and play crucial roles in nutrient dynamics and the carbon cycle of forest ecosystems [20]. Soil microbes are sensitive to soil disturbance and forest management practices [21]. Several studies have shown that thinning alters soil microbial communities and structure [22,23]. Thinning reduces tree density and canopy cover, provides more light for the understory, and increases microbial populations [32,33]. Microbial populations affect ecosystem processes and plant populations [34]. Following tree thinning, an increase in litterfall decomposition and nitrogen mineralization rates is related to the increase in soil temperature, available soil moisture, and soil microbial activity [35,36]. To date, most studies have focused on short-term responses following thinning treatments. The long-term (>10 years) effects of forest harvest on soil fungal and bacterial composition and diversity have rarely been studied [37]. Recently, a study reported that the creation of clear-cut openings in forests may increase the abundance and richness of AM fungal propagules, and soil bacterial communities were resilient 40 years after the thinning treatments [2].

Previous studies have indicated that fungi play a dominant role in WSA formation and stability [38,39] by producing polysaccharides [40] and enmeshing soil particles [41], and the role of bacteria is mostly related to polysaccharide production [42]. Some studies have focused on the role of specific soil microorganisms in aggregate formation and stability [43,44]. Little information is available on how microbial diversity is influenced by soil aggregates. Kihara et al. [45] reported that bacterial diversity decreased with increasing soil content of free silt + clay, and fungal diversity negatively correlated with the soil macro-aggregates in tillage and cropping systems in semi-arid

Kenya. Therefore, studying the changes in microbial communities following thinning could help us understand soil WSA and SOC stability.

Chinese fir is native to China, Northern Vietnam, and Laos, and is one of the most important timber species in Southern China [46]. Chinese fir is a fast-growing tree species that accounts for 16% of all plantations in China, covering approximately 12 million ha [47]. A number of studies have mainly focused on Chinese fir growth and timber production [46,48,49]. Several studies have reported the effect of thinning on SOC stocks and soil properties in the top soil (0–20 cm) [6,50]. However, information on the long-term effects of thinning on WSA and soil microbial diversity is limited. Therefore, the objective of this study was to evaluate how different thinning treatments affect WSA, aggregate-associated carbon and nitrogen, and soil microbial diversity and composition in Chinese fir stands. We hypothesized that (1) long-term thinning has a negligible effect on the WSA composition and aggregate-associated carbon and nitrogen during one rotation period (approximately 25 years) and (2) soil bacterial and fungal community diversity and structure might respond differently to thinning during one rotation period.

#### 2. Materials and Methods

#### 2.1. Study Site

The study was performed at the Kaihua Forest Farm (118°25′ E, 29°09′ N, mean altitude 234 m a.s.l.), which is located in the southwest of Zhejiang Province, China. The forest coverage rate of Kaihua County was 80.54% in 2013, and the plantation area of Chinese fir was over 90,000 ha, covering almost 50% of the total forest area. This study area has a subtropical marine monsoon climate. The mean annual temperature is 16.4 °C and the mean annual precipitation is 1814 mm. The slope of the experimental area was approximately 20% in the planted stands, and all plots had a northeastern aspect. The soil type is silt loam with a pH of 4.0–4.9.

#### 2.2. Experimental Design

The experimental stands were planted in 1991 with one-year-old Chinese fir seedlings, and the initial stand density was 3000 trees ha<sup>-1</sup>. The thinning experiment was randomly designed using three blocks. Each block included three thinning plots (20 m × 20 m) with the following treatments: conventional management model (as a control treatment), moderate thinning (MT), and heavy thinning (HT) treatments. The control treatment included a single, light thinning treatment (stand density reduced by approximately 15%), which was carried out approximately in the middle (the 14th year) of the rotation period (approximately 25 years). The MT treatment plots were thinned in the seventh year (stand density reduced by 20%) and the 14th year (stand density reduced by 16%). The HT treatments plots were also thinned in the seventh year (stand density reduced by 18%). In each plot, the trees were marked with red paint for thinning and then cut using a chainsaw. The branches of downed trees were removed, and randomly scattered in the thinned stands. The felled logs were transported by manual skidding, and no skid trails were created for thinning the stands.

#### 2.3. Vegetation Measurements

The tree height, diameter at breast height (DBH), canopy coverage, volume, floor biomass, and understory vegetation biomass of each plot were measured in November 2015. The canopy coverage of each plot was measured using Tracing Radiation and Architecture of Canopies (TRAC; Third Wave Engineering Inc., Nepean, Ontario, Canada). Six 1 m  $\times$  1 m subplots were randomly selected within each plot, the understory vegetation was harvested, and all litterfall on the ground of each subplot was collected. Dry biomass of the understory vegetation and litterfall samples was calculated by drying the samples at 70 °C for 48 h, and then estimated as total dry biomass per unit area. The concentration of litterfall total organic carbon (TOC) was measured using the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> method and

the content of total nitrogen (TN) was determined using the Kjeldahl digestion method [51]. The basic stand characteristics of each stand are shown in Table 1.

**Table 1.** Stand characteristics of the three thinning treatments; Control: conventional management model, MT = moderate thinning, HT = heavy thinning, TOC = total organic carbon, SOC = soil organic carbon, TN = total nitrogen; n = 3, data are shown as mean  $\pm$  standard deviation (SD). Different lowercase letters indicate significant differences among the three treatments (p < 0.05).

Treatments	Control	MT	HT
Stem density (tree $ha^{-1}$ )	$1960\pm107~^{\rm a}$	$1530\pm81~^{\rm b}$	$1334\pm88~^{\rm c}$
Canopy coverage (%)	$98.5\pm1.3$	$98.1 \pm 1.7$	$97.8 \pm 1.9$
Height (m)	$17.5\pm0.6$	$18.0\pm0.7$	$18.6\pm0.5$
Diameter at breast height (cm)	$20.3\pm0.5$ <sup>b</sup>	$22.5\pm0.8~^{\rm a}$	$23.5\pm0.8~^{a}$
Volume (m <sup>3</sup> ha <sup><math>-1</math></sup> )	$562.4\pm31.7$	$547.2\pm28.6$	$531.8\pm33.9$
Forest floor biomass (t ha <sup><math>-1</math></sup> )	$7.35 \pm 1.28$	$7.83 \pm 1.62$	$8.06\pm0.84$
Understory vegetation biomass (t ha $^{-1}$ )	$1.41\pm0.47$	$1.55\pm0.72$	$1.72\pm0.40$

## 2.4. Soil Sampling and Measurements

In each plot, soil samples were collected randomly from six points (0–20 cm), after removing the surface litter before sampling. The soil cores in each plot were mixed as one sample. Each sample was divided into three sub-samples. One sub-sample of fresh soil was used to analyze the soil microbial community, another sub-sample was used to determine WSA fractions, and the third sub-sample was used to determine SOC and total nitrogen concentrations and pH. SOC was measured using the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> method. Total nitrogen was determined using the Kjeldahl digestion method. Soil pH was determined in a 1:2.5 (soil: water) soil dilution using a glass electrode potentiometer (PHS-3C; Leici Instruments, Shanghai, China). For details on how we conducted these measurements, see Bao [51].

#### 2.5. WSA Fractions Separation

The WSA fractions were measured using the wet sieving method [12]. Two sieve sizes, i.e., 0.250 mm and 0.053 mm, were used to collect macro-aggregates (>0.250 mm), micro-aggregates (0.053 mm–0.250 mm), and clay + silt (<0.053 mm). Soil samples (50 g, passed through a 10 mm sieve) were placed on top of the 0.250 mm sieve, and the 0.053 mm sieve was nested below it. The two sieves were submerged in water for 30 min. The nested sieves were moved up and down within a column of water with a range of 4 cm (30 strokes min<sup>-1</sup>) for 30 min. Free floating particulate organic matter was removed during the process [52]. All WSA fractions were washed in different aluminum pans and dried at 45 °C until the sample reached a constant mass. The mean weight diameter (MWD) of the soil aggregate fractions was determined using the method described by Le Guillou et al. [53].

#### 2.6. Soil Microbial Community Measurements

Total DNA was extracted from fresh soil samples (passed through a 2 mm sieve) using a MO BIO Power Soil<sup>®</sup> DNA Isolation Kit (MO BIO, Laboratories, Carlsbad, CA, USA). The concentration of the extracted DNA was tested by Pico Green using a FLUO star OPTIMA fluorescence plate reader (BMG LABTECH, Jena, Germany). PCR amplification was performed in an ABI Gene Amp1 9700 type PCR machine (USA). For bacteria, a 16S rDNA gene fragment was amplified by PCR using the following primers: 338F (5'-GTGCCAGCMGCCGCGGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [54]. For fungi, an 18S rDNA gene fragment was amplified using the primers 0817F (5'-TTAGCATGGAATAATRRAATAGGA-3') and 1196R (5'-TCTGGACCTGGTGAGTTTCC-3') [55]. Three replicates of each DNA extract were used for the PCR amplification, and then combined into one PCR product, which was examined on 2% agarose gel and purified with an AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, Inc., Union, CA, USA). The products of purified PCR were examined using a Quanti Fluor TM-ST system (Promega Corporation, Madison, WI, USA) and the

equimolar mixtures of multiple amplicons were obtained. An MiSeq library was constructed, and the sequencing was processed on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) at Majorbio Bio-Pharm Technology, Co., Ltd., Shanghai, China.

#### 2.7. Data Analyses

Raw sequences were assigned to different sample libraries and were trimmed using Trimmomatic, with a minimum overlap of 10 base pairs (bp) [56]. The quality-filtered sequences were clustered into operational taxonomic units (OTUs) using the Usearch program (version 7.1) at a 97% similarity level [57]. Taxonomic assignment was performed using the Ribosomal Data-base Project (RDP) classifier [58], with a minimal 70% confidence estimate. The diversity of bacterial and fungal communities was assessed using the Chao1 index, Shannon index, and Simpson index based on the OTUs. The relative abundance was estimated by calculating the ratio of the gene copy numbers for each microbial population to the total community gene copy number (i.e., the sum of gene copy numbers for bacteria). The effects of thinning intensities on WSA fractions, aggregate-associated carbon, and bacterial and fungal diversity indices were examined by one-way analysis of variance (ANOVA) (after confirming that our data met the assumptions of homogeneity of variance and normal distribution of residuals) and a post hoc Tukey's test. We tested for differences in the bacterial and fungal community composition between these treatments with permutational multivariate ANOVA (PERMANOVA) using the 'adonis' function in the VEGAN package (v.2.4-1) with 999 permutations in R. The Bray-Curtis  $\beta$  diversity index was used in this test. The relationship between tree growth, soil properties, aggregate-associated carbon and nitrogen, and soil microbial diversity was tested by Pearson correlation in R. Hierarchically clustered heat-map analysis was conducted with Past 3 (v.3.18). The effects were significantly different if p < 0.05. All data were analyzed using R statistical software (R Core Team, v.3.3.2, 2015) and Past 3.

#### 3. Results

#### 3.1. Composition, SOC, and TN Concentration of WSA

The relative proportion of macro-aggregates showed an increasing trend with increased thinning, and the relative proportion of clay + silt decreased slightly with thinning intensity, although there were no statistically significant differences among the control, MT, and HT treatments (p > 0.05) (Table 2). The pattern of distribution of the WSA fractions was similar among these treatments, and the proportion of macro-aggregates was higher than that of clay + silt, which was higher than that of micro-aggregates. There were also no significant differences in the MWD among the three treatments (p > 0.05).

**Table 2.** Relative proportion of water-stable aggregates (WSA) and mean weight diameter (MWD) between the treatments in the 0–20 cm soil layer; Control: conventional management model, MT = moderate thinning, and HT = heavy thinning; n = 3, mean  $\pm$  SD.

Treatment	>0.250 mm	0.053–0.250 mm	<0.053 mm	MWD
Control MT	$55.89 \pm 5.81$ $62.15 \pm 3.85$	$\begin{array}{c} 17.63 \pm 2.13 \\ 15.79 \pm 1.75 \end{array}$	$26.48 \pm 5.05$ $21.06 \pm 2.68$	$0.52 \pm 0.05 \\ 0.58 \pm 0.04$
HT	$63.53 \pm 5.46$	$17.27 \pm 1.82$	$19.20 \pm 4.84$	$0.58\pm0.05$

The SOC concentration in the WSA fractions decreased with decreasing aggregate size (Figure 1A). No significant differences in the SOC concentration in macro-aggregates, micro-aggregates, and clay + silt were observed among the control, MT, and HT treatments (p > 0.05). The pattern of the total nitrogen concentration in the WSA fractions was similar to that of the SOC concentration (Figure 1B). The total nitrogen concentration in macro-aggregates was significantly higher in the HT treatment

than that in the control treatment (p < 0.05), and there were no significant differences between the MT and control treatments (p > 0.05) (Figure 1B).



**Figure 1.** (**A**): SOC concentration in each aggregate size fraction between the treatments; (**B**): TN concentration in each aggregate size fraction between the treatments; Control: conventional management model, MT = moderate thinning, and HT = heavy thinning; n = 3, Figure shows means and standard error bars. Different lowercase letters above bars indicate significant differences among the three treatments (p < 0.05).

## 3.2. Diversity of the Soil Bacterial and Fungal Communities

The diversity of the soil bacterial community was assessed by high-throughput sequencing of the 16S rDNA gene, and the diversity of the soil fungal community was assessed using the 18S rDNA gene. After filtering out the low-quality reads, trimming the barcodes and primers, and removing the ambiguous bases and chimera, the total number of qualified reads was 371,013 for soil bacteria and 355,871 for soil fungi. Based on the OTUs at 97% similarity, the OTU number, Chao1 index, Shannon index, and Simpson index of soil bacterial and fungal communities were not significantly different among the control, MT, and HT treatments (p > 0.05) (Table 3).

Microbial Community	Treatment	OTUs	Chao1	Shannon	Simpson
Bacteria	Control MT HT	$825 \pm 58 \\ 818 \pm 52 \\ 884 \pm 61$	$916 \pm 77 \\ 949 \pm 71 \\ 985 \pm 52$	$\begin{array}{c} 5.40 \pm 0.23 \\ 5.29 \pm 0.31 \\ 5.45 \pm 0.27 \end{array}$	$\begin{array}{c} 0.010 \pm 0.004 \\ 0.014 \pm 0.007 \\ 0.011 \pm 0.003 \end{array}$
Fungi	Control MT HT	$217 \pm 74 \\ 253 \pm 67 \\ 230 \pm 30$	$218 \pm 75 \\ 259 \pm 64 \\ 255 \pm 37$	$\begin{array}{c} 3.24 \pm 0.47 \\ 3.20 \pm 0.42 \\ 2.80 \pm 0.19 \end{array}$	$\begin{array}{c} 0.104 \pm 0.036 \\ 0.098 \pm 0.026 \\ 0.109 \pm 0.017 \end{array}$

**Table 3.** Bacterial and fungal diversity indices in the three thinning treatments; Control: conventional management model, MT = moderate thinning, and HT = heavy thinning; n = 3, mean  $\pm$  SD.

## 3.3. Composition of the Soil Bacterial and Fungal Community

The composition of the bacterial community under different treatments was further analyzed. Twenty-two phyla were identified based on the OTUs of all the samples. In all treatments, Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, and Chloroflexi were the dominant phyla, and the relative abundance of these five bacterial phyla accounted for 88%–89% of the total bacterial community (Table 4). Other minor phyla (>1% for any treatment) included Planctomycetes (1.32%–2.48%), Verrucomicrobia (1.56%–2.01%), Bacteroidetes (2.63%–3.35%), Gemmatimonadetes (1.80%–2.14%), and Fusobacteria (0.50%–1.01%) (Table 4). Compared with that of the control treatment, the relative abundance of Actinobacteria was 33.9% higher (p < 0.05) and the relative abundance

of Planctomycetes was 43.7% lower in the HT treatment (p < 0.05). At the genus level (Figure A1), *Nitrosovibrio* was the most dominant genus in all the treatments, followed by *Holophaga*. Several genera such as *Acidisphaera, Burkholderia, Chitinophaga, Clostridium, Gemmata, Rhodothermus,* and *Roseiarcus* showed greater variation between the treatments, and the relative abundance of most of these genera was below 1%. Venn diagrams illustrating the overlapping and unique OTUs (at 97% similarity) between treatments are shown in Figure 2. The soil bacterial communities of the control, MT, and HT treatments shared 971 OTUs, which accounted for 82.8% of the total OTUs. The number of unique OTUs in the three bacterial communities was low; the proportion of unique OTUs of the control, MT, and HT treatments was 1.5%, 0.7%, and 2.7%, respectively (Figure 2A). The PERMANOVA analyses revealed that the composition of bacterial communities was not significantly different between the treatments (p = 0.783).

The composition of the fungal community was also explored in the three treatments. The fungus sequences in all treatments were affiliated with five phyla, namely Ascomycota, Basidiomycota, Glomeromycota, Entomophthoromycota, and Chytridiomycota (Table 4). The relative abundance of Ascomycota and Basidiomycota was 78%–86% and the relative abundance of the unclassified fungal community varied from 11% to 18%. Compared with that of the control treatment, the relative abundance of Ascomycota was 18.04% lower in the MT treatment, and it was 19.50% higher in the HT treatment; whereas the relative abundance of Basidiomycota was 71.9% higher in the MT treatment and 21.8% lower in the HT treatment. No rank and unclassified fungi also varied among the three treatments. At the genus level (Figure A2), the genera *Archaeorhizomyces* and *Cryptococcus* were dominant in all the treatments, and the former was the most dominant genus in these treatments. The soil fungal communities of the control, MT, and HT treatments shared 152 OTUs, which accounted for 22.4% of the total OTUs (Figure 2B). The number of unique OTUs increased with increasing thinning intensity. The control, MT, and HT treatments had 99 (14.6%), 107 (15.8%), and 142 (20.9%) unique OTUs, respectively (Figure 2B). PERMANOVA analyses revealed that the composition of fungal communities was not significantly different between the three treatments (p = 0.118).



**Figure 2.** The unique and overlapped OTUs detected in the different treatments for bacterial communities (**A**) and fungal communities (**B**); Control: conventional management model, MT: moderate thinning, and HT: heavy thinning.

**Table 4.** Relative abundance of bacterial (relative abundances  $\geq$  1%) and fungal communities at the phylum level; Control: conventional management model, MT = moderate thinning, and HT = heavy thinning; *n* = 3, mean  $\pm$  SD. Different lowercase letters above bars indicate significant differences among the three treatments (*p* < 0.05).

	Bacteria			Fungi						
Phylum	Control	MT	HT	Phylum	Control	MT	HT			
Acidobacteria (%)	$17.29\pm3.60$	$20.24\pm0.50$	$19.96 \pm 1.40$	Ascomycota (%)	$61.05 \pm 11.15$	$50.03 \pm 7.79$	$72.95 \pm 10.10$			
Actinobacteria (%)	$5.70\pm0.79~^{\rm b}$	$5.66\pm1.13$ <sup>b</sup>	$7.63\pm2.15$ $^{\rm a}$	Basidiomycota (%)	$17.04\pm4.67^{\text{ b}}$	$29.31\pm7.69~^{\rm a}$	$13.33\pm4.39$ <sup>b</sup>			
Bacteroidetes (%)	$3.14 \pm 1.17$	$2.63\pm0.61$	$3.35\pm0.32$	Chytridiomycota (%)	$0.04\pm0.03$	$0.06\pm0.03$	$0.02\pm0.01$			
Chloroflexi (%)	$11.85\pm5.56$	$9.70\pm3.01$	$9.75\pm7.17$	Entomophthoromycota (%)	$0.20\pm0.18$	$0.01\pm0.01$	$0.02\pm0.01$			
Firmicutes (%)	$12.86\pm8.81$	$17.90\pm11.56$	$8.96 \pm 4.36$	Fungi_norank (%)	$0.49\pm0.26$ <sup>b</sup>	$2.14 \pm 1.24$ a	$0.53\pm0.45$ <sup>b</sup>			
Fusobacteria (%)	$0.66\pm0.43$	$0.50\pm0.32$	$1.01\pm0.62$	Glomeromycota (%)	$3.36 \pm 1.56$	$3.63\pm2.07$	$2.59\pm3.65$			
Gemmatimonadetes (%)	$2.14\pm0.61$	$1.80\pm0.60$	$1.95\pm0.32$	Unclassified (%)	$17.81\pm8.74$	$14.83\pm 6.09$	$10.55\pm6.45$			
Planctomycetes (%)	$2.35\pm1.16~^{\rm a}$	$2.48\pm0.69$ <sup>a</sup>	$1.32\pm0.84$ <sup>b</sup>							
Proteobacteria (%)	$40.33\pm7.94$	$35.24 \pm 10.38$	$42.68\pm10.59$							
Verrucomicrobia (%)	$1.88\pm0.72$	$2.01\pm0.65$	$1.56\pm0.34$							
Others (%)	$1.80\pm0.45$	$1.84\pm0.59$	$1.83\pm0.26$							

#### 4. Discussion

## 4.1. WSA Change

Changes in soil structure influence the transportation and supply of water and oxygen to plants, and these changes are caused by different disturbances [59]. Forest thinning affects soil structure by soil compaction [29] and changes aboveground vegetation and belowground environments [30]. It is well-reported that the formation and stability of soil aggregates are primarily related to the roots, SOM, and soil microbial community (especially fungi) [60–62]. Mechanized harvest operations led to soil compaction, with a negative effect on aggregate stability, and WSA required a long time to recover after harvest operations in boreal forests [26]. In the current study, the WSA fractions and aggregate-associated carbon were not significantly different among the three thinning treatments. This finding suggests that WSA composition and aggregate-associated carbon recovered 18 years after thinning treatments. The smaller variation in WSA fractions might be related to the thinning operations carried out manually, leading to negligible soil disturbance compared with that by mechanized harvesting operations. Marchi et al. [63] also found that bulk density, total porosity, and WSA did not significantly change after thinning with vehicle-less methods in a pine plantation in Central Italy. However, Liu et al. [30] reported that the average WSA sizes increased with an increase in thinning intensities after ten years of treatment in Chinese fir plantations in Central China. This can be attributed to different stand characteristics and recovery times. In the study of Liu et al. [30], canopy coverage was reduced, and understory vegetation biomass and forest floor biomass were enhanced with an increase in thinning intensity, which indicated an increase in organic matter input into the soil, promoting an increase in WSA. In our study, the production or biomass of dominant tree and understory vegetation was not significantly different between the treatments, suggesting that root biomass was similar among these treatments. Additionally, the fact that there was little difference in aggregate-associated carbon and microbial diversity between the treatments also demonstrates that all treatments resulted in a similar organic matter input and microbial community structure. This result is partially consistent with our first hypothesis, and thinning had little effect on WSA composition and aggregate-associated carbon during one rotation period. However, limited information on the effect of thinning or clearcutting on WSA is available in the current literature. Further research is needed to explore the variations in WSA and aggregate-associated carbon following forest thinning.

The availability of soil nitrogen greatly influences the growth of trees and stand productivity. In this study, the concentration of soil total nitrogen in mineral soil and macro-aggregates increased with an increase in thinning intensity. This result was not consistent with our first hypothesis. Fang et al. [8] also reported that heavy thinning treatments increased annual net soil nitrogen accumulation in a lowland poplar (Populus deltoides) plantation in Eastern China three years after thinning. However, Bravo-Oviedo et al. [12] found that long-term thinning had no effect on soil nitrogen stocks in a Scots pine (Pinus sylvestris) forest in Southern Europe. The difference in results might be attributed to different tree species, thinning regimens, and soil environments. Aboveground vegetation is one of the primary sources of the soil nitrogen pool, and plant species affect nitrogen mineralization rates [64]. Some studies have shown that thinning increased the composition and biomass of the understory [47,65], which subsequently altered litter decomposition and nutrient status, and enhanced nitrogen input into soil [66]. Similarly, in the present study, we found that understory vegetation biomass, forest floor biomass, and nitrogen concentration associated with the thinning intensity had a positive correlation with soil nitrogen concentration (Table A1). Therefore, an increase in soil nitrogen concentration of the mineral soil and macro-aggregates in the heavily thinned stands was likely related to changes in understory vegetation after thinning treatments.

Forest disturbances alter the soil micro-environment, which may change soil microbial biomass and soil microbial community compositions. In the current study, the diversity and composition of bacteria and fungi showed no difference among the three treatments 18 years after thinning treatments. Overby et al. [2] also reported that the richness and abundance of soil bacterial communities, based on the phospholipid fatty acids method, were not affected 40 years after thinning treatment in a ponderosa pine (Pinus ponderosa) forest in the Southwestern United States, while the creation of clear-cut openings in forests increased the abundance and richness of AM fungal propagules. Several short-term studies have reported that soil microbial biomass and community structure are significantly altered one to five years after thinning treatments [18,65,67,68]. A recent meta-analysis of soil microbial biomass responses to forest disturbances indicated that partial harvesting (thinning) had no significant effect on soil bacterial and fungal biomass, while clear-cutting significantly reduced soil bacterial and fungal biomass [37]. Although no significant differences were observed in the bacterial and fungal composition with a PERMANOVA analysis of the Bray-Curtis  $\beta$  index, the effect of thinning might be greater on the structure of the fungal community than on the structure of the bacterial community, because the fungal community was more varied than the bacterial community. The proportion of overlapping total OTUs among the three treatments was lower in the fungal communities (22.4%) than that in the bacterial communities (82.8%) (Figure 2A,B). This finding partially supports our second hypothesis, and soil bacterial and fungal community diversity responded differently to thinning treatments. Fungi are considered the primary decomposers of dead plant biomass in terrestrial ecosystems [69]. Fungi can produce various extracellular enzymes to perforate plant cell walls, and they are assumed to be better decomposers of recalcitrant organic matter than bacteria [70]. In the study site, the proportion of recalcitrant organic carbon varied from 79.8% to 84.5% in the three treatments 15 years after thinning [14]. This might partially explain the relatively higher variation in the fungal community than in the bacterial community post-thinning. Previous studies indicated that the diversity and structure of soil microbial communities were markedly changed by clear-cutting disturbances after 10 or 40 years [2,71]. However, the disturbance intensity from partial harvesting (thinning) was lower than that of clear-cutting disturbance, which implies that soil microbial communities might need less time to recover from thinning than from clear-cutting.

Soil microbial communities are affected by many factors. They are highly sensitive to vegetation changes due to their close associations with plants [72]. In forest ecosystems, tree species are considered the "most important filter" for shaping the microbial community in soils surrounding roots [73]. Especially in plantations, monoculture management practices mean there is only one host species, which provides a single type of litter, which may also result in slight shifts in microbial communities [74]. In our study, we also found that canopy coverage and aboveground biomass of Chinese firs recovered after repeated thinning treatments, and we found no differences in forest floor biomass between treatments. Most of the tree growth parameters and soil properties had no significant correlation with soil bacterial and fungal diversity (Table A2). In a previous study, understory vegetation diversity increased temporarily following thinning, but diversity and biomass did not differ between the treatments over 15 years [75]. Additionally, minor differences in microbial communities between the treatments could also be attributed to similar soil structure and nutrient properties, especially SOC status, because the diversity and composition of the microbial community mainly depends on carbon availability [45,76]. These results indicate that bacterial and fungal communities recovered following repeated thinning, suggesting that there was no effect of the thinning practices on soil microbial community structures during one rotation of the Chinese fir plantation.

#### 4.3. WSA and Microbial Diversity And Composition

The WSAs are influenced by SOM and microbial activity, and they can protect SOC in aggregates from mineralization. Le Guillou et al. [53] reported that microorganisms might play a dual role (as producers and degraders) in regulating WSA dynamics, which depend on the quality of carbon substrate and availability of mineral nitrogen. The dynamics of WSA could be more strongly related to the microbial community structure than to the total microbial biomass. Tardy et al. [77] demonstrated that carbon dynamics can be explained by microbial diversity and that it depends on the quality of carbon substrates. In their study, the recalcitrance of carbon substrates affected the diversity of bacteria and fungi [77]. In the current study, WSA stability, aggregate-associated carbon, and microbial diversity and composition did not differ significantly between the thinning treatments. This indicates that the changes in WSA stability and aggregate-associated carbon following thinning might be accompanied by dynamic changes in soil microbial diversity and composition. The rate of recovery of soil properties from disturbance (e.g., thinning) may vary, depending on the degree of soil disturbance [37,78]. However, dynamic information on the recovery of soil properties after forest disturbance is scarce. Therefore, further studies on the WSA and microbial community structure in response to short-term and long-term thinning are needed.

# 5. Conclusions

In conclusion, minor changes were detected in the WSA fractions, stability, and aggregate-associated carbon in the repeated thinning stands with the recovery of aboveground trees and understory vegetation. Thinning was beneficial to soil nitrogen accumulation. Although the abundance of some bacteria or fungi species was different between the treatments, no difference in the overall bacterial and fungal community composition and diversity was observed between the treatments. This study demonstrates that WSA stability and soil microbial communities are resilient to repeated thinning in Chinese fir plantations.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

## Appendix A

	V	FFB	UVB	FTOC	FTN	SOC	STN	pН	MACC	MICC	CSC	MACN	MICN
FFB	0.012												
UVB	-0.037	0.954 **											
FTOC	-0.775 *	0.058	-0.019										
FTN	-0.776 *	0.271	0.381	0.698 *									
SOC	-0.484	0.043	0.095	0.009	0.230								
STN	-0.244	0.709 *	0.744 *	0.283	0.372	-0.218							
pН	0.009	0.121	0.219	-0.164	0.389	0.331	-0.330						
MACC	-0.159	-0.008	0.158	-0.364	0.093	0.836 **	-0.227	0.399					
MICC	-0.623	-0.013	0.183	0.191	0.586	0.709 *	0.095	0.314	0.766 *				
CSC	-0.361	-0.003	0.230	0.101	0.692 *	0.243	0.077	0.671 *	0.428	0.733 *			
MACN	-0.551	0.620	0.677 *	0.540	0.888 **	0.108	0.529	0.419	-0.018	0.339	0.529		
MICN	-0.398	0.630	0.611	0.385	0.302	-0.038	0.936 **	-0.497	-0.193	0.116	-0.109	0.419	
CSN	-0.085	0.309	0.241	0.047	0.274	0.285	-0.277	0.646	0.090	-0.111	0.064	0.488	-0.290

Table A1. Pearson correlation between tree growth and soil properties.

V = volume, UVB = understory vegetation biomass, FTOC = total organic carbon in forest floor, FTN = total nitrogen in forest floor, SOC = soil organic carbon, STN = soil total nitrogen, MACC = carbon concentration in the macro-aggregates, MICC = carbon concentration in the micro-aggregates, CSC = carbon concentration in the clay + silt, MACN = nitrogen concentration in the macro-aggregates, MICN = nitrogen concentration in the micro-aggregates, CSN = nitrogen concentration in the clay + silt. \* Significant correlation at 0.05 level; \*\* Significant correlation at 0.01 level.

	V	FFB	UVB	FTOC	FTN	SOC	STN	pН	MACC	MICC	CSC	MACN	MICN	CSN
OUT1	-0.674 *	-0.333	-0.251	0.487	0.311	0.283	0.232	-0.421	0.149	0.558	0.180	-0.061	0.389	-0.631
Chao1	-0.705 *	-0.219	-0.174	0.585	0.366	0.281	0.258	-0.424	0.062	0.424	0.032	0.036	0.426	-0.453
Shannon1	-0.349	-0.262	-0.212	0.350	0.153	0.128	0.185	-0.300	0.060	0.394	0.149	-0.193	0.261	-0.710 *
Simpson1	0.118	0.289	0.175	-0.187	-0.119	0.080	-0.161	0.245	-0.089	-0.391	-0.283	0.210	-0.111	0.800 **
OTU2	-0.391	0.044	0.056	0.496	0.421	-0.180	0.429	-0.015	-0.392	0.085	0.270	0.269	0.411	-0.294
Chao2	-0.516	0.129	0.170	0.554	0.590	-0.008	0.447	0.132	-0.219	0.292	0.441	0.425	0.421	-0.205
Shannon2	0.321	-0.362	-0.401	-0.171	-0.218	-0.408	-0.376	0.263	-0.472	-0.565	-0.090	-0.226	-0.454	0.201
Simpson2	0.209	0.138	0.212	-0.240	-0.136	-0.041	0.180	-0.238	0.308	0.271	0.096	-0.074	0.113	-0.357

**Table A2.** Pearson correlation between tree growth, soil properties, and soil microbial diversity.

V = volume, UVB = understory vegetation biomass, FTOC = total organic carbon in forest floor, FTN = total nitrogen in forest floor, SOC = soil organic carbon, STN = soil total nitrogen, MACC = carbon concentration in the macro-aggregates, MICC = carbon concentration in the micro-aggregates, CSC = carbon concentration in the clay + silt, MACN = nitrogen concentration in the macro-aggregates, MICN = nitrogen concentration in the micro-aggregates, CSN = nitrogen concentration in the clay + silt, 1 indicates the diversity of bacterial community, 2 indicates the diversity of fungal community. \* Significant correlation at 0.05 level; \*\* Significant correlation at 0.01 level.



**Figure A1.** Hierarchically clustered heat-map analysis of the bacterial communities (top 40) at the genus level. Control: conventional management model, MT = moderate thinning, and HT = heavy thinning, n = 3.



**Figure A2.** Hierarchically clustered heat-map analysis of the fungal communities (top 40) at the genus level. Control: conventional management model, MT = moderate thinning, and HT = heavy thinning, n = 3.

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