Effects of Thinning on Microbial Community Structure in the Organic Horizon of Chinese Pine Plantations in Badaling, Beijing, China

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Received: 12 September 2019; Accepted: 17 September 2019; Published: 20 September 2019

Abstract: Research Highlights: Moderate thinning can effectively improve forestry production and change the microenvironment of understory vegetation. Background and Objectives: Microbial communities control the decomposition and transformation of forest organic matter; however, the influence of thinning on microbes in the organic horizon remains unclear. Materials and Methods: In this study, we subjected four plots of Chinese pine plantations in Badaling, Beijing to different thinning intensities, including no thinning (T0), low-intensity thinning (T10), medium-intensity thinning (T20), and high-intensity thinning (T50). The changes in chemical properties and microbial community compositions observed in the organic horizon, which comprised undecomposed litter (L layer) and half-decomposed litter (F layer), were analyzed after thinning. Microbial community compositions were evaluated using phospholipid fatty acid (PLFA) methods. Results: The results showed that the abundances of gram-negative bacteria (GN) and total bacteria (B) under the T10 thinning condition were the highest among the four thinning intensities, and the abundance of arbuscular mycorrhizal fungi (AMF) in T20 was higher than under other thinning intensities. The abundance of gram-positive bacteria (GP) and actinobacteria (ACT) in T10 was lower than in both T0 and T50. The abundance of total PLFAs and fungi (FU) was higher in the L layer, whereas the abundance of GP, GN, B, ACT, and AMF was higher in the F layer. Conclusions: Our results demonstrated that the L layer better reflects the influence of thinning on litter. Redundancy analysis (RDA) results indicated that the organic carbon (LOC), dissolved organic carbon (DOC), and ammonium nitrogen (NH$_4^+$-N) contents of litter were primarily responsible for the observed changes in microbial community structure, with LOC alone explaining 62.6% of the total variance among the litter substrate factors selected. Overall, moderate-intensity thinning of Pinus tabulaeformis Carr. plantations created more favorable conditions for microbial communities in the organic horizon.

Keywords: Pinus tabulaeformis Carr.; plantations; thinning intensity; litter; phospholipid fatty acid

1. Introduction

Selective thinning through human intervention is a vital management measure for adjusting forest density and, as such, it has been widely implemented in forestry production [1–5]. It can directly or indirectly influence changes in the microclimatic conditions of forest stands, such as light levels, temperature, and water content [6–8], as well as the stocks and dynamics of soil organic carbon [9]. Soil microbial community plays a fundamental role in the process of biogeochemical cycles by controlling the transformation of organic matter [10–12], and act as a quality indicator reflecting the effect of forest management on soil and litter in forest ecosystem [13]. With the development of biological
technologies, a series of methods for analyzing the soil microbial community have been used, such as profiling of soil phospholipid fatty acids (PLFA), community level physiological profiles (CLPPs), next-generation DNA sequencing, high-throughput sequencing technologies, and metabarcoding techniques [14–16]. The PLFA method is particularly rapid and sensitive [17,18]. In addition, several PLFA biomarkers have been defined, PLFA soil profiling has been established [19–21], and the specific ratios of PLFA groups’ responses to the shifts in environmental conditions have been determined. Therefore, the PLFA method acts as a powerful tool to provide both functional and structural information on microbial communities. It has thus become one of the most commonly used methods to study microbial community structure since the early 1990s [20,22–24].

Several studies have indicated that thinning or clear-cutting can significantly decrease gram-negative bacteria, fungi, and actinomycete populations [25,26], whereas the creation of clear-cut openings in forests may increase the richness of arbuscular mycorrhiza fungal and bacterial communities [27]. However, other researchers have asserted that the same thinning intensity causes a decrease in the richness of soil fungal communities and an increase in the richness of bacterial communities [28]. Therefore, thinning can influence soil microbial functional diversity in a variety of ways [29].

In forest ecosystems, the topmost layer of the soil profile is called the organic horizon and is a key factor in the control of ecosystem productivity. It serves as a vital nutrient pool for tree growth, and an energy and nutrient source for microbial growth and activity [30]. Research on the chemical and biological processes that occur in leaf litter is crucial for an understanding of forest soil ecology and forest management processes [31]. As leaf litter and soil have different responses to environmental stresses, they should be considered separately when investigating changes in microbial communities [32]. Considering the complexity of the influence of thinning on soil microbes, it is essential to explore the response of the organic horizon to thinning.

Quantitative research on the influence of thinning on litter decomposition was first reported in the first decade of the 21st century [33]. Previous studies have indicated that thinning significantly reduces annual litter biomass [34]; at the same time, thinning intensity influences litter decomposition rate [33] and results in changes to the chemical composition of the organic horizon [35–37]. Further studies have shown that the thinning intensity significantly influences the availability of substrate resources to microbes, which leads to changes in microbial richness and enzymatic activity [38,39]. Furthermore, thinning has been shown to significantly alter the chemical properties and enzymatic activity of litter, with different types of change being observed in litter layers of different initial compositions [40].

The Chinese pine is a major conifer and is often planted for ecological restoration in northern China. Due to a lack of effective forest management, artificial Chinese pine plantations usually show poor growth and are unable to provide the forest ecosystem functions they were planted for. From 2000 onwards, due to an increased emphasis placed on the provision of forest ecosystem services, the adjustment of stand density through thinning has become a key measure in the management of artificial Chinese pine plantations. To determine the effects of thinning in Chinese pine plantations, Dang et al. [41] and Ma et al. [42] studied the changes in plant diversity and seedling regeneration after thinning, while other studies have reported the influence of thinning on soil chemical properties, soil microbes, and enzymatic activity [41,43,44].

Although research on thinning has achieved substantial advances to date, the effects of thinning vary with vegetation type and climatic zone [45]. Therefore, a favorable microenvironment for understory vegetation [3] and soil microbes [29] can only be created with appropriate thinning intensities within specific regions. However, at present, the influence of thinning on microbial litter populations in Chinese pine plantations remains unclear. To bridge this knowledge gap and advance the state-of-art, in this study, we explored the changes in understory vegetation, chemical properties, and microbial community compositions in leaf litter in Chinese pine plantations induced by different thinning treatments. We hypothesized that the thinning of Chinese pine plantations would change
litter microbial community structures, and that the microbial communities would be a function of the understory plant communities, litter mass, and the chemical properties of the organic horizon.

2. Materials and Methods

2.1. Study Area

This study was conducted in the Badaling Forest Farm (115°55' E, 40°17' N), which is located in the Yanqing district, approximately 60 km from Beijing, China. The Badaling Forest Farm is situated in the Yanshan mountain range, and has an average elevation of 780 m, with minimum and maximum elevations of 1238 and 450 m, respectively. The region has a warm-temperate, semi-humid, continental monsoon climate. According to observational records from Badaling Monitoring Station (Yanqing Meteorological Bureau), the average annual precipitation and average annual temperature across the 1981–2010 period were 435 mm and 9.7 °C, respectively. The existing vegetation mainly consists of artificial forests established since the 1950s, with the Chinese pine being the dominant species. The other tree species that are present include the black locust (*Robinia pseudoacacia* L.), Shantung maple (*Acer truncatum* Bge), and Oriental arborvitae (*Platycladus orientalis* (L.) Franco.). The understory shrubs mainly consist of the three-lobed spirea (*Spiraea trilobata* var. *pubescens*), *Lespedeza floribunda* Bge., *Leptopus chinensis* (Bge.) Pojark, and *Deutzia grandiflora* Bge. The soil is classified as Hapli-Ustic Cambosol, according to Chinese Soil Taxonomy [46], which is derived from a granite parent material; the soil layer thickness is 30–70 cm.

2.2. Experimental Design and Sample

A Chinese pine plantation approximately 60 years old planted on shady slopes at an elevation of approximately 700 m was selected as the experimental site. The forest had a mean height and mean diameter at breast height of 10.5 m and 15.25 cm, respectively. In August 2015, 12 plots with an area of 20 m × 30 m were established, at least 10 m apart. To minimize the effect of slope on the sample, the long side of each plot (30 m) was orthogonal to the slope.

The 12 plots were subjected to one of the following four thinning treatments:

1. Control plots with no thinning (henceforth, T0) had a typical stand density of 1600 individuals hm\(^{-1}\);
2. Low-intensity thinning (10% of the trees removed, henceforth T10) created plots with a density of 1440 individuals hm\(^{-1}\);
3. Medium-intensity thinning (20% of the trees removed, henceforth T20) created plots with a density of 1280 individuals hm\(^{-1}\);
4. High-intensity thinning (50% of the trees removed, henceforth T50) created plots with a density of 800 individuals hm\(^{-1}\).

Three replicates were established for each thinning intensity. Individual Chinese pines which possessed good external stem quality were retained during the thinning process, and the remaining trees were thinned to retain as even a distribution as possible.

In August 2017, five quadrats of 5 m × 5 m and 1 m × 1 m were randomly established in each plot to assess plant diversity within the shrub and herb layers, respectively. In each quadrat, the number, coverage, frequency, and height of each plant were investigated.

The belt transect method was used for sampling the litter. In each plot, three sample belts with a width of 50 cm were established. On each sample belt, five 20 cm × 20 cm sampling points were established 3 m apart. In the organic layer, needle leaves with a loose web-like stratification pattern, and a withered-yellow or yellow-brown appearance were classified as part of the undecomposed litter layer (henceforth L layer); needle leaves with a dark brown to brown-black appearance with traces of white mycelia and a fragmented stratification pattern were classified as part of a partially intact, partially decomposed litter layer (henceforth F layer) [47].
After removing shrub-grass vegetation, samples of both the L layer and F layer were collected. Similar layer samples from each sample belt were mixed and weighed. A total of 24 samples were collected for analysis (4 thinning intensities × 2 litter layers × 3 replicates). Two sub-samples were obtained using the quartering method, with one part being placed in a kraft paper envelope for chemical property analysis, and the other being stored at 4 °C before being transported to the laboratory for microbial community analysis.

2.3. Laboratory Analysis

2.3.1. Chemical Properties

The water content of the litter samples was measured by weighing after drying at 70 °C for 48 h. Litter pH was determined using an FE20K pH meter (Mettler Toledo, Zurich, Switzerland), with a 1:20 ratio of litter to water (m/v). Litter organic carbon (LOC) and total nitrogen (TN) were measured using a TOC/TN analyzer (LiquiTOC II, Elementar Analysensysteme GmbH, Hanau, Germany). The litter sample was digested by sulfuric acid-hydrogen peroxide, and the total phosphorus (TP) content was determined following the molybdenum-antimony-scandium colorimetric method [48]. The litter sample was mixed with ultrapure water using a 1:20 ratio (m/v), centrifuged and filtered using a 0.45 μm membrane filter, before the dissolved organic carbon (DOC) in the filtrate was determined using a Multi N/C 3100 analyzer (Analytik Jena AG, Jena, Germany). Ammonium nitrogen (NH$_4^+$-N) and nitrate nitrogen (NO$_3^-$-N) were extracted from the litter using 2 mol/L potassium chlorate solution and measured with an AA3 Continuous Flow Analyzer (Seal Analytical Corporation, Germany).

2.3.2. Microbial Community Structure

The microbial community structure of the litter sample was evaluated using the PLFA analysis method. PLFA contents of the litter samples were extracted following the procedure described by Kourtev et al. [49]. Essentially, the lipid content of 2 g of litter was extracted using a chloroform:methanol:phosphate buffer (1:2:0.8 v/v), and lipid classes were separated by solid phase extraction (SPE) chromatography using a silica gel column. Fatty acid methyl esters were formed through mild acid methanolysis. With 19-alkyl acid as the internal standard, we used gas chromatography (Agilent 6850N, Agilent Technologies, Santa Clara, CA, USA) and the Sherlock MIS 4.5 system (MIDI company, Newark, DE, USA) to analyze the conversion of PLFA content in nmol per gram dry weight (DW) of litter samples.

We grouped total PLFAs according to specific microbial community markers: gram-positive bacteria (GP) were the sum of fatty acids i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, and i18:0 [49–53]; gram-negative bacteria (GN) were the sum of fatty acids 16:1ω7c, 16:1ω9c, 18:1ω5c, and 18:1ω7c [50,53]; total bacteria (B) were the sum of GP and GN markers together with cy17:0 and cy19:0 [54,55]. Fungi (FU) were the sum of fatty acids 18:2ω6c and 18:1ω9c [20,49,52]; Actinobacteria (ACT) were the sum of fatty acids 10Me16:0, 10Me17:0, and 10Me18:0 [49,52,53]. The PLFA labelled 16:1ω5 was used as an important marker for arbuscular mycorrhizal fungi (AMF) [56,57]. All of the PLFA mentioned above were used to calculate the total PLFAs (totPLFAs) of the litter microbial community.

2.4. Statistical Analysis

Species richness (R) was described according to the types of plant species or individual PLFA present. The diversity of the understory plants and individual PLFAs were calculated with the Shannon index (H), using the following formula [10,40,58]:

$$ H = - \sum_{i=1}^{n} P_i \ln P_i $$  (1)
In Equation (1), \( P_i \) is the relative abundance of each plant and \( n \) is the number of plants detected. The diversity of the individual PLFAs was calculated using the same formula, where \( P_i \) is the relative abundance of each PLFA in the sum of all individual PLFAs, and \( n \) is the number of individually detected PLFAs.

One-way analysis of variance (ANOVA) was used for comparing plant characteristics among different thinning intensities, litter chemical properties, the diversity indices of PLFAs, and the microbial community structure and totPLFAs in different organic layers under different thinning intensities. Duncan’s test was used to determine the significance of differences between means. Two-way ANOVA (general linear model) was used to analyze the influence of different thinning intensities (T0, T10, T20, and T50), different organic layers (L layer and F layer), and the interactions of these two factors on microbial community structures. Pearson’s correlation analysis was also used to examine the correlations between litter chemical properties and microbial communities. All statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL, USA).

Principal component analysis (PCA) was used to determine and analyze the differences between microbial community structures as a whole under different thinning intensities. Redundancy analysis (RDA) was used to investigate the relationships between the chemical properties and microbial community structures of the litter samples. Prior to RDA, the Monte Carlo permutation test was used to identify factors significantly correlated with changes in microbial community structures. These statistical analyses were performed using Canoco 5.0 for Windows (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. Plant Characteristics

Significant differences in understory plant characteristics were found under different thinning intensities, as shown in Table 1. In the shrub layers, the parameters R, \( H \), coverage, and height in T50 were significantly higher than that in T0. The coverage in T20 was significantly lower than that in T50, and R and \( H \) in T20 were significantly higher than in T10. In the herb layers, R in T50 and T20 was significantly higher than that in T10. The coverage in T50 was the highest, followed by T20 and T10, which were both significantly higher than the coverage in T0.

Table 1. Plant characteristics in different understory layers under different thinning intensities.

<table>
<thead>
<tr>
<th>Layer</th>
<th>Thinning Intensity</th>
<th>R</th>
<th>( H )</th>
<th>Coverage</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrub</td>
<td>T0</td>
<td>5.67 ± 0.882b</td>
<td>1.13 ± 0.68c</td>
<td>0.19 ± 0.03c</td>
<td>0.39 ± 0.29c</td>
</tr>
<tr>
<td></td>
<td>T10</td>
<td>7.67 ± 1.76b</td>
<td>1.81 ± 0.31bc</td>
<td>0.30 ± 0.01b</td>
<td>0.52 ± 0.10bc</td>
</tr>
<tr>
<td></td>
<td>T20</td>
<td>13.33 ± 0.67a</td>
<td>3.05 ± 0.29a</td>
<td>0.34 ± 0.04b</td>
<td>0.79 ± 0.17ab</td>
</tr>
<tr>
<td></td>
<td>T50</td>
<td>16.00 ± 1.53a</td>
<td>2.77 ± 0.38ab</td>
<td>0.41 ± 0.02a</td>
<td>0.93 ± 0.17a</td>
</tr>
<tr>
<td>Herb</td>
<td>T0</td>
<td>10.33 ± 1.48b</td>
<td>1.72 ± 0.57a</td>
<td>0.15 ± 0.02c</td>
<td>0.08 ± 0.17a</td>
</tr>
<tr>
<td></td>
<td>T10</td>
<td>7.00 ± 1.53b</td>
<td>2.55 ± 0.63a</td>
<td>0.32 ± 0.03b</td>
<td>0.08 ± 0.16a</td>
</tr>
<tr>
<td></td>
<td>T20</td>
<td>15.67 ± 0.67a</td>
<td>2.76 ± 0.45a</td>
<td>0.35 ± 0.02b</td>
<td>0.07 ± 0.10a</td>
</tr>
<tr>
<td></td>
<td>T50</td>
<td>13.33 ± 0.89a</td>
<td>2.22 ± 0.90a</td>
<td>0.48 ± 0.01a</td>
<td>0.06 ± 0.12a</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error. In each column, different lower-case letters indicate significant differences (\( p < 0.05 \)). R: Species richness; \( H \): Shannon index.

3.2. Litter Mass and Chemical Characteristics

Litter mass composition differed significantly in different organic layers, Table 2. Among the different thinning intensities, the L layer litter mass was highest in T20. The litter mass in both T0 and T10 was significantly higher than in T50. In the F layer, the litter mass in T0 was similar to that in T20, which were both significantly higher than the one in T10; at the same time, litter mass in T50 was the lowest. However, total litter mass in the organic layers did not differ significantly under different thinning intensities.
Table 2. Mass and chemical properties of litter in different forest floor layers under different thinning intensities for *Pinus tabuliformis* plantations.

<table>
<thead>
<tr>
<th>Thinning Intensity</th>
<th>L Layer</th>
<th>F Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T10</td>
</tr>
<tr>
<td>Litter Mass/(t/hm²)</td>
<td>6.17 ± 0.58b</td>
<td>6.00 ± 0.75b</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>4.92 ± 0.04a</td>
<td>5.13 ± 0.09a</td>
</tr>
<tr>
<td>LOC/(g/kg)</td>
<td>466.10 ± 7.22a</td>
<td>476.10 ± 10.38a</td>
</tr>
<tr>
<td>DOC/(g/kg)</td>
<td>1.55 ± 0.07c</td>
<td>2.37 ± 0.05a</td>
</tr>
<tr>
<td>TN/(g/kg)</td>
<td>13.17 ± 1.34a</td>
<td>13.40 ± 1.79a</td>
</tr>
<tr>
<td>TP/(g/kg)</td>
<td>1.07 ± 0.08a</td>
<td>0.63 ± 0.34b</td>
</tr>
<tr>
<td>NH₄⁺-N/(mg/kg)</td>
<td>49.77 ± 1.56b</td>
<td>56.92 ± 3.41a</td>
</tr>
<tr>
<td>NO₃⁻-N/(mg/kg)</td>
<td>65.17 ± 0.65b</td>
<td>59.66 ± 0.75b</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± standard error (n = 3). Different letters indicate significant differences (p < 0.05) among four thinning treatments based on one-way ANOVA followed by a Duncan test. LOC: litter organic carbon; DOC: dissolved organic carbon; TN: total nitrogen; TP: total phosphorus; NH₄⁺-N: ammonia nitrogen; NO₃⁻-N: nitrate nitrogen.
The results in Table 2 also demonstrate that litter chemical properties were strongly influenced by thinning. In the L layer, no significant difference in the content of LOC or TN was found among the different thinning intensities. The values of pH and DOC content were lowest in T20, whereas the NO$_3^-$ content was highest in T20, among the four thinning intensities. The TP contents in T50 and T0 were significantly higher than those in T10 and T20.

In the F layer, the value of pH was highest in T10, DOC content was highest in T50, and NO$_3^-$ was highest in T20. Compared with T0, the contents of NH$_4^+$-N in T10, T20, and T50 increased significantly.

### 3.3. Litter Microbial Communities

#### Diversity of Litter PLFA

Compared with T0, the R and H of litter PLFA in T20 increased significantly by 16.98% and 40.18%, respectively, in the L layers (Table 3); however they were significantly lower in T50 in the F layers than those from the other thinning intensities. According to the two-way ANOVA analysis, R and H in T20 were higher than those in the other thinning intensities. Both R and H in the L layer of T20 were significantly higher than that in the F layer.

#### Composition of Litter Microbial Community Structure

The composition of the litter microbial community structure was different among the four thinning intensities (Table 4). In the L layers, the abundances of GP, GN, B, FU, and AMF were significantly higher in T10 than those in other thinning intensities. Only the GN abundance in T20 was higher than in T50. No differences in totPLFAs and ACT were found among the four thinning intensities. In the F layer, the abundances of totPLFAs, GN, B, FU, and AMF were highest in T20, whereas the abundance of GP and ACT in T10 was significantly lower than in other thinning intensities.

### Table 3. The diversity of litter PLFA in different forest floor layers under different thinning intensities for *Pinus tabulaeformis* plantations.

<table>
<thead>
<tr>
<th>Organic Layer</th>
<th>Thinning Intensity</th>
<th>R</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>L layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>53 ± 2b</td>
<td>2.24 ± 0.13b</td>
<td></td>
</tr>
<tr>
<td>T10</td>
<td>54 ± 2b</td>
<td>2.26 ± 0.03b</td>
<td></td>
</tr>
<tr>
<td>T20</td>
<td>62 ± 1a</td>
<td>3.14 ± 0.07a</td>
<td></td>
</tr>
<tr>
<td>T50</td>
<td>51 ± 3b</td>
<td>2.21 ± 0.23b</td>
<td></td>
</tr>
<tr>
<td>F layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>60 ± 2a</td>
<td>3.09 ± 0.25a</td>
<td></td>
</tr>
<tr>
<td>T10</td>
<td>62 ± 4a</td>
<td>3.17 ± 0.25a</td>
<td></td>
</tr>
<tr>
<td>T20</td>
<td>61 ± 2a</td>
<td>3.28 ± 0.08a</td>
<td></td>
</tr>
<tr>
<td>T50</td>
<td>53 ± 4b</td>
<td>2.38 ± 0.08b</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error. In each column, different lower-case letters indicate significant differences (p < 0.05).

### Table 4. The composition of litter microbial communities’ structures in different forest floor layers under different thinning intensities for *Pinus tabulaeformis* plantations.

<table>
<thead>
<tr>
<th>Organic Layer</th>
<th>Thinning Intensity</th>
<th>totPLFAs</th>
<th>GP</th>
<th>GN</th>
<th>B</th>
<th>FU</th>
<th>ACT</th>
<th>AMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>L layer</td>
<td>T0</td>
<td>301.56 ± 16.72a</td>
<td>8.88 ± 0.09ab</td>
<td>3.96 ± 0.15c</td>
<td>14.67 ± 0.68b</td>
<td>55.64 ± 1.60b</td>
<td>1.51 ± 0.06a</td>
<td>0.55 ± 0.04b</td>
</tr>
<tr>
<td></td>
<td>T10</td>
<td>339.67 ± 46.74a</td>
<td>10.26 ± 1.14a</td>
<td>56.93 ± 0.69a</td>
<td>70.89 ± 7.59a</td>
<td>94.76 ± 8.53a</td>
<td>1.52 ± 0.27a</td>
<td>1.69 ± 0.06a</td>
</tr>
<tr>
<td></td>
<td>T20</td>
<td>293.62 ± 67.61a</td>
<td>7.90 ± 1.63b</td>
<td>7.07 ± 1.64b</td>
<td>16.98 ± 0.57b</td>
<td>69.81 ± 11.08b</td>
<td>1.47 ± 0.22a</td>
<td>0.45 ± 0.43b</td>
</tr>
<tr>
<td></td>
<td>T50</td>
<td>321.56 ± 39.46a</td>
<td>8.55 ± 0.66ab</td>
<td>3.83 ± 0.32c</td>
<td>14.66 ± 0.35b</td>
<td>57.97 ± 4.66b</td>
<td>1.48 ± 0.04a</td>
<td>0.58 ± 0.06b</td>
</tr>
<tr>
<td>F layer</td>
<td>T0</td>
<td>185.53 ± 0.77b</td>
<td>23.43 ± 3.06b</td>
<td>20.40 ± 1.34b</td>
<td>64.16 ± 2.44b</td>
<td>26.60 ± 0.93b</td>
<td>8.95 ± 0.43a</td>
<td>3.39 ± 0.29b</td>
</tr>
<tr>
<td></td>
<td>T10</td>
<td>147.50 ± 19.64c</td>
<td>18.21 ± 1.59c</td>
<td>16.12 ± 0.64c</td>
<td>43.72 ± 0.61c</td>
<td>19.52 ± 2.72c</td>
<td>5.72 ± 0.28c</td>
<td>2.62 ± 0.09b</td>
</tr>
<tr>
<td></td>
<td>T20</td>
<td>243.25 ± 16.32a</td>
<td>25.61 ± 1.18a</td>
<td>36.84 ± 3.99a</td>
<td>75.64 ± 2.97a</td>
<td>53.99 ± 5.01a</td>
<td>7.88 ± 0.18b</td>
<td>6.26 ± 0.73a</td>
</tr>
<tr>
<td></td>
<td>T50</td>
<td>181.20 ± 7.29b</td>
<td>26.43 ± 2.82a</td>
<td>21.40 ± 2.24b</td>
<td>62.82 ± 3.96b</td>
<td>25.93 ± 1.38b</td>
<td>8.68 ± 0.64a</td>
<td>3.52 ± 0.47b</td>
</tr>
</tbody>
</table>

totPLFAs: total phospholipid fatty acids; GP: gram-positive bacteria; GN: gram-negative bacteria; B: bacteria; FU: fungi; ACT: actinobacteria; AMF: arbuscular mycorrhizal fungi.
According to the two-way ANOVA analysis, thinning intensities can strongly affect the composition of the litter microbial communities (Table 5). The abundances of GN and B in T10 were highest among the four thinning intensities, and the AMF in T20 was higher than in other thinning intensities. The abundances of GP and ACT in T10 were lower than those in both T20 and T50. The abundances of totPLFAs and FU in the L layer were significantly higher than in the F layer, while the opposite was true for the abundances of GP, GN, B, ACT, and AMF.

Table 5. A two-way ANOVA for analysis of litter microbial communities’ compositions. The categorical factors are thinning intensity (T0, T10, T20, T50) and forest floor layer (L layer, F layer).

<table>
<thead>
<tr>
<th>Microbial Communities</th>
<th>Thinning Intensity</th>
<th>Organic Layer</th>
<th>Thinning Intensity × Organic Layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>Sig.(Ti)</td>
</tr>
<tr>
<td>totPLFAs</td>
<td>0.710</td>
<td>0.560</td>
<td>a,a,a,a</td>
</tr>
<tr>
<td>GP</td>
<td>3.639</td>
<td>0.036</td>
<td>ab,b,a,a</td>
</tr>
<tr>
<td>GN</td>
<td>276.051</td>
<td>0.000</td>
<td>c,a,b,c</td>
</tr>
<tr>
<td>B</td>
<td>39.886</td>
<td>0.000</td>
<td>c,a,b,c</td>
</tr>
<tr>
<td>FU</td>
<td>18.376</td>
<td>0.000</td>
<td>b,a,a,b</td>
</tr>
<tr>
<td>ACT</td>
<td>30.538</td>
<td>0.000</td>
<td>a,c,b,a</td>
</tr>
<tr>
<td>AMF</td>
<td>19.466</td>
<td>0.000</td>
<td>b,b,a,b</td>
</tr>
</tbody>
</table>

Data are expressed as F-values with the level of significance. Different lower-case letters in Sig.(Ti) indicate significant differences among four thinning intensities a > b > c > d. Different capital letters in Sig.(Li) indicate significant differences between the L and the F layer of the forest floor layer, respectively, A > B.

3.5. PCA Analysis of Litter Microbial Community Structure

The different varieties of litter microbial community structures occurred across a spectrum and differed among the different thinning intensities (Figure 1). For litter microbial communities in the L layer, the first principal component (PC1) and second principal component (PC2) accounted for 68.5% and 30.5% of the total variance, respectively (Figure 1A). The entire composition of all microbial communities was positively correlated with PC1, except for ACT, which was negatively correlated with PC1. PC2 was primarily negatively driven by totPLFAs and positively driven by GP, GN, B, FU, ACT, and AMF. T10 and T0 were separated completely by PC2, and the separation between T0 and T50 was weak.
For litter microbial communities in the F layer, PC1 and PC2 explained 95.9% and 2.2% of the total variance, respectively (Figure 1B). All the litter microbial communities were positively correlated with PC1, whereas PC2 was positively correlated with the abundances of totPLFAs, AMF, GN, and FU, and negatively correlated with the abundances of B, GP, and ACT. The separation between T0 and T50 was weak, whereas T20 and the other three thinning intensities were separated almost completely by PC1. T10 and both T0 and T50 were separated almost completely by PC2.

3.6. Relationships between Litter Chemical Properties and Microbial Communities

Redundancy analysis indicated that all litter chemical properties were significantly correlated with the variation in composition of the litter microbial community structure as a whole (Figure 2).

![Figure 2. RDA of the correlations between litter chemical properties and the microbial communities’ structures under different thinning intensities for Pinus tabulaeformis plantations.](image)

For litter microbial communities, PC1 and PC2 accounted for 69.3% and 9.2% of the total variance, respectively. The RDA results showed that parameters LOC, DOC, and NH$_4^+$-N of the litter chemical properties were the main factors that affected microbial community structure. The variation in LOC, DOC, and NH$_4^+$-N alone explained 62.6%, 47.9%, and 44.7% of the total variance, respectively.

Litter chemical properties showed significant correlations with microbial community structure (Table 6). Litter pH, DOC, and NH$_4^+$-N showed significant positive correlations with totPLFAs and FU, and negative correlations with GP, ACT, and AMF. LOC was positively related to totPLFAs and FU, and negatively related to GP, B, ACT, and AMF. Litter TN and NO$_3^-$-N were significantly correlated with FU and AMF, respectively.

Table 6. Correlations between litter chemical properties and microbial communities’ structures.

<table>
<thead>
<tr>
<th>Microbial Communities</th>
<th>pH (H$_2$O)</th>
<th>LOC</th>
<th>DOC</th>
<th>TN</th>
<th>TP</th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>totPLFAs</td>
<td>0.548 **</td>
<td>0.837 **</td>
<td>0.731 **</td>
<td>0.404</td>
<td>-0.039</td>
<td>0.731 **</td>
<td>-0.129</td>
</tr>
<tr>
<td>GP</td>
<td>-0.733 **</td>
<td>-0.923 **</td>
<td>-0.684 **</td>
<td>-0.182</td>
<td>0.152</td>
<td>-0.849 **</td>
<td>0.307</td>
</tr>
<tr>
<td>GN</td>
<td>0.079</td>
<td>-0.074</td>
<td>0.392</td>
<td>0.150</td>
<td>-0.367</td>
<td>0.105</td>
<td>0.197</td>
</tr>
<tr>
<td>B</td>
<td>-0.404</td>
<td>-0.596 **</td>
<td>-0.148</td>
<td>-0.032</td>
<td>-0.167</td>
<td>-0.440 *</td>
<td>0.289</td>
</tr>
<tr>
<td>FU</td>
<td>0.538 **</td>
<td>0.803 **</td>
<td>0.796 **</td>
<td>0.453 *</td>
<td>-0.261</td>
<td>0.788 **</td>
<td>0.010</td>
</tr>
<tr>
<td>ACT</td>
<td>-0.801 **</td>
<td>-0.940 **</td>
<td>-0.738 **</td>
<td>-0.264</td>
<td>0.140</td>
<td>-0.875 **</td>
<td>0.249</td>
</tr>
<tr>
<td>AMF</td>
<td>-0.652 **</td>
<td>-0.766 **</td>
<td>-0.556 **</td>
<td>0.111</td>
<td>0.128</td>
<td>-0.693 **</td>
<td>0.627 **</td>
</tr>
</tbody>
</table>

* Significant correlations (p < 0.05). ** Significant correlations (p < 0.01).
4. Discussion

4.1. Change Tendencies of Microbial Communities due to Thinning

The two-way ANOVA results suggest that thinning does not induce changes in totPLFAs. Nevertheless, appropriate thinning intensities were shown to increase the richness and diversity of individual PLFAs, which provided the conditions for the changes to microbial community structures that were observed to occur at different thinning intensities. For instance, low-intensity (T10) and medium-intensity (T20) thinning led to notable increases in the abundances of GN, B, and FU, and a decrease in the abundance of ACT. The abundance of AMF was only observed to increase with medium-intensity thinning. In other words, no significant differences in microbial community structure were observed between high thinning intensity (T50) and the control (T0). This result is partially consistent with our first hypothesis. A study by Grayston et al. [59] provided partial support for our research results, asserting that thinning had no significant influence on the microbial communities of litter, except on AMF abundance. Our result conflicts with previous work that indicated that forest thinning had decreased total microbial abundance in decomposing litter [38,60].

Variation in thinning intensity induces different extents of change in environmental conditions, resulting in complex change trends in different microbial communities. The microclimate has been changed with the stand developed after thinning, and this may have induced equivalent change trends in microbial communities. Maassen et al. [61] reported that no significant differences between the organic layer and mineral layer for biomasses of B, F, and ACT, whereas early evidence of change in PLFA patterns was detected five years after thinning; at the same time, Dang et al. [41] reported that thinning intensity did not significantly affect microbial community diversity indices even 11 years after thinning. These inconsistent results on the variation of microbial communities after thinning may be a result of differences in thinning intensity and differences in sampling time. Our results were obtained based on observations made one year after thinning at the site under study; hence, long-term investigation and additional studies on microbial communities are required soon afterwards.

Although high-speed analysis methods were presented, the soil microbial community is a complex “blackbox”, that requires a multi-conceptual approach. PLFA have been used for investigating the variation of microbial communities, although limitations in PLFA interpretation have been demonstrated [20]. Metabarcoding provides a much greater level of detail than PLFA profiling does. The methods are also complementary, i.e., each method can provide different information on the structure and function of microbial communities [62,63]. Clearly, to quantify meaningful changes, using other methods besides PLFA will be needed.

4.2. The Effects of Organic Layer Changes on Microbial Community Structure

Litter which covers the soil is an important component for supporting microbial community growth, as well as investigations of the effects of forest management protocols on ecosystem processes. Substantial variations in microbial biomass, community composition, and microbial functions with litter decomposition occur across small spatial scales [64]. In the present study, different thinning intensities influenced the L and F layers to different extents. Specifically, the abundances of totPLFAs and FU were higher in the L layer, while the abundances of GP, GN, B, ACT, and AMF were higher in the F layer. Hence, the observed variation in microbial communities was related to the specific microbial species composition and the decomposition stage of needles [65]. Our findings supported the results of Šnajdr et al. [66], who reported that fungal biomass was largest in the L layer among the different organic layers, and partly supported the findings of Zheng et al. [67], who reported that the abundance of GP, GN, B, FU, and microbial biomass of the organic layer were significantly higher than those of the mineral layer in a coniferous forest. These results imply that different litter layers should be investigated separately when assessing changes to forest soil microbial communities.

In the present study, different thinning intensities had different influences on the microbial community structures of different litter layers. Furthermore, PCA results indicated that the different
influences were more distinct in the F layer than in the L layer. We infer from this observation that in the L layer, UV levels and the physical effects of repeated wetting-drying may increase the susceptibility of litter to microbial processing [68], whereas the F layer may be kept under relatively constant environmental conditions. Because of this, we suggest that the F layer can better reflect the effect of thinning intensities on litter microbial communities.

4.3. Factors Associated with the Microbial Community Variation

Thinning significantly increased both the R and H of understory plants in this study. In particular, the coverage of shrub and herb layers was highest in T50. This result is consistent with previous research [6,41,68]. The development of understory plant diversity may in turn improve the diversity of the associated microbial community, by altering the eco-physiological environment of the forest stand [69–71]. In our study, the richness and diversity of shrubs were higher in both T20 and T50 plots, whereas the variation in individual microbial community abundance did not show a consistent trend. Specifically, GN and B in T10, and AMF in T20 were higher than those at other thinning intensities. These results were not completely in accordance with the results of previous studies, where AMF was found to have the highest species diversity [72], and the microbial functional diversity of litter was greater with greater plant production and litter reserves [73]. Nutrients secreted by different plants have different influences on microbial development [74]. Therefore, the influence of plant diversity changes on microorganisms in litter after thinning requires further study. In addition, the lower diversity of microbial communities observed in T50 may be related to inadequate soil water content in arid and semi-arid regions [33].

As litter is the energy and nutrient source for shaping microbial community, changes in litter properties inevitably lead to changes in microbial communities [75,76]. Our results showed that LOC, DOC, and NH$_4^+$-N were primarily responsible for changes in microbial community structure. In particular, LOC alone explained 62.6% of the total variance. DOC content of the litter was highest in T10, and the content of TN and NO$_3^-$-N were significantly higher in T20 than in other thinning intensities—these changes increased the variation in litter microbial communities. These results were consistent with our hypothesis, and supported the findings of Fanin et al. [77] who asserted that litter with high dissolved C content lead to an increase in microbial biomass and to a structural shift towards a relatively greater abundance of GN. Chen et al. [29] reported that TN explained the greatest amount of variation in the FU of litter.

High fungal diversity is essential to support litter decomposition and resilience of the forest ecosystem [78,79]. In the present study, litter pH, and the contents of LOC, DOC, TN, and NH$_4^+$-N showed significant positive correlations with FU. This correlation indicates that the changes in litter carbon and nutrient content in both T10 and T20 improved the composition changes in the fungal community.

In forest ecosystems, biotic and abiotic factors interact, and can strongly affect the variation of microbial community structure. The shift of abiotic factors such as litter moisture and temperature after thinning may have a more drastic impact than litter nutrient [80,81]. In this paper, only the effect of plant vegetation and the reaction to the alteration of litter substrate properties were involved. For future investigations, microclimate measurements and the assessment of their contribution to microbial communities after thinning should be performed. Besides the effect of plant vegetation and the reaction to the alteration of litter substrate properties, microclimate measurements after thinning, such as water content and temperature of litter, can strongly contribute to determining the composition of microbial communities.

5. Conclusions

Thinning is a necessary measure for the enhancement of forest land quality in the relatively high stand densities of artificial Chinese pine plantations. In this study, four plots of Chinese pine plantations in Badaling, Beijing were subjected to different thinning intensities and the changes in
chemical properties and microbial community compositions observed in the organic horizon. Based on the results obtained in the study, the following conclusions are drawn:

1. One year after thinning, the richness and Shannon index of understory vegetation both increased with increasing thinning intensity.

2. Low-intensity and medium-intensity thinning led to increases in the abundance of gram-negative bacteria, total bacteria, and fungi, and resulted in a decrease in the abundance of actinobacteria, while the abundance of arbuscular mycorrhizal fungi was only increased by medium-intensity thinning. The influences of different thinning intensities on microbial communities were more distinct in the semi-decomposed layer. Furthermore, the abundances of total PLFAs and fungi were higher in undecomposed organic layers, while the abundances of gram-positive bacteria, gram-negative bacteria, total bacteria, actinobacteria, and arbuscular mycorrhizal fungi were higher in the semi-decomposed layer.

3. In the litter substrate, the litter contents of organic carbon, dissolved organic carbon, and ammonium nitrogen were determined to be primarily responsible for changes in microbial community structure.

This study was conducted by using PLFA method, and the investigation lasted only one year after thinning; this might result in uncertainty in some conclusions. More time and further analyses using combinations of multiple methods are needed to fully investigate the effect of different thinning intensities on microbial communities in the organic horizon of Chinese pine plantations.

**Author Contributions:** Methodology, Y.Y.; software, L.W.; validation, H.Z.; investigation, Y.Y. and H.Z.; resources, L.W. and G.Z.; data curation, H.Z.; writing—original draft preparation, L.W.; writing—review and editing, G.Z. and Y.S.; project administration, G.Z.; funding acquisition, Y.Y. and H.Z.

**Funding:** This research was funded by FINANCIAL PROJECT OF BEIJING, grant number PXM2016-154309-000006. The APC was covered by the same grant.

**Acknowledgments:** We thank Editage for English language revision.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

**References**


