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Effects of Forest Gaps on Forest Floor Microbial Community Composition in *Pinus tabulaeformis* Forests in a Rocky Mountain Area, Beijing, China

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Abstract: Forest gaps induce environmental heterogeneity, but their effects on the local forest floor microbial communities are not fully understood. This research investigated the impact of forest gap positions on the forest floor microbial community composition to provide baseline information for projects to accelerate nutrient cycling and forest regeneration and enhance ecosystem services. A one-year-old forest gap and an area of 40–50 m^2 in *Pinus tabulaeformis* plantations were selected in the Beijing mountainous area. Forest floor samples were collected from the following positions: gap center, gap border, and adjacent closed canopy. Our study demonstrated that gap positions significantly influenced the forest floor microbial community composition. The Gram-positive bacteria, Gram-negative bacteria, and total bacteria, as well as the fungi, were significantly greater in the forest gap center and gap border compared to those in the closed canopy, and the dissolved organic carbon, readily oxidized organic carbon, ammonia nitrogen, and nitrate nitrogen followed the same trend. Compared with those of the closed canopy, the Gram-positive bacteria, Gram-negative bacteria, total bacteria, and fungi in the gap center were markedly greater by 23%, 25%, 22%, and 24% and by 14%, 14%, 11%, and 16% in the gap border, respectively ($p < 0.05$). Redundancy analysis demonstrated that shifts in the litter microbial community composition were predominantly predicted by litter moisture and β-1,4-glucosidase. In addition, we discovered that the microbial community composition was greater in the undecomposed forest layer than that in the semi-decomposed layer. In summary, gap positions and forest floor layers have a significant impact on microbial community composition. Nevertheless, additional long-term investigations are needed. Our study provides a reference for the promotion of nutrient cycling to guide future ecological management.

Keywords: *Pinus tabulaeformis*; gap position; forest floor layer; microbial community composition; phospholipid fatty acid; forest management

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1. Introduction

Forest canopy gaps open due to mortality or disturbances [1,2]; induce changes in the microenvironments such as net radiation, temperature, and humidity [3,4]; preserve bio– and pedodiversity [5,6]; and play essential roles in facilitating nutrient cycling [7,8], regenerating forests [9,10], and improving ecosystem services [11]. As a result, forest gaps have received widespread attention [12,13]. With the increase in forest gap research on aboveground aspects, such as plant diversity, vegetation restoration, and succession [14], forest soil and litter have also received increasing attention [13,15–18].

Gap position is an important indicator of forest gap characteristics [4], and litter decomposition status is an important indicator of soil quality variation [19]. Different gap positions lead to the redistribution of light, heat, and water in the forest canopy [7,20], thereby affecting the litter decomposition rate [21–24], nutrient cycling [25], microbial

activity, and enzyme activity [25–29]. Studies have demonstrated that a closed canopy has high litter decomposition and humification rates [21,30] and accelerates leaf litter mass loss (lignin, cellulose, hemicellulose, total phenols, and condensed tannins) [22–24] and nutrient release (availability of carbon, nitrogen, and phosphorus) [8,31,32]. However, some studies have reported that litter total carbon and phenol were not notably different among different gap positions [33,34]. Microbial community composition varies substantially, with shifts in soil properties associated with gap position [35]. Soil microbial biomass and total phospholipid fatty acids are substantially more abundant in gap centers than in gap edges [36] or the understory [37]. However, some studies have found that the fungal community has no significant difference among gap positions [25].

Numerous studies have suggested that the soil microbial community is a sensitive indicator of soil quality [38–40] and plays an essential role in a biogeochemical cycle by regulating the conversion of organic matter [41,42]. Understanding the variations in litter microbial communities is essential for elucidating litter decomposition characteristics, nutrient cycling, and forest regeneration. Although litter chemical properties at different gap positions have been well–studied [33–35], the microbial community mechanisms that reflect variations in litter chemical properties remain unclear [4]. Therefore, strengthening the knowledge of the relationship between gap position and litter microbial communities is necessary.

Chinese pine (*Pinus tabulaeformis*), which is drought–resistant and highly adaptable, is one of the most vital afforestation species in mountain coniferous forests in North China. Forest gaps in Chinese pine plantations have enhanced not only the management of effective forests but also the litter microbial community. Nevertheless, there are few studies on litter microbial communities under different gap positions. In this research, we examined the shifts in litter microbial communities at different gap positions in a Chinese pine plantation using the phospholipid fatty acid (PLFA) method. The main objectives were to examine (i) litter microbial communities, physicochemical properties, and enzyme activity responses to forest gap positions and describe (ii) the main drivers affecting litter microbial community composition.

2. Materials and Methods

2.1. Site Description

Our research was located at Badaling Forest Park (40°17′ N, 115°55′ E), a rocky mountainous area 60 km north of Beijing, Northern China. The average elevation is approximately 780 m. The region is characterized by a temperate semi–arid climate [43], and a mean air temperature of 9.7 °C. The yearly average precipitation is 435 mm, mainly concentrated from June to August, and the annual total evaporation was 1585.9 mm from 1981 to 2010 [44]. The park is dominated by artificial forests, most of which were planted in the 1950s and 1970s. These forests are dominated by *P. tabulaeformis*. Other species included *Acer truncatum*, *Robinia pseudoacacia*, and *Platycladus orientalis*. The soils are derived from granite parent material and classified as Hapli–Ustic Cambosols [44].

In 2015, we selected a ~60–year–old *P. tabulaeformis* plantation with an elevation of approximately 700 m, a slope of 18–24°, and an eastward and northeastern direction. *P. tabulaeformis* accounted for 90%, with a canopy density of 0.7–0.8. The mean diameters at breast height and tree height were 14.89 cm and 9.36 m, respectively. The understory vegetation cover was 25%–30%, and the main shrubs were *Spiraea trilobata*, *Leptopus chinensis*, and *Deutzia grandiflora*. The herbs were *Saussurea nivea*, *Carex lanceolata* var. *subpediformis*, and *Calamagrostis arundinacea*.

2.2. Experimental Design and Litter Samples

Due to the high planting density, which limited tree growth, measures were implemented to thin and remove felled trees from the forest in June 2015. Moreover, the canopy gap in artificial coniferous forests is typically small in the mountainous areas of Beijing [45]. In June 2016, three canopy gaps with similar conditions—slope, aspect, and boundary

wood—were selected. The canopy gap was 1 year old, and was created by felling 1–3 trees, approximately elliptical in a short north–south direction and a long east–west direction (ca. 6 m × 8 m in size), with an area of 40–50 m^2; moreover, the boundary distance of the gap was greater than 10 m.

The gap center (GC) and gap border (GB) positions were set in each gap, and the adjacent closed canopy (CC) was used as the control. The GC plots were circular areas formed by the intersection of the two axes and extended a 1/2 area outward along the center point. The GB plots were circular areas from the vertical line of the forest gap boundary trees to the base of the surrounding boundary trees and extended a 3/4 area outward along the center point. The CC plots were a canopy forest adjacent to the forest gap (area 100–120 m^2). A schematic is presented in [25].

Three replicates were established for each forest gap. In June 2016, litter samples were collected from three positions (GC, GB, and CC) in each gap. At each position, four plots of 1 m × 1 m were established along the four directions of the gap. In each plot, three sampling points with areas of 20 cm × 20 cm were collected randomly from the undecomposed and semi–decomposed layers. All 12 litter samples (from one layer and one gap position) were pooled to form a composite sample. In total, 18 composite samples were collected for analysis (3 gaps × 3 positions × 2 forest floor layers). We obtained two subsamples by using the quartering method. One part was stored in a box at 4 °C for microbial community and enzyme activity analysis, and the other part was placed in a Ziplock bag for analyzing litter physicochemical properties.

2.3. Laboratory Analysis

2.3.1. Litter Physicochemical Properties

Litter moisture and mass were obtained following drying at 70 °C for 48 h and weighing. Litter pH (H_2O) was measured using a litter suspension (litter/water = 1/20) and an S210 Seven Compact pH meter (Mettler–Toledo, Zurich, Switzerland). Litter organic carbon (LOC) and total nitrogen (TN) were quantified with an elemental analyzer (Elementar, Hanau, Germany) [46]. Litter dissolved organic carbon (DOC) was centrifuged, filtered, and measured by a Multi N/C 3100 TOC/TN analyzer (Analytik Jena, Jena, Germany). Litter readily oxidized organic carbon (ROC) was measured using the 0.02 mol/L $KMnO_4$ oxidation method. The ammonium nitrogen (NH_4^+–N) and nitrate nitrogen (NO_3^-–N) content indicated the available nitrogen and was determined using an AA3 continuous flow analyzer (Seal Analytical Corporation, Norderstedt, Germany) using an extracted solution with 2 mol/L KCL (litter/solution = 1/4). Litter total phosphorus (TP) was quantified using sulfuric acid–hydrogen peroxide digestion and the molybdenum–antimony–scandium colorimetric method [47].

2.3.2. Litter Microbial Community

The PLFA content of the litter samples was analyzed with a modified procedure [48]. PLFAs were extracted from 2 g of lyophilized litter, separated, and methylated [49]. The lipid content was extracted with a pH 4.0 chloroform/methanol/phosphate buffer (1/2/0.8 $v/v/v$). The resulting fatty acid methyl esters were selected using solid–phase extraction chromatography on a silica gel column. Fatty acid methyl esters were prepared via mild acid methanolysis. By using 19–alkyl acid as the internal standard, the litter PLFA content was analyzed using gas chromatography (Agilent 6850N, Santa Clara, CA, USA) and the Sherlock MIS 4.5 system (MIDI, Newark, DE, USA).

The PLFA content (nmol/g dry weight) represents the biomass of different types of litter microorganisms. The biomass of the major microbial groups was estimated through the total amounts of the following PLFAs [50]: Gram–positive bacteria (GP) (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, and i18:0), Gram–negative bacteria (GN) (16:1ω7c, 16:1ω9c, 18:1ω5c, and 18:1ω7c), bacteria (GP, GN, cy17:0, and cy19:0), fungi (18:2ω6c and 18:1ω9c), actinobacteria (ACT) (10Me16:0, 10Me17:0, and 10Me18:0), and arbuscular mycorrhizal fungi (AMF)

(16:1ω5). All the aforementioned PLFAs were employed to calculate the total PLFAs for the litter microbial community.

2.3.3. Litter Enzyme Activities and Hydrolase–Based Vector Lengths and Angles

Five types of litter enzyme activities were evaluated. These three hydrolytic enzymes are related to C–cycling (BG, β–1,4–glucosidase), N–cycling (NAG, β–1,4–N–acetylglucosaminidase), and P–cycling (AP, acid phosphatase). Two oxidative enzymes, phenol oxidase (POX) and peroxidase (PER), are involved in recalcitrant C–cycling. The enzyme activity of the litter samples was evaluated using soil enzyme activity. The protocol used is described in a previous study [26]. Different substrates were added to the suspension (the ratio of litter mass to buffer volume was 1:16), and the product content was measured after culturing [26].

To characterize litter carbon and nutrient limitation status, the protocol used in previous studies [51,52] was performed for the vector analysis of litter enzyme activity. Because the relative enzyme activities participating in the carbon and phosphorus cycles in the soil are X, and the relative enzyme activities engaged in the carbon and nitrogen cycles are Y, the coordinate (X, Y) is connected to the origin (0, 0) to form a vector. Vector length characterizes the extent to which energy is limited relative to nutrients (C vs. nutrients) in the soil, and vector angle is used to characterize the extent to which P is limited relative to N (P vs. N) in the soil.

$$\text{vector length} = \text{SQRT}\ (X^2 + Y^2)$$

$$\text{vector angle} = \text{DEGREES}\ (\text{ATAN2}(X, Y))$$

The formula for calculating X and Y is as follows:

$$X = BG/(BG + AP)$$

and

$$Y = BG/(BG + NAG)$$

2.4. Data Analysis

One–way analysis of variance (ANOVA) and Duncan's test ($p < 0.05$) were employed to compare the litter microbial community composition, physicochemical properties, enzyme activities, and hydrolase–based vector lengths and angles among different gap positions (GC, GB, and CC). A paired samples *t*-test ($p < 0.05$) was employed to examine the litter microbial community structure, physicochemical properties, enzyme activities, and hydrolase–based vector lengths and angles in the different forest floor layers (undecomposed and semi–decomposed layers). A two–way ANOVA was performed to evaluate the influence of different gap positions, different forest floor layers, and the interactions of these two factors on the litter microbial community composition. To explore the influence of the litter microbial community composition on physicochemical properties, enzyme activities, and hydrolase–based vector lengths and angles, we performed Mantel tests using the vegan R package. A detrended correspondence analysis was performed on the litter microbial community composition in different gap positions. The maximum length of the gradient (0.172) was less than 3.0, and the linear model characteristics were evident. Therefore, redundancy analysis (RDA) was used to investigate correlations between the litter microbial community composition and physicochemical properties, enzyme activities, and hydrolase–based vector lengths and angles. All statistical analyses were conducted with SPSS 26 (IBM Corporation, Chicago, IL, USA), and pictures were run through Origin 9.1 (Origin Lab, Northampton, MA, USA).

3. Results

3.1. Litter Physicochemical Properties

Litter physicochemical properties showed significant responses to different forest gap positions (Figure 1). Litter DOC, ROC, NH_4^+–N, and NO_3^-–N were significantly higher in the GC than in the GB and CC (Figure 1G–J), while moisture was significantly lower

in the GC than in the GB and CC ($p < 0.05$) (Figure 1C). Values for LOC and TN were significantly larger in the GB than in the GC and CC ($p < 0.05$) (Figure 1D,E), and pH (H_2O) and TP was not significantly different among the gap positions (Figure 1B,F). Litter DOC, ROC, NH_4^+–N, and NO_3^-–N were markedly greater by 72%, 23%, 45%, and 60%, in the GC and by 26%, 10%, 32%, and 45%, respectively, in the GB, compared with the respective values in the CC ($p < 0.05$). In addition, different forest floor layers considerably affected the physicochemical properties. The values of physicochemical properties in the undecomposed layer were significantly greater than those in the semi–decomposed layer ($p < 0.001$), except for the mass, moisture, and NO_3^-–N.

3.2. Litter Microbial Community Composition

The forest floor microbial community composition showed several significant responses to different forest gap positions (Figure 2). Litter GN, bacteria, and total PLFAs were present in significantly greater quantities in the GC than in the GB and CC (Figure 2B,C,G), while AMF were significantly less abundant in the GC than in the GB and CC ($p < 0.05$) (Figure 2F). Litter GP and fungi were significantly smaller in the CC than in the GC and GB ($p < 0.05$) (Figure 2A,D). Litter ACT were significantly less in the GB than in the GC and CC ($p < 0.05$) (Figure 2E). Compared with those in the CC, the GP, GN, bacteria, and fungi in the GC were markedly greater by 23%, 25%, 22%, and 24% and by 14%, 14%, 11%, and 16% in the GB, respectively ($p < 0.05$). Moreover, the microbial community composition of the different forest floor layers varied significantly. Specifically, the microbial community composition was significantly greater in the undecomposed layer than that in the semi–decomposed layer ($p < 0.001$).

3.3. Litter Enzyme Activities and Hydrolase–Based Vector Lengths and Angles

The litter enzyme activity and hydrolase–based vector lengths and angles showed significant responses at different forest gap positions (Figure 3). The activity of litter BG and NAG were significantly greater in the GC than in the GB and CC (Figure 3A,B), and the angle was significantly less in the GC than in the GB and CC ($p < 0.05$) (Figure 3F). The trend in AP activity was as follows: GB > GC > CC (Figure 3C). Compared to the CC, the activity of POX + PER was significantly greater in the GC and GB ($p < 0.05$) (Figure 3D). The length was significantly less at the GB than that at the GC and CC ($p < 0.05$) (Figure 3E). The activity for litter BG, NAG, AP, and POX + PER was markedly greater by 31%, 32%, 11%, and 9% in the GC and by 11%, 22%, 23%, and 9%, respectively, in the GB, compared with the respective activity in the CC ($p < 0.05$). Additionally, the enzyme activity and hydrolase–based vector lengths and angles varied significantly at different forest floor layers. Litter enzyme activity was greater at the undecomposed layer than those at the semi–decomposed layer ($p < 0.001$), except for the POX + PER activity.

3.4. Factors Affecting Litter Microbial Community Composition

The Mantel test analysis revealed that all litter physicochemical properties, enzyme activities, and hydrolase–based vector lengths and angles strongly corresponded to variations in the litter microbial community composition ($p < 0.01$) (Figure 4).

The RDA of the litter microbial community composition and physicochemical properties revealed that RDA1 and RDA2 explained 98.96% and 0.23% of the variation, respectively (Figure 5A). Values for moisture ($F = -273$, $p = 0.002$), NO_3^-–N ($F = -12.8$, $p = 0.002$), TP ($F = -5.2$, $p = 0.038$), and NH_4^+–N ($F = -4.9$, $p = 0.042$) significantly affected the litter microbial community composition. Shifts in moisture, NO_3^-–N, TP, and NH_4^+–N accounted for 94.47%, 2.57%, 0.81%, and 0.59% of the overall variance, respectively. The RDA of the litter microbial community composition, enzyme activities, and hydrolase–based vector lengths and angles showed that RDA1 and RDA2 explained 98.98% and 0.22% of the variation, respectively (Figure 5B). The activity of BG ($F = 758$, $p = 0.002$), POX + PER ($F = -6.8$, $p = 0.006$), and angle ($F = -4.5$, $p = 0.036$) had marked effects on the litter microbial community composition. The variations in BG, POX + PER, and angle explained 97.93%, 0.65%,

and 0.31% of the overall variance, respectively. Therefore, litter moisture and BG were the main contributors to the litter microbial community composition.

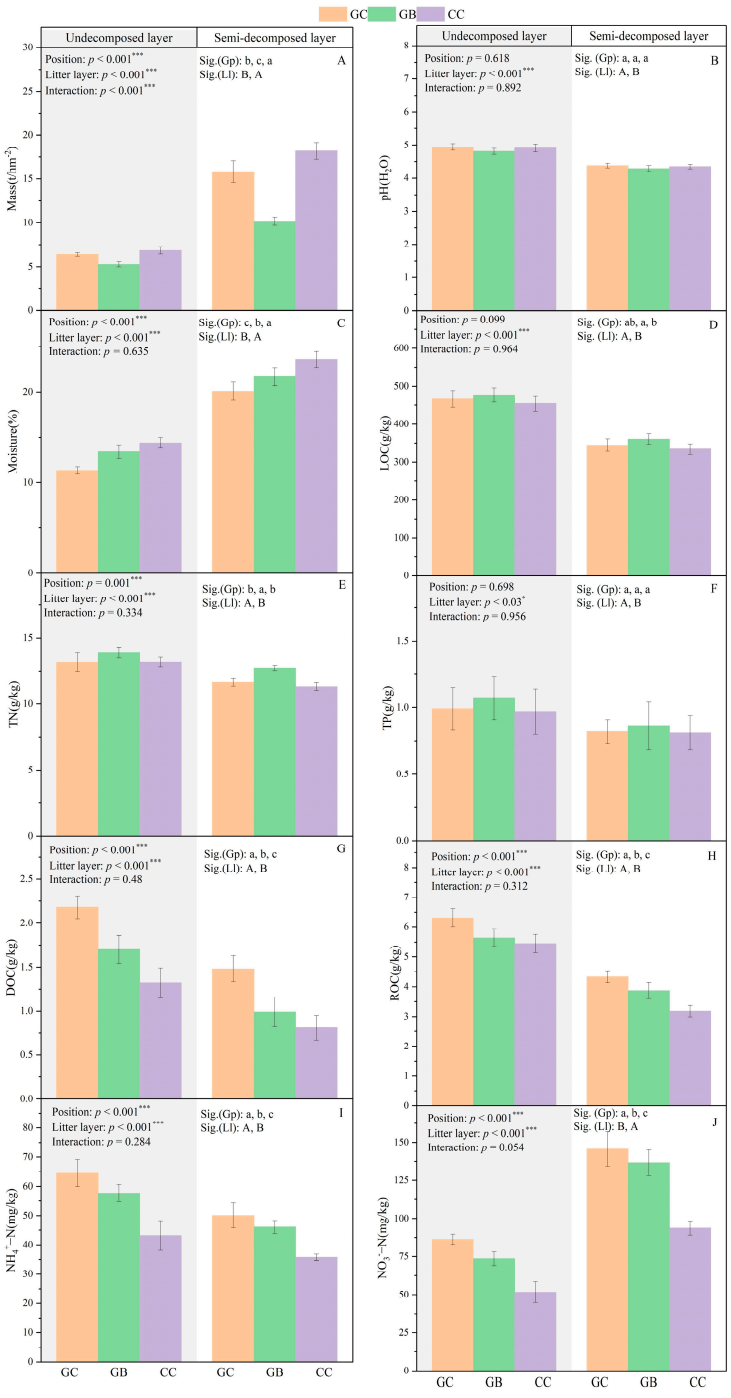

Figure 1. Litter physicochemical properties (mass, (**A**); pH (H_2O), (**B**); moisture, (**C**); LOC, (**D**); TN, (**E**); TP, (**F**); DOC, (**G**); ROC, (**H**); NH_4^+–N, (**I**); NO_3^-–N, (**J**)) under different gap positions in different forest floor layers for *Pinus tabulaeformis* plantations. Different lower–case letters in Sig. (Gp) indicate significant differences among the GC, GB, and CC, respectively. a > b > c. Different capital letters in Sig. (Ll) indicate significant differences between the litter layers, respectively. A > B. ($p < 0.05$). * $p < 0.05$; *** $p < 0.001$. Abbreviations: GC, gap center; GB, gap border; CC, closed canopy; LOC, litter organic carbon; TN, total nitrogen; TP, total phosphorus; DOC, dissolved organic carbon; ROC, readily oxidized organic carbon; NH_4^+–N, ammonia nitrogen; NO_3^-–N, nitrate nitrogen.

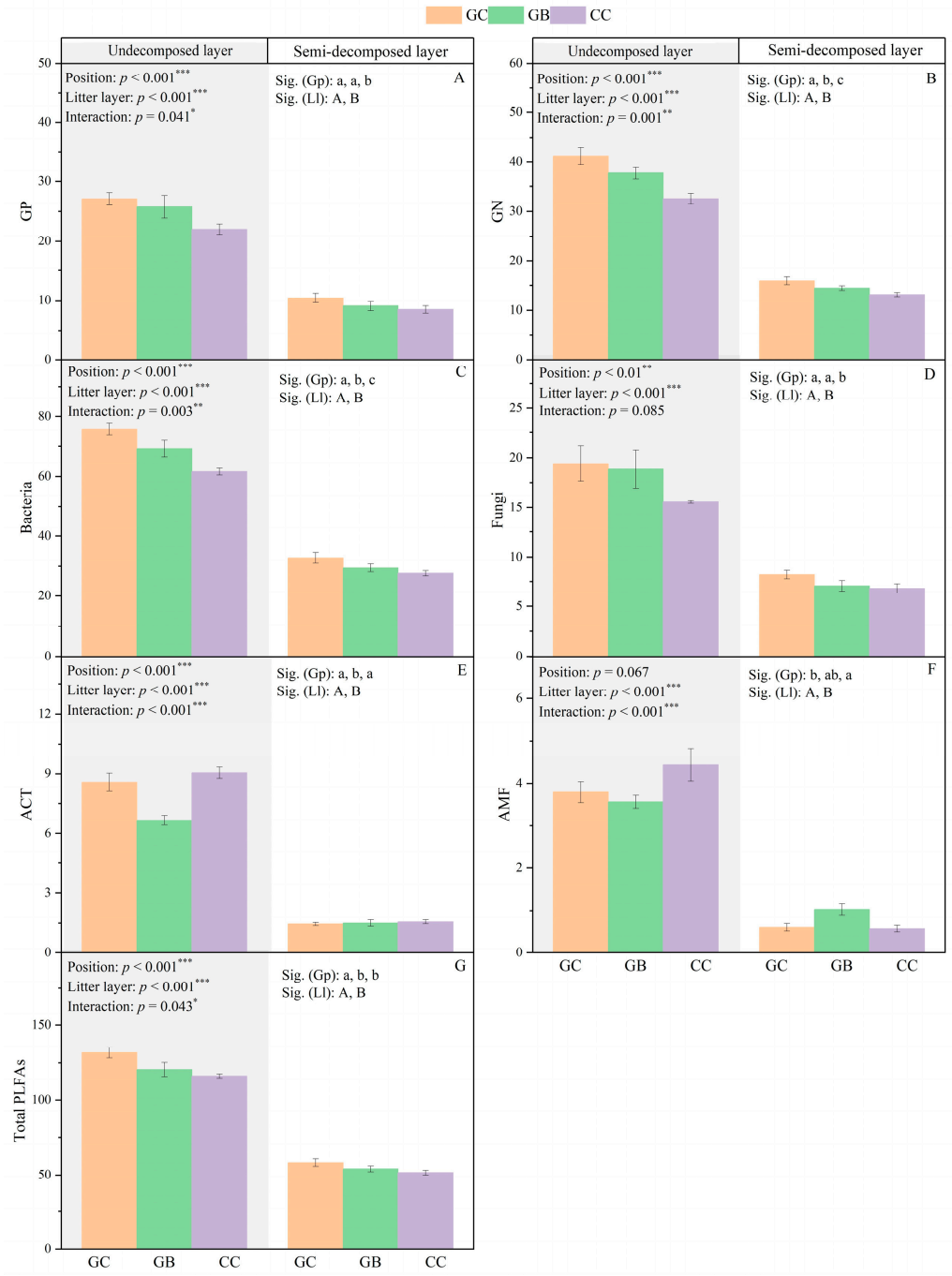

Figure 2. Litter microbial community composition (GP, (**A**); GN, (**B**); bacteria, (**C**); fungi, (**D**); ACT, (**E**); AMF, (**F**); total PLFAs, (**G**)) under different gap positions in different forest floor layers for *Pinus tabulaeformis* plantations. Different lower–case letters in Sig. (Gp) indicate significant differences among the GC, GB, and CC, respectively. a > b > c. Different capital letters in Sig. (Ll) indicate significant differences between the litter layers, respectively. A > B. ($p < 0.05$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Phospholipid fatty acid content was measured in nmol/g dry weight. Abbreviations: GC, gap center; GB, gap border; CC, closed canopy; GP, Gram–positive bacteria; GN, Gram–negative bacteria; ACT, actinobacteria; AMF, arbuscular mycorrhizal fungi; total PLFAs, total phospholipid fatty acids.

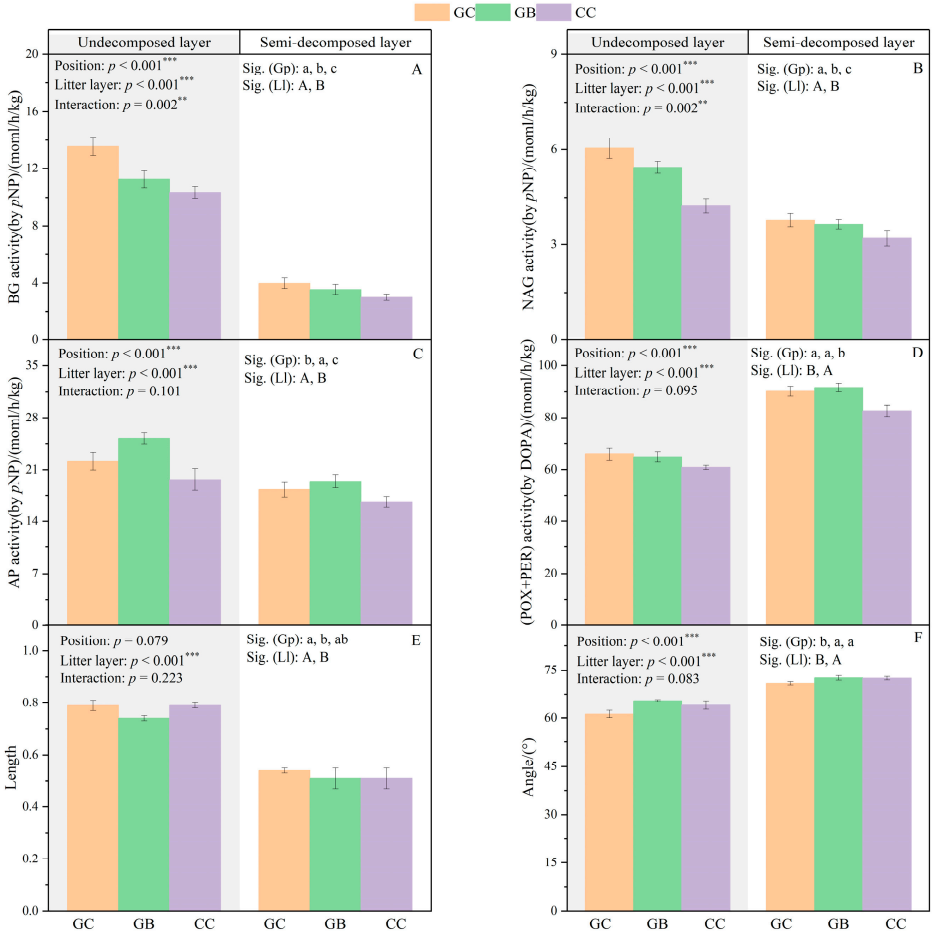

Figure 3. Litter enzyme activities (BG, (**A**); NAG, (**B**); AP, (**C**); POX +PER, (**D**)) and hydrolase–based vector length (**E**) and angle (**F**) under different gap positions in different forest floor layers for *Pinus tabulaeformis* plantations. Different lower–case letters in Sig. (Gp) represent significant differences among the GC, GB, and CC, respectively. a > b > c. Different capital letters in Sig. (Ll) indicate significant differences between the litter layers, respectively. A > B. ($p < 0.05$). ** $p < 0.01$; *** $p < 0.001$. Abbreviations: GC, gap center; GB, gap border; CC, closed canopy; BG, β–1,4–glucosidase; NAG, β–1,4–N–acetylglucosaminidase; AP, acid phosphatase; POX + PER, phenol oxidase and peroxidase.

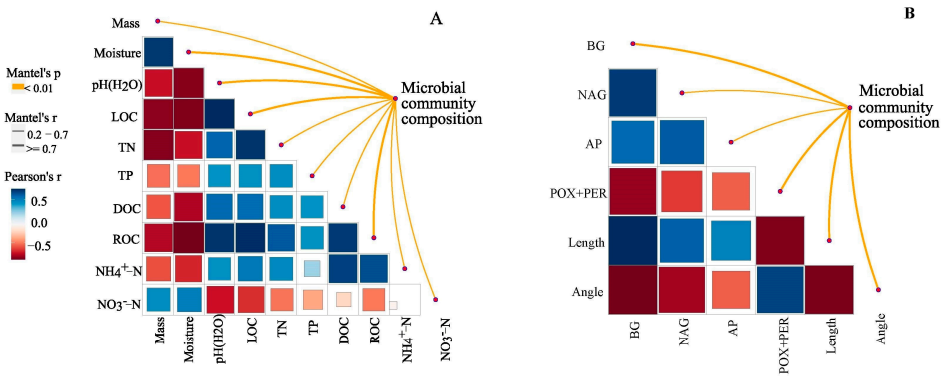

Figure 4. Mantel test analysis of litter microbial community composition and litter physicochemical properties (**A**), enzyme activities, and hydrolase–based vector lengths and angles (**B**) under different gap positions for *Pinus tabulaeformis* plantations. Abbreviations: LOC, litter organic carbon; TN, total nitrogen; TP, total phosphorus; DOC, dissolved organic carbon; ROC, readily oxidized organic carbon; NH_4^+–N, ammonia nitrogen; NO_3^-–N, nitrate nitrogen; BG, β–1,4–glucosidase; NAG, β–1,4–N–acetylglucosaminidase; AP, acid phosphatase; POX + PER, phenol oxidase and peroxidase.

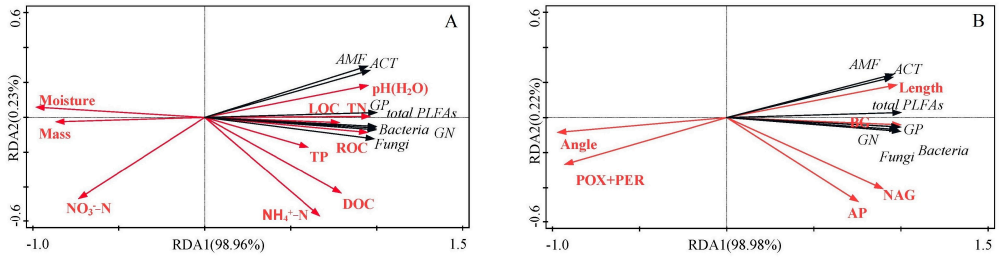

Figure 5. Redundancy analysis of correlations between litter physicochemical properties (**A**), enzyme activities, hydrolase–based vector lengths and angles (**B**), and the microbial community composition among gap positions for *Pinus tabulaeformis* plantations. Abbreviations: GP, Gram–positive bacteria; GN, Gram–negative bacteria; ACT, actinobacteria; AMF, arbuscular mycorrhizal fungi; total PLFAs, total phospholipid fatty acids; LOC, litter organic carbon; TN, total nitrogen; TP, total phosphorus; DOC, dissolved organic carbon; ROC, readily oxidized organic carbon; NH_4^+–N, ammonia nitrogen; NO_3^-–N, nitrate nitrogen; BG, β–1,4–glucosidase; NAG, β–1,4–N–acetylglucosaminidase; AP, acid phosphatase; POX + PER, phenol oxidase and peroxidase.

4. Discussion

4.1. Effect of Gap Position on Litter Microbial Community Composition

Due to solar radiation and thermal effects at the forest edge, different gap positions can induce different microenvironments (temperature and moisture) [53,54], which can change litter input and organic matter dynamics; thus, the microbial community quantity and composition can also change accordingly [7,22–24]. However, the extent of this change is affected by forest gap characteristics. Studies have revealed that the gap age can lead to microbial changes at different gap locations. In gaps existing for one–two years, the soil microbial biomass was apparently greater in the GC than in the gap edges [36] or the understory [37]. In contrast, in a gap existing for six–nine years, the microbial biomass was significantly larger at the gap edge than at the GC or the understory [55,56]. The literature also reports that gap size affects soil microbial communities at different gap positions. When gaps were small, soil total PLFAs were notably higher, and fungi, GP, GN, and AMF were significantly lower in the GC than those at the gap edges. However, when the gap was large, the results were the opposite: total PLFAs were significantly higher, and fungi, GP, GN, and AMF were significantly lower at the gap edges than those in the GC. When the gap was medium–sized, the GP showed no significant differences among the various gap positions [4,25]. In addition, during the sampling period, mid–June, the forest was in the early stages of vigorous vegetation growth, and the biological cycle processes were relatively strong. Thus, a relatively suitable environmental temperature and rich substrate caused the soil microbial biomass to peak [37]. Therefore, the effect of gap position on microbial biomass in this study was representative of the tree–growing season. However, the sampling season causes variations in soil microbial biomass at different forest gap positions [7,37,53]. Therefore, the influence of gap position on litter microbial biomass should be studied in combination with the dynamic changes in soil microorganisms in the future.

The gap size in this study is representative of coniferous forest in the mountainous area of Beijing [45], when the gap age is one year. We observed that different forest gap positions affected the litter microbial community composition. Notably, total PLFAs, GN, and bacteria were significantly greater in the GC than in the GB and CC (Figure 2), which is consistent with the findings of previous research [36]. This trend may occur because the gap center receives a greater intensity and duration of light and rainfall [57]. This results in an increase in the amount of litter DOC, ROC, NH_4^+–N, and NO_3^-–N (Figure 1) and promotes the growth and reproduction of microorganisms. In addition, the RDA demonstrated that increasing litter NH_4^+–N and NO_3^-–N promoted an increase in the microbial population (Figure 5). Compared with the CC, total PLFAs, GP, GN, bacteria, and fungi in the GC and GB were significantly greater, and litter mass was less, which

accelerated litter decomposition at the gap center and edge. Therefore, creating forest gaps during forest management should improve the biological properties of litter and accelerate nutrient cycling in forest ecosystems.

4.2. Effect of Litter Layers on Microbial Community Composition

Litter is an essential component that supports the entry of organic matter into the soil environment and microbial community growth. With litter decomposition, there are substantial variations in litter chemical composition [22–24,58], which lead to changes in the litter microbial biomass, community composition, and functions [27,53]. Different forest floor layers represent different stages of forest floor decomposition. Some researchers have discovered that the amount of total PLFAs, fungi [26,59], GP, GN [60], and bacteria [27] in the undecomposed layer is higher than that in the semi–decomposed layer, but others have reported that the amount of GP, GN, bacteria, ACT, and AMF [59] in the semi–decomposed layer is greater than that in the undecomposed layer. Our results have revealed that the litter microbial community composition in the undecomposed layer was greater than that in the semi–decomposed layer. This finding is attributed to the fact that the undecomposed layer contains more microbial nutrient sources, such as carbon, nitrogen, and phosphorus, than the semi–decomposed layer does [58]. The improvement in resource availability in the undecomposed layer (DOC, ROC, and NH_4^+–N; Figure 1) probably stimulates microorganisms [60], accelerating the litter decomposition rate, further facilitating the nutrient cycling of forest soil ecosystems, and providing favorable conditions for forest plant regeneration. The two–way ANOVA showed that the variations in microorganisms in the litter layers were more prominent than those in gap positions, indicating that microorganisms may be more sensitive to changes in litter layers. Therefore, the role of litter layers should not be ignored in studies of the gap effect.

4.3. Factors Affecting Microbial Community Composition

As litter is a source of energy and nutrients for microbial communities, variations in litter nature inevitably result in variations in microbial communities. The RDA revealed that the shifts in microbial community composition were principally predicted by moisture and BG (Figure 5). Among the measured litter physicochemical properties, LM, NO_3^-–N, TP, and NH_4^+–N were the most relevant factors in determining the composition of the microbial community, and BG, POX + PER, and angle were the major factors among the measured litter enzyme activities and hydrolase–based vector lengths and angles. We found that litter moisture alone explained 94.47% of the overall variance in the microbial community composition (Figure 5). Consistent with our findings, the moisture content was the optimal indicator of microbial activity [30,60–62]. In addition, NH_4^+–N and NO_3^-–N played essential roles in explaining the changes in the structure of microbial functional potential [59].

The activity of BG is related to the conversion of the organic carbon involved in cellulose hydrolysis. Hydrolysis products are the principal sources of energy and nutrients for soil microorganisms [26,27]. We observed that litter BG alone explained 97.93% of the overall variance in microbial community composition (Figure 5). Moreover, the angles of enzyme activities were all greater than 45°, indicating that the content of soil phosphorus was the main nutrient element limiting microorganisms in *P. tabulaeformis* plantations. Consistent with our results, another study [63] found a soil phosphorus deficiency at Badaling Mountain, Beijing. The PLFA method could quickly and effectively quantify shifts in microbial community dynamics at the genus level. With the development of biomonitoring, high–throughput sequencing, community–level physiological profiling, and next–generation DNA sequencing techniques have become widespread [64–66]. Therefore, shifts in litter microbial community under different gap positions should be investigated in combination with other monitoring techniques.

In this study, we investigated how the different gap positions significantly influence forest floor microbial community composition, which provides baseline information for

projects to improve forest quality management and enhance ecosystem services. However, the shift in plant vegetation and root characteristics under different forest gap positions may have a substantial influence on forest floor substrate properties and microorganisms. For future studies, investigations of plant vegetation and root characteristics under different forest gap positions should be performed, and their contribution to microbial communities should be evaluated. In addition, the forest gap duration was only one year and only one gap–size sampling site was used; emphasis should be placed on long–term dynamic monitoring of the influence of different forest gap characteristics on litter microorganisms in the future.

5. Conclusions

In this study, litter microbial community compositions under different gap positions were investigated in Chinese pine forests using the PLFA method. The amounts of GP, GN, bacteria, and fungi were greater in the GC and GB than in the CC, and the DOC, ROC, NH_4^+–N, and NO_3^-–N followed the same trend, indicating that the availability of nutrient sources accelerates the litter decomposition rate, thereby further facilitating microorganism growth. Redundancy analysis demonstrated that shifts in litter microbial community composition were predominantly predicted by litter moisture and BG. Furthermore, we also discovered that the microbial community composition in the undecomposed layer was greater than that in the semi–decomposed layer. Because the variation in forest gap characteristics and microorganisms is dynamic, and this study only analyzed the one–year–old forest gap and was conducted using one sample, further investigations should concentrate on the long–term dynamic monitoring of the influence of the forest gap position on litter microorganisms. Collectively, our study provides guidelines for evaluating the quality of soil in the forest quality management of rocky mountain areas.

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