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Shifts in the Soil Microbial Community and Enzyme Activity Under *Picea crassifolia* Plantations and Natural Forests

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Abstract: Soil microbes are crucial for regulating biogeochemical cycles and maintaining forest ecosystem sustainability; however, the understanding of microbial communities and enzyme activity under natural and plantation forests in plateau regions remains limited. Using soil samples from 15-, 30-, and 50-year-old *Picea crassifolia* plantations and a natural forest (NF) in eastern Qinghai, China, this study assessed physicochemical properties, microbial communities, and enzyme activity across three soil layers. Microbial composition was characterized using the phospholipid fatty acid (PLFA) method, which is sensitive to structural changes. The PLFAs of bacteria, fungi, and actinomycetes accounted for 58.31%–74.20%, 8.91%–16.83%, and 3.41%–10.41% of the total PLFAs in all forests, respectively. There were significant differences between the NF and plantations, with the NF exhibiting higher PLFA abundance and enzyme activities than plantations, except for fungal PLFAs. PLFAs in plantations increased with the plantation age. However, the fungi-to-bacteria ratio was lower in the NF than in plantations. Finally, a redundancy analysis revealed that soil properties influence microbial composition and enzyme functionality significantly. These findings highlight the influence of stand age on microbial communities and structure, offering valuable insights for forest management practices aimed at conserving natural forests.

Keywords: forest age; fungi-to-bacteria ratio; microbial abundance; phospholipid fatty acid analysis; plateau region

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1. Introduction

As an integral ecosystem component, soil is vital in maintaining forest health and the sustainability of forest ecosystems [1]. Soil microbes are essential mediators in the transformation of organic matter and biogeochemical cycles, influencing the overall functionality and resilience of forest ecosystems [2,3]. Microbial communities are sensitive indicators of environmental changes, reflecting shifts in soil conditions and forest management practices [4]. The enzymes produced by soil microbes are pivotal for soil quality, as they facilitate organic matter movement and highlight changes in microbial community composition [5,6]. Hence, gaining insight into the regeneration and progressive development of subterranean microbial communities is pivotal for preserving soil quality and forest ecosystem health [7].

Natural forests composed of indigenous tree taxa that regenerate naturally have higher soil carbon sequestration rates [8], higher biodiversity [9], and enhanced ecosystem func-

tions [10] than plantations with relatively simple stand structures [11]. The shift from natural forests to plantations due to anthropogenic disturbances influences microclimates, plant diversity, and biological microcirculation [12]. Broad-leaved natural forests promote microbial growth and reproduction compared to deciduous larch plantation soil and can support more robust microbial communities [13]. Similarly, a study found that microbial biomass, fungal diversity, and enzyme dynamics decreased during the replacement of natural broad-leaved forests with coniferous plantations [14]. Natural forests have higher enzyme activity than plantations, despite similar soil microbial taxonomic compositions [15]. These results highlight the negative effects of converting natural forests to plantations. However, these results were obtained under different tree taxa and there were taxon-specific impacts on microbial diversity and enzymatic functionality through litter and root exudates influencing soil organic nutrients [16,17]. Processes driving shifts in microbial communities between plantations and natural forests with identical tree taxa remain inadequately understood.

Afforestation contributes to the global protection of native forests. Microbial diversity is integral to advancing afforestation initiatives, as it bolsters soil fertility and facilitates nutrient cycling, thereby promoting the establishment and growth of trees [18]. Moreover, microbial communities enhance trees' capacity to withstand environmental challenges, ensuring the enduring success of afforestation efforts [19]. Forest type and microbe communities are established with forest ecosystem development [20]. Stand age is associated with the soil microbial community; nevertheless, no consensus regarding the precise effects of age was found. Some researchers have revealed that the complexity, abundance, and enzymatic functionality of soil microbial communities tend to enhance with stand age [21–23]. Wang et al. [24] reported that bacterial and fungal populations, along with β-glucosidase activity, initially declined and subsequently increased as stand age increased under plantation conditions. Wu et al. [25] showed that phenol oxidase activity is inversely proportional to stand age. In contrast, no prominent trend in the microbial community composition regarding succession was observed by Odriozola et al. [26]. These findings highlight the impacts of stand age on soil microbial dynamics. Further, the effects may vary among sites and with respect to various factors, including soil type, plantation taxa, and management practices [27]. Soil microbial characteristics are related to sustained forest productivity by impacting soil quality, and the unpredictable changes within microbial community composition across stand ages may undermine its reliability as a soil quality indicator, presenting challenges for sustainable forest management strategies.

The eastern region of the Qinghai Province, situated in the western section and belt of transition from the Loess Plateau to the Qinghai–Tibet Plateau, is ecologically vulnerable because of its distinctive geography and climate, which is further exasperated by human activities altering forest organisms [28]. Natural forest resources have decreased sharply with agricultural exploitation over several decades [29]. To improve the ecological environment and enhance the ecosystem services of forests, the Chinese government has launched the Three North Shelterbelt Project [30,31], Natural Forest Conservation Program, and Grain for Green Program. *Picea crassifolia*, belonging to the cold–temperate conifer ecosystem, is predominant in the Qilian mountains, within the range of 33°35′ N–39°38′ N and 97°43′ E–111°16′ E. It is a common afforestation tree, owing to its important functions in maintaining ecological safety and economic construction [32]. Previous studies of the region have focused primarily on soil moisture [33,34] and soil carbon stocks [35]. Shifts in soil fungal diversity with stand age in *Picea* spp. forests have also been examined [36]. However, research on microbial community abundance, structural composition, and enzyme activity across different stand stages in subalpine regions is limited.

Various soil microbe analysis methods have been developed. Phospholipid fatty acid (PLFA) analyses can identify key soil microbial functional groups [37]. This method demonstrates high sensitivity to fluctuations in the structure of microbial communities, as modifications in the fatty acids of microbial membranes serve as indicators of environmental forces [38]. This study employed PLFA analyses to differentiate soil microbial communities in Qinghai Province. This study aimed to (1) investigate differences in soil microbial communities and enzyme activity across three soil layers between natural forests and *Picea crassifolia* plantations at different stand ages and (2) identify major driving factors for the differences in soil microbial characteristics. These findings provide a scientific foundation for promoting soil microbial health and forest management strategies aimed at conserving forest ecosystems.

2. Materials and Methods

2.1. Site Description and Sampling

This study was conducted in Huzhu Tu Autonomous County, Qinghai Province (36°30′–37°9′ N and 101°46′–102°45′ E; Figure 1a), at an altitude range of 2100–3500 m. This region has an alpine continental mountain climate with monsoonal characteristics. The average annual temperature is 5 °C, annual precipitation is 500 mm, and average annual evaporation is 1260 mm [39]. The original forests of the region were destroyed by prolonged anthropogenic disturbances, with the current vegetation primarily consisting of naturally regenerated forests, including *P. crassifolia*, *Juniperus przewalskii*, *Populus cathayana*, and *Betula albosinensis*, and planted forests, including *P. crassifolia*, *Pinus tabuliformis*, and *Larix gmelinii*. The soil mainly has loess-like sediment parent materials. According to the Chinese Soil Taxonomy, the primary soil type is Typic Hapli–Ustic Iso Humisol.

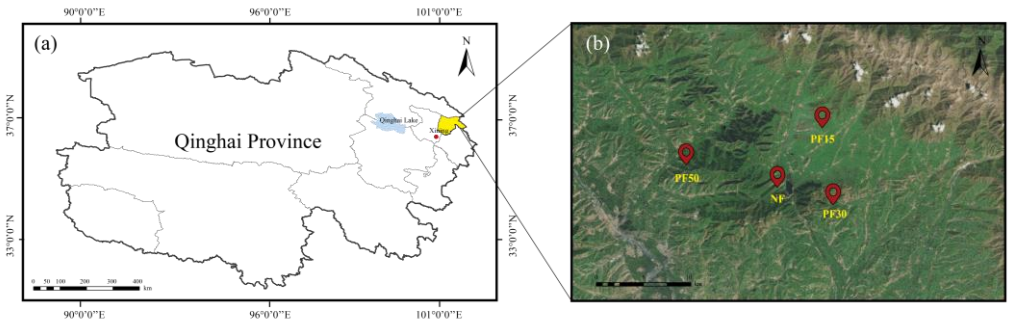

Figure 1. (**a**) Sample site map of Eastern Qinghai Province, China, and (**b**) location of the study area.

Four stand types were evaluated in this study: 15-, 30-, and 50-year-old *P. crassifolia* plantation forests (hereafter abbreviated as PF15, PF30 and PF50, respectively), as well as a nearly 100-year-old natural forest (NF). All were located at similar altitudes, with comparable slope aspects and gradients (Figure 1b). Three plots (20 × 20 m) were selected for each stand type, with a minimum ground interval of 100 m between plots. Geographical data and above-ground vegetation communities were also investigated (Table S1). Within each plot, three 20 × 20 cm subplots were randomly chosen to collect ground litter samples. For each subplot, soil cutting ring samples were collected from three soil layers at three random points and soil samples were collected using the five-point method. Soil from the 0–5 cm (topsoil), 5–10 cm, and 10–20 cm layers was sampled after removing surface litter and combined into composite samples for each layer, yielding 36 soil analysis samples. Each soil sample was divided into three subsamples: one part was kept at −20 °C for a microbial PLFA analysis, one was preserved at 4 °C for dissolved organic carbon and enzymatic activity analyses, and one was dried at ambient temperature for evaluating soil physicochemical characteristics.

2.2. Litter and Soil Properties

2.2.1. Litter Chemical Traits

Total concentrations of carbon (LOC), nitrogen (LTN), and phosphorus (LTP) in the litter were determined using the oxidation method, sulfuric acid digestion, and sulfuric acid digestion–molybdenum antimony colorimetric methods, respectively [40].

2.2.2. Soil Physicochemical Properties

Soil bulk density (SBD) and field water capacity (FWC) were determined using the soil cutting ring method, according to LY/T 1215–1999 (National Forestry Administration of China, 1999) [41]. Soil pH and electrical conductivity (EC) were determined using a PHS-3E pH meter and a conductivity meter. Organic carbon (SOC), total nitrogen (TN), and alkaline hydrolysis nitrogen (AN) were assessed using the oxidation, sulfuric acid digestion, and alkaline hydrolysis diffusion methods, respectively. Dissolved organic carbon (DOC) and nitrogen (DON) were extracted using a 1:5 soil-to-water solution [42]. Active organic carbon (AOC) was assessed using potassium permanganate [43]. Nitrate-nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) levels were determined using a SmartChem 200 Discrete Auto Analyzer (AMS, Rome, Italy). The available phosphorus (AP) was determined using the Olsen method using a spectrophotometer (Purkinje General, Beijing, China).

2.3. Microbial Community Composition

The microbial community was analyzed using signature fatty acid biomarkers. Briefly, soil microbial PLFA was extracted using a chloroform–methanol–phosphate buffer solution [44]. Phospholipids were isolated via solid-phase extraction, methylated with methanolic KOH to form fatty acid methyl esters, and analyzed using a gas chromatograph (Agilent 6890N, Agilent Technologies Inc., Santa Clara, CA, USA) equipped with the MIDI Sherlock MIS 4.5 system (MIDI, Newark, DE, USA). Esterified C19:0 was used as the internal standard, and PLFA abundances were expressed as nmol per gram of dry soil. PLFAs were classified into microbial groups, including bacteria (BAC), fungi (FUN), gram-positive bacteria (GP), gram-negative bacteria (GN), actinomycetes (ACT), and arbuscular mycorrhizal fungi (AMF), based on biomarker specificity and stability (Table S2) [45–48].

2.4. Enzyme Activity

The colorimetric method under optimal reaction conditions was adopted to determine soil enzyme activity using microplate substrates [49]. β-glucosidase (BGL), *N*-acetyl-glucosaminidase (NAG), and phosphatase (ALP) were determined based on *ρ*-nitrophenol (*ρ*NP) substrates in an MUB buffer. Oxidative enzymes were determined in acetate buffer (pH 4.5) with an l-dihydroxyphenylalanine (DOPA) substrate for phenol oxidase (PHO) and DOPA and hydrogen peroxide for peroxidase (PEO). For all enzymes, the soil suspension containing the buffer was mixed and the substrates were added, followed by incubation at 25 °C for a specified time [50]. After incubation, enzyme activity was determined using a microplate reader (TECAN Spark, Männedorf, Switzerland). The results are expressed as μmol·*ρ*NP for hydrolytic enzymes and mmol DOPA for oxidative enzymes converted per gram of dry-weight soil per hour.

2.5. Statistical Analysis

A one-way analysis of variance (ANOVA) with LSD tests ($p < 0.05$) was used to compare variances and evaluate differences in litter and soil properties, microbial communities, and enzyme activities across stand types and soil depths. A two-way ANOVA was used to assess the effects of stand type, soil depth, and their interactions, with results presented as *F*-values and significance levels (** $p < 0.01$; * $p < 0.05$). Statistical analyses were performed

using SPSS 26 (IBM Corporation, Chicago, IL, USA). Redundancy analysis (RDA) in Canoco 5.0 identified drivers of microbial community and enzyme activity changes, and other analyses were conducted using R 4.4.1 software [51].

3. Results

3.1. Litter and Soil Physicochemical Properties

The litter chemical qualities varied markedly among stand types (Table 1). The concentrations of LOC and LTN were highest under the NF. In the plantations, LOC increased significantly with increasing stand age. PF15 exhibited the lowest LTN, whereas the difference between PF30 and PF50 was insignificant. LTP was highest under PF30 and lowest under PF15. The ratio of LOC to LTN (LOC/LTN) did not differ significantly among stands. Therefore, no pronounced differences in litter chemical quality were found among different stand types.

Table 1. Litter chemical properties under different forests.

Stand Type	LOC (g kg^{-1})	LTN (g kg^{-1})	LTP (g kg^{-1})	LOC/LTN
PF15	203 ± 6.24 ^{d}	8.73 ± 0.55 ^{c}	2.31 ± 0.14 ^{d}	23.33 ± 2.06 ^{a}
PF30	227 ± 7.02 ^{c}	9.97 ± 0.57 ^{bc}	3.84 ± 0.08 ^{a}	22.90 ± 1.69 ^{a}
PF50	275 ± 12.50 ^{b}	11.17 ± 0.31 ^{ab}	2.85 ± 0.10 ^{c}	24.65 ± 0.47 ^{a}
NF	332 ± 20.40 ^{a}	12.67 ± 1.46 ^{a}	3.41 ± 0.08 ^{b}	26.54 ± 4.02 ^{a}

Notes: LOC, LTN, and LTP stand for litter organic carbon, litter total nitrogen, and litter total phosphorus, respectively. PF15, PF30, PF50, and NF stand for 15-, 30-, 50-year-old *P. crassifolia* plantation forests, and natural forest, respectively. Values are presented as means ± standard error. Different lowercase letters indicate significant differences among stand types ($p < 0.05$).

The results indicate that stand type affected soil properties (Table 2). SBD and pH were highest under PF15 and lowest under the NF. FWC, EC, SOC, NO_3^--N, and NH_4^+-N were higher under the NF than in the plantations. FWC and NO_3^--N increased significantly with increasing plantation age. The AOC and DOC concentrations peaked under PF50, markedly exceeding the levels observed under PF30 and PF15. The differences in TN, AN, and DON concentrations between PF50 and the NF were small, and these values were higher than those in early plantations. The soil AP concentrations under PF30 and the NF were notably higher than those under PF50.

Table 2. Two-way ANOVA of the effects of stand types, soil depths, and their interactions on soil properties.

Factor	SBD	FWC	pH	EC	SOC	AOC	DOC	TN	AN	DON	NO_3^--N	NH_4^+-N	AP
Stand (S)	320.01 **	1623.65 **	50.51 **	134.85 **	200.06 **	5.89 **	16.26 **	17.22 **	182.91 **	147.10 **	23.62 **	8.42 **	3.99 *
S (sig)	a, b, c, d	d, c, b, a	a, b, d, c	b, c, b, a	c, c, b, a	c, bc, a, ab	c, bc, a, b	b, b, a, a	b, b, a, a	b, b, a, a	d, c, b, a	b, b, b, a	ab, a, b, a
Layer (L)	47.76 **	258.38 **	13.95 **	1.52	12.92 **	4.09	1.99 *	6.61 **	11.63 **	12.99 **	2.23	3.40	0.33
L (sig)	c, b, a	a, b, c	b, b, a	a, a, a	a, b, c	a, a, a	a, a, b	a, a, b	a, a, b	a, a, b	a, a, a	a, ab, b	a, a, a
S × L	3.13 *	55.17 **	1.74	2.03	1.34	0.53	0.84	0.80	1.56	1.094	0.92	0.52	1.43

Notes: SBD, FWC, EC, SOC, AOC, DOC, TN, AN, DON, NO_3^--N, NH_4^+-N, and AP stand for soil bulk density, field water capacity, electrical conductivity, soil organic carbon, active organic carbon, dissolved organic carbon, total nitrogen, alkaline hydrolysis nitrogen, dissolved organic nitrogen, nitrate nitrogen, ammonium nitrogen, and available phosphorus, respectively. ** $p < 0.01$; * $p < 0.05$. Different letters in the table represent significant ($p < 0.05$) differences, with a > b > c > d. S (sig) and L (sig) denote significant differences among different stand types (PF15, PF30, PF50, and NF) and three soil layers, respectively.

Soil properties were also significantly influenced by soil depth, particularly SBD, FWC, and SOC. SBD decreased with soil depth, whereas FWC and SOC showed the opposite trend. DOC, TN, AN, and DON were significantly reduced in the 10–20 cm layer compared to upper soil layers (0–5 cm to 10 cm). Stand type exerted a more pronounced influence on soil properties compared to soil layer, as indicated by *F*-statistics. Additionally, the interaction between stand type and soil depth significantly affected SBD and FWC.

3.2. Soil Microbial Community

The BAC PLFA dominated the total PLFAs in all samples, accounting for 58.31%–74.20%, followed by FUN (8.91%–16.83%) and ACT (3.41%–10.41%) PLFAs. The total PLFAs and microbial community structure showed significant variance among the four stand types (Figure 2). Except for the FUN PLFA, PLFA abundances under the NF were significantly higher than those in the plantations in all three layers. In the topsoil, the total PLFAs under PF15, PF30, and PF50 were reduced by 85.34%, 60.01%, and 33.84%, respectively, compared with those under the NF. The FUN PLFA in the topsoil was most abundant under PF50, with 442.64% and 65.46% higher estimates than those under PF15 and PF30, respectively. Beneath the topsoil, the PLFA quantity showed a similar trend in the comparison among stand types. The GP PLFA showed a significant difference among the four stands in all three soil layers.

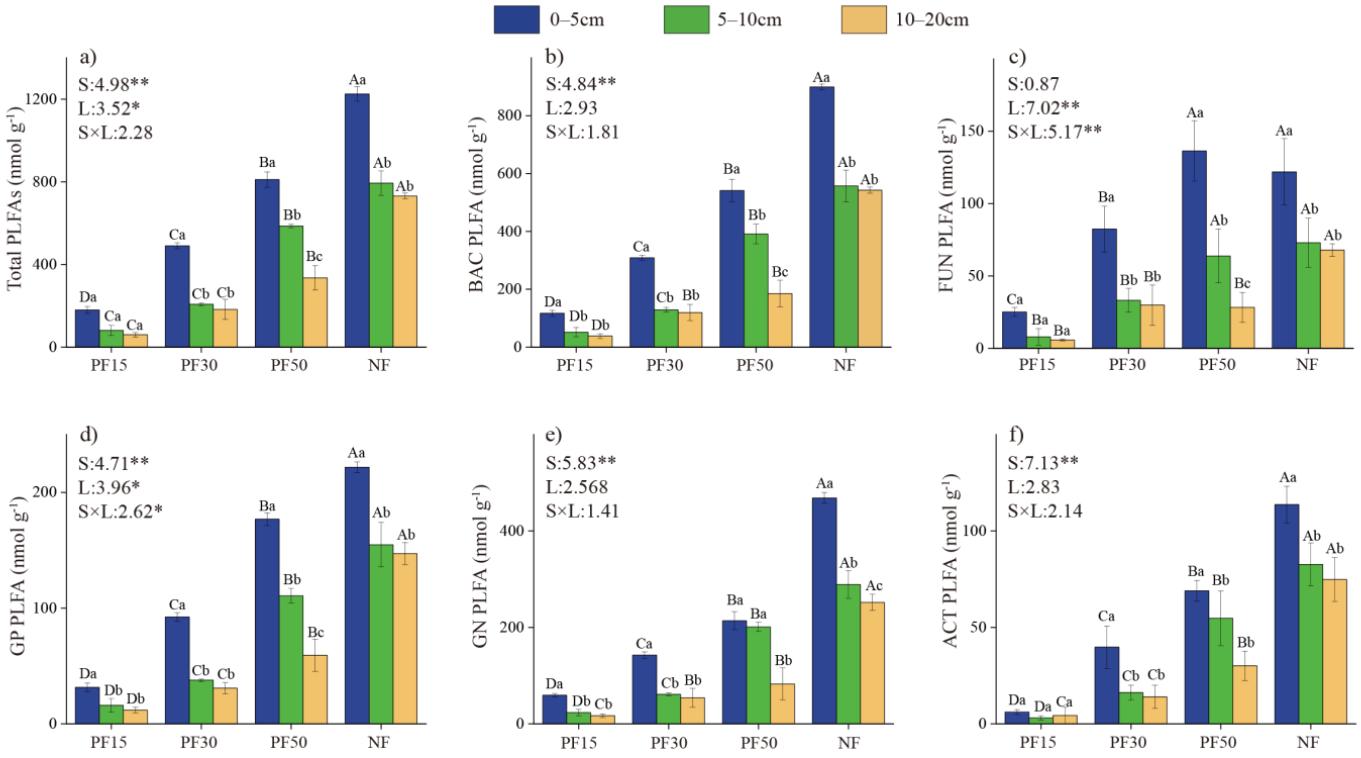

Figure 2. *Cont.*

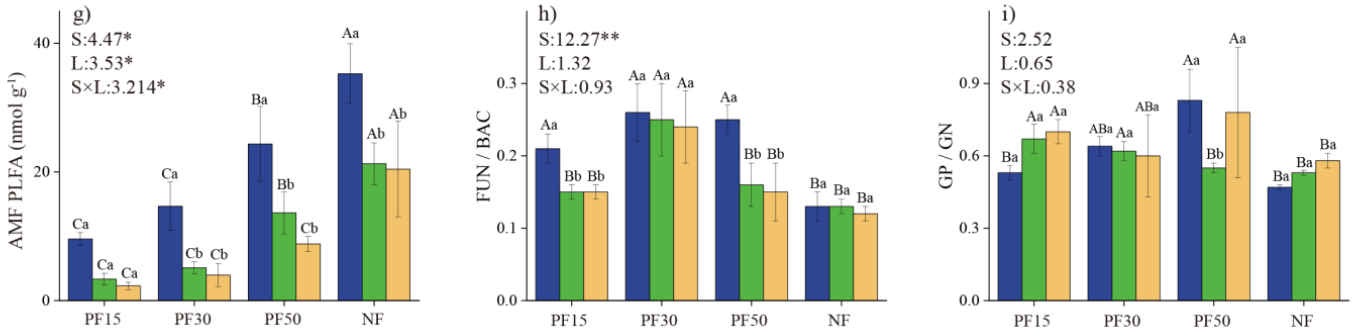

Figure 2. Microbial phospholipid fatty acid (PLFA) abundances and community structure under four stand types: (**a**) total PLFAs, total concentration of PLFAs; (**b**) BAC PLFA, bacterial PLFA; (**c**) FUN PLFA, fungal PLFA; (**d**) GP PLFA, gram-positive PLFA; (**e**) GN PLFA, gram-positive PLFA; (**f**) ACT PLFA, actinomycetal PLFA; (**g**) AMF PLFA, arbuscular mycorrhizal fungal PLFA; (**h**) FUN/BAC, the ratio of fungal PLFA to bacterial PLFA, and (**i**) GP/GN, the ratio of gram-positive PLFA to gram-negative PLFA. Error bars indicate the standard error of the mean (n = 3). Distinct superscript upper- and lowercase letters indicate significant differences among stand types at the same soil depth, as well as among three soil depths for the same stand type, respectively. PF15, PF30, PF50 and NF stand for 15-, 30-, 50-year-old *P. crassifolia* plantation forests and natural forest, respectively. S, L, and S × L indicate the F-values for the effects of stand type (S), soil depth (L), and their interaction (S × L) based on two-way ANOVA. ** $p < 0.01$; * $p < 0.05$.

The total PLFAs and all microbial community PLFAs decreased with layer addition under different stands. Except for PF15, the PLFA abundances in the topsoil were substantially greater than those in the 5–10 cm and 10–20 cm soil layers. Moreover, in the topsoil layer, the FUN/BAC ratio under the NF was significantly lower than those under the plantations and was highest under PF30. Similarly, the FUN/BAC ratio under PF30 was significantly higher than those under PF15, PF50, and the NF in both layers. The ratio of GP/GN varied from 0.47 to 0.83, while the NF showed a lower GP/GN than those in plantations. In the topsoil, the GP/GN ratio under PF50 was significantly higher than those under PF15 and NF. However, in the lower layer, the GP/GN ratio under PF15 was higher than that under the NF.

A two-way ANOVA showed that stand type had a marked effect on the abundances of BAC, GP, GN, ACT, AMF, and total PLFAs. Except for the FUN PLFA, the effect of stand type on total and other PLFAs was greater than that of soil depth. The abundance of PLFAs increased significantly with stand age. Stand type influenced the FUN/BAC ratio significantly; however, it had no notable effect on the GP/GN ratio. FUN and AMF PLFAs were influenced by the cooperation of stand age and soil depth.

3.3. Soil Enzyme Activity

Soil enzyme activity differed significantly among stand types (Table 3). The BGL levels were significantly higher under PF50 and the NF than under PF15 and PF30. ALP and PHO levels were higher under the NF than under plantations and showed a significant increase with increasing stand age in plantation soils across all three layers. Enzyme activity was highest in the topsoil, followed by the 5–10 cm and 10–20 cm soil layers. The two-way ANOVA showed that stand type considerably impacted five kinds of enzyme activities; in particular, enzyme activity levels increased significantly with increasing plantation stand age. Stand type had stronger effects on ALP, PHO, and PEO levels than on levels of other enzymes. It had weaker effects on BGL and NAG activity levels than those of soil depth. The interaction between stand type and soil depth had a significant effect on NAG and ALP activity levels.

Table 3. Soil enzyme activity under *Picea crassifolia* forests with different stand types (mean ± standard error).

Stand Type	Soil Depth (cm)	BGL	NAG	ALP	PHO	PEO
PF15	0–5	2.52 ± 0.26 ^{Ca}	0.82 ± 0.10 ^{Da}	1.25 ± 0.14 ^{Da}	0.66 ± 0.10 ^{Da}	0.99 ± 0.10 ^{Ca}
PF30	0–5	3.28 ± 0.18 ^{Ba}	1.35 ± 0.15 ^{Ca}	4.46 ± 0.48 ^{Ca}	1.20 ± 0.07 ^{Ca}	1.89 ± 0.06 ^{Ba}
PF50	0–5	4.11 ± 0.12 ^{Aa}	1.89 ± 0.06 ^{Ba}	8.79 ± 0.57 ^{Ba}	1.87 ± 0.10 ^{Ba}	2.04 ± 0.12 ^{Ba}
NF	0–5	4.46 ± 0.32 ^{Aa}	2.53 ± 0.28 ^{Aa}	10.74 ± 0.64 ^{Aa}	2.68 ± 0.17 ^{Aa}	2.64 ± 0.18 ^{Aa}
PF15	5–10	1.52 ± 0.39 ^{Cb}	0.50 ± 0.05 ^{Cb}	0.85 ± 0.10 ^{Db}	0.47 ± 0.10 ^{Dab}	0.84 ± 0.21 ^{Da}
PF30	5–10	2.34 ± 0.13 ^{Bb}	1.23 ± 0.17 ^{Ba}	2.23 ± 0.13 ^{Cb}	0.72 ± 0.06 ^{Cb}	1.57 ± 0.15 ^{Cb}
PF50	5–10	3.35 ± 0.18 ^{Ab}	1.30 ± 0.18 ^{ABb}	6.65 ± 0.56 ^{Bb}	1.42 ± 0.33 ^{Bb}	1.86 ± 0.03 ^{Ba}
NF	5–10	3.47 ± 0.32 ^{Ab}	1.53 ± 0.15 ^{Ab}	8.22 ± 0.24 ^{Ab}	2.12 ± 0.15 ^{Ab}	2.19 ± 0.08 ^{Ab}
PF15	10–20	0.49 ± 0.02 ^{Bc}	0.32 ± 0.08 ^{Cb}	0.57 ± 0.10 ^{Db}	0.27 ± 0.15 ^{Db}	0.50 ± 0.12 ^{Cb}
PF30	10–20	0.84 ± 0.07 ^{Bc}	0.71 ± 0.02 ^{Bb}	1.45 ± 0.15 ^{Cc}	0.62 ± 0.04 ^{Cb}	1.17 ± 0.17 ^{Bc}
PF50	10–20	1.31 ± 0.17 ^{Ac}	0.80 ± 0.07 ^{Bc}	5.67 ± 0.27 ^{Bc}	1.14 ± 0.34 ^{Bb}	1.31 ± 0.10 ^{Bb}
NF	10–20	1.57 ± 0.24 ^{Ac}	1.10 ± 0.15 ^{Ac}	7.47 ± 0.32 ^{Ac}	2.11 ± 0.07 ^{Ab}	1.75 ± 0.10 ^{Ac}
F-value	Stand (S)	98.08 **	106.87 **	906.33 **	205.76 **	170.28 **
	Layer (L)	381.16 **	124.35 **	152.26 **	36.42 **	83.93 **
	S × L	3.55 n.s.	8.81 **	9.46 **	1.51 n.s.	1.09 n.s.

Notes: BGL, NAG, ALP, PHO, and PEO stand for β-glucosidase, *N*-acetyl-glucosaminidase, phosphatase, phenol oxidase, and peroxidase, respectively. Distinct superscript upper- and lowercase letters indicate significant differences among stand types at the same soil depth, as well as among three soil depths for the same stand type, respectively. PF15, PF30, PF50, and NF stand for 15-, 30-, 50-year-old *P. crassifolia* plantation forests and natural forest, respectively. S, L, and S × L indicate the *F*-values for the effects of stand type (S), soil depth (L), and their interaction (S × L) based on two-way ANOVA. ** $p < 0.01$; n.s.: not significant.

3.4. Correlation Analyses

Figure 3a showed that RDA1 and RDA2 explained approximately 95.9% of the variation in microbial communities. The abundances of total PLFAs and microbial community PLFAs were positively correlated with LOC, LTN, LTP, FWC, SOC, TN, AN, AOC, DON, NO_3^--N, and NH_4^+-N, and were negatively correlated with SBD and pH. The FUN/BAC and GP/GN ratios were negatively correlated with soil chemical properties. A pseudo-canonical test showed that FWC made the largest contribution to soil microbial communities, with an explanatory rate of 87.4% (Table 4), followed by SBD, SOC, LOC, AN, DON, and other soil properties.

Table 4. Variation explained by various terms in RDA of soil microbial communities and abiotic factors.

Factor	Explains %	Pseudo-F	*p*
FWC	87.4	237	0.002
SBD	84.4	184	0.002
SOC	81.4	148	0.002
LOC	72.8	91	0.002
AN	71.5	85.4	0.002
DON	69.3	76.7	0.002
LTN	63.3	58.7	0.002
TN	53.9	39.8	0.002
LOC/LTN	52.5	37.5	0.002
NO_3^--N	51.5	36.1	0.002
EC	49.9	33.9	0.002
pH	44.7	27.5	0.002
NH_4^+-N	28.2	13.3	0.002
AOC	18.4	7.7	0.006
DOC	15.8	6.4	0.016
LTP	10.7	4.1	0.062
AP	<0.1	<0.1	0.962

Notes: Abbreviations are as defined in Tables 1 and 2.

Similar to the soil microbial communities, RDA1 and RDA2 together accounted for approximately 96.19% of the variation in soil enzyme activity (Figure 3b). LOC, LTN, LTP, FWC, SOC, TN, AN, AOC, DOC, NO_3^--N, and NH_4^+-N were positively related to

enzyme activity and negatively correlated with LOC/LTN, SBD, and pH. Among these soil properties, SBD was the principal factor, explaining 88.7% of the variation (Table 5). The main determinants of differences in microbial community structures and enzymatic activity levels were similar, including SBD, FWC, SOC, AN, and DON.

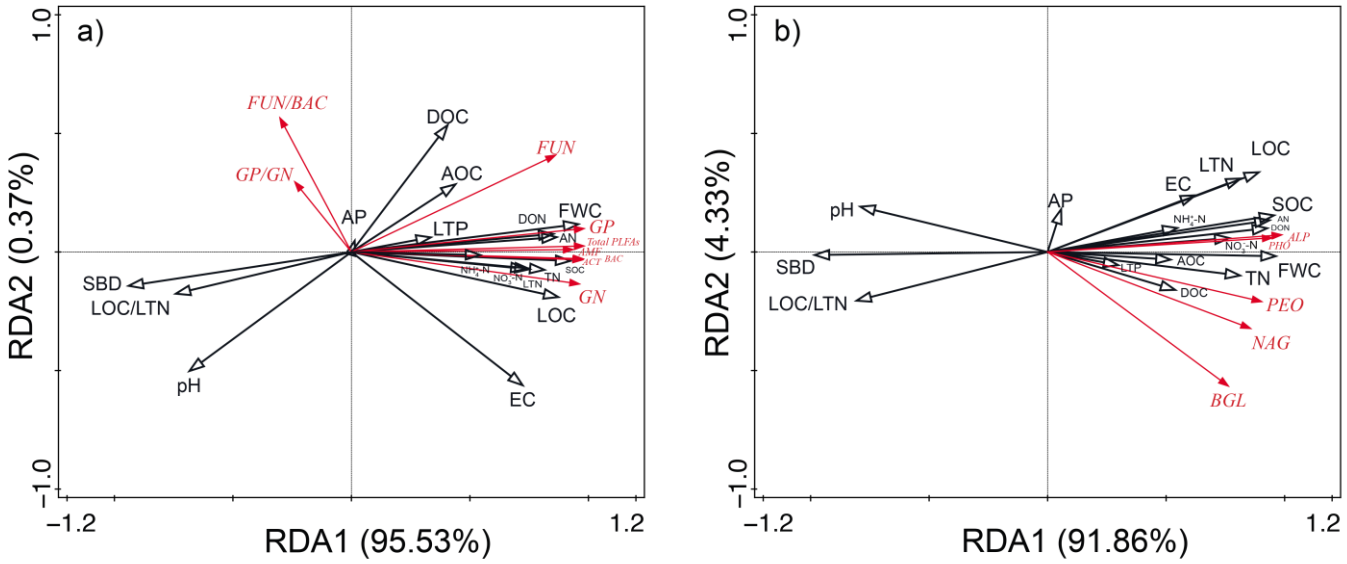

Figure 3. RDA of (**a**) soil microbial communities and abiotic factors, with black arrows representing abiotic factors and red arrows representing microbial communities, and (**b**) soil enzyme activities and abiotic factors, with black arrows indicating abiotic factors and red arrows indicating enzyme activities. Abbreviations are as defined in Tables 1 and 2 and Figure 2.

Table 5. Variation explained by various terms in RDA of enzyme activity and abiotic factors.

Factor	Explains %	Pseudo-F	*p*
SBD	88.7	266	0.002
FWC	85	193	0.002
SOC	83.9	177	0.002
AN	80.5	141	0.002
DON	78.8	127	0.002
LOC	73.4	93.9	0.002
LTN	61.4	54	0.002
TN	60.6	52.2	0.002
LOC/LTN	59.9	50.9	0.002
pH	57.5	46	0.002
NO_3^--N	54	40	0.002
EC	35.9	19	0.002
NH_4^+-N	27.5	12.9	0.002
DOC	26.8	12.4	0.002
AOC	24.7	11.2	0.008
LTP	8.4	3.1	0.084
AP	0.5	0.2	0.792

Notes: Abbreviations are as defined in Tables 1 and 2.

Based on Pearson's correlation coefficients, different microbial communities and enzyme activity levels were sensitive to changes in various abiotic factors (Figure 4). LOC, LTN, SBD, FWC, SOC, AOC, TN, AN, DON, NO_3^--N, and NH_4^+-N were significantly related to PLFA abundance and enzyme activity levels.

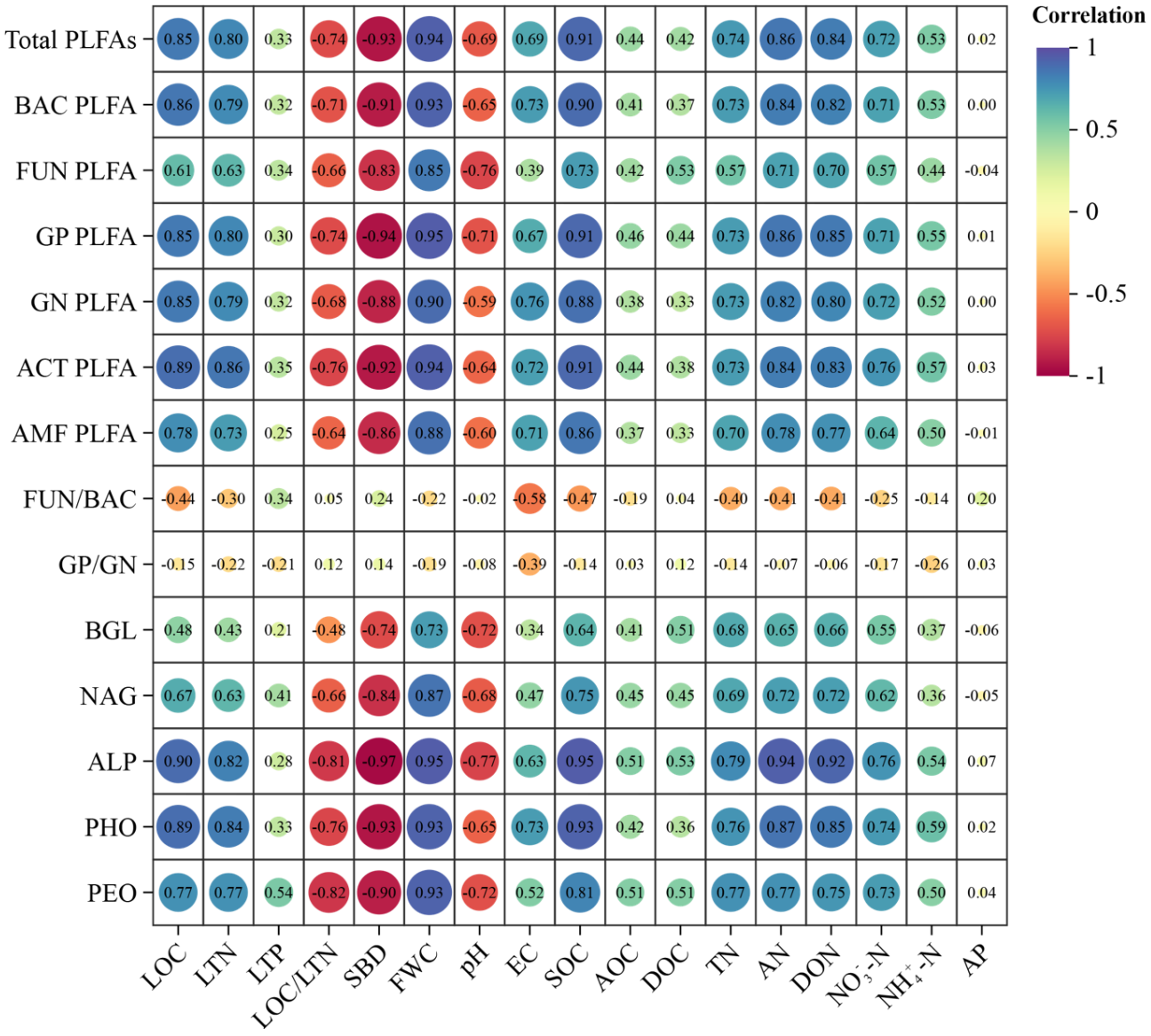

Figure 4. Heatmap of Pearson correlation coefficients between abiotic factors and soil microbial community and enzyme activities. Color intensity represents the strength of the correlation, with blue indicating a positive correlation and red indicating a negative correlation.

4. Discussion

4.1. Variation in Soil Microbial Community and Enzyme Activity Under Various Stand Types

The forest ecological environment can influence microbial community features. Natural forests have an elevated abundance of microbes compared with that in plantations. Monkai et al. [52] demonstrated that natural forests have a higher biomass, estimated as all microbial PLFAs, than plantations. Zi et al. [45] found that *P. crassifolia* plantations tended to have simpler microbial community compositions than natural forests. A similar result was observed in this study. The microbial PLFA, representing BAC, FUN, GP, GN, AMF, and ACT, was markedly more abundant in the natural forest compared to the plantations. Our findings align with prior research, showing that natural forests typically exhibit higher enzyme activity levels than those of plantations [12,53].

Soil microbial communities and enzymatic functions exhibit notable variations across different plantation ages. Kang et al. [21] reported a significant increase in PLFA abundance and enzyme activity levels, such as BGL and ALP levels, with advancing forest age,

potentially attributed to the progressive amassing of surface litter and roots over time. The soil fungal community composition within subalpine *Picea asperata* plantations gradually converged with that of natural forests as time progressed [36]. These past results support our findings that as the stand age of the *P. crassifolia* plantation increased, the abundance of soil microbes and enzyme activity increased significantly. This implies that forest evolution varies, driving the advancement of soil microbial communities. No soil degradation was observed as the stand age increased. Therefore, forest management can minimize human intervention.

The FUN/BAC ratio is an indicator of the stability of soil microbial communities [54] and reflects alterations in their structural dynamics [55], with a higher ratio indicating a more stable microbial community [56]. Natural forests have higher FUN/BAC ratios and more resilient microbial communities than plantations and the ratio increases significantly with increasing plantation age because of the accumulation of fungal substrates [13,57,58]. However, in this study, plantations had higher FUN/BAC ratios than the natural forest, with the highest observed in the 30-year-old plantation. These findings partially support those of Zi et al. [45], who found that plantations exhibited a higher FUN/BAC ratio than natural forests.

The GP/GN ratio was utilized to evaluate the relative carbon availability for soil bacterial communities, reflecting energy constraints within natural ecosystems. GN bacteria predominantly metabolize easily degradable carbon derived from plant materials, whereas GP bacteria rely on more recalcitrant carbon sources originating from soil organic matter [27]. GP bacteria tend to dominate under nutrient-poor conditions and can withstand environmental stress [59]. Natural forests had a higher GP/GN ratio than plantations [12]. This study revealed no significant variation in the GP/GN ratio when comparing natural forests and plantations. This means that microbes may acclimatize to the plateau in a cold–temperate environment where the contrast between GP and GN bacteria is weak during the initial stages of stand development.

4.2. Driving Factors for the Soil Microbial Community and Enzyme Activity

The growth and reproduction of soil microbes and their induction require suitable spatial and nutrient conditions such as water content, pH, carbon, and nitrogen [60]. Based on the RDA analysis (Figure 3) and Pearson correlation coefficients (Figure 4), microbial community composition and enzymatic functionality were profoundly affected by abiotic factors, such as litter quality and soil physiochemical properties.

BD and FWC, as soil physical properties, contributed to shaping soil microbial community characteristics. A higher BD reduces pore space and limits the diffusion of moisture [61], which is an essential physiological resource for microbial cellular functions [62]. An increase in soil water is favorable for the growth and reproduction of soil microbes [63]. It also promotes microbial activities because it supplies the necessary conditions for microbial enzyme synthesis [64]. In contrast, a reduction in soil moisture in forests significantly decreases microbial community abundance [65]. These results align with our observation of a direct correlation between FWC and microbial PLFA. The study area has a high evaporation rate, and soil microbes are subjected to drought stress and are sensitive to shifts in moisture. Therefore, the rise in FWC with advancing stand age enhanced the soil microbial community and enzymatic activity in *P. crassifolia* natural forests and its plantations.

Organic C and N contents have been recognized as key determinants of microbial structure and enzyme activity in forest soils [66,67]. Carbon provides energy and substrates for microbial reproduction [68]. Nitrogen is essential for protein synthesis and other metabolic processes [69]. The RDA showed that SOC and AN accounted for up to 70% of the variation in microbial communities (Table 4) and enzyme activity levels (Table 5).

This implies that SOC and AN are the main factors influencing microbial communities and their functions. Increases in C and N contents enhance microbial PLFA abundance, particularly by promoting the growth of microbes [70,71], consistent with our results. A higher nutrient content can stimulate enzyme activity by supporting greater microbial biomass [72]. Higher soil enzymatic activities, such as BGL, in natural forests result from diverse understory vegetation and complex litter layers, driving carbon and nitrogen cycling processes essential for soil fertility and ecosystem stability [73]. The litter input supplies essential organic carbon and nutrients. As stand age increases, significant shifts can occur in the microenvironmental properties of both litter and soil [74], enhancing microbial processes and reshaping soil microbial communities [75]. With increasing forest age in plantations, the accumulation of litter accelerates the build-up of soil nutrients and increases microbial community dynamics and functionality, thereby promoting more robust carbon and nitrogen cycling [76]. Lucas-Borja et al. [77] found that soil C and N contents in young forest stands were lower than those in older forest stands. Similar findings were obtained in *P. crassifolia* plantations in the present study.

Reductions in soil moisture and C and N inputs from litter generally limit microbial growth and reproduction. For example, Yao et al. [47] found that nutrient and environmental gradients resulted in a reduction in the abundance of the soil microbial community. This pattern was consistently observed throughout the soil profile in the present study, with the topsoil having the highest microbial community abundance and enzyme activity, both of which decreased with increasing depth.

The FUN/BAC ratio in plantations exceeded that of natural forests, contrasting with the results of many studies. EC was identified as a key edaphic factor in regulating the microbial community structure, exhibiting an inverse relationship with the FUN/BAC ratio (Figure 4). These results agreed with those of Kamble et al. [78]. Elevated EC enhances the osmotic potential of water, adversely impacting microbial survival [79]. Bacterial communities exhibit greater sensitivity than fungal communities [80] and fungi decrease with increasing EC while bacterial residues increase [81]. This indicates that the dominant role of fungi in organic matter transport in *P. crassifolia* natural forests has declined. This is because the contents of cellulose and hemicellulose, the main substrates decomposed by fungi [82], decrease significantly with an increasing degree of litter decomposition [83]. Therefore, natural forests with a higher EC provide a better environment for bacteria. Because the effects of LOC and LTN on BAC are greater than those on FUN, the FUN/BAC ratio decreases with increasing nutrient levels during the conversion from plantations to natural forests [84]. In addition, the FUN/BAC ratio showed negative correlations with the C and N contents and soil moisture influenced the FUN/BAC ratio significantly (Figure 4). These findings suggest that multiple environmental factors drive shifts in the FUN/BAC ratio, highlighting the critical role of fungi function diversity in maintaining forest ecosystem health [85]. Advanced methods such as metagenomics could provide more detailed insights into the shift mechanisms of fungi by directly identifying microbial taxa and functional genes [86].

In this study, the influences of abiotic factors on GP and GN bacteria generally showed consistent trends and litter or soil parameters were not related to the GP/GN ratio. The key parameters must be determined. Litter was an essential nutrient reservoir for tree development and supplied both energy and nutrients to support microbial growth and activity within forest soils. LOC and LON can promote microbial community abundance in soil, though the influence of the LOC/LON ratio on these microbial populations was relatively limited. Soil microbial properties change during the year and with plant growth. To gain a comprehensive understanding of microbial processes and their ecological significance, future research should explore C and N dynamics during litter decomposition using

advanced methods such as metagenomics to reveal microbial functional roles in nutrient cycling. In addition, seasonal sampling is essential to capture microbial dynamics and their ecosystem contributions.

5. Conclusions

The shift in microbial communities between plantations and natural forests of *P. crassifolia* was investigated using the PLFA method. The BAC PLFA dominated the total PLFAs, followed by FUN and ACT PLFAs. Except for the FUN PLFA, the abundance of taxa based on PLFAs was significantly higher in the natural forest than in the plantations. The abundance of the soil microbial community exhibited a significant increase with the advancing plantation stand age. The FUN/BAC ratio varied significantly with stand type, with higher values in plantations. The GP/GN ratio did not change significantly during conversion. Soil enzyme activity levels differed significantly between the natural forest and plantations and increased with plantation age. The soil layer had a significant effect on total PLFA, mainly on FUN, GP, and AMF PLFAs. In natural forests, the PLFA abundance in the topsoil was higher than those in the 5–10 cm and 10–20 cm layers. SBD, FWC, LOC, SOC, and AN emerged as the primary driving factors in soil microbial communities and enzymatic activity across different forest types. Overall, plantation stand age and the conversion of natural forests and plantations influence microbial communities and their structure. Conserving natural forests and implementing adaptive management practices in plantations are vital for maintaining high microbial abundance and functional diversity. The results serve as a scientific basis for improving soil quality and ecosystem sustainability in afforestation efforts, particularly in regions dominated by *P. crassifolia*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f16010014/s1, Table S1: Study plot information; Table S2: Characterization of PLFA markers of various microbial community composition.

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