

Article

Responses of Soil Microbial Community Composition and Enzyme Activities to Land-use Change in the Eastern Tibetan Plateau, China

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Abstract: The land-use change is a major determinant influencing ecosystem carbon (C) patterns and nutrient cycling in subalpine forests in the Eastern Tibetan Plateau. While some results have been obtained in relation to the influence of land-use change on aboveground components, less is known about the belowground microbial communities and related processes. We assessed the structure and function of soil microbial communities following land-use change from old-growth forest (OF) to secondary forest (SF), plantation forest (PF), and grass land (GL) in the Eastern Tibetan Plateau, China. Phospholipid fatty acid profiles and enzyme activity analysis were used to determine the composition and activities of microbial communities, respectively. Significant differences in physicochemical characteristics, microbial communities and extracellular enzyme activities in soils under different land uses were observed in this study. OF and SF showed the highest soil organic C (SOC), total nitrogen (TN) and pH, followed by PF, and then for GL. Whereas PF showed the highest total phosphorus (TP) compared with the other land uses. The anaerobic bacteria, and saprotrophic and ectomycorrhizal (SEM) fungi were significantly higher in OF and SF when compared to GL. The actinobacteria increased by 9.4% and decreased by 6.3%, respectively, after land-use changes from OF to SF and PF. Land-use changes from OF to PF and GL shifted enzyme production toward a decrease in β -glucosidase and a significant increase in polyphenoloxidase related to depolymerization of recalcitrant organic compounds. Anaerobic bacteria, arbuscular mycorrhizal fungi and SEM fungi were positively correlated with SOC and TP, Gram+ bacteria were correlated with C/P and N/P ratio. N-acetylglucosaminidase activity was negatively correlated with anaerobic bacteria, while polyphenoloxidase activity was positively related to actinobacteria. Furthermore, redundancy analysis revealed that the microbial community composition was primarily regulated by TN and pH. This suggested that altered land-use type initiated changes in the physicochemical characteristics of the soils, which affected the composition of microbial communities and microbial enzyme activities related to nutrient cycling in this area. This provides a scientific basis for the influence mechanism of land use on composition and function of microbial communities, as well as the rational utilization and management of land resources.

Keywords: microbial community composition; extracellular enzyme activity; phospholipid fatty acid; soil physicochemical properties; subalpine forest; land-use change

1. Introduction

Interactions between aboveground and belowground communities may strongly affect ecosystem functioning by regulating plant community dynamics and biogeochemical processes [1,2]. Belowground, soil microbial communities are treated as decomposers of organic materials, playing important roles in mediating carbon (C) and nitrogen (N) processes and nutrient availability that are critical for the growth and maintenance of plants [3,4]. Aboveground plant communities can substantially alter composition, diversity and functions of soil microbial communities by changing root system, rhizodeposition, litter chemistry, canopy structure, and subsequent influences on soil microclimate conditions and physicochemical properties [5,6].

In the human-influenced landscapes, land-use changes are accompanied by changes in aboveground plant communities and soil characteristics from which aboveground and belowground interactions develop [7]. It has been recognized that such land-use driven change is a major determinant influencing soil microbial community [8,9]. Slight changes in microbial community structure and specific microbial functions, such as extracellular enzyme activities, have been considered to alter the processes of C and N cycling [10]. Deeper understanding of the responses of soil microbial communities and processes in relation to land-use change therefore would greatly benefit land management and ecosystem restoration.

Recent studies, focused mainly on forest conversion or land-use change, have reported the impacts on the composition and activities of soil microbial communities [1,11–15]. ~~However, there appears to be no consistent effects of land-use change.~~ Liu et al. [14], for example, investigated the influence of forest regeneration patterns on belowground microbial community composition and activities and found that soil enzyme activities significantly varied among forest types while bacterial and fungal community did not vary significantly. Ahmed et al. [11] showed that soil microbial properties and enzyme activities differed significantly among land-use types. Krashevskaya et al. [13] examined the effects of land-use change in terms of conversion from rainforests into rubber plantations and observed significant increase in the abundance of arbuscular mycorrhizal (AM) fungi and a decrease in bacteria abundance. Guo et al. [12] found significant changes in the relative abundance of anaerobic bacteria and AM fungi (16:1w5c) due to conversion of native broadleaf forests to mixed plantations, or to bamboo forests. A recent study ~~conducted in an oak forest showed persisted for more than 100 years reported~~ that both microbial biomass and enzymatic activity levels were distinctly lower in ancient forests when compared to historically cultivated sites [1]. In contrast, there have been no significant differences observed in microbial community composition or enzymatic activities in the “Montado ecosystem” [16] or between shrublands and broadleaf forests over 27 years of reforestation [17].

The subalpine forest ecosystems in the Eastern Tibetan Plateau located at the transition zone from Qinghai-Tibetan plateau to Sichuan basin, could be very sensitive to global climate change, with important consequences for biodiversity conservation and the global C and N balance. In the last century, the subalpine regions in southwestern China were largely covered by old-growth forest (OF) dominated by firs. With the widespread commercial logging and increased human activities during the second half of the 20th century, OF were deforested and replaced by natural secondary forest (SF), plantation forest (PF), or grass land (GL). Recently, forest restoration has been emphasized to prevent against any further deforestation disturbances in this region. Therefore, studies on belowground communities in response to land-use change can provide a good opportunity to improve the understanding of C patterns and nutrient cycling in the subalpine forests. To date, studies on land-use change in this region have focused mainly on aboveground components, with less focus on soils and belowground community processes [18–21]. The size, structure, and function of microbial communities in soils may rapidly response to any changes in plant and soil characteristics because of different nutrient availability, substrate’s quality and

quantity, root exudates, and rhizodeposits [22–24]. However, soil microbial communities and related processes of subalpine land-use changes in the Eastern Tibetan Plateau were rarely studied.

This study aimed to assess potential effects of different land-use types (OF, SF, PF, GL) on (i) soil physicochemical characteristics and microbial community composition, and (ii) microbial extracellular enzyme activities. Furthermore, (iii) can we explain any variation in microbial community composition through the variations in soil physicochemical properties? We hypothesized that soil microbial community composition and extracellular enzyme activities would vary among land-use types. Specifically, we expected that differences in microbial community composition can be explained by differences in soil physicochemical properties.

2. Materials and methods

2.1. Study Site

This study was conducted in the Miyaluo Experimental Forest Region (31°24′–31°55′ N, 102°35′–103°04′ E), located in Li County, Sichuan Province and in the Eastern Tibetan Plateau, China. This region has a typical subalpine climate (cold winter and cool summer), and altitude ranges from 2700 to 3600 m above sea level (a.s.l.). Mean air temperatures in January and July are −8 °C and 12.6 °C, respectively, and annual mean air temperature is 6.1 °C. Annual accumulated temperature (≥ 10 °C) is 1200–1400 °C. Annual mean rainfall is approximately 864 mm, concentrated (about 70.0%) between May and September, with an average ~~potential evapotranspiration–evaporation~~ ranging between 1000 and 1900 mm y^{-1} . ~~The soil is classified as belonging to the mountain brown soil. The soils at the study area are formed from weathered granite and classified as Cambisols and Regosols according to IUSS Working Group WRB [25].~~

Historically, the primary subalpine forests predominantly contained firs (*Abies faxoniana* Rehd. et Wils) which were harvested from the 1940s until 1998 when the Natural Forest Protection Program started. The deforested lands were subsequently planted with spruce (*Picea asperata* Mast.) saplings ~~after site preparation without any fertilization applied~~. Spruce plantations had been established in some places of the harvested areas with sparse understory vegetation, and eventually formed monoculture forest (*P. asperata* plantation forest). Meanwhile, natural restoration was also carried out in areas without cultivation. Nevertheless, some of the deforested sites failed to form secondary forest and turned into ~~rye grass land (GL)~~. Thus, OF, SF, PF, and GL now present on the subalpine area. Currently, OF is typically dominated by old growth *A. faxoniana*, SF is typically dominated by *A. faxoniana* and *Betula albo-sinensis* Burk., PF is *P. asperata* monoculture forest. The main understory species were *Carex tristachya* Thunb, *Cystopteris montana* (Lam.) Bernh ex Desv, *Polygonum viviparum* Linn and *Trisetum sibiricum* Rupr, etc. The coverage of undergrowth vegetation was about 60% in OF and SF, and 25% in PF. ~~GL was mainly characterized as *Lolium perenne* L. and *Thalictrum alpinum* L., and the coverage was about 95%.~~

2.2. Experimental Design, Sampling and Processing

In the studied site, four different land-use types (i.e., OF, SF, PF, and GL) were selected based on criteria of similar altitude ~~and soil~~. The land-use types exhibited a mosaic distribution pattern. We chose three representative stands for each land-use type. The stands were hundreds of meters to kilometres apart. In each stand, one plot of 20 m $\times$ 20 m was randomly established for sample collection and was approximately 200 m from the edge of the stand. Each plot served as the replicate, and consequently there were three replicates in each of the four land-use types. All the plots were on the southeastern slope.

To determine soil physicochemical properties, together with microbial community composition and microbial extracellular enzyme activities, in each plot, fallen litters were removed and soil samples (0–10 cm) were collected from five cores (6 cm diameter). The soil samples collected from each plot ~~in the same period~~ were thoroughly mixed to obtain one homogeneous and representative sample. After the composite samples were sieved < 2 mm to remove visible roots, stones, plant debris, and soil animals, all samples were placed in polyethylene bags in triplicate and immediately

transported to the laboratory. One was air-dried and passed through a 0.25 mm sieve for soil physicochemical analysis, another was stored at -20°C prior to phospholipid fatty acid (PLFA) profiles, and a third was used for microbial extracellular enzyme activity analysis.

2.3. Soil Physicochemical Analysis

Soil samples were air-dried (at room temperature) and stored in air-tight plastic bags, and later analyzed for physicochemical analysis. Soil pH was determined using a glass electrode meter in a 1:2.5 soil:deionized water solution. Soil organic carbon (SOC) was measured using the wet oxidation method with $\text{K}_2\text{Cr}_2\text{O}_7$ and H_2SO_4 , and FeSO_4 titration. Total nitrogen (TN) content was measured using the Kjeldahl method. One gram of soil samples was digested with 15 mL of 72% perchloric acid (HClO_4) at 203°C until heavy fumes appeared and its contents turned to a white color like sand. After digestion the samples were filtered with filter paper, and 2 mL supernatant solution was neutralized with 4 N NaOH using p-nitrophenol as an indicator, and analyzed for total phosphorus (TP) [26]. Soil samples were extracted with 2 M potassium chloride (KCl) solution, and ammonium-N ($\text{NH}_4^+\text{-N}$) and nitrate-N ($\text{NO}_3^-\text{-N}$) in extracts were determined with a flow injection auto-analyzer (FIA, Lachat Instruments, USA). The C/N ratio, C/P ratio, and N/P ratio were also calculated.

2.4. PLFA Analysis

PLFA analysis was used to determine microbial biomass and microbial community composition [27,28]. For the analysis of microbial lipids, fresh soil equivalent to 8 g dry weight were extracted with 23 mL of chloroform:methanol:phosphate buffer (1:2:0.8) for 2 h. The chloroform layer was decanted and dried under N_2 at 32°C . The extracts were sequentially fractionated into neutral lipids, glyceride, and phospholipids with chloroform, acetone, and methanol, respectively, using silica gel-filled solid-phase extraction cartridges. The samples were then subjected to mild alkaline methanolysis by dissolving them into 1 mL of methanol:toluene (1:1) and 1 mL of 0.2 mol L^{-1} potassium hydroxide (KOH) solutions, at 37°C for 15 min. Subsequently, 2 mL of water and 0.3 mL of 1.0 mol L^{-1} acetic acid were added. The resulting fatty acid methyl esters were separated, quantified, and identified by gas chromatography (GC, N6890, Agilent, USA) and fitted with a MIDI Sherlock microbial identification system (Version 4.5, MIDI, USA). Lipid peaks were determined manually using the associated Agilent FAME identification library, based on retention time, mass spectra, and comparison with standards. Peak areas were converted into nmol lipid g^{-1} dry soil based on the methyl nonadecanoate (19:0) internal standard concentrations.

The total nmol lipid g^{-1} dry soil (sum of all lipids present) was used as an index of total microbial biomass. In addition, chemically similar lipid indicators were used to represent ecological groups of microorganisms. These included the following: Gram-positive (Gram+) bacteria (sum of i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative (Gram-) bacteria (sum of 16:1 ω 11c, 16:1 ω 7c, 17:1 ω 8c, 16:1 2OH, and 18:1 ω 7c), anaerobic bacteria (sum of cy17:0 and cy19:0), actinobacteria (sum of 10Me 16:0, 10Me 17:0, 10Me 18:0, and 10Me 19:0), ~~arbuscular mycorrhizal (AM)~~ fungi (16:1 ω 5c), and saprotrophic and ectomycorrhizal (SEM) fungi (18:2 ω 6,9c and 18:1 ω 9c). Ratios of fungal/bacterial (F/B ratio) and Gram+/Gram- bacterial lipids (Gram+/Gram- ratio) were also included in the data analysis [1].

2.5. Microbial Extracellular Enzyme Activity Analysis

The function of the microbial communities in soils was assessed by analyzing the potential activities of three microbial extracellular enzymes. The activities of β -glucosidase and N-acetylglucosaminidase were determined following the method of German et al. [29]. Briefly, soil sample suspensions were prepared by adding 4.0 g soil to 10 mL of 50 mM sodium acetate buffer (pH 5.0) and homogenizing for 0.5 h by oscillator. Then, 0.2 mL of 50 mM p-nitrophenyl β -D-glucopyranoside (for β -glucosidase assay) or 0.2 mL of 10 mM p-nitrophenyl N-acetyl- β -D-glucosaminidase (for N-acetylglucosaminidase assay) was added to 0.8 mL of

prepared suspensions. The mixtures were incubated at 37 °C in the dark for 1 h. To stop the enzymatic reactions, 0.2 mL of 0.5 M calcium chloride (CaCl₂) and 0.8 mL of 0.1 M Trishydroxymethylaminomethane-sodium hydroxide (THAM-NaOH, pH 12) buffer were added after incubation. In the control, the respective substrates were added before the addition of CaCl₂ and THAM-NaOH. Polyphenoloxidase activity was determined according to Sinsabaugh et al. [30], using L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate. All enzyme activities were expressed as nmol substrate g⁻¹ dry soil h⁻¹.

2.6. Statistical Analysis

To test if soil physicochemical properties (pH, SOC, TN, NH₄⁺-N, NO₃⁻-N, TP, C/N ratio, C/P ratio, N/P ratio), microbial groups (total microbial biomass, Gram+ bacteria, Gram- bacteria, anaerobic bacteria, actinobacteria, AM fungi, SEM fungi, Gram+/Gram- ratio, F/B ratio), and microbial extracellular enzyme activities (β-glucosidase, N-acetylglucosaminidase, polyphenoloxidase) varied with land-use type, we conducted a series of one-way analysis of variance (ANOVA) followed by Tukey-HSD tests with land-use type as a fixed factor, and each attribute of soil physicochemical properties, microbial community groups, or microbial extracellular enzyme activities as responses. All data were checked for normality and homoscedasticity using Shapiro-Wilk and Bartlett's test, respectively. When these assumptions were not met, non-parametric Kruskal-Wallis tests were used. Additionally, Pearson's correlation coefficients were used to explore the relationship between the microbial groups and soil physicochemical properties, as well as microbial groups and enzyme activities. All statistical tests were performed using SPSS 19.0 (SPSS Inc., Chicago, USA). Statistical significance was established at *P* values < 0.05. Microbial PLFA biomarkers obtained from the sampled soils were standardized before performing principal component analysis (PCA) to ensure each PLFA had the same weight in the analysis. Redundancy analysis (RDA) was used to analyze the responses of soil microbial community composition to physicochemical properties using CANOCO software (version 4.5, Microcomputer Power, Inc., Ithaca, NY) for Windows. Automatic selection of means by Monte Carlo permutations was used to test the significance of the variables (*P* values < 0.05). Figures were generated using SigmaPlot Version 10.0.

3. Results

3.1. Soil Physicochemical Properties

Soil physicochemical properties varied significantly according to land-use types (Table 1). Notably, OF and SF soils showed the highest pH and TN, followed by PF soil, and then for GL soil. SOC in OF and PF soils was higher than in SF soil (*P* > 0.05), and significantly higher than in GL soil (*P* < 0.05). The highest NH₄⁺-N was found for SF soil, while the lowest was observed for GL soil. GL soil had significantly higher NO₃⁻-N than OF and SF soils (*P* < 0.05). Additionally, TP and C/N ratio in PF soil were significantly higher than the other land-use types (*P* < 0.05). C/P ratio and N/P ratio, however, were significantly lower in PF soil than the other land-use types (*P* < 0.05).

Table 1. Soil physicochemical properties of the upper 0–10 cm soil in different land-use types. Values are means ± SE. Different letters indicate statistically significant differences among the land-use types (Tukey HSD test, 5% level).

	Old-growth Forest	Secondary Forest	Plantation Forest	Grass Land
pH	5.98 ± 0.18 a	5.82 ± 0.03 a	5.34 ± 0.05 ab	5.02 ± 0.27 b
SOC (g kg ⁻¹)	61.79 ± 3.97 a	52.32 ± 2.42 ab	63.34 ± 4.75 a	40.39 ± 4.04 b
TN (g kg ⁻¹)	4.35 ± 0.44 a	4.22 ± 0.39 ab	3.24 ± 0.12 bc	2.54 ± 0.10 c
NH ₄ ⁺ -N (mg kg ⁻¹)	10.99 ± 2.03 ab	13.03 ± 0.12 a	11.06 ± 0.07 ab	8.61 ± 1.47 b
NO ₃ ⁻ -N (mg kg ⁻¹)	2.88 ± 0.88 b	3.17 ± 1.21 b	4.22 ± 1.63 ab	8.38 ± 1.84 a
TP (mg kg ⁻¹)	76.07 ± 4.65 b	70.19 ± 4.21 b	116.31 ± 3.21 a	43.46 ± 5.23 c
C/N ratio	14.35 ± 0.69 bc	12.52 ± 0.64 c	19.50 ± 0.97 a	15.82 ± 1.08 b
C/P ratio	812.24 ± 17.61 ab	746.79 ± 15.01 b	547.63 ± 57.06 c	938.16 ± 68.36 a

N/P ratio	56.96 ± 3.77 a	59.89 ± 2.36 a	27.96 ± 1.64 b	59.96 ± 6.46 a
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3.2. Soil Microbial Group PLFAs

Land-use type significantly affected the relative abundance of anaerobic bacteria ($F = 8.949$, $P < 0.05$), actinobacteria ($F = 4.264$, $P < 0.05$), SEM fungi ($F = 8.231$, $P < 0.05$), and F/B ratio ($F = 47.5$, $P < 0.001$). The relative abundance of anaerobic bacteria was significantly higher in OF (11.7%) soil than in SF (10.9%) and GL (9.9%) soils ($P < 0.05$) (Figure 1c). Actinobacterial abundance was increased by 9.4% and decreased by 6.3%, respectively, due to the conversion from OF into SF and PF ($P < 0.05$) (Figure 1d). PF soil was associated with the highest SEM fungi (8.1%) and F/B ratio (0.12), and significantly higher than in GL (5.3% and 0.08, respectively) soil ($P < 0.05$) (Figure 1f, h). There was no significant difference among land-use types for the other microbial groups examined ($P > 0.05$; Figure 1a, b, e, g).

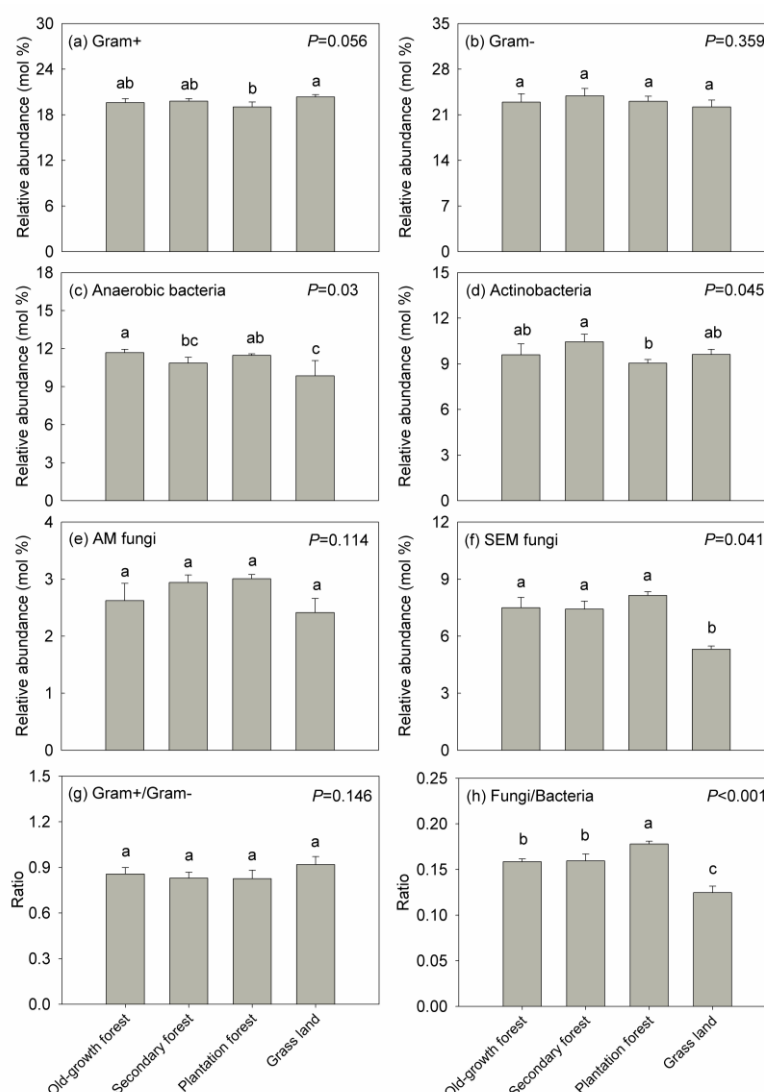


Figure 1. Relative abundance of different microbial group PLFAs in different land-use types. Error bars represent the standard error of the mean. Different letters indicate statistically significant differences among the land-use types (Tukey's HSD: $P < 0.05$).

We found that SOC and TP were significantly and positively correlated with anaerobic bacteria, AM fungi, and SEM fungi. NO_3^- -N was negatively correlated with SEM fungi. TP, C/P ratio and N/P ratio were correlated with Gram+ bacteria. Additionally, C/P ratio appeared to be negatively correlated with anaerobic bacteria and SEM fungi (Table 2).

Table 2. Pearson's correlation coefficient (*R*) testing association between microbial group PLFAs and soil physicochemical properties. *P*-values are shown in the parenthesis.

	Gram+ Bacteria	Gram− Bacteria	Anaerobic Bacteria	Actinobacteri a	AM Fungi	SEM Fungi
pH	−0.14 (0.67)	0.39 (0.21)	0.49 (0.11)	0.32 (0.31)	0.25 (0.43)	0.44 (0.16)
SOC	−0.41 (0.19)	0.18 (0.57)	0.60 (0.04)	−0.06 (0.86)	0.59 (0.04)	0.76 (<0.01)
TN	−0.10 (0.77)	0.20 (0.54)	0.44 (0.15)	0.36 (0.26)	0.34 (0.28)	0.49 (0.11)
NH ₄ ⁺ -N	−0.07 (0.83)	0.25 (0.44)	0.49 (0.11)	0.06 (0.86)	0.15 (0.65)	0.46 (0.14)
NO ₃ [−] -N	0.42 (0.17)	−0.25 (0.43)	−0.53 (0.07)	−0.12 (0.72)	−0.31 (0.32)	−0.68 (0.02)
TP	−0.70 (0.01)	0.12 (0.72)	0.63 (0.03)	−0.38 (0.22)	0.65 (0.02)	0.81 (<0.01)
C/N ratio	−0.32 (0.31)	−0.09 (0.78)	0.14 (0.68)	−0.53 (0.08)	0.20 (0.54)	0.19 (0.55)
C/P ratio	0.76 (<0.01)	−0.05 (0.89)	−0.68 (0.02)	0.43 (0.16)	−0.53 (0.08)	−0.75 (<0.01)
N/P ratio	0.63 (0.03)	0.03 (0.92)	−0.49 (0.11)	0.55 (0.06)	−0.40 (0.20)	−0.54 (0.07)

SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; C/N: the ratio of soil organic carbon to total nitrogen; C/P: the ratio of soil organic carbon to total phosphorus; N/P: the ratio of total nitrogen to total phosphorus.

3.3. Soil Microbial Community Composition

The data concerning the individual relative abundance of the 24 most common PLFAs were subjected to a principal component analysis (PCA; Figure 2). Results showed that the first principal component (PC1) and the second principal component (PC2) together accounted for 66.7% of total variation of the soil microbial community composition. The PCA biplots revealed that the soil microbial community composition from OF was like that of SF. While the soil microbial communities from PF, GL, and SF were compositionally distinct from each other. GL with higher PC1 scores were observed on the right of the axes 1. By their loading values it is evident that the microbial PLFA biomarkers associated with gram-positive bacteria, including i14:0, i17:0, a17:0, were all most important for the separation of the GL. The higher PC2 scores were observed for PF on the upper of the axes 2. Specifically, PF soil was abundant in AM [fungal](#) PLFA biomarkers (16:1ω5c) and one of SEM [fungal](#) PLFAs biomarkers (represented by 18:2ω6,9c).

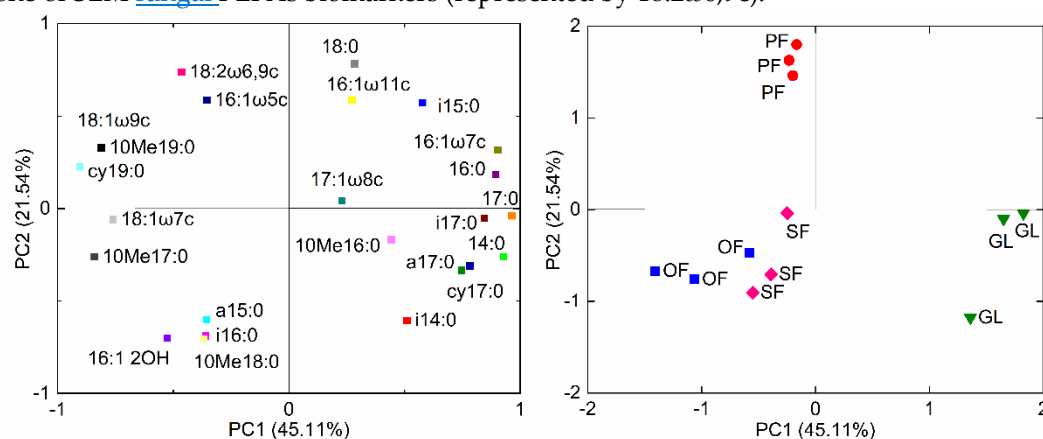


Figure 2. Principal component analysis (PCA) biplots of the phospholipid fatty acid (PLFA) composition of microbial community in the soil samples from different land-use types. OF: Old-growth forest; SF: Secondary forest; PF: Plantation forest; GL: Grassland.

Redundancy analysis (RDA) of relationships between soil microbial community composition and soil physicochemical properties showed that the first axes, RD1, explained 45.44% of the total variance in soil microbial communities, while the second axes (RD2) explained 21.74% of the total variance (Figure 3).

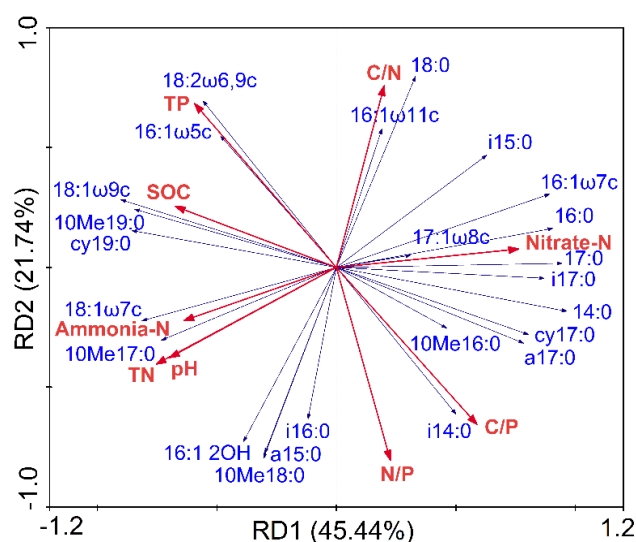


Figure 3. Redundancy analysis (RDA) of relationships between soil microbial community composition and physicochemical properties. The dashed lines with blue color represent the microbial PLFA biomarkers and the solid lines with red color represent the soil physicochemical variables. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; C/N: the ratio of soil organic carbon to total nitrogen; C/P: the ratio of soil organic carbon to total phosphorus; N/P: the ratio of total nitrogen to total phosphorus.

Nine variables, which included pH, SOC, TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TP, C/N ratio, C/P ratio, and N/P ratio, were found to be significantly correlated to soil microbial community composition (Table 3). These variables jointly explained 88% of the variations of the soil microbial community. The forward selection of the variables in the RDA showed that the soil microbial community composition across these four land-use types was primarily affected by the TN and pH, which explained 30% and 23% of the variations, respectively.

Table 3. Marginal and conditional effects of forward selection through redundancy analysis (RDA).

Variables	Explained α^a	Explained β^b	P^c	F-ratio ^d
TN	0.30	0.30	0.008	4.25
pH	0.26	0.23	0.002	4.47
TP	0.27	0.07	0.194	1.43
N/P	0.18	0.07	0.190	1.52
Ammonia-N	0.21	0.05	0.436	1.02
C/P	0.26	0.04	0.452	0.89
SOC	0.24	0.06	0.382	1.16
Nitrate-N	0.27	0.03	0.556	0.73
C/N	0.17	0.03	0.656	0.50

^a Marginal effect, which show the variance explained when the variable is used as the only factor. ^b Conditional effects, which show the additional variance each variable explains when it is include in the model. ^c Level of significance corresponding to b when performing Monte Carlo test at the 0.05 significance level. Bold values indicate significant effects at $P < 0.05$. ^d Monte Carlo test statistics corresponding to b at the 0.05 significance level.

3.4. Soil Microbial Extracellular Enzyme Activities

Land-use type had a marked impact on total microbial biomass ($F = 23.717$, $P < 0.001$), β -glucosidase ($F = 6.347$, $P < 0.05$) and polyphenoloxidase activities ($F = 11.565$, $P < 0.01$). Total microbial biomass, N-acetylglucosaminidase and β -glucosidase activities were consistently higher in OF and SF soils than in GL soil (Figure 4a-c). However, differences in N-acetylglucosaminidas

activities were not statistically significant. Additionally, GL soil had significantly higher polyphenoloxidase activities than the other land-use types (Figure 4d).

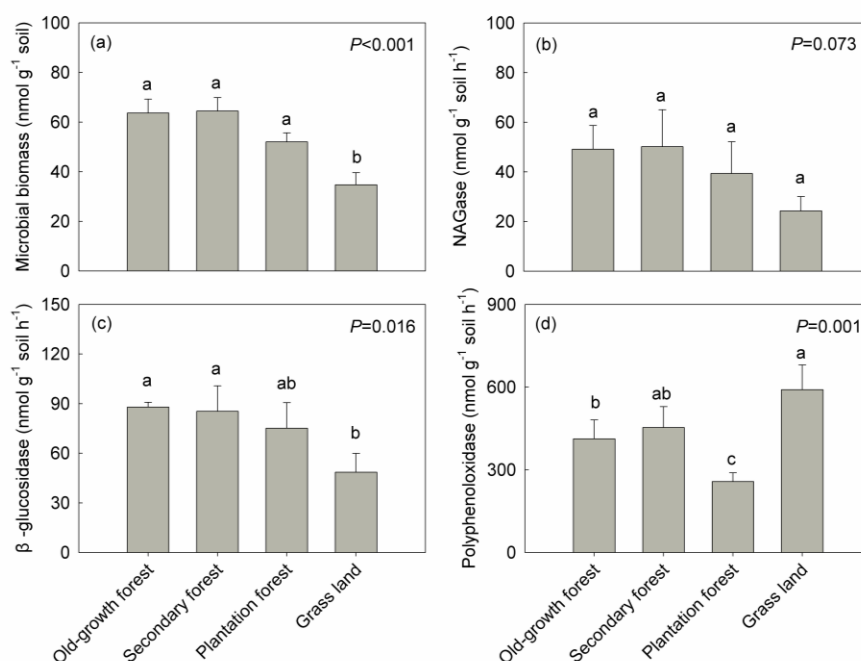


Figure 4. Soil total microbial biomass and microbial extracellular enzyme activities in different land-use types. Error bars represent the standard error of the mean. Different letters indicate statistically significant differences among the land-use types (Tukey HSD test, 5% level). NAGase: N-acetylglucosaminidase.

In a correlation analysis, N-acetylglucosaminidase and β-glucosidase activities significantly increased with increasing total microbial biomass. N-acetylglucosaminidase activity was negatively correlated with anaerobic bacteria, while polyphenoloxidase activity was positively related to actinobacteria. No other microbial variables showed any significant correlations with enzyme activities (Table 4).

Table 4. Pearson's correlation coefficient (*R*) and *P* values of regression between soil microbial group PLFAs and microbial extracellular enzyme activities.

	N-acetylglucosaminidase		β-glucosidase		Polyphenoloxidase	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Gram+ bacteria	0.104	0.748	0.242	0.449	0.399	0.199
Gram− bacteria	0.439	0.153	0.183	0.570	0.205	0.523
Anaerobic bacteria	−0.702	<0.05	−0.545	0.067	0.014	0.965
Actinobacteria	0.470	0.123	0.300	0.344	0.578	<0.05
AM fungi	0.173	0.591	0.007	0.982	−0.162	0.614
SEM fungi	−0.340	0.280	−0.346	0.271	−0.131	0.685
Gram+:Gram− ratio	−0.262	0.411	−0.005	0.987	0.042	0.897
Fungi:Bacteria ratio	−0.289	0.362	−0.315	0.319	−0.263	0.408
Total microbial biomass	0.624	<0.05	0.718	<0.01	−0.443	0.149

4. Discussion

The response of soil microbial community composition to land-use changes or disturbances is commonly assessed by community level PLFA profiles. For example, quite a few studies observed that soil microbial community showed some tree specificity [1,12]. Consistent with our hypothesis and findings from previous studies [7,12,13], we found that land-use type had a significant influence

on soil microbial communities and microbial extracellular enzyme activities (Figures 1 and 4), in large part, due to modified soil physicochemical properties and plant community structure. Contrarily, Bezemer et al. [31] and Lucas-Borja et al. [32] did not find any relationship between soil microbial community and land-use type.

Different microbial group PLFAs seemed to prefer different soil conditions [12,33–35]. Gram+ bacteria, for example, were positively correlated with increasing soil TP [12], while actinobacteria and AM fungi were strongly associated with $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ [23]. In the present study, we found that anaerobic bacteria, SEM fungi, and β -glucosidase activity were distinctly higher in OF and SF soils, the most fertile soils examined in our experimental sites, when compared to GL soil (Figure 1). OF and SF that we examined had persistently more fertile soils with approximately 1.5-fold higher concentrations of SOC, TN and TP (Table 1). Therefore, in this case, land-use change from OF to GL has resulted in soils with lower nutrient concentrations, and thus the potential to suppress the growth and enzyme activities of those microbial groups. Additionally, our correlation analysis also showed that anaerobic bacteria, AM fungi and SEM fungi were significantly and positively correlated with SOC and TP (Table 2). A previous study in an oak forest showed that fertile soils favored highly microbial groups after land-use changes [1], which supported our findings. In addition, our observed trend towards lower Gram+ bacteria in PF soil may be related to higher TP, lower C/P ratio or N/P ratio, since significant correlations were found between them (Table 2).

In the present study, RDA showed that the soil microbial community composition from OF soil was like that of SF soil (Figure 2), which consistent with previous findings that soil microbes under similar vegetation showed more similar community structure [32]. In addition, we found that microbial communities from OF (or SF), PF and GL soils were compositionally distinct from each other (Figure 2), and the soil microbial community composition was mainly controlled by TN and pH (Figure 3 and Table 3). Previous studies also concluded that high soil N availability could stimulate microbial growth in forest soils [36,37]. In this study, we found that OF and SF increased the Gram-, anaerobic, and actinobacteria abundance (Figure 1). One of the explanations is that bacteria generally have higher N demand (microbial C/N = 4 for bacteria, and 10 for fungi) [38], therefore might develop well under nutrient-rich conditions. Soil pH is one of the major factors influencing the soil microbial community composition [39,40]. Nevertheless, it was also reported that there was no significant relationship between soil microbial community and pH [41]. Soil types and their associated soil characteristics, such as nutrient availability, texture, and soil moisture have varied in different studies, which might account for the inconsistent relationships.

Plant community structure and species identity has been shown to have significant influence on microbial communities in soils [42–44]. In this study, changes in plant communities associated with distinct land uses resulted in different microbial communities in soils (Figure 1). At least two aspects could be explained for this situation. First, different plant communities often select microorganisms differentially through affecting access to nutrients and plant rhizodeposits [22]. Second, the increase of plant community diversity can provide more types of food supply for belowground microbial communities, leading to more niche availability for soil microbes [1]. In this study, the plant community composition and structure were more complicated of mixed forests (OF and SF) than that of monoculture PF and GL, meanwhile, mixed leaf litters that contained broad-leaved tree species (SF) often decompose at a faster rate than monoculture coniferous litters (PF), such differences often affect the structure and activities of belowground microbial communities [45,46].

Land-use driven changes are likely to feedback on ecosystem C and nutrient cycling through the effects on soil microbial communities [47]. Microorganisms produce extracellular enzymes in order to degrade complex organic substrates into monomers for nutrient acquisition [48,49]. Extracellular enzyme activities can, therefore, represent microbial nutrient limitation and decomposition potential in response to changes in soil quality or land-use change [1]. Soil enzymes can be divided into two broad groups: (i) hydrolytic enzymes responsible for the acquisition of C, N, and P to support primary metabolism; and (ii) oxidative enzymes, produced primarily by fungi, which degrade poor-quality and chemically complex compounds like lignin in cometabolic

acquisition of nutrients [30,50]. These groups of enzymes may respond differently to land-use change. In our study, land-use changes from OF to PF and GL shifted enzyme production toward decrease in N-acetylglucosaminidase and β -glucosidase activities (Figure 4), which may reduce nutrient cycling rates and thus decrease nutrient acquisition for metabolism. However, such land-use changes decreased the fungi abundance (Figure 1) but dramatically increased the production of polyphenoloxidase activity (Figure 4), which is consistent with the results from a *Casuarina equisetifolia* L. plantation ecosystem [51]. Oxidative enzymes may contribute to humification and are typically linked to the depolymerization of recalcitrant organic compounds and poor-quality C compounds, such as aromatic C (found in lignin), as observed in Puerto Rico montane forests [52] and northern United States hardwood forests [53]. Environmental conditions that restrict in situ oxidative activities sustain an activity-limited feedback cycle that increases the C storage [32]. These results indicate that there is likely to be significant variability in microbial community structural and functional responses to land-use change, with potential effects on soil C cycling.

5. Conclusions

In summary, we found strong evidence that land-use change has significant impacts on the structure and function of soil microbial communities in subalpine forests in the Eastern Tibetan Plateau. The microbial communities from OF (or SF), PF and GL soils were compositionally distinct from each other. However, microbial community composition from OF soil was like that of SF soil. Differences in microbial community composition and microbial enzyme activities among sites with contrasting land use types could be attributed to differences in soil physicochemical properties. OF and SF with fertile soils and diverse plant species favored highly total microbial biomass, anaerobic bacterial, SEM fungal communities, and β -glucosidase activities, whilst less fertile GL soil favored highly polyphenoloxidase activities. The microbial community composition was primarily regulated by soil physicochemical characteristics. These findings suggested that altered land-use type initiated changes in the physicochemical characteristics of the soils, which affected the composition of microbial communities and microbial enzyme activities related to nutrient cycling in this area. This provides a scientific basis for the influence mechanism of land use on composition and function of microbial communities, as well as the rational utilization and management of land resources.

Author Contributions: D.L. performed the experiments, analyzed the data, and wrote and revised the manuscript. R.-M.C. and S.L. performed the experiments and analyzed the data. Z.-M.S. conceived and designed the experiments, supervised the study and revised the paper. Q.-H.F. contributed to the experimental tools and conducted the field experiments. All authors contributed critically to the manuscript and gave final approval for publication.

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