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Effect of 40 and 80 Years of Conifer Regrowth on Soil Microbial Activities and Community Structure in Subtropical Low Mountain Forests

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Academic Editors: Rodney J. Keenan and Timothy A. Martin
Received: 5 September 2016; Accepted: 18 October 2016; Published: 21 October 2016

Abstract: The effects of long-term reforestation on soil microbial communities and biomass are poorly understood. This study was conducted on two coniferous plantations: *Cunninghamia konishii* Hayata, planted 40 years ago (CONIF-40), and *Calocedrus formosana* (Florin) Florin, planted 80 years ago (CONIF-80). An adjacent natural broadleaf forest (BROAD-Nat) was used as a control. We determined microbial biomass C and N contents, enzyme activities, and community composition (via phospholipid fatty acid [PLFA] assessment). Both microbial biomass and PLFA content were higher in the summer than in the winter and differed among the forests in summer only. Total PLFA, total bacterial, gram-positive bacterial, gram-negative bacterial, and vesicular arbuscular mycorrhizal fungal contents followed the same pattern. Total fungal content and the ratios of fungi to bacteria and of gram-positive to gram-negative bacteria were highest in CONIF-40, with no difference between the other forests. Principal component analysis of PLFA contents revealed that CONIF-40 communities were distinct from those of CONIF-80 and BROAD-Nat. Our results suggest that vegetation replacement during reforestation exerts a prolonged impact on the soil microbial community. The understory broadleaf shrubs and trees established after coniferous plantation reforestation may balance out the effects of coniferous litter, contributing to bacterial recovery.

Keywords: PLFA; microbial community; soil enzymes; forest

1. Introduction

The management of forest ecosystems, such as harvesting, burning, deforestation, and reforestation, results not only in quantitative and qualitative changes in soil C and N contents but also in eventual changes in the microbial community [1,2]. Tree type is a primary factor affecting the microbial community structure in forest soils as it controls the input of substrate type to soil [3] and soil chemical properties, such as the C content, C/N ratio, and pH [4]. Reforestation with exotic and mostly single species at original natural forest sites has changed the soil microbial community in subtropical areas [5,6]. Ushio et al. [7] indicated that tree species had a direct impact on the soil microbial community in tropical montane forests. Likewise, these authors suggested that coniferous forest soils exhibited higher ratios of gram-positive (G+) to gram-negative (G−) bacteria and fungi to bacteria than did broadleaf forests.

After reforestation, the microbial community structure evolves over time to approach that in native forest soils. Using phospholipid fatty acids (PLFA) analysis, Moore-Kucera and Dick [8] found that soil total PLFA, as well as total bacteria and fungi were lower in an 8-year-old replanted forest than in old-growth forests. However, those in a 25-year-old replanted site were similar to those of an old-growth forest. Considerable research has been conducted to assess the response of the soil microbial community under secondary forests in temperate regions [9]. PLFA has been used to estimate changes in the soil microbial community after forest management [10]. Comparatively less attention has been paid to this subject in subtropical forests, where higher temperatures and precipitation may elicit a different response from the soil microbial community under secondary forests [11,12]. Furthermore, little information is available on the extent to which microbial communities differ between native forests and 50 to 100-year-old reforested areas.

Many studies have reported that coniferous and broadleaf tree species exert strong influences on soil microbial communities [7,13]. However, a recent study by Wang et al. [14] showed that differences in bacterial community structure between coniferous and broadleaf forests disappeared due to changes in soil properties. In an attempt to promote the economical production of timber, reforested conifers replaced several native broadleaf hardwood forests decades ago in a low- to high-elevation subtropical montane region in central Taiwan. Our previous study suggested that disturbances during forest conversion influence the soil bacterial community, eliciting differences among three forest ecosystems in the same climate [15]. The results of Lin et al. [15] also revealed that soil bacterial community diversity in 40-year-old coniferous forest was greater than that in 80-year-old coniferous forest, which was in turn greater than that in natural broadleaf forest, reflecting a trend of disturbance.

We hypothesized that the soil microbial community of secondary forests would establish a similar structure to that of a natural system of a different vegetation type after a sufficient period of time. We therefore compared the soil microbial community structure in a 40-year-old and an 80-year-old secondary coniferous forest with that of a native broadleaf forest. We used PLFA analysis to provide quantitative data on the structure of microbial communities [16,17] and evaluated similarities in community structure across the different forest types. We found that while soil microbial communities differed between native broadleaf forests and 40-year-old coniferous forests, such differences were reduced between native broadleaf and 80-year-old coniferous forests. The understory broadleaf shrubs and trees established after the long-term reforestation of coniferous plantations may contribute to the recovery of bacteria in these secondary forests.

2. Materials and Methods

2.1. Site Description

This study was conducted at the Lienhuachi Experimental Forest in a subtropical low mountain area in central Taiwan (23°54′ N, 120°54′ E). The elevation ranges from 600 to 950 m above sea level, with a mean annual precipitation of 2200 mm and a mean annual temperature of 20.8 °C. Soils are moderately well drained and classified as Typic Dystrudept (U.S. Soil Taxonomy). As one of the few remaining low-elevation, natural, undisturbed forests in central Taiwan, the site is part of a long-term ecological study covering 461 ha of a low-altitude forest ecosystem, including 261 ha belonging to evergreen natural broadleaf hardwood forest dominated by Fagaceae and Lauraceae species. Coniferous plantations established 80 and 40 years ago with *Calocedrus formosana* (Florin) Florin and *Cunninghamia konishii* Hayata, respectively, replaced approximately 50% of the native forests on this study site.

The study included the two coniferous plantations, i.e. the 40-year-old *C. konishii* plantation (CONIF-40) and the 80-year-old *C. formosana* plantation (CONIF-80), as well as a natural broadleaf forest (BROAD-Nat) as the control. The CONIF-80 and CONIF-40 plantations, which were established by reforestation with coniferous trees after the native forest was cleared, encompass 5 and 100 ha, respectively. The BROAD-Nat forest exhibits a high diversity of plant species and an even species

composition dominated by tree species in the families Lauraceae and Fagaceae. BROAD-Nat trees exhibit a mean height of 10–18 m, a density of 1450 stems/ha, and a diameter at breast height (DBH) of 10–30 cm (Table 1). Several hardwood tree species in the BROAD-Nat forest form a canopy structure with multiple layers.

Table 1. Indices of natural broadleaf forest (BROAD-Nat), 80-year-old *Calocedrus* (CONIF-80) plantation, and 40-year-old *Cunninghamia* (CONIF-40) plantation at Lienhuachi Experimental Forest.

Forest type	Species	Density (stems·ha^{-1})	Plant Height (m)	Average DBH [1] (cm)	Average Basal Area (m^2·ha^{-1})	Wood Volume (m^3·ha^{-1})
BROAD-Nat	*Cryptocarya chinensis* (Hance) Hemsl.	250	10.9	15.1	7.89	77.28
	Litsea acuminatae (Blume) Kurata	175	12.5	14.7	4.74	41.85
	Prunus phaeosticta (Hance) Maxim.	175	13.2	16.7	4.64	36.20
	Lithocarpus amygdalifolius (Skan) Hayata	100	17.5	18.5	3.84	33.20
	Schefflera octophylla (Lour.) Harms	100	13.5	16.8	2.56	16.45
	Cinnamomum subavenium Miq.	175	13.1	10.5	2.44	18.44
	Cyclobalanopsis pachyloma (O. Seem.) Schott.	75	10.7	20.3	2.44	19.17
	Styrax suberifolia Hook. & Arn.	75	16.3	17.2	1.98	16.44
	Pasania harlandii (Hance) Oerst.	25	16.0	31.1	1.90	13.75
	Machilus zuihoensis Hayata	50	18.0	20.4	1.85	16.20
	Others	250	9.5	9.1	1.92	9.35
	Total	1450			36.20	298.3
CONIF-80	*Calocedrus formosana* (Florin) Florin	275	26.6	48.0	51.44	644.9
	Randia cochinchinensis (Lour.) Merr	625	8.6	6.2	1.95	7.80
	Schefflera octophylla (Lour.) Harms	575	8.7	6.2	1.79	7.24
	Prunus phaeosticta (Hance) Maxim.	275	10.2	7.5	1.26	5.96
	Others	375	8.0	5.9	1.15	4.39
	Total	2125			57.59	670.3
CONIF-40	*Cunninghamia lanceolata* (Lamb.) Hook.	900	15.8	21.4	36.01	289.6
	Styrax formosana Matsum	80	10.6	10.7	0.79	4.14
	Schefflera octophylla (Lour.) Harms	40	12.5	15.1	0.75	4.53
	Others	140	12.0	10.6	1.62	8.75
	Total	1160			39.17	307.0

[1] DBH: diameter at breast height.

In the CONIF-80 forest, *C. formosana* is the major species, with a mean height of 27 m, a density of 275 stems/ha, and a DBH of 48 cm. With a space of 6 m × 6 m, the forest has an open canopy allowing sunshine to pass through. Regrowth of broadleaf trees, including *Randia cochinchinensis* (Lour.) Merr., *Schefflera octophylla* (Lour.) Harm, and *Prunus phaeosticta* (Hance) *Maxim.*, forms the second layer of the canopy, with a mean height of 10 m and a density of 1850 stems/ha. Although the density of broadleaf regrowth trees in CONIF-80 is quite high, broadleaf trees make up much less of the canopy than do conifers.

In the CONIF-40 plantation, *C. lanceolata* grows to a mean height of 15.8 m, a density of 900 stems/ha, and a mean stand basal area of 36.0 m^2/ha. This plantation also consists of regrowth of broadleaf trees, such as *Styrax formosana* Matsum. and *S. octophylla,* to a height of 10–12 m and a density of 260 stems/ha. The density of broadleaf tree regrowth in the CONIF-40 plantation is lower than that in the CONIF-80 plantation and therefore, there is not a multiple-canopy structure in the CONIF-40 plantation as there is in the CONIF-80 plantation and BROAD-Nat (Figure 1).

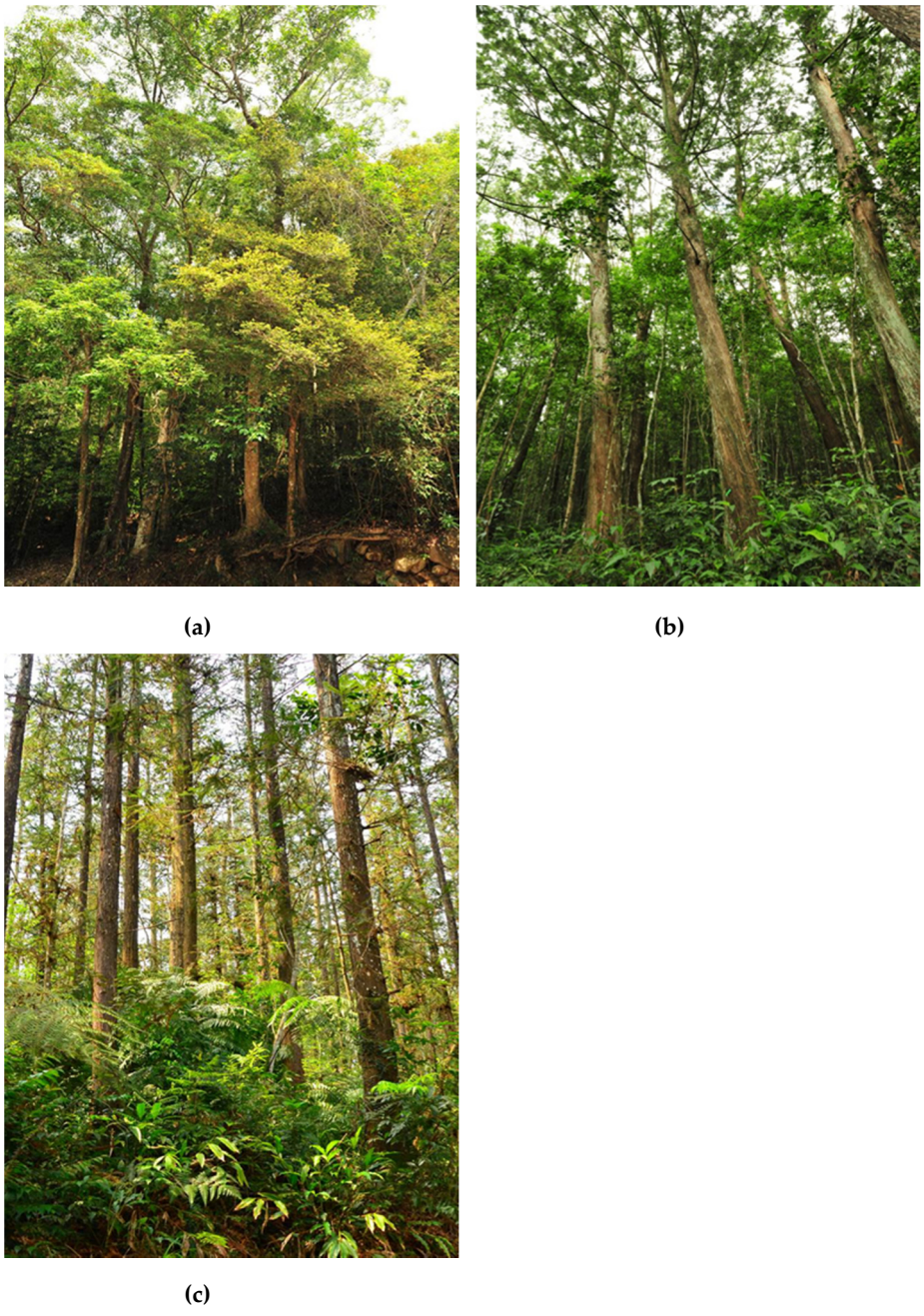

Figure 1. Photographs of (**a**) natural broadleaf (BROAD-Nat), (**b**) 80-year-old coniferous (CONIF-80), and (**c**) 40-year-old coniferous (CONIF-40) forests at the study site.

2.2. Soil Sampling and Analysis

Five plots (50 m × 50 m each) were established in each forest site for collection of soil samples. Three soil core samples were collected in each plot to create a composite sample. The soil samples were collected in February 2011 during the winter (dry) season and in August 2011 during the summer (wet) season at a depth of 0–10 cm using a soil auger that was 8 cm in diameter. Roots and litter were manually removed before samples were sieved through a 2-mm sieve. The samples were maintained at 4 °C in the dark. Several subsamples were air-dried and ground in order to analyze their physicochemical properties, such as total organic C and total N. Subsamples of fresh soil were also freeze-dried at −20 °C immediately after field sampling for PLFA analysis. Additional subsamples were weighed

and oven-dried at 105 °C to determine moisture content in order to provide an oven-dried basis for comparison of analytical results. Soil organic C (C_{org}) and total N (N_{tot}) contents were determined using a Fisons NA1500 elemental analyzer (ThermoQuest Italia, Milan, Italy). Soil pH values of air-dried soil were measured using a combination glass electrode (soil:water ratio 1:2.5; [18]).

2.3. Biochemical Assays

The microbial biomass of each soil sample was determined using the chloroform fumigation extraction method [19]. Organic C retrieved through K_2SO_4 extraction was measured using a total organic carbon analyzer (Model 1010, OI Analytical, College Station, TX, USA) and converted to microbial biomass C (C_{mic}) assuming a conversion factor of 2.22 [20]. The microbial biomass N (N_{mic}) content was calculated from the ninhydrin-reactive N released from the biomass and determined colorimetrically at 560 nm [21].

Urease (EC 3.5.1.5) activity was determined as described in Kandeler and Gerber [22]. Phosphatase (EC 3.1.3.2) activity was determined using the method of Tabatabai and Bremner [23]. Cellulase (EC 3.2.1.4) and xylanase (EC 3.2.1.8) activities were determined using the method of Schinner and von Mersi [24].

PLFA extraction and analysis was performed using the methods described by Frostegard et al. [25]. Lipids were extracted using a single-phase mixture of the chloroform-methanol-citrate system. Phospholipids were then divided into neutral, glycol-, and phospholipids using a solid-phase extraction column and eluting with chloroform, acetone, and methanol, respectively. Next, phospholipids underwent methylation to form fatty acid methyl esters (FAMEs). The FAMEs were analyzed using capillary gas chromatography (GC) and flame ionization detection with the Thermo Fisher TRACE chromatography system (Thermo Fisher Scientific, Waltham, MA, USA). GC was performed according to the method of Chang et al. [26]. The fatty acid nomenclature was established according to Frostegard et al. [25]. The position of double bond is defined by symbol ω from the methyl end of the molecule. The prefix c refers to cis configuration. Iso and anteiso branching are designated by the prefix i or a, respectively. The prefix cy refers to cyclopropane fatty acids. The fatty acids i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 17:0, i17:0, cy17:0, 18:1ω7c, and cy19:0 were categorized as being derived from bacteria: i15:0, a15:0, i16:0, and i17:0 from G+ bacteria; 16:1ω7c, cy17:0, 18:1ω7c, and cy19:0 from G− bacteria; and 10Me18:0 from actinomycetes. The fatty acid 18:2ω6c was categorized as being derived from fungi, and 16:1ω5c was categorized as being derived from vesicular-arbuscular mycorrhizal (VAM) fungi [16,27].

2.4. Statistical Analysis

Two-way analysis of variance was used to assess vegetation-type and seasonal effects. Principal component analysis (PCA) was used to determine the similarities between succession and native systems according to fatty acid concentration (mole %). Pearson's correlation coefficients were used to assess correlations between microbial parameters and soil properties. Statistical analyses were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS v12.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Chemical and Physical Properties of Soils

Soil moisture was higher in the summer than in the winter (Table 2) because winters are dry in this area. The CONIF-40 forest contained the highest soil moisture content in the summer. The soil organic C and total N contents were highest in the CONIF-40 forest, with no significant differences between the CONIF-80 and BROAD-Nat forests. All soils were strongly acidic.

Table 2. Soil chemical and physical properties and microbial biomass in 40-year-old (CONIF-40) and 80-year-old (CONIF-80) coniferous plantations and a natural broadleaf forest (BROAD-Nat) in two seasons.

Season	Forest Type	Soil Moisture (%)	pH	C_{org} [1] (%)	N_{tot} (%)	C_{mic} (mg·kg^{-1})	N_{mic} (mg·kg^{-1})	C_{mic}/C_{org} (%)	N_{mic}/N_{tot} (%)
Summer	BROAD-Nat	27.6 a [2]	3.78 b	3.45 b	0.29 ab	1668 a	140 a	4.83 a	4.82 b
	CONIF-80	23.1 b	3.74 b	3.23 bc	0.24 bc	1314 b	94.5 c	4.21 a	3.93 c
	CONIF-40	29.2 a	3.62 c	4.45 a	0.31 a	1229 b	116 b	2.82 b	3.74 c
Winter	BROAD-Nat	19.3 c	3.87 a	2.62 c	0.22 c	763 c	119 b	2.92 b	5.40 a
	CONIF-80	21.9 c	3.87 a	3.31 bc	0.25 bc	748 c	121 ab	2.28 c	4.84 b
	CONIF-40	27.3 a	3.75 b	3.79 b	0.29 ab	812 c	117 b	2.16 c	3.98 c
ANOVA results [3]									
Forest type		***	***	**	**	***	***	***	***
Sample season		***	***	ns	*	***	ns	***	***
Forest × season		***	ns	ns	*	***	***	ns	ns

[1] C_{org}: soil organic C, N_{tot}: soil total N, C_{mic}: microbial biomass C, N_{mic}: microbial biomass N; [2] Values in each column followed by different lowercase letters are significantly different at $p < 0.05$, based on Duncan's multiple test; [3] * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

3.2. Soil Microbial Biomass

Soil C_{mic} and N_{mic} contents were significantly higher in the summer than in the winter. Soil C_{mic} and N_{mic} contents did not differ significantly among the three forests in the winter. During the summer, both soil C_{mic} and N_{mic} contents were higher in the BROAD-Nat forest than in the CONIF-40 and CONIF-80 forests. Ratios of C_{mic}/C_{org} and N_{mic}/N_{tot} were significantly different among the different forest soils, being higher in the BROAD-Nat forest than in the CONIF-40 and CONIF-80 forests (Table 2).

3.3. Soil Enzyme Activities

Soil xylanase, phosphatase, and urease activities were significantly affected by forest type and season. Summer soil cellulase, xylanase, and phosphatase activities were significantly higher in the CONIF-40 forest than in the BROAD-Nat forest (Table 3), with no significant difference between the CONIF-80 and BROAD-Nat forests. Soil enzyme activities in the winter did not differ among forest types, except for a lower xylanase activity in the CONIF-80 forest. Additionally, enzymatic activities were higher in the summer than in winter, except for xylanase.

Table 3. Soil enzymatic activities in 40-year-old (CONIF-40) and 80-year-old (CONIF-80) coniferous forest plantations and a natural broadleaf forest (BROAD-Nat) in two seasons.

Season	Forest Type	Cellulase (μg glucose g^{-1}·h^{-1})	Xylanase (μg glucose g^{-1}·h^{-1})	Urease (mmol NH_4^+-N g^{-1}·h^{-1})	Acid Phosphatase (μg nitrophenol g^{-1}·h^{-1})
Summer	BROAD-Nat	31.7 c [1]	65.8 d	10.6 a	569 b
	CONIF-80	63.8 bc	70.8 cd	11.9 a	628 b
	CONIF-40	126 a	109 ab	9.47 ab	850 a
Winter	BROAD-Nat	36.7 bc	116 a	5.83 b	338 c
	CONIF-80	46.7 bc	92.1 bc	9.84 ab	318 c
	CONIF-40	76.2 b	118 a	6.45 b	361 c
ANOVA results					
Forest type		**	*	*	ns
Sample Season		ns	***	**	***
Forest × season		ns	ns	Ns	ns

[1] Values in each column followed by different lowercase letters are significantly different at $p < 0.05$, based on Duncan's multiple test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

3.4. Microbial Community Structure

Phospholipid acid biomarkers were significantly affected by forest type and season. In the summer, the total soil PLFA content as well as contents of bacteria, VAM fungi, actinomycetes, and G+ and G− bacteria were significantly lower in CONIF-40 and CONIF-80 samples than in BROAD-Nat samples

(Table 4). Ratios of fungi to bacteria and of G+ to G− bacteria were also higher in CONIF-40 forest than in BROAD-Nat forest. Contents of total soil PLFA, bacteria, fungi, VAM fungi, actinomycetes, G+ bacteria, and G− bacteria were all lower in the winter than in the summer for all forest types, and values did not significantly differ among the three forests in the winter. However, as in summer, the ratio of fungi to bacteria was higher in the CONIF-40 forest than in the BROAD-Nat forest in the winter, while this ratio was similar between the CONIF-80 and BROAD-Nat forests during both seasons. The ratio of soil G+ to G− bacteria was higher in the winter than in the summer.

The PCA based on PLFA soil microbial communities in the summer indicated that the first principal component (PC1) and second principal component (PC2) accounted for 62% of the forest soil variation. The high loadings in PC1 included G+ bacteria (i15:0, a15:0, i16:0, and 10Me16:0; positive loadings) and G− bacteria (18:1ω7c and cy19:0; negative loadings) (Figure 2). The high loading in PC2 was a result of the actinomycetes (10Me17:0; positive loading). The majority of the variability was due to the differences between the CONIF-40 forest samples and those of CONIF-80 and BROAD-Nat forest. In this study, PC1 explains 43% of variability, and separates the CONIF-40 samples (positive loadings) from the CONIF-80 and BROAD-Nat samples (negative loadings). The PC2, which explains 19% of the variability, separates the samples CONIF-80 (negative loadings) from BROAD-Nat (positive loadings) (Figure 3). The CONIF-80 forest differed from the BROAD-Nat forest; however, this difference was smaller than that between CONIF-80 and CONIF-40. In winter, differences in the PLFA profiles of the three forest types were not as clear (Figure 4).

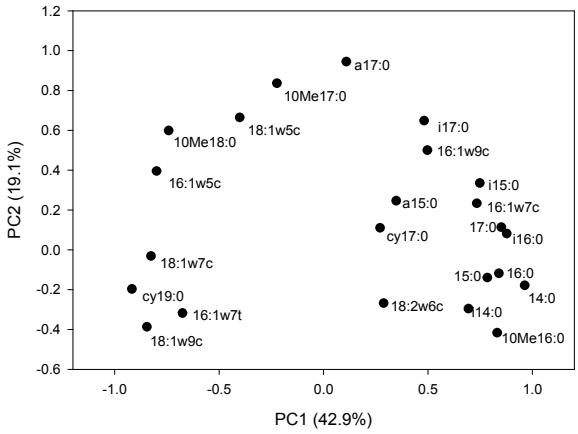

Figure 2. Corresponding loading values from the principal component (PC) analysis of the mole % microbial phospholipid fatty acid content in soil samples from three forest types in summer.

Table 4. Contents of phospholipid acid biomarkers (nmol·g^{-1} soil) and biomarker content ratios in 40-year-old (CONIF-40) and 80-year-old (CONIF-80) coniferous forest plantations and a natural broadleaf forest (BROAD-Nat) in two seasons.

Season	Forest Type	Total PLFA [1]	Bacteria	Fungi	VAM Fungi	Actino-mycetes	G+	G−	G+/G−	Fungi/ Bacteria
Summer	BROAD-Nat	115 a [2]	50.4 a	1.92 a	2.59 a	3.53 a	23.4 a	34.1 a	0.69 c	0.038 b
	CONIF-80	80.7 b	34.5 b	1.63 a	1.68 b	1.99 b	16.6 b	23.4 b	0.71 c	0.047 ab
	CONIF-40	71.5 bc	29.8 b	1.59 a	1.38 b	1.80 b	17.0 b	16.4 c	1.16 b	0.052 a
Winter	BROAD-Nat	50.7 d	12.5 c	0.270 b	0.611 c	0.781 c	6.92 d	5.24 d	1.32 ab	0.021 c
	CONIF-80	58.7 d	17.4 c	0.420 b	0.650 c	0.790 c	9.80 c	7.14 d	1.37 ab	0.024 c
	CONIF-40	64.1 d	18.4 c	0.701 b	0.705 c	0.777 c	10.7 c	7.02 d	1.53 a	0.038 b
ANOVA results [3]										
Forest type		*	*	ns	**	***	*	**	***	***
Sampling season		***	***	***	***	***	***	***	***	***
Forest × season		**	**	ns	***	***	***	***	ns	ns

[1] PLFA: phospholipid fatty acids, VAM: vesicular-arbuscular mycorrhizal, G+: gram-positive bacteria, G−: gram-negative bacteria; [2] Values in each column followed by different lowercase letters are significantly different at $p < 0.05$, based on Duncan's multiple test; [3] * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.

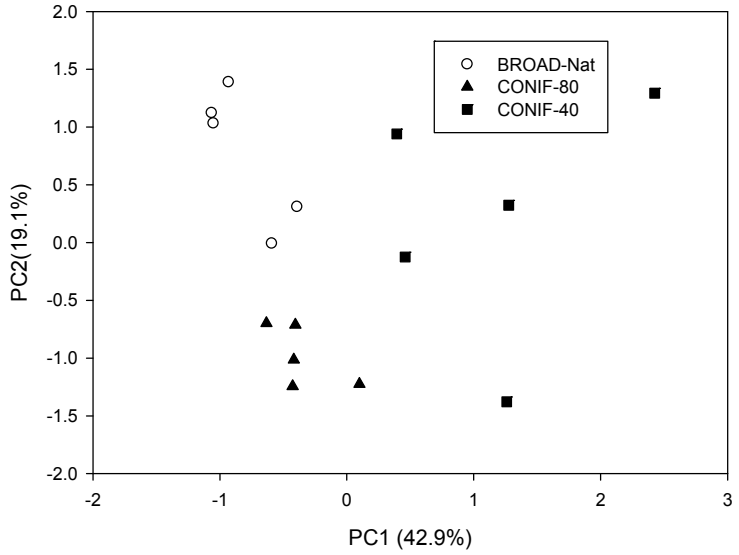

Figure 3. Plots of the two first principal components (PCs) from the principal component analysis of the mole % microbial phospholipid fatty acid content in soil samples from three forest types in summer.

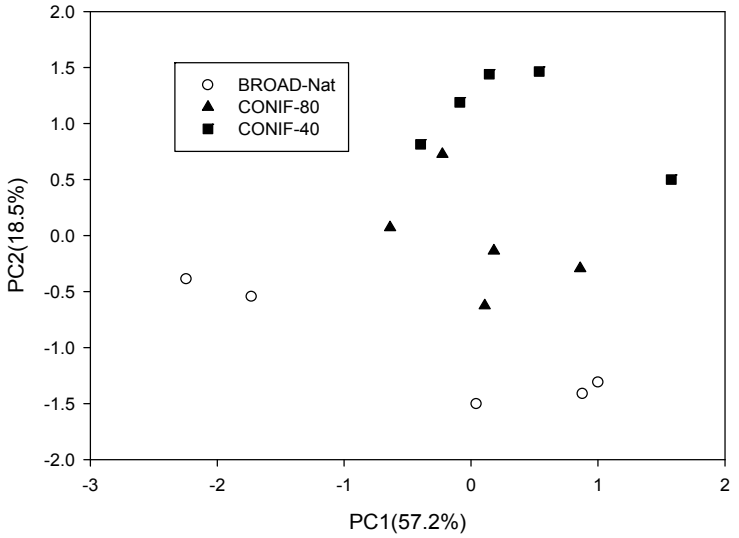

Figure 4. Plots of the two first principal components (PCs) from the principal component analysis of the mole % microbial phospholipid fatty acid content in soil samples from three forest types in winter.

3.5. Correlations

Soil cellulase and phosphatase activities were positively correlated with C_{org} content ($p < 0.05$; Table 5). The total PLFA and G+ bacterial contents as well as soil enzyme activities, except xylanase, were positively correlated with soil N_{tot} content. Additionally, total PLFA content was positively correlated with C_{mic} content, while xylanase activity was negatively correlated with C_{mic} content. PLFA marker content, excluding fungal markers, was positively correlated with the C_{mic}/C_{org} ratio and soil moisture.

Table 5. Pearson's correlation coefficients between soil properties and microbial variables.

Soil Properties	Cellulase	Xylanase	Urease	Phosphatase	Total PLFA	G+	G−	G+/G−	Fungi	VAM Fungi	Fungi/ Bacteria
C	0.643 ***	0.292	0.366	0.454 *	0.320	0.376	0.177	−0.050	0.132	0.186	0.159
N	0.476 **	0.118	0.406 *	0.394 *	0.464 *	0.498 **	0.325	−0.050	0.132	0.339	0.206
C_{mic}	−0.039	−0.606 ***	0.503 **	0.619 ***	0.895 ***	0.917 ***	0.964 ***	−0.902 ***	−0.651 ***	0.952 ***	0.888 ***
C_{mic}/C_{org}	−0.365	−0.753 **	0.268	0.363	0.711 ***	0.711 ***	0.843 ***	−0.884 ***	−0.672 ***	0.821 ***	0.776 ***
C_{mic}/N_{mic}	0.112	−0.584 **	0.459 *	0.676 ***	0.665 ***	0.743 ***	0.819 ***	−0.889 ***	0.670 ***	0.779 ***	0.919 ***
pH	−0.678 ***	−0.148	−0.273	−0.682 ***	−0.419 *	−0.513 **	−0.381 *	0.247	0.202	−0.389 *	−0.492 **
Moisture	0.428 *	−0.039	0.291	0.529 **	0.637 ***	0.680 **	0.529 **	−0.252	−0.023	0.556 **	0.440 *

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

Previous studies involving soil organic matter characterization using ^{13}C nuclear magnetic resonance (NMR) at the same study site showed greater recalcitrant tannin and polyphenol contents in coniferous plantations (*C. konishii* and *C. formosana*) than in BROAD-Nat forest [28]. The resistance of coniferous litter to decomposition may contribute to the accumulation of C and N in soil. However, a decrease in the quality of organic matter input after the replacement of the native broadleaf vegetation with a pure coniferous plantation led to reductions in microbial C and N biomasses (Table 2). Swift et al. [29] showed that litter with a high C/N ratio, such as in coniferous forests, was a poor substrate for microbial growth. Both the C_{mic} and N_{mic} contents were high in the summer, low in the winter, and positively correlated with soil moisture (Table 5). Cool temperatures combined with low rainfall were responsible for the low microbial biomass in winter. Liu et al. [30] reported that the lack of water and low temperatures in winter reduced the microbial biomass in a tropical forest in China.

Several studies have shown that enzyme activities depend on the quality of organic matter [31,32]. Thus, differences in enzyme activities among forest types may have been due to differences in organic matter quality. The leaf litter in native broadleaf forests contains fewer components that are resistant to decomposition, leading to a faster decomposition rate than that of conifer litter [33]. The cellulase activity was positively correlated with the soil organic matter content and negatively correlated with pH (Table 5), in agreement with the results of Safari and Sharifi [34].

Community-level PLFA profiles are useful for detecting soil microbial community responses to various land uses or disturbances in ecosystems [35,36]. The litter derived from coniferous forests decomposes slowly; therefore, coniferous forests may provide fewer labile compounds than the native broadleaf forest. The G− bacterial content has been reported to increase with higher substrate availability [37,38]. Additionally, Kelly et al. [39] reported faster growth of G− compared to that of G+ bacteria in fertile environments. Not surprisingly, the G− bacterial content in our study was lower in coniferous soil than in broadleaf soil. G+ bacteria may be able to adapt to inferior conditions more effectively than G− bacteria, resulting in higher G+/G− ratios. The clearing of native forests and the conversion to coniferous species leads to environmental stress, which may explain the higher G+/G− ratio in CONIF-40 soil compared with that in BROAD-Nat soil. Likewise, Ushio et al. [7,40] reported a higher G+/G− ratio in coniferous soils than in hardwood soils. Similarities in soil G+/G− ratios between CONIF-80 and BROAD-Nat forests, however, confirm our hypothesis that the microbial community structure can be restored with long-term succession despite the use of a different vegetation type through the development of an understory. Ample litter from broadleaf tree regrowth in the CONIF-80 forest may have reduced differences in litter quality between CONIF-80 and BROAD-Nat forests. Seasonally, the higher G+/G− ratio in the winter than in the summer is also due to the greater adaption of G+ bacteria to unfavorable conditions. Generally, G− bacteria are more sensitive to changes in soil moisture [41]. Schimel et al. [42] indicated that drought-tolerant microbes, such as G+ bacteria, are inherently protected from low moisture during drought stress.

The ratio of fungi to bacteria increased in coniferous forest soils after the conversion from BROAD-Nat forest (Table 4). Several studies have shown that the microbial community structure responds to soil fertility, with fertile soils favoring bacteria while less fertile soils favor fungi [43,44]. High availability of labile C, for which bacteria are more competitive than fungi, may restrain fungal growth in natural broadleaf forest soil [33]. Greater concentrations of tannins in coniferous forests may also contribute to a higher ratio of fungi to bacteria, as fungi have a higher tannin tolerance [40]. Additionally, fungi can use recalcitrant compounds as C sources [45]. Ushio et al. [7] found the ratio of fungi to bacteria to be significantly higher in coniferous compared to broadleaf soils in tropical mountain areas. In this study, the higher soil fungal content in CONIF-40 than in the BROAD-Nat forest and the similarities in the soil fungal contents between CONIF-80 and the BROAD-Nat forest suggest an evolution of the microbial community that coincides with forest clearing, reforestation, and aging of secondary forest. Thus, forest clearing and reforestation with coniferous species changes the

microbial community by increasing soil fungi and decreasing soil bacteria, resulting in a high ratio of fungi to bacteria in the soil. The ratio of soil fungi to bacteria, however, returns to levels similar to that of the native forest system with the long-term (~80 years) growth of secondary forest. Understory broadleaf shrubs and trees that gradually become established over time eventually contribute sufficient litter to balance out the effects of the coniferous litter, favoring the recovery of bacteria.

The 16:1ω5 PLFA peak is an indicator of VAM fungi [46]. The highest soil VAM fungal content was observed in the BROAD-Nat forest in the summer, suggesting that broadleaf plants support mycorrhizal fungi [47]. Priha et al. [48] and Djukic et al. [49] also found higher contents of 16:1ω5 PLFA peak in broadleaf- than in conifer-dominated forest soils. However, one should be careful in using 16:1ω5 PLFA peak as the marker for AM fungi. Olsson [50] indicated this PLFA peak could be also found in some bacteria. Priha et al. [51] noted the 16:1ω5 PLFA peak content may respond to changes in the soil available C. Thus, the high 16:1ω5 content in the BROAD-Nat forest may be due to the high labile soil C in the broadleaf forest.

The PCA of the PLFA content clearly differentiated between the CONIF-40 forest soil and the other two forest soils, largely due to the PC1 axis. The bacterial biomarkers contributed the most to the PC1 score, suggesting that bacterial content is an important factor in determining the microbial community structure of forests in this ecosystem. Our results indicate that the soil microbial community under the CONIF-40 forest was still in a successional phase, while the soil microbial community under the CONIF-80 forest more reached that of the BROAD-Nat forest.

5. Conclusions

Soil microbial biomass and enzyme activities remain lower in secondary coniferous forests than in a natural broadleaf forest even after several decades of vegetation replacement. However, 80 years of growth of a secondary conifer forest led to a soil microbial community structure similar to that under the natural broadleaf forest. We found that the soil microbial community changes along with the growth of coniferous forest in a subtropical region. The conversion of broadleaf to coniferous forests leads to increased ratios of fungi to bacteria and of G+ to G− bacteria. Following the long-term aging of the coniferous plantation, however, bacterial and G− bacterial contents increased, returning the soil microbial community to lower ratios of fungi to bacteria and of G+ to G− bacteria and restoring values similar to those in the native broadleaf forest. The understory broadleaf shrubs and trees established after the reforestation of coniferous plantations may balance out the effects of the coniferous litter, contributing to the recovery of bacteria. Such a mechanism may improve soil restoration after reforestation in subtropical areas.

Acknowledgments: This work was funded by the Ministry of Science and Technology, Taiwan (MOST 104-2621-B-001-005) and by Academia Sinica.

Author Contributions: E.-H.C., T.-H.C., and C.-Y.C. conceived and designed the experiments; E.-H.C. performed the experiments; E.-H.C. and G.T. analyzed the data; C.-K.H. contributed plant inventory; E.-H.C. wrote the original draft; G.T. and C.-Y.C. reviewed and edited the manuscript.

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

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