Response of Humic Acids and Soil Organic Matter to Vegetation Replacement in Subtropical High Mountain Forests

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Abstract Reforestation can alter the chemical composition of soil organic matter (SOM) and humification; however, information on how specific plant types impact SOM lability and humification is not well documented. In this study, we used solid-state 13C nuclear magnetic resonance spectroscopy, photometric analysis, and chemical fractionation to examine carbon (C) components and lability of SOM in a Japanese cedar (Cryptomeria japonica) forest and bamboo (Phyllostachys edulis) plantation that reforested a cutover primary broadleaf forest. The 2logK value of soil humic acids, the inverse index of SOM humification, was lowest in the bamboo plantation, suggesting a higher SOM humification stage in the bamboo plantation. The soil labile C/Total C ratio was highest in the bamboo plantation, and this can be attributed to low aromaticity and alkyl-C/O-alkyl-C ratio (A/O-A) in the bamboo litter. Intensive cultivation of the bamboo plantation accelerated litter breakdown in the strongly acidic soil, resulting in the depletion of SOM. Cedar coniferous leaves, with their high recalcitrant substances and slow decomposition, only slightly lowered SOM humification due to the substantial broadleaf understory. Our results suggest that the type of plants involved in reforestation and understory reestablishment is critical to how SOM humification and lability change during the reforestation through the control of litter C components. Further research into the interaction between microclimate change and forest type in forest conversion will be useful for increasing understanding on the impact of forest conversion on SOM lability and humification in subtropical high mountain forest ecosystems.

1. Introduction

Soil organic matter (SOM) comprises microbial, plant, and animal residues. As the largest terrestrial organic carbon (C) pool, it can be both a CO2 sink and source (Stockmann et al., 2013). This substantial pool of soil C is the result of decomposition and humification processes that affect stable soil C accumulation and efflux, thus regulating the overall C budget (Bayer et al., 2002; Cheng et al., 2007). The changes in SOM stability and decomposability impact the local and regional C cycles, and the CO2 exchange process between the soil and atmosphere (Larionova et al., 2007; Stockmann et al., 2013). SOM humification and soil C accumulation are sensitive to climatic and local environmental fluctuations and changes in land use and soil management (Wang et al., 2016; Wang et al., 2016).

Humification of SOM is largely dependent on its chemical properties (e.g., labile and recalcitrant residue content) and molecular structure (e.g., lignin, lipids, proteins, and saccharides) (Guggenberger, 2005). Both factors also exert complex and interactive effects on the function and quality of the soil (Feller & Beare, 1997; Tiessen et al., 1994). Specifically, the chemical composition of SOM affects the proportion of the labile (rapid turnover) to recalcitrant (slow turnover) C fractions (Rovira & Vallejo, 2002). Since the former is highly bioactive, it dominates the C and nitrogen (N) fluxes as a reservoir of readily available nutrients, whereas the latter is more relevant to long-term C storage (Belay-Tedla et al., 2009; Cheng et al., 2007). Thus, C availability to microorganisms in both the long and short term relies on the proportion of the labile to recalcitrant fractions and, hence, on the SOM chemical composition (Belay-Tedla et al., 2009; Hu et al., 1997).
In general, changes in litter input due to vegetation conversion alter microbial species composition, soil nutrient availability, and moisture supply, and SOM chemical structure, and these changes affect microbial processes and litter decomposition rates (Chang et al., 2016; Jien et al., 2011; Song et al., 2016; Xu et al., 2014). From 1990 to 2010, countries in East Asia, particularly China, underwent considerable reforestation in which primary forests were replaced with one or two even-aged, fast-growing, and short-rotation tree species (FAO, 2010). Among these, bamboo was widely planted in East Asia (5.9 million hectares in 2010), and China has 15.4% of bamboo in the world (FAO, 2010; Fu, 2001; Song et al., 2011). The total area of bamboo plantations increased by 1.8 million hectares in China from 1990 to 2010 (FAO, 2010). The plant’s unique rhizome and root system enables the effective establishment of a plantation. The herbaceous litter from bamboo leaves and branches with a low C/N ratio is easily decomposed and likely to alleviate environmental stress to the soil bacterial communities (Chang & Chiu, 2015).

Ono et al. (2011) noted that initial litter components (aromatic and aliphatic C groups) of reforested Japanese cedar and Hinoki cypress and humification processes could affect organochemical compositions of the SOM. Reforestation with cedar secondary forest decreased soil microbial biomass, soil enzymatic activities, and the abundance of total phospholipid fatty acid content (Chang et al., 2018; Lin et al., 2017); these changes could slow the SOM humification process. Reforestation of the strongly acidic natural forest was found to drive microbial community structure to favor gram-negative bacteria due to an enhancement in SOM decomposition and increase in nutrient availability after forest conversion (Chang et al., 2018). Disturbance to forests undergoing forest conversion reduces SOM stabilization and accelerates SOM turnover (Jandl et al., 2007).

Although a large body of data is available on how changes in land use affect SOM turnover and humification, little is known about the impact of reforestation (e.g., cedar and bamboo plantation) using high-altitude natural forests on SOM chemical properties and the degree of humification. The impact of reforestation on SOM was controlled not only by vegetation type but also by climatic factors. Schmidt et al. (2011) indicated that biotic and abiotic environment (environmental properties), rather than the molecular structure of plant input and organic matter, has a major role in determining carbon residence time. Wang, Chou, et al. (2016) suggested that SOM humification decreases with increasing elevation (600–1400 m) in moso bamboo plantations. Less adapted to low temperature than a cedar plantation, the impact of reforestation with a bamboo plantation on SOM humification in high altitude needs further study.

We hypothesized that changes in litter composition and environment properties from a broadleaf forest to bamboo plantation or cedar forest would affect the degree of SOM humification and lability. In the current study, we tested this hypothesis using both physical and chemical analyses of SOM, including solid-state $^{13}$C cross-polarized magic angle spinning nuclear magnetic resonance (NMR) spectroscopy, which is widely used to investigate SOM functional groups associated with the humification index (Kögel-Knabner, 1997). In addition, we discuss the relevance of labile and recalcitrant soil C fractions in the context of soil nutrient availability and long-term organic C storage during vegetation change.

### 2. Materials and Methods

#### 2.1. Study Site

The study was conducted in the Shitou Experimental Forest of National Taiwan University in Nantou County, central Taiwan (23°39′17″N, 120°48′29″E; 1,800 m asl). No endangered or protected species were involved, and all appropriate permissions were obtained from the university prior to the study. The site is in a mild and humid subtropical climate, and the altitude is approximately 1,800 m. The average annual temperature (1941–2009) is 16.6 °C; lowest monthly temperature is 7.7 °C in January and highest is 24.2 °C in July (Wang et al., 2010). Approximately 77% of the annual precipitation (mean precipitation of 2600 mm) occurs during the rainy season (May to September) (Wang et al., 2010). Soils in the hardwood forest and bamboo plantation were Typic Dystrudepts, while the soil in the cedar plantation was Humic Dystrudepts; all soils had a clay loam texture (Chang et al., 2018).

Even-aged reforested Japanese cedar (Cryptomeria japonica) replaced some areas of the primary broadleaf forest at the study site 40–60 years ago. In addition, the temperate, giant timber moso bamboo (Phyllostachys edulis) was planted in some areas originally covered by the broadleaf forest about 25–40
years ago. Land use changes were recorded in the maps of the Chinese-American Joint Commission on Rural Reconstruction from 1956, instructions from the Agricultural and Forestry Aerial Survey Institute from 1977, and instructions from the Agricultural and Forestry Aerial Survey Institute from 1995 (Aerial Survey Office, Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan) (Figure 1).

The region’s main vegetation is primary broadleaf forest, which includes the following dominant species: *Osmanthus matsumurana*, *Litsea acuminata*, *Illicium arborescens*, *Castanopsis cuspidata*, and *Michelia compressa*. Due to the low number of disturbances and regrowth of broadleaf forest plants, several broadleaf species were observed in the cedar secondary plantation, including *Strobilanthes flexicaulis*, *Illicium arborescens*, *Meliosma callicarpifolia*, *Eurya gnaphalocarpa*, *Neolitsea sericea (BL) Koidz.*, and *Diplazium kawakamii Hayata* (Pai et al., 2014). The 2-year litterfall data from March 2016 to February 2018 showed that broadleaf plants (e.g., broadleaf foliage, branches, seeds, flowers, and bracts) and other residual matter (e.g., insects) accounted for 32.0–42.1% of annual litterfall in the cedar secondary plantation, but only 6.9–7.3% of annual litterfall in the bamboo plantation (C.P. Chen, unpublished data). In contrast to the dense understory plants in broadleaf primary forests and cedar secondary forests, bamboo plantations have nearly no undergrowth. The leaves of bamboo have alkaloids and dense culms, and the rhizome system of the bamboo can cause allelopathy to restrict understory growth.

In addition, bamboo shoots are consumed as a vegetable and are considered as a traditional food in East Asia. Moso bamboo shoots emerge out of the ground in the winter and spring, and forest floors are removed to dig into subsoil more than 10 cm deep to harvest bamboo shoots annually. Frequent harvesting cycles and methods for extracting the shoots from culms are used to manage bamboo plantations, resulting in a greater soil disturbance than in the other two types of forest.

### 2.2. Soil Sampling and Chemical Analysis

Three 50 × 50 m sampling plots for each vegetation type, separated by more than 50 m, were randomly established in February 2014. In each plot, a composite soil sample 0–10 cm deep, consisting of 15 soil cores randomly distributed across the entire plot, was collected with a soil auger (3 cm diam.). The 15 soil samplings were mixed into a composite sample from each plot, and each forest type had three (replicates) composite samples. After removing stones and visible plant residues such as roots, shoots, leaves, the fresh soil was weighed and measured for soil water to determine its bulk density. Subsamples of the fresh soil were air dried, ground to powder for chemical analysis. The volume of stones was measured and excluded when calculating soil bulk density. Subsamples of the fresh soil were air-dried, and sieved through 2 mm mesh. Litter was also sampled in each plot using a 0.5 × 0.5 m quadrat. The litter sample was dried at 70 °C and ground to powder for chemical analysis.

The soil water content was determined by oven drying at 105 °C to constant weight (w/w, %). A combination glass electrode was used to determine pH of 1:1 soil water extracts. Organic C and total N (TN) were measured using 13C NMR spectroscopy.

### 2.3. Extraction of Humic Acids

For NMR spectroscopy, humic substances were extracted using 0.1 M NaOH under an atmosphere of N2 using a ratio of 10:1 (extractant: soil). Extraction was centrifuged (15,000 × g for 20 min) to collect the alkaline supernatant, which was acidified using 6 M HCl for 12–16 hr to pH = 1.0. The supernatant was centrifuged again (5,500 × g for 15 min) to obtain a precipitate, which was then washed with a mixture of 0.3 M KCl and 0.1 M KOH, followed by centrifugation (10,000 × g for 10 min). Next, the supernatant was acidified using 6 M HCl to pH = 1.0 and centrifuged (5,500 × g for 15 min). Further extraction was performed overnight in a mixture of 0.3 M HF and 0.1 M HCl, followed by centrifugation (5,500 × g for 15 min) (Swift, 1996). Humic acids were put into the freezer at −20 °C in freeze-dry aluminum containers for 24 hr and were kept in the ultralow temperature freezer at −80 °C for 24 hr. The freeze dryer (Freeze Dryer CT series, Panchum com.) with an oil vacuum pump was operated to freeze dry the resultant at 40 °C for 96 hr. The components of the freeze-dried resultant were determined by 13C NMR spectroscopy.
Figure 1. Maps showing land use changes from primary broadleaf forest (black circles) to Japanese cedar coniferous secondary forest (white circles) and bamboo plantation (black triangles). (a) Study site location in Taiwan; (b) land use in 1956 (according to the Chinese-American Joint Commission on Rural Reconstruction, Land Use and Forest Resource Maps, JCRR, Taipei); (c) land use in 1977 (according to the second forest resource and land use inventory in Taiwan, Aerial Survey Office, Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan); (d) land use in 1995 (according to the third forest resource and land use inventory in Taiwan, Aerial Survey Office, Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan). According to the cultivation history, the bamboo was cultivated for 25–40 years and Japanese cedar was cultivated for 40–60 years.
centrifugation. Precipitates were dissolved using 0.01 M NaOH (30 ml), and the ratio of humic acid absorbance was determined using a spectrophotometer (Hitachi U-2000) (Gerzabek et al., 1996). The ΔlogK value was then calculated as log(A400/A600), where A400 and A600 are the humic acid absorbances at 400 and 600 nm, respectively (Kumada, 1987). The ΔlogK value was used as the index to evaluate the SOM humification stage. A lower ΔlogK indicates a higher SOM humification stage (Ikeya & Watanabe, 2003).

2.4. 13C NMR Spectroscopy

Organic C functional groups were measured for soil humic acid and litter by 13C NMR spectroscopy. Freeze-dried soil humic acid samples were placed in a sample tube (7 mm diameter) to determine the soil chemical shift composition using 13C NMR spectroscopy (BRUKER DSX 400 MHz solid-state NMR, Germany). Powdered litter collected from the study site was analyzed using 13C NMR under the same conditions. The following spectrometer settings were used: spectrum frequency (100.46 MHz), spinning speed (7000 Hz), bandwidth (20,000 Hz), contact time (6 ms), delay time (1 s), and scans (8,200). Chemical shifts were divided into alkyl-C (0–50 ppm), O-alkyl-C (50–90 ppm), di-O-alkyl-C (90–110 ppm), aromatic-C (110–165 ppm), and carboxyl-C (165–190 ppm) bands (Kögel-Knabner, 1997). The area under the spectrum curve was calculated for the relative content of different functional group classes, which was showed as the proportion of peak area to total spectrum curve area. During decomposition, alkyl-C levels increase while O-alkyl-C levels decrease; thus, the degree of SOM humification was calculated as the ratio of alkyl-C to O-alkyl-C and di-O-alkyl-C fractions (A/O-A) (Baldock et al., 1997). In addition, because aromatic-C content increases during decomposition, aromaticity was selected as another SOM humification index and calculated as the proportion of 110–165 ppm spectrum area (aromatic-C) to 0–165 ppm total spectrum area (the sum of alkyl-C, O-alkyl-C, di-O-alkyl-C, and aromatic-C) (Hatcher et al., 1981).

2.5. Quantification of Labile and Recalcitrant C Pools

The acid hydrolysis technique is a chemical separation method that divides labile C (acid-hydrolyzable) and recalcitrant C (unhydrolyzable) pools (McLaughlan & Hobbie, 2004). A composite soil sample (0.5 g) and extractant (20 ml) of 2.5 M H2SO4 were placed in Pyrex flasks with Allihn condensers and hydrolyzed at 105 °C for 30 min (Rovira & Vallejo, 2002). Hydrolyzed SOM samples were then centrifuged (20,000 × g for 10 min) and decanted to collect the C-containing supernatant. The precipitated residue was washed with deionized water (20 ml) and rinsate was combined with the supernatant (hydrolyzate). C content of hydrolyzable and recalcitrant C pools were determined by one-way analysis of variance ANOVA. To meet the ANOVA test assumptions, the normality of residues of each factor level were tested by Shapiro-Wilk test, and homoscedasticity was checked using Levene's test. A nonparametric Kruskal-Wallis test was used when the assumptions of the ANOVA test were not met. Tukey's Honestly Significant Difference test and the Pairwise Wilcoxon rank sum test were used to calculate significant differences in group means among vegetation types. Significance threshold was set at p < 0.05. All statistical data were analyzed using R programming (R Core Team).

3. Results

3.1. Soil Physical and Chemical Properties

The soil water content did not differ significantly among vegetation types. The pH significantly differed among the forest types, being highest in the bamboo plantation and lowest in the broadleaf forest (Table 1). Reforestation with Japanese cedar and bamboo decreased total soil organic C (TC) and TN concentrations, though TC mass did not differ significantly among vegetation types. The ΔlogK values of soil humic acids for
the sites were lowest for bamboo, indicating a higher SOM humification stage in the bamboo plantation, followed by the Japanese cedar secondary and broadleaf forests.

3.2. Carbon Functional Groups

The spectra of litter and surface soils suggested the presence of five C functional groups at 33, 72, 103, 129, and 173 ppm (Figure 2). The O-alkyl-C compounds were predominant in all types of litter (cellulose and hemicellulose) (Table 2). Di-O-alkyl-C content tended to be highest in the bamboo litter and lowest in the Japanese cedar litter. The aromatic-C content in litter tended to be lowest for bamboo, moderate for the broadleaf forest, and highest for the cedar secondary forest. The litter alkyl-C, O-alkyl-C, and carboxyl-C contents did not differ significantly among vegetation types. The bamboo litter exhibited the lowest A/O-A ratio and aromaticity, though there was no significant difference among vegetation types.

Soil O-alkyl-C and alkyl-C content did not significantly differ among forest types (Table 2). Soil aromatic-C content tended to be highest for the bamboo plantation, moderate for the broadleaf forest, and lowest for the cedar forest. Soil carboxyl-C content did not differ significantly among vegetation types. There was no difference in soil A/O-A ratios among the three forest types. The aromaticity was highest in the bamboo soil, moderate in the broadleaf forest soil, and the lowest in the Japanese cedar soil.

3.3. Labile and Recalcitrant C Components

Neither soil labile (LPII-C and LPII-C) nor recalcitrant (RP-C) C pools differed significantly among vegetation types (Table 3). However, the soil LPII-C/TC ratio was highest in the bamboo plantation, followed by the

| Vegetation          | Soil water content (%) | Bulk density (g cm⁻³) | pH (H₂O)       | TC (%)  | TN (%)  | TC mass (Mg C ha⁻¹) | ΔlogK  |
|---------------------|------------------------|-----------------------|----------------|---------|---------|--------------------|--------|
| Broadleaf           | 44.7 ± 3.2             | 0.46 ± 0.03 a         | 3.67 ± 0.12 b  | 18.2 ± 0.9 a | 1.06 ± 0.09 | 78.3 ± 4.8       | 5.60 ± 0.13 a |
| Japanese cedar      | 60.4 ± 9.1             | 0.61 ± 0.07 b         | 4.34 ± 0.29 a  | 11.7 ± 1.0 b | 0.83 ± 0.08 | 71.8 ± 14.3      | 5.45 ± 0.33 ab |
| Bamboo              | 49.3 ± 6.9             | 0.38 ± 0.15 b         | 4.77 ± 0.05 a  | 11.8 ± 2.2 b | 0.92 ± 0.16 | 64.2 ± 12.6      | 4.71 ± 0.41 b  |
| p value             | 0.073                  | 0.073                 | <0.001***      | 0.003**  | 0.118   | 0.372              | 0.028*** |

Note. Different letters within a column indicate significant differences among forest types (Tukey’s HSD test, p = 0.05) based on the one-way ANOVA test. Data from three replicate samples (n = 3 for each forest type) are presented as mean ± standard deviation.

Figure 2. (a) Solid-state [C] CP-MAS NMR spectra of litter from the primary broadleaf forest (BL), Japanese cedar secondary forest (JCD), and bamboo plantation (BM). The following chemical shift regions are indicated: 0–50 ppm (alkyl-C), 50–90 ppm (O-alkyl-C), 90–110 ppm (di-O-alkyl-C), 110–165 ppm (aromatic-C), and 165–190 ppm (carboxyl-C). (b) Solid-state [C] CP-MAS NMR spectra of soil humic acids from BL, JCD, and BM.
Table 2

| Vegetation          | Functional group (%)      |
|---------------------|---------------------------|
|                     | Alkyl-C  | O-alkyl-C | Di-O-alkyl-C | Aromatic-C | Carboxyl-C | A/O-A ratio | Aromaticity |
| Litter              |          |           |              |            |            |             |             |
| Broadleaf           | 22.89 ± 1.33 | 45.52 ± 0.71 | 10.60 ± 0.21 b | 15.88 ± 0.70 a | 5.12 ± 0.21 | 0.41 ± 0.03 | 16.74 ± 0.71 |
| Japanese cedar      | 23.61 ± 0.81 | 45.24 ± 1.01 | 9.91 ± 0.40 c | 16.55 ± 0.72 a | 4.64 ± 0.17 | 0.43 ± 0.03 | 17.36 ± 0.72 |
| Bamboo              | 13.07 ± 0.52 | 58.07 ± 0.90 | 12.63 ± 0.08 a | 11.62 ± 0.55 b | 4.61 ± 0.30 | 0.19 ± 0.01 | 12.19 ± 0.61 |
| Soil (0–10 cm)      | 32.41 ± 2.07 | 32.34 ± 1.15 a | 5.76 ± 0.59  | 18.86 ± 1.23 a | 10.64 ± 0.93 | 0.85 ± 0.09 | 21.10 ± 1.19 |

Note. Data from three replicate samples (n = 3 for each forest type) are presented as mean ± standard deviation. Different letters within a column indicate significant differences among forest types (Tukey's HSD test, p = 0.05) based on the one-way ANOVA test. kw = Kruskal-Wallis test, which was used because the data had due to nonnormal distribution of the data. A/O = Aromaticity.

4. Discussion

The data presented indicate that reforestation of the broadleaf forest resulted in reduced soil TC content. A meta-analysis of data from 30 published studies indicates that soil C stock declines (−13%) after land use changes from native forest to plantations (Guo & Gifford, 2002). Compared to the litter, soil contained relatively lower percentages of O-alkyl-C and di-O-alkyl-C compounds and higher percentages of alkyl-C. Alkyl-C and aromatic-C compounds—which contain high concentrations of recalcitrant substances such as surface waxes, cutins, lignin, and tannins—increased with the decomposition of easily degradable compounds of organic inputs (Baldock et al., 1997; Kögel-Knabner, 1997). These patterns indicate that, regardless of vegetation type, recalcitrant substances are enriched in SOM as litter decomposes (Kögel-Knabner, 1997; Wang, Tian, & Chiu, 2016). However, forest type can affect SOM composition through differences in organic C groups in the litter and roots (Schmidt et al., 2011). For example, the O-alkyl-C content was highest in the bamboo soil because of the input of the bamboo litter, which contained relatively more O-alkyl-C than broadleaf and Japanese cedar litter. We also observed that, of the three vegetation types, the aromatic-C and ΔlogK were lowest in bamboo herbaceous litter, and aromaticity was highest in bamboo soil. Such C functional groups in the bamboo litter seem to favor litter decomposition, facilitate SOM humification, and lead to greater humification stage of SOM in the bamboo plantation. Schmidt et al. (2011) indicated that root-derived C is retained in soils much more efficiently than aboveground inputs of plant litter; thus, fine roots also play an important role in the humification of SOM. The fine root biomass, annual fine root production, annual fine root decomposition, and turnover rate in the bamboo plantation were 2.30, 2.84, 5.88, and 1.23 times larger, respectively, than those at the cedar plantation study site (Chen, 2017). This

Table 3

| Vegetation          | LPII-C (g C/kg) | LPII-C (g C/kg) | RP-C (g C/kg) | LPII-C/TC | LPII-C/TC | RP-C/TC |
|---------------------|-----------------|-----------------|--------------|-----------|-----------|---------|
| Broadleaf           | 52.92 ± 8.46    | 19.23 ± 2.31    | 124.9 ± 15.88 | 0.290 ± 0.038 b | 0.106 ± 0.014 | 0.684 ± 0.054 |
| Japanese cedar      | 42.04 ± 5.78    | 12.25 ± 3.26    | 84.80 ± 20.10 | 0.363 ± 0.081 ab | 0.106 ± 0.036 | 0.732 ± 0.212 |
| Bamboo              | 58.18 ± 10.39   | 14.85 ± 4.50    | 110.1 ± 27.71 | 0.498 ± 0.089 a | 0.130 ± 0.052 | 0.957 ± 0.323 |
| p value             | 0.134           | 0.119           | 0.154         | 0.033      | 0.686      | 0.352    |

Note. Data from three replicate samples (n = 3 for each forest type) are presented as mean ± standard deviation. Ratios of labile and recalcitrant carbon to total organic carbon (TC) are shown in the last three columns. Different letters within a column indicate significant differences among forest types (Tukey's HSD test, p = 0.05) based on the one-way ANOVA test.
might partially explain the high SOM humification under bamboo through root-derived C. Further research on the C composition of roots is necessary to better understand the contribution of litter and roots to SOM humification at this site.

High lability of SOM in the bamboo plantation, as revealed by acid hydrolysis, should also be linked to relatively high contributions of soil O-alkyl-C and di-O-alkyl-C compounds in the bamboo litter. A decrease in the G+/G− bacteria ratio was also observed after reforestation of the same site in the broadleaf forest with bamboo in another study (Chang et al., 2018). It confirms that the microbial community experienced low C and N stress in the bamboo soil, which is consistent with the high labile C content in the bamboo soil revealed by acid hydrolysis. Some studies have indicated that vegetation conversion from a natural forest to monoculture plantations could reduce the soil labile fraction of C (Sheng et al., 2015; Yang et al., 2009); the inconsistent result in this study should be due to the high input of rhizomes and fine roots leading to increase in lability of SOM in the bamboo plantation.

The SOM stability or lability during the processes of litter decomposition and humification in various vegetation types could be related to climatic conditions. The bamboo plantation at this study site (1,800 m) showed higher soil aromaticity than at low elevation sites (800–1,400 m) (Wang, Chou, et al., 2016), indicating that increases in elevation could reduce the lability of bamboo soil SOM. The lower temperature at higher altitudes could restrict the microbial decomposition, resulting in the increase in recalcitrant SOM (Wang, Chou, et al., 2016). Human management could also affect the SOM lability and stability during forest conversion. For instance, compared to the broadleaf forest and cedar secondary forest, the bamboo plantation received intensive field management—such as regular bamboo shoot harvesting, tilling, and fertilizer use—and this could have accelerated litter and SOM decomposition (Li et al., 2013) and resulted in a higher SOM humification stage and lower soil organic C (Li et al., 2013; Liu et al., 2011; Wang, Tian, & Chiu, 2016).

Even though the Japanese cedar secondary forest was older in this site than the bamboo plantation, the Japanese cedar secondary forest showed only a slightly change from broadleaf forest in SOM composition and degree of SOM humification. The Japanese cedar litter was characterized by the highest aromatic-C, implying that the coniferous litter was less susceptible to degradation than the broadleaf litter. Several studies have indicated that cedar litter is associated with low nutrient (N and P) content and the presence of a high amount of resistant substances (lignin and waxes) (Laganière et al., 2010; Prescott et al., 2004; Wang et al., 2013). In addition, C components of the Japanese cedar litter—characterized by NMR—were similar to those of the broadleaf litter, but different from the bamboo litter. The relatively few changes in the modifications to SOM composition and humification that occurred after forest conversion with the Japanese cedar secondary forest is also due to the decreased density and groundcover of cedar, which allowed remnant broadleaf species to regenerate in the understory. The 2-year observation data showed that broadleaf species accounted for 32–42% of annual litterfall in the cedar plantation (C.P. Chen, unpublished data). The roots of understory plants that derived from broadleaf plants accounted for about 50% of fine root biomass and turnover (Chen, 2017). The good regrowth of the remnant broadleaf species understory in the Japanese cedar secondary forest may have alleviated the influence of needle litter and roots on SOM composition after the forest conversion by conifers. A study on reforestation with native pine in China also found that understory fern expansion increased soil C storage and improved soil N and phosphorus (P) status (Lyu et al., 2019). Thus, maintaining a substantial broadleaf understory in Japanese cedar plantations could mitigate the impacts of conifer reforestation on the SOM composition.

5. Conclusions

The current study demonstrates that reforestation impacts soil C content, lability and recalcitrance, and humification stage. Forest conversion to a bamboo plantation with high input of labile C via plant litter and fine roots and frequent shoot harvesting accelerated litter decomposition and turnover, leading to a lower amount of SOM and higher humification stage of SOM. The SOM lability, recalcitrance, and humification stage remained almost unchanged when the broadleaf forest was converted into a Japanese cedar forest, possibly due to the similarity in litter C groups between the Japanese cedar and broadleaf forests. Cedar secondary forests contain a substantial broadleaf understory, which could have alleviated the influence of conifer needle litter and roots on SOM composition after reforestation. Overall, in a given ecosystem, the type of trees used for reforestation is a critical factor affecting SOM lability, recalcitrance, and
humification stage. In forest management and reforestation projects, a lower frequency of disturbances will allow remnant species to regenerate in the understory, which might be beneficial in terms of reducing the negative impact of afforestation on soil properties. Further research is needed to understand the changes in microclimate after the forest conversion for high mountain ecosystems and look into the interplay between changes in microclimatic condition and SOM lability and humification.

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