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Conversion of Natural Evergreen Broadleaved Forests Decreases Soil Organic Carbon but Increases the Relative Contribution of Microbial Residue in Subtropical China

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Abstract: It has been recognized that land use change affects soil organic carbon (SOC) dynamics and the associated microbial turnover. However, the contribution of microbial residue to SOC storage remains largely unknown in land use change processes. To this end, we adopted a "space for time" approach to examine the dynamics of SOC and amino sugars, which was a biomarker of microbial residue C, in different natural forest conversions. Three typical converted forests were selected: an assisted natural regeneration (ANR) and two coniferous plantations of *Cunninghamia lanceolata* (Lamb.) Hook (Chinese fir) and *Pinus massoniana* Lamb. (pine) each. All of these were developed at the same time after the harvest of an old natural forest and they were used to evaluate the effects of forest conversions with contrasting anthropogenic disturbance on SOC and microbial residue C, along with the natural forest. Natural forest conversion led to an approximately 42% decrease in SOC for ANR with low anthropogenic disturbance, 60% for the Chinese fir plantation, and 64% for the pine plantation. In contrast, the natural forest conversion led to a 32% decrease in the total amino sugars (TAS) for ANR, 43% for the Chinese fir plantation, and 54% for the pine plantation at a soil depth of 0–10 cm. The ratios of TAS to SOC were significantly increased following natural forest conversion, with the highest ratio being observed in the Chinese fir plantation, whereas the ratios of glucosamine to muramic acid (GluN/MurA) were significantly decreased in the two plantations, but not in ANR. The contents of SOC, individual amino sugar, or TAS, and GluN/MurA ratios were consistently higher at a soil depth of 0–10 cm than at 10–20 cm for all of the experimental forests. Redundancy analysis showed that microbial residue C was significantly correlated with SOC, and both were positively correlated with fine root biomass, annual litterfall, and soil available phosphorus. Taken together, our findings demonstrated that microbial residue C accumulation varied with SOC and litter input, and played a more important role in SOC storage following forest conversion to plantations with higher anthropogenic disturbance.

Keywords: soil organic carbon; soil microbial residue; forest conversion; natural forest; assisted natural regeneration; plantation

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1. Introduction

The decomposition, transformation, and stabilization of soil organic carbon (SOC) are the consequence of microbial growth and activity, which is a process that is associated with proliferation, metabolism, and mortality of microorganisms. More and more attention has been paid to microbial residue in recent studies, which demonstrated that senesced microbial biomass may play a much greater role in the stabilization of soil C pools than that previously considered [1–4]. It is critical to elucidate the response of soil microbial residue to global change, as well as the underlying mechanisms driving its transformation, to better understand global biogeochemical cycles and improve current global C cycle models [5,6]. There are a number of studies exploring the effects of global change on soil microbial residue. For example, Zhang et al. reported land use effects on amino sugars in soil particle size fractions [7]. Liang and Balser observed that warming and N deposition reduced microbial residue contribution to the soil C pool [8]. To the best of our knowledge, studies investigating the effects of forest conversion on microbial residue contribution to soil C sequestration are lacking.

Space for time substitution is still used as a reasonable method to evaluate the legacy of forest conversion on ecosystem properties. In general, the reestablished forests, such as plantation forests, are taken as experimental units and adjacent native forest as a reference. The forest conversions are usually subjected to different management coupled with anthropogenic disturbances of various intensity, which alter the structure of the forest ecosystem [9–11]. Consequently, changes in forest structure and management practices likely lead to the alteration, not only of input rates and organic matter decomposability, but also of soil moisture and temperature regimes [12,13]. The adaptation of soil microbial abundance, community composition, and activity following these changes can shift the soil biogeochemical cycling processes regulated by microorganisms [14,15], eventually affecting the contribution of microbial residue C to the soil C pool [16].

It is difficult to establish the correlation between living microbial biomass and long-term SOC sequestration [17] or to directly measure C that is bound in microbial residues. Alternatively, amino sugars, which are important microbial residue biomarkers in soil [18,19], can represent the legacy of microbial-derived constituents and be used to estimate the contribution of dead microbial cells to soil organic C pools [20,21]. Glucosamine (GluN), muramic acid (MurA), galactosamine (GalN), and mannosamine (ManN) are the most important amino sugars in soils [22,23]. GluN is predominantly derived from the chitin of fungal cell walls and it is also found in bacterial peptidoglycan [18,24]. MurA is solely derived from bacterial cell walls [19,24]. GalN is generally considered to originate from bacteria [19]. The concentration and ratios of the above amino sugars can be used to evaluate the microbial (bacterial versus fungal) contribution to soil C sequestration [18,20,23,25].

Subtropical forests provide an important contribution to global terrestrial ecosystem C storage [26,27]. In southern China, the majority of natural forests were cleared by the late 1970s and first converted into two coniferous plantations, Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) and pine (*Pinus massoniana* Lamb.), to satisfy the high demand for timber, fuel, and other forest products due to rapid human population growth. The conversion not only has a profound effect on the ecosystem C budget [11,28,29], but it also leads to other ecological consequences, such as a loss in species diversity [30–33] and soil erosion [34]. As an alternative to conversion to plantations, forests with assisted natural regeneration (ANR) have been introduced to China for more than five decades [35,36], with the goal of protecting and nurturing mother trees and their seedlings that are inherently present in the area, rather than the establishment of entirely new forest plantations [37]. Our previous study showed that, when compared to monoculture plantations, ANR significantly increased the plant biomass and diversity, and more effectively promoted ecosystem services, including the mitigation of runoff and soil erosion, and the exportation of dissolved organic C [34]. However, the effects of forest conversions on these forest types with various intensities of anthropogenic disturbance to soil C dynamics, and, in particular, that of microbial residue, have rarely been assessed and compared.

In the present study, an ANR forest and two dominant plantations (Chinese fir and pine) with contrasting intensity of anthropogenic disturbance were selected to examine the effects of forest conversions on the dynamics of soil microbial residues. All of the selected forests were developed from a natural forest clearing in subtropical China, with similar soil properties and forest age. Following the conversion of native forest to plantations or ANR forests, different litter inputs exhibited a remarkable influence on the response of bacterial and fungal groups [38], which possibly resulted in distinct microbial residue retention patterns and different microbial functions during SOC accumulation [39,40]. We hypothesize that (1) soil amino sugar concentrations are lower in all of the converted forests than the native forest, with the lowest concentration in one of the two plantations. Mature and undisturbed ecosystems have a higher ratio of K-strategists to r-strategists than did young and disturbed ecosystems, according to the theory of Odum on ecosystem succession and disturbance [41,42]. Furthermore, a recent meta-analysis showed that converted forests consistently shifted from fungal to bacterial dominance with increasing land degradation [43]. Thus, we hypothesize that (2) natural forest conversion leads to a decrease of fungal relative to the bacterial residue C ratios, and these ratios, in descending order, will be as follows: native forest > ANR > plantations. Previous studies showed that labile C fractions were sensitive indicators of SOC dynamics that resulted from forest conversion [44], whereas microbial residue C was incorporated into the recalcitrant C pool [6], which may not respond equally to environmental change, as does the total soil C. Therefore, we hypothesize that (3) forest conversions increase the ratios of TAS (total amino sugars) to total SOC, with highest ratio in one of the two plantations, because of the entirely new forest establishment, resulting in rapid loss of labile C fractions, whereas a lower ratio than the plantations will occur in the ANR forest.

2. Materials and Methods

2.1. Site Description

This study was conducted in the Chenda Observation Study Site (26°19′55′′ N, 117°36′53′′ E, 300 m a.s.l.) of Sanming Forest Ecosystem and Global Change Research Station in Fujian Province, China. This study site borders the Daiyun Mountains on the southeast and the Wuyi Mountains on the northwest. A typical maritime subtropical monsoon climate characterizes the study site. The mean annual temperature (MAT) is 19.1 °C with low temperatures occurring in January and high temperatures occurring in July. The mean annual relative humidity is 81% and the mean annual precipitation (MAP) is 1750 mm. Approximately 75% of the total precipitation occurs from March to August. The mean annual potential evapotranspiration is 1585 mm. The soil at the study site is formed from granite and is classified as red soil according to the China soil classification system and it is equivalent to Ultisol in the USDA Soil Taxonomy [45]. Soil texture in the natural forest is sandy with sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (<0.002 mm), being 16.4%, 37.3%, and 46.3%, respectively, which does not significantly change after forest conversions. The soil depth exceeds 1.0 m.

The natural forest represents an old-growth, evergreen broadleaved *Castanopsis carlesii* (Hemsl.) forest in mid-subtropical China, which has been protected for more than 200 years, according to the record of local forest management department and it is characterized with high biodiversity, widespread old trees, snags, and downed wood. In addition to *Castanopsis carlesii*, the overstory contained other tree species, such as *Castanopsis kawakamii* Hayata, *Schima superba* Gardner & Champ., *Litsea subcoriacea* Y.C. Yang & P.H. Huang, and *Elaeocarpus decipiens* Hemsl. Two types of regeneration were adopted following the deforestation of the natural *Castanopsis carlesii* forests in 1975. One was natural regeneration, in which only the overstory was harvested and the understory and harvest residue remained. The other was to reestablish entirely new plantations following the forest being clear-cut, slashed, and burned. In 1976, the soil was prepared by digging holes. Afterwards, one-year-old seedlings of *C. lanceolata* or *P. massoniana* were planted at 3000 trees per hectare. The plantations were managed with similar practices, such as weeding and fertilization during the first three years and thinning twice between 10 and 15-years-old. The distances between the selected experimental forests fell within 1 km. Table 1 presents the general characteristics of the forests.

Table 1. Forest characteristics in a natural forest of *Castanopsis carlesii* (Hemsl.) (NF), assisted natural regeneration (ANR) and two plantation forests of *Cunninghamia lanceolata* (Lamb.) Hook (Chinese fir, CF) and *Pinus massoniana* Lamb. (PM).

Variable	NF	ANR	CF	PM
Altitude (m)	315	315	301	303
Slope (°)	35	28	30	35
Canopy coverage (%)	89	90	65	70
Mean tree height (m)	11.9	10.8	18.2	18.4
Mean tree diameter at breast height (cm)	20.0	14.3	15.6	16.3
Stand density (stem ha^{-1})	1955	3788	2858	1500

2.2. Soil Sampling, Litterfall, and Fine Root Biomass Measurements

Three replicate plots (20 m × 50 m) were set up for each forest type, and the distance between plots was kept at 20 m to assess the effects of forest conversion on microbial residue. Soil samples (0–10 cm and 10–20 soil layer) were collected in April 2017; 15 cores (5 cm in diameter) were randomly collected from each plot in a plastic bag as a composite sample. After removing the stones, pebbles, roots, and large pieces of plant residues, the soil was ground and sieved (<2 mm sieve), and then combined, homogenized, and divided into three subsamples. The first samples were kept at 4 °C for the determination of soil enzyme activity and microbial biomass. The second samples were kept at −20 °C and they were freeze-dried at −80 °C, and then ground to pass through a 0.149 mm sieve for the determination of amino sugar. The third soil samples were air dried for analysis of soil physical and chemical properties. Five rectangle litter traps (0.5 m × 1.0 m) with 1 mm nylon mesh were randomly arranged about 0.25 m above the soil surface in each plot. Litterfall was semimonthly collected from October 2010 to September 2016 using the method that was described by Yang et al. [46]. For the fine root sampling, 15 soil cores were randomly taken in each plot with a soil corer (5 cm in diameter) in April of 2017. The thick roots (>2 mm) were carefully removed from the soil samples with forceps and then the soils were wet-sieved with a mesh size of 0.5 mm. The sieved soils were put into a beaker with deionized water at a temperature of 1 °C and repeatedly stirred to float the fine root segments to water surface for collection [47]. The fine roots were placed into an oven at a temperature of 65 °C for 48 h and then weighed.

2.3. Soil Analysis

Soil pH was measured with a soil:water ratio of 1:2.5. SOC and total N were determined while using a CN auto analyzer (Elementar Vario MAX, Germany). For nitrate and ammonium analyses, 5 g of fresh soil from each sample was extracted using a 2 mol/L KCl solution. The solutions were shaken for 40 min. and then filtered for nitrate and ammonium determination while using a continuous flow analyzer (SKALAR San++, Breda, The Netherlands). The soil cation exchange capacity (CEC) was assessed by the ammonium acetate extraction method at pH 7. The soil available phosphorus (P) was detected while using the ion exchange resin method that Sibbesen developed [48].

2.4. Determination of Soil Amino Sugars

Amino sugar content in soils was detected while using the method of Zhang and Amelung [22]. Briefly, the finely ground soil samples (containing approximately 0.3 mg N) were hydrolyzed with 10 mL 6 M HCl at 105 °C for 8 h, and 0.1 mL myo-inositol (internal standard) was added to the hydrolysate solution, which was filtered through a glass fiber membrane filter (0.45 diameter), dried using a rotary evaporator, re-dissolved with deionized water, and then transferred into a 50 mL Teflon tube. The pH of the sample solutions was adjusted to 6.6–6.8 with 1 M KOH and 0.01 M HCl and the samples were then centrifuged. After the solution was freeze-dried, 5 mL methanol was added to dissolve the residues, after which the methanol solution was transferred to a vial and dried with N_2 at

45 °C. Finally, 1 mL deionized water and 0.1 mL recovery standard (*N*-methylglucamine) were added to the residues and freeze-dried.

The freeze-dried residues were dissolved with 0.3 mL derivatization reagent containing 32 mg mL^{-1} hydroxylamine hydrochloride and 40 mg mL^{-1} 4-dimethylamino-pyridine in pyridine-methanol (4:1 *v/v*) were heated at 78 °C for 35 min. in a water bath. After cooling, 1 mL acetic anhydride was added and then reheated to 78 °C for 25 min. Next, 1.5 mL dichloromethane and 1 mL 1 M HCl were added to achieve liquid–liquid separation. After water phase removal, the organic phase was washed three times with 1 mL deionized water. The remaining organic phase was dried by N_2 gas at 45 °C. Finally, 0.2 mL ethyl acetate-hexane (1:1) was added to dissolve the derivative for final analysis. The amino sugar derivatives were separated using an Agilent 6890A gas chromatography (GC, Agilent Tech. Co. Ltd., USA) that was equipped with DB-1 fused silica column (25 m × 0.32 mm × 0.25 mm) with a flame ionization detector. The concentrations of individual amino sugars were quantified based on the internal standard myo-inositol.

2.5. Statistics

Each forest was considered as an experimental unit, and the data were averaged across the three plots from each forest. Due to using pseudo-replication in this study, all of the standard errors were pseudo-replication errors as were mean comparisons. Results must be carefully interpreted and only trends in the data by forest type can be discussed, with an appreciation of the problems of pseudo-replication.

Before analysis, all the variables were checked for normal distribution (Kolmogorov–Smirnov test) and homogeneity (Levene test). One-way ANOVA with Tukey's HSD test was performed to test for differences of litter mass, fine root biomass, soil physicochemical properties, and soil amino sugars among forests. Statistical significance was established at the 5% level, unless otherwise mentioned. Redundancy analysis (RDA) was applied to elucidate the relationships among total amino sugar (TAS), GluN, GalN, MurA, GluN/MurA, GluN/GalN, the proportion of total amino sugar to total C, and the corresponding soil environmental variables (total litterfall, fine root biomass, pH, soil organic C, total N, total P, soil texture) among different forest types. SPSS 18.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses, except for RDA, which was performed while using CANOCO software for Windows 4.5 (Ithaca, NY, USA). Forward selection was based on Monte Carlo permutation ($n = 999$). Before RDA, we conducted forward selection of the environmental variables that were significantly correlated with variations of amino sugars while using the Monte Carlo permutation test ($p < 0.05$).

3. Results

3.1. Soil Properties

The SOC content was high in the natural forest (51.8 ± 3.0 mg g^{-1}). Following conversion of the natural forest, there was an approximately 42% reduction in SOC for the ANR forest (30.0 ± 2.7 mg g^{-1}), 60% for the Chinese fir plantation (20.9 ± 1.3 mg g^{-1}), and 64% for the pine plantation (18.6 ± 0.9 mg g^{-1}) at 0–10 cm soil layer, with over 50% reductions at the 10–20 cm soil layer for all the converted forests. Similarly, the soil N content was 2.75 ± 0.10 mg g^{-1} at 0–10 cm soil layer in the natural forest, and there was an approximately 30% reduction in the ANR (1.91 ± 0.12 mg g^{-1}) and an approximately 50% decrease in the two plantations (1.13 ± 0.09 and 1.29 ± 0.07 mg g^{-1} for the Chinese fir and pine plantations, respectively) after forest conversions, as well as more than a 50% decrease at the 10–20 cm soil layer for all of the converted forests (Table 2). Soil pH was increased for all of the converted forests (Table 2).

Table 2. Soil properties under four different forest types.

Soil Property	0–10 cm Layer				10–20 cm Layer			
	NF	**ANR**	**PM**	**CF**	**NF**	**ANR**	**PM**	**CF**
SOC (mg g^{-1})	51.8 ± 3.0 A	30.0 ± 2.7 B	18.6 ± 0.9 C	20.9 ± 1.3 bC	27.0 ± 1.4 a	12.7 ± 0.1 b	10.7 ± 0.5 b	11.7 ± 0.7 b
TN (mg g^{-1})	2.75 ± 0.10 A	1.91 ± 0.12 B	1.13 ± 0.09 C	1.29 ± 0.07 C	1.47 ± 0.03 a	1.06 ± 0.03 b	0.75 ± 0.01 c	0.86 ± 0.05 bc
C/N	18.8 ± 0.6 A	15.6 ± 0.4 B	16.5 ± 1.1 BC	16.2 ± 0.2 C	18.3 ± 0.8 a	12.0 ± 0.3 b	14.3 ± 0.4 b	13.7 ± 0.2 b
CEC (cmol kg^{-1})	15.8 ± 1.7 A	7.1 ± 1.2 B	6.7 ± 0.6 B	6.5 ± 0.5 B	9.8 ± 0.4 a	5.5 ± 0.2 b	5.7 ± 0.3 b	6.0 ± 0.2 b
pH	4.08 ± 0.07 B	4.44 ± 0.09 A	4.47 ± 0.03 A	4.52 ± 0.06 A	4.10 ± 0.03 a	4.28 ± 0.04 ab	4.39 ± 0.02 b	4.35 ± 0.02 b
NH_4^+ (mg kg^{-1})	27.3 ± 0.8 A	26.5 ± 4.7 A	11.6 ± 0.9 B	12.8 ± 1.0 B	12.2 ± 0.2 a	9.6 ± 0.8 b	6.4 ± 0.1 b	6.8 ± 0.2 b
NO_3^- (mg kg^{-1})	0.86 ± 0.09 BC	1.98 ± 0.33 A	0.26 ± 0.03 C	1.38 ± 0.18 AB	1.19 ± 0.04 a	1.14 ± 0.06 a	0.32 ± 0.03 c	0.73 ± 0.05 b
Sand (2–0.05 mm, %)	16.4 ± 2.7 A	15.2 ± 3.1 A	11.1 ± 0.3 A	12.3 ± 0.2 A	16.3 ± 1.1 a	17.5 ± 3.0 a	13.3 ± 0.5 b	16.6 ± 0.5 a
Silt (0.05–0.002 mm, %)	37.3 ± 1.9 A	34.9 ± 1.9 A	39.0 ± 1.6 A	37.5 ± 1.2 A	40.6 ± 1.8 a	36.6 ± 1.8 a	40.8 ± 1.2 a	41.1 ± 0.2 a
Clay (<0.002 mm, %)	46.3 ± 3.3 A	50.0 ± 1.9 A	50.0 ± 1.9 A	50.3 ± 1.4 A	43.1 ± 2.8 a	45.8 ± 1.7 a	45.8 ± 1.7 a	42.4 ± 0.6 a
Available P (mg kg^{-1})	5.47 ± 0.45 A	6.55 ± 0.56 A	2.57 ± 0.52 B	3.46 ± 0.35 B	1.10 ± 0.14 b	1.96 ± 0.20 a	1.17 ± 0.10 b	1.22 ± 0.06 b

The effects were significant at $p < 0.05$; different uppercase letters indicate significant differences at 0–10 cm among different forests, and different lowercase letters indicate significant differences at 10–20 cm among different forests. Values are means ± standard errors ($n = 3$). ANR: Assisted natural regeneration; CEC: cation exchange capacity; CF: Chinese fir forest; NF: Natural forest; PM: *Pinus massoniana* Lamb. forest; SOC: Soil organic carbon; TN: Total nitrogen.

The average annual litterfall from 2011 to 2016 was found to be the highest in the natural forest (8.04 Mg hm^{-2}), followed by the ANR forest (6.15 Mg hm^{-2}), pine plantation (5.03 Mg hm^{-2}), and Chinese fir (4.63 Mg hm^{-2}) plantation. The conversion of the natural forest led to a significant decrease in annual leaf litterfall in the pine and Chinese fir plantations, but not in the ANR, with the lowest annual litterfall observed in the Chinese fir plantation. Similarly, the conversion of the natural forest led to a significant decrease in fine root biomass at the 0–10 cm soil layer in all the converted forests, with the lowest biomass being observed in the pine plantation (Figure 1).

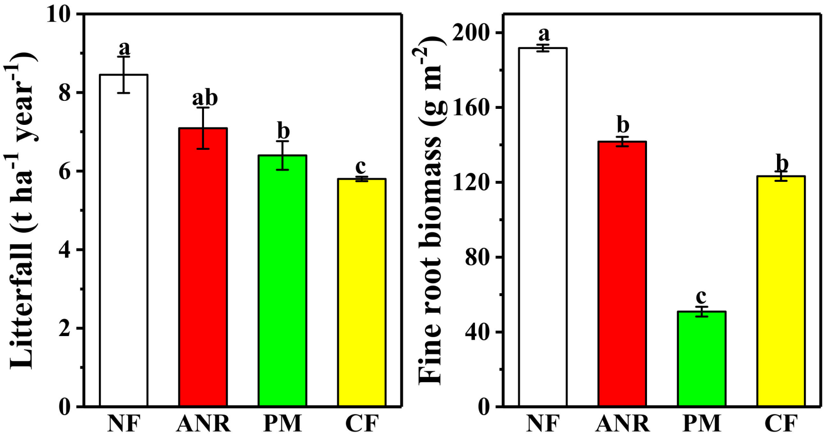

Figure 1. Litterfall and fine root biomass (0–10 cm) under different forest types; different lowercase letters indicate significant differences among the different forests. Values are means ± standard errors ($n = 3$), Natural forest: NF; Natural regeneration forest: ANR; *Pinus massoniana* Lamb. forest: PM; Chinese fir forest: CF.

3.2. Concentrations of Amino Sugars in the Different Forests

Conversions of natural forest to either ANR forest or plantations led to significant decreases in the concentrations of GluN, MurA, and TAS at the 0–10 cm soil layers, whereas, for GalN, significant decreases were only observed in the pine plantation. The concentration of TAS was high in the natural forest (1850 mg kg^{-1}), which was reduced by 32% in the ANR forest (1250 mg kg^{-1}), by 43% in the Chinese fir plantation (1050 mg kg^{-1}), and by 54% in the pine plantation (850 mg kg^{-1}). All of the individual amino sugar and total amino sugar concentrations were significantly higher at the 0–10 cm than at the 10–20 cm soil layer for all forest types (Figure 2).

In the converted forests, there were significantly higher concentrations of GluN and TAS at the 0–10 cm soil layer in the ANR forest than in the pine plantation, but there were no significant differences in the concentrations of individual amino sugars or TAS at the 10–20 cm soil layers (Figure 2).

3.3. Amino Sugar Biomarker Ratios

Following the conversions of the natural forest, there was an approximately 20% decrease in the GluN/MurA ratios for the two plantations at the 0–10 cm soil layer, but there was no significant decrease at the 10–20 cm soil layer for all the converted forests (Figure 3).

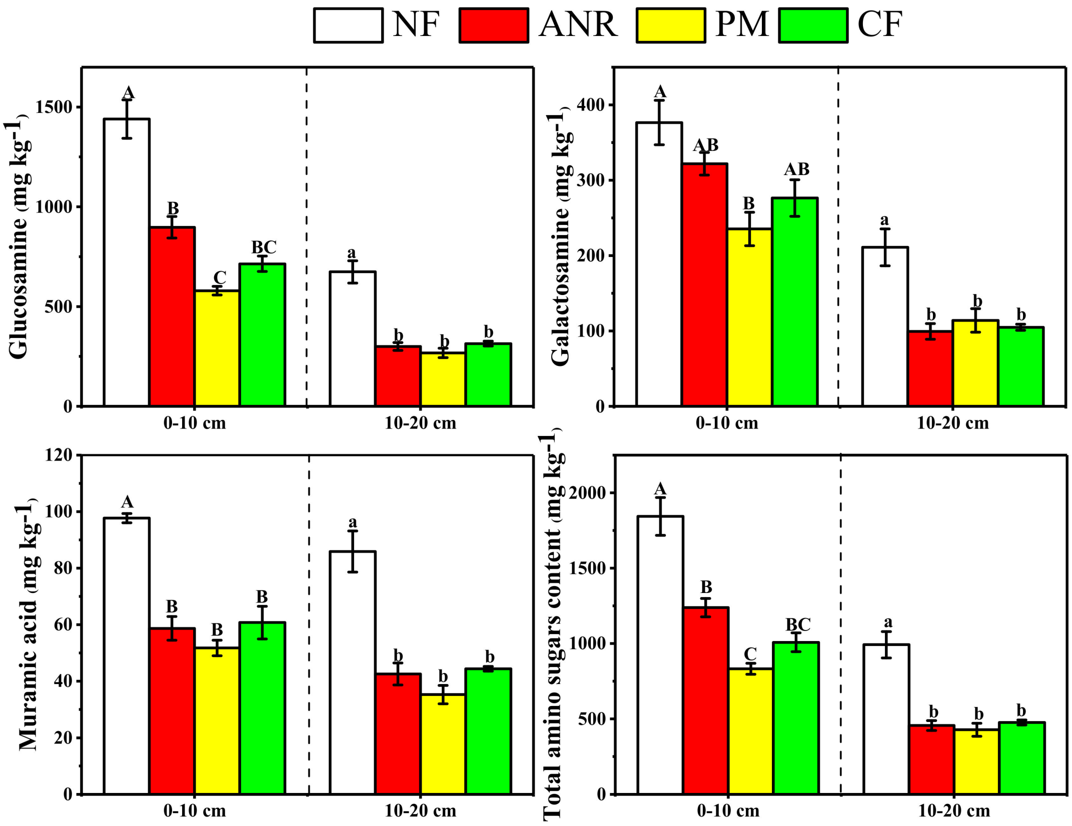

Figure 2. The concentration of soil amino sugars at different soil layers in different forest types; different uppercase letters indicate significant differences at 0–10 cm among the different forests, and different lowercase letters indicate significant differences at 10–20 cm among the different forests. Values are means ± standard errors ($n = 3$), Natural forest: NF; Natural regeneration forest: ANR; *P. massoniana* forest: PM; Chinese fir forest: CF.

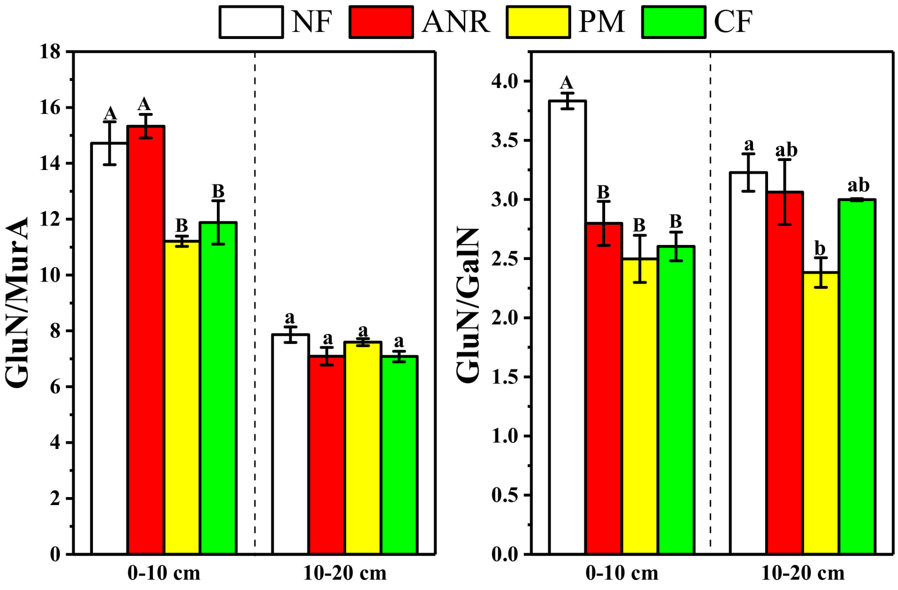

Figure 3. Ratios of glucosamine (GluN) to muramic acid (MurA) or to galactosamine (GalN) at different soil layers in different forest types; different uppercase letters indicate significant differences at 0–10 cm among the different forests, and different lowercase letters indicate significant differences at 10–20 cm among the different forests. Values are means ± standard errors ($n = 3$), Natural forest: NF; Natural regeneration forest: ANR; *P. massoniana* forest: PM; Chinese fir forest: CF.

Conversions of the natural forest led to significant decreases in the GluN/GalN ratios for all of the converted forest types at the 0–10 cm soil layer. At the 10–20 cm soil layer, the natural forest conversion to pine plantation led to a significant decrease in the GluN/GalN ratio (Figure 3).

3.4. Ratios of TAS to SOC in the Different Forest Type

Conversions of the natural forest led to a significant increase in the ratios of TAS to SOC at the 0–10 cm soil layer, but there was no significant difference among the converted forests. No differences were observed in the ratios of TAS to SOC following conversions of the natural forest at the 10–20 cm soil layer, but there was a significantly higher ratio of TAS to SOC in the pine plantation than in the ANR (Figure 4).

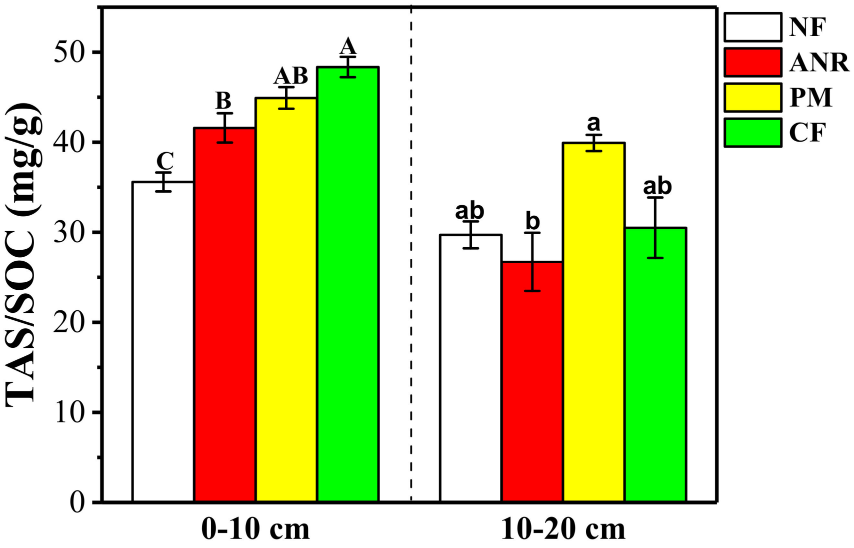

Figure 4. The proportion of total amino sugars to total soil organic carbon (TAS/SOC) in two soil layers under different forest types; different uppercase letters indicate significant differences at 0–10 cm among the different forests, and different lowercase letters indicate significant differences at 10–20 cm among the different forests. Values are means ± standard errors (*n* = 3), Natural forest: NF; Natural regeneration forest: ANR; *P. massoniana* forest: PM; Chinese fir forest: CF.

3.5. Correlation between Amino Sugars and Soil Properties

Redundancy analysis indicated that the environmental factors explained 87.9% of the variance of soil amino sugars across four different forests, with axis 1 explaining 83.1% and axis 2 explaining 4.8% of the variance (Figure 5). The ordination biplot from RDA was clearly distinguished among the four forests. Forward selection of factors in the RDA ordinations revealed that variations in soil amino sugars were closely related to SOC, fine root biomass, litter fall, and available P.

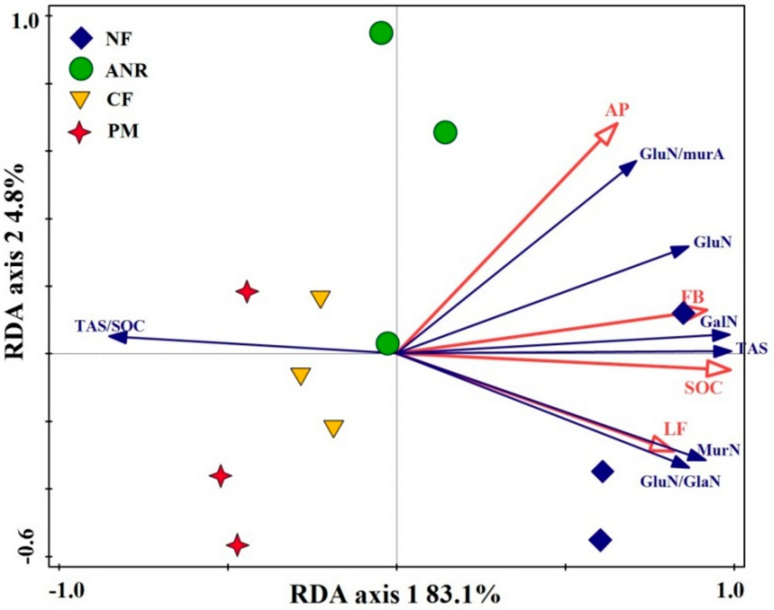

Figure 5. Correlations of soil amino sugar data to soil environmental factors determined by redundancy analysis (RDA). The amino sugar data included glucosamine (GluN), galactosamine (GalN), muramic acid (MurA), total amino sugar (TAS), ratios of glucosamine (GluN) to muramic acid, and proportion of total amimo sugar to SOC (TAS/SOC). AP, available P; SOC, soil organic carbon, FB, fine root biomass, LF, litterfall, Natural forest: NF; Natural regeneration forest: ANR; *P. massoniana* forest: PM; Chinese fir forest: CF.

4. Discussion

4.1. *Effects of Natural Forest Conversion on Concentrations of Soil Total C and Amino Sugars*

Although a considerable disagreement remains regarding the effects of land-use change on soil C stocks [49], it is generally considered that the preservation of natural old-growth forests may be related to a much higher C sequestration than that by the promotion of forest regrowth in long-term C pools, in particular, in recalcitrant soil organic matter [50–52]. Our previous studies in the subtropics also showed a much higher soil C concentration in the older natural forest than in the secondary forests or plantations [44,53]. Consistent with this finding, in the present study, conversions of old natural forest result in over 40% decreases in soil C concentration in all of the converted forests (Table 1). Replacing old-growth forest by young stands will lead to massive soil C losses to the atmosphere, mainly by reducing the flux into a permanent pool of soil organic matter and by redistributing C between pools with different turnover times because of the disturbance effects of various intensities [54]. The converted forests had experienced several forest management disturbances before our experiment, and the present study showed that natural forest conversion has a profound effect on soil C stocks in the subtropical forest, which cannot be recovered to a pre-harvest level, even after over 40 years of forest regrowth.

We anticipate that forest conversion may affect not only the SOC dynamics, but also the variation of microbial residues, because microbial C residues are regarded as a significant contributor to SOC, owing to their relatively long residence time in soils [19,55–58]. As in hypothesis (1), we observed a generally significant reduction in the concentration of microbial residues, including GluN, GalN, MurA, and TAS, in the converted forests, with the lowest decrease of TAS being observed in the ANR forest and the highest decrease in the pine plantation (Figure 1). These results are consistent with the results of previous studies, which also show that land use and management substantially affected amino sugar concentrations [2,59,60]. High temperature and rainfall, steep slopes, and fragile soil characterize South China. Following natural forest conversion, increases in soil temperature and erosion due to less forest cover in the initial forest regrowth generally led to accelerated SOC loss in the subtropics [44,61]. In particular, increased and intensive anthropogenic disturbance, such as slash burning and site preparation, contributed to higher SOC reduction in forest plantations [61]. Likewise, these aforementioned factors may also affect microbial residue accumulation in the different forest types, which the redundancy analysis that showed soil amino sugar concentrations were closely correlated with SOC and soil available P was confirmed (Figure 5). Most tropical and subtropical forests occur in highly weathered soil, where the available P is low [62,63]. The soil P availability could influence the activities, biomass, and compositions of soil microbial communities [64–66], which may also explain the amino sugar dynamics during the forest conversions.

Further, Liang et al. reported there was a tree species-specific effect on soil microbial residue accumulation in an old-growth forest ecosystem, with recalcitrant Eastern Hemlock litter making a relatively lower contribution of microbial residue to the total soil C [67]. When compared with the plantations, the ANR forest mostly consisted of broad leaf tree species, which, when compared to coniferous litter, could be conducive to microbial residue retention by increasing soil amino sugars [68]. Correlation analysis showed that soil amino sugar concentrations were positively correlated with fine root biomass and annual leaf litterfall (Figure 5). These factors could again explain the higher GluN, GalN, and TAS in the ANR forest than in the pine plantations (Figure 1). Unexpectedly, the absence of significant differences in all amino sugars was observed between the ANR forest and the Chinese fir plantation in the present study. This could result from the high fine root biomass in the Chinese fir plantations, mainly owing to a higher abundance of herbs and shrubs than those in other two converted forests [69]. The herbs and shrubs are ephemeral, which could lead to high input of fine root litter and eventually contribute to amino sugar accumulation.

The higher concentration of SOC in surface soil than in deeper soil may support more abundant microorganisms [70,71]. Thus, larger amounts of microbial residue accumulated at the 0–10 cm than at 10–20 cm soil depth in each forest that was observed in this study (Table 1). This was consistent with the results of previous studies [68,72,73].

4.2. Amino Sugar Biomarker Ratios

Ratios of GluN/MurA and GluN/GalN are normally used to indicate the contribution of fungal versus bacterial residues to SOC accumulation [20,23,25,57]. Land use change may affect the formation of microbial residues [7]. In this study, forest conversions resulted in dramatic declines in all of the examined amino sugars, including GluN, GalN, and MurA, but at different rates (Figure 1). The decrease of GluN/MurA ratios in the two plantations indicated a slower recovery of fungal communities relative to bacteria following natural forest conversion, which is in agreement with Hedlund et al., who argued that fungi, in particular, mycorrhizal fungi, may have less efficient dispersal and colonizing abilities after vegetation removal [74]. Among the converted forests, although there was no difference in TAS, different GluN/MurA ratios (Figure 2) may reflect the effect of land use intensification on amino sugar partition. Our finding of higher GluN/MurA ratios in the ANR forest indicated that slight anthropogenic disturbance is conducive to the accumulation of fungal derived microbial residue, which is in agreement with our hypothesis (2).

When compared to the 0–10 cm soil depth, at 10–20 cm, there was an approximately 40% decrease in GluN to MurA ratios (Figure 2). The reduction in fungal biomass and residues in subsoil might be related to the accumulation of less decomposed plant residues with an increasing depth. Moritz et al. [75] and Liang and Balser [17] observed similar decreases in the GluN to MurA ratio with depth. These decreases indicated that bacteria are more important in subsoil organic C turnover than are fungi. Fungi are aerobic organisms that typically utilize fresh litter as their preferred C source and generally successfully outcompete bacteria in the surface soils, particularly under acidic conditions [76]. With increasing depth, studies have typically shown a decrease in the relative abundance of fungi [70,75,77].

The absence of a difference in GluN/GalN rather than GluN/MurA ratios between the two soil depths was observed in this study (Figure 3), which is consistent with the result of Liang et al. [66] and

Moritz et al. [75]. Liang et al. argued that GluN/MurA and GluN/GalN indicated different aspects of the relative fungal to bacterial contribution or the relative retention times of various amino sugars in soil C dynamics [67].

4.3. Contribution of TAS to SOC in the Different Forest Types

Microbial residues are important constituents of SOC and they do not always change in the same pattern, as does the SOC dynamics [68]. Our previous studies showed a more pronounced loss of labile C than total SOC following the conversion of natural forest into plantations or secondary forests [44]. The present study shows a significant increase in the ratios of TAS to total SOC at the 0–10 cm soil depth in the converted forest, whereas no significant difference between the natural forest and converted forests occurred for the 10–20 cm soil depth (Figure 3). These results indicate the slower loss of microbial residue C than other soil C fractions following forest conversions, which suggests that microbial residue, as a refractory C, plays an important role in SOC storage, particularly in the surface 0–10 cm soil. Furthermore, lower increases of the TAS to total SOC ratio in the ANR forest than those in the two plantations may reflect that microbial residue is of greater importance in soil storage with the intensity of anthropogenic disturbance. Our findings support hypothesis (3), that forest conversions increase the ratios of microbial residue C to total C, with the highest increase in the two plantations.

We compared the effects of forest conversions that are based on the experiment of three replicate plots in each stand, which is not truly replicated. Therefore, our results should be cautiously interpreted because of an intrinsic error of pseudo-replication [78]. Furthermore, we should be critical of the scope of possible interpretation due to inevitable temporal and spatial heterogeneity derived from space for time substitution. It deserves special notice that all mean comparisons were expected to be a best-case scenario, because the error terms should be larger and mean separations should have a wider difference to allow for a true separation.

5. Conclusions

In the subtropics, natural forest conversions result in dramatic reductions, not only in SOC, but also in microbial residue C, which cannot be recovered, even after more than 40 years of forest regrowth. Microbial residue C accumulation varies with SOC and litter input constrained by forest management with various anthropogenic disturbances. As a recalcitrant C, microbial residue plays an important role in soil C storage following forest conversions. When comparing with tree plantations, natural forest conversion to the ANR forest had a significant effect in maintaining SOC, but not necessarily a significant effect on microbial residue accumulation.

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