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Contribution of Arbuscular Mycorrhizal Fungal Communities to Soil Carbon Accumulation during the Development of *Cunninghamia lanceolata* Plantations

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Abstract: Arbuscular mycorrhizal (AM) fungi can establish mutual association with most land plants, and impact a series of important ecological processes, including plant productivity, ecological succession and soil carbon (C) accumulation. Understanding the AM fungal diversity and community assembly, and their associated soil C sequestration, could be a crucial interest for the forest ecologist. In this study, the AM fungal abundances and community structure as well as glomalin-related soil protein (GRSP) concentrations were investigated in typical development stages (young, middle and mature) of *Cunninghamia lanceolate* plantations, which are widely distributed species in subtropical regions. The mycorrhizal colonization, spore density, AM fungal biomass and diversity were higher in mature than younger stands. The development of *C. lanceolata* also increased soil GRSP concentrations, and enhanced their C contribution to soil organic C. Soil difficulty extractable (DE) GRSP demonstrated a greater C contribution to soil organic C relative to easily extractable (EE) GRSP. Linkage analyses found that AM fungal biomass demonstrated a positive correlation with GRSP concentrations, and soil organic C positively related to DE-GRSP and total (T) GRSP. Soil AM fungal community structure differed dramatically across all studied *C. lanceolata* plantations with a decrease in Gigasporaceae and increase in Acaulosporaceae. Soil AM fungal community assembly was more phylogenetic clustering than expected by chance and primarily shaped by deterministic processes, with a non-shift during the development of *C. lanceolata*. Collectively, *C. lanceolata* development shaped the AM fungal communities and enhanced their biomass and GRSP contents, which might, in turn, partially contribute to soil C accumulation.

Keywords: stand age; arbuscular mycorrhizal fungal community; glomalin-related soil protein; soil C sequestration

Citation: Liu, Z.; Han, Y.; Lai, F.; Zhao, H.; Cao, J. Contribution of Arbuscular Mycorrhizal Fungal Communities to Soil Carbon Accumulation during the Development of *Cunninghamia lanceolata* Plantations. *Forests* **2022**, *13*, 2099. https://doi.org/10.3390/f13122099

Academic Editor: Choonsig Kim

Received: 21 October 2022
Accepted: 5 December 2022
Published: 9 December 2022

1. Introduction

Cunninghamia lanceolata (Lamb.) Hook is an important and widely distributed species in subtropical China with 1000 years of cultivation history. Due to its fast growth rate and great economic values, the area of *C. lanceolata* plantation has increased rapidly during the past several decades. There are approximately 9 million ha of *C. lanceolata* plantations, accounting for 6.5% of total global plantations [1]. The increased planting activities in the past decades have made *C. lanceolata* plantations consist of a series of plots of different ages. Forest plantations of different ages have different canopy density, stand basal area aboveground and belowground production, which can subsequently cause variation in biogeochemical cycling and soil microbial characteristics [2,3]. Previous studies have reported that plant carbon (C) stocks increase with secondary forest age, while root biomass, soil nutrients and C pools are variable over time-scales [2–4]. Yu et al. [5] observed that soil organic C decreased and then increased from 7-year to 29-year-old *C. lanceolata* plantations. Soil microbial biomass and diversity was reduced firstly and then enhanced, whereas

soil-dissolved C increased with stand ages of *C. lanceolata* plantations [6]. Soil bacterial community composition were different across a chronosequence of *C. lanceolate* plantations and tallgrass prairies [7,8]. Many factors could lead to the changes of soil C pools, including a variation in C input from plants to soils, litter quantity and quality, organic matter decomposition rate, and protection of C decomposition by soil aggregates [9–11]. Understanding the processes mediating soil C accumulation and sequestration is therefore crucial for the sustainable management of *C. lanceolata* plantations.

Arbuscular mycorrhizal (AM) fungi are ubiquitous plant symbionts having mutual relationships with most plant species. They not only transfer nutrients to plants but also participate in soil biogeochemical cycling. Studies reported that AM fungi could facilitate soil C accumulation by holding soil organic matter from escaping via forming soil aggregates or by depositing recalcitrant organic C into soils [12,13]. Other studies, however, documented that AM fungi might go against soil C storage via promoting organic matter decomposition to supply nutrients for their hosts [14]. Whether AM fungi facilitate soil C storage is dependent on the sum of formation, maintenance and decomposition by AM fungi [11]. Glomalin-related soil protein (GRSP), a glycoprotein secreted by AM fungi, is an important slow-cycling part of soil C (accounts for 4%–5%) and plays important role in preventing soil C decomposition due to its sticky nature [15]. The responses of GRSP to stand ages varied with ecosystems, plant species and managements. Carrington et al. [16] observed a higher GRSP content at 30-year-old sites compared to those in younger tallgrass prairies. The content of GRSP also varied significantly with tea plantation development and reached to its highest in the 25-year-old plantation [17]. In bamboo forests, soil total GRSP contents decreased with stand ages, coupled with shifted AM fungal community structure among different stands [18]. Moreover, in *C. lanceolata* plantations, soil extractable GRSP contents were significantly decreased by the nitrogen and phosphorus addition [19]. However, how GRSP respond to the development of *C. lanceolata* plantations in the subtropical region is still limitedly studied.

Previous studies have evaluated the changing pattern of plant growth, soil C and nitrogen dynamics during the development of *C. lanceolata* plantations [2,20], but AM fungal communities and functions were seldom focused upon. It was reported that the AM fungal communities were closely related to host plant species and ages [21,22]. Therefore, we predicted that the soil AM fungal communities would be shifted across different stand ages. In addition, mycorrhizal colonization was higher in mature-aged *C. lanceolata* plantation relative to young-aged plantation [23]. Thus, we hypothesized that soil GRSP concentrations would also be higher in mature-aged *C. lanceolata* plantations, since plants might allocate more C to mycorrhizas to meet mycorrhizal growth demand. Furthermore, previous studies have demonstrated that the AM fungal-associated soil C sequestration was closely related to their community and biomass [10,12]. Hence, we predicted that the changed soil AM fungal communities and biomass would provide an underlying mechanism driving the soil C sequestration during *C. lanceolata* development. In the present work, the AM fungal biomass, community structure and soil GRSP concentrations were examined in three typical development stands. The results will aid a deeper understanding of the responses of AM fungal-associated C sequestration to the development of subtropical *C. lanceolata* plantations, which would be important for plantation sustainable management.

2. Materials and Methods

2.1. Study Site

The study was performed on the national forest farm station in Fujian Province, China. The site is in a subtropical region of southern China, with an average annual temperature of 19.1 °C and precipitation of 1750 mm. The soil is characterized as oxisol formed from sandstone according to the Chinese soil taxonomy.

We used the geological map of Sanming (http://www.ngnc.org.cn (accessed on 1 November 2017)) and selected three typical development stages of *C. lanceolata* stands (Figure S1) with 5-year (Young-aged, hereafter Young), 18-year (Middle-aged, hereafter

Middle), and 40-year (Mature) old trees based on their growth traits [24]. All selected plantations were in their first rotation, which were planted on natural forests after clear cutting. The understory vegetation in the young-aged plantation was sparse through weeding control. The plantation age and history were provided by the local forest management department. Distance between two stands was approximately 1 km. These plantations were established at 200 m elevation, with slopes varying from 30° to 35° and with similar parent material, soil texture and topography. More descriptions of the selected plantations are provided in Table S1.

2.2. Sample Collection

In each of the three stands, five plots (50 m × 50 m) were selected for sampling tree roots and rhizosphere soils. In November 2017, surface soils (0–20 cm) were sampled from the four sides of the trunks of five similar trees. Fine roots (<2 mm in diameter) were taken carefully by hands and scissors. Rhizosphere soils were collected from the fine roots by gentle shaking. In total, 15 rhizosphere soil samples and 15 root samples were obtained and then transported immediately to the lab. Rhizosphere soil samples were passed (2 mm mesh) and then stored for soil chemical determination and molecular analyses. Fine root samples were rinsed and stored for the measurement of mycorrhizal colonization.

2.3. Soil Chemical Analyses

Soil pH (H_2O) was tested with an electrode. Soil moisture was analyzed gravimetrically (105 °C, 12 h). Soil organic carbon (SOC) was measured using the K_2CrO_7 titration with oil bath [25]. Soil dissolved organic carbon (DOC) and total nitrogen (TN) were measured using an elemental analyzer (Elementar, Langenselbold, Germany). Soil available N (AN, NO_3^--N+NH_4^+-N) were measured by a continuous flow analyzer (San^{++}, Breda, The Netherlands). Soil available phosphorus (AP) and potassium (AK) concentrations were tested with a spectrophotometer and flame photometer, respectively [26].

2.4. Mycorrhizal Colonization, Spore Density, Phospholipid Lipid Fatty Acids and Glomalin-Related Soil Proteins Analyses

Fresh fine roots were cleared, stained with trypan blue and the mycorrhizal structures were recorded with a microscope (Olympus BX51, Japan) using the gridline intersect technique to measure the total mycorrhizal colonization [27]. Spores were extracted with wet sieving, spread on a filter paper, and counted using a dissecting microscope (Olympus BX51, Japan).

Lipid acids were extracted from 3.0 g soils using chloroform:methanol:citrate, and then classified into phospholipid fatty acids, neutral lipids and glycolipids using silica acid columns. The phospholipid fatty acids (PLFA) were methylated to free fatty acid methyl esters (FAMEs) and then analyzed with gas chromatography (6890N, Agilent, Santa Clara, CA, USA) by fitting with an identification platform (Sherlock, MIDI, Newark, DE, USA) using FAME 19:0 as internal quantitative standard. The PLFA 16:1ω5c contents were calculated to represent the AM fungal biomass [28].

Easily extractable (EE) GRSP and total GRSP (T-GRSP) were measured as previously reported [29]. In brief, EE-GRSP or T-GRSP were extracted from 1.0 g soils using the sodium citrate at 121 °C. Extraction of T-GRSP was conducted for several times until the supernatant is straw-colored. Soil GRSP contents were measured using the Bradford protein assay. Difficultly extractable (DE) GRSP concentration was obtained as: DE-GRSP = T-GRSP − EE-GRSP. In addition, GRSP-C contents were calculated with a coefficient of 45% C, as previously reported [30,31].

2.5. Soil DNA Extraction and High-Throughput Sequencing

Total genomic DNA was collected from a 0.25 g soil with Power Soil Kit (MO BIO, Carlsbad, CA, USA). Target 18S rDNA gene fragment was amplified by the primers' sets AMV4.5NF/AMDGR [32] in a 50-μL mixture of Taq DNA polymerase (2 U, 25 μL), each

primer (20 μmol L^{-1}, 0.5 μL), extracted template DNA (1 μL) and sterile deionized water (23 μL). Amplifications were conducted on a thermal cycler (Bio-Rad, Hercules, CA, USA) with the program: 5 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C; and a final 7 min at 72 °C. Sequences were sequenced on an Illumina platform in an equimolar mixture. Reads were uploaded to the online database (NCBI: PRJNA532954).

2.6. Bioinformatics and Statistics

All sequenced raw reads were analyzed with QIIME pipeline [33]. Reads were firstly filtered by removing low quality sequences (<200 bp or <25), and then binned into operational taxonomic units (OTUs) with a 97% similarity. The AM fungal taxonomic assignments were conducted using the MaarjAM database [34].

Statistical analyses were performed with SPSS 18.0. Quantitative comparisons among different plantations were conducted by one-way analysis of variance (ANOVA) with Tukey's analysis at $p = 0.05$. Observed OTUs, Chao1, ACE, and Shannon indices were calculated to represent the AM fungal diversity. Nonmetric multidimensional scaling analysis (NMDS) plots were generated with the 'metaMDS' function using the vegan package to display AM fungal community composition taxonomically (Bray-Curtis) and phylogenetically (unweighted UniFrac). Permutational multivariate analysis of variance (PerMANOVA) was conducted using the 'adonis' function (permutations = 999) of the vegan package in R to examine the dissimilarity of AM fungal communities among different stands. In addition, multivariate generalized linear models (MV-GLMs) were employed to evaluate the changes in AM fungal OTUs during stand development [35]. Only OTUs that existed in two or more soil samples were used for the MV-GLM analyses. The relative abundances of AM fungal OTUs were regressed by the Random Forest (RF) model to test the performances of different AM fungal taxa across different stand ages. The marker taxa number was calculated using the 'rfcv' function in R. Correlation between AM fungal community and various environmental factors was analyzed by mantel tests using the vegan package in R. Linkage between GRSP and PLFA, soil organic C, communities and relative abundances was tested using linear regression analyses in SPSS 18.0. Relationships between soil characteristics and AM fungal abundances and diversity were evaluated with the corrplot package in R. Mean nearest taxon distance (MNTD), and the nearest relative index (NRI) were analyzed with the 'mntd' and 'ses.mntd' functions of the picante package in R [36]. These were meant to estimate the non-random phylogenetic assembly of the AM fungal community. More positive (negative) NRI indicate phylogenetic clustering (phylogenetic over-dispersion). βMNTD and βNTI were estimated using 'comdistnt' with the picante package in R. If βNTI > +2 or <−2 between one pair of samples, which means the dominance of deterministic processes and higher or lower turnover relative to null model. βNTI < +2 and >−2 between one pair of samples indicates stochastic processes shaped the microbial communities and non-shift from the null model [37].

3. Results

3.1. Soil Physicochemical Properties and AM Fungal Biomass

Compared to the young plantation, *C. lanceolata* plantation development did not significantly influenced soil pH and available N contents. In contrast, soil total N and available P concentrations decreased from young to middle plantations. Soil moisture, available K and DOC concentrations were higher in middle-aged plantations compared to young and mature stands. Soil organic C concentration decreased and then increased from young to middle-aged plantations ($p < 0.05$; Table S2).

3.2. Mycorrhizal Colonization, Spore Density, AM Fungal Biomass and Glomalin-Related Soil Protein Content

Total mycorrhizal colonization, spore density and EE-GRSP content increased significantly along with the plantation development. Soil DE-GRSP and T-GRSP concentrations were higher in mature plantations relative to younger plantations. Soil AM fungal

biomass (as indicated by PLFA 16:1ω5c) was greater in middle and mature stands than in young plantations, with no significant changes between middle and mature plantations (Figure 1). Soil EE-GRSP/SOC and EE-GRSP/T-GRSP were higher in middle-aged plantation than young and mature plantations. In contrast, *C. lanceolata* plantation development significantly increased DE-GRSP/SOC and T-GRSP/SOC by 49.0% and 37.2%, respectively (Figure 2). Soil T-GRSP and DE-GRSP contents had a positive correlation with soil organic C. There are also significant correlations between GRSP contents and AM fungal biomass (PLFA) (Figure 3).

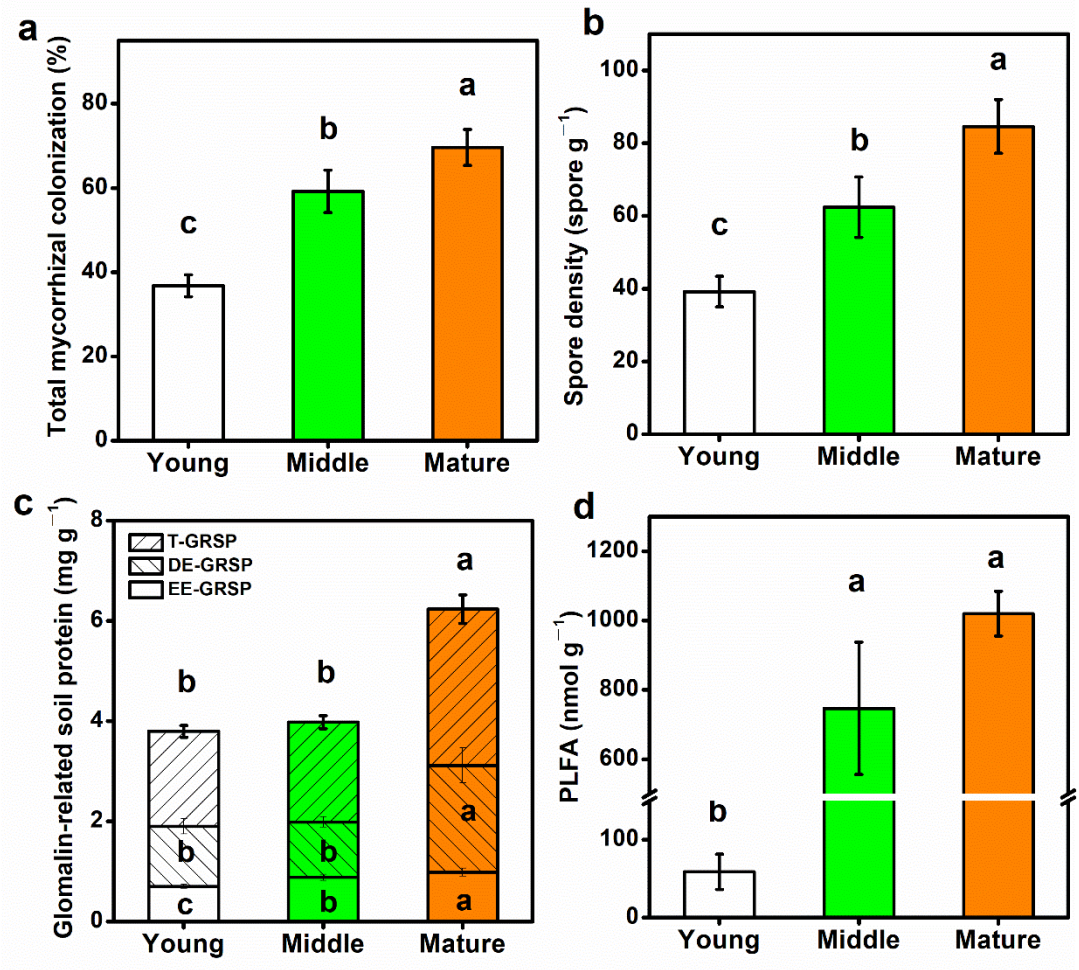

Figure 1. Total colonization rate (**a**), spore density (**b**), GRSP contents (**c**) and AM fungal biomass (**d**) in different *C. lanceolata* plantations. Bars topped by different letters indicate significant difference ($p < 0.05$).

3.3. Soil AM Fungal Diversity and Community Composition

In total, the sequencing generated 406,103 high-quality reads of AM fungi, ranging from 3506 to 49,280 of all soil samples (Table S3). All rarefaction curves reached a stable plateau (Figure S2a), indicating that the analyzed sequences were adequate to examine AM fungal community. In addition, Good's coverage were greater than 0.995 for the three studied plantations, indicating that most of the AM fungal community was recorded at this sequencing depth (Figure S2b). Based on 97% similarity, AM fungal sequences were classified into four families: Acaulosporaceae (~0.31%), Ambisporaceae (~0.03%), Gigasporaceae (~0.94%), and Glomeraceae (~98.72%), with Glomeraceae being a dominant family in all the samples (Figure S3).

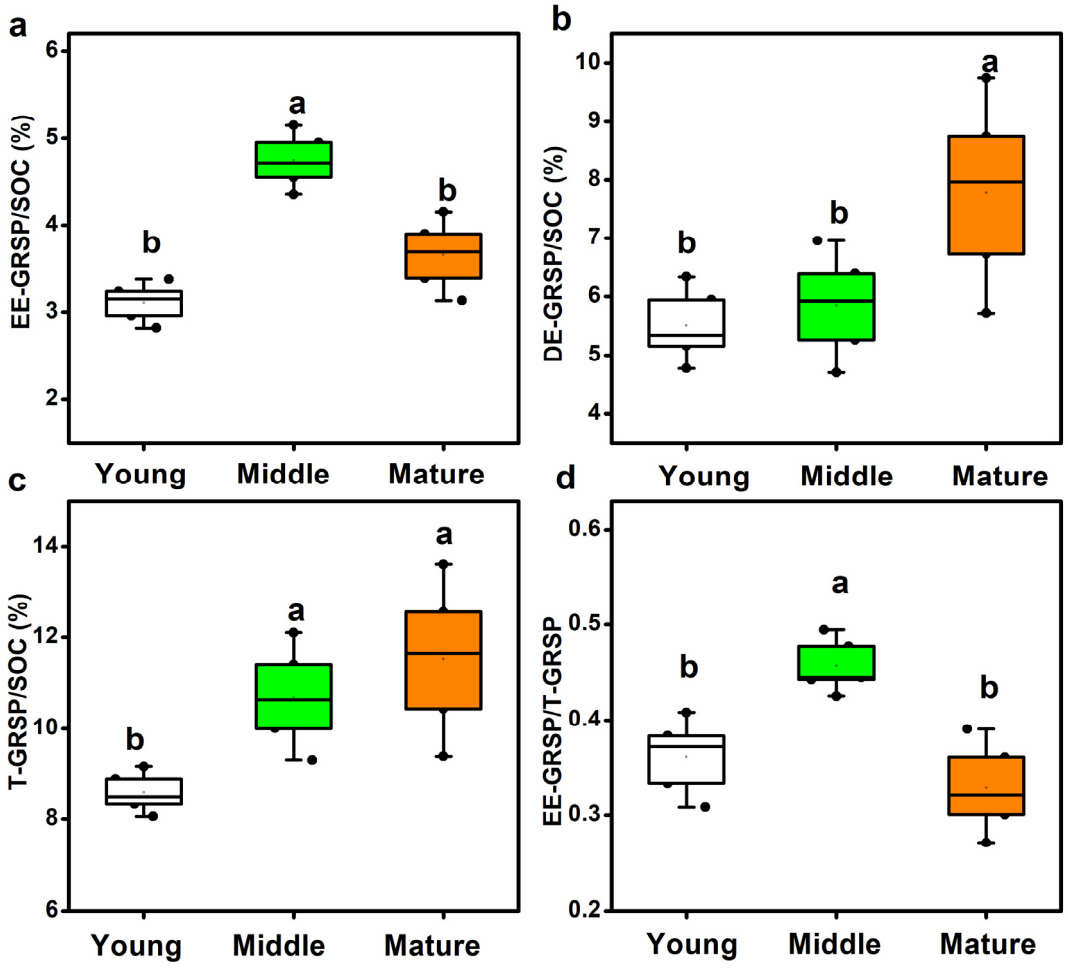

Figure 2. Contribution of EE-GRSP (**a**), DE-GRSP (**b**), T-GRSP (**c**) to soil organic C (SOC) and ratios of EE-GRSP that account for T-GRSP (**d**) during development of *C. lanceolata* plantations. Bars topped by different letters indicate significant difference ($p < 0.05$).

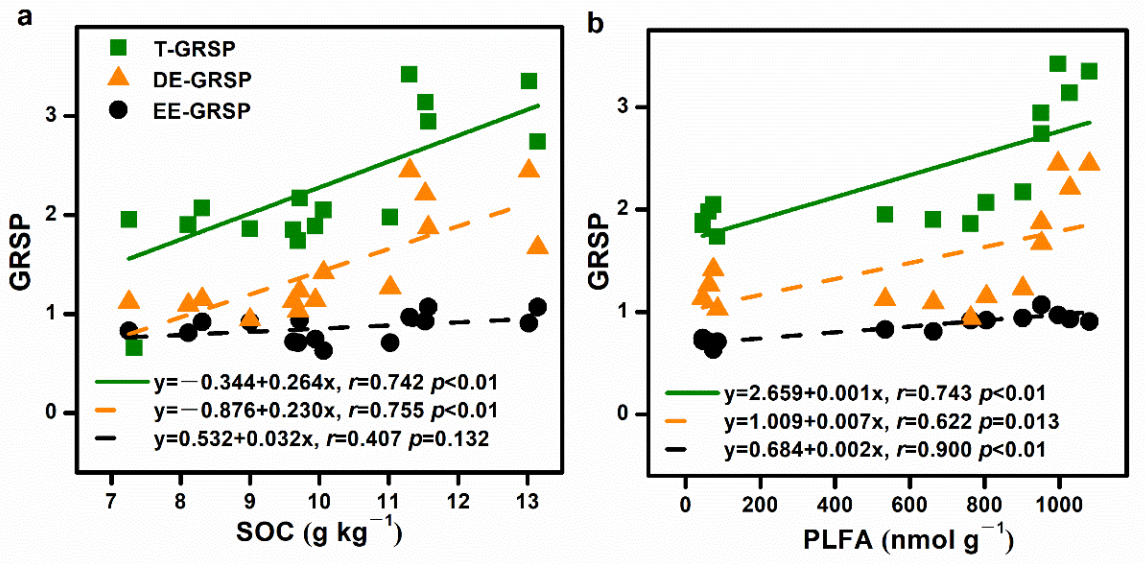

Figure 3. Relationship between soil glomalin-related soil protein (GRSP) and soil organic C (SOC) (**a**), soil AM fungal PLFA biomass and GRSP content (**b**).

One-way ANOVA analysis indicated that AM fungal diversity of estimated richness (Chao1), observed richness (observed OTUs), ACE, and Shannon index were higher in middle and mature plantations compared to the young plantation. There were no significant changes in AM fungal diversity between middle and mature plantations (Table 1).

The AM fungal communities were significantly shifted by the development of *C. lanceolata* plantations. They were clustered into different groups based on their stand age (Figure 4a). Results indicated significant differences in both taxonomic and phylogenetic composition of the AM fungal community (Figure 4b,c). PerMANOVA analysis results demonstrated significant dissimilarities among the three studied plantations (Figure S4).

Table 1. Soil AM fungal diversity in different *C. lanceolata* plantations.

	Observed OTUs	Chao1	ACE	Shannon
Young	20.4 ± 3.36 b	21.8 ± 2.28 b	23.2 ± 2.09 b	2.06 ± 0.17 b
Middle	36.25 ± 1.26 a	34.9 ± 7.39 a	36.0 ± 8.11 a	2.66 ± 0.20 a
Mature	37.6 ± 3.78 a	38.7 ± 3.45 a	39.40 ± 3.82 a	2.53 ± 0.36 a

Young: young-aged, Middle: middle-aged, Mature: mature; values are mean ± SD. Different letters in the same column indicate significant difference ($p < 0.05$).

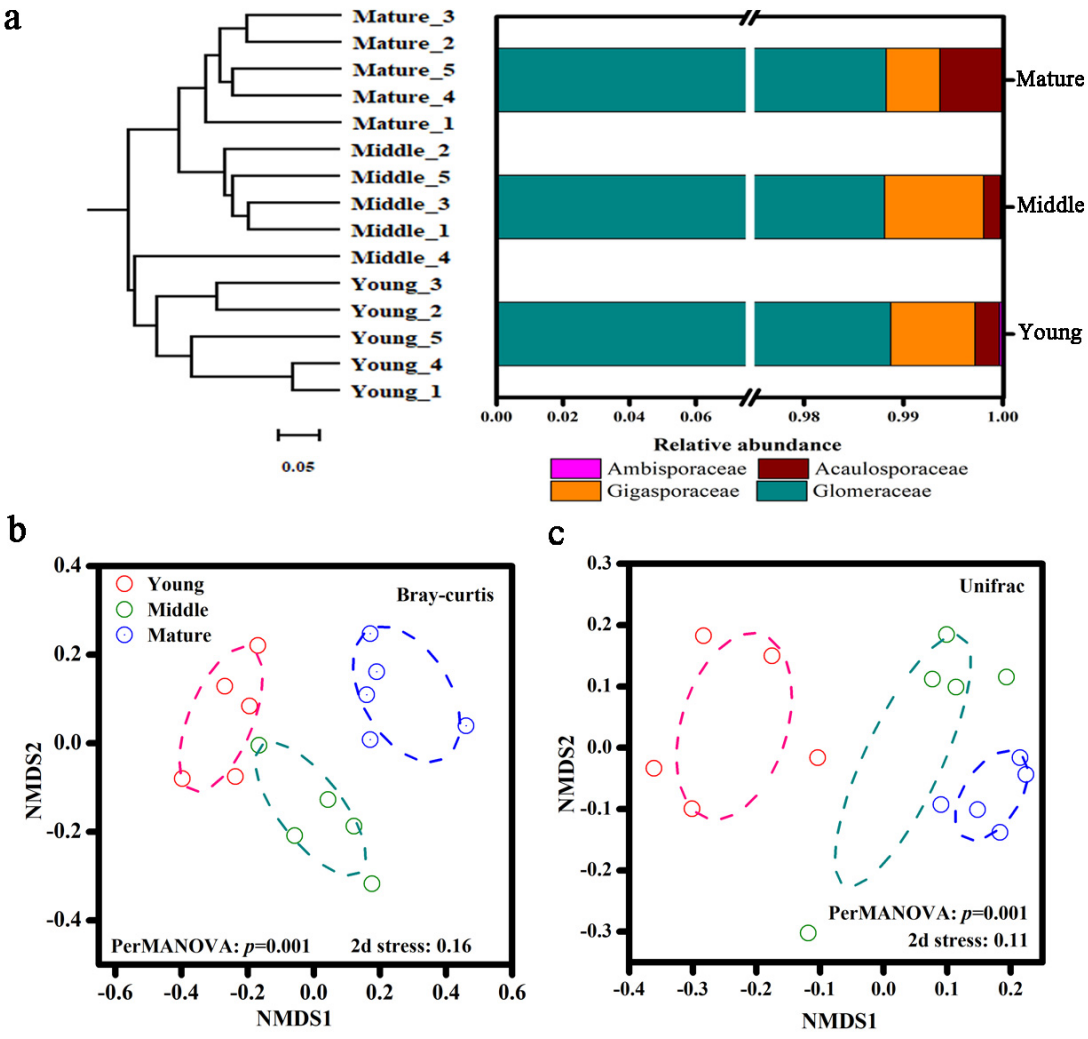

Figure 4. Relative abundances at family level (**a**), taxonomic composition (**b**) and phylogenetic composition (**c**) of soil AM fungal communities during the development of *C. lanceolata* plantations.

Among the three plantations, the relative abundance of Gigasporaceae was lowest in the mature plantation. In contrast, the mature plantation had a higher abundance of Acaulosporaceae than younger plantations. We found no significant difference in Glomeraceae among the three studied plantations (Figure 5a). At the OTUs level, AM fungal OTUs showed fluctuations during the development of the *C. lanceolata* plantation. For example, five Glomeraceae OTUs significantly changed in the middle-aged plantation in comparison with the young plantation, six OTUs differed between mature and middle-aged plantations and five OTUs differed between mature and young plantations. Interestingly, no significant OTUs changed in Gigasporaceae and Acaulosporaceae among the young, middle and mature plantations (Figure 5b–d).

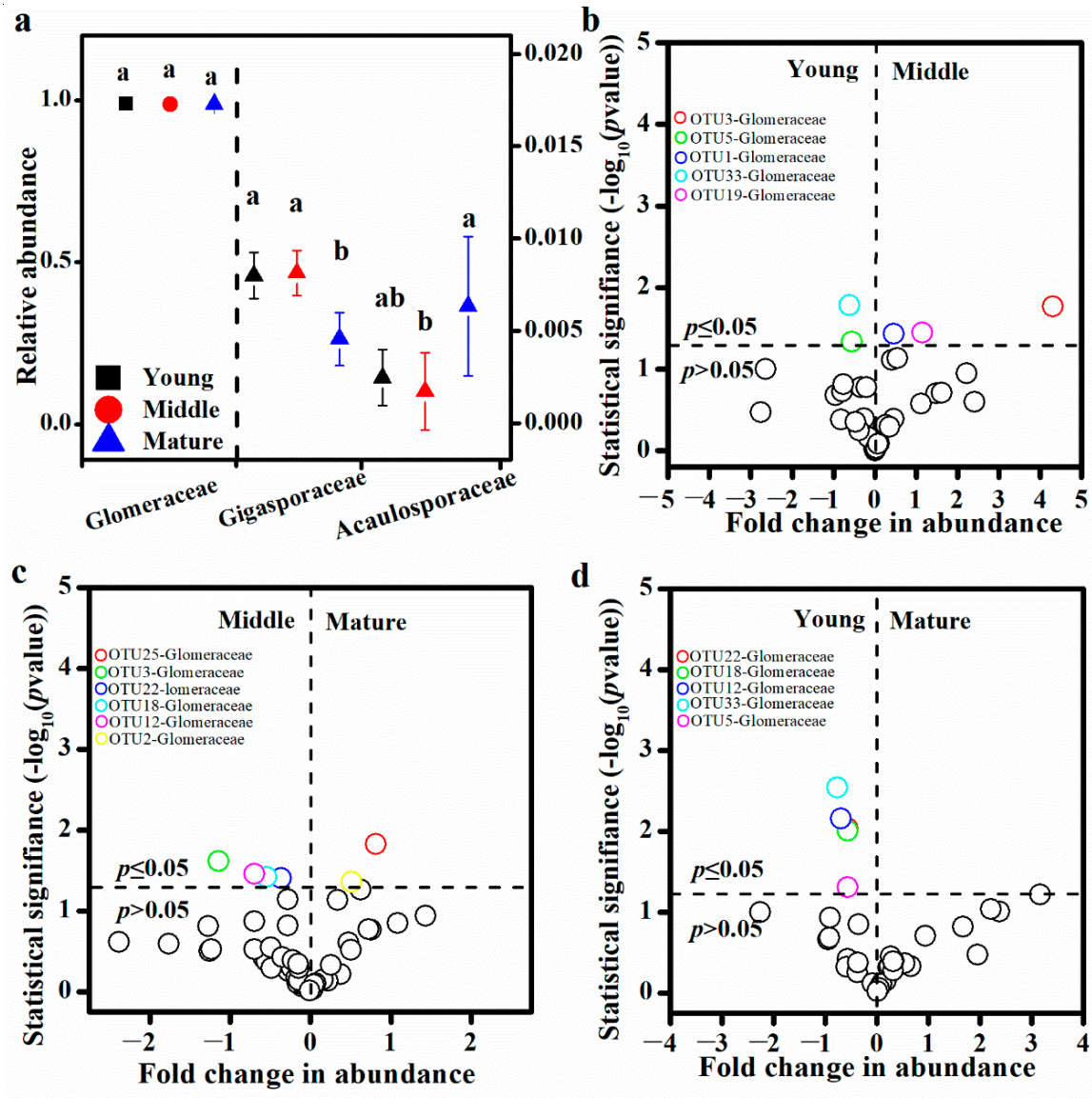

Figure 5. Relative abundance of AM fungal families under different plantations (**a**), Volcano plot of MV-GLM modelled shifts in AM fungal abundances in middle-aged plantation relative to young plantation (**b**), mature plantation relative to middle-aged plantation (**c**) and mature plantation relative to young plantation (**d**). Colored points represent OTUs abundances were significantly shifted ($p < 0.05$), whereas hollow points representing OTUs abundances were not significantly altered ($p > 0.05$). AM fungal OTUs in the left were more abundant in the left treatment, while OTUs in the right were more abundant in the right treatment.

3.4. Potential Key AM Fungal Taxa

Important AM fungal guilds as biomarker taxa for the different ages of *C. lanceolata* plantations were further examined with the RF model. The top 20 AM fungal taxa were chosen as the representative biomarker taxa. The most important biomarker species shifted from OTU72 under young plantation to OTU42 under middle-aged plantation to OTU48 under mature plantation. There were 17 Glomeraceae OTUs and three Gigasporaceae OTUs as a biomarker for the young plantation. Two Gigasporaceae OTUs and Acaulosporaceae OTUs were recorded as important biomarkers for the middle-aged plantation. Six Gigasporaceae OTUs and two Acaulosporaceae OTUs were noted as important biomarkers for the mature plantation. At the family level, Glomeraceae was the most important AM fungi in the studied *C. lanceolata* plantations. However, the importance of Acaulosporaceae was higher in middle and mature plantations than in the young plantation. In contrast, the importance of Gigasporaceae was lower in the middle and mature-aged plantations than in young plantations (Figure 6).

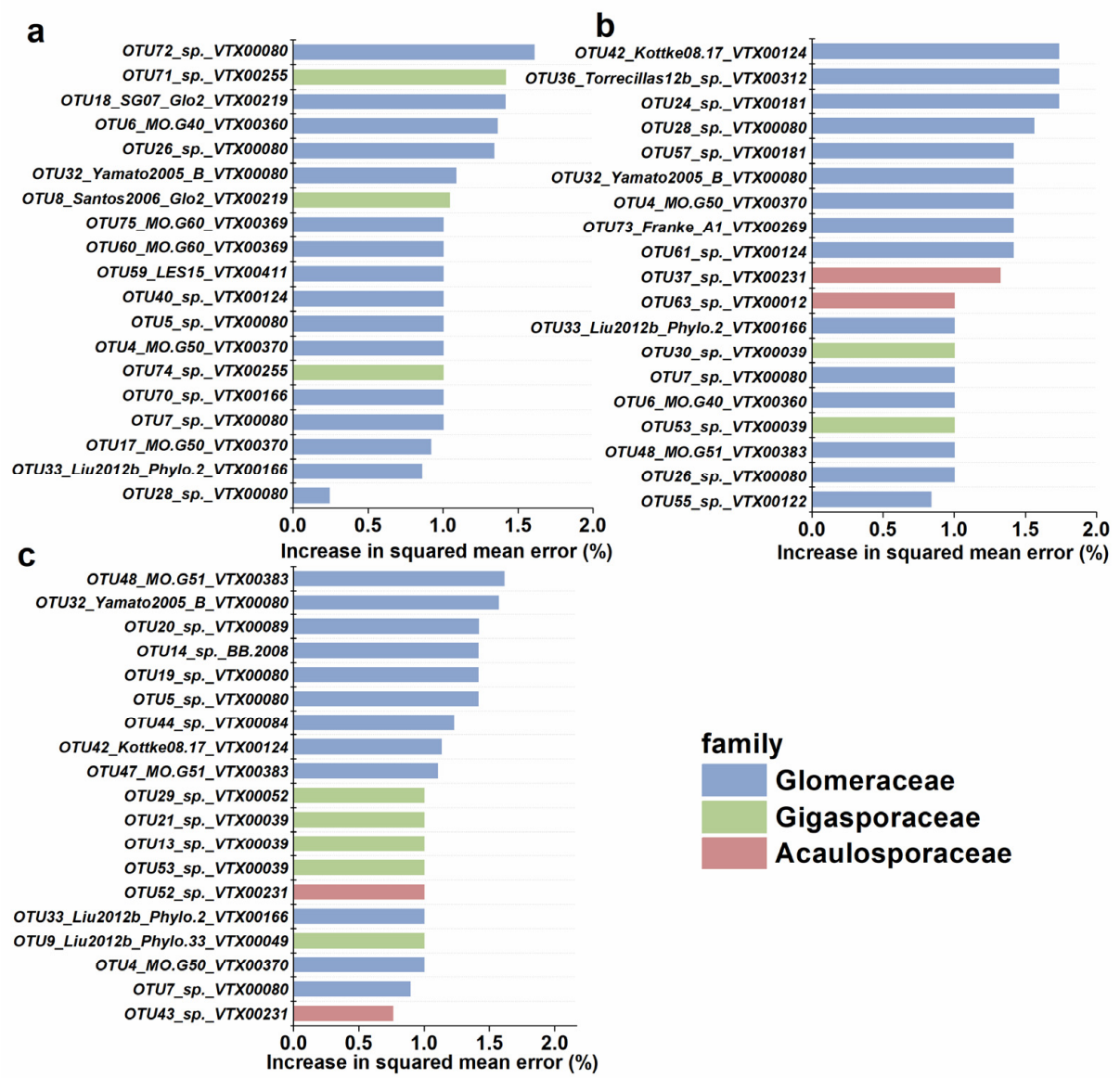

Figure 6. Predictor importance (Percentages of increase in mean square error (MSE) between observations and OOB predications) of the top AM fungal taxonomic biomarkers in young plantation (**a**), middle plantation (**b**) and mature plantation (**c**).

3.5. Linkage between AM Fungal Community and Soil Environmental Factors

The stand age exerts significant effects on AM fungal communities and the relative abundances (Table 2, Figure S5). In addition, SOC, TN, AN, and AP also have significant correlation with the community composition (Table 2). Specifically, the relative abundance of Acaulosporaceae was positively related to stand age and SOC, and negatively related to DOC (Figure S5). Furthermore, AM fungal diversity indices demonstrated different correlations with the soil chemical properties. The observed OTUs, Chao1, and ACE index positively changed with the stand age, soil moisture, and AP, but were negatively related to TN (Figure S5). Moreover, significant correlations were also observed between AM fungal community composition and GRSP contents, and between Acaulosporaceae abundance and T-GRSP content (Table S4).

Table 2. Correlation between AM fungal community composition and environmental variables measured by Mantel test.

Variable	*r*	*p*
Stand age	0.5965	0.001
Soil moisture	0.0842	0.173
pH	−0.008	0.487
DOC	0.0286	0.364
SOC	0.2889	0.009
TN	0.2500	0.037
AN	−0.086	0.767
AP	0.2534	0.041
AK	0.1071	0.184

SOC, soil organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; AP, available phosphorus.

3.6. Phylogenetic Structure of Soil AM Fungal Community

The mean NTI values for AM fungi of all the studied plantations were higher than zero, indicating the AM fungal community structure was more phylogenetic clustered than that expected by the null model in *C. lanceolata* plantations. All βNRI values for AM fungi were larger than +2 from young to mature plantations. The development of *C. lanceolata* plantation did not alter the proportion of NTI and βNRI vaules (Figure 7).

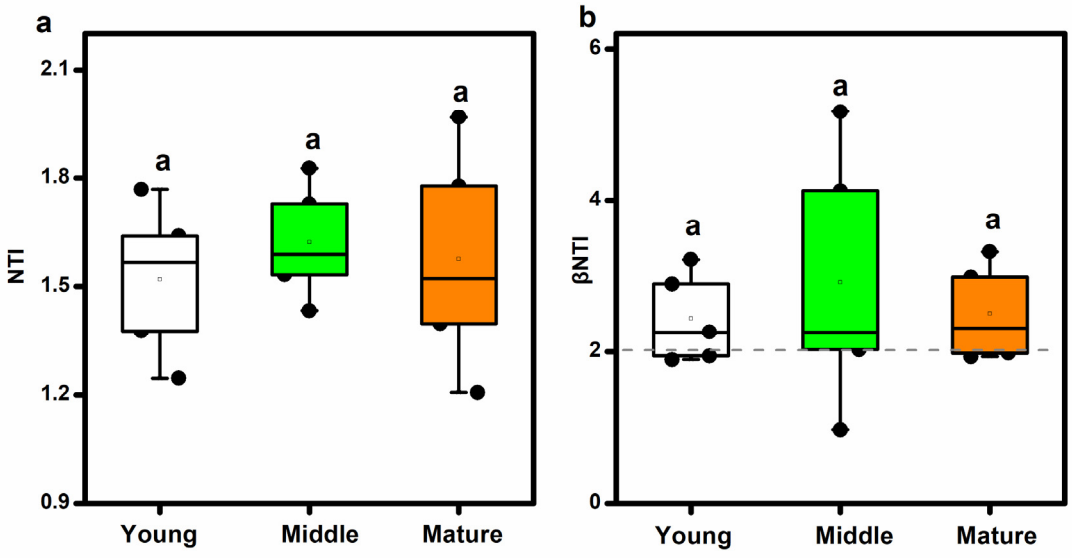

Figure 7. Soil AM fungal community assembly based on phylogenetic relatedness (nearest relative index, NRI) (**a**) and βNTI (**b**) during the development of *C. lanceolata* plantations. Bars topped by different letters indicate significant difference ($p < 0.05$).

4. Discussion

4.1. Increased AM Fungal Diversity and Altered Community Composition during the Development of C. lanceolata Plantations

Although the symbiotic association between mycorrhiza and *C. lanceolata* has long been recognized, the knowledge of changing patterns of AM fungal growth and community structure as well as their related C sequestration roles during the development of *C. lanceolata* is still limited. Here, we observed that both root colonization and AM fungal biomass (as assessed by PLFA 16:1ω5c) increased from young to mature *C. lanceolata* plantations (Figure 1). Such phenomena might imply that mature *C. lanceolata* had a greater dependency on AM fungi than younger trees. It has been well documented that P is a limiting factor for subtropical plant growth due to the highly weathered soil and recalcitrant P formation [38]. In such a situation, *C. lanceolata* might have a strong dependency on AM fungi for growth demand, especially under lower P availability conditions, such as the mature plantations. It was found that soil available P concentration was lower in the mature plantation than young plantation (Table S2). Correspondingly, AM fungal diversity was greater in mature plantations compared to those in young plantations, which is corroborated with the findings of Lu [22], who found greater AM fungal diversity in older *C. lanceolata* plantations than the younger plantations, as well as those in the *Artocarpus altilis Fosberg* forests [39]. However, in *Populus-salix* forests, AM fungal richness decreased significantly from young to old trees [40]. In *Robinia pseudoacacia* plantations, AM fungal colonization and richness also decreased, along with stand ages [41]. These discrepancies suggested a variable effect of stand age on mycorrhizas, which may be correlated to host-specific characteristics of AM fungi and context-dependent differences in C allocation from plant to AM fungi [42].

Development of *C. lanceolata* dramatically shifted the soil in AM fungal communities (Figure 3), similar to those of tallgrass and black locust plantations [39,41]. Such a variation in AM fungal communities reflects changes in specific mycorrhizal groups rather than formation of a new AM fungal community that is compositionally different from those occurring in younger plantations. At the family level, Glomeraceae was the most common group in the AM fungal community across the three investigated stands, similar to the dominance of Glomeraceae in managed agricultural lands and natural forests [22,43]. The AM fungi of this family could colonize and propagate via mycorrhizal roots or extramycelium [44]. Although the Glomeraceae abundance did not change among different plantations, the responses of Glomeraceae OTUs to stand age were complex with decreasing, increasing or no significant changes (Figure 4), implying that the survival mechanisms and functional traits are not common among AM fungal taxa within a family. In contrast, the mature plantation demonstrated a higher relative abundance of Acaulosporaceae, which can effectively survive under abiotic or biotic stresses [45]. This finding demonstrates that the development of *C. lanceolata* plantations may bring potential stresses to soil AM fungi; hence, those stress-tolerant AM fungi groups such as Acaulosporaceae were favored. Gigasporaceae were depleted in mature plantation, which often respond positively to low P conditions to increase plant root P uptake due to their high P acquisition traits [45]. It is known that *C. lanceolata* trees grow rapidly with high nutrient requirements during the young and middle stages according to their growth properties [24]. In this situation, younger trees may tend to favor AM fungal partners that provide nutritional benefits, such as Gigasporaceae. In mature plantations, the nutrient requirements of *C. lanceolata* trees would differ from younger trees, which could have resulted in decreased abundance of Gigasporaceae relative to the younger plantations.

The context- or host-dependent characteristics of mycorrhizas may also be responsible for the shifts in AM fungal community structure. A significant correlation was recorded between AM fungal communities and stand age (Table 2), similar to many studies that have documented a strong correlation between stand age and taxonomic community composition for AM fungi [22,41]. Stand age is a complex factor, which may cause changes in many environmental characteristics. Soil nutrient status has been commonly recognized as a strong driver of bacterial and AM fungal communities during the development of stand

age. For instance, Sheng et al. [41] revealed that the variation of AM fungal communities was attributed to the soil available nutrient status in *Robinia pseudoacacia* plantations. In *C. lanceolata* plantations, we observed that both the AM fungal community composition and diversity were significantly related to TN, SOC, and AP (Table 2, Figure S3). It is commonly known that AM fungal members possess distinct niches and are apt to exist in different environments. Based on the functional equilibrium model, soil nutrient (N:P) status can influence the C trade from plant to AM fungi resulting in changes in the AM fungi species [42]. Furthermore, plants are known to directly interact with AM fungal communities. Different plant species and/or growth ages hosted different AM fungal communities [21]. The vegetation characteristics may affect the mycorrhizal fungal community; however, we cannot evaluate the correlation of fungal community with vegetation characteristics due to the lack of plant data, and thus cannot exclude the possibility that certain AM fungal members were selected by the plants. Nevertheless, our results highlight that the mycorrhizal fungal communities were different across the development of subtropical *C. lanceolata* plantations, which might be partially correlated with the changed soil environmental factors.

Unlike the composition and diversity, the AM fungal phylogenetic structure was not changed during the development of *C. lanceolata* plantations. Phylogenetic metrics indicate that the mycorrhizal fungal community was phylogenetically clustered, and AM fungal groups within the community were closely related in all studied plantations. This suggests that environmental filtering and deterministic processes primarily governed the AM fungal community assembly due to the high phylogenetically conservatism of AM fungal traits [46]. Such phylogenetic clustered assembly for AM fungi was consistent with the previous reports from natural and managed forests and grasslands [47,48]. Both biotic and abiotic factors may exert forces on ecological phylogenetic structures. Soil AM fungi serve as nutrient transporters to their hosts; their communities could be largely shaped by plant-AM fungi interactions, as different AM fungal groups have different nutrient uptake abilities. For example, plants can select AM fungi members with high P uptake function in low P soils [49]. In the studied subtropical region, soil P is a key factor limiting plant growth due to high weathering and recalcitrant P compound formation [38]. Therefore, the clustered pattern in our study (available P content lower than 3 mg g^{-1} soil) might be largely attributed to environmental (host plant) filtering, which supports the environmental selection effect under low P conditions in alpine meadows and grasslands [47,50]. Although variations in the fungal community were recorded during the development of the *C. lanceolata* plantation, Glomeraceae was the dominant group across all studied plantations, which might be one possible mechanism of the non-shift in the community assembly among different stages. Collectively, it is speculated that the community assembly could be less sensitive to the development of *C. lanceolata* plantations than their composition and diversity.

4.2. Stimulated Contribution of AM Fungi-Associated Soil C to Soil C Pools during the Development of C. lanceolata Plantations

Previous studies documented that different AM fungi pose different abilities in forming hyphae and excreting glomalin [51,52]. Therefore, it is conceivable that different subsets of the AM fungal community may result in changes in their associated C sequestration [12,53]. Here, we found that the mycorrhizas significantly correlated with soil GRSP contents, and PLFA 16:1ω5c abundance and soil GRSP content were also positively correlated. These findings confirmed our hypothesis that changes in mycorrhizal abundance and communities might lead to different associated C sequestration. The higher proportion of Acaulosporaceae in older *C. lanceolata* plantations may contribute to the higher soil GRSP content. It was reported that Acaulosporaceae species can secrete higher glomalin than Gigasporaceae and Glomeraceae [54]. Similarly, the AM fungal inoculation study also proved that the AM fungi increased the soil GRSP concentrations in trifoliate orange and poplar plantations, and the improvement of GRSP was dependent on AM fungal species [55]. The correlation analyses found the increase in GRSP significantly correlated

with SOC in *C. lanceolata* plantations, suggesting that GRSP was an important C source for the buildup of SOC.

GRSP is known to account for 3%–5% of soil C pools with 30% aromatic C and 40% carboxyl C [10,56]. Soil GRSP-C accounted for approximately 10% of SOC in *C. lanceolata* plantations, similar to those in tropical forests (~12.95%) [31], Hawaiian rainforests (~9.9%) [10] and marine sediments (~10.8%) [57], suggesting that AM fungi are also important C source in *C. lanceolata* plantations compared to other ecosystems. Among the two parts of GRSP, DE-GRSP represented a higher proportion of soil C than that of EE-GRSP, suggesting an important function of DE-GRSP in soil C sequestration. Soil EE-GRSP is a lately secreted and unstable glomain relative to DE-GRSP, a recalcitrant and less active GRSP fraction transformed from EE-GRSP [58]. It was exemplified that DE-GRSP rather than EE-GRSP positively correlated with soil C pools in *C. lanceolata* plantations. Similarly, DE-GRSP made a greater contribution to soil C stocks than EE-GRSP in tropical orange forests [58]. The present work also detected that the C contribution of all GRSP fractions to soil C was higher in mature versus young *C. lanceolata* plantations. The increased C contribution of GRSP to soil C might be related to an excretion of soil enzymes (i.e., hydrolases), accompanied with stimulated C input from plants [59]. In addition, GRSP could contribute strongly to soil aggregation and thus subsequently protect soil C from decomposition via physical protection [15]. Forest plantation enhanced GRSP concentrations and promoted soil aggregates' formation, which ultimately contributed to soil C storage [60]. Hence, AM fungi would directly and indirectly contribute to soil C sequestration in *C. lanceolata* plantations.

5. Conclusions

The study describes how the subtropical *C. lanceolata* plantation development impact on AM fungal communities and their associated soil C sequestration. Our results reveal that the *C. lanceolata* development caused significant variations in the AM fungal community composition and increased the mycorrhizal colonization, biomass and diversity, and exhibit information regarding the contribution of AM fungal biomass to GRSP, which possessed a close correlation with soil organic C. The development of the *C. lanceolate* plantation increased soil GRSP concentrations and the C contribution to soil organic C. Our findings have implications of AM fungal growth and GRSP on soil C stocks in *C. lanceolata* plantations. This information highlights that further studies should focus on the associated soil C functions of AM fungi when exploring the sustainable management of *C. lanceolata* plantation ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13122099/s1, Table S1: Details of the sampling sites of *C. lanceolata* plantations; Table S2: Soil chemical properties in different *C. lanceolata* plantations; Table S3: Soil AM fungal sequences and OTUs in each soil sample at different age of *C. lanceolata* plantations; Table S4: Correlations between the AM fungal community composition and AM fungal groups and GRSP contents; Figure S1: Location of study area in Chenda Town, Fujian Province, China; Figure S2: Rarefaction curves of observed bacterial OTUs and good coverage among the different age of *C. lanceolata* plantations; Figure S3: Taxonomic composition of soil bacterial communities at the phylum level under the different age of *C. lanceolata* plantations; Figure S4: Significant test of bacterial community composition between different ages of *C. lanceolata* plantations based on the PerMANOVA analysis. Figure S5 Correlation analysis between environmental factors and AM fungal diversity and relative abundances of AM fungal families in different ages of *C. lanceolata* plantations.

Author Contributions: Conceptualization and methodology, Z.L.; software and formal analysis, Z.L. and Y.H.; validation and investigation, F.L.; data curation, Y.H. and H.Z.; writing—original draft preparation, Z.L. and J.C.; writing—review and editing, Z.L. and J.C.; visualization, Z.L. and Y.H.; supervision, J.C.; project administration, J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Natural Science Foundation of China (31800520) and the startup foundation for introducing the talent of Jiangxi agricultural university (9232308147).

Acknowledgments: We are grateful to the staff of the State-owned Forest Farm Station and Climate Change and Forest Ecosystem Experimental Station for their kind management and great help with the sample collection.

Conflicts of Interest: The authors declare no conflict of interest.

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