forests

MDPI

Article

Accumulation of Glomalin-Related Soil Protein Regulated by Plantation Types and Vertical Distribution of Soil Characteristics in Southern China

Miaolan Wu [1], Shaochun Zhang [1], Xiaojuan Gu [1], Zhihang He [1], Yue Liu [1] and Qifeng Mo [1,2,*]

1 College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China; 20222159024@stu.scau.edu.cn (M.W.); zhangsc22@stu.scau.edu.cn (S.Z.); 20232159005@stu.scau.edu.cn (X.G.); hzh@stu.scau.edu.cn (Z.H.); bgly@stu.scau.edu.cn (Y.L.)

2 China Forest Ecosystem Research Network, Guangdong E'huangzhang National Field Observation and Research Station, Yangjiang 529631, China

* Correspondence: moqifeng@scau.edu.cn

Abstract: The glomalin-related soil protein (GRSP) is an important component of soil organic carbon (SOC), which plays an important role in maintaining soil structural stability, soil carbon (C), and nitrogen (N) fixation. However, little is known about the GRSP content in soil and its contribution to soil nutrients in plantations of different tree species. In this study, we determined the soil physicochemical characteristics and GRSP contents in different soil layers of four kinds of plantations, including *Acacia mangium* (AM), *Pinus caribaea* (PC), *Eucalyptus urophylla* (EU), and *Magnoliaceae glanca* (MG), to address how the plantation types affected the GRSP in different layers of soil in southern China. The results showed that with an increase in soil depth, the GRSP content decreased linearly, and the contribution rate of GRSP to SOC and total nitrogen (TN) in deep soil was 1.08–1.18 times that in surface soil. The tree species significantly affected the vertical distribution of GRSP in soil. Among the four plantations, the conifer species PC had the highest level of GRSP, while the N-fixing species AM had the lowest level. However, SOC, soil capillary porosity (CP), TN, soil water content (SWC), and total phosphorus (TP) were important factors regulating soil GRSP content. Additionally, the regulation effects of soil properties on GRSP were various in surface and deep soil among different plantations. In order to improve soil quality and C sequestration potential, conifer species can be planted appropriately, or conifer species and N-fixing species can be mixed to increase soil nutrient content and enhance soil structure and function in afforestation of southern China.

Keywords: glomalin-related soil protein (GRSP); plantation; N-fixing tree species; vertical distribution; subtropical China

check for updates

Citation: Wu, M.; Zhang, S.; Gu, X.; He, Z.; Liu, Y.; Mo, Q. Accumulation of Glomalin-Related Soil Protein Regulated by Plantation Types and Vertical Distribution of Soil Characteristics in Southern China. *Forests* **2024**, *15*, 1479. https://doi.org/10.3390/f15081479

Academic Editor: Wenjie Liu

Received: 18 July 2024
Revised: 15 August 2024
Accepted: 21 August 2024
Published: 22 August 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Glomalin-related soil protein (GRSP) is a kind of hydrophobic glycoprotein with metal ions, and produced by the mycelium of arbuscular mycorrhizal fungi (AMF) [1]. According to the complexity of extraction, GRSP can be divided into easily extractable glomalin (EE-GRSP) and total glomalin-related soil protein (T-GRSP) [2]. Generally, EE-GRSP is considered as the newly produced fraction, while T-GRSP represents the total amount of long-term accumulation in soil [3]. Moreover, the ratio of EE-GRSP to T-GRSP can reflect the proportion of newly generated GRSP to a certain extent. Soil carbon (C) and nitrogen (N) are important indicators for evaluating soil quality and fertility. It is believed that GRSP contains 3%–5% N with a mean C of nearly 37% [4]. As an important part of soil C and N pools, the contribution of GRSP to soil C and N pools is much larger than microbial biomass [5]. Therefore, studying the contribution of GRSP to soil organic carbon (SOC) and total nitrogen (TN) fixation and its influencing factors can provide data support and practical reference for improving soil quality and C sink capacity.

As a cementing material of soil aggregate structure, GRSP can enhance the stability of soil structure and promote soil nutrient cycling [6,7]. The change in GRSP in soil has been widely studied. Most researchers have focused on the effects of soil physicochemical properties, land use patterns, environmental stress, fertilization, and other factors on GRSP [8–11]. Previous studies found that there was a significantly positive correlation between GRSP content and SOC and TN contents in surface soil [8,12]. In addition, a national survey in France found that there was no significant difference between GRSP and other soil parameters under different land use patterns [13]. However, the current studies on GRSP mainly focus on surface soil or shallow soil, especially in the upper 40 cm soil layer, based on the assumption that the GRSP in deep soil tends to be stable over time [8,14,15]. To date, studies have confirmed that the GRSP content gradually decreases with an increase in soil depth, and the depth of GRSP accumulation in the soil can reach 100 cm [16]. However, the current research on GRSP changes in vertical profiles is still limited. In order to clarify the importance of GRSP for the C and N cycles, it is necessary to sample soil and explore the relationship between GRSP and soil physicochemical factors in deeper soil.

Forests play an important role in improving the soil C sequestration of ecosystems [17]. China has the largest area of plantations in the world, and plantations in the subtropics account for about two-thirds of the area of plantations [18]. Afforestation can promote the accumulation of soil GRSP by affecting multiple variables in the soil, thereby alleviating soil degradation [19]. Changes in soil nutrients and soil physical properties are crucial to the growth of trees, and the alteration of tree species composition and disturbance of silvicultural activities in plantations greatly drives the biogeochemical cycle of ecosystems [20]. Previous studies found that different trees affected the characteristics of soil GRSP through the interaction with soil, and the concentration of soil GRSP varies with tree species and root depth [21,22]. Rotter et al. [23] studied the soil GRSP of five forest types, including coniferous forest, deciduous forest, mixed forest, and mountain rainforest, and found that the coniferous forest soil had the highest GRSP content. In a tropical arid forest in central India, Singh et al. [24] reported that forest communities significantly affected the concentration of GRSP in soil, and the GRSP concentration of exotic tree species was the lowest; meanwhile, the content of GRSP in the upper soil was higher than that in the deeper soil. However, there are few studies focusing on the effects of afforestation of different tree species on soil GRSP content and its contribution to soil nutrients. In plantation practices, the selection of suitable tree species according to their characteristics and their interactions with environmental factors is conducive to the improvement of the soil quality and the nutrient cycle of plantation ecosystems.

In this study, we hypothesize that the contribution of GRSP to soil C and N nutrient accumulation depends on soil depth, and the characteristics of soil GRSP are strongly regulated by tree species and soil properties. Herein, broad-leaved and coniferous, N-fixing and non-N-fixing tree species, were selected as the research objects for plantations, and the GRSP content of different plantations was analyzed to evaluate its contribution to soil C and N pools. Combining tree species and soil physicochemical factors to explore the influencing factors on the vertical distribution pattern of GRSP in soil, we aimed to address the following questions: (1) What are the differences in soil GRSP characteristics (including EE-GRSP, T-GRSP, EE-GRSP/T-GRSP, and the contribution rates of EE-GRSP and T-GRSP to SOC and TN) in 1 m depth soil for different tree species? (2) In the case of different tree species, what factors lead to the vertical variation in GRSP, and the differences in GRSP between surface and deep soil? The results of this study clarify the importance of GRSP to the C and N cycles in the deep soil, and support the construction of subtropical plantations in terms of tree species selection and soil quality improvement.

2. Materials and Methods

2.1. Study Sites and Soil Sampling

The experimental area is located in the state-owned Huatan Forest Farm (22°15′05″ N, 111°43′15″ E) in Yangjiang City, Guangdong Province, China. The region belongs to the subtropical monsoon climate, with a mean annual temperature (MAT) of 22.3 °C. The mean annual precipitation (MAP) is 2392.3 mm, and most of the precipitation is distributed from April to September. In this area, Chinese fir, masson pine, and eucalyptus are the main tree species for afforestation. According to the soil classification system in China, the soil in this area is classified as acidic lateritic red soil developed from granite. The soil is loose, with a pH value of 4.0–4.5.

In this study, four kinds of pure plantations with similar conditions in the experimental area were selected, including *Acacia mangium* (AM), *Pinus caribaea* (PC), *Eucalyptus urophylla* (EU), and *Magnoliaceae glanca* (MG) (Figure 1). According to a survey in 2022, the stand ages of the four plantations were 11, 12, 8, and 12 years, respectively, while the stand densities were 800, 1275, 1525, and 1475 individual hm^{-2}, respectively. The diameters at breast height (DBH) were 18.15, 17.09, 14.54, and 17.08 cm, respectively, while the heights were 15.28, 15.18, 16.81, and 16.56 m, respectively. In December 2022, three plots of 10 m × 10 m were randomly established in each plantation, and the distance between the neighboring plots was greater than 50 m. Three soil profiles of 100 cm depth were set up in each plot by the three-point sampling method, and the profiles were divided into five layers from top to bottom: 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, and 60–100 cm. Soil samples were collected for analysis and cutting ring samples (100 cm^3) were used to determine soil bulk density (BD), soil water content (SWC), and other physical properties [25]. A total of 60 soil samples were collected by fully mixing three samples from the same plots with the same layer of soil samples to form a mixed soil sample, and then, brought back to the laboratory to determine other physical and chemical properties of the soil. The samples were dried in a cool and dry place after removing debris including gravel and plant roots, and then, sieved for determination.

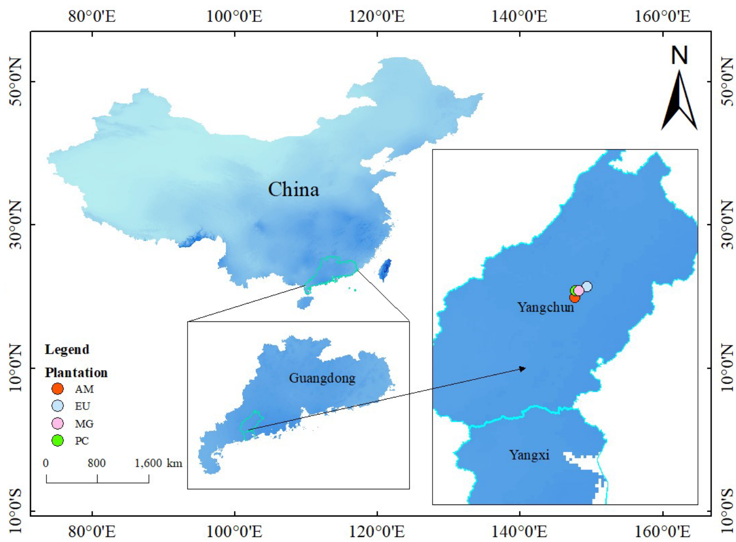

Figure 1. Geographical location information of the study site. The four points are the locations of the four plantations we studied. AM, *Acacia mangium*; PC, *Pinus caribaea*; EU, *Eucalyptus urophylla*; MG, *Magnoliaceae glanca*. The map was generated by the ArcGIS 10.6 software.

2.2. Determination of Soil Physicochemical Properties

The determination of soil physicochemical properties followed the method of Bao [26]. The soil pH value was determined by the potentiometric method (water:soil = 2.5:1); soil organic carbon (SOC) was determined by the potassium dichromate–concentrated sulfuric acid external heating method. Soil total nitrogen (TN) was determined by the concentrated sulfuric acid–catalyst digestion–indophenol blue colorimetric method. Soil total phosphorus (TP) was determined by the concentrated sulfuric acid–catalyst digestion–molybdenum antimony anti-colorimetric method. Soil available phosphorus (AP) was extracted with an ammonium fluoride–hydrochloric acid solution and determined by the molybdenum antimony colorimetric method.

The soil bulk density (BD) was tested by the cutting ring method [25], and the soil water content (SWC) was determined by the oven drying method, calculated by the mass loss after drying at 105 °C for 24 h. The soil capillary water holding capacity (CC) was measured by the water absorption method of undisturbed soil in the cutting ring. The soil capillary porosity (CP) was calculated based on BD and CC, where CC = water weight in the cutting ring/dry soil weight in the cutting ring × 100%; CP = CC × BD × 100%.

2.3. Extraction and Determination of GRSP in Soil

The extraction and determination of GRSP in soil were performed according to the methods described by Wright and Upadhyaya [2]. Briefly, EE-GRSP was extracted from 0.5 g soil with 4 mL of sodium citrate solution (pH = 7.0, 20 mmol L^{-1}) and autoclaved at 121 °C for 30 min, and then, centrifuged at 5000 r min^{-1} for 6 min to collect the supernatant. T-GRSP was extracted from 0.5 g soil with 4 mL of sodium citrate solution (pH = 8.0, 50 mmol L^{-1}) and autoclaved at 121 °C for 60 min, and then, centrifuged at 5000 r min^{-1} for 6 min to collect the supernatant. The procedure of the extraction of T-GRSP was repeated until the supernatant was basically colorless, and the supernatant from the same sample was combined into one sample. The protein amount in the crude extract was determined by the Bradford assay using bovine serum albumin as a standard.

GRSP characteristics include EE-GRSP content, T-GRSP content, EE-GRSP/T-GRSP, EE-GRSP-C/SOC (the proportion of EE-GRSP carbon to soil SOC), T-GRSP-C/SOC (the proportion of T-GRSP carbon to soil SOC), EE-GRSP-N/TN (the proportion of EE-GRSP nitrogen to soil TN), and EE-GRSP-N/TN (the proportion of T-GRSP nitrogen to soil TN). The C and N contents in extracted glomalin are 37% and 4%, respectively, from the study of Lovelock et al. [4].

2.4. Data Analysis

Excel 2021 was used to sort the data, and SPSS 27.0 was used for variance analysis, including one-way ANOVA and two-way ANOVA. Duncan's multiple comparison test was used to test the differences in GRSP content and soil physicochemical properties in the vertical soil profiles. Origin was used for Pearson correlation analysis of the data to test the correlation between the GRSP content and soil physicochemical properties of different tree species. Stepwise regression analysis was used to further explore the linear relationship between physicochemical factors and GRSP in surface and deep soils.

3. Results

3.1. Effects of Tree Species and Soil Depth on GRSP Content and Distribution

Tree species and soil depth and their interaction significantly affected soil EE-GRSP and T-GRSP contents (Table 1). With an increase in soil depth, the EE-GRSP and T-GRSP contents of the four plantations showed a decreasing trend (Figure 2). There were significant differences among different tree species in the 0–20 cm soil layer. The EE-GRSP content of N-fixing tree species (AM) in the 0–10 cm soil layer was significantly higher than that of non-N-fixing tree species (EU and MG), but the EE-GRSP content in the 10–20 cm soil layer was significantly lower than that of native tree species (MG). In the deep 40–100 cm soil layer, the EE-GRSP content of the conifer species (PC) in the 40–60 cm soil layer was significantly higher than that of the other three tree species (AM, EU, and MG), and the EE-GRSP content of the exotic tree species EU in the 60–100 cm soil layer was significantly higher than that of the other three tree species (AM, PC, and MG). However, there was no significant difference in the T-GRSP content of the four tree species in the 0–40 cm soil layer. Additionally, in the deep 40–100 cm soil layer, the T-GRSP content of the exotic tree species

(EU) was significantly higher than that of the N-fixing tree species (AM) and non-N-fixing tree species (Figure 2).

Table 1. Two-way analysis of variance of GRSP characteristics of four forest stands in Huatan Forest Farm.

Parameter		EE-GRSP	T-GRSP	EE-GRSP/ T-GRSP	EE-GRSP-C/SOC	T-GRSP-C/SOC	EE-GRSP-N/TN	T-GRSP-N/TN
Tree	F	2.867	4.976	7.049	7.478	15.375	2.522	11.305
	p	0.048	0.005	<0.001	<0.001	<0.001	0.071	<0.001
Depth	F	66.830	49.071	0.928	4.988	1.178	3.927	1.825
	p	<0.001	<0.001	0.458	0.002	0.335	0.009	0.143
Tree × Depth	F	3.961	2.615	1.968	2.052	3.197	2.900	2.677
	p	<0.001	0.011	0.055	0.044	0.003	0.006	0.010

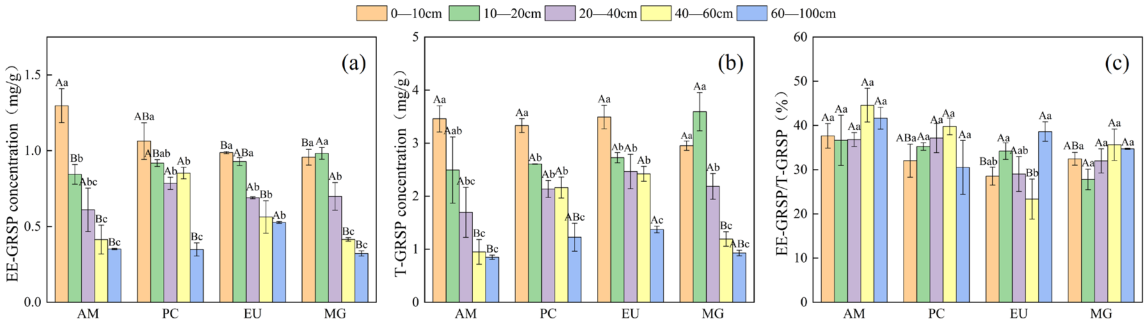

Figure 2. Vertical distribution of easily extractable glomalin-related soil protein (EE-GRSP), total GRSP (T-GRSP), and EE-GRSP/T-GRSP ratio in soil of different plantations. AM, *Acacia mangium*; PC, *Pinus caribaea*; EU, *Eucalyptus urophylla*; MG, *Magnoliaceae glanca*. Different capital letters indicate that there are significant differences between different tree species at the same soil depth, and different lowercase letters indicate that there are significant differences between the same tree species at different soil depths ($p < 0.05$). The graphic (**a**) is EE-GRSP concentration, (**b**) is T-GRSP concentration, (**c**) is the ratio of EE-GRSP to T-GRSP.

The proportions of EE-GRSP and T-GRSP to SOC and TN increased with increasing soil depth (Table S1), indicating that GRSP contributed more to SOC and TN in deep soil. Specially, the ratio of GRSP to SOC in deep 40–100 cm soil was 1.17 times that in surface 0–40 cm soil.

The contribution rate of GRSP content to soil SOC was significantly affected by tree species (Table 1). In different plantations, the contribution rates of soil EE-GRSP and T-GRSP to soil SOC were 1.38%–2.15% and 3.34%–7.61%, respectively; with contributions from the different tree species in the order EU > PC > MG > AM (Table S2). The contribution rate of EE-GRSP to SOC was significantly affected by tree species, soil depth, and their interaction, while the contribution rate of T-GRSP to SOC was significantly affected by tree species and their interaction with soil depth (Table 1).

In the surface 0–40 cm soil layer, the contribution rate of EE-GRSP to SOC in the 0–20 cm soil layer of EU was significantly higher than that of AM. In the deep 40–100 cm soil layer, the contribution rate of EE-GRSP to SOC in the 60–100 cm soil layer of PC and EU was significantly higher than that of AM and MG. Compared with EU and MG, the contribution rate of T-GRSP to SOC in the 0–40 cm soil layer of AM and PC was lower. In deep soil, the contribution rate of T-GRSP to SOC in the 40–60 cm soil layer of EU was significantly higher than that of the other three forests (Figure 3).

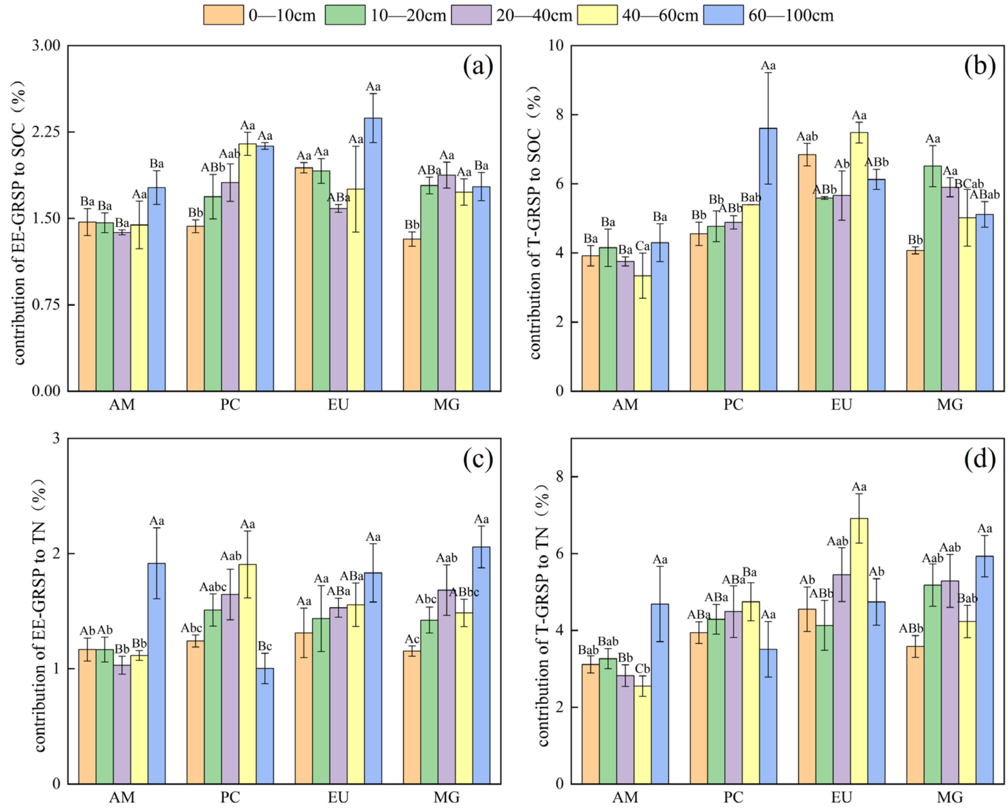

Figure 3. Contributions of easily extracted glomalin-related soil protein (EE-GRSP) and total GRSP (T-GRSP) to SOC and TN in soils of different tree species plantations at different soil depths. AM, *Acacia mangium*; PC, *Pinus caribaea*; EU, *Eucalyptus urophylla*; MG, *Magnoliaceae glanca*. Different capital letters indicate that there are significant differences between different tree species at the same soil depth, and different lowercase letters indicate that there are significant differences between the same tree species at different soil depths ($p < 0.05$). The graphic (**a**) is the contribution of EE-GRSP to SOC, (**b**) is the contribution of EE-GRSP to SOC, (**c**) is the contribution of EE-GRSP to TN, (**d**) is the contribution of T-GRSP to TN.

The contribution rate of the two GRSP components to TN showed different characteristics depending on the tree species and soil depth. The contribution rate of EE-GRSP to TN was significantly affected by soil depth, while the contribution rate of T-GRSP to TN was only significantly influenced by tree species, but the interaction between tree species and soil depth significantly affected the contribution rate of both to soil TN (Table 1). The contribution rates of soil EE-GRSP and T-GRSP to TN were 1.28%–1.56% and 3.29%–5.16%, respectively. Among different tree species, the contribution rates of soil EE-GRSP and T-GRSP to TN in the EU plantation were higher than those of other tree species. On the contrary, the contribution rates of two GRSP components of AM to TN were the lowest (Table S2).

Similar to GRSP-C/SOC, EE-GRSP-N/TN and T-GRSP-N/TN increased linearly with increasing soil depth (Table S1). The soil EE-GRSP-N/TN of PC, EU, and MG showed an obvious increasing trend with increasing soil depth. The change in GRSP-N/TN of the N-fixing tree species AM in the 0–40 cm surface soil tended to be stable, and was significantly lower than that of the non-N-fixing tree species (Figure 3). In the deep soil, the soil T-GRSP-N/TN of EU in the 40–60 cm layer was significantly higher than that of other tree species.

3.2. Correlation between Soil Physicochemical Properties and GRSP

Regression analysis showed that the soil physicochemical properties and nutrients changed linearly in the vertical profile (Table S5). Soil BD increased linearly with soil depth, and soil SWC, pH, SOC, TN, TP, and AP decreased linearly with soil depth (Table S5). The SWC content of surface soil was the highest at 30.99%. In the deep 40–100 cm soil, the soil bulk density was 1.21 times that of the surface 0–40 cm soil. Soil depth had no significant effect on soil pH (p = 0.917), but had a significant effect on soil C, N, and P nutrients. SOC and TN in the 0–40 cm soil were about 2.3 times higher than those in the 40–100 cm soil, while the difference in P content between the surface and deep soil was about 0.1–0.5 times (Table S5).

The correlation analysis showed that there was a significant negative correlation between soil EE-GRSP and T-GRSP and soil BD in the AM and PC plantations, and a significant negative correlation between soil EE-GRSP and T-GRSP and soil BD in the MG plantation (Figure 4). There was a positive correlation between soil GRSP and multiple soil physicochemical factors except soil BD in the AM, PC, and MG plantations. There was a significant positive correlation between SWC, CC, SOC, TN, and soil GRSP content. The correlation between soil GRSP and soil physicochemical factors in EU was similar to that in the other stands, but the positive correlation between soil physical factors, such as SWC and CC, and GRSP content was not significant, while the extremely significant positive correlation between SOC, TN, and GRSP was consistent with the other three stands. It is worth noting that there was a significant positive correlation between soil pH and GRSP content in MG, while there was a negative correlation between GRSP and pH in the other three forests, and the correlation was not significant (Figure 4).

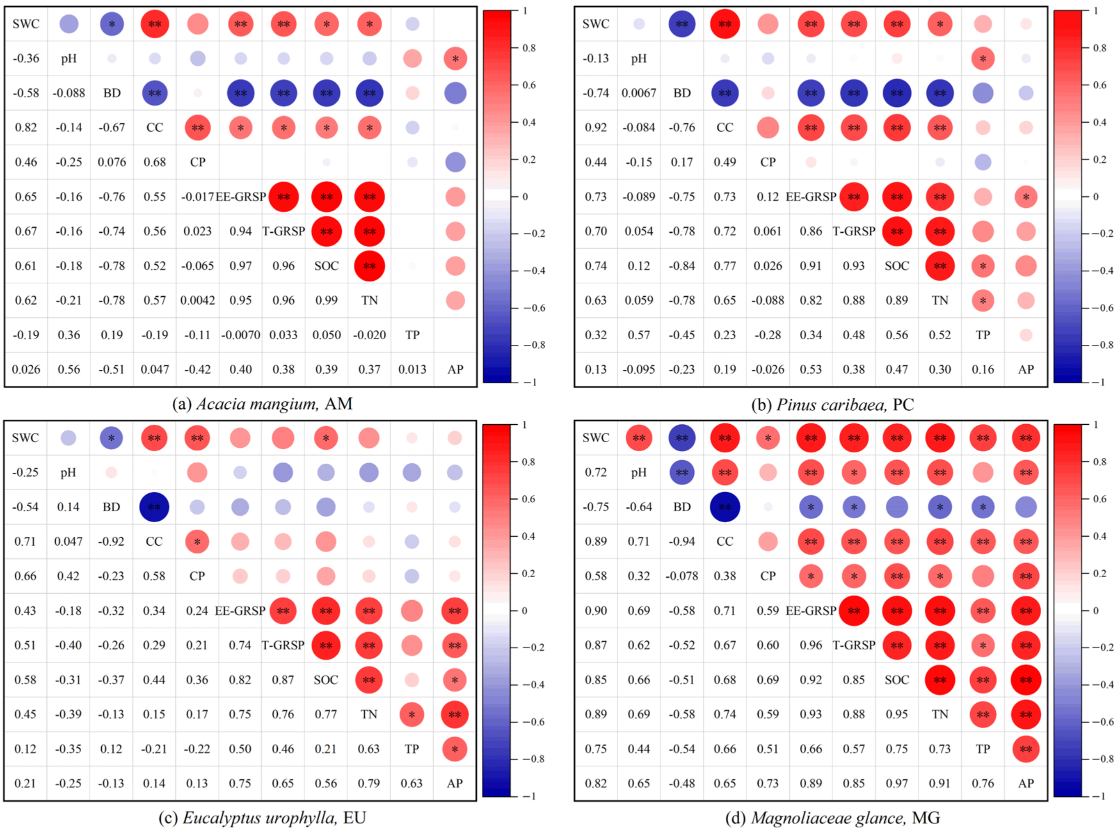

Figure 4. Correlation heat map of soil GRSP and soil physical and chemical factors of four tree species. '**' indicates that the difference is extremely significant ($p < 0.01$); '*' means significant difference ($p < 0.05$).

Because GRSP had the same change trend with multiple soil physicochemical properties (Tables S1 and S5), correlation analysis showed that there was a significant or extremely significant correlation between GRSP and various physicochemical properties among the four plantations (Figure 4). Therefore, the relationship between them was further explored by stepwise regression analysis.

The stepwise regression analysis showed that the multiple correlation coefficients between GRSP and soil physicochemical factors in surface soil and deep soil were between 0.961 and 0.996, and reached a significant level ($p < 0.05$). Therefore, stepwise regression analysis could reflect the correlation between GRSP and soil physical and chemical factors. Soil SOC, CP, TN, SWC, and TP are important factors in regulating soil GRSP content (Table 2). The effects of soil physicochemical properties on GRSP content in surface soil were mainly regulated by SOC, SWC, TP, and TN, while in deep soil, GRSP content was only regulated by SOC and CP.

Table 2. Stepwise regression of GRSP with soil physical and chemical properties of different tree species and their differences between surface and deep soils.

Plantation	Y	Depth	Parameter	Unstandardized Coefficient, B	Standardized Coefficient, β	Sig.	R^2
AM	EE-GESP	Top soil, 0–40 cm	SOC	0.039	0.995	<0.001	0.990
		Deep soil, 40–100 cm	CP	0.010	0.984	<0.001	0.968
	T-GRSP	Top soil, 0–40 cm	TN	0.789	0.993	<0.001	0.985
		Deep soil, 40–100 cm	CP	0.028	0.987	<0.001	0.974
PC	EE-GESP	Top soil, 0–40 cm	SWC TN	0.017 0.163	0.531 0.473	0.002 0.004	0.995
		Deep soil, 40–100 cm	SOC	0.057	0.998	<0.001	0.996
	T-GRSP	Top soil, 0–40 cm	SOC TP	0.070 1.072	0.559 0.442	0.016 0.041	0.992
		Deep soil, 40–100 cm	SOC	0.154	0.980	<0.001	0.961
EU	EE-GESP	Top soil, 0–40 cm	SOC	0.049	0.995	<0.001	0.991
		Deep soil, 40–100 cm	CP	0.015	0.989	<0.001	0.978
	T-GRSP	Top soil, 0–40 cm	TP	2.521	0.993	<0.001	0.987
		Deep soil, 40–100 cm	SOC	0.182	0.990	<0.001	0.979
MG	EE-GESP	Top soil, 0–40 cm	SWC TN	0.018 0.122	0.630 0.373	0.002 0.039	0.992
		Deep soil, 40–100 cm	SOC	0.046	0.995	<0.001	0.990
	T-GRSP	Top soil, 0–40 cm	SWC	0.096	0.983	<0.001	0.966
		Deep soil, 40–100 cm	CP	0.025	0.983	<0.001	0.965

The regulation of soil GRSP content by soil physicochemical factors was different among different tree species. Except for EU, the soil GRSP content of AM, PC, and MG was regulated by TN, and TN had the greatest influence on the T-GRSP content of the AM surface layer (β = 0.993). The soil GRSP content of both the PC and EU stands was affected by TP, and the T-GRSP content of the EU surface soil (β = 0.993) was more affected by TP. SWC also affected the surface soil GRSP content of PC and MG, and had a higher effect on the surface soil T-GRSP content of MG (β = 0.983).

The regulation of SOC and CP on the four tree species in deep soil was also different. Among them, CP and SOC alone affected the GRSP content in deep soil of AM and PC, respectively, and had the opposite effect in the stepwise regression models of EE-GRSP and T-GRSP in deep soil of EU and MG. In EU, CP had the highest impact on the EE-GRSP content (β = 0.989), and SOC had the highest impact on T-GRSP (β = 0.983). In MG, on the contrary, the higher the soil CP, the higher the T-GRSP content, and the higher the soil SOC, the higher the EE-GRSP content (Table 2).

4. Discussion

4.1. Effects of Tree Species on the Accumulation of GRSP in Soil

In the subtropical plantation ecosystem of this study, the concentration of EE-GRSP ranged from 0.27 to 1.51 mg·g^{-1}, while the concentration of T-GRSP ranged from 0.55 to 4.08 mg·g^{-1}, which was close to the average level of EE-GRSP in other subtropical regions of China, but the level of GRSP in tropical and temperate zones is higher than that in this study [4,19,27]. Previous studies have shown that plant community composition could affect the content of GRSP in soil, and the dominant species of plant communities have a significant effect on GRSP [28,29]. In this study, the tree species significantly affected the GRSP content in southern China (Table 1). Specifically, the EE-GRSP of the coniferous species PC was significantly higher than that of the broad-leaved species MG, and the T-GRSP content of the coniferous species PC was significantly higher than that of the broad-leaved species AM (Table S2), which is consistent with the results of previous studies [23]. Our study found that the surface layer of the coniferous forest had a higher level of GRSP than that of the broad-leaved forest, which was supported by the results from Lovelock et al. [4]. This may be due to the higher litter biomass of coniferous tree species and the larger input of SOC to the surface layer [27], and that was related to the characteristics of coniferous tree species. In addition, a study also found that the initial lignin content of litter in coniferous forest was higher, which was not conducive to the growth and reproduction of microbial communities [30]. The litter decomposition rate of coniferous trees is lower than that of broad-leaved trees [31]. Therefore, the higher GRSP level in the surface layer of coniferous forests compared with broad-leaved forests is also related to the differences in leaf traits between the coniferous and broad-leaved trees in this study.

In this study, compared with other tree species the soil C and N nutrient contents in the AM plantation (N-fixing tree species) were at a high level, while the T-GRSP content of AM was at a low level among the four plantations. Previous studies have shown that the effects of different abiotic factors, host tree species, forest management methods, and human activities on forests lead to differences in AMF community composition and different GRSP decomposition rates in soil [28,32,33]. Singh et al. [32] showed that the root infection rate of AMF was significantly different among various tree species, and trees could directly change the concentration of GRSP in soil by affecting the release of GRRP in their roots. In leguminous N-fixing tree species, AMF could form symbionts with N-fixing bacteria, and their interaction might further promote the N fixation efficiency of the ecosystem, increase the content of SOC and TN in the soil, and thus, facilitate the soil C and N cycles [34,35]. When the nutrients in the soil increased and the host plants were less restricted, the AMF infection rate decreased, and the host plants would supply less C to AMF [36,37]. In this case, AMF may reduce the secretion of GRSP. In this study, the EE-GRSP-N/TN and T-GRSP-N/TN of the N-fixing tree species AM were significantly lower than those of the non-N fixing tree species (Table S2), which was related to the promoting effect of N-fixing tree species on soil N accumulation. Jia et al. [38] studied the responses of soil GRSP content to N addition in a 3-year-old Chinese fir plantation and found that N addition inhibited the content of GRSP. These results also explained the reason why the soil GRSP level of the N-fixing tree species AM in this study was lower than that of the non-N-fixing tree species. Due to the N fixation of the leguminous tree species AM, the N supply of soil was increased, so the infection rate of AMF in the root system of AM was reduced, resulting in a decrease in GRSP secreted by AM, which finally manifested as a lower GRSP content and lower contribution rate of GRSP to TN in soil.

4.2. Variation in Concentration and Contribution to Carbon and Nitrogen of GRSP in Deep Soil

In this study, the contribution rates of EE-GRSP and T-GRSP to SOC were 1.43%–2.15% and 3.34%–7.61%, respectively, and the contribution rates to TN were 1.03%–2.06% and 2.55%–6.91%, respectively. We also found that the GRSP decreased linearly from surface soil to deep soil, and the accumulation of GRSP in deep soil was much lower than that in surface soil, but its contribution rate to SOC and TN was 1.08–1.18 times that in surface soil

(Table S1), suggesting that GRSP is more important than surface soil nutrient fixation in deep soil C and N fixation, which is similar to other studies [16,17,39]. Previous studies have shown that in the 0–30 cm soil profile of different types of secondary forests, GRSP decreases with increasing soil depth, showing an obvious vertical distribution pattern, but its contribution to soil SOC increases [40]. The soil GRSP of poplar shelterbelt in northeast China decreased linearly in the 1 m vertical profile, but its contribution to the soil C and N pools increased with the increase in soil depth. The contribution rate of surface soil GRSP to soil nutrients was only 60%–67% of that in deep soil [39]. Another study also showed that GRSP gradually decreased with the increase in soil depth, but its contribution to the soil SOC pool was 1.2 times higher in deep soil than that in surface soil [17]. Therefore, deep soil is an important part of terrestrial ecosystems, and SOC has an average residence time of up to thousands of years in deep soil [41].

Additionally, Zhang et al. [7] studied the relationship between the chemical structure of GRSP and SOC in tropical forests and revealed that GRSP contained a high proportion of aromatic-C and a considerable proportion of alkyl-C. Compared with the high proportion of easily degradable O-alkyl-C in SOC, recalcitrant alkyl-C and aromatic-C were beneficial to maintaining the long-term stability of soil GRSP. It has been proved that SOC is the soil parameter with the strongest effect in controlling GRSP content [27]. In this study, SOC played an important role in regulating GRSP in surface and deep soil among different tree species (Table 2), as verified by the results of this study. Among different tree species, GRSP, SOC, and TN decreased with increasing soil depth, but the contribution rate of GRSP to both increased with increasing soil depth. A previous study indicated that the SOC in deep soil was enriched in microbial-derived compounds [41]. The enrichment of SOC in GRSP in deep soil may be the result of microbial activities including fungi, and microbial activities increase the production of GRSP in deep soil [27]. Studies have shown that soil dissolved organic carbon is more prone to mineralization and is lost in the form of CO_2 [41–43]. There is a significant positive correlation between C mineralization and net N mineralization in surface soil. The lack of active C and degradable organic N in deep soil limits soil net N mineralization [44], while GRSP is more stable in soil [6,7], which leads to a greater decrease in SOC and TN than GRSP, which in turn leads to the vertical distribution of GRSP in soil and its contribution to soil SOC and TN.

4.3. Effects of Soil Properties on the Vertical Distribution of GRSP

Many studies have shown that soil GRSP content is affected by many factors, including climate, soil physicochemical properties, and microbial community characteristics [19,39,45]. In this study, GRSP was positively correlated with soil SWC, SOC, and TN, and negatively correlated with soil BD, partly consistent with previous studies [46–48]. We also found that the effect of soil physicochemical properties on GRSP content could reach 1 m depth. Additionally, there was a negative correlation between BD and soil GRSP, and the negative correlation was particularly significant in the N-fixing tree species AM and the coniferous tree species PC. This finding is consistent with other research results [49,50], indicating that BD is an antagonistic factor affecting soil GRSP content. Previous studies have shown that high soil compaction inhibits plant growth to a certain extent, and increases in GRSP content in soil are conducive to soil structure improvement [39,48]. Therefore, with the increase in soil depth, soil BD increased linearly, while EE-GRSP and T-GRSP decreased linearly.

Stepwise regression analysis can objectively reflect the correlation between GRSP content and soil physicochemical properties in surface and deep soil [16]. In this study, SOC, TN, and TP were important nutrient factors regulating GRSP content, indicating that GRSP played an important role in the enrichment of soil main nutrients (C, N, etc.) [1,29], which is consistent with the results of most studies, which have also shown the contribution of GRSP to maintaining the soil C and N pools [51,52].

In addition to nutrient factors, SWC and CP were important soil factors affecting the GRSP content. Among them, SWC significantly affected the accumulation of GRSP in surface soil, while the accumulation of GRSP in deep soil was mainly determined by CP. The content of GRSP increased with an increase in the SWC, suggesting that the SWC can significantly affect GRSP [53,54]. It is believed that GRSP is a product of AMF secretion, and the content of GRSP is closely related to the abundance of AMF. It was found that AMF mediated the metabolism of GRSP [55]. Therefore, SWC was an important factor related to AMF metabolism, and maintaining a high SWC content in the surface layer was conducive to increasing the soil GRSP content. However, soil CP was an indicator related to soil water retention capacity and soil aeration. Previous studies have shown that ventilation and water conditions in deep soil inhibited the accumulation of GRSP in deep soil by affecting the growth and reproduction of AM fungi [56]. Therefore, increasing soil CP, soil water retention, and permeability and aeration are conducive to plant growth and microbial activity, thereby promoting the accumulation of soil GRSP and improving soil structure in subtropical plantations.

5. Conclusions

In this study, the vertical distribution of GRSP in four typical plantations in southern China showed significant tree species differences. With an increase in soil depth, the GRSP contents decreased linearly, and the contribution rate of GRSP to SOC and TN in deep soil was 1.08–1.18 times that in surface soil. Additionally, soil GRSP, SOC, and TN decreased linearly with the increase in soil depth, indicating that soil GRSP was an important part of soil C and N, and will play an important role in the C and N cycles of plantation ecosystems. However, the content of T-GRSP was ranked in the order EU > PC > MG > AM, while the contribution rate of soil GRSP to soil SOC was ranked as EU > PC > MG > AM. Also, there was a significant positive correlation between GRSP and various soil physicochemical properties. Specifically, the SOC, CP, TN, SWC, and TP were important factors regulating GRSP content in soil. The regulation effect of soil properties on GRSP varied in surface and deep soil among different tree species. Therefore, our results recommend that plantations with coniferous tree species play a greater role in soil structure improvement than broad-leaved tree species. Although plantations with N-fixing species play an important role in soil nutrient enhancement, they are at a disadvantage in GRSP accumulation. Thus, larger scales and further study are needed to investigate the effects and underlying mechanisms of plantation GRSP accumulation and C sequestration in subtropical China.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15081479/s1, Table S1: Variation of soil GRSP at different depths; Table S2: Variation of soil GRSP in four plantations; Table S3: Two-way ANOVA of soil physicochemical properties; Table S4: Variation of soil physicochemical properties among four plantations; Table S5: Variation of soil physicochemical properties in different soil depths.

Author Contributions: Conceptualization, M.W. and Q.M.; methodology, M.W., S.Z. and X.G.; software, M.W.; validation, Y.L. and Z.H.; formal analysis, M.W., Y.L., Z.H. and X.G.; investigation, M.W. and Z.H.; resources, Z.H.; data curation, Z.H.; writing—original draft preparation, M.W. and Q.M.; writing—review and editing, Q.M.; visualization, Y.L. and Z.H.; supervision, Q.M.; project administration, Q.M.; funding acquisition, Q.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Innovation Foundation of Guangdong Forestry (2022KJCX017).

Data Availability Statement: All data relevant to the study are included in the article.

Acknowledgments: We sincerely thank the staff of the State-owned Huatan Forest Farm in Yangjiang City, Guangdong Province for their assistance in field sampling. We also appreciate the anonymous reviewers for their helpful and detailed comments and advices for improving the manuscript.

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

References

1. Wright, S.F.; Upadhyaya, A. Extraction of an Abundant and Unusual Protein from Soil and Comparison with Hyphal Protein of Arbuscular Mycorrhizal Fungi. *Soil Sci.* **1996**, *161*, 575. [CrossRef]
2. Wright, S.F.; Upadhyaya, A. A Survey of Soils for Aggregate Stability and Glomalin, a Glycoprotein Produced by Hyphae of Arbuscular Mycorrhizal Fungi. *Plant Soil* **1998**, *198*, 97–107. [CrossRef]
3. Rillig, M.C. Arbuscular Mycorrhizae, Glomalin, and Soil Aggregation. *Can. J. Soil Sci.* **2004**, *84*, 355–363. [CrossRef]
4. Lovelock, C.E.; Wright, S.F.; Clark, D.A.; Ruess, R.W. Soil Stocks of Glomalin Produced by Arbuscular Mycorrhizal Fungi across a Tropical Rain Forest Landscape. *J. Ecol.* **2004**, *92*, 278–287. [CrossRef]
5. Rillig, M.C.; Wright, S.F.; Nichols, K.A.; Schmidt, W.F.; Torn, M.S. Large Contribution of Arbuscular Mycorrhizal Fungi to Soil Carbon Pools in Tropical Forest Soils. *Plant Soil* **2001**, *233*, 167–177. [CrossRef]
6. Barbosa, M.V.; Pedroso, D.D.F.; Curi, N.; Carneiro, M.A.C. Do Different Arbuscular Mycorrhizal Fungi Affect the Formation and Stability of Soil Aggregates? *Ciênc. Agrotec.* **2019**, *43*, e003519. [CrossRef]
7. Zhang, J.; Tang, X.; Zhong, S.; Yin, G.; Gao, Y.; He, X. Recalcitrant Carbon Components in Glomalin-Related Soil Protein Facilitate Soil Organic Carbon Preservation in Tropical Forests. *Sci. Rep.* **2017**, *7*, 2391. [CrossRef]
8. Yang, Y.; He, C.; Huang, L.; Ban, Y.; Tang, M. The Effects of Arbuscular Mycorrhizal Fungi on Glomalin-Related Soil Protein Distribution, Aggregate Stability and Their Relationships with Soil Properties at Different Soil Depths in Lead-Zinc Contaminated Area. *PLoS ONE* **2017**, *12*, e0182264. [CrossRef]
9. Singh, A.K.; Rai, A.; Singh, N. Effect of Long Term Land Use Systems on Fractions of Glomalin and Soil Organic Carbon in the Indo-Gangetic Plain. *Geoderma* **2016**, *277*, 41–50. [CrossRef]
10. Gao, W.-Q.; Wang, P.; Wu, Q.-S. Functions and Application of Glomalin-Related Soil Proteins: A Review. *JSM* **2019**, *48*, 111–119. [CrossRef]
11. Wu, F.; Dong, M.; Liu, Y.; Ma, X.; An, L.; Young, J.P.W.; Feng, H. Effects of Long-Term Fertilization on AM Fungal Community Structure and Glomalin-Related Soil Protein in the Loess Plateau of China. *Plant Soil* **2011**, *342*, 233–247. [CrossRef]
12. Wang, X.; Cao, Q.; Yang, W.; Zhu, X. Spatial Changes in Glomalin-Related Soil Protein and Their Correlation with Soil Properties in the Black Soil Region of Northeast China. *Agronomy* **2022**, *12*, 2165. [CrossRef]
13. Staunton, S.; Saby, N.P.A.; Arrouays, D.; Quiquampoix, H. Can Soil Properties and Land Use Explain Glomalin-Related Soil Protein (GRSP) Accumulation? A Nationwide Survey in France. *Catena* **2020**, *193*, 104620. [CrossRef]
14. Li, T.; Yuan, Y.; Mou, Z.; Li, Y.; Kuang, L.; Zhang, J.; Wu, W.; Wang, F.; Wang, J.; Lambers, H.; et al. Faster Accumulation and Greater Contribution of Glomalin to the Soil Organic Carbon Pool than Amino Sugars Do under Tropical Coastal Forest Restoration. *Glob. Chang. Biol.* **2023**, *29*, 533–546. [CrossRef] [PubMed]
15. Preger, A.C.; Rillig, M.C.; Johns, A.R.; Du Preez, C.C.; Lobe, I.; Amelung, W. Losses of Glomalin-Related Soil Protein under Prolonged Arable Cropping: A Chronosequence Study in Sandy Soils of the South African Highveld. *Soil Biol. Biochem.* **2007**, *39*, 445–453. [CrossRef]
16. Wang, W.; Zhong, Z.; Wang, Q.; Wang, H.; Fu, Y.; He, X. Glomalin Contributed More to Carbon, Nutrients in Deeper Soils, and Differently Associated with Climates and Soil Properties in Vertical Profiles. *Sci. Rep.* **2017**, *7*, 13003. [CrossRef]
17. Wang, Q.; Wang, W.; Zhong, Z.; Wang, H.; Fu, Y. Variation in Glomalin in Soil Profiles and Its Association with Climatic Conditions, Shelterbelt Characteristics, and Soil Properties in Poplar Shelterbelts of Northeast China. *J. For. Res.* **2020**, *31*, 279–290. [CrossRef]
18. *National Forestry and Grassland Administration China Forest Resources Report (2014–2018)*; Chinese Forestry Publishing House: Beijing, China, 2019.
19. Gu, R.; Xiao, K.; Zhu, Z.; He, X.; Li, D. Afforestation Enhances Glomalin-Related Soil Protein Content but Decreases Its Contribution to Soil Organic Carbon in a Subtropical Karst Area. *J. Environ. Manag.* **2024**, *356*, 120754. [CrossRef]
20. Liao, C.; Luo, Y.; Fang, C.; Chen, J.; Li, B. The Effects of Plantation Practice on Soil Properties Based on the Comparison between Natural and Planted Forests: A Meta-Analysis. *Glob. Ecol. Biogeogr.* **2012**, *21*, 318–327. [CrossRef]
21. Zhang, X.; Ji, Q.; Cheng, G.; Zhu, M.; Zhang, Z.; Jing, L.; Wang, L.; Li, Q.; Tao, Q.; Zhang, X.; et al. Tree Growth and Density Enhanced, While Diversity and Spatial Clustering Reduced Soil Mycorrhizal C and N Sequestration: Strong Interaction with Soil Properties in Northeastern China. *Sci. Total Environ.* **2024**, *912*, 169131. [CrossRef]
22. Singh, A.K.; Zhu, X.; Chen, C.; Wu, J.; Yang, B.; Zakari, S.; Jiang, X.J.; Singh, N.; Liu, W. The Role of Glomalin in Mitigation of Multiple Soil Degradation Problems. *Crit. Rev. Environ. Sci. Technol.* **2022**, *52*, 1604–1638. [CrossRef]
23. Rotter, P.; Malý, S.; Sáňka, O.; Sáňka, M.; Čižmár, D.; Zbíral, J.; Čechmánková, J.; Kalábová, T. Is Glomalin an Appropriate Indicator of Forest Soil Reactive Nitrogen Status? *J. Plant Nutr. Soil Sci.* **2017**, *180*, 694–704. [CrossRef]
24. Singh, A.K.; Rai, A.; Banyal, R.; Chauhan, P.S.; Singh, N. Plant Community Regulates Soil Multifunctionality in a Tropical Dry Forest. *Ecol. Indic.* **2018**, *95*, 953–963. [CrossRef]
25. *LY/T 1215–1999*; Forestry Industry Standards of China. Determination of Forest Soil Water-Related Physical Properties. Chinese Academy of Forestry Sciences: Beijing, China, 1999.
26. Bao, S. *Soil and Agricultural Chemistry Analysis*; China Agricultural Publishing House: Beijing, China, 2000.
27. Cissé, G.; Essi, M.; Kedi, B.; Nicolas, M.; Staunton, S. Accumulation and Vertical Distribution of Glomalin-Related Soil Protein in French Temperate Forest Soils as a Function of Tree Type, Climate and Soil Properties. *Catena* **2023**, *220*, 106635. [CrossRef]
28. Treseder, K.K.; Turner, K.M. Glomalin in Ecosystems. *Soil Sci. Soc. Am. J.* **2007**, *71*, 1257–1266. [CrossRef]

29. Rillig, M.C.; Wright, S.F.; Eviner, V.T. The Role of Arbuscular Mycorrhizal Fungi and Glomalin in Soil Aggregation: Comparing Effects of Five Plant Species. *Plant Soil* **2002**, *238*, 325–333. [CrossRef]
30. Ashagrie, Y.; Zech, W.; Guggenberger, G. Transformation of a Podocarpus Falcatus Dominated Natural Forest into a Monoculture Eucalyptus Globulus Plantation at Munesa, Ethiopia: Soil Organic C, N and S Dynamics in Primary Particle and Aggregate-Size Fractions. *Agric. Ecosyst. Environ.* **2005**, *106*, 89–98. [CrossRef]
31. Prescott, C.E.; Zabek, L.M.; Staley, C.L.; Kabzems, R. Decomposition of Broadleaf and Needle Litter in Forests of British Columbia: Influences of Litter Type, Forest Type, and Litter Mixtures. *Can. J. For. Res.* **2011**, *30*, 1742–1750. [CrossRef]
32. Singh, A.K.; Jiang, X.J.; Yang, B.; Li, H.; Liu, W.; Singh, N. Effect of Root-Glomalin on Soil Carbon Storage in Trees' Rhizosphere and Interspace of a Tropical Dry Forest. *Land Degrad. Dev.* **2021**, *32*, 5281–5291. [CrossRef]
33. Zhang, J.; Tang, X.; He, X.; Liu, J. Glomalin-Related Soil Protein Responses to Elevated CO2 and Nitrogen Addition in a Subtropical Forest: Potential Consequences for Soil Carbon Accumulation. *Soil Biol. Biochem.* **2015**, *83*, 142–149. [CrossRef]
34. Wang, F.; Li, Z.; Xia, H.; Zou, B.; Li, N.; Liu, J.; Zhu, W. Effects of Nitrogen-Fixing and Non-Nitrogen-Fixing Tree Species on Soil Properties and Nitrogen Transformation during Forest Restoration in Southern China. *Soil Sci. Plant Nutr.* **2010**, *56*, 297–306. [CrossRef]
35. Yu, H.; Liu, X.; Yang, C.; Peng, Y.; Yu, X.; Gu, H.; Zheng, X.; Wang, C.; Xiao, F.; Shu, L.; et al. Co-Symbiosis of Arbuscular Mycorrhizal Fungi (AMF) and Diazotrophs Promote Biological Nitrogen Fixation in Mangrove Ecosystems. *Soil Biol. Biochem.* **2021**, *161*, 108382. [CrossRef]
36. Frater, P.N.; Borer, E.T.; Fay, P.A.; Jin, V.; Knaeble, B.; Seabloom, E.; Sullivan, L.; Wedin, D.A.; Harpole, W.S. Nutrients and Environment Influence Arbuscular Mycorrhizal Colonization Both Independently and Interactively in *Schizachyrium scoparium*. *Plant Soil* **2018**, *425*, 493–506. [CrossRef]
37. Zhang, Y.; Wang, Q.; Xu, L.; Ma, S.; Cui, D.; Zhu, K.; Feng, W. Mixed Conifer-Broadleaf Trees on Arbuscular Mycorrhizal and Ectomycorrhizal Communities in Rhizosphere Soil of Different Plantation Stands in the Temperate Zone, Northeast China. *Front. Microbiol.* **2022**, *13*, 986515. [CrossRef] [PubMed]
38. Jia, S.; Liu, X.; Lin, W.; Zheng, Y.; Li, J.; Hui, D.; Guo, J. Decreased Glomalin-Related Soil Protein with Nitrogen Deposition in a 3-Year-Old Cunninghamia Lanceolata Plantation. *J. Soils Sediments* **2022**, *22*, 931–941. [CrossRef]
39. Cheng, G.; Zhu, M.; Zhang, X.; Guo, Y.; Yang, Y.; Yun, C.; Wu, Y.; Wang, Q.; Wang, W.; Wang, H. Northeastern China Shelterbelt-Farmland Glomalin Differences Depend on Geo-Climates, Soil Depth, and Microbial Interaction: Carbon Sequestration, Nutrient Retention and Implication. *Appl. Soil Ecol.* **2023**, *191*, 105068. [CrossRef]
40. Sun, X.; Xing, Y.; Yan, G.; Liu, G.; Wang, X.; Wang, Q. Dynamics of Glomalin-Related Soil Protein and Soil Aggregates during Secondary Succession in the Temperate Forest. *Catena* **2024**, *234*, 107602. [CrossRef]
41. Rumpel, C.; Kögel-Knabner, I. Deep Soil Organic Matter—A Key but Poorly Understood Component of Terrestrial C Cycle. *Plant Soil* **2011**, *338*, 143–158. [CrossRef]
42. Lorenz, K.; Lal, R. The Depth Distribution of Soil Organic Carbon in Relation to Land Use and Management and the Potential of Carbon Sequestration in Subsoil Horizons. In *Advances in Agronomy*; Academic Press: Cambridge, MA, USA, 2005; Volume 88, pp. 35–66.
43. Ahrens, B.; Guggenberger, G.; Rethemeyer, J.; John, S.; Marschner, B.; Heinze, S.; Angst, G.; Mueller, C.W.; Kögel-Knabner, I.; Leuschner, C.; et al. Combination of Energy Limitation and Sorption Capacity Explains 14C Depth Gradients. *Soil Biol. Biochem.* **2020**, *148*, 107912. [CrossRef]
44. Tian, Q.; Wang, X.; Wang, D.; Wang, M.; Liao, C.; Yang, X.; Liu, F. Decoupled Linkage between Soil Carbon and Nitrogen Mineralization among Soil Depths in a Subtropical Mixed Forest. *Soil Biol. Biochem.* **2017**, *109*, 135–144. [CrossRef]
45. Wang, Q.; Wang, W.; He, X.; Zhang, W.; Song, K.; Han, S. Role and Variation of the Amount and Composition of Glomalin in Soil Properties in Farmland and Adjacent Plantations with Reference to a Primary Forest in North-Eastern China. *PLoS ONE* **2015**, *10*, e0139623. [CrossRef]
46. Gałązka, A.; Niedźwiecki, J.; Grządziel, J.; Gawryjołek, K. Evaluation of Changes in Glomalin-Related Soil Proteins (GRSP) Content, Microbial Diversity and Physical Properties Depending on the Type of Soil as the Important Biotic Determinants of Soil Quality. *Agronomy* **2020**, *10*, 1279. [CrossRef]
47. Zhong, Z.; Wang, W.; Wang, Q.; Wu, Y.; Wang, H.; Pei, Z. Glomalin Amount and Compositional Variation, and Their Associations with Soil Properties in Farmland, Northeastern China. *J. Plant Nutr. Soil Sci.* **2017**, *180*, 563–575. [CrossRef]
48. Chojnacka, A.; Jonczak, J.; Oktaba, L.; Pawłowicz, E.; Regulska, E.; Słowińska, S.; Olejniczak, I.; Oktaba, J.; Kruczkowska, B.; Jankiewicz, U. Dynamics of Fungal Community Structure in a Silver Birch (Betula Pendula Roth) Succession Chronosequence on Poor-Quality Post-Arable Soil. *Agric. Ecosyst. Environ.* **2023**, *342*, 108225. [CrossRef]
49. Zhang, Z.; Wang, Q.; Wang, H.; Nie, S.; Liang, Z. Effects of Soil Salinity on the Content, Composition, and Ion Binding Capacity of Glomalin-Related Soil Protein (GRSP). *Sci. Total Environ.* **2017**, *581–582*, 657–665. [CrossRef]
50. Wang, Q.; Wu, Y.; Wang, W.; Zhong, Z.; Pei, Z.; Ren, J.; Wang, H.; Zu, Y. Spatial Variations in Concentration, Compositions of Glomalin Related Soil Protein in Poplar Plantations in Northeastern China, and Possible Relations with Soil Physicochemical Properties. *Sci. World J.* **2014**, *2014*, 160403. [CrossRef]
51. Nautiyal, P.; Rajput, R.; Pandey, D.; Arunachalam, K.; Arunachalam, A. Role of Glomalin in Soil Carbon Storage and Its Variation across Land Uses in Temperate Himalayan Regime. *Biocatal. Agric. Biotechnol.* **2019**, *21*, 101311. [CrossRef]

52. Wang, Y.; Ji, C.; Shi, Z.; Yin, X.; Liu, C. Spatial Distribution of Glomalin-Related Soil Proteins in Coniferous and Broadleaf Mixed Temperate Forest. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2019**, *47*, 1087–1093. [CrossRef]
53. Ji, L.; Tan, W.; Chen, X. Arbuscular Mycorrhizal Mycelial Networks and Glomalin-Related Soil Protein Increase Soil Aggregation in Calcaric Regosol under Well-Watered and Drought Stress Conditions. *Soil Tillage Res.* **2019**, *185*, 1–8. [CrossRef]
54. Liu, G.; Duan, X.; Yan, G.; Sun, X.; Jiang, S.; Xing, Y.; Wang, Q. Changes in Soil Aggregates and Glomalin-Related Soil Protein Stability During the Successional Process of Boreal Forests. *J. Soil Sci. Plant Nutr.* **2024**, *24*, 1335–1348. [CrossRef]
55. Wu, Q.-S.; Cao, M.-Q.; Zou, Y.-N.; He, X. Direct and Indirect Effects of Glomalin, Mycorrhizal Hyphae and Roots on Aggregate Stability in Rhizosphere of Trifoliate Orange. *Sci. Rep.* **2014**, *4*, 5823. [CrossRef] [PubMed]
56. Voets, L.; Dupré de Boulois, H.; Renard, L.; Strullu, D.-G.; Declerck, S. Development of an Autotrophic Culture System for the in Vitro Mycorrhization of Potato Plantlets. *FEMS Microbiol. Lett.* **2005**, *248*, 111–118. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.