






RESEARCH ARTICLE

Faster accumulation and greater contribution of glomalin to the soil organic carbon pool than amino sugars do under tropical coastal forest restoration

Tengteng Li^{1,2,3,4} | Ye Yuan^{1,2,5} | Zhijian Mou^{1,2,5} | Yue Li^{1,2,3,4} | Luhui Kuang^{1,2,3,4} |
 Jing Zhang^{1,2,3,4}  | Wenjia Wu^{1,2,3,4} | Faming Wang^{1,2,3,4}  | Jun Wang^{1,2,4} |
 Hans Lambers⁶ | Jordi Sardans^{7,8}  | Josep Peñuelas^{7,8}  | Hai Ren^{1,2,4} |
 Zhanfeng Liu^{1,2,3,4} 

¹Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems & CAS Engineering Laboratory for Vegetation Ecosystem Restoration on Islands and Coastal Zones, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

²South China National Botanical Garden, Guangzhou, China

³Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou, China

⁴Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Guangzhou, China

⁵College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China

⁶School of Biological Sciences, University of Western Australia, Perth, Western Australia, Australia

⁷CSIC, Global Ecology Unit CREAM-CSIC-UAB, Catalonia, Spain

⁸CREAF, Catalonia, Spain

Correspondence

Zhanfeng Liu, Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems & CAS Engineering Laboratory for Vegetation Ecosystem Restoration on Islands and Coastal Zones, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.
 Email: liuzf@scbg.ac.cn

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Abstract

Microbial metabolic products play a vital role in maintaining ecosystem multifunctionality, such as soil physical structure and soil organic carbon (SOC) preservation. Afforestation is an effective strategy to restore degraded land. Glomalin-related soil proteins (GRSP) and amino sugars are regarded as stable microbial-derived C, and their distribution within soil aggregates affects soil structure stability and SOC sequestration. However, the information about how afforestation affects the microbial contribution to SOC pools within aggregates is poorly understood. We assessed the accumulation and contribution of GRSP and amino sugars within soil aggregates along a restoration chronosequence (Bare land, *Eucalyptus exserta* plantation, native species mixed forest, and native forest) in tropical coastal terraces. Amino sugars and GRSP concentrations increased, whereas their contributions to the SOC pool decreased along the restoration chronosequence. Although microaggregates harbored greater microbial abundances, amino sugars and GRSP concentrations were not significantly affected by aggregate sizes. Interestingly, the contributions of amino sugars and GRSP to SOC pools decreased with decreasing aggregate size which might be associated with increased accumulation of plant-derived C. However, the relative change rate of GRSP was consistently greater in all restoration chronosequences than that of amino sugars. The accumulation of GRSP and amino sugars in SOC pools was closely associated with the dynamics of soil fertility and the microbial community. Our findings

Tengteng Li and Ye Yuan contributed equally to this work.

suggest that GRSP accumulates faster and contributes more to SOC pools during restoration than amino sugars did which was greatly affected by aggregate sizes. Afforestation substantially enhanced soil quality with native forest comprising species sequestering more SOC than the monoculture plantation did. Such information is invaluable for improving our mechanistic understanding of microbial control over SOC preservation during degraded ecosystem restoration. Our findings also show that plantations using arbuscular mycorrhizal plants can be an effective practice to sequester more soil carbon during restoration.

KEYWORDS

afforestation, amino sugars, glomalin-related soil protein, soil aggregates, soil microbial community, tropical coastal terrace

1 | INTRODUCTION

Forests play an important role in maintaining coastal ecosystem functioning such as biodiversity, typhoon sheltering, and carbon (C) sequestration (Snäll et al., 2021). However, about 25%–50% of coastal vegetation habitats (such as mangroves, seagrasses, salt marshes, and kelp forests) have become degraded worldwide in the past 50 years due to land-use and climate change, which is threatening their health and causing considerable soil C loss (Duarte et al., 2013). Of the natural coastal habitats in China, more than 50% have been lost in the past 60 years (Ma et al., 2014), specifically as a result of severe forest degradation in south China (Ren et al., 2007). Forest restoration is an effective way to mitigate the degradation and improve ecosystem services (Canadell & Raupach, 2008). Soil C cycling is central to the re-establishment of belowground ecosystem structure and functioning during restoration processes; however, most previous studies focused on the aboveground biodiversity and its functioning, and less attention has been paid to the microbial role in mediating soil C preservation during forest restoration (Capellesso et al., 2021; Crouzeilles et al., 2016; Hisano et al., 2018).

As the largest terrestrial C pool, soils contain more C than the vegetation and atmosphere combined and play a vital role in the terrestrial C cycle and climate change mitigation (Lal et al., 2021). The formation and stabilization of soil organic carbon (SOC) is a complex process of microbial transformation of plant residues via *ex vivo* (mainly exoenzymatic decomposition, catabolism) modification and *in vivo* (anabolism) turnover pathways (Liang, 2020). The process is affected by various biotic and abiotic factors (Jackson et al., 2017). For example, plant diversity could increase soil microbial activity and soil C storage (Lange et al., 2015); yet, the stability of SOC is affected by tree species and characteristics through the composition of their aboveground organs and roots (Angst et al., 2019). The physical protection by soil aggregates and the formation of organo-mineral associations are thought to stabilize SOC pools (Liang et al., 2020; Schmidt et al., 2011; Wang et al., 2017). Due to the variation of physicochemical conditions among aggregate fraction sizes (Rillig et al., 2017), greater microbial diversity and potential functions are

associated with microaggregates than with macroaggregates (Bach et al., 2018; Navas et al., 2021; Upton et al., 2019) which may result in the accumulation of different microbial-derived C within them (Murugan et al., 2019). Specifically, microbial metabolic products such as amino sugars and glomalin-related soil proteins (GRSP) have been widely investigated for their effects on SOC preservation (Irving et al., 2021; Joergensen, 2018) due to their relatively greater recalcitrance and benefit for soil aggregation (Agnihotri et al., 2022; Buckeridge et al., 2020). However, the information related to their accumulation and contribution to SOC during vegetation restoration is not well understood.

Soil microbial community is a core driver of SOC transformation and is sensitive to land degradation and climate change (Xiao et al., 2016). Phospholipid fatty acids (PLFAs) are widely used as biomarkers to indicate the “fingerprint” profile of the living soil microbial community (Vestal & White, 1989; Zelles, 1999). By contrast, amino sugars are primarily derived from the cell walls of dead microbes and are commonly used as biomarkers to quantify the contribution of microbial necromass to SOC accumulation (Liang et al., 2019). Glucosamine is present in both fungal and bacterial cell walls, whereas muramic acid exclusively occurs in the cell walls of bacteria (Joergensen, 2018). As microbial metabolic products, amino sugars reflect a time-integrated microbial community (Glaser et al., 2004). The ratio of total amino sugars to total PLFAs provides some information on microbial necromass C-transformation efficiency (Xu et al., 2022). Furthermore, there is no consensus on the contribution of necromass to the SOC pool during vegetation restoration (Guo et al., 2021). A recent study showed that the concentrations of PLFAs and amino sugars respond differently to forest restoration in a subtropical region with divergent contributions of fungi or bacteria to the SOC pool via physical protection pathways (Zhang et al., 2021). Especially microbial communities and biomasses might vary with aggregate sizes due to their difference in microhabitats (Gupta & Germida, 2015), hence influencing the accumulation of amino sugars in soil aggregates. Further exploration is needed to clarify the role of soil aggregates on the accumulation and contribution

of amnio sugars in the SOC pool during vegetation restoration (Murugan et al., 2019).

GRSP are microbial products produced by arbuscular mycorrhizal fungi (AMF) and characterized as hydrophobic sticky and recalcitrant glycoproteins (Wright et al., 1998). GRSP is composed of a broad range of elements (e.g., C/N/H/O/Fe/Al), functional groups (e.g., aromatic- and carboxyl-C), and composite substances such as proteins and carbohydrates (Agnihotri et al., 2022). Recently produced GRSP (EE-GRSP, easily extractable GRSP) are more labile in soil than total GRSP (T-GRSP; Wright & Upadhyaya, 1996). GRSP generally increases with AMF colonization and biomass (Agnihotri et al., 2021) during vegetation restoration (Qiao et al., 2019) and is affected by land-use change, nutrient availability, and tillage (Agnihotri et al., 2022). The turnover of glomalin is slower than that of AMF hyphae (Rillig et al., 2001). Zhang, Zhang, et al. (2017); Zhang, Tang, et al. (2017); Zhang et al. (2022) found that aromatic and alkyl-C in glomalin are more recalcitrant, with benefits for aggregate stability, jointly enhancing SOC persistence in tropical forests. Iron is an important element in the composition of GRSP, converting monomeric GRSP units into a multimeric complex, thus promoting GRSP stabilization. Other metal ions such as Al^{3+} , Ca^{2+} , and Mg^{2+} probably have similar effects on GRSP. GRSP may contribute to SOC content not only owing to their recalcitrant chemistry but also due to their stabilizing effect on soil aggregates (Rillig & Mummey, 2006). GRSP sorbed onto organic substances, clays, and silt particles, facilitates adsorption between and within microaggregates and AMF hyphae could bind particles or microaggregates thereby promoting the formation and stabilization of soil macroaggregates (Agnihotri et al., 2022). The distribution of GRSP in soil macroaggregates may influence aggregate stability (Xie et al., 2015). Although it is acknowledged that both GRSP and amino sugars play important roles in mediating SOC sequestration, the information about the dynamics of their relative contribution to the SOC pool during forest restoration is poorly understood. Simultaneous measurements of GRSP and amino sugars make it possible to compare their relative contribution to SOC accumulation and stability and provide valuable information for developing a restoration strategy in terms of carbon sequestration. To obtain a comprehensive understanding of microbial-driven SOC preservation during vegetation restoration, it is worth investigating how GRSP and amino sugars accumulate and contribute to SOC across soil aggregate fraction sizes during restoration.

Starting from the 1950s, forest restoration was conducted by planting pioneer plant species and mixtures of native plant species on bare land on tropical coastal terraces in south China (Ren et al., 2007). After 60 years of afforestation, native species mixtures have recovered and restored plant communities, soil biodiversity, and soil fertility (Wu et al., 2021). However, less attention was paid to how microbial metabolic products accumulate and contribute to the SOC pool, which is central to the re-establishment of soil structure and functioning during restoration. Such information is valuable for guiding restoration practices in the study area and mitigating climate change. In this study, we aimed to investigate the accumulation

dynamics of glomalin and amino sugars within aggregates and to evaluate their relative contribution to the SOC pool following forest restoration. We hypothesized that: (1) the accumulation of GRSP and amino sugars would increase with forest restoration due to enhanced plant C inputs benefiting AMF and other microorganisms, which leads to the accumulation of microbial metabolic products (Guo et al., 2021; Qiao et al., 2019); (2) the accumulation rate and contribution of GRSP to the SOC pool would be greater than those of amino sugars due to the differences in their chemical recalcitrance and propensity to mineral protection (Agnihotri et al., 2022; Rillig et al., 2001).

2 | MATERIALS AND METHODS

2.1 | Site description and experimental design

The present study was carried out at the Xiaoliang Tropical Coastal Ecosystem Research Station, Chinese Academy of Sciences, in Guangdong province of China (21°27'N; 110°54'E). The climate in this region is typically tropical monsoon, with a mean annual temperature of 23°C and mean annual precipitation of 1400–1700mm with wet (April–September) and dry (November–March) seasons. The soil is classified as a latosol that originated from granite (Yao et al., 1984). Evergreen broad-leaved seasonal rainforest was the climax vegetation in this region before the 1850s, while soils became severely degraded resulting from massive deforestation and soil erosion by the 1950s, and only a small part of native forest (NF) was protected for more than 200 years (Ren et al., 2007; Yu & Pi, 1985). A *Eucalyptus exerta* plantation (EP) was established on bare land in the early 1960s, it can associate with both arbuscular and ectomycorrhizal fungi. Forests using mixed native species (MF) were promoted from EP after clear-cutting in 1974 and are dominated by AMF plants including *Carallia brachiata*, *Aphanamixis polystachya*, *Schefflera octophylla*, *Carallia brachiata*, *Symplocos chunii*, *Acacia auriculiformis*, *Photinia benthamiana*, and *Cinnamomum burmanni*, *Lygodium japonicum*, *Ophiopogon japonicus*, and *Nephrolepis cordifolia*, and ectomycorrhizal plants including *Acacia auriculiformis* and *Calamus tetradactylus*. NF is dominated by AMF plants including *Sterculia lanceolata*, *Cinnamomum camphora*, *Cryptocarya chinensis*, *Syzygium levinei*, *Syzygium hancei*, *Schefflera octophylla*, *Aquilaria sinensis* (Wu et al., 2021). Bare land (BL) was used as a reference system in this study (Ren et al., 2007; Wang et al., 2017). Four treatments (BL, EP, MF, and NF) were a randomized block design in the study and the distance between plots was over 50m; five replicated plots (10 × 10 m) in each treatment (Figure 1). More detailed information on the study site and forest restoration is included in Yao et al. (1984) and Ren et al. (2007).

2.2 | Soil sampling and analyses

In May 2019, five soil cores (0–20 cm depth) were randomly sampled and then mixed into one composite sample in each plot. After

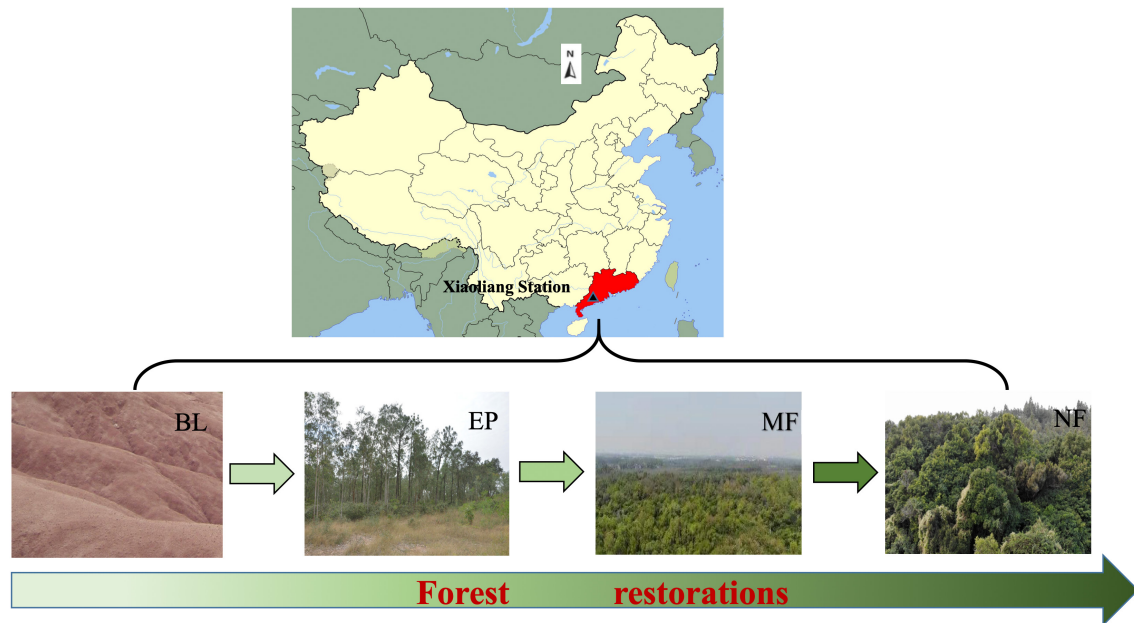


FIGURE 1 Degraded coastal land and forest restorations in the study area. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16467)]

removing litter, roots, and stones by sieving through a 4-mm sieve, fresh soils were fractionated into three aggregate-size classes: >2.0mm large macroaggregate, LMA; 0.25–2.0mm small macroaggregate, SMA; <0.25mm microaggregate, MA, by using a dry sieving method (Yuan et al., 2021). Then, every aggregate fraction was divided into two parts. One part was air-dried for determining soil physicochemical characteristics, and the other was stored at -20°C for analyzing microbial properties. The concentrations of SOC, total nitrogen (TN), and total phosphorus (TP) in each aggregate fraction were determined following the protocols described by Lu (2000).

2.3 | Soil microbial community composition

Microbial community composition in soil was measured using the PLFA method (Bossio & Scow, 1998). The extracted lipids from soil samples, after separation and transformation into free methyl esters, were analyzed using a gas chromatograph (7890B, Agilent Technologies) and identified by MIDI peak identification software (MIDI Inc.). Specific fatty acids were used to represent Gram-positive (GP) bacteria (i15:0, α 15:0, i16:0, i17:0, and α 17:0), Gram-negative (GN) bacteria (16:1 ω 9c, 16:1 ω 7c, 18:1 ω 7c, cy17:0, cy19:0), saprotrophic fungi (18:2 ω 6c), arbuscular mycorrhizal fungi (AMF; 16:1 ω 5c), and actinomycetes (10Me16:0, 10Me17:0, and 10Me18:0). The sum of GP and GN bacteria was represented as the total bacterial biomass. Total microbial biomass represented by total PLFAs concentration was calculated as well. The sum of PLFAs representing fungi was divided by the sum of PLFAs representing bacteria to get the ratio of fungal to bacterial PLFA (F:B ratio). The amount of all PLFAs was expressed as nmol g^{-1} dry soil.

2.4 | Glomalin-related soil proteins

Total extractable GRSP (T-GRSP) and easily extractable GRSP (EE-GRSP) in each aggregate fraction was determined according to Wright and Upadhyaya (1996). In brief, 1.0 g of air-dried soil sample was added to an autoclavable centrifuge tube, with a mixture of 8 ml of 20mM sodium citrate solution at pH 8.0, and vortexed for 30s. Then, the mixture was autoclaved for 30min at 121°C before being centrifuged at 4200 g for 5 min, and the supernatant was determined as EE-GRSP. The residue in the tube was mixed with 8 ml of 50mM sodium citrate at pH 8.0 and then autoclaved at 121°C for 90min, and centrifuged at 4200 g for 5 min. The supernatant was transferred to another tube. Extractions would be repeated till the supernatants had a pale straw color, indicating that GRSP was completely extracted. The concentrations of GRSP in the two extracts were summed as T-GRSP and determined at 595 nm by an enzyme microplate reader (Multiskan FC, Thermo Fisher Scientific) according to the Bradford (1976) method. Both T-GRSP and EE-GRSP were expressed as mg g^{-1} dry soil. The detailed measurements are included in supplementary materials and methods.

2.5 | Soil amino sugars

Amino sugars (ASs), including muramic acid (MurN), galactosamine (GalN), and glucosamine (GlcN), were measured as described in Indorf et al. (2011), with minor modification (Mou et al., 2020). In brief, ASs were hydrolyzed, extracted, and derivatized with ortho-phthalaldehyde, determined by high-performance liquid chromatography (Dionex Ultimate 3000, Thermo Fisher Scientific). The detailed relevant information and calculation are shown in supplementary materials and methods. The concentrations of amino sugars in soil were expressed as $\mu\text{g g}^{-1}$ dry soil.

2.6 | Statistical analyses

All data were compiled in Excel software and analyzed in *SPSS* 26.0, R 3.5.1, and Graphpad prism 8.0. Before analysis, normal distribution and homogeneity were tested for all data. One-way ANOVA with Duncan's test was utilized to identify significant differences in soil physicochemical properties, microbial community composition, GRSP, and amino sugars among forest restorations and aggregate fraction sizes ($p < .05$). Linear regression models were used to display the correlations between the contributions and relative changes of soil amino sugars and T-GRSP to SOC across aggregate fraction sizes. Random forest models were used to assess the average importance of all soil abiotic and biotic properties for the concentration of total GRSP (T-GRSP) and total amino sugars (Total ASs) and their contribution to SOC. The percentage increase in the MSE (mean squared error) of variables was used and higher MSE% values indicated greater importance. With the "rfPermute" package, the significance of predictors for the response variables was calculated, and with the "A3" package, the significance of the models and cross-validated R^2 was estimated with 1000 permutations of each response variable (Breiman, 2001; Jiao et al., 2018).

3 | RESULTS

3.1 | Soil physicochemical properties

Soil physicochemical properties were consistently affected by forest restoration across aggregate fraction sizes ($p < .05$, Table 1). Afforestation on bare land significantly enhanced soil nutrient accumulation, and the concentrations of SOC, TN, and TP in EP, MF, and NF were much greater than those in BL. In addition, native species

mixtures accumulated about three times more total soil nutrients than EP over 60 years. Afforestation also changed soil stoichiometry; for example, soil C:P and N:P ratios were greatly increased and soil C:N ratio was decreased along the restoration chronosequence. Soil pH decreased constantly during the restoration process. The overall effects of aggregate fraction sizes on soil nutrients and stoichiometry were not statistically significant.

3.2 | Soil microbial community composition

Soil microbial community biomass was significantly affected by aggregate fractions and afforestation ($p < .05$, Table 2). Microbial biomass in MA was much greater than that in LMA and SMA. Afforestation consistently increased soil microbial biomass compared with bare land and the recovery of the soil microbial community in MF was much faster than that in EP. Afforestation significantly decreased the F:B ratio, whereas the GP:GN ratio increased, regardless of aggregate fraction sizes.

3.3 | Soil microbial-derived product concentrations and contribution to SOC

Afforestation on bare land greatly enhanced the accumulation of amino sugars and GRSP ($p < .05$, Figure 2). Greater accumulation of total GRSP than total amino sugars was observed with restoration. On average, the concentration of total amino sugars in EP, MF, and NF significantly increased by 102%, 263%, and 249% compared with bare land, and the concentration of total glomalin also significantly increased by 268%, 859%, and 922%, respectively. Native species

TABLE 1 Selected physicochemical characteristics within soil aggregate fractions along a restoration chronosequence

Aggregate fraction	Treatment	SOC	TN	TP	pH	C:N	C:P	N:P
		(g kg ⁻¹)						
LMA	BL	1.5 ± 0.2 c	0.2 ± 0.0 d	0.0 ± 0.0 d	4.6 ± 0.1 a	12.3 ± 2.9 ab	38.0 ± 7.3 b	3.5 ± 0.7 b
	EP	7.1 ± 1.3 b	0.4 ± 0.1 c	0.1 ± 0.0 c	4.6 ± 0.0 a	16.7 ± 0.8 a	96.1 ± 18.4 a	5.8 ± 1.1 a
	MF	19.7 ± 3.7 ab	1.6 ± 0.1 b	0.2 ± 0.0 b	4.4 ± 0.1 b	12.4 ± 2.3 ab	83.1 ± 11.8 a	6.9 ± 0.5 a
	NF	29.7 ± 2.0 a	3.0 ± 0.1 a	0.5 ± 0.0 a	4.5 ± 0.1 b	9.8 ± 0.5 b	64.2 ± 4.9 a	6.5 ± 0.2 a
SMA	BL	2.7 ± 0.6 c	0.3 ± 0.1 b	0.1 ± 0.0 c	4.5 ± 0.0 b	11.9 ± 3.6 ns	54.5 ± 15.4 b	7.5 ± 3.4 ns
	EP	8.6 ± 0.8 b	0.5 ± 0.1 b	0.1 ± 0.0 c	4.7 ± 0.0 a	17.5 ± 1.5 ns	127.4 ± 21.2 a	7.7 ± 1.5 ns
	MF	24.0 ± 4.4 a	1.8 ± 0.1 a	0.3 ± 0.0 b	4.3 ± 0.1 c	13.1 ± 2.4 ns	85.5 ± 12.8 ab	6.7 ± 0.5 ns
	NF	23.6 ± 1.5 a	2.2 ± 0.2 a	0.4 ± 0.0 a	4.4 ± 0.0 b	10.8 ± 0.8 ns	65.6 ± 5.5 b	6.1 ± 0.1 ns
MA	BL	4.7 ± 0.4 c	0.1 ± 0.1 c	0.1 ± 0.0 c	4.5 ± 0.1 a	196.3 ± 143.0 a	48.7 ± 3.6 c	1.4 ± 0.8 b
	EP	14.8 ± 2.2 b	1.0 ± 0.2 b	0.1 ± 0.0 c	4.7 ± 0.0 a	15.5 ± 1.1 b	130.4 ± 23.8 a	8.6 ± 1.7 a
	MF	28.2 ± 4.6 a	2.7 ± 0.4 a	0.4 ± 0.0 b	4.0 ± 0.1 b	10.2 ± 0.3 c	80.5 ± 11.2 b	7.8 ± 0.9 a
	NF	29.7 ± 1.0 a	3.1 ± 0.1 a	0.5 ± 0.0 a	4.1 ± 0.1 b	9.7 ± 0.3 c	62.2 ± 1.9 bc	6.4 ± 0.1 a

Note: Values in the table are shown as means ± SE ($n = 5$). Different lowercase letters indicate significant differences among the different forest restorations within the same aggregate fraction at $p < .05$.

Abbreviations: BL, bare land; EP, *Eucalyptus* plantation; LMA, large macroaggregates; MA, microaggregates; MF, native species mixed forest; NF, native forest; SMA, small macroaggregates.

mixtures showed a faster accumulation of microbial-derived products than *Eucalyptus* plantations and reached the level of native forests after 60 years of afforestation. In addition, native species mixtures accumulated more fungal-derived amino sugars in soil with the F-GluN:MurN ratio being much greater than that in EP and NF (Figure S1). The effects of aggregate fraction sizes on the concentrations of amino sugars and GRSP were overall not significant.

The contributions of amino sugars and GRSP to the SOC pool were significantly affected by afforestation, aggregate fractions, and their interaction ($p < .05$, Figure 3). On average, the contribution of soil total amino sugars to the SOC pool in EP, MF, and NF significantly decreased by 50%, 59%, and 68% compared with bare land, and the contribution of total glomalin to the SOC pool also declined by 16%, 12%, and 19%, respectively, but this was not significant. Although the contributions of GluN and total amino sugars in LMA were significantly greater than those in SMA and MA, the contributions of specific amino sugars and total amino sugars decreased constantly along the restoration chronosequence. The contribution of GRSP to the SOC pool in LMA was generally greater than that in SMA and MA. The contribution of total GRSP to SOC significantly decreased in LMA, whereas it increased in MA along the restoration chronosequence. The contribution of EE-GRSP to the SOC pool was greater in SMA than that in LMA and MA, and in MA, the contribution was much greater in EP than that in other treatments.

3.4 | Relative changes in soil microbial-derived products compared with SOC

Afforestation led to a faster accumulation of GRSP than of amino sugars in soil, with the relative change rate of GRSP four times greater than that of amino sugars ($p < .05$, Figure 4). The relative change rates of microbial-derived products in SMA and MA were much greater than those in LMA ($p < .05$, Figure 4). The relative change rate of microbial-derived products within aggregate fractions did not significantly vary with afforestation types. The relative change in total ASs was positively correlated with the relative change in T-GRSP in SMA and total (including LMA, SMA, and MA; $p < .01$, Figure 5). The contribution of total ASs to SOC was positively correlated with the contribution of T-GRSP to SOC in LMA and total ($p < .001$, Figure 5). The change ratio of total ASs relative to SOC was positively correlated with the change ratio of T-GRSP relative to SOC in SMA ($p < .01$, Figure 5) and in total ($p < .05$, Figure 5).

3.5 | Predictors of the accumulation and contribution of soil microbial-derived products to SOC

Random forest models suggested that soil fertility (e.g., SOC, TN, TP, and their ratios) and microbial community explained most of the variation in the accumulation of amino sugars in soil (Figure 6a,c, $p < 0.001$). The contribution of total GRSP to SOC was mainly

affected by fungal biomass and SOC (Figure 6b, $p < .001$). The contribution of amino sugars to SOC was mainly affected by SOC, actinomycete biomass, GP biomass, and soil C:P ratio (Figure 6d, $p < .001$).

4 | DISCUSSION

4.1 | The accumulation of GRSP and amino sugars increased constantly by afforestation

In this study, the accumulation of glomalin and amino sugars in soil increased along the afforestation chronosequence, with greater accumulation of GRSP than amino sugars, which confirmed our first hypothesis (Figure 2). The enhanced accumulation of microbial-derived products in soil was ascribed to the synchronized increases in microbial biomasses and soil fertility. Vegetation restoration could enhance plant-C inputs and in tandem stimulate soil microbial activities (Hu et al., 2020). Microbial products mainly accumulate in soils via microbial decomposition and turnover (Liang, 2020; Zhang et al., 2021). However, the contribution of GRSP and amino sugars to the SOC pool decreased along the afforestation chronosequence (Figure 3), suggesting an increase in the contribution of plant-derived C to the SOC pool with forest restoration. This was in line with previous studies that microbial-derived C contribution to SOC declined along a forest restoration chronosequence (Shao et al., 2019). SOC in forest soil may be dominated by particulate organic matter (plant-derived; Cotrufo et al., 2019) and decompose less owing to unfavorable soil conditions (e.g., lower pH) for bacterial growth with afforestation chronosequence (Table 1; Angst et al., 2021).

The overall effects of aggregate fractions on the accumulation of microbial-derived products in soil were not significant. Although microbial biomass tended to increase with decreasing size of aggregate fraction, lower transformation efficiency from living microbial biomass to necromass within microaggregates might inhibit the accumulation of amino sugars and GRSP there (Figure S2; Xu et al., 2022). Furthermore, microaggregates might harbor more diverse and abundant microbial communities relative to macroaggregates (Bach et al., 2018), which favors faster microbial metabolic activities and further promotes the recycling of microbial by-products. Our results suggest that necromass recycling might be a vital mechanism for mediating microbial metabolism and soil C cycling (Cui et al., 2020), and forest restoration may strengthen this effect in microaggregates. Additionally, the accumulation of GRSP and amino sugars was positively correlated with aggregate stability (Tables S1 and S2, Zhang et al., 2022).

Regression analysis shows that the accumulation of GRSP and amino sugars during forest restoration was synergistic (Figure 5). AMF could not only delay the turnover of macroaggregates providing more time and space for metabolic interactions between AMF and their associated microbiota (Rillig & Mummey, 2006) but also accelerate the turnover of microaggregates (Morris et al., 2019). Macroaggregates contain larger pore spaces, more AMF hyphae, and more GRSP which might facilitate the synergistic accumulation

TABLE 2 Soil microbial community composition within soil aggregate fractions along a restoration chronosequence

Aggregate fraction	Treatment	Bacteria (nmol g ⁻¹)	Fungi	AMF	Actinomycetes	GP	GN	Total PLFAs	F:B	GP:GN
LMA	BL	4.3 ± 0.9 c	0.4 ± 0.1 c	0.1 ± 0.0 c	1.2 ± 0.3 c	2.2 ± 0.4 d	2.2 ± 0.6 c	6.1 ± 1.3 c	0.1 ± 0.0 a	1.1 ± 0.1 b
	EP	10.0 ± 2.6 b	0.5 ± 0.1 bc	0.4 ± 0.1 b	3.1 ± 0.8 b	6.0 ± 1.5 c	4.0 ± 1.1 bc	14.0 ± 3.5 b	0.1 ± 0.0 b	1.6 ± 0.1 a
	MF	14.9 ± 1.9 b	0.7 ± 0.1 ab	0.7 ± 0.1 b	4.3 ± 0.6 b	9.5 ± 1.2 b	5.4 ± 0.7 b	20.6 ± 2.6 b	0.1 ± 0.0 bc	1.8 ± 0.1 a
	NF	25.5 ± 2.1 a B	0.9 ± 0.1 a B	1.2 ± 0.1 a B	7.2 ± 0.5 a B	15.9 ± 1.4 a B	9.6 ± 0.7 a B	34.8 ± 2.8 a B	0.0 ± 0.0 c	1.7 ± 0.1 a
SMA	BL	5.5 ± 0.9 c	0.5 ± 0.0 c	0.1 ± 0.0 c	1.5 ± 0.2 c	2.9 ± 0.6 b	2.6 ± 0.4 b	7.5 ± 1.1 b	0.1 ± 0.0 a	1.1 ± 0.1 c
	EP	10.7 ± 1.5 b	0.7 ± 0.0 b	0.4 ± 0.1 b	3.3 ± 0.4 b	6.4 ± 1.0 b	4.3 ± 0.5 b	15.0 ± 2.0 b	0.1 ± 0.0 b	1.5 ± 0.1 b
	MF	18.9 ± 3.5 a	1.1 ± 0.2 a	0.8 ± 0.2 a	5.8 ± 1.1 a	12.4 ± 2.3 a	6.5 ± 1.2 a	26.6 ± 5.0 a	0.1 ± 0.0 b	1.9 ± 0.1 a
	NF	21.7 ± 1.7 a B	1.1 ± 0.1 a B	1.0 ± 0.1 a B	6.0 ± 0.5 a B	13.8 ± 1.1 a B	7.8 ± 0.6 a B	29.6 ± 2.3 a B	0.1 ± 0.0 b	1.8 ± 0.0 a
MA	BL	10.1 ± 2.3 b	0.8 ± 0.1 ns	0.4 ± 0.1 b	2.6 ± 0.6 c	5.4 ± 1.1 b	4.7 ± 1.2 b	13.8 ± 3.0 b	0.1 ± 0.0 a	1.2 ± 0.1 b
	EP	16.2 ± 3.3 b	1.3 ± 0.3 ns	0.6 ± 0.1 b	5.4 ± 1.1 b	10.4 ± 2.2 b	5.8 ± 1.1 b	23.5 ± 4.9 b	0.1 ± 0.0 a	1.8 ± 0.1 a
	MF	33.2 ± 6.7 a	1.8 ± 0.6 ns	1.7 ± 0.3 a	10.3 ± 2.2 a	21.0 ± 4.1 a	12.2 ± 2.6 a	46.9 ± 9.9 a	0.1 ± 0.0 b	1.8 ± 0.1 a
	NF	32.7 ± 1.7 a A	1.3 ± 0.1 ns A	1.6 ± 0.1 a A	9.0 ± 0.5 a A	21.2 ± 1.1 a A	11.5 ± 0.7 a A	44.7 ± 2.3 a A	0.0 ± 0.0 b	1.8 ± 0.0 a

Note: Values in the table are shown as means ± SE (n = 5). Different uppercase letters indicate significant differences among the different sizes of aggregate fractions at $p < .05$. Different lowercase letters indicate significant differences among the different forest restorations within the same aggregate fraction at $p < .05$. ns indicates no significant difference among the different forest restorations within the same aggregate fraction at $p < .05$.

Abbreviations: AMF, arbuscular mycorrhizal fungi; B, bacteria; BL, bare land; EP, *Eucalyptus* plantation; F, fungi; GN, Gram-negative bacteria; GP, Gram-positive bacteria; LMA, large macroaggregates; MA, microaggregates; MF, native species mixed forest; NF, native forest; SMA, small macroaggregates; total PLFAs, total microbial biomass.

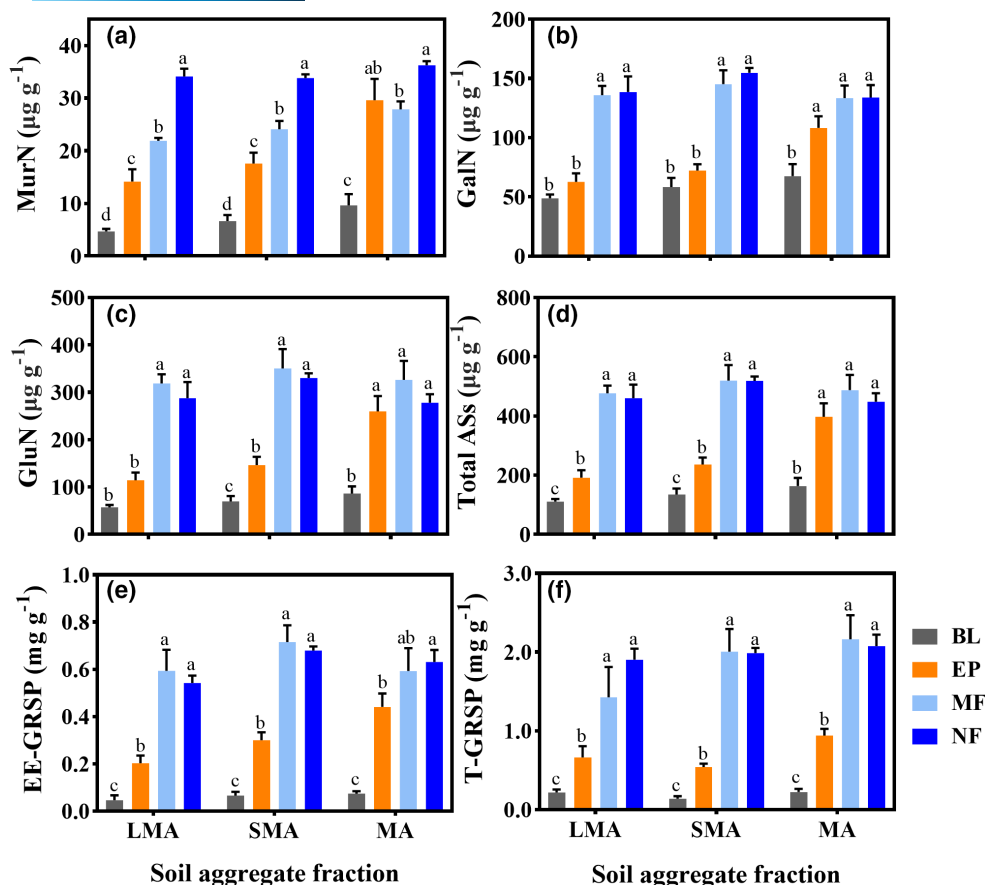


FIGURE 2 Concentrations of muramic acid (MurN), galactosamine (GalN), glucosamine (GluN), total amino sugars (ASs), easily (EE-GRSP), and total (T-GRSP) extractable glomalin-related soil proteins within soil aggregate fractions along a restoration chronosequence. BL, bare land; EP, *Eucalyptus* plantation; LMA, large macroaggregates; MA, microaggregates; MF, native species mixed forest; NF, native forest; SMA, small macroaggregates. Different lowercase letters indicate significant differences among the different forest restorations at $p < 0.05$. Vertical bars denote standard errors of mean values ($n = 5$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

of amino sugars and glomalin (Lovelock et al., 2004). However, the underlying mechanism should be explored via manipulative experiments and microscopic observation in future research.

4.2 | Faster accumulation and greater contribution of GRSP to the SOC pool than of amino sugars during forest restoration

Our data support our second hypothesis that GRSP comprised a larger component of SOC than amino sugars did and therefore accumulated faster during forest restoration (Figures 2–4). This can be explained as follows. First, GRSP is likely more stable than soil amino sugars (Agnihotri et al., 2022; Rillig et al., 2001). GRSP is composed of >50% recalcitrant components such as aromatic and alkyl-C and has greater chemical recalcitrance than amino sugars (Agnihotri et al., 2022). GRSP has a higher propensity to form stable aggregates via binding mineral and organic particles than amino sugars do (Gunina & Kuzyakov, 2015). Conversely, amino sugars may establish relatively fewer bonds with minerals than glomalin does. Second, AMF may reduce bacterial biomass

and soil amino sugar concentrations due to nutrient deficiency (He et al., 2020), which would intensify the different accumulation rates of GRSP and amino sugars. Third, GRSP concentrations are positively correlated with net primary productivity (NPP) globally and higher plant productivity can provide more available C to AM fungi for glomalin production (Treseder & Turner, 2007). NPP may increase with vegetation restoration on barren land if AM host plants are more abundant for the fungi that would benefit the accumulation of glomalin (Treseder & Turner, 2007) and promote the microbial-derived C in soil. In the study, *Eucalyptus exserta* (EP) associates with both arbuscular and ectomycorrhizal fungi, all dominant tree species in NF are arbuscular mycorrhizal, while *Acacia auriculiformis* as a dominant species in MF is ectomycorrhizal, and *Calamus tetradactylus* is ectomycorrhizal as a dominant herb in MF. However, Guo et al. (2021) found that the contribution of amino sugars to SOC in karst soils increased with vegetation restorations and more bacterial-derived C accumulated, which might be associated with the difference in microbial C transformation efficiency driven by pH (Malik et al., 2018).

The relative change rate of microbial-derived products was mediated by soil aggregate fraction, with values much greater in

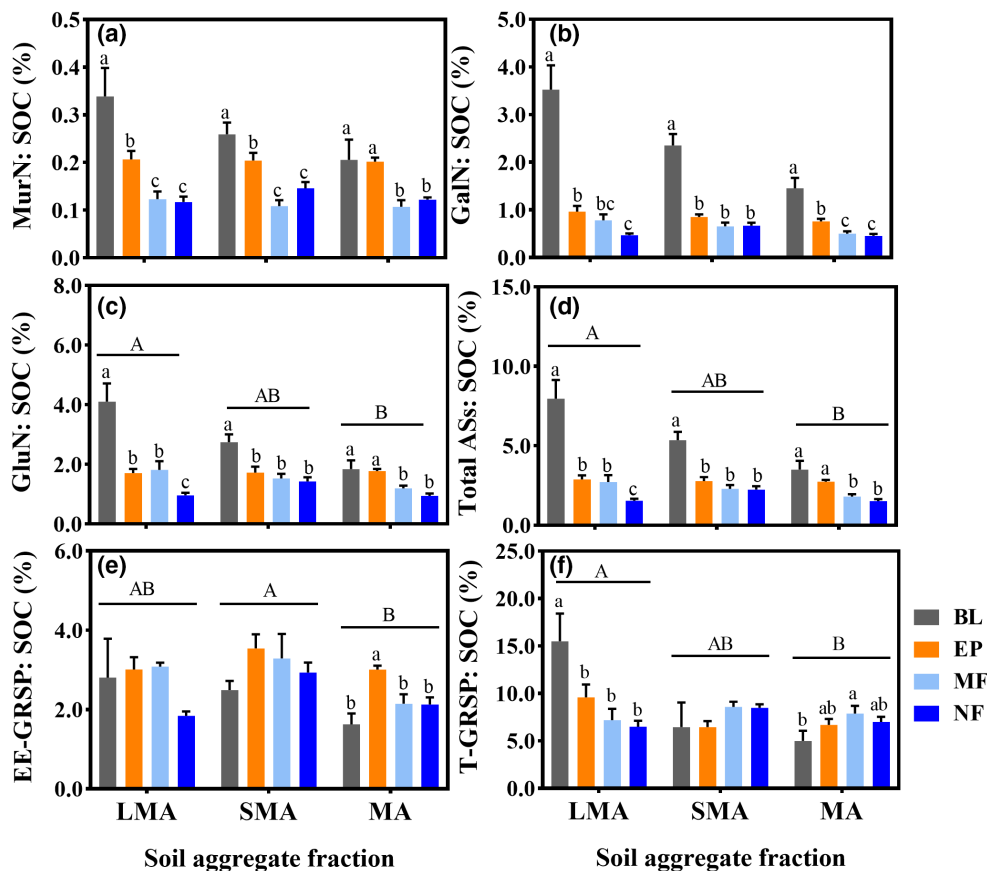


FIGURE 3 Contributions of muramic acid (MurN), galactosamine (GalN), glucosamine (GlcN), total amino sugars (ASs), easily (EE-GRSP), and total (T-GRSP) extractable glomalin-related soil proteins to soil organic carbon (SOC) within soil aggregate fractions along a restoration chronosequence. BL, bare land; EP, *Eucalyptus* plantation; LMA, large macroaggregates; MA, microaggregates; MF, native species mixed forest; NF, native forest; SMA, small macroaggregates. Different uppercase letters indicate significant differences among the different sizes of aggregates fractions at $p < .05$. Different lowercase letters indicate significant differences among the different forest restorations at $p < .05$. Vertical bars denote standard errors of mean values ($n = 5$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.14947)]

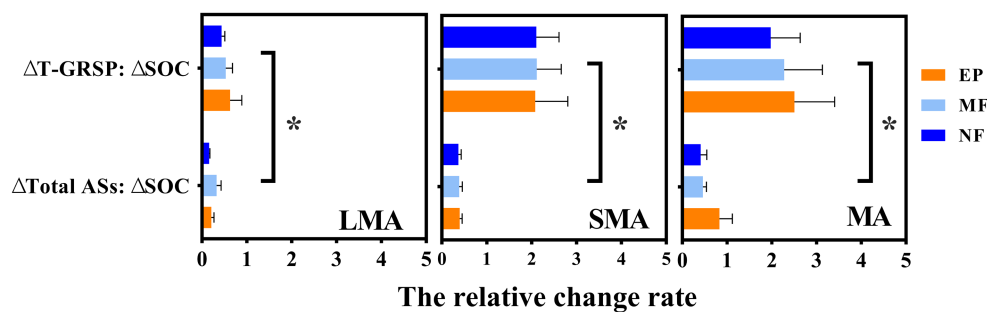


FIGURE 4 Relative changes in total amino sugars (ASs) and total extractable glomalin-related soil proteins (T-GRSP) compared with soil organic carbon (SOC) within soil aggregate fractions along a restoration chronosequence. EP, *Eucalyptus* plantation; LMA, large macroaggregates; MA, microaggregates; MF, native species mixed forest; NF, native forest; SMA, small macroaggregates. *Indicates significant differences between the relative change rate of T-GRSP and total ASs at $p < .05$. Vertical bars denote standard errors of mean values ($n = 5$). [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.14947)]

microaggregates than in macroaggregates (Figure 4). Minerals within microaggregates have a huge surface area to adsorb microbial-derived C to form stable organo-mineral complexes (Liang, 2020). In addition, Macroaggregates are more vulnerable to disturbances and

environmental changes than microaggregates are (Ye et al., 2020), whereas microaggregates maintain a relatively stable microenvironment. The contribution of microbial-derived products to SOC pools decreased with decreasing aggregate fraction size, suggesting that

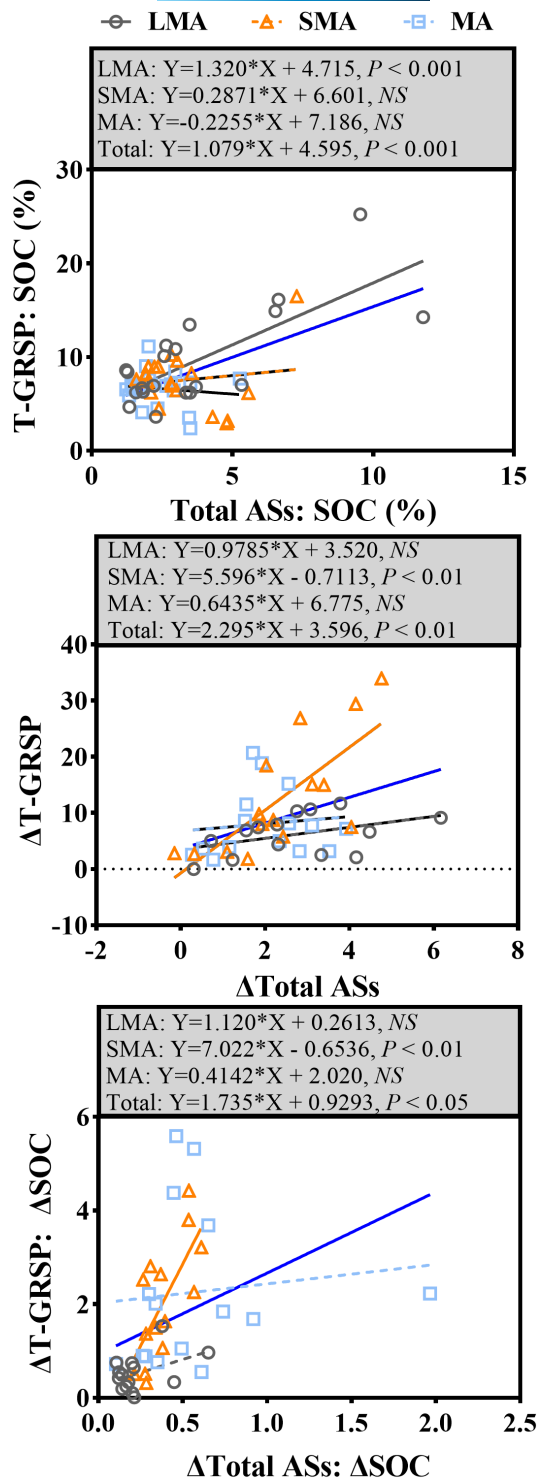


FIGURE 5 The correlations between contributions or relative change rates of total extractable glomalin-related soil proteins (T-GRSP) and amino sugars (ASs) within the large macroaggregates (LMA), small macroaggregates (SMA), and microaggregates (MA) fractions and in total. The solid blue line represents the correlations for all three sizes of aggregates; NS indicates no significance at $p < .05$. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16467)]

microaggregates maintain a faster accumulation rate or greater sequestration efficiency of microbial-derived products, and greater potential storage capacity relative to macroaggregates. As argued by

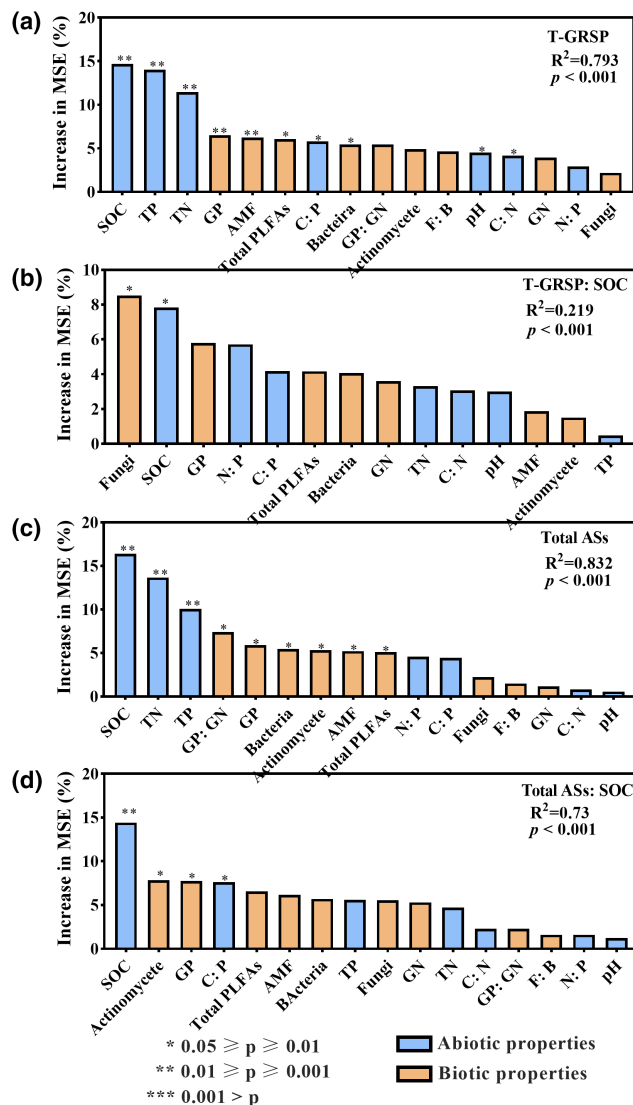


FIGURE 6 The average predictive importance (mean square error [MSE] increase percentage) for all soil abiotic and biotic properties for concentrations and contributions of total amino sugars (ASs) and total extractable glomalin-related soil proteins (T-GRSP) to soil organic carbon (SOC). AMF, arbuscular mycorrhizal fungi; N:P, TN:TP; B, bacteria; C:N, SOC:TN; C:P, SOC:TP; F, fungi; GN, Gram-negative bacteria; GP, Gram-positive bacteria; TN, total nitrogen; total PLFAs; total microbial biomass; TP, total phosphorus [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.16467)]

Six et al. (2002) and Stewart et al. (2008), when SOC is further from its maximum content (C saturation point), its C-sequestration rate is greater. The farther away from its C saturation point, the more efficient the C-sequestration rate. In addition, the decreased contribution of microbial-derived products to SOC pools in smaller aggregate fractions might be accompanied by increased plant-derived C accumulation. The decreased microbial-derived product contributions to SOC pools along the afforestation chronosequence further indicate that the role of the soil microbial community shifted from in vivo turnover (reducing C-use efficiency) to ex vivo modification (decomposing and incorporating more plant-C into the stable C pool; Liang, 2020).

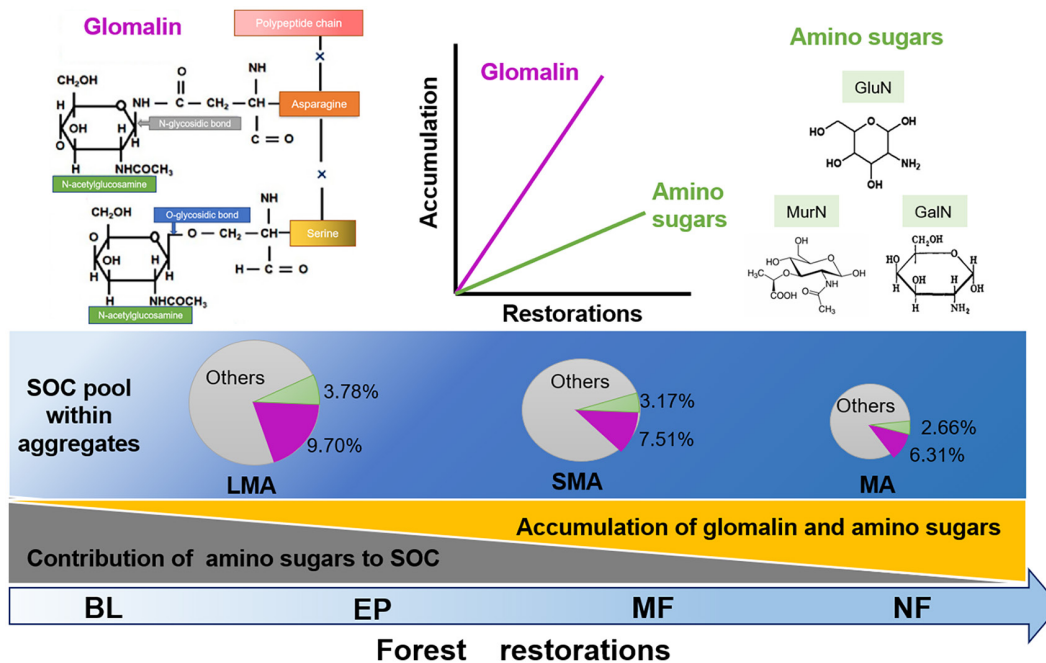


FIGURE 7 A conceptual diagram illustrating the accumulation and contribution of glomalin and amino sugars to soil organic carbon (SOC) within soil aggregate fractions along a restoration chronosequence. BL, bare land; EP, *Eucalyptus* plantation; LMA, large macroaggregates; MA, microaggregates; MF, native species mixed forest; NF, native forest; SMA, small macroaggregates [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/gcb.14949)]

4.3 | Soil fertility and microbial community mediated the accumulation and contribution of microbial-derived products during forest restoration

Random forest modeling revealed that the accumulation and contribution of GRSP and amino sugars were mainly affected by soil fertility and soil microbial community during forest restoration in our study (Figure 6; Agnihotri et al., 2022; Chen et al., 2020). Soil fertility and microbial biomass explained most of the variation of GRSP and amino sugars along the afforestation chronosequence. The contribution of GRSP to SOC was mainly affected by fungal biomass and SOC, and the contribution of amino sugars was affected by SOC, actinomycete biomass, GP biomass, and soil C:P ratio. SOC was the major substrate for microbial metabolism; in tandem, the microbial metabolites also contributed to the soil C pool. Hence, the accumulation of microbial byproducts (glomalin and amino sugars) and SOC preservation were enhanced synergistically. Especially, glomalin and amino sugars bind with minerals to form mineral-associated organic C (Agnihotri et al., 2022; Liang et al., 2020) which can be stabilized in soils for decades to centuries (Lavelle et al., 2020). The important role of TN and TP in explaining the variation of the concentrations of total glomalin and amino sugars indicates that soil fertility plays a vital role in mediating microbial byproduct accumulation, which can be explained by the beneficial effect of vegetation restoration on fungi in P-limited ecosystems. Indeed, our data also support the contention that fungal biomass plays an important role in mediating the contribution of GRSP to SOC. Vegetation restoration offered favorable conditions for the growth and development of

mycorrhizal fungi, with a positive correlation between AMF biomass and its diversity (Figure S3), and further facilitated the release and accumulation of glomalin in soils. Actinomycetes are aerobic spore-forming GP bacteria characterized by substrate and aerial mycelium growth (Bhatti et al., 2017), whereas GP bacteria have a thicker peptidoglycan cell wall (Joergensen, 2018; Liang et al., 2019). Hence, the proliferation of GP bacteria was also an important contributor to microbial-derived byproducts to SOC. The soil C:P ratio may affect the microbial community composition and its activities and thus influence soil C cycling (Shen et al., 2019). Yuan et al. (2021) also demonstrated in the same study area that P addition decreases the contribution of amino sugars to SOC via increasing microbial biomass and enzyme activities.

The present results highlight the greater benefits for soil C-accumulation and nutrient fertility associated with afforestation with native versus exotic fast-growing species. The capacity for symbiotic establishment (in this case with arbuscular mycorrhizal fungi) and the increased SOC were due to fungi and native tree species. The stability of SOC has even been demonstrated to be affected by tree species (Angst et al., 2019). The results also show a higher P-use efficiency (higher soil C:P) with native than with exotic species, indicating that native species with self-adaptation to P-limit soil conditions should be considered a priority in restoration. The native forests improve soil C storage capacity and nutrient retention and use efficiency, maintaining greater soil microbial populations and diversity than fast-growing non-native species. All of this suggests greater biodiversity conservation and service provision such as mitigation of climate change with native reforestation (Wu et al., 2021).

5 | CONCLUSIONS

Our study provides new insight into the accumulation and contribution of glomalin and amino sugars to the SOC pool during forest restoration (Figure 7). Afforestation of bare land greatly enhanced the accumulation of GRSP and amino sugars, but it decreased their contribution to SOC. The faster accumulation and greater contribution of GRSP to SOC compared with those of amino sugars highlights the important role of AMF in mediating soil C cycling during forest restoration, despite the proportional contribution of GRSP and amino sugars to the SOC pool diminishing with forest restoration. Such information is valuable for improving our mechanistic understanding of the microbial control of SOC preservation during the restoration of degraded ecosystems. Our findings also suggest that favoring arbuscular mycorrhizal plants can be an effective option to sequester more soil C during restoration practices. The importance of the soil C:P ratio in mediating the accumulation of microbial-derived products suggests that appropriate fertilization may also play an important role in mediating soil C sequestration and stabilization, particularly in a P-limited ecosystem. Meanwhile, it also highlights the importance of native species mixture in regenerating/restoring forests because of their long-term adaptation to P limitation, greater capacity to store and conserve P, and higher P use efficiency. These results together provide important guidance for management practices considering belowground microbial processes and functions during coastal restoration, benefiting both aboveground and belowground biodiversity and multifunctionality.

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CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data that support the findings of this study are available in Zenodo (<https://doi.org/10.5281/zenodo.6860872>).

ORCID

Jing Zhang  <https://orcid.org/0000-0003-1712-0255>

Faming Wang  <https://orcid.org/0000-0002-7543-6779>

Jordi Sardans  <https://orcid.org/0000-0003-2478-0219>

Josep Peñuelas  <https://orcid.org/0000-0002-7215-0150>

Zhanfeng Liu  <https://orcid.org/0000-0002-6602-6196>

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SUPPORTING INFORMATION

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