**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†], Ye Yuan[1,4†], Zhijian Mou[1,4], Yue Li[1,2,3], Luhui Kuang[1,4], Jing Zhang[1,2,3], Wenjia Wu[1,2,3], Faming Wang[1,2,3], Jun Wang[1,2], Hans Lambers[5], Jordi Sardans[6,7], Josep Peñuelas[6,7], Hai Ren[1,2], Zhanfeng Liu[1,2,3*]

[1]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

[2]Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Guangzhou 510650, China

[3]Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 511458, China

[4]College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China

[5] School of Biological Sciences, University of Western Australia, Perth, WA 6009, Australia

[6]CSIC, Global Ecology Unit CREAF-CSIC-UAB, Cerdanyola del Valles, Catalonia 08193, Spain;

[7]CREAF, Cerdanyola del Valles, Catalonia 08193, Spain;

[†] These authors contributed equally to this work.

***Corresponding author:**

**Zhanfeng Liu** **Email**: liuzf@scbg.ac.cn; **Tel**: +86-20-37252592

**Running title:** Afforestation and microbial-derived products

**Type of article:** Primary research article

**Number of words:** 5640 words in the main text

**Number of figures and tables:** 7 figures and 2 tables

**Supporting information:** 2 figures

**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 and mitigate climate change. Glomalin-related soil proteins and amino sugars are regarded stable microbial-derived C, and their distribution within soil aggregate fractions 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 glomalin-related soil proteins (GRSP, produced by arbuscular mycorrhizal fungi) and amino sugars (originating from microbes) within soil aggregate fractions along a restoration chronosequence (Bare land (BL), *Eucalyptus exserta* plantation (EP), native species mixed forest (MF) and native forest (NF)) in tropical coastal terraces. The concentrations of amino sugars and GRSP increased, whereas their contributions to the SOC pool decreased along the restoration chronosequence. Although microaggregates harbored greater microbial abundances, the concentrations of amino sugars and GRSP were not significantly affected by aggregate sizes. Interestingly, the contributions of amino sugars and GRSP to SOC pools decreased with decreasing aggregate fraction size which might be associated with increasing accumulation of plant-derived carbon. 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 suggest that glomalin accumulates faster and contributes more to SOC pools during forest restoration than amino sugars did which was greatly affected by soil aggregate size. 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:** glomalin-related soil protein, amino sugars, soil aggregates, soil microbial community, afforestation, tropical coastal terrace

## 1. INTRODUCTION

Forests play an important role in maintaining coastal ecosystem functioning such as biodiversity, typhoon sheltering and carbon sequestration (Snäll et al., 2021). However, about 25-50% of coastal vegetation habitats (such as mangroves, seagrasses, salt-marshes, kelp forests) have become degraded worldwide in the past 50 years due to land-use and climate change, which are threatening their health and causing major $CO_2$ emission (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 in the early 1950s, affecting ecosystem functioning (Ren et al., 2007). Forest restoration is an effective way to mitigate the degradation and improve its ecosystem services (Canadell & Raupach, 2008). Soil carbon (C) cycling is central to the reestablishment 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, 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, 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 the 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 and 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).

Glomalin-related soil proteins (GRSP) are microbial products produced by arbuscular mycorrhizal fungi (AMF) and characterized as hydrophobic sticky and recalcitrant glycoproteins (Wright et al., 1998). GRSP are 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 increase 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 et al. (2017; 2022) found that aromatic and

121 alkyl-C in glomalin are more recalcitrant, with benefits for aggregate stability, jointly enhancing SOC
122 persistence in tropical forests. Iron is an important element in the composition of GRSP, converting
123 monomeric GRSP units into a multimeric complex, thus promoting GRSP stabilization. Other metal ions
124 such as $Al^{3+}$, $Ca^{2+}$ and $Mg^{2+}$ probably have similar effects on GRSP. GRSP may contribute to SOC content
125 not only owing to their recalcitrant chemistry, but also due to their stabilizing effect on soil aggregates
126 (Rillig and Mummey, 2006). GRSP sorbed onto organic substances, clays and silt particles, facilitates
127 adsorption between and within microaggregates and AMF hyphae could bind particles or
128 microaggregates, thereby promoting the formation and stabilization of soil macroaggregates (Agnihotri
129 et al., 2022). The distribution of GRSP in soil macroaggregates may influence aggregate stability (Xie et
130 al., 2015). Although it is acknowledged that both GRSP and amino sugars play important roles in
131 mediating SOC sequestration, the information about the dynamics of their relative contribution to the
132 SOC pool during forest restoration is poorly understood. Simultaneous measurements of GRSP and
133 amino sugars make it possible to compare their relative contribution to SOC accumulation and stability,
134 and provide valuable information for developing a restoration strategy in terms of carbon sequestration.
135 To obtain a comprehensive understanding of microbial-driven SOC preservation during vegetation
136 restoration, it is worth investigating how GRSP and amino sugars accumulate and contribute to SOC
137 across soil aggregate fraction sizes during restoration.

138 Coastal vegetation habitats have been degraded extensively in south China and this has caused a
139 series of ecological problems, such as biodiversity loss and soil erosion (Ren et al., 2007). Starting from
140 the 1950s, forest restoration was conducted by planting pioneer plant species and mixtures of native plant
141 species on bare land on tropical coastal terraces (Ren et al., 2007). After 60 years of afforestation, native
142 species mixtures have recovered and restored plant communities, soil biodiversity and soil fertility (Wu
143 et al., 2021). However, less attention was paid to how microbial metabolic products accumulate and
144 contribute to the SOC pool, which is central to the reestablishment of soil structure and functioning
145 during restoration. Such information is valuable for guiding restoration practices in the study area and
146 mitigating climate change . In this study, we aimed to investigate the accumulation dynamics of glomalin
147 and amino sugars within aggregates and to evaluate their relative contribution to the SOC pool following
148 forest restoration. We hypothesized that: (1) greater accumulation of GRSP contrasted with that of amino
149 sugars during forest restoration due to enhanced plant C inputs and microbial transformation (Guo et al.,
150 2021; Qiao et al., 2019); (2) the accumulation rate and contribution of GRSP to the SOC pool would be

151 greater than those of amino sugars due to the differences in their chemical recalcitrance and propensity
152 to mineral protection (Agnihotri et al., 2022; Rillig et al., 2001).
153
154 **2. MATERIALS AND METHODS**
155 **2.1. Site description and experimental design**
156 The present study was carried out at the Xiaoliang Tropical Coastal Ecosystem Research Station, Chinese
157 Academy of Sciences, in Guangdong province of China (21°27′N; 110°54′E). The climate in this region
158 is typical tropical monsoon, with a mean annual temperature of 23°C and mean annual precipitation of
159 1,400-1,700 mm with wet (Apr.-Sept.) and dry (Nov.-Mar.) seasons. The soil is classified as a latosol that
160 originated from granite (Yao et al., 1984). Evergreen broad-leaved seasonal rainforest was the climax
161 vegetation in this region before the 1850s, while soils became severely degraded resulting from massive
162 deforestation and soil erosion by the 1950s and only a small part of native forest (NF) was protected for
163 more than 200 years (Yu et al., 1985; Ren et al., 2007). A *Eucalyptus exserta* plantation (EP) was
164 established on bare land in the early 1960s; it can associate with both arbuscular and ectomycorrhizal
165 fungi. Forests using mixed native species (MF) were promoted from EP after clear-cutting in 1974 and
166 are dominated by AMF plants including *Carallia brachiata*, *Aphanamixis polystachya*, *Schefflera*
167 *octophylla*, *Carallia brachiata*, *Symplocos chunii*, *Acacia auriculaeformis*, *Photinia benthamiana, and*
168 *Cinnamomum burmanni*, *Lygodium japonicum, Ophiopogon japonicus* and *Nephrolepis cordifolia*, and
169 ectomycorrhizal plants including *Acacia auriculaeformis* and *Calamus tetradactylu*. NF is dominated by
170 AMF plants inculding *Sterculia lanceolata*, *Cinnamomum camphora*, *Cryptocarya chinensis*, *Syzygium*
171 *levinei*, *Syzygium hancei, Schefflera octophylla*, *Auquilaria sinensis* (Wu et al., 2021). Bare land (BL)
172 was used as a reference system in this study (Ren et al., 2007; Wang et al., 2017). Four treatments (BL,
173 EP, MF and NF) were a randomized block design in the study and the distance between plots was over
174 50 m; five replicated plots (10 ×10 m) in each treatment (Fig. 1). More detailed information on the
175 study site and forest restoration is included in Yao et al. (1984) and Ren et al. (2007).

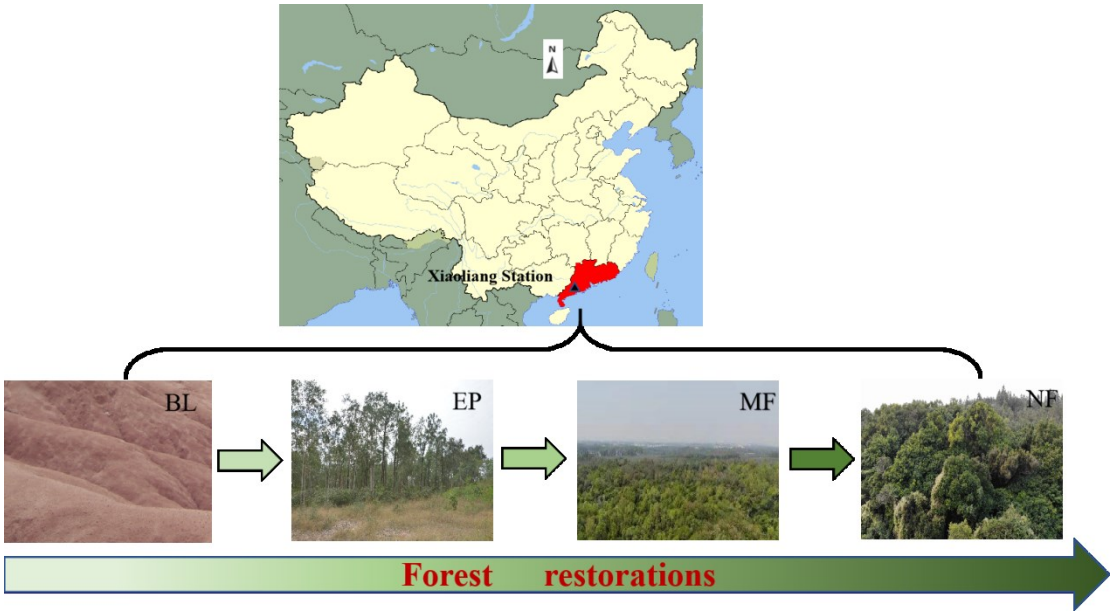


**Figure 1.** Degraded coastal land and forest restorations in the study area.

### 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 removing litter, roots and stones by sieving through a 4-mm sieve, fresh soils were fractionated into three aggregate-size classes: > 2.0 mm large macroaggregate, LMA; 0.25-2.0 mm small macroaggregate, SMA; < 0.25 mm 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 analysing 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 phospholipid fatty acid (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, Wilmington, USA) and identified by MIDI peak identification software (MIDI Inc., Newark, USA). Specific fatty acids were used to represent Gram-positive (GP) bacteria (i15:0, α15:0, i16:0, i17:0 and α17:0), Gram-negative (GN) bacteria (16:1ω9c, 16:1ω7c, 18:1ω7c, cy17:0, cy19:0), saprotrophic fungi (18:2ω6c), arbuscular mycorrhizal fungi (AMF) (16:1ω5c), 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 were determined according to Wright & 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 20 mM sodium citrate solution at pH 8.0, and vortexed for 30 s. Then, the mixture was autoclaved for 30 min 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 50 mM sodium citrate at pH 8.0 and then autoclaved at 121°C for 90 min, 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, Waltham, USA) according to the Bradford (1976) method. Both T-GRSP and EE-GRSP were expressed as mg $g^{-1}$ dry soil. The detailed meaurements 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-phthaldialdehyde, determined by high-performance liquid chromatography (Dionex Ultimate 3000, Thermo Fisher Scientific, Waltham, USA). The detailed relevant information and calculations are shown in supplementary materials and methods. The concentrations of amino sugars in soil were expressed as μg $g^{-1}$ dry soil.

**2.6. Statistical analyses**

All data was 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 ($P < 0.05$). 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 (Brieman, 2001; Jiao et al., 2018).

## 3. RESULTS

### 3.1. Soil physiochemical properties

Soil physicochemical properties were consistently affected by forest restoration across aggregate fraction sizes ($P < 0.05$, 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; e.g., 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.

**Table 1** Soil physicochemical characteristics within soil aggregate fractions along a restoration chronosequence. LMA, large macroaggregates; SMA, small macroaggregates; MA, microaggregates; BL, bare land; EP, *Eucalyptus* plantation; MF, native species mixed forest; NF, native forest. Values are the means (n=5). Different lowercase letters indicate significant differences among the different forest restorations within the same aggregate fraction at $P < 0.05$.

| 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 |

### 3.2. Soil microbial community composition

Soil microbial community biomass was significantly affected by aggregate fractions and afforestation ($P < 0.05$, 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.

**Table 2** Soil microbial community composition within soil aggregate fractions along a restoration chronosequence. LMA, large macroaggregates; SMA, small macroaggregates; MA, microaggregates; BL, bare land; EP, *Eucalyptus* plantation; MF, native species mixed forest; NF, native forest; AMF, arbuscular mycorrhizal fungi; GP, Gram-positive bacteria; GN, Gram-negative bacteria; F, fungi; B, bacteria and total microbial biomass (total PLFAs). Values are the means (n=5). Different uppercase letters indicate significant differences among the different sizes of aggregate fractions at $P < 0.05$. Different lowercase letters indicate significant differences among the different forest restorations within the same aggregate fraction at $P < 0.05$. ns indicates no significant difference among the different forest restorations within the same aggregate fraction at $P < 0.05$.

| Aggregate fraction | Treatment | Bacteria | Fungi | AMF | Actinomycetes | GP | GN | Total PLFAs | F: B | GP: GN |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (nmol $g^{-1}$) | | | | | | | | |
| 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 ± 11 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 ± 01 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 ± 02 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 |

### 3.3. Soil microbial-derived product concentrations and contribution to SOC

Afforestation on bare land greatly enhanced the accumulation of amino sugars and glomalin-related soil proteins (GRSP) ($P < 0.05$, Fig. 2). 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 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 (Fig. S1). The effects of aggregate fraction sizes on the concentrations of amino sugars and GRSP were overall not significant.

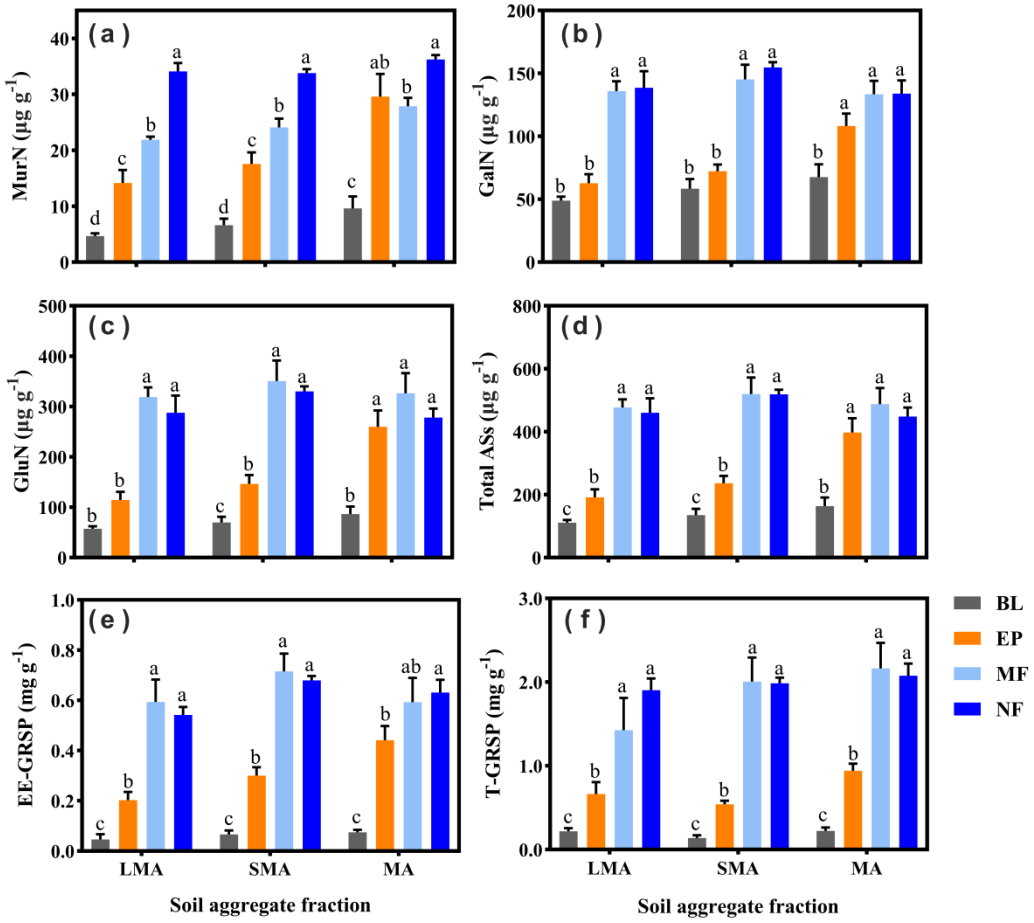


**Figure 2.** Concentrations of muramic acid (MurN), galactosamine (GalN), glucosamine (GlcN), total amino sugars (ASs), easily (EE-GRSP) and total (T-GRSP) extractable glomalin-related soil proteins within soil aggregate fractions along a restoration chronosequence. LMA, large macroaggregates; SMA, small macroaggregates; MA microaggregates; BL, bare land; EP, *Eucalyptus* plantation; MF, native

species mixed forest; NF, native forest. 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).

The contributions of amino sugars and GRSP to the SOC pool were significantly affected by afforestation, aggregate fractions and their interaction ($P < 0.05$, Fig. 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.

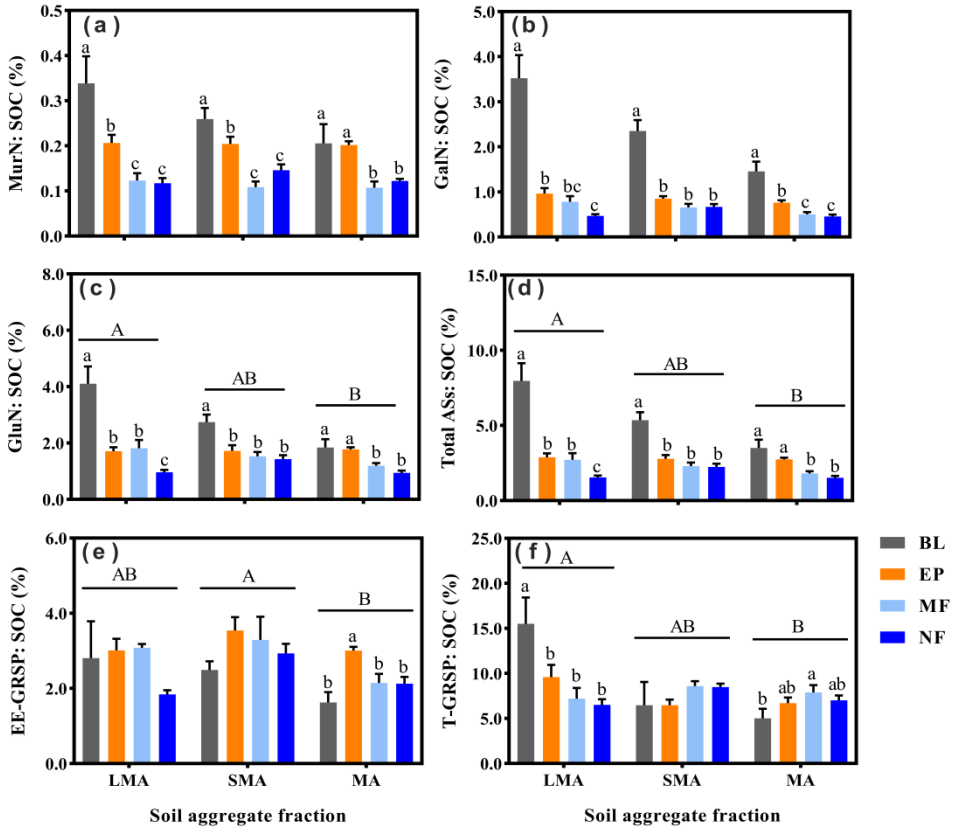


**Figure 3.** Contributions of muramic acid (MurN), galactosamine (GalN), glucosamine (GlcN), total amino sugars (ASs), easily (EE-GRSP) and total (T-GRSP) extractable glomalin-related soil proteins to

soil organic carbon (SOC) within soil aggregate fractions along a restoration chronosequence. LMA, large macroaggregates; SMA, small macroaggregates; MA microaggregates; BL, bare land; EP, *Eucalyptus* plantation; MF, native species mixed forest; NF, native forest. Different uppercase letters indicate significant differences among the different sizes of aggregates fractions at $P < 0.05$. 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).

### 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 ($P < 0.05$, Fig. 4). The relative change rates of microbial-derived products in SMA and MA were much greater than those in LMA ($P < 0.05$, Fig. 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 in total (including LMA, SMA and MA) ($P < 0.01$, Fig. 5). The contribution of total ASs to SOC was positively correlated with the contribution of T-GRSP to SOC in LMA and in total ($P < 0.001$, Fig. 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 ($P < 0.01$, Fig. 5) and in total ($P < 0.05$, Fig. 5).

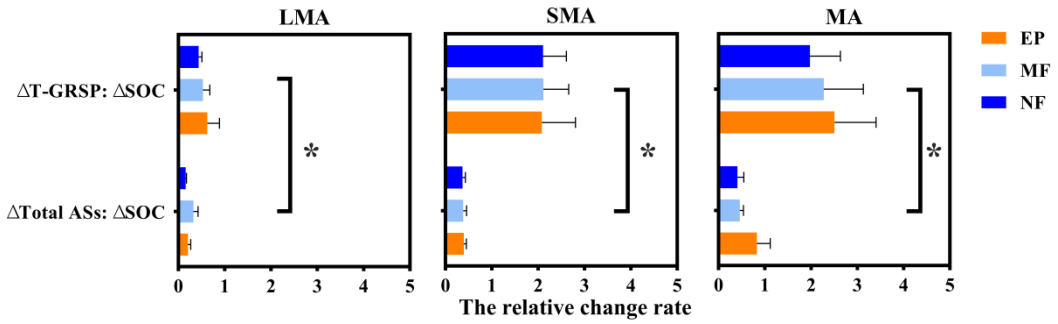


**Figure 4.** Relative changes in total amino sugars (ASs) and total extractable glomalin-related soil proteins (T-GRSP) compared with soil organic carbon (SOC) within soil aggregate fractions along a restoration chronosequence. LMA, large macroaggregates; SMA, small macroaggregates; MA microaggregates; EP, *Eucalyptus* plantation; MF, native species mixed forest; NF, native forest. * indicates significant differences between the relative change rate of T-GRSP and total ASs at $P < 0.05$. Vertical bars denote standard errors of mean values (n = 5).

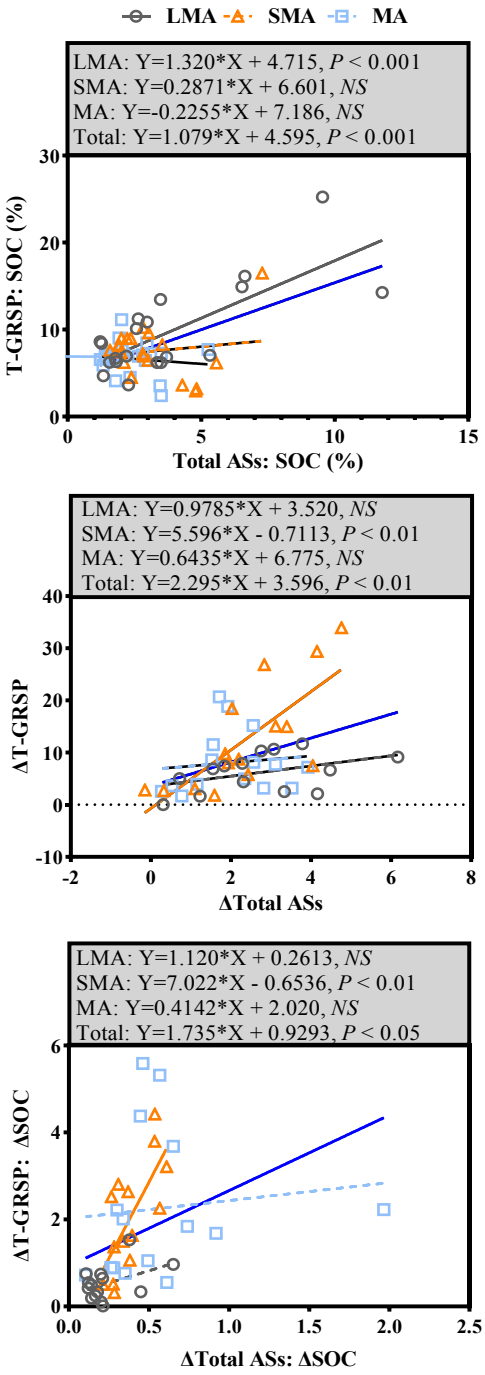


**Figure 5.** The correlations between contributions or relative change rates of total extractable glomalin-related soil proteins (T-GRSP) and amino sugars (ASs) within the large macroaggregates (LMA), small macroaggregates (SMA) and microaggregates (MA) fractions and in total. The solid blue line represents the correlation for all three sizes of aggreagtes; NS indicates no significance at $P < 0.05$.

### 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 of the accumulation of amino sugars in soil (Fig. 6a,c, $P < 0.001$). The contribution of total GRSP to SOC was mainly affected by fungal biomass and SOC (Fig. 6b, $P < 0.001$). The contribution of amino sugars to SOC was mainly affected by SOC, actinomycete biomass, GP biomass and soil C:P ratio (Fig. 6d, $P < 0.001$).

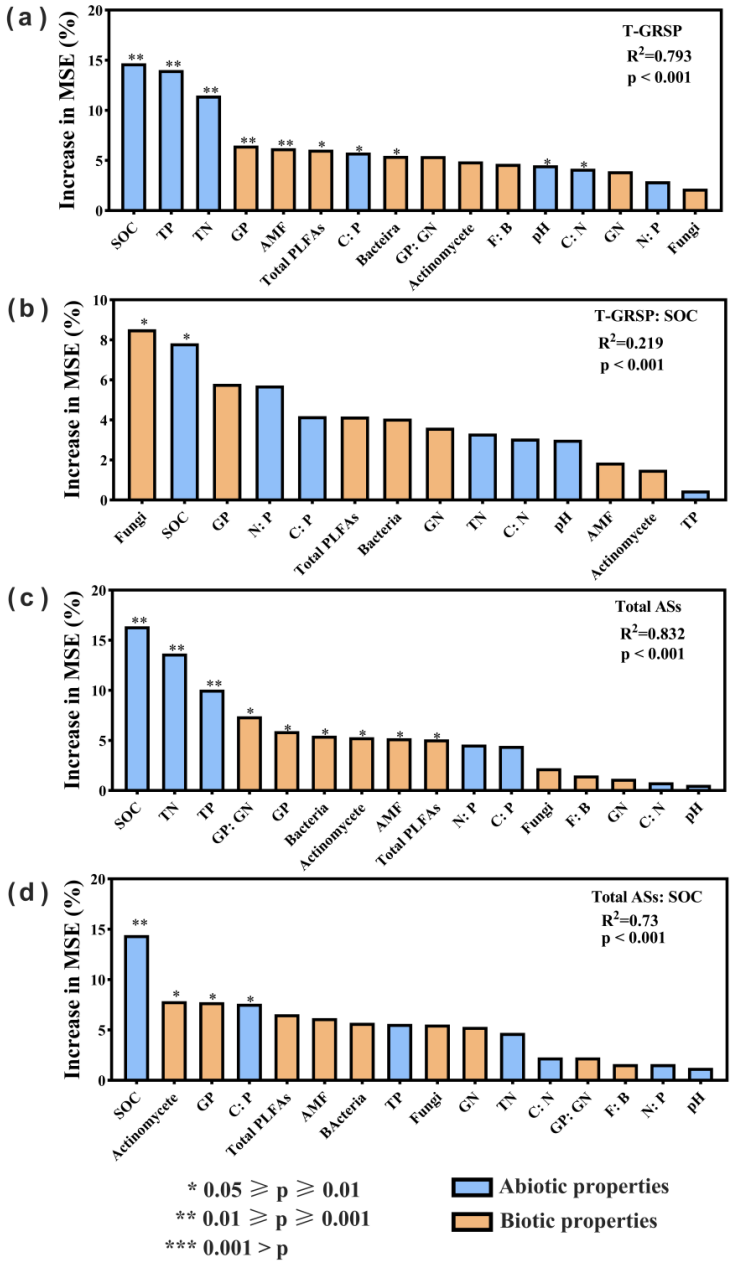


**Figure 6.** The average predictive importance (mean square error (MSE) increase percentage) for all soil abiotic and biotic properties for concentrations and contributions of total amino sugars (ASs) and total extractable glomalin-related soil proteins (T-GRSP) to soil organic carbon (SOC). TP, total phosphorus; TN, total nitrogen; C:P, SOC:TP; C:N, SOC:TN; AMF, N:P, TN:TP; arbuscular mycorrhizal fungi; GP,

Gram-positive bacteria; GN, Gram-negative bacteria; F, fungi; B, bacteria and total microbial biomass (total PLFAs).

## 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 which confirmed our first hypothesis (Fig. 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 (Fig. 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 conditions (e.g., low pH) for bacterial growth in subtropical forest soils compared with cropland (Angst et al., 2021). The contribution of different microorganisms to SOC with succession changes was also observed in other studies (Shao et al., 2017).

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 (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

371 restoration was synergistic (Fig. 5). AMF could not only delay the turnover of macroaggregates providing
372 more time and space for metabolic interactions between AMF and their associated microbiota (Rillig &
373 Mummey, 2006) but also accelerate the turnover of microaggregates (Morris et al., 2019).
374 Macroaggregates contain larger pore spaces, more AMF hyphae and more GRSP which might facilitate
375 the synergistic accumulation of amino sugars and glomalin (Lovelock et al., 2004). However, the
376 underlying mechanism should be explored via manipulative experiments and microscopic observation in
377 future research.
378
379 **4.2. Faster accumulation and greater contribution of GRSP to the SOC pool than of amino sugars**
380 **during forest restoration**
381 Our data support our second hypothesis that GRSP comprised a larger component of SOC than amino
382 sugars did and therefore accumulated faster during forest restoration (Figs 2, 3 and 4). This can be
383 explained as follows. First, GRSP is likely more stable than soil amino sugars (Agnihotri et al., 2022;
384 Rillig et al., 2001). GRSP is composed of > 50% recalcitrant components such as aromatic- and alkyl-C,
385 and has a greater chemical recalcitrance than amino sugars (Agnihotri et al., 2022). GRSP has a higher
386 propensity to form stable aggregates via binding mineral and organic particles than amino sugars do
387 (Gunina & Kuzyakov, 2015). Conversely, amino sugars may establish relatively fewer bonds with
388 minerals than glomalin does. Second, AMF may reduce bacterial biomass and soil amino sugar
389 concentrations due to nutrient deficiency (He et al., 2020), which would intensify the different
390 accumulation rates of GRSP and amino sugars. Third, GRSP concentrations are positively correlated
391 with net primary productivity (NPP) globally and higher plant productivity can provide more available
392 C to AM fungi for glomalin production (Treseder & Turner, 2007). NPP may increase with vegetation
393 restoration on barren land, if AM host plants are more abundant for the fungi that would benefit the
394 accumulation of glomalin (Treseder & Turner, 2007) and promote the microbial-derived C in soil. In the
395 study, *Eucalyptus exserta* (EP) associates with both arbuscular and ectomycorrhizal fungi, all dominant
396 tree species in NF are arbuscular mycorrhizal, while *Acacia auriculaeformis* as a dominant species in
397 MF is ectomycorrhizal, and *Calamus tetradactylus* is ectomycorrhizal as a dominant herb in MF.
398 However, Guo et al. (2021) found that the contribution of amino sugars to SOC in karst soils increased
399 with vegetation restorations and more bacterial-derived C accumulated, which might be associated with
400 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 microaggregates than in macroaggregates (Fig. 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 micro-environment. The contribution of microbial-derived products to SOC pools decreased with decreasing aggregate fraction size, suggesting that 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 Six et al. (2002) and Stewart et al. (2008), when SOC was 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 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 indicates 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).

### 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 (Fig. 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 (Lavallee 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, and further facilitated the release and accumulation of glomalin in soils (Li et al., 2020; Wright & Anderson, 2000). Actinomycetes are aerobic spore-forming Gram-positive bacteria characterized by substrate and aerial mycelium growth (Bhatti et al., 2017), whereas Gram-positive bacteria have a thicker peptidoglycan cell wall (Joergensen, 2018; Liang et al., 2019). Hence, proliferation of Gram-positive 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 enzymes activities.

The present results highlight the greater benefits for soil C-accumutation and nutrient fertility associated afforestation with native versus exotic fast-growing species. The capacity to 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. 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 this suggests greater biodiversity conservation and service provision such as mitigation of climate chage with native reforestation (Wu et al., 2021).

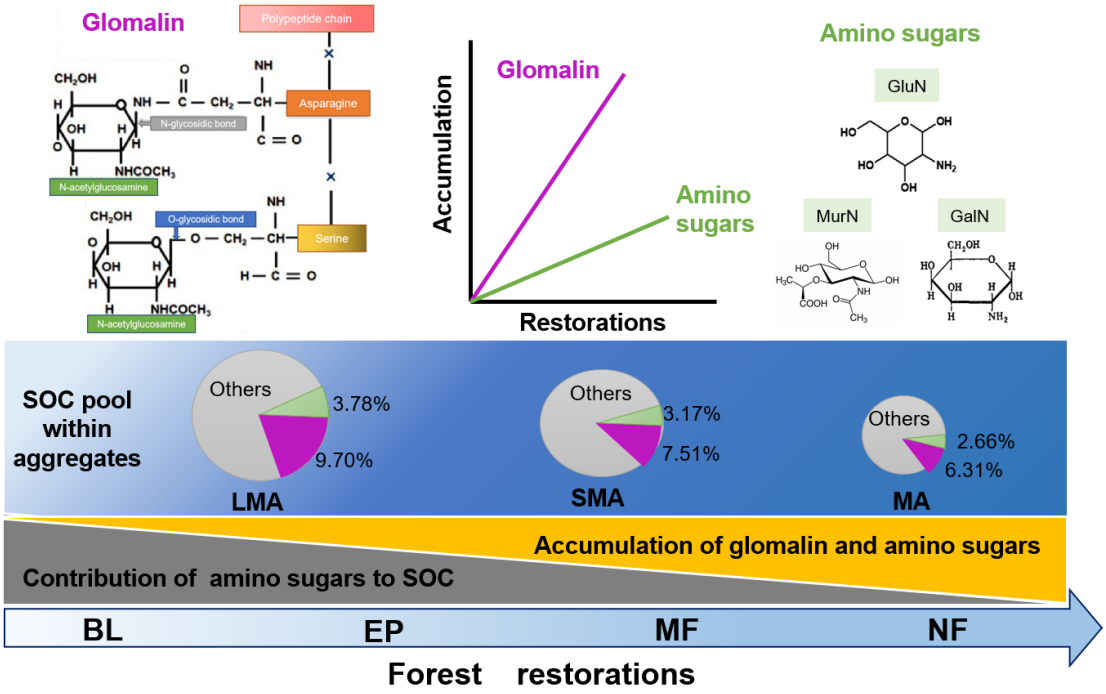


**Figure 7.** A conceptual diagram illustrating the accumulation and contribution of glomalin and amino sugars to soil organic carbon (SOC) within soil aggregate fractions along a restoration chronosequence. LMA, large macroaggregates; SMA, small macroaggregates; MA, microaggregates; BL, bare land; EP, *Eucalyptus* plantation; MF, native species mixed forest; NF, native forest.

## 5. CONCLUSIONS

Our study provides new insight into the accumulation and contribution of glomalin and amino sugars to the SOC pool during forest restoration (Fig. 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. 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.

**ACKNOWLEDGMENTS**

This work was supported by the National Key Research and Development Program of China (No. 2021YFC3100405), the National Natural Science Foundation of China (42177289), the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (Grant No. GML2019ZD0408), the Youth Innovation Promotion Association CAS (2019340), the Spanish Government projects PID2019-110521GB-I00 and PID2020115770RB-I, Fundación Ramón Areces project CIVP20A6621 and Catalan government project SGR2017-1005.

**CONFLICT OF INTEREST**

The authors declare to have no conflict of interest.

**REFERENCES**

Agnihotri, R., Bharti, A., Ramesh, A., Prakash, A. & Sharma, M. P. (2021). Glomalin related protein and C16:1 ω 5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. *European Journal of Soil Biology, 103*, 103286. https://doi.org/10.1016/j.ejsobi.2021.103286

Agnihotri, R., Sharma, M. P., Prakash, A., Ramesh, A., Bhattacharjya, S., Patra, A. K., Manna, M. C., Kurganova, I. & Kuzyakov, Y. (2022). Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. *Science of The Total Environment, 806*, 150571. https://doi.org/10.1016/j.scitotenv.2021.150571

Angst, G., Mueller, K. E., Eissenstat, D. M., Trumbore, S., Freeman, K. H., Hobbie, S. E., Chorover, J., Oleksyn, J., Reich, P. B. & Mueller, C. W. (2019). Soil organic carbon stability in forests: Distinct effects of tree species identity and traits. *Global Change Biology, 25*, 1529-1546. https://doi.org/10.1111/gcb.14548

Angst, G., Mueller, K. E., Nierop, K., G. J. & Simpson., M. J. (2021). Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry, 156*, 108189. https://doi.org/10.1016/j.soilbio.2021.108189

503 Bach, E. M., Williams, R. J., Hargreaves, S. K., Yang, F. & Hofmockel, K. S. (2018). Greatest soil
504 microbial diversity found in micro-habitats. *Soil Biology and Biochemistry, 118*, 217-226.
505 https://doi.org/10.1016/j.soilbio.2017.12.018
506 Bhatti, A. A., Haq, S. & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health.
507 *Microbial Pathogenesis, 111*, 458-467. https://doi.org/10.1016/j.micpath.2017.09.036
508 Bossio, D. & Scow, K. (1998). Impacts of carbon and flooding on soil microbial communities:
509 phospholipid fatty acid profiles and substrate utilization patterns. *Microbial Ecology, 35*, 265-278.
510 https://doi.org/10.1007/s002489900082
511 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of
512 protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248-254.
513 https://doi.org/10.1006/abio.1976.9999
514 Breiman, L. (2001). Random forests. *Machine Learning, 45*, 5-32.
515 https://doi.org/10.1023/A:1010933404324
516 Buckeridge, K. M., La Rosa, A. F., Mason, K. E., Whitaker, J., Mcnamara, N. P., Grant, H. K. & Ostle,
517 N. J. (2020). Sticky dead microbes: Rapid abiotic retention of microbial necromass in soil. *Soil*
518 *Biology and Biochemistry, 149*, 107929. https://doi.org/10.1016/j.soilbio.2020.107929
519 Canadell, J. G. & Raupach, M. R. (2008). Managing forests for climate change mitigation. *Science, 320*,
520 1456-7. https://doi.org/10.1126/science.1155458
521 Capellesso, E. S., Cequinel, A., Marques, R., Sausen, T. L., Bayer, C. & Marques, M. C. M. (2021). Co-
522 benefits in biodiversity conservation and carbon stock during forest regeneration in a preserved
523 tropical landscape. *Forest Ecology and Management, 492*, 119222.
524 https://doi.org/10.1016/j.foreco.2021.119222
525 Chen, G. P., Ma, S. H., Tian, D., Xiao, W., Jiang, L., Xing, A. J., Zou, A. L., Zhou, L. H., Shen, H. H.,
526 Zheng, C. Y., Ji, C. J., He, H. B., Zhu, B., Liu, L. L. & Fang, J. Y. (2020). Patterns and determinants
527 of soil microbial residues from tropical to boreal forests. *Soil Biology and Biochemistry, 151*,
528 108059. https://doi.org/10.1016/j.soilbio.2020.108059
529 Cotrufo, M. F., Ranalli, M. G., Haddix, M. L. Six, J. & Lugato, E. (2019). Soil carbon storage informed
530 by particulate and mineral-associated organic matter. *Nature Geoscience, 12*, 989-994.
531 https://doi.org/10.1038/s41561-019-0484-6
532 Crouzeilles, R., Ferreira, M. S. & Curran, M. (2016). Forest restoration: a global dataset for biodiversity

and vegetation structure. *Ecology, 97*, 2167. https://doi.org/10.1002/ecy.1474

Cui, J., Zhu, Z. K., Xu, X. L., Liu, S. L., Jones, D. L., Kuzyakov, Y., Shibistova, O., Wu, J. S. & Ge, T. (2020). Carbon and nitrogen recycling from microbial necromass to cope with C: N stoichiometric imbalance by priming. *Soil Biology and Biochemistry, 142*, 107720. https://doi.org/10.1016/j.soilbio.2020.107720

Duarte, C. M., Losada, I. J., Hendriks, I. E., Mazarrasa, I. & Marbà, N. (2013). The role of coastal plant communities for climate change mitigation and adaptation. *Nature Climate Change, 3*, 961-968. https://doi.org/10.1038/nclimate1970

Glaser, B., Turrión, M.-B., Alef, K. (2004). Amino sugars and muramic acid-biomarkers for soil microbial community structure analysis. *Soil Biology and Biochemistry, 36*, 399-407. https://doi.org/10.1016/j.soilbio.2003.10.013

Gunina, A. & Kuzyakov, Y. (2015). Sugars in soil and sweets for microorganisms: Review of origin, content, composition and fate. *Soil Biology and Biochemistry, 90*, 87-100. https://doi.org/10.1016/j.soilbio.2015.07.021

Guo, Z. M., Zhang, X. Y., Dungait, J. A. J., Green, S. M., Wen, X. F. & Quine, T. A. (2021). Contribution of soil microbial necromass to SOC stocks during vegetation recovery in a subtropical karst ecosystem. *Science of The Total Environment, 761*, 143945. https://doi.org/10.1016/j.scitotenv.2020.143945

Gupta V. V. S. R. & Germida, J. J. (2015). Soil aggregation: Influence on microbial biomass and implications for biological processes. *Soil Biology and Biochemistry, 80*, A3-A9. https://doi.org/10.1016/j.soilbio.2014.09.002

He, X. X., Li, X. B., Liu, T., Yang, X. J., Cao, J. B., Tao, L. B., Wang, X. L., Liu, Z. F., Yao, Q., Li, Y. T., Zou, X. M., Shao, Y. H., Li, J. X., Zhang, W. X. & Fu, S. (2020). Earthworms negate the adverse effect of arbuscular mycorrhizae on living bacterial biomass and bacterial necromass accumulation in a subtropical soil. *Soil Biology and Biochemistry, 151*, 108052. https://doi.org/10.1016/j.soilbio.2020.108052

Hisano, M., Searle, E. B. & Chen, H. Y. H. (2018). Biodiversity as a solution to mitigate climate change impacts on the functioning of forest ecosystems. *Biological Reviews, 93*, 439-456. https://doi.org/10.1111/brv.12351

Hu, P. L., Xiao, J., Zhang, W., Xiao, L. M., Yang, R., Xiao, D., Zhao, J. & Wang, K. L. (2020). Response

of soil microbial communities to natural and managed vegetation restoration in a subtropical karst region. *Catena, 195*, 104849. https://doi.org/10.1016/j.catena.2020.104849

Indorf, C., Dyckmans, J., Khan, K. S. & Joergensen, R.G. (2011). Optimisation of amino sugar quantification by HPLC in soil and plant hydrolysates. *Biology and Fertility of Soils, 47*, 387-396. https://doi.org/10.1007/s00374-011-0545-5

Irving, T. B., Alptekin, B., Kleven, B. & Ane, J. M. (2021). A critical review of 25 years of glomalin research: A better mechanical understanding and robust quantification techniques are required. *New Phytologist, 232,* 1572-1581. https://doi.org/10.1111/nph.17713

Jackson, R. B., Lajtha, K., Crow, S. E., Hugelius, G., Kramer, M. G. & Piñeiro, G. (2017). The ecology of soil carbon: Pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology, Evolution, and Systematics, 48*, 419-445. https://doi.org/10.1146/annurev-ecolsys-112414-054234

Jiao, S., Chen, W. M., Wang, J. L., Du, N. N., Li, Q. P. & Wei, G. H. (2018). Soil microbiomes with distinct assemblies through vertical soil profiles drive the cycling of multiple nutrients in reforested ecosystems. *Microbiome, 6*, 146. https://doi.org/10.1186/s40168-018-0526-0

Joergensen, R. G. (2018). Amino sugars as specific indices for fungal and bacterial residues in soil. *Biology and Fertility of Soils, 54*, 559-568. https://doi.org/10.1007/s00374-018-1288-3

Lal, R., Monger, C., Nave, L. & Smith, P. (2021). The role of soil in regulation of climate. *Philosophical Transactions of the Royal Society B: Biological Sciences, 376*, 20210084. https://doi.org/10.1098/rstb.2021.0084

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E. & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications, 6*, 6707. https://doi.org/10.1038/ncomms7707

Lavallee, J. M., Soong, J. L. & Cotrufo, M. F. (2020). Conceptualizing soil organic matter into particulate and mineral□associated forms to address global change in the 21st century. *Global Change Biology, 26*, 261-273. https://doi.org/10.1111/gcb.14859

Liang, C. (2020). Soil microbial carbon pump: Mechanism and appraisal. *Soil Ecology Letters, 2*, 241-254. https://doi.org/10.1007/s42832-020-0052-4

Liang, C., Amelung, W., Lehmann, J. & Kästner, M. (2019). Quantitative assessment of microbial necromass contribution to soil organic matter. *Global Change Biology, 25*, 3578-3590.

https://doi.org/10.1111/gcb.14781

Liang, C., Kästner, M. & Joergensen, R. G. (2020). Microbial necromass on the rise: The growing focus on its role in soil organic matter development. *Soil Biology and Biochemistry, 150*, 108000. https://doi.org/10.1016/j.soilbio.2020.108000

Lovelock, C. E., Wright, S. F. & Nichols, K. A. (2004). Using glomalin as an indicator for arbuscular mycorrhizal hyphal growth: An example from a tropical rain forest soil. *Soil Biology and Biochemistry, 36*, 1009-1012. https://doi.org/10.1016/j.soilbio.2004.02.010

Lu, R. K. (2000). *Analytical methods for soils and agricultural chemistry*. Beijing: Scientific and Technology Press.

Ma, Z., Melville, D. S., Liu, J., Chen, Y., Yang, H., Ren, W., Zhang, Z., Piersma, T. & Li, B. (2014). Rethinking china's new great wall. *Science, 346*, 6212. https://doi.org/10.1126/science.1257258

Malik, A. A., Puissant, J., Buckeridge, K. M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H. S., Peyton, J. M., Mason, K. E., van Agtmaal, M., Blaud, A., Clark, I. M., Whitaker, J., Pywell, R. F., Ostle, N., Gleixner, G. & Griffiths, R. I. (2018). Land use driven change in soil pH affects microbial carbon cycling processes. *Nature Communications, 9*, 3591. https://doi.org/10.1038/s41467-018-05980-1

Morris, E. K., Morris, D. J. P., Vogt, S., Gleber, S. C., Bigalke, M., Wilcke, W. & Rillig, M. C. (2019). Visualizing the dynamics of soil aggregation as affected by arbuscular mycorrhizal fungi. *The ISME Journal, 13*, 1639-1646. https://doi.org/10.1038/s41396-019-0369-0

Mou, Z. J., Kuang, L. H., Yan, B. Y., Zhang, X. Y., Wang, Y. Q. & Liu, Z. F. (2020). Influences of sample storage and grinding on the extraction of soil amino sugars. *Soil Ecology Letters, 20*, 157-163. https://doi.org/10.1007/s42832-020-0031-9

Murugan, R., Djukic, I., Keiblinger, K., Zehetner, F., Bierbaumer, M., Zechmeister-Bolternstern, S. & Joergernsen, R. G. (2019). Spatial distribution of microbial biomass and residues across soil aggregate fractions at different elevations in the Central Austrian Alps. *Geoderma, 339*, 1-8. https://doi.org/10.1016/j.geoderma.2018.12.018

Navas, M., Martín Lammerding, D., Hontoria, C., Ulcuango, K. & Mariscal Sancho, I. (2021). The distinct responses of bacteria and fungi in different□sized soil aggregates under different management practices. *European Journal of Soil Science, 72*, 1177-1189. https://doi.org/10.1111/ejss.12997

623 Qiao, L. L., Li, Y. Z., Song, Y. H, Zhai, J. Y, Wu, Y., Chen, W. J., Liu, G. B. & Xue, S. (2019). Effects of
624 vegetation restoration on the distribution of nutrients, glomalin-related soil protein, and enzyme
625 activity in soil aggregates on the loess plateau, china. *Forests, 10*, 796.
626 https://doi.org/10.3390/f10090796
627 Ren, H., Li, Z. A., Shen, W. J., Yu, Z. Y., Peng, S. L., Liao, C. H., Ding, M. M. & Wu, J. G. (2007).
628 Changes in biodiversity and ecosystem function during the restoration of a tropical forest in south
629 China. *Science in China Series C: Life Sciences, 50*, 277-284. https://doi.org/10.1007/s11427-007-
630 0028-y
631 Rillig, M. C. & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist, 171*, 41-53.
632 https://doi.org/10.1111/j.1469-8137.2006. 01750.x
633 Rillig, M. C., Muller, L. A. & Lehmann, A. (2017). Soil aggregates as massively concurrent evolutionary
634 incubators. *The ISME Journal, 11*, 1943-1948. https://doi.org/10.1038/ismej.2017.56
635 Rillig, M. C., Wright, S. F., Nichols, K. A., Schmidt, W. F. & Torn, M. S. (2001). Large contribution of
636 arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant and Soil, 233*, 167-
637 177. https://doi.org/10.1023/A:1010364221169
638 Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M.,
639 Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S. &
640 Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature, 478*,
641 49-56. https://doi.org/10.1038/nature10386
642 Shao, P. S., Liang, C., Lynch, L., Xie, H. T. & Bao, X. L. (2019). Reforestation accelerates soil organic
643 carbon accumulation: Evidence from microbial biomarkers. *Soil Biology and Biochemistry, 131*,
644 182-190. https://doi.org/10.1016/j.soilbio.2019.01.012
645 Shao, S., Zhao, Y., Zhang, W., Hu, G. Q., Xie, H. T., Yan, J. H., Han, S. J., He, H. B. & Zhang, X. D.
646 (2017). Linkage of microbial residue dynamics with soil organic carbon accumulation during
647 subtropical forest succession. *Soil Biology and Biochemistry, 114*, 114-120.
648 https://doi.org/10.1016/j.soilbio.2017.07.007
649 Shen, F. F., Wu, J. P., Fan, H. B., Liu, W. F., Guo, X. M., Duan, H. L., Hu, L., Lei, X. M. & Wei, X. H.
650 (2019). Soil N/P and C/P ratio regulate the responses of soil microbial community composition and
651 enzyme activities in a long-term nitrogen loaded Chinese fir forest. *Plant and Soil, 436*, 91-107.
652 https://doi.org/10.1007/s11104-018-03912-y

Six, J., Conant, R. T., Paul, E. A. & Paustian, K. (2002). Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil, 241*, 155-176. https://doi.org/10.1023/A:1016125726789

Snäll, T., Triviño, M., Mair, L., Bengtsson, J. & Moen, J. (2021). High rates of short-term dynamics of forest ecosystem services. *Nature Sustainability, 4*, 951-957. https://doi.org/10.1038/s41893-021-00764-w

Stewart, C. E., Plante, A. F., Paustian, K., Conant, R. T. & Six, J. (2008). Soil carbon saturation: Linking concept and measurable carbon pools. *Soil Science Society America Journal, 72*, 379-392. https://doi.org/10.2136/sssaj2007.0104

Treseder, K. K. & Turner, K. M. (2007). Glomalin in ecosystems. *Soil Science Society of America Journal, 71*, 1257-1266. https://doi.org/10.2136/sssaj2006.0377

Upton, R. N., Bach, E. M. & Hofmockel, K. S. (2019). Spatio-temporal microbial community dynamics within soil aggregates. *Soil Biology and Biochemistry, 132*, 58-68. https://doi.org/10.1016/j.soilbio.2019.01.016

Vestal, J. R. & White, D. C. (1989). Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. *Bioscience, 39*, 535-541. https://doi.org/10.2307/1310976

Wang, W. J., Zhong, Z. L., Wang, Q., Wang, H. M., Fu, Y. J. & He, X. Y. (2017). Glomalin contributed more to carbon, nutrients in deeper soils, and differently associated with climates and soil properties in vertical profiles. *Scientific Reports, 7*, 13003. https://doi.org/10.1038/s41598-017-12731-7

Wright, S. F., Upadhyaya, A. & Buyer, J. S. (1998). Comparison of N-linked oligosaccharides of glomalin from arbuscular mycorrhizal fungi and soils by capillary electrophoresis. *Soil Biology and Biochemistry, 30*, 1853-1857. 10.1016/S0038-0717(98)00047-9

Wright, S.F. & Upadhyaya, A. (1996). Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science, 161*, 575-586. http://dx.doi.org/10.1097/00010694-199609000-00003

Wu, W. J., Kuang, L. H., Li, Y., He, L. F., Mou, Z. J., Wang, F. M., Zhang, J., Wang, J., Li, Z. A., Lambers, H., Sardans, J., Peñuelas, J., Geisen, S. & Liu, Z. F. (2021). Faster recovery of soil biodiversity in native species mixture than in Eucalyptus monoculture after 60 years afforestation in tropical degraded coastal terraces. *Global Change Biology, 27*, 5329-5340. https://doi.org/10.1111/gcb.15774

683 Xiao, L., Liu, G. B., Zhang, J. Y. & Xue, S. (2016). Long-term effects of vegetational restoration on soil
684 microbial communities on the Loess Plateau of China. *Restoration Ecology, 24*, 794-804.
685 https://doi.org/10.1111/rec.12374
686 Xie, H. T., Li, J. W., Zhang, B., Wang, L F.., Wang, J. K., He, H. B. & Zhang, X. D. (2015). Long-term
687 manure amendments reduced soil aggregate stability via redistribution of the glomalin-related soil
688 protein in macroaggregates. *Scientific Reports, 5*, 14687. 10.1038/srep14687
689 Xu, Y. D., Gao, X. D., Liu, Y. L., Li, S. Y., Liang, C., Lal, R. & Wang, J. K. (2022). Differential
690 accumulation patterns of microbial necromass induced by maize root vs. Shoot residue addition in
691 agricultural Alfisols. *Soil Biology and Biochemistry, 164*, 108474.
692 https://doi.org/10.1016/j.soilbio.2021.108474
693 Yao, Q., Zheng, L., & Peng, S. (1984). The erosion of weathering crust in granite tableland and its
694 remediation at Xiaoliang. *Tropical and Subtropical Forest Ecosystem, 2*, 91-106.
695 Ye, Y. Y., Xiao, S. S., Liu, S. J., Zhang, W., Zhao, J., Chen, H. S., Guggenberger, G. & Wang, K. L.
696 (2020). Tillage induces rapid loss of organic carbon in large macroaggregates of calcareous soils.
697 *Soil and Tillage Research, 199*, 104549. https://doi.org/10.1016/j.still.2019.104549
698 Yu, Z., & Pi, Y. (1985). The path and its effects of rehabilitating vegetation on the tropical littoral eroded
699 land in Guangdong province. *Tropical and Subtropical Forest Ecosystem Research, 3*, 97-108.
700 Yuan, Y., Li, Y., Mou, Z. J., Kuang, L. H., Wu, W. J., Zhang, J., Wang, F. M., Hui, D. F., Peñuelas, J.,
701 Sardans, J., Lambers, H., Wang, J., Kuang, Y. W., Li, Z. A. & Liu, Z. F. (2021). Phosphorus addition
702 decreases microbial residual contribution to soil organic carbon pool in a tropical coastal forest.
703 *Global Change Biology, 27*, 454-466. https://doi.org/10.1111/gcb.15407
704 Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of
705 microbial communities in soil: A review. *Biology and Fertility of Soils, 29*, 111-129,
706 https://doi.org/10.1007/s003740050533
707 Zhang, C., Zhang, X. Y., Zou, H. T., Kou, L., Yang, Y., Wen, X. F., Li, S. G., Wang, H. M. & Sun, X. M.
708 (2017). Contrasting effects of ammonium and nitrate additions on the biomass of soil microbial
709 communities and enzyme activities in subtropical China. *Biogeosciences, 14*, 4815-4827.
710 https://doi.org/10.5194/bg-14-4815-2017
711 Zhang, J., Li, J., Ma, L. L., He, X. H., Liu, Z. F., Wang, F. M., Chu, G. W. & Tang, X. L. (2022).
712 Accumulation of glomalin‐related soil protein benefits soil carbon sequestration with tropical

coastal forest restoration. *Land Degradation & Development*. https://doi.org/10.1002/ldr.4192

Zhang, J., Tang, X. L., Zhong, S. Y., Yin, G. C., Gao, Y. F. & He, X. H. (2017). Recalcitrant carbon components in glomalin-related soil protein facilitate soil organic carbon preservation in tropical forests. *Scientific Reports, 7*, 2391. https://doi.org/10.1038/s41598-017-02486-6

Zhang, S., Deng, Q., Wang, Y. P., Chen, J., Yu, M. X., Fang, X., He, H. B., Chen, J. L., Xu, P. P., Wang, S. H. & Yan, J. H. (2021). Linkage of microbial living communities and residues to soil organic carbon accumulation along a forest restoration gradient in southern China. *Forest Ecosystems, 8*, 57. https://doi.org/10.1186/s40663-021-00334-8