Accumulation of glomalin-related soil protein benefits to soil carbon sequestration with tropical coastal forest restoration

Running title: Glomalin-related soil protein benefits to soil carbon sequestration

Jing Zhang[1,2†], Jian Li[1,3†], Lingling Ma[1,4], Xinhua He[5], Zhanfeng Liu[1,2], Faming Wang[1], Guowei Chu[1], Xuli Tang[1*]

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

[3]*Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen, 361021, China*

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

[5]*Department of Land, Air and Water Resources, University of California at Davis, Davis, CA 95616, USA*

† These authors contributed equally to this work.

[*]**Corresponding author:**

Dr. Xuli Tang

Phone: +86-20-37089712

E-mail: xltang@scbg.ac.cn

Address: Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Abstract

Reforestation is widely used to restore degraded infertile soils in the coastal area. Substantial attention has been paid to the functioning of AMF in vegetation restoration because arbuscular mycorrhizal fungi (AMF) are considered beneficial to this process. However, little is known about the effect of AMF product, glomalin-related soil protein (GRSP), on soil organic carbon (SOC) sequestration during the forest restoration. We conducted a study in a tropical region where the native forest has been seriously deforested with only a few grasses and then a series of restoration approaches have been made to restore the forest ecosystem. The study sites include a barren land (BL), a *Eucalyptus exserta* planted forest (EF), a mixed broadleaved forest (MF) and a secondary natural forest (SF), which represents the un-, early-, middle- and late-restoration stage, respectively. The results showed that the restoration increased EE-GRSP and T-GRSP by 3.9-12.3 times and 1.9-4.6 times compared with the barren land, respectively. The proportion of GRSP in SOC is 1.6-2.0% (EE-GRSP/SOC) and 6.5-15.8% (T-GRSP/SOC), respectively. Also, a significantly positive relationship was found between the proportion of GRSP in SOC and recalcitrant SOC composition percentage (aromatic C), as well as between GRSP and soil aggregate stability. These results together suggest that the restoration of the degraded tropical forest is beneficial to soil C sequestration with the accumulation of GRSP, most likely, through an improvement of the soil aggregate stability and increase of the proportion of recalcitrant soil C chemical composition.

Keywords: mixed broadleaved forest, *Eucalyptus exserta* forest, secondary natural forest, soil aggregate, soil carbon chemical composition

1. Introduction

Forests cover one-third of the total land area and store over half of the global soil carbon (C), which occupies an indispensable position in the global C cycle (Kuuluvainen & Gauthier, 2018; Zhu et al., 2017). Forest ecosystems can be readily managed to either increase or decrease C sequestration by restoring or degrading vegetation (Tang et al., 2018). Compared with biomass C, more C is stored in the soil, mostly as soil organic carbon (SOC). A recent study has found that on average soil C is about three times higher than vegetation C in Chinese forests (Tang et al., 2018). Therefore, even a small change in SOC of forest ecosystems could have far-reaching impacts on the global climate. Reforestation and afforestation are common practices that can contribute to negative CO_2 emissions since the growth of additional plants sequesters atmospheric CO_2 and naturally sinks it in plant biomass and soil. Large afforestation and reforestation programs have been undertaken, and the afforestation area is 26.7 Mha (1 Mha = 10^6 ha) from 2000 to 2019 (FAO, 2020). For example, the existing afforestation projects in China have increased the forest area by 2 Mha per year during the 1990s and 3 Mha per year since 2000. Such afforestation has resulted in an estimated total emission reduction of 4.4 Mt CO_2 equivalent (FAO, 2020; Zhou, Gong, & Gao, 2017).

Arbuscular mycorrhizal fungi (AMF) play an important role in forest restoration in the erosion area with infertility soils at the early/middle restoration stage. For instance, they colonize approximately 80% of terrestrial vascular plants (Smith & Read, 2008), provide benefits for their hosts and contribute to plant vigor by improving nutrient uptake and increasing their survival in poor soil (Asmelash, Bekele, & Birhane, 2016; Bonfim,

Vasconcellos, Sturmer, & Cardoso, 2013). AMF can also improve the stability of soil aggregates (Morris et al., 2019) and contribute to soil C stock because plants allocate approximately 4-20% of photosynthates to AMF (Bago, Pfeffer, & Shachar-Hill, 2000). Furthermore, glomalin, mostly produced by AMF, is an important SOC component and remains in the soil after the hyphae senescence (Treseder, Turner, & Mack, 2007).

Glomalin is a kind of hydrophobic glycoprotein. Due to the difficulty in obtaining a pure compound, glomalin is often operationally defined as glomalin-related soil protein (GRSP) according to its extraction method (Rillig, 2004a). GRSP contains ~20-35% C and accounts for even up to 40% of SOC (He, Li, & Zhao, 2010; Lovelock, Wright, Clark, & Ruess, 2004), as well as riches in recalcitrant aromatic C that can reside in the soil for decades (Schindler, Mercer, & Rice, 2007; Q. Wang et al., 2020). GRSP also increases with soil chronosequence (Kumar, Singh, & Ghosh, 2018; Matthias C. Rillig, Wright, Nichols, Schmidt, & Torn, 2001). A global-scale study has shown that GRSP reserves are affected by the ecosystem's net primary productivity and increase with the plant community diversity in specific ecosystems (Treseder et al., 2007). However, to our knowledge, studies on GRSP have been mainly focused on grassland, farmland or wetland ecosystems (H. F. Liu et al., 2020; Tian et al., 2020; S. Wright, Green, & Cavigelli, 2007), but not in forest ecosystems. In addition, it is not clear how changes in GRSP and/or the accumulation of GRSP can relate to soil C storage in the forest restoration process in erosion areas.

Previous studies have shown that soil C stock increased markedly in forest restoration in southern China (F. Wang et al., 2013; H. Zhang et al., 2019). Therefore, to investigate the relationship between the accumulation of GRSP and SOC sequestration in the forest ecosystem restoration in erosion areas, a series of restoration approaches that have been

conducted for nearly 60 years (1959-2016) were selected in this study. The restoration series included a barren land (BL, reference), a *Eucalyptus exserta* plantation forest (EF), a mixed broadleaved forest (MF) and a secondary natural forest (SF), which represent the un-, early-, middle- and late-restoration stage of the forest ecosystem, respectively. The objective of the present study was to compare the differences in GRSP, SOC, soil aggregate stability and soil C chemical composition among different forest restoration stages. Firstly, we hypothesized that GRSP would be increased along with the increase of AMF biomass during forest restoration (Zhong, Zhang, Chu, Xia, & Tang, 2017). Secondly, we hypothesized that the proportion of GRSP in SOC would also be increased with the forest restoration since GRSP is a recalcitrant component in SOC. Finally, we hypothesized that GRSP would be beneficial to soil C sequestration since GRSP plays important role in soil aggregate stability during forest restoration (S. Wright & Upadhyaya, 1998; J. Zhang et al., 2017).

2. Materials and Methods

2.1 Study sites and soil sampling

This study was performed in a restoration sequence of tropical coastal forest ecosystems at the Xiaoliang Station in southwest Guangdong, China (2l°27′49″N, 110°54′18″E, 10 m above the sea level). The station has a tropic monsoon climate and a mean annual temperature of 23°C. The precipitation is 1,400-1,700 mm with a contrasting dry (October-April) and wet season (May-September). Climax vegetation is a secondary monsoon evergreen broadleaved forest, however, which at present rarely exists due to human logging before the 1950s. The zonal soil is latosol, which has developed from granite but being endured heavy erosion for more than 70 years (Ren et al., 2007). The maximum monthly temperature in July on the soil surface is 47.5 °C due to extremely eroded and bareness, which is about 17 °C higher than the

ambient air temperature. The soil moisture was 17.6% at the top 20 cm soil layer and soil total C and nitrogen concentration was only 0.6% and 0.03%, respectively (Ren et al., 2007). These extreme soil conditions preventing from natural vegetation restoration. Hence, a series of afforestation practices have been conducted since 1959 with a barren land (BL) as the reference and three restoration treatments. The BL (3.7 ha) was then left alone without any human interference, and still no abundant plants except a few kinds of grass such as *Dicranopteris pedata* and *Eriachne pallescens* existing. The restoration campanign was firstly planted with *Eucalyptus exserta* seedlings (2 years old, 3.9 ha) with a rotation cyle of 5-8 years. Half of the *Eucalyptus exserta* plantation was clear-cut during 1964-1975 and replanted with 12 tree species to build a mixed forest (MF). We did not directly create an MF on the barren land because the native species could not be established in extreme soil if there was no successful growth of this pioneer *Eucalyptus* (Ren et al. 2007). The MF could develop into secondary natural evergreen broadleaved forests that are similar to the undisturbed secondary natural forest (SF) ambient (Ren et al., 2007). As a result, the restoration stage treatments including 1) the barren land as the reference (BL), 2) *Eucalyptus exserta* plantation forest (EF), 3) a planted mixed broadleaved forest (MF) and 4) a secondary natural forest (SF) nearby without clear-cut and erosion (Fig. 1). See Ren, Chen, Li, and Han (2010) and Table 1 for more detailed information about soil and microbial properties in this study site.

Five arranged plots (20 × 20 m each) with a > 20 m distance of each other were set for each of the four restoration stage treatments, and seven soil cores (2.5 cm diameter at 10 cm depth) from each plot were randomly collected and mixed as one composite sample in June 2016. Besides, a block of 1.0 kg undeformed soil (0-10 cm) was removed from each plot and stored in a hard plastic box for soil aggregates testing. A total of 20 (4 restoration stages treatments

147 × 5 replicates) samples were thus collected for the measurement of soil aggregates, and
148 measurement of GRSP and soil C chemical composition, etc., respectively. After transferred
149 all the samples to the lab, each composite sample was sifted through a 2 mm mesh sieve and
150 then divided into 2 subsamples, one was for microbial analysis immediately, and another was
151 air-died for GRSP, SOC, etc measurement.
152
153 *2.2 Laboratory measurements*
154 *2.2.1 Glomalin-related soil proteins*
155 Both the easily extracted GRSP (EE-GRSP) and total GRSP (T-GRSP) were measured by a
156 modified method mentioned by Zhang, Tang, Zheng, Tong, and Chen (2014) based on the
157 Bradford protein assay (S. Wright & Upadhyaya, 1998). Briefly, EE-GRSP or T-GRSP was
158 extracted by 8 mL of 20 mmol L^{-1} sodium citrate (pH = 7.0) or 50 mmol L^{-1} of sodium citrate
159 (pH = 8.0) from 1.00 g DW soil. Then the extractions were autoclaved for 30 (EE-GRSP) or
160 60 (T-GRSP) minutes at 121 °C and centrifuged with 10,000 × g for 10 minutes later. The T-
161 GRSP extraction process was performed 4 times and then all supernatant pooled together and
162 stored at 4 °C before the Bradford analysis. The optical density (OD) value of the GRSP was
163 measured at 595 nm by using bovine serum albumin (BSA) as the standard with an enzyme
164 microplate reader (Thermo Multiskan FC, USA).
165
166 *2.2.2 Soil organic carbon concentration and chemical composition*
167 The content of SOC was tested by titration with $FeSO_4$ (0.2 mol l^{-1}) after dichromate
168 oxidation (G. Liu, Jiang, Zhang, & Liu, 1996). The soil C chemical composition was
169 measured by ^{13}C cross-polarization magic angle spinning (CPMAS) nuclear magnetic
170 resonance (NMR). In brief, 8 g air-dried soil was pretreated with a hydrofluoric acid solution
171 (HF) 8 times as described in Mathers, Xu, Blumfield, Berners-Price, and Saffigna (2003).

The NMR signals were collected on an AVANCE III spectrometer (Bruker Ascend™ 300 WB, Bruker, Karlsruhe, Germany) as described in Zhang et al. (2017) with a minor modification of 12,000 scans. The area below each spectrum was integrated and separated into 4 different C functional groups based on their chemical shift values, including alkyl C (0-50 ppm), O-alkyl C (50-110 ppm), aromatic C (110-160 ppm) and carbonyl C (160-220 ppm) (Mao et al., 2000; Ono, Hiradate, Morita, Ohse, & Hirai, 2011). In this study, the soil C concentration in the BL was too low to obtain useful NMR signals.

2.2.3 Soil aggregates

Soil aggregates were measured by the wet sieve method according to Cambardella and Elliott (1993). Soil aggregates were divided into 4 classes of particular sizes: <0.053 mm, 0.053-0.25 mm, 0.25-2.00 mm and >2.00 mm. Briefly, 200 g air-dried soil sample was gently placed on the first sieve (2.00 mm) and then capillary rewetted with distilled water and incubated for 5 minutes, and then put the sieves into the bucket for mechanical wet sieving. Sieves oscillated at a frequency of 50 cycles per minute for 30 minutes. Materials on the sieves were washed gently into pre-weighed aluminum specimen boxes, dried at 105 °C and weighed after cooling down. Mean weight diameter (MWD) was used to evaluate soil aggregation stability and calculated as follows (B. Zhang & Horn, 2001):

$$\mathrm{MWD} = \sum_{n=1}^{n+1} \frac{r_{i-1}+r_i}{2} \times m_i = \sum_{n=1}^{n+1} \frac{r_{i-1}+r_i}{2} \times m_i$$

Where, r_i is the aperture of the i_{th} mesh (mm), $r_0=r_1$ and $r_n=r_{n+1}$; m_i is the fraction of aggregation remaining on the i_{th} sieve and n is the number of the soil aggregates size fraction (n = 4, represents <0.053 mm, 0.053-0.25 mm, 0.25-2.00 mm and >2.00 mm).

2.2.4 Soil microbial biomass and arbuscular mycorrhizal fungi diversity

196 Soil microbial and arbuscular mycorrhizal fungi biomass were characterized by using the
197 phospholipid fatty acid (PLFA) method as described in Frostegård and Bååth (1996) with
198 minor modifications. Selected PLFA biomarkers were used to present different soil microbes
199 (J. Li, Zhou, Alaei, and Bengtson., 2020) and the specific biomarker 16:1ω5 as the AMF
200 biomarker (J. Zhang, Ekblad, Sigurdsson, & Wallander, 2020). Microbial and AMF biomass
201 was calculated as nmol g^{-1} based on the internal standard (19:0) concentration. AMF diversity
202 was analyzed by high throughput sequencing on an Illumina HiSeq2000 platform as
203 described in J. Zhang et al. (2020). AMF α-diversity was calculated by the Shannon-Wiener
204 index. The α-diversity could not be presented in the BL due to the low DNA concentration of
205 AMF.
206

207 *2.3 Statistical analysis*

208 Statistical analyses were conducted after all data had been checked for normal distribution
209 and homogeneity. With an SPSS 24.0 statistical software (IBM, Armonk, NY, USA), one-
210 way ANOVA was applied to compare the significant difference ($P < 0.05$) of soil nutrient
211 status, GRSP, soil aggregates and soil C chemical composition among different restoration
212 stages treatments. Linear regressions were used to test the relationship between the
213 proportion of GRSP (GRSP/SOC) and the percentage of different soil C chemical groups, and
214 between GRSP and SOC. Correlation analyses were used to test the relationship between
215 GRSP and different soil aggregates.
216

3. Results

3.1 The concentration of GRSP and SOC

EE-GRSP concentration ranged from 0.14 to 1.91 mg g^{-1} dry soil and significantly increased by 3.9-12.3 times from EF to SF compared with the BL ($P < 0.05$, Fig. 2A). Similarly, T-GRSP concentration also increased ($P < 0.05$) with the forest restoration and its highest value (7 mg g^{-1}) was found in the SF, which was 4.6 times higher than the BL (Fig. 2A). The ratio of EE-GRSP to T-GRSP (EE-GRSP/T-GRSP), ranging from 0.1 to 0.3, increased from BL to MF ($P < 0.05$), but decreased slightly from MF to SF ($P > 0.05$, Fig. 2B). The SOC concentration significantly increased with the vegetation restoration process. Particularly, SOC in the vegetation restoration sites (EF, MF and SF) was 5 to 13 times higher than that in the BL ($P < 0.05$, Fig. 3A).

3.2 Soil carbon chemical and aggregates composition

^{13}C NMR analysis showed that SOC had a relatively high percentage of O-alkyl C (~40% of the total), alkyl C (~32% of the total) and carbonyl C (~22% of the total), while the percentage of aromatic C was relatively low (~5.02% of the total, Fig. 3B) in all sites. The proportion of the relatively easily degradable carbonyl C was similar in all the restoration sites, and the proportion of the relatively easily degradable O-alkyl C was higher in the MF than in other restoration treatments. While the relative percentage of the recalcitrant alkyl C and recalcitrant aromatic C was highest under SF and EF, respectively (Fig. 3B).

The proportion of large macroaggregate (> 2 mm) increased, while the proportions of other sizes of aggregates (0.25-2 mm, 0.053-0.25 mm and < 0.053 mm) decreased with the forest vegetation restoration ($P < 0.05$, Table 2). Based on the MWD values, the significantly

241 greater aggregate stability among restoration treatments ranked as under SF ≈ MF > EF > BL
242 (Table 2). Among different aggregate sizes for the same forest restoration treatment,
243 significantly greater aggregate proportions patterned as 0.25-2 mm > 0.053-0.25 mm >
244 <0.053 ≈ 2 mm under BL, and as 2 mm > 0.25-2 mm > 0.053-0.25 mm ≈ <0.053 under EF,
245 MF and SF (Table 2).
246

247 *3.3 Relationships between GRSP and SOC, soil carbon chemical composition, and soil*
248 *aggregate stability*

249 According to previous studies, 37% of C in GRSP was used to estimate the C in GRSP
250 proportion to SOC (Lovelock et al., 2004). GRSP comprised a large proportion of SOC with
251 EE-GRSP and T-GRSP, which accounted for 1.7% and 9.6% of SOC on average,
252 respectively (Fig 4A). There were no significant differences in the proportion of EE-GRSP to
253 SOC among all vegetation restoration treatments (Fig 4A). Whereas the proportion of T-
254 GRSP to SOC significantly decreased with the forest vegetation restoration treatments, the
255 lowest and highest proportion were respectively found under SF and BL (Fig. 4A).
256 Regression analyses showed that both EE-GRSP and T-GRSP had a strongly positive
257 relationship with SOC ($P < 0.01$, Fig. 4B). Both EE-GRSP and T-GRSP were significantly
258 positively correlated with the proportion of large macroaggregate (>2 mm) and MWD, but
259 significantly negatively with smaller particle sizes of aggregates (0.25-2 mm, 0.053-0.25 mm
260 and <0.053 mm) (Table 2).
261

262 Different percentages of soil C chemical composition displayed different correlations with
263 the proportion of GRSP. Specifically, the percentage of the recalcitrant aromatic C or
264 recalcitrant alkyl C was increased or decreased with the proportion of T-GRSP (Fig. 5A and
265 5C), but both of them did not show any relationship with the proportion of EE-GRSP (Fig.

5A and 5C). In addition, neither the percentage of the easily degradable O-alkyl C nor carbonyl C did show any significant relationship with the proportion of T-GRSP (Fig. 5B and 5D).

4. Discussion

4.1 Changes in GRSP concentration among different forest restoration stages

T-GRSP represents the old fraction of GRSP that is difficult to be decomposed (M. Rillig, 2004a; S. F. Wright & Upadhyaya, 1996). The increases of T-GRSP with forest vegetation restoration probably resulted from the tradeoff between the production of GRSP by AMF and the microbial decomposition of GRSP as both the AMF and microbial biomass increased from BL to SF (Table 1). Specifically, Only several kinds of herbaceous plants and small xeric shrubs were grown in the ditches under BL where the topsoil had been eroded completely (Ren et al., 2007). Therefore, the degree of AMF colonization would not be intensive under BL. In contrast, AMF could be preserved and propagated by *Eucalyptus exserta* under EF since AMF could benefit for *E. exserta* growth (Adjoud, Plenchette, Halli-Hargas, & Lapeyrie, 1996). Similarly, AMF biomass increased under MF and SF and so did GRSP (Table 1 and Fig. 2A). The restoration climax is similar between MF and SF after approximately 40-50 years restoration (Ren et al., 2007), the similar GRSP under MF and SF partly revealed that the successful restoration after 30 years plantation of several different tree species. This was in coincidence with previous studies, which showed that AMF increased with the increase of plant diversity and biomass production (Hiiesalu et al., 2014; van der Heijden et al., 1998). Soil microorganisms under MF and SF would prefer to use the easily decomposed soil organic matters rather than to degrade the relatively recalcitrant T-GRSP (Wright & Upadhyaya, 1996), although the decomposition rate could also be increased with restoration due to an increase of the total microbial biomass by the restoration.

EE-GRSP represents glomalin that is the newly produced or readily decomposed ones in soil (Steinberg & Rillig, 2003). The increase of EE-GRSP could be attributed to the increased AMF and microbial biomass with the restoration (Table 1) as we discussed above, or probably because such increased EE-GRSP had not been decomposed by microbes since soil nutrients were also significantly improved with the forest vegetation restoration (Table 1). Furthermore, the increased ratio of EE-GRSP/T-GRSP from BL to MF indicated that the restoration process increased the fraction of labile GRSP, while the ratio of EE-GRSP/T-GRSP slightly decreased under SF (Table 1), suggested that restoration climax could be beneficial for the recalcitrant GRSP reservation. Considering the different stability of different fraction of GRSP, we speculate that although EE-GRSP and T-GRSP have already reached the climax highest under MF, it is probably still not enough for benefiting the reservation from the recalcitrant GRSP.

In this study, the increased AMF biomass can well explain the changes in GRSP during the forest restoration process. It should, however, be noticed that the PLFA biomarker 16:1 ω5 also includes the bacterial taxa, but it is still a good indicator of AMF biomass (McKinley, Peacock, & White, 2005; Olsson, 1999). Furthermore, another interesting point was that the AMF diversity increased with the forest restoration as the GRSP did (see Table 1 for detail). Nevertheless, these results demonstrated our first hypothesis that GRSP increased with forest restoration.

4.2 Changes in soil organic carbon, chemical composition and soil aggregates among different forest restoration stages

SOC significantly increased with forest restoration (Fig. 3A), which is in line with previous

316 studies (D. Li, Niu, & Luo, 2012; X. Liu, Yang, Wang, Huang, & Li, 2018). The increase of
317 SOC might mainly be due to more plant residues input into the soil during the forest
318 restoration (Post & Kwon, 2000). The similar soil organic C in the MF and SF restoration
319 stages (Fig. 3A) is in line with a meta-analysis that showed that the secondary and
320 undisturbed tropical forest has a similar capacity of SOC sequestration (Martin, Newton, &
321 Bullock, 2013). In addition, studies in the same site as ours found that plant biomass and soil
322 C sequestration capacity under MF and SF were comparable after over 50 years of vegetation
323 restoration (Tang et al., 2018; H. Zhang et al., 2019). Soil MWD also increased with forest
324 vegetation restoration (Table 2). The increased SOC and plant diversity is the possible
325 underlying mechanism that improved soil aggregate stability in the process of vegetation
326 restoration in the study area (Wei, Li, Zou, Tan, & Ding, 2007). These results indicated that
327 the restoration of vegetation could improve the formation and stabilization of soil
328 macroaggregates, and hence improved the stability of soil structure and soil C storage.
329
330 The solid-state ^{13}C NMR spectra of SOC showed a similar recalcitrant C percentage
331 (aromatic C + alkyl C) under EF and SF, which was higher under MF (Fig. 3B). However,
332 the reason for the higher recalcitrant C percentage under EF and SF might be different since
333 the aromatic C ratio under EF was higher than under the other two restoration treatments,
334 while the SF had the highest proportion of alkyl C (Fig 3B). In the EF, the riched aromatic C
335 because the *Eucalyptus* leaves were probably rich in aromatic hydrocarbon, as lots of
336 aromatic C could be returned to the soil through fallen leaves (Rodriguez et al., 2012). While
337 the SF had the highest microbial biomass among all the restoration sites (Table 1), and soil
338 microorganisms would prefer to use the labile C other than the resistant components, the
339 recalcitrant C could be deposited and gradually sequestrated in soil. Our results were in line
340 with the results measured by SOC density fractionations methods, which also showed that the

proportion of resistant C in the soil under EF and SF was higher than that under MF at our study site (Zhang et al., 2019). As a result, the climax stage (SF) of forest restoration or special tree species, like *Eucalyptus,* could enhance the ratio of soil recalcitrant C fraction and thus benefit soil C sequestration.

4.3 Relationships between GRSP and SOC sequestration among different forest restoration stages

The higher proportion of T-GRSP in SOC in the early-restoration (BL and EF) than in the late-restoration (MF and SF) stage (Fig. 4A) disagreed with our second hypothesis, which was caused by the increasing magnitude of SOC higher than the T-GRSP (the insert plot in Fig. 2A and 3A). The disproportionate increase of T-GRSP and SOC could be attributed to the turnover of GRSP slower than that of SOC due to the recalcitrant index of SOC was significantly lower than that of GRSP (Zhang et al., 2017), and also a higher SOC concentration under MF and SF (Fig. 3A). However, the percentage of EE-GRSP with SOC was insignificant among different restoration stages (Fig. 4A), which might be due to a similar increase in the magnitude of EE-GRSP and SOC among all restoration stages (Fig. 2A and 3A). In addition, the positive correlation between GRSP and SOC (Fig.4B) was in line with studies in the grassland (Zhang et al., 2020) and subtropical forest (Zhang et al. 2017). The increase of GRSP while the decreased percentage of GRSP in SOC in the forest vegetation restoration process might suggest that GRSP was more important for SOC accumulation in the earlier forest restoration stage.

GRSP has about 37% C and is difficult to be decomposed in nature (Lovelock et al., 2004). The positive correlations between the proportion of GRSP and aromatic C percentage (Fig. 5C) in soil revealed that GRSP probably related to the recalcitrant C sequestration at our

restoration sites as aromatic C was enriched in GRSP (Zhang et al. 2017). While the percentage of the alkyl C in GRSP was comparable or significantly lower than that in SOC (Zhang et al., 2017), which might partly explain why there was a negative correlation between the proportion of T-GRSP and alkyl C percentage (Fig. 5A). Also, our results showed that EE-GRSP and T-GRSP were significantly positively correlated with large macroaggregate (>2 mm) and MWD (Table 2), which were consistent with the results from a path analysis that indicated the GRSP was significantly promoted water-stable aggregates in grassland in California (Rillig, Wright, & Eviner, 2002). Miller and Jastrow (2000) had suggested that most of the increase in large macroaggregates was due to the binding of small particles into macroaggregates by GRSP. GRSP functioned as a "super glue" and form a 'sticky-string-bag' with mycelia of AMF to make them important for soil structure in the long term (Rillig, 2004a; Wright & Upadhyaya, 1998). Besides, GRSP promoted soil aggregate formation and benefited for SOC accumulation since they provided physical protection for liable C within aggregates from microbial degradation (Rillig, 2004b). In summary, the accumulation of GRSP would beneficial to the soil C sequestration in the tropical coastal degraded forest ecosystem restoration process, like in other natural and agricultural ecosystems (Liu et al., 2018; Wright et al., 2007).

5. Conclusion

After decades of reforestation in tropical coastal degraded land, we found that forest reforestation could significantly increase the content of GRSP because of the increase of AMF biomass. Accompany with the accumulation of GRSP, forest restoration also improved the soil aggregate stability and proportion of soil recalcitrant C fraction. Together with an increased GRSP, but a decrease proportion of GRSP in SOC among different forest restoration stages, such results suggested that GRSP may play a more important role for SOC

391 accumulation in the early stages than the late stages during forest restoration. In conclusion,
392 GRSP could be beneficial to soil C sequestration due to its accumulation during forest
393 restoration, which would increase the proportion of recalcitrant C component and soil
394 aggregate stability. These findings provide our better understanding of increasing soil C
395 sequestration through forest restoration approaches.
396
397 **Conflict of Interest**
398 The authors declare that they do not have a conflict of interest.
399
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589 # Tables

590 **Table 1.** Soil nutrient and microbial indexes among different forest restoration stage
591 treatments.

	BL[†]	EF	MF	SF
Total N (mg g^{-1})[§]	0.35±0.03 d[‡]	1.03±0.06 c	2.15±0.09 b	3.02±0.08 a
Total P (mg g^{-1})	0.09±0.01 c	0.10±0.01 c	0.18±0.01 b	0.43±0.01 a
NO_3-N (mg kg^{-1})	2.64±0.33 c	1.22±0.24 c	14.86±1.24 b	36.99±2.58 a
NH_4-N (mg kg^{-1})	3.30±0.09 b	11.21±0.71 b	38.20±3.43 a	31.14±4.37 a
Available P (mg kg^{-1})	0.50±0.20 c	2.39±0.31 b	5.98±0.35 a	6.98±0.70 a
AMF diversity	_[¶]	1.56±0.13 b	1.83±0.11 b	2.37±0.05 a
AMF biomass (nmol g^{-1})	0.04±0.02 c	0.18±0.02 c	0.57±0.15 b	1.08±0.08 a
Microbial biomass (nmol g^{-1})	7.40±0.99 b	10.52±0.67 b	32.83±4.90 a	38.14±3.43 a

592 [†]BL, barren land; EF, *Eucalyptus* forest; MF, mixed broadleaved forest; SF; secondary natural forest.

593 [§]Total N, soil total nitrogen; Total P, soil total phosphorus; NO_3-N, nitrate nitrogen; NH_4-N, ammonial nitrogen;
594 AMF, arbuscular mycorrhizal fungi.

595 [‡]Different lowercase letters in the same row represent significant differences among different forest restoration
596 stage treatments (P<0.05).

597 [¶]Data did not obtained due to the AMF is scarce in the BL.

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605 **Table 2.** Soil aggregate relative proportion in different forest restoration sites and the
606 correlations between the relative proportion of soil aggregates and GRSP content.

	> 2 mm[†]	**0.25-2 mm**	**0.053-0.25 mm**	**<0.053 mm**	**MWD**[‡]
BL[§]	9.24±2.75 c,z[¶]	52.19±4.86 a,x	27.04±0.97 a,y	12.54±2.40 a,z	1.16±0.13 c
EF	58.24±5.63 b,x	23.79±3.38 b,y	13.63±1.95 b,yz	4.32±0.66 b,z	3.78±0.30 b
MF	79.03±0.71 a,x	13.72±2.44 cd,y	4.78±0.97 c,z	2.46±1.11 b,z	4.86±0.05 a
SF	72.69±2.01 a,x	22.36±1.04 bc,y	3.95±0.94 c,z	1.10±0.16 b,z	4.62±0.11 a
EE-GRSP	0.81**	-0.68**	-0.72**	-0.69**	0.89**
T-GRSP	0.72**	-0.66**	-0.83**	-0.75**	0.79**

607 [†] >2mm, 0.25-2mm, 0.053-0.25mm and <0.053 mm are different soil aggregates size.

608 [‡]MWD, mean weight diameter.

609 [§]BL, barren land; EF, *Eucalyptus* forest; MF, mixed broadleaved forest; SF; secondary natural forest;

610 [¶]a, b and c represent significant differences among different forest restoration stage treatments for the same
611 aggregate size ($P < 0.05$), and x, y and z represent significant differences among aggregate sizes for the same
612 forest restoration stage treatment ($P < 0.05$). ** represent $P < 0.01$.

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Figure Legends

Figure 1. Status of forest restoration treatments at the Xiaoliang Station in the southwest of Guangdong Province, China.

Figure 2. Easily extracted glomalin-related soil protein (EE-GRSP), total GRSP (T-GRSP), and EE-GRSP/T-GRSP ratio among four forest restoration stage treatments. BL represents barren land, EF represents *Eucalyptus* forest, MF represents mixed broadleaved forest, SF represents secondary natural forest. The insert plot is the relative increase of restoration stage treatments compare to the barren land. Different lowercases represent significant differences among forest restoration stage treatments ($P< 0.05$).

Figure 3. The concentrations of SOC (A) and the relative percentage of different soil chemical groups (B) among different forest restoration stage treatments. BL represents barren land, EF represents *Eucalyptus* forest, MF represents mixed broadleaved forest, SF represents secondary natural forest. The insert plot is the relative increase of restoration stage treatments compare to the barren land. Different lowercase letters (a,b,c,d) represent the significant difference of different soil chemical groups in the same forest restoration stage treatment ($P< 0.05$); while bars of the same color in (B) sharing different letters (x,y,z) indicate the significant difference in the relative proportion of the same functional group among different forest restoration stage treatments ($P< 0.05$). The soil carbon concentration in the BL was too low to obtain useful NMR signals.

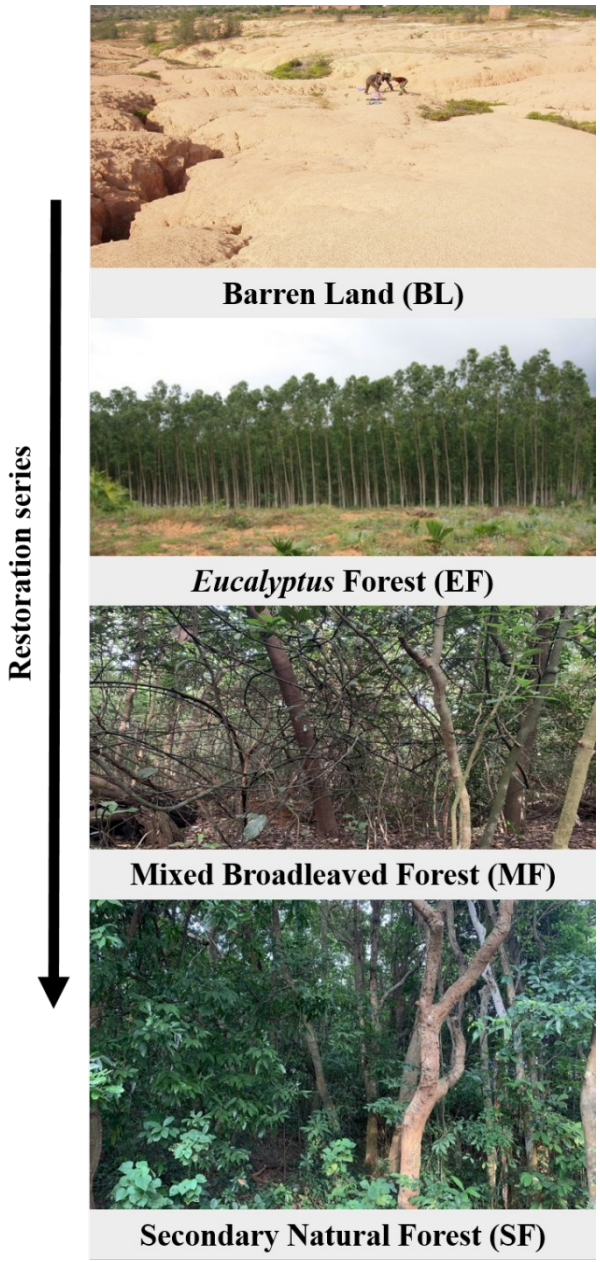
Figure 4. The proportion of EE-GRSP and T-GRSP in SOC (A), and the relationship of EE-GRSP and T-GRSP on the SOC content (B) among different forest restoration stage treatments. BL represents barren land, EF represents *Eucalyptus* forest, MF represents mixed

broadleaved forest, SF represents secondary natural forest. The insert plot is the relative increase of restoration stage treatments compare to the barren land. Different lowercase letters above bars of the same filling color represent significant differences among different forest restoration stage treatments ($P< 0.05$).

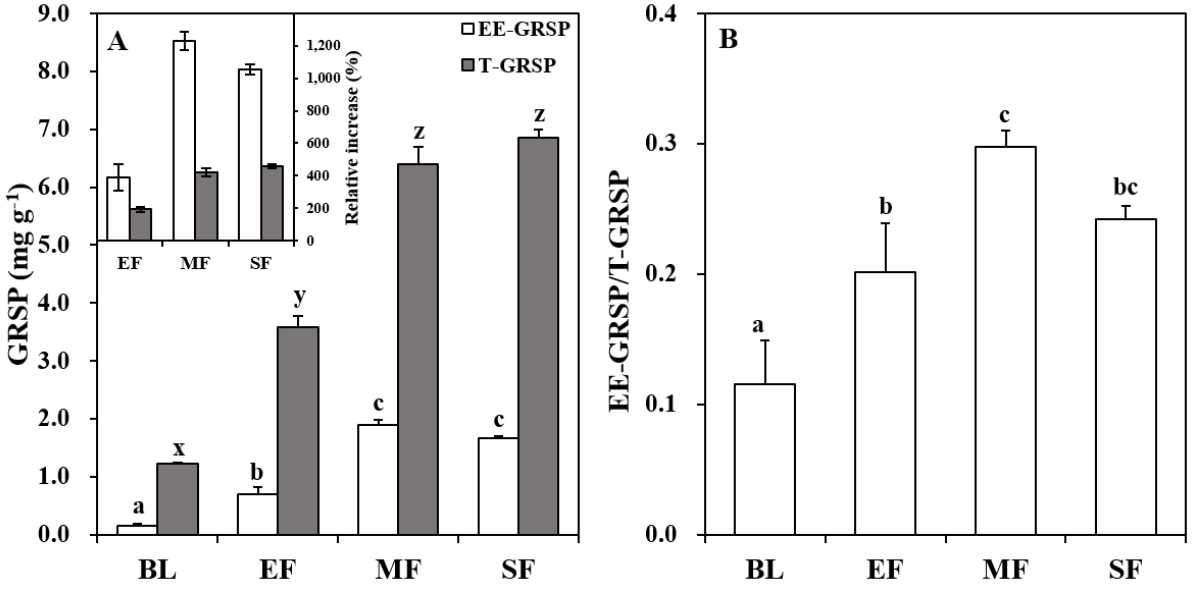
Figure 5. The correlation of the relative proportions of each soil chemical composition and the proportion of GRSP in SOC among different forest restoration stage treatments. The trendline is present if significant ($P< 0.05$).

Figures

Fig. 1



650 **Fig.2**



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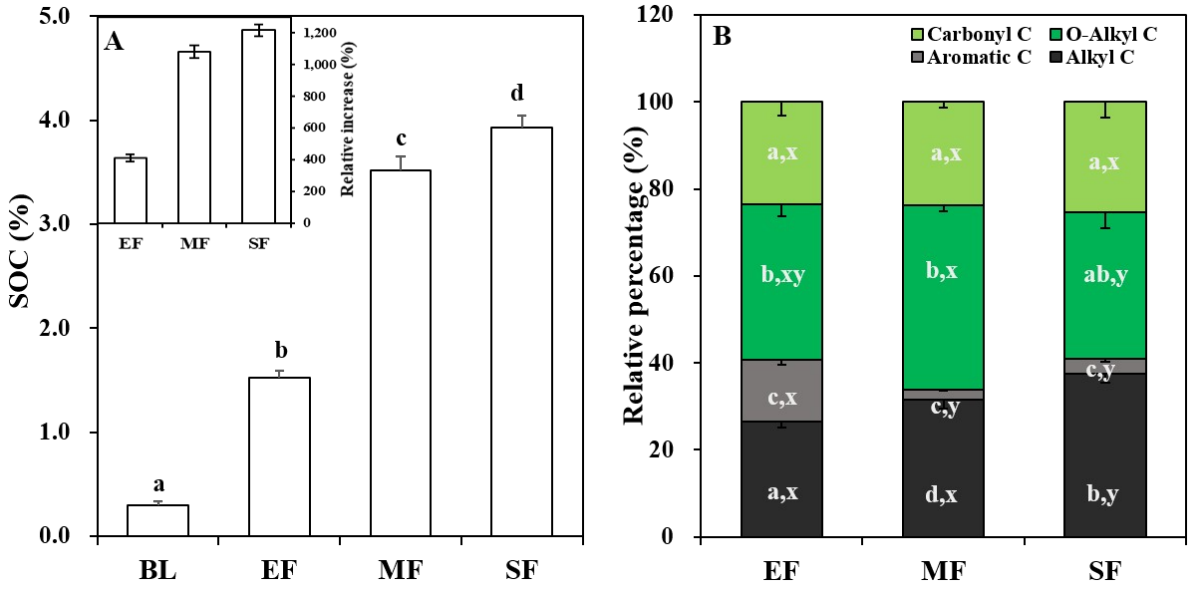
653 **Fig. 3**


A
SOC (%)
5.0
4.0
3.0
2.0
1.0
0.0
a
b
c
d
BL
EF
MF
SF
Relative increase (%)
1,200
1,000
800
600
400
200
0
EF
MF
SF
B
Relative percentage (%)
120
100
80
60
40
20
0
Carbonyl C
O-Alkyl C
Aromatic C
Alkyl C
a,x
b,xy
c,x
a,x
a,x
b,x
c,y
d,x
a,x
ab,y
c,y
b,y
EF
MF
SF

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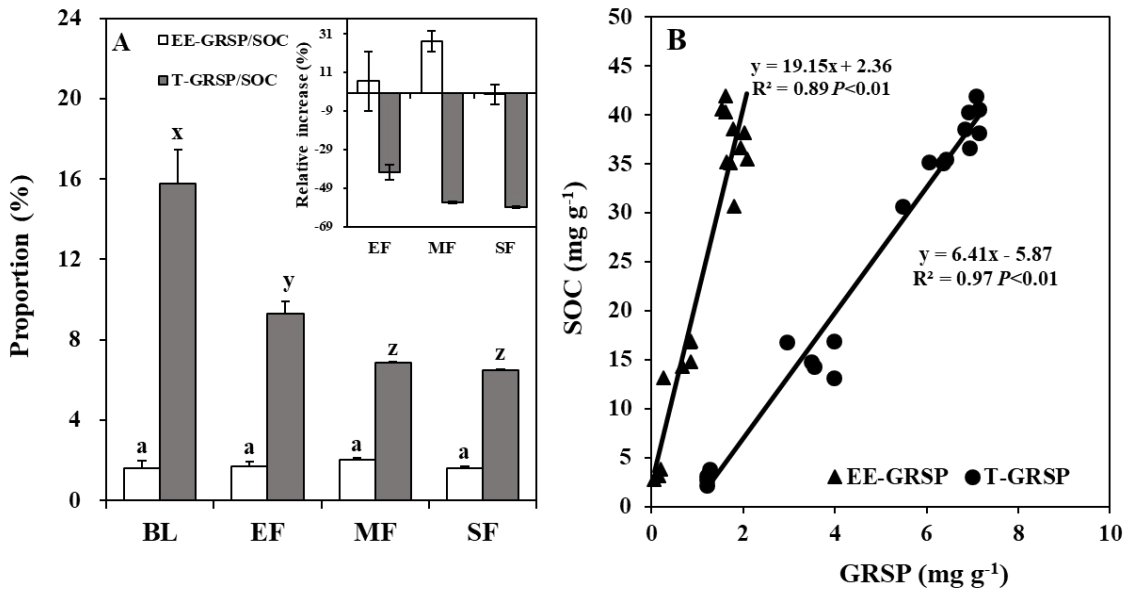
655 **Fig. 4**



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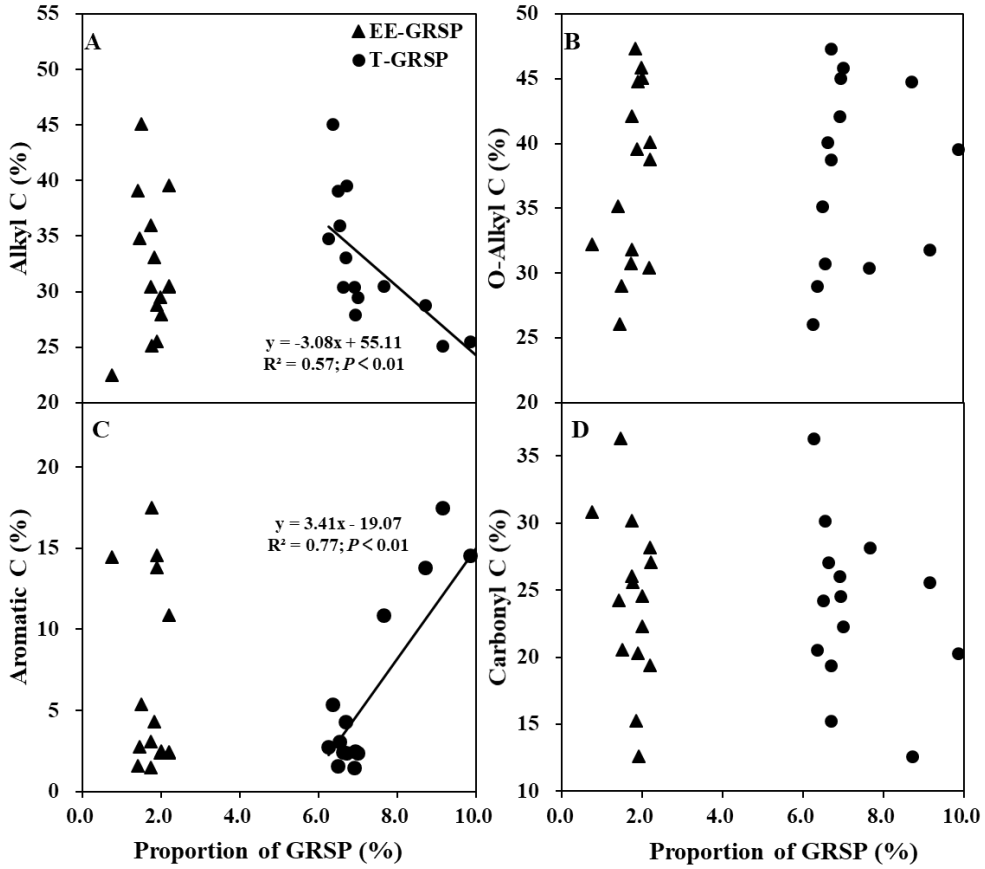
657 **Fig. 5**



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