

RESEARCH ARTICLE

Plant-mycorrhizae association affects plant diversity, biomass, and soil nutrients along temporal gradients of natural restoration after farmland abandonment in the Loess Plateau, China

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Abstract

The interaction between plants and arbuscular mycorrhizal fungi in the rhizosphere plays a vital role in driving vegetation recovery and restoration of soil nutrients. However, how this interaction affects vegetation succession and how soil nutrient recovery is driven by vegetation restoration and rhizosphere processes are still largely unknown. In this study, a well-documented grassland restoration chronosequence on the Loess Plateau, China (fields at 0, 7, 12, 17, 22, and 32 years after farmland abandonment and a natural grassland reference) was selected. The species richness and diversity reached maximum values between 17 and 22 years after farmland abandonment, whereas the plant total above and belowground biomasses simultaneously peaked at 22 years and then remained stable. In the dominant plant rhizosphere and bulk soil concentrations of total glomalin-related soil protein (including both old and recently produced fungal proteins) substantially increased from 3.58 to 4.87 g kg⁻¹ and from 2.67 to 3.86 g kg⁻¹, respectively, between 12 and 32 years after farmland abandonment. The concentrations of soil organic carbon (SOC) and total nitrogen (TN) in the plant rhizosphere and bulk soil significantly increased between 17 and 32 years and reached the levels of the natural grassland. The aboveground plant biomass, soil SOC, and TN concentrations were positively correlated with the glomalin-related soil protein (GRSP) concentration ($p < .05$). Our study suggested that interactions among plant-mycorrhizae association, plant diversity, and biomass promote GRSP and nutrient accumulation in the plant rhizosphere and bulk soil, and GRSP largely contributes to SOC stabilization and the accumulation of SOC and TN.

KEYWORDS

glomalin-related soil protein, rhizosphere, soil organic carbon, total nitrogen, vegetation recovery

1 | INTRODUCTION

Natural restoration without human interference can effectively be used to recover ecosystems with degraded soil and to maintain soil

fertility (Prach & Walker, 2011). Natural restoration helps to promote the recovery of vegetation cover and plant diversity and is conducive to increasing soil carbon and nitrogen contents (Chang et al., 2017; Deng, Wang, Liu, & Shangguan, 2016; Liu, Wu, Ding, Tian, & Shi,

A list of abbreviations: AMF, arbuscular mycorrhizal fungi; GRSP, glomalin-related soil protein; GRSPt, total GRSP; GRSPe, easily extractable GRSP; SOC, soil organic carbon; TN, total nitrogen; MR, mycorrhizal colonization rate; TP, total phosphorus; AP, available phosphorus; KMnO₄-C, KMnO₄-oxidizable carbon

2017). The rhizosphere is a biologically active zone where complex interactions among plant roots, soil, and microbes occur (Puglisi et al., 2008; Zoysa, Loganathan, & Hedley, 1999). Rhizodeposition accounts for approximately 10% of fixed net carbon and nitrogen or 25% of belowground allocated carbon, which is highly available to the microbial community and further promotes the cycling of soil carbon and nitrogen in the rhizosphere, (Jones, Nguyen, & Finlay, 2009; Kidd et al., 2009; Pausch & Kuzyakov, 2018). Therefore, the rhizosphere process of interaction between roots and soil following natural restoration is important for the recovery of soil carbon and nitrogen.

Mutualism between plants and arbuscular mycorrhizal fungi (AMF) that links the root and the soil systems together is one of the most ubiquitous interactions between plants and soil organisms (Koide & Mosse, 2004). Strong correlations between the composition of plant and AMF communities have been observed (Hiiesalu et al., 2014; Landis, Gargas, & Givnish, 2004). AMF have the potential to affect interspecific competition and, consequently, the plant community composition (Lin, McCormack, & Guo, 2015; van der Heijden, Bardgett, & van Straalen, 2008). Herbs and especially legumes benefit more from AMF colonization than grasses, and AMF colonization increases the ability of herbs to compete with grasses (Scheublin, Van Logtestijn, & Van Der Heijden, 2007; van der Heijden et al., 2006). Garcia de Leon et al. (2016) suggested that AMF arrived faster than plants and drove vegetation dynamics along secondary vegetation succession. The reintroduction of AMF can drive plant community dynamics by promoting the growth of desirable late successional plant species, while simultaneously inhibiting less desirable weedy plants (Kozioł & Bever, 2017; Middleton & Bever, 2012). Soil nutrient stoichiometry is a determinant factor influencing plant-mycorrhizae associations, as previous studies observed that the addition of nitrogen (N) and phosphorus (P) to the soil reduced mycorrhizal colonization (Frater et al., 2018; Johnson, 2010; Liu et al., 2012). However, all these studies ignored how environmental factors regulate plant-mycorrhizae associations in natural restoration and how plant-mycorrhizae associations, in turn, influence vegetation succession.

Glomalin is usually quantified as glomalin-related soil protein (GRSP), which is a glycoproteinaceous substance that is produced by AMF and released into the soil with hyphal turnover (Driver, Holben, & Rillig, 2005; Treseder & Turner, 2007; Wright & Upadhyaya, 1998); therefore, GRSP can be considered the result of the interaction between plants and AMF. GRSP is usually classified into easily extractable glomalin-related soil protein (GRSPe) and total glomalin-related soil protein (GRSPt; Wright & Upadhyaya, 1996). GRSPe is recently produced fungal protein, and GRSPt is both old and recently produced fungal protein glomalin (Rillig, 2004; Wright & Upadhyaya, 1996). Glomalin largely contributes to long-term carbon (C) and nitrogen (N) storage at levels of 4–5% of soil C and 3–5% of soil N, which even exceeds the contribution by the soil microbial biomass (Rillig, Wright, Nichols, Schmidt, & Torn, 2001; Lovelock, Wright, Clark, & Ruess, 2004). In addition, GRSP may improve the stability of soil organic carbon (SOC) because it is resistant to heat degradation and is a stable compound with a turnover time of many years (Fokom et al., 2012; Rillig et al., 2001; Treseder & Turner, 2007; Vasconcellos, Bonfim,

Baretta, & Cardoso, 2016). Net primary production (NPP) determines the availability of plant C to AMF, AMF can allocate a higher proportion of their resources to glomalin, where NPP is greater (Treseder & Cross, 2006; Treseder & Turner, 2007). Additionally, turnover of arbuscular mycorrhizal hyphae is faster where NPP is greater, which promotes glomalin production (Rillig, Wright, & Eviner, 2002; Wu, Cao, Zou, & He, 2014; Yang, He, Huang, Ban, & Tang, 2017). Previous studies revealed that the content of GRSP after 20 years of natural recovery from sugar cane plantation to forest in Brazil still did not reach the level of the native forest (Bonfim, Vasconcellos, Stuermer, & Cardoso, 2013; Vasconcellos et al., 2016), and the GRSP content dramatically decreased after pasture was converted into farmland (Spohn & Giani, 2010, 2011). The rhizosphere process of GRSP production following natural restoration in grasslands and the contribution of this process to the recovery of soil carbon and nitrogen contents and the stability of SOC are important for clarifying the mechanism by which AMF promotes the restoration of soil carbon and nitrogen. Although grassland soils represent approximately 30% of the land surface area of Earth (Chaplot, Dlamini, & Chivenge, 2016), the role of these processes following the natural restoration of grasslands has been neglected.

The Loess Plateau in the upper and middle regions of the Yellow River in China features an arid and semiarid climate and is known for its long agricultural history and severe soil erosion (Zhang, Liu, Xue, & Wang, 2016). The native vegetation in the slope areas that are prone to erosion has been destroyed and converted into farmland to meet the food supply needs of a growing population, which has caused serious soil erosion and degradation (Fu, Shao, Wei, & Horton, 2010; Wei, Zhou, Tian, He, & Tang, 2006). A series of conservation projects have been launched to control soil erosion and restore the ecological environment on the Loess Plateau in the last several decades. Many farmlands with slopes $>15^\circ$ were abandoned, and natural vegetation succession followed without human disturbance. The objectives of this study were (a) to evaluate how plant-mycorrhizae associations promote the recovery of plant biomass and diversity, (b) to determine the relationship of soil GRSP accumulation and plant-mycorrhizae associations, and (c) to clarify how soil nutrient recovery was driven by vegetation restoration and GRSP accumulation. We hypothesized that (a) close plant-mycorrhizae associations promote the recovery of plant biomass and diversity by enhancing the ability of host plant in absorbing nutrients; (b) close plant-mycorrhizae associations drive soil carbon and nitrogen recovery by promoting soil GRSP accumulation.

2 | MATERIALS AND METHODS

2.1 | Study area

The sloped farmland and five sloped farmlands that had been abandoned for 7, 12, 17, 22, and 32 years that were selected as the study areas were located in the Zhifanggou Watershed ($36^\circ46'N$, $109^\circ16'E$) in Ansai County on the northern Loess Plateau, China. The natural grassland was located in the Songjiagou Watershed ($N36^\circ44'N$, $109^\circ16'E$) in Ansai County (Figure 1). This area has a temperate

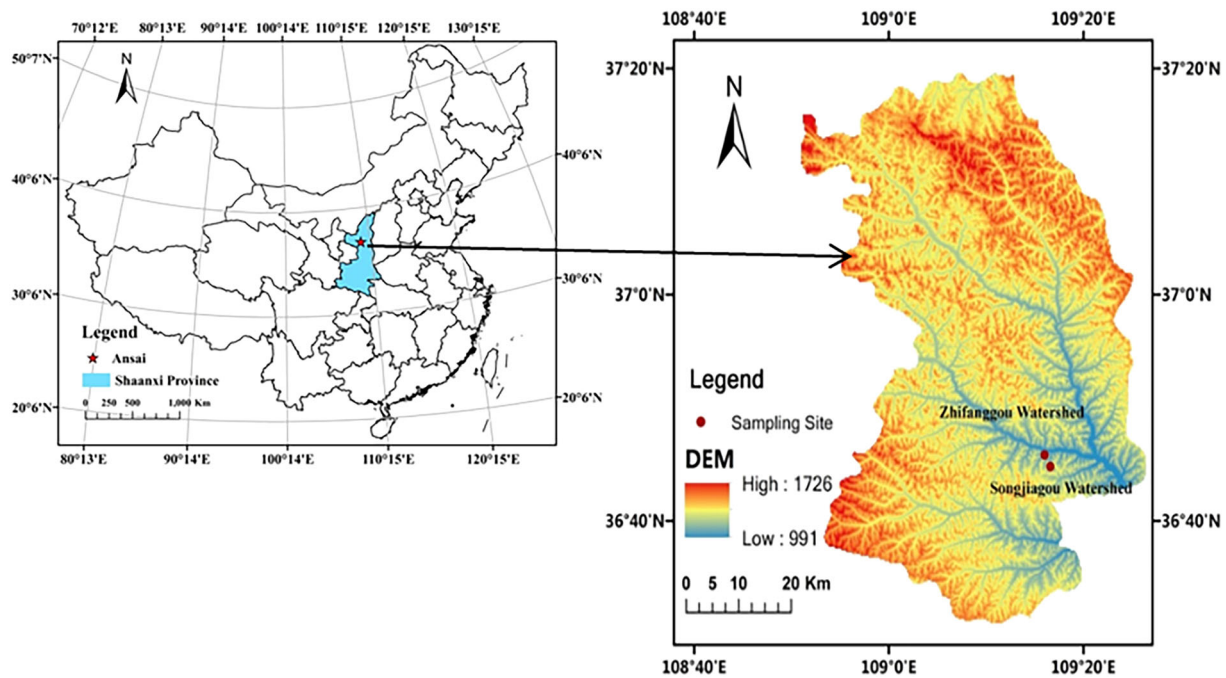


FIGURE 1 Location of the Zhifanggou and Songjiagou Watershed on the Loess Plateau, China [Colour figure can be viewed at wileyonlinelibrary.com]

semiarid climate, with an annual temperature of 8.8°C and precipitation of 549.1 mm. The landscape in this area is a typical hilly gullied loessial landscape with deeply carved gullies. The soil is mainly Huangmian soil (Calcaric Cambisols, Food and Agriculture Organization), which is derived from wind-blown deposits and is characterized by an absence of bedding, a silty texture, looseness, and wetness-induced collapsibility (Zhang, Liu, Xue, & Xiao, 2013).

2.2 | Experimental design and soil sampling

The space-for-time method that is a common method used to study the changes in the plant community and soil conditions following natural restoration was used to investigate the changes in plant biomass and diversity and soil GRSP and nutrient contents in the plant rhizosphere and bulk soil (Deng, Shangguan, & Sweeney, 2013; Walker, Wardle, Bardgett, & Clarkson, 2010). This field experiment was conducted in September 2016 when the plant community biomass peaked. The farming system and fertilization measures in the sloped cropland have been presented in Zhang et al. (2016), and the same farming system and fertilization measures were applied in these study areas before abandonment. A natural grassland that has existed for at least 50 years with no farm history was used as references. The study sites were systematically selected, and the geographical conditions of these sites (altitude, slope, aspect, etc.) along the chronosequence were similar. The properties of the study areas are showed in Table 1.

Three 20 m × 20 m plots were set up at each site, and each plot was at least 30 m from the other plots. Three 1 m × 1 m quadrats were randomly selected in each plot to measure the vegetation characteristics. In each quadrat, the plant species were classified into three groups:

dominant species, companion species, and minor species. The coverage and above and belowground biomasses were measured according to these three groups. The plant was extracted from the soil, separated into above and belowground parts, and oven dried at 70°C for 48 hr to determine the above and belowground biomasses. Eight plants of the dominant and companion species were randomly selected from each plot. These plants were extracted from soil, and the roots of these plants were shaken to detach loose soil and were then carefully brushed to collect rhizosphere soil. The soil strongly adhering to the roots and collected within the space used by the roots was considered the rhizosphere soil (Zhang, Liu, Xue, & Wang, 2015). The roots of these plants were also collected, and the aboveground parts were abandoned. Bulk soils were sampled from a location at least 15 cm from the roots in the 0–20 cm soil layer in each plot. Roots, stones, and debris were removed from the rhizosphere and bulk soils. All of these samples were transported to the laboratory on ice. One part of the soil samples was air-dried for the soil nutrient and GRSP analyses. The root samples were stored in a freezer at –20°C prior to the measurement of MR.

2.3 | Laboratory analysis

Mycorrhizal colonization rate (MR) was assessed by clearing and staining the roots with Trypan blue in lactic acid (0.05%), and the percentages of AM colonization were estimated under a microscope with the gridline intersect method (Giovannetti & Mosse, 1980). SOC and total nitrogen (TN) were determined using the Walkley-Black method (Nelson & Sommers, 1982) and the Kjeldahl method (Bremner & Mulvaney, 1982), respectively. Total phosphorus (TP) was measured using molybdenum antimony blue colorimetry (Murphy & Riley,

TABLE 1 Geographic features and floristic compositions of the sampling sites

| Study sites | Slope aspects | Slope gradient | Geographical coordinates | Altitude (m) | Dominant species | Companion species | Minor species |
|-------------------|---------------|----------------|--------------------------|--------------|-------------------|-------------------|--|
| Farmland | E15°N | 25° | 36°44'39"N, 109°14'35"E | 1274 | SET ITA + GLY MAX | | SAL COL; PHR AUS |
| 7-y | W10°N | 20° | 36°44'47"N, 109°15'12"E | 1303 | ART CAP | | HET ALT; SAL COL; SON OLE |
| 12-y | E40°N | 26° | 36°44'02"N, 109°16'31"E | 1276 | ART CAP | ART SAC | HET ALT; SET VIR; STI BUN; SAL COL; CIR SET; LES DAV; POA ANN; CLE SQU |
| 17-y | E25°N | 28° | 36°44'09"N, 109°16'14"E | 1307 | ART SAC | STI BUN | ART CAP; HET ALT; SET VIR; CIR SET; LES DAV; POA ANN; VIC SEP; OXY BIC; POT CHI; CLE FLO; SPI SAL; LEY SEC; POT TAN; CLE SQU |
| 22-y | E10°N | 30° | 36°44'05"N, 109°16'27"E | 1267 | ART SAC | STI BUN | POT TAN; HET ALT; ART CAP; SET VIR; CLE SQU; CIR SET; LES DAV; POA ANN; VIC SEP; LEY SEC |
| 32-y | E16°N | 30° | 36°44'15"N, 109°15'55"E | 1246 | ART SAC | | LES DAV; POT TAN; VIC SEP; POT CHI; POA SPH; CLE SQU; ART ARG; CIR SET |
| Natural grassland | E34°N | 26° | 36°44'59"N, 109°16'28"E | 1183 | ART SAC | BOT ISC | LES DAV; POT TAN; STI BUN; ART ARG; CIR SET; CLE SQU |

Abbreviations: NG: natural grassland; ART ARG: *Artemisia argyi*; ART CAP: *Artemisia capillaries*; ART SAC: *Artemisia sacrorum*; BOT ISC: *Bothriochloa ischaemum*; CLE FLO: *Clematis florida*; CLE SQU: *Cleistogenes squarrosa*; CIR SET: *Cirsium setosum*; GLY MAX: *Glycine max*; HET ALT: *Heteropappus altaicus*; LES DAV: *Lespedeza davurica*; LEY SEC: *Leymus secalinus*; OXY BIC: *Oxytropis bicolor*; POA ANN: *Poa annua*; PHR AUS: *Phragmites australis*; POT CHI: *Potentilla chinensis*; POA SPH: *Poa sphondylodes*; POT TAN: *Potentilla tanacetifolia*; SET ITA: *Setaria italica*; STI BUN: *Stipa bungeana*; SAL COL: *Salsola collina*; SON OLE: *Sonchus oleraceus*; SPI SAL: *Spiraea salicifolia*; SET VIR: *Setaria viridis*; VIC SEP: *Vicia sepium*.

1962), and available phosphorus (AP) was extracted with 0.5 M NaHCO₃ (pH 8.5; Olsen & Sommers, 1982). KMnO₄-oxidizable carbon (KMnO₄-C) was determined following the methods of Blair, Lefroy, and Lise (1995). Three grams of soil was added to centrifuge tubes containing 25 ml of a 333, 167, or 33 mM KMnO₄ solution, so that KMnO₄-C was divided into four different fractions according to its decreasing order of lability. KMnO₄-C under 33 mM KMnO₄ was termed very labile C (C_{VL}); the difference in the amount of SOC extracted between 33 and 167 mM KMnO₄ was termed moderate labile C (C_{ML}); the difference in the amount of SOC extracted between 167 and 333 mM KMnO₄ was termed less labile C (C_{LL}); and the difference between the levels of total SOC and SOC oxidized by 333 mM KMnO₄ was termed recalcitrant C (C_{NL}).

GRSP fractions were obtained using the methods proposed by Wright and Upadhyaya (1996) as follows. GRSPe was obtained by autoclaving 1 g of 2-mm-sieved soil with 8 ml of a 20 mM citrate solution (pH 7.0) at 121°C for 30 min, and GRSPt was obtained by successive autoclave extractions of 1 g of soil with 8 ml of a 50 mM citrate solution at pH 8.0 at 121°C. The protein content was determined by the Bradford assay using bovine serum albumin as a standard.

2.4 | Statistical analysis

The sum of C_{VL}, C_{ML}, and C_{LL} was considered to be the labile carbon (C_L) of the soil (Blair et al., 1995). The following indices were calculated

based on the differences in the C content between the grasslands and farmland:

$$\begin{aligned} \text{Carbon pool index (CPI)} &= \text{SOC}_{\text{grassland}} / \text{SOC}_{\text{farmland}} \cdot \text{Lability (L)} \\ &= C_L / C_{NL} \cdot \text{Lability Index (LI)} \\ &= L_{\text{grassland}} / L_{\text{farmland}} \end{aligned}$$

Carbon management index (CMI) = CPI × LI × 100 (Blair et al., 1995).

The rhizosphere effect on SOC, TN, TP, AP, GRSPt, and GRSPe was calculated as follows.

Rhizosphere effect on A = (A concentration in the rhizosphere—A concentration in the bulk soil)/A concentration in the rhizosphere.

Normal distribution and homogeneity test of variance have been conducted before ANOVA. One-way analysis of variance (ANOVA) was used to analyse the effects of the natural restoration and plant group on the vegetation properties, MR, concentrations of GRSP, and other soil nutrients, followed by Duncan's test. A correlation analysis was conducted based on Pearson's correlation coefficients. All ANOVAs and correlation analyses were tested for significance at $p < .05$ using SPSS 20.0 (SPSS Inc., Chicago, IL, United States), and the structural equation models (SEMs) were analysed using the SPSS Amos expansion pack RDA. The graphs were drawn using SigmaPlot 10.0 (Systat Software, San Jose, CA, United States).

3 | RESULTS

3.1 | Dynamics of plant composition, diversity, and biomass with restoration time

Artemisia capillaries dominated the community within the first 12 years and then the coverage of *A. capillaries* significantly decreased between 12 and 17 years after farmland abandonment (Figure 2a). Two perennial species, *Artemisia sacrorum* and *Stipa bungeana*, emerged at the 12-year site; coverage of *A. sacrorum* and *S. bungeana* significantly increased between 12 and 22 years. As a result, *A. sacrorum* and *S. bungeana* became the dominant and companion species between 17 and 22 years after farmland abandonment. In the natural grassland, *S. bungeana* and *Bothriochloa ischaemum* codominated the plant community, covering 40.7% and 30.0%, respectively, of the area.

Both the above and belowground biomasses of the dominant plant significantly decreased between 7 and 12 years after farmland

abandonment and then considerably increased between 12 and 22 years after farmland abandonment (Figure 2b,c). However, the above and belowground biomasses of companion plants significantly decreased between 12 and 17 years. The plant total above and belowground biomasses simultaneously reached a peak value at 22 years after farmland abandonment (Figure 2b,c), and the root-to-shoot ratio dramatically increased from 0.58 to 2.04 between 7 and 32 years after farmland abandonment (Figure 2d). The root-to-shoot ratio of the dominant plant continuously increased with time and overtook that of the total plant at 17 years. Total plant coverage largely increased following natural restoration (Table 2), with 65.3% in the 32-year site. The total plant coverage (Table 2) and above-ground biomass (Figure 2b) at the 32-year site reached the levels of the natural grassland, and the belowground biomass and the total plant root-to-shoot ratio exceeded those of the natural grassland (Figure 2c,d). The species richness and Shannon-Wiener diversity index increased over the first 17 and 22 years, respectively and then decreased with time (Table 2).

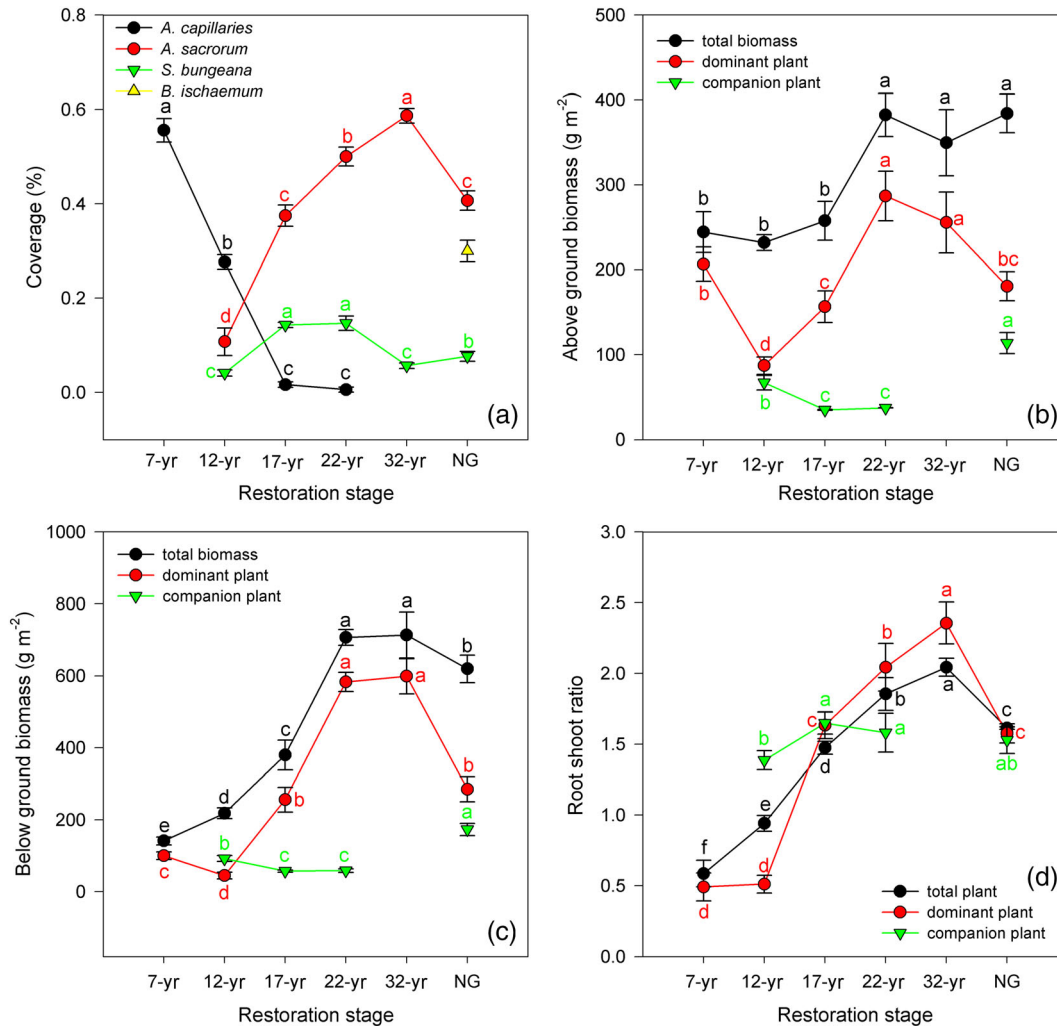


FIGURE 2 Changes of the coverage of main plant species during the succession (a). Changes of plant above (b) and below-ground biomass (c), and root shoot ratio (d) during the succession. Error bars indicate standard deviations. Different lowercase letters with the same color indicate significant differences between sites at the $p < .05$ level [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Characteristics of the vegetation during the succession

| Study site | Coverage (%) | Litter (g m ²) | R | H | E |
|------------|--------------|----------------------------|-------------|--------------|---------------|
| Farmland | -- | -- | -- | -- | -- |
| 7 year | 66.0 ± 2.3a | 43.5 ± 5.5c | 5.3 ± 0.6e | 1.55 ± 0.11d | 0.59 ± 0.07d |
| 12 year | 42.7 ± 1.4d | 44.7 ± 10.0c | 11.0 ± 1.0c | 2.23 ± 0.13b | 0.90 ± 0.03bc |
| 17 year | 47.3 ± 1.5c | 62.3 ± 11.5bc | 17.7 ± 1.6a | 2.48 ± 0.05a | 0.84 ± 0.02c |
| 22 year | 56.0 ± 3.5b | 70.1 ± 8.4b | 15.0 ± 1.0b | 2.64 ± 0.10a | 0.96 ± 0.05ab |
| 32 year | 65.3 ± 2.5a | 99.3 ± 10.6a | 10.7 ± 0.6c | 2.28 ± 0.08b | 0.90 ± 0.03bc |
| NG | 71.7 ± 7.6a | -- | 8.0 ± 1.0d | 2.05 ± 0.07c | 1.00 ± 0.03a |

Abbreviations: NG: natural grassland; R: species richness; H: Shannon-Wiener diversity index; E: evenness index.

Note. Different letters denote statistically significant differences at $p < .05$.

3.2 | Changes of soil carbon, nitrogen, and phosphorus with restoration time

The dynamics of SOC, TN, and TP in the bulk soil (Figure 3) and C_{VL} , C_{ML} , and C_{NL} in the bulk soil (Figure 4) were capable of fitting a quadratic model ($p < .001$). The concentrations of SOC and TN in both

the plant rhizosphere and the bulk soil were rapidly restored to the levels of the natural grassland between 22 and 32 years after farmland abandonment (Figure 3a,b). The concentrations of SOC and TN in the rhizosphere of the companion plant were lower than those of the dominant plant, and the concentrations of SOC and TN in the plant rhizosphere were significantly higher than those in the bulk soil

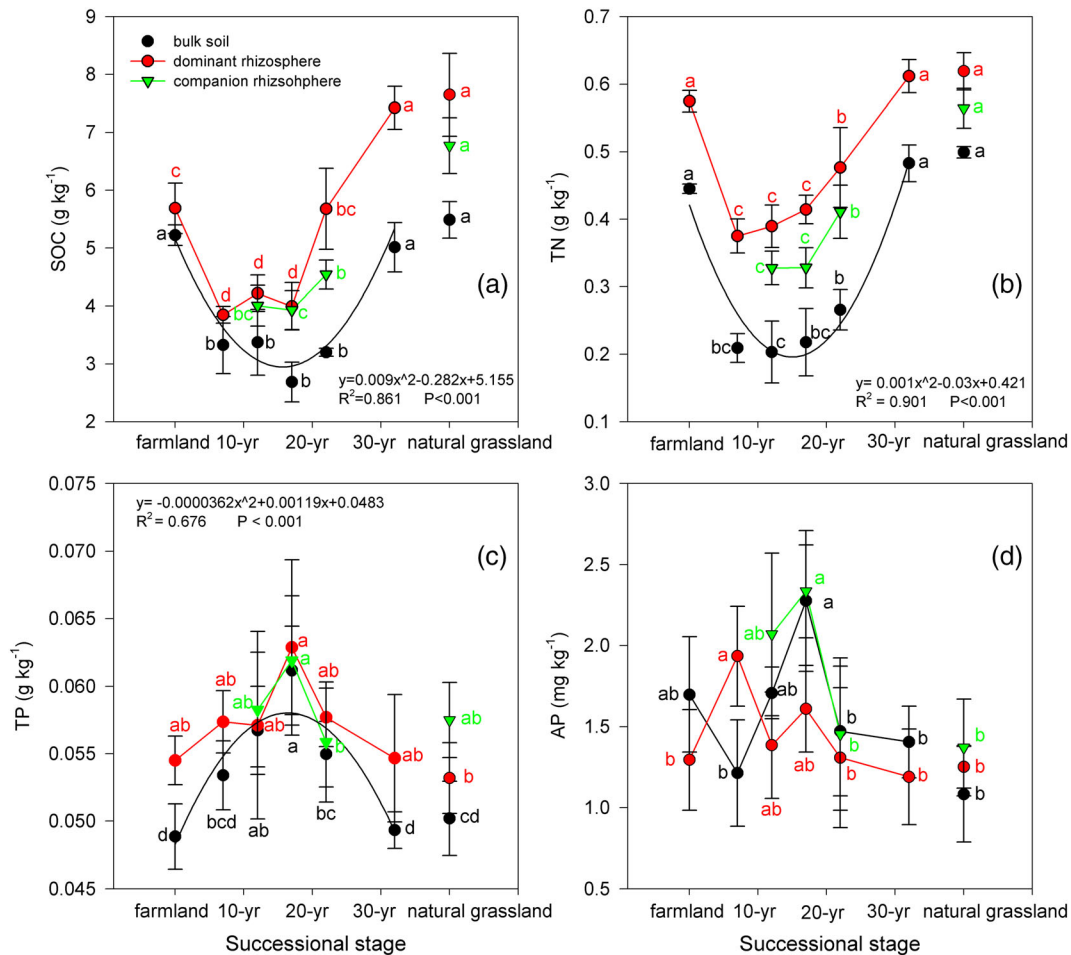


FIGURE 3 Dynamics of soil organic carbon (SOC; a), total nitrogen (TN; b), total phosphorus (TP; c), and available phosphorus (AP; d) in the bulk soil and rhizosphere of dominant plant and companion plant during the succession. Error bars indicate standard deviations. Different lowercase letters with the same color indicate significant differences between sites at the $p < .05$ level [Colour figure can be viewed at wileyonlinelibrary.com]

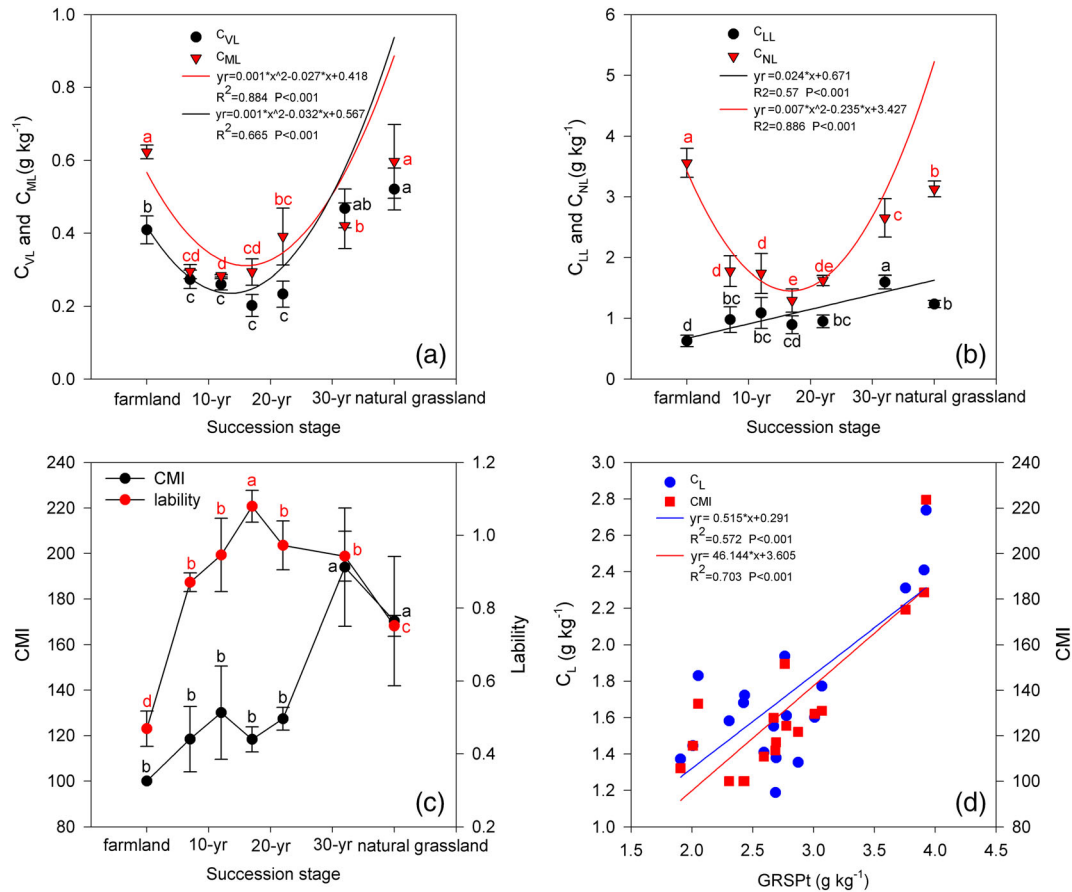


FIGURE 4 Dynamics of C_{VL} (a), C_{ML} (a), C_{LL} (b), C_{NL} (b), carbon management index (CMI; c), and lability of soil organic carbon (SOC; c) in the bulk soil during the succession. Linear relationship between C_L , CMI, and total glomalin-related soil protein (GRSpt) along the succession (d). Error bars indicate standard deviations. Different lowercase letters with the same color indicate significant differences between sites at the $p < .05$ level [Colour figure can be viewed at wileyonlinelibrary.com]

(Figure 3a,b). The rhizosphere effect on SOC increased within the first 22 years (Figure S1a), whereas the rhizosphere effect on TN increased within the first 17 years and then decreased with time (Figure S1b). Moreover, the SOC and TN concentrations in the bulk soil were linearly correlated with litter biomass (Figure S2a) and the concentrations of SOC and TN in the rhizosphere of the dominant plant (Figure S2b and S2c). The TP concentration initially showed an increasing trend after farmland abandonment but then rapidly decreased between 17 and 32 years after farmland abandonment (Figure 3c). In addition, the AP concentration in the bulk soil significantly increased from 1.21 to 2.28 $mg\ kg^{-1}$ between 7 and 17 years, after which it dramatically decreased to 1.41 $mg\ kg^{-1}$ in the 32-year site (Figure 3d). The concentrations of C_{VL} , C_{ML} , and C_{NL} were still lower than those in the natural grassland after 32 years of restoration, but the concentration of C_{LL} at the 32-year site exceeded that at the natural grassland (Figure 4a,b). CMI rapidly increased between 22 and 32 years and surpassed the level in the natural grassland at 32 years (Figure 4c). The lability of SOC dramatically decreased between 17 and 32 years, and it was significantly higher than that in the natural grassland between 7 and 32 years (Figure 4c). Additionally, the GRSpt concentration in the bulk soil was positively correlated with C_L and CMI (Figure 4d, $p < .001$).

3.3 | Changes of MR and GRSP with restoration time

The dynamics of GRSpt in the bulk soil with natural succession fit a quadratic model (Figure 5a, $p < .001$). The GRSpt concentrations in the bulk soil substantially increased from 2.67 to 3.86 $g\ kg^{-1}$ between 12 and 32 years. Conversely, GRSPe in the bulk soil significantly decreased from 0.397 to 0.237 $g\ kg^{-1}$ between 7 and 17 years and then remained constant between 17 and 32 years (Figure 5b). The concentrations of GRSpt and GRSPe and the GRSPe/GRSpt ratio in the bulk soil at the 32-year site were significantly lower than those at the natural grassland. GRSPe/GRSpt in the bulk soil significantly increased within the first 7 years and then considerably decreased between 7 and 32 years (especially 7 to 12 years; Figure 5c). The GRSpt concentration in the rhizosphere of the dominant plant decreased from 4.17 to 3.58 $g\ kg^{-1}$ within the first 12 years and the dramatically increased to 4.87 $g\ kg^{-1}$ between 12 and 32 years; however, and no significant differences were observed between the rhizosphere of the dominant plant and the companion plant (Figure 5a). Significantly, higher MR and GRSPe concentrations in the rhizosphere of dominant plants were observed relative to those of the companion plants (Figure 5b, $p < .01$, $F = 11.692$; Figure 5d, $p < .01$, $F = 8.698$). The MR and GRSPe concentration in the rhizosphere of *A. capillaries*

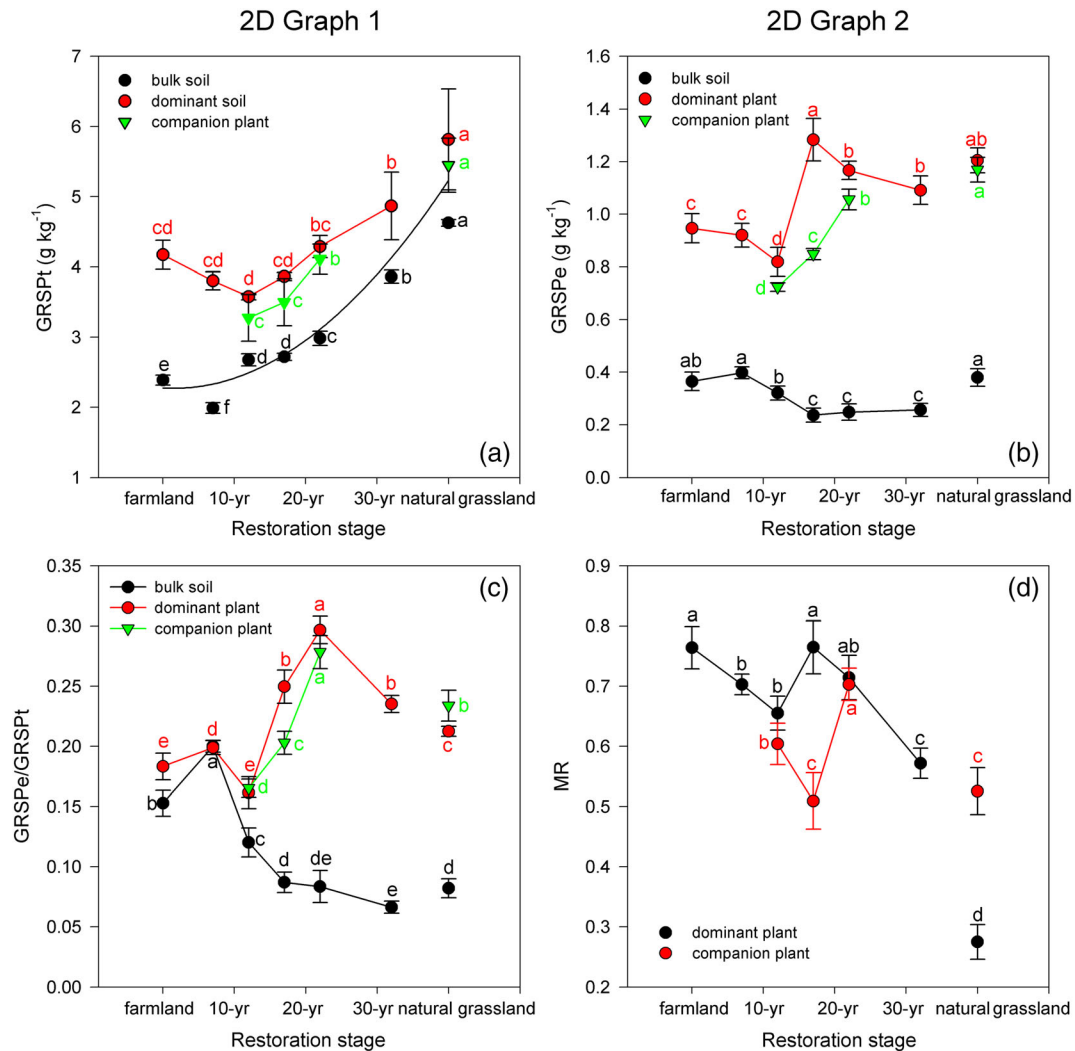


FIGURE 5 Dynamics of total glomalin-related soil protein (GRSPt; a), easily extractable glomalin-related soil protein (GRSPe; b), and GRSPe/GRSPt (c) in the bulk soil and rhizosphere of dominant plant and companion plant during the succession. Dynamics of mycorrhizal colonization rate (MR) of dominant plant and companion plant during the succession (d). Error bars indicate standard deviations. Different lowercase letters with the same color indicate significant differences between sites at the $p < .05$ level [Colour figure can be viewed at wileyonlinelibrary.com]

significantly decreased between 7 and 12 years after farmland abandonment and that of *A. sacrorum* rapidly increased between 12 and 17 years after farmland abandonment and then decreased between 17 and 32 years after farmland abandonment (Figure 5b and 5d). The GRSPe concentration in plant rhizosphere was positively correlated with plant above and belowground biomasses (Figure 6a). GRSPe, GRSPt, and GRSPe/GRSPt in the plant rhizosphere were significantly higher than those in the bulk soil ($p < .05$). Significantly, higher rhizosphere effects of the dominant plant on GRSPt and GRSPe were observed relative to that of the companion plant (Figure S1e and S1f).

3.4 | Relationship between plant-mycorrhizae association and the plant and soil conditions

The results of correlation analysis showed that MR was positively correlated with concentrations of GRSPe and TP in plant rhizosphere

(Figure 6b). According to the indices, the SEM Models 1, 2, and 3 adequately fit the data to describe the role of dominant plant-mycorrhizae association on shaping the dominant plant biomass and rhizosphere soil nutrients ($\chi^2_{12} = 15.239, 0.229$, root square mean errors of approximation [RMSEA] = 0.04, Figure 7a), the role of companion plant-mycorrhizae association on shaping the companion plant biomass and rhizosphere soil nutrients ($\chi^2_5 = 7.087, 0.182$, RMSEA = 0.04, Figure 7 b), and the relationships among GRSP, vegetation characteristics, and bulk soil nutrients ($\chi^2_{14} = 17.298, 0.242$, RMSEA = 0.04, Figure 7c). MR of dominant plant was negatively correlated with TN in plant rhizosphere but was positively correlated with TP ($p < .001$). MR of companion plant was positively correlated with TP in plant rhizosphere ($p < .001$). The TN in the rhizosphere of the dominant plant and bulk soil had a positive effect on the belowground biomass of the dominant and total plants ($p < .001$). The dominant plant aboveground biomass exhibited a positive relationship with the GRSP in the rhizosphere of the dominant plant ($p < .05$), and plant diversity had a positive relationship

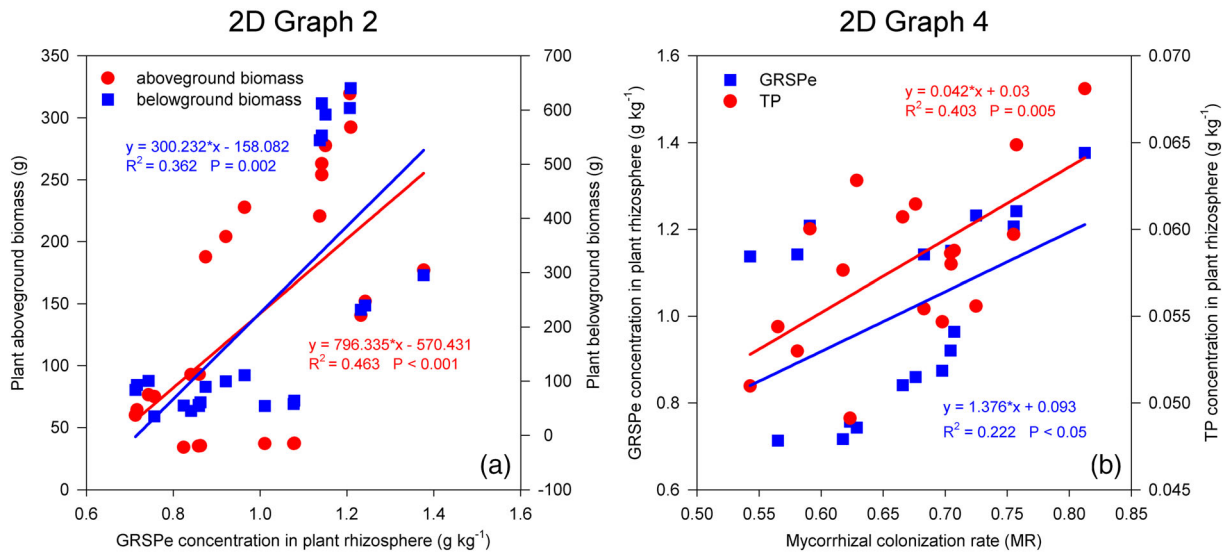


FIGURE 6 Linear relationship between glomalin-related soil protein concentration in plant rhizosphere and plant above and belowground biomasses (a) and between mycorrhizal colonization and easily extractable glomalin-related soil protein (GRSPe) and total phosphorus (TP) in plant rhizosphere (b) [Colour figure can be viewed at wileyonlinelibrary.com]

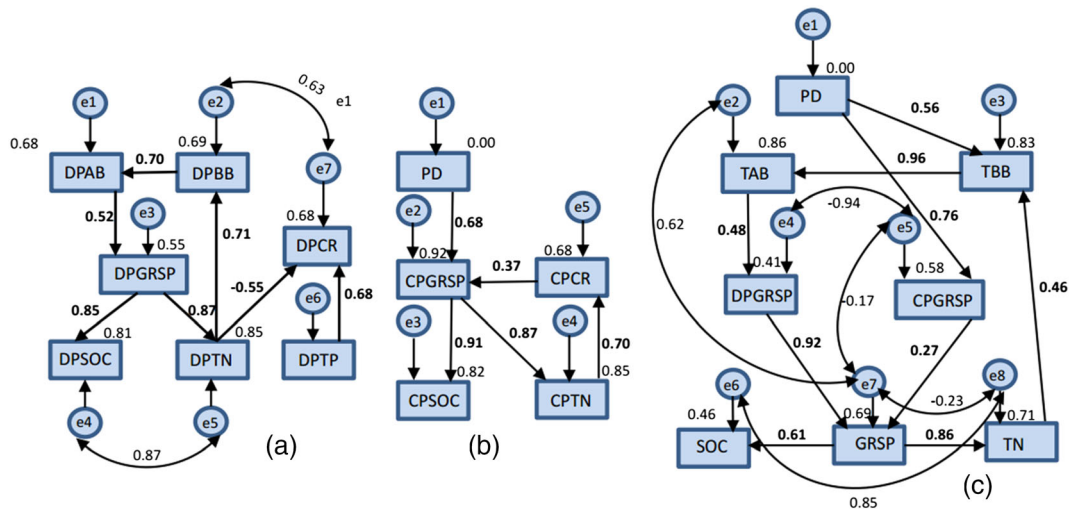


FIGURE 7 Structural equation model results for the effects of environment factors on total glomalin-related soil protein, soil organic carbon (SOC), and total nitrogen (TN) contents in (a) the rhizosphere of dominant plant, (b) the rhizosphere of companion plant, and (c) the bulk soil. Numbers on arrows represent the standardized path coefficients (equivalent to correlation coefficients). Circles indicate error terms. Percentages close to endogenous variable. A list of abbreviations: DPAB—dominant plant aboveground biomass, DPBB—dominant plant belowground biomass, DPGRSP—GRSPt in dominant plant rhizosphere, DPCR—mycorrhizal colonization rate of dominant plant, DPSOC—SOC in dominant plant rhizosphere, DPTN—TN in dominant plant rhizosphere, DTP—TP in dominant plant rhizosphere, PD—plant diversity, CPGRSP—GRSPt in companion plant rhizosphere, CPCR—mycorrhizal colonization rate of companion plant, CPSOC—SOC in companion plant rhizosphere, CPTN—TN in companion plant rhizosphere, TAB—total plant aboveground biomass, TBB—total plant belowground biomass [Colour figure can be viewed at wileyonlinelibrary.com]

with the GRSP in the rhizosphere of the companion plant ($p < .001$). The GRSP was positively correlated with SOC and TN ($p < .001$).

4 | DISCUSSION

4.1 | Interactions among plant-mycorrhizae association, plant diversity, and biomass

The plant-mycorrhizae association is dependent upon numerous factors, including soil nutrient availability and environmental variables

(Frater et al., 2018). Soil nutrient stoichiometry was a determinant factor influencing MR, as previous studies observed that the addition of nitrogen (N) and phosphorus (P) that are plant-limiting nutrients, reduced MR (Johnson, Rowland, Corkidi, Egerton-Warburton, & Allen, 2003; Johnson, 2010; Liu et al., 2012; Frater et al., 2018). In this study, MR was restricted by soil TP as natural restoration progressed (Figure 6 b); therefore, the decreased TP at the late stage of natural restoration resulted in a decreased plant-mycorrhizae association. The MR of the dominant plant was negatively correlated with the TN content, whereas the MR of the companion plant was positively correlated with the TN content (Figure 7), indicating that only the MR of the companion plant

was restricted by soil TN. Specifically, the dominant plant had a tighter association with AMF relative to the companion plant, as the MR of the dominant plant was significantly higher than that of the companion plant (Figure 5d). Therefore, the dominant plant had a higher competitive ability in absorbing nitrogen relative to that of the companion plant, contributing to the higher biomass of dominant plant. In addition, the dominant plants accumulated more carbon and nitrogen in their rhizosphere with the help of GRSP production from AMF than the companion plants, which enhanced the microbial diversity and activity in the dominant plant rhizosphere and promoted the performance of the dominant plants, as plant growth-promoting bacteria can produce hormones that increase plant growth or antibiotics that suppress harmful fungi (Ramirez, Craine, & Fierer, 2012; Vinale et al., 2008; Zhong et al., 2010).

The dynamics of species richness and diversity of the grassland vegetation, which reached maximum values between 17 and 22 years after farm abandonment (Table 2), were in agreement with the hump-backed model proposed by Grime (1973). The increased competition for resources with vegetation succession excluded species with lower competitive capacities, for example, *Setaria viridis*, *Heteropappus altaicus*, and *A. capillaries* (Table 1), resulting in a net decrease in species richness and diversity between 22 and 32 years after farm abandonment (Arroyo, Pueyo, Saiz, & Alados, 2015; Liu et al., 2017; Tilman, 1985; Zhang, Wang, Liu, Song, & Fang, 2019). Plant diversity has been considered an important determinant of grassland productivity (Chen et al., 2018; Tilman, Reich, & Isbell, 2012; Zhang, Liu, Song, Wang, & Guo, 2018). In this study, plant diversity also had a direct positive influence on the plant belowground biomass and had an indirect positive effect on the plant aboveground biomass (Figure 7). Similar to species richness and diversity, plant total above and belowground biomasses simultaneously reached peaks value at 22 years after farm abandonment and then remained stable, which is consistent with the results from previous studies showing that species diversity and biomass simultaneously peaked and reached equilibrium after approximately 20 years of natural restoration (Jing, Cheng, & Chen, 2013; Liu et al., 2017). Therefore, the increased plant diversity at the middle stage of vegetation succession is an important factor promoting plant production, and the coverage and above and belowground biomasses at the late stage of natural restoration (22 to 32 years) had been restored to the levels of the natural grassland, indicating that abandoning farmland is an effective way to restore vegetation.

4.2 | Effects of vegetation characteristics and plant-mycorrhizae association on recovery of GRSP

The plant-mycorrhizae association and above and belowground biomasses determined the GRSPe concentration in the plant rhizosphere, as MR was positively correlated with the GRSPe concentration (Figure 6b), and the above and belowground biomasses were also positively correlated with GRSPe in the plant rhizosphere (Figure 6a). A previous study suggested that net primary production determines the upper bound of C that is available for glomalin production and the turnover of AM hyphae (Treseder, 2004; Treseder & Turner, 2007).

Therefore, the significantly improved plant-mycorrhizae association and the above and belowground biomasses at the middle stage of vegetation succession rapidly promoted GRSPe production in the plant rhizosphere. The structural equation models showed that GRSPt in the rhizosphere of the dominant plant was mainly controlled by the aboveground biomass, whereas GRSPt in the rhizosphere of the companion plant was mainly controlled by plant diversity and MR (Figure 7). Previous studies suggested that the availability of plant C to AMF is a determinant of GRSP storage (Treseder & Cross, 2006; Treseder & Turner, 2007) and that AMF status efficiently mediates the metabolism of GRSP (Rillig et al., 2002; Wu et al., 2014; Yang et al., 2017). The close plant-mycorrhizae association and high above and belowground biomasses of the dominant plant contributed to accumulating more GRSPe and GRSPt in the rhizosphere of the dominant plant than that of the companion plant. Distinct from the GRSPe dynamics, the GRSPt contents in the plant rhizosphere substantially increased between 12 and 32 years after farm abandonment (Figure 5). The GRSPt content is the result of GRSPe accumulation and GRSP decomposition by soil microbes, and GRSP is a refractory compound with a turnover time of many years, so its content increases with soil age (Rillig, Wright, Allen, & Field, 1999; Treseder & Turner, 2007). The GRSPt concentrations in the plant rhizosphere after 32 years of restoration were still lower than in those in the natural grassland; GRSPe/GRSPt can reflect the potential increase in GRSP between soils (Jorge-Araujo, Quiquampoix, Matumoto-Pintro, & Staunton, 2015), and GRSPe/GRSPt in the plant rhizosphere at 32 years after farm abandonment was higher than that in the natural grassland. Therefore, a strong potential for GRSP accumulation during succession was observed.

The GRSPe concentration in the bulk soil decreased by nearly half between 7 and 17 years after farm abandonment and remained stable afterwards. Although AMF in the plant rhizosphere produce a great quantity of glomalin, glomalin is insoluble in water and hard to transport in soil (Wright & Upadhyaya, 1996), which limits the accumulation of GRSPe in the bulk soil far away from the plant rhizosphere. The GRSPt concentrations in the bulk soil substantially increased, and the difference in GRSPt between the plant rhizosphere and bulk soil rapidly decreased at the late stage of natural restoration (Figure 5). AMF generally form mutualistic associations with the roots of the majority of higher plants (Rillig, 2004) and produce glycoprotein glomalin within their hyphal walls (Driver et al., 2005); therefore, the GRSPt concentration in the bulk soil is determined by the plant rhizosphere process. Our results suggested that the GRSPt concentration in the rhizosphere of the dominant (standardized path coefficient was 0.92) and companion plants (standardized path coefficient was 0.27) positively contributed to the GRSPt in the bulk soil and that the dominant plants had a higher contribution than the companion plants.

4.3 | Effect of vegetation characteristics and GRSP on soil SOC stabilization and nutrient recovery

The dominant plant played a more important role in accumulating SOC and TN than the companion plant because higher SOC and TN

contents were observed in the rhizosphere of the dominant plant than in the rhizosphere of the companion plant (Figure 2), which is consistent with the results of previous studies (Zhang et al., 2013; Zhang et al., 2015). The difference in the level of GRSP production between dominant and companion plant rhizospheres is an important factor resulting in the difference in the ability of soil carbon and nitrogen to accumulate in dominant and companion plants. GRSP plays an important role in contributing to the accumulation of carbon and nitrogen, as positive correlations between GRSP and SOC as well as TN have been observed in numerous studies (Fokom et al., 2012; Singh, Rai, & Singh, 2016; Vasconcellos et al., 2016; Wang et al., 2016), and GRSP contributes 4–5% of soil C and 3–5% of soil N (Rillig et al., 2001; Lovelock et al., 2004). The structural equation model showed that GRSPt in the plant rhizosphere equally contributed to the contents of SOC (standardized path coefficients were 0.85 and 0.91) and TN (standardized path coefficients were 0.87 and 0.87) in the plant rhizosphere. Therefore, the rapid increase in GRSP at the late stage of natural succession was an important factor promoting the accumulation of SOC and TN, as the concentrations of SOC and TN in the dominant plant rhizosphere were restored to the levels of the natural grassland after 32 years of natural restoration. In addition, a lower AP content was observed in the rhizosphere of the dominant plant than in the rhizosphere of the companion plant (Figure 2). P has unique properties, including low mobility and solubility and is highly fixed in the soil matrix, so it can be rapidly depleted in the rhizosphere by root uptake, leading to a gradient of P concentrations in a radial direction away from the root surface (Shen et al., 2011). The strong competition among plant species prompted more AP to be absorbed by dominant plants than by the companion plants. The TP concentrations in the plant rhizosphere rapidly increased between 12 and 17 years after farm abandonment and then significantly decreased between 17 and 22 years after farm abandonment (Figure 2), indicating that the middle stage of vegetation succession is important for soil P recovery.

The concentrations of SOC and TN in the bulk soil rapidly increased at the late stage of vegetation succession and reached the levels of the natural grassland (22 to 32 years), indicating that natural restoration is an efficient way to recover SOC and TN storage. Specifically, natural succession mostly promoted the accumulation of carbon with less lability (C_{L1}) and enhanced the lability of SOC. Moreover, the CMI was a sensitive index to evaluate changes in the soil organic matter content and soil quality (Blair et al., 1995), and this index indicated that although the plant community remained stable at the late stage of natural restoration, this stage is important for SOC sequestration. In this study, a positive correlation between plant litter and SOC ($R^2 = 0.579$ $p < .001$, Figure S2A), TN ($R^2 = 0.8665$ $p < .001$, Figure S2A) was observed. The fate of litter degradation determines soil organic matter sequestration (Kogel-Knabner, 2002; Pries, Bird, Castanha, Hatton, & Torn, 2017; Tamura, Suseela, Simpson, Powell, & Tharayil, 2017). Previous studies observed positive correlations between plant litter biomass and the contents of SOC and TN, and the contribution of litter biomass to SOC was greater than that to TN as succession progressed (Tateno et al., 2017; Wu, Liu, Tian, & Shi, 2017). In addition, the rhizosphere process largely contributed to SOC and TN accumulation in the bulk

soil, as the SOC and TN concentrations in the plant rhizosphere were positively correlated with those in the bulk soil (Figure S2B and S2C). This result is primarily attributed to rhizodeposition, which made up approximately 25% of the belowground allocated C (Jones et al., 2009) and constituted approximately 0.5–10% of net fixed carbon (Farrar, Hawes, Jones, & Lindow, 2003). The initial increase in the TP concentration in the bulk soil after farmland abandonment is probably related to the increase in soil organic phosphorus, as AP is taken up by the vegetation following cessation of agricultural production, resulting first in an increase in the plant P concentration and later in an increase in the soil organic phosphorus due to litter inputs (Spohn, Novak, Incze, & Giani, 2016). The AP and TP concentrations at the 32-year site were similar to those at the natural grassland site, indicating that long-term secondary succession had a negligible effect on the soil P concentration.

GRSPt also largely promoted the accumulation of SOC and TN in the bulk soil, as GRSPt was positively correlated with the concentrations of SOC and TN (Figure 7). Because the coefficient of correlation between GRSPt and SOC (0.61) was lower than that between GRSPt and TN (0.86), GRSPt contributed more to SOC than to TN. GRSP is an important component of dissolved organic carbon, and $KMnO_4$ -C mainly consists of hot water-extractable carbon and undefined labile organic matter, so GRSP promotes the accumulation of labile carbon (Strosser, 2010; Fokom et al., 2012; Singh, Rai, Pandey, & Singh, 2017). Additionally, a positive correlation was observed between GRSPt and CMI, confirming that GRSP is important for the improvement of SOC quality. The soil TN concentration had a direct positive effect on the plant belowground biomass and an indirect positive effect on the plant aboveground biomass (Figure 7), indicating that nitrogen instead of phosphorus is the limiting nutrient for plant growth in the Loess Plateau, which is consistent with the results from previous studies on the Loess Plateau (Zeng et al., 2017).

5 | CONCLUSIONS

Vegetation restoration in the Loess Plateau is mainly limited by soil nitrogen, and significantly increased plant diversity promotes the production of plant biomass. The plant-mycorrhizae association is mainly dependent on soil TP. The significantly increased plant-mycorrhizae association and plant biomass after natural restoration rapidly promote the accumulation of GRSPe and GRSPt in the plant rhizosphere. Although a 32-year natural restoration is not long enough for the GRSPt concentration to return to the level of the natural grassland, a large potential for GRSP accumulation is shown with a longer recovery time. The increased litter input and GRSP content after vegetation restoration largely promotes the accumulation of SOC and TN. Specifically, GRSP plays a vital role in SOC stabilization. Dominant plants rather than companion plants benefit more from AMF by acquiring nitrogen and accumulating carbon and nitrogen in the rhizosphere largely from GRSP production, leading to dominant plants having higher biomass and coverage than companion plants. The results of this research highlight the importance of plant-mycorrhizae association,

plant species, and restoration time in determining the restoration of soil nutrients along temporal gradients of natural restoration after farmland abandonment.

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CONFLICT OF INTEREST

No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my coauthors that the work described was original research that has not been published previously and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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