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Glomalin-related soil protein affects soil aggregation and recovery of soil nutrient following natural revegetation on the Loess Plateau

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ABSTRACT

The glomalin-related soil protein (GRSP) is an important fraction of soil organic carbon (SOC) and soil nitrogen (N), and is important for stabilization of SOC and soil aggregates. However, the effects of natural restoration on the concentration and allocation of GRSP differ for different soil aggregate sizes, and how size further affects SOC and soil N restoration, and stabilization of SOC and soil aggregates is not well understood. Here, we present the first characterization of the distribution of GRSP fractions and soil nutrients in soil aggregates following natural restoration by choosing fields of 0, 7, 12, 17, 22, 32 years after cropland abandonment, and a natural grassland as reference. GRSP concentration increased most in microaggregates after 32 years of natural restoration. The processes of rapid accumulation of GRSP (22 to 32 years) occurred simultaneously with the formation of macroaggregates, reduction of microaggregates, and rapid increase of mean weight diameter (22 to 32-years). The soil aggregate stability and contents of GRSP, SOC, labile carbon, total N and phosphorus in each soil aggregate fraction significantly increased in the late stage of natural restoration (22 to 32 years). The most recalcitrant carbon fraction in microaggregates significantly increased between 7 and 32 years (0.887 g kg^{-1}). Our study suggests that abandoning farmland is effective for the restoration of GRSP, soil nutrients and structure and that microaggregates promote the accumulation of recalcitrant carbon and increase the stability of SOC largely through its ability to retain GRSP.

1. Introduction

Natural revegetation without further anthropogenic disturbance is an efficient way for soil rehabilitation (Deng et al., 2013; Walker et al., 2010; Zhang et al., 2016), and it has been implemented on the Loess Plateau in China which is well known for its long agricultural history and severe soil erosion (Chen et al., 2007). The native vegetation on steep slopes were destroyed and converted to farmland to satisfy food supply needs for an increasing population, leading to serious soil erosion and soil deterioration (Fu et al., 2010; C. Zhang et al., 2012). In order to control soil erosion and improve the quality of the degraded soil, Chinese government launched "Grain for Green" project for vegetation restoration by converting farmland to forests and grassland (Deng et al., 2013). Most farmlands with slopes > 15° on the Loess Plateau were abandoned, where ploughing and sowing were ceased, to allow plant secondary succession (Chang et al., 2011). This implement has greatly promoted vegetation restoration (Zhang et al., 2016), and improved soil organic matter and soil aggregate stability (An et al., 2013; Deng et al., 2016; Zhao et al., 2017). Abandoned farmlands in Loess Plateau that have been colonized by natural vegetation for different lengths of time offer a good opportunity for investigating the effects of natural revegetation on the recovery of the quality of the degraded soil (Zhang et al., 2016).

Glomalin is known as soil glycosylated protein, produced by arbuscular mycorrhizal fungi and released into soil with hyphal turnover (Treseder and Turner, 2007; Wright and Upadhyaya, 1998). Glomalin is

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operationally defined and extracted from soils as glomalin-related soil protein (GRSP), which has been classified into two fractions, i.e., easily extractable GRSP (GRSPe) and total GRSP (GRSPt). GRSPe represents the newly produced fraction and GRSPt is the sum of both recent and old fungal protein production (Rillig, 2004; Wright and Upadhyaya, 1998). GRSP can promote the formation of water-stable aggregates by acting as an aggregate binding agent (Fokom et al., 2012; Zhu et al., 2019), which binds soil particles to form aggregates through a "gluing" action (Gispert et al., 2013). Numerous studies have reported that the contents of GRSP in both bulk soil and aggregates were highly correlated with the stability of water-stable aggregates (Fokom et al., 2012; Zhang et al., 2014; Zhu et al., 2019). Conservation tillage, no tillage, and organic amendment can promote the formation and stabilization of soil aggregates via redistribution of GRSP in various aggregate sizes which contribute to binding within microaggregates and macroaggregates (Dai et al., 2015; S. Zhang et al., 2012; Zhang et al., 2014). Additionally, GRSP facilitates soil organic carbon (SOC) and soil nitrogen (N) accumulation given the amount of carbon (C) and N it retains; the contents of C and N in glomalin have been estimated to be 36–59% and 3–5%, respectively, comprising 3% of soil C and 5% of soil N, which even exceeds the contribution from soil microbial biomass (Sousa et al., 2012; Wang et al., 2015; Wang et al., 2017). Positive correlations between the contents of SOC and GRSP in aggregates across particle sizes have been observed in numerous studies (Dai et al., 2015; Xiao et al., 2019; Zhang et al., 2014; Zhu et al., 2019), so that GRSP plays an important role in the concentration and allocation of SOC in different aggregate sizes. Given that GRSP plays a vital role in soil carbon and nitrogen storage and structural stability, GRSP is considered an important indicator of soil quality to monitor soil degradation (Fokom et al., 2012; Vasconcellos et al., 2016). Natural-succession recovery on abandoned farmland significantly increased the GRSP concentrations in both bulk soil and each aggregate fraction (Xiao et al., 2019). However, little is known about the effects of GRSP on the concentration and allocation of soil N and stabilization of SOC in the aggregate fractions following natural revegetation.

Soil aggregates significantly affect the dynamics of soil organic matter and nutrient cycling, as well as their responses to revegetation (Six et al., 2000; Yao et al., 2019). The incorporation of soil organic matter in aggregates can physically protect soil organic matter from mineralization by preventing it from accessing to microorganisms and enzymes (Six and Paustian, 2014; Wei et al., 2013). SOC and soil N accumulation mainly depends on the accumulation of SOC in macroaggregates rather than microaggregates after natural revegetation (Deng et al., 2018; Wang et al., 2018; Yao et al., 2019; Zhu et al., 2017). The short-term storage of SOC and N in the soil concentrates in macroaggregates and long-term sequestration concentrates in microaggregates (Haile et al., 2008; Sainju et al., 2009), because microaggregates mainly contain old organic carbon and macroaggregates contain a considerable amount of younger organic material (Blanco-Canqui and Lal, 2004; Six et al., 2004). Y. Liu et al. (2018) applied the technique of ^{13}C natural abundance to study C incorporation into soil aggregates and suggested that SOC sequestration of fresh organic matter generally starts in macroaggregates, and the resulting degraded organic matter after disaggregation and microbiological consumption is sequestered in microaggregates. Additionally, the oxidization of labile carbon, which has a rapid turnover rate, determines the flux of CO_2 to the atmosphere, and labile organic carbon plays an important role in regulating aggregate organic carbon (Deng et al., 2018; Majumder et al., 2007). Phosphorous (P) plays an important role in plant growth and the biogeochemical cycles of SOC and soil N by maintaining the molecular structure and mediating the energy transfer during the activities of plant and soil microbes (Richardson et al., 2011; Singh and Satyanarayana, 2011), so that it significantly affects ecosystem functionality (Ellsworth et al., 2017). Revegetation had a negligible effect on P concentrations in bulk soil and aggregates in an agro-pastoral ecotone of northern China (Yao et al., 2019). Therefore, a comprehensive study of the concentration and allocation of GRSP in aggregate fractions and its influence on soil aggregate stability, concentration and allocation of SOC fractions with different labilities, soil N and P in the aggregate fractions would contribute to a better understanding of the underlying mechanisms of soil aggregate and SOC stabilization, and recovery of SOC, soil N and P during natural revegetation on the Loess Plateau.

The present study quantified soil aggregate concentrations and compared GRSP, SOC, soil N and P in soil aggregates along a well-dated grassland restoration chronosequence on the Loess Plateau. Our objectives were (1) to evaluate the effects of natural revegetation on concentration and allocation of soil N and P, and the dynamic and stabilization of SOC in different soil aggregate sizes, and how this effect further affects soil aggregate stabilization. (2) To investigate the effects of natural revegetation on concentration and allocation of GRSP, and how this effect further affects soil aggregate stabilization. (3) To clarify the contribution of GRSP to recovery of SOC and soil N, and SOC stabilization in different soil aggregate sizes.

2. Materials and methods

2.1. Study area

The study sites of sloped farmland and five sloped farmlands abandoned for 7, 12, 17, 22 and 32-years were located in the Zhifanggou watershed (the coordinates of each study site were shown in Table 1) in Ansai County on the northern Loess Plateau, China. Each study site was at least 500 m from the other sites. The study site of natural grassland was located in the Songjiagou watershed (the coordinate was shown in Table 1) in Ansai County (Fig. S1). This study area has a temperate semiarid climate and a deeply incised hilly-gully Loess landscape. The mean annual temperature is 8.8 °C, and the mean annual precipitation is 510 mm. The soil, which is developed on wind-deposited loessial parental material, is classified as a Calcic Cambisol (IUSS Working group WRB, 2014) with silty loam texture. The detailed geographical information of study sites is presented in Table 1. The soil chemical and physical properties of study sites were provided in Tables 2 and 3, respectively.

Table 1
Geographic features and floristic compositions of the sampling sites.

Study site	Slope aspect	Slope gradient	Altitude (m)	Coordinates	Vegetation community
Farmland	E15°N	25°	1274	36°44′39″N, 109°14′35″E	*S. + Gmax*
7-years	W10°N	20°	1303	36°44′47″N, 109°15′12″E	*A.capillarie*
12-years	E40°N	26°	1276	36°44′02″N, 109°16′31″E	*A.sacrorum + Acapillaries*
17-years	E25°N	28°	1307	36°44′09″N, 109°16′14″E	*A.sacrorum + Sbungeana*
22-years	E10°N	30°	1267	36°44′05″N, 109°16′27″E	*A.sacrorum + Sbungea*
32-years	E16°N	30°	1246	36°44′15″N, 109°15′55″E	*Asacrorum*
Natural grassland	E34°N	26°	1183	36°44′59″N, 109°16′28″E	*A.sacrorum + B. ischaemum* (L.) Keng.

S.italic:*Setaria italic*;*G.max*:*Glycine max*;*A.capillaries*:*Artemisia capillaries*;*A.sacrorum*:*Artemisia sacrorum*;*S.bungeana*:*Stipa bungeana*;*B. ischaemum* (L.) Keng:*Bothriochloa ischaemum* (L.) Keng.

Table 2
Soil chemical properties following natural revegetation.

Study sites	Organic C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	Total P ($g\ kg^{-1}$)	NH_4^+-N ($g\ kg^{-1}$)	NO_3^-N ($g\ kg^{-1}$)	Available P ($g\ kg^{-1}$)	pH
Farmland	5.22 ± 0.18a	0.45 ± 0.01a	0.49 ± 0.02d	7.90 ± 0.95a	16.56 ± 3.04ab	1.70 ± 0.36ab	8.48 ± 0.12a
7-years	3.33 ± 0.49b	0.21 ± 0.02bc	0.53 ± 0.03bcd	6.47 ± 0.49a	12.83 ± 2.46bcd	1.21 ± 0.33b	8.53 ± 0.04a
12-years	3.37 ± 0.57b	0.20 ± 0.05c	0.57 ± 0.03ab	7.29 ± 0.71a	15.41 ± 1.37abc	1.71 ± 0.16ab	8.55 ± 0.08a
17-years	2.69 ± 0.34b	0.22 ± 0.05bc	0.61 ± 0.03a	6.29 ± 0.16ab	19.02 ± 2.82a	2.28 ± 0.44a	8.49 ± 0.05a
22-years	3.20 ± 0.07b	0.27 ± 0.03b	0.55 ± 0.02bc	4.52 ± 0.36c	11.17 ± 1.42bc	1.47 ± 0.40b	8.51 ± 0.01a
32-years	5.01 ± 0.43a	0.48 ± 0.03a	0.49 ± 0.01d	4.79 ± 0.25bc	13.00 ± 2.84bcd	1.41 ± 0.22b	8.48 ± 0.07a
Natural grassland	5.49 ± 0.31a	0.50 ± 0.01a	0.50 ± 0.03cd	7.64 ± 0.93a	9.25 ± 1.85d	1.08 ± 0.30b	8.48 ± 0.08a

Values are means ± standard deviation (n = 3).
Different letters indicate significant differences ($P < 0.05$) among soils for the individual variables.

2.2. Sampling design

In our study, we used the "space" for "time" method to study the effect of GRSP content in different soil aggregate sizes and the aggregate stability of abandoning farmland for natural restoration, which is a common method used to investigate soil recovery during natural restoration (Chang et al., 2017).

We conducted this field experiment in September 2016 when the plant biomass peaked. The millet (*Setaria italica*) and soybean (*Glycine max*) in rotation were the main crops cultivated in the farmlands of this area. The sloped farmland and natural grassland with no farm history for at least 40 years were used as references. Each study site was at least 400 m from the other sites. Similar slope gradients, slope aspects and elevations of these sites were ensured to reduce experimental error induced by geographical conditions. The sloped farmland was fertilized annually with 6.0 t ha^{-1} goat manure, 60 kg ha^{-1} nitrogen ($CO(NH_2)_2$) and 45 kg ha^{-1} phosphorus pentoxide (P_2O_5).

Three 20 m × 20 m plots were established at each site. Each plot was at least 40 m from the other plots. Soil samples were collected from the 0–20 cm depth interval using a soil auger (diameter, 4 cm). Before soil sampling, the litter horizons were removed. Five sampling points were selected in each plot. One point was in the center of the plot and the other four points were in the center points between the center and each corner of the plot. Soil samples were collected from these points on the same day and thoroughly mixed to make a composite sample for each plot. All samples were air-dried, ground, and passed through 2-, 1- and 0.25-mm sieves. Soil sections were excavated in each plot, and the undisturbed soil samples were taken from 0 to 20 cm soil layer, and were kept in the crispers before analysis of water-stable aggregates, soil particle size distribution, and soil bulk density.

2.3. Water-stable aggregate fractionation

Water stable aggregates were separated by wet sieving following the procedure by Elliott (1986). Briefly, 100 g undisturbed soil was submerged in distilled water for 30 min and subsequently transferred to a 2-mm sieve that was submerged at the highest point of oscillation. The sample was subject to oscillation for 5 min. Water-stable aggregates that remained on the 2-mm sieve were transferred to an aluminium case. The material passing through the sieve was further separated using the same protocol with a 0.25-mm sieve and a 0.053-mm sieve. This process ultimately yielded four aggregate fractions comprising soil particles with diameters of > 2-mm (large macroaggregate, LAGA), 0.25–2 mm (small macroaggregate, SMGA), 0.053–0.25 mm (microaggregate, MIGA), and < 0.053 mm (silt + clay, SICL). After oven drying at 50 °C, the four classes of aggregates were weighed and stored at room temperature for future analysis of the chemical properties. The proportional mass (%) of water-stable aggregates in each fraction was based on the oven-dried weights. The mean weight diameter (MWD) represents the fraction of the sample on the sieve times the mean inter-sieve aperture.

2.4. GRSP determination

GRSP was extracted based on the method described by Wright and Upadhyaya (1998). Briefly, the easily extractable GRSP (GRSPe) was obtained by autoclaving 1 g of 2-mm-sieved soil in a 8 mL of 20 mM citrate solution at pH 7.0 at 121 °C for 30 min. The total GRSP (GRSPt) was extracted by six successive autoclave extractions with 8 mL of a 50 mM citrate solution at pH 8.0 by autoclaving at 121 °C for 60 min and centrifuged at 10,000 ×*g* for 5 min to remove the supernatant. After each cycle, the 8-mL citrate solution was re-added to repeat the extraction until the solution was straw coloured. The supernatants were combined to form the total extract. Extracts were frozen until required for analysis. After thawing, samples were centrifuged at 10,000 ×*g* for 20 min to remove precipitate. The protein content was assayed using the Bradford assay (Rillig, 2004; Wright and Upadhyaya, 1998). Controlled sample dilutions with colour correction were performed according to Moragues-Saitua et al. (2018), the final dilutions were 1:2 for all the samples based on the dilution curves and calculations were made with correction for sample blank colour (at the same dilution and pH).

Table 3
Soil physical properties following natural revegetation.

Study sites	Bulk density ($g\ cm^{-3}$)	Particle size distribution (%)				Erodibility	Particle fractal dimension
		Clay < 0.002 mm	Fine silt 0.002–0.02 mm	Coarse silt 0.02–0.05 mm	Sand 0.05–1 mm		
Farmland	1.40 ± 0.18a	9.68 ± 0.98a	14.26 ± 0.69a	45.52 ± 0.90ab	30.54 ± 1.23c	0.411 ± 0.002b	2.62 ± 0.02a
7-years	1.41 ± 0.03a	6.63 ± 0.41b	10.90 ± 0.17c	45.05 ± 0.65b	37.43 ± 0.45a	0.408 ± 0.002c	2.56 ± 0.02b
12-years	1.24 ± 0.09b	7.12 ± 0.70b	13.41 ± 1.53ab	44.78 ± 0.90b	34.69 ± 1.56b	0.413 ± 0.001ab	2.57 ± 0.02bc
17-years	1.23 ± 0.09b	7.67 ± 0.05b	12.34 ± 0.24bc	46.19 ± 0.37a	33.80 ± 0.41b	0.414 ± 0.002a	2.58 ± 0.02bc
22-years	1.23 ± 0.05b	7.85 ± 0.94b	11.91 ± 1.34bc	44.61 ± 0.42b	35.62 ± 1.88ab	0.408 ± 0.001c	2.59 ± 0.02b
32-years	1.27 ± 0.04b	7.48 ± 0.44b	12.74 ± 0.20ab	44.77 ± 0.22b	35.01 ± 0.61b	0.408 ± 0.001c	2.58 ± 0.02bc
Natural grassland	1.35 ± 0.09ab	7.69 ± 0.40b	12.50 ± 0.63bc	44.90 ± 0.46b	34.90 ± 0.61b	0.406 ± 0.001c	2.58 ± 0.02b

Values are means ± standard deviation (n = 3).
Different letters indicate significant differences ($P < 0.05$) among soils for the individual variables.

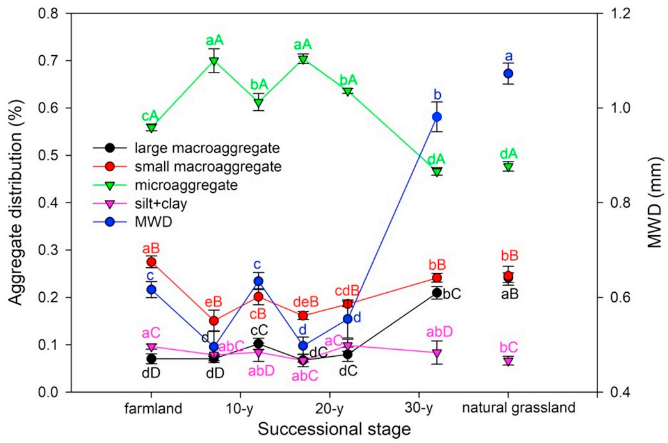

Fig. 1. Distribution of soil aggregates (%) and MWD following natural succession in the Loess Plateau, China. Error bars denote standard deviations (n = 3). Different capital letters indicate significant differences among different aggregate sizes in same study site. Different small letters indicate significant differences among study sites in same aggregate size.

2.5. Determination of SOC, C fractions, soil N and P contents

Soil organic carbon (SOC) content was measured using the Walkley and Black dichromate oxidation method (Nelson and Sommers, 1982). Soil oxidizable organic carbon (OC) fraction contents were determined using a modified Walkley–Black method (Walkley and Black, 1934) described by Chan et al. (2001) and H. Liu et al. (2018). In brief, the amount of SOC was oxidized at three H_2SO_4 concentrations (3, 6, and 9 mol/L) and a constant concentration of dichromate. Thus, SOC was separated into four fractions with different degrees of lability: Fraction 1 (C_{f1}): oxidized OC in 3 mol/L; Fraction 2 (C_{f2}): oxidized OC between 6 and 3 mol/L; Fraction 3 (C_{f3}): oxidized OC between 9 and 6 mol/L, and Fraction 4 (C_{f4}): SOC-oxidized OC in 9 mol/L. C_{f1} represented the labile carbon (LC) of soil, and the non-labile carbon (NLC) was determined as the difference between SOC and LC (NLC = SOC − LC) (Maia et al., 2007). Soil total nitrogen (TN) content was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). Soil total phosphorus (TP) content was determined using the molybdenum antimony blue colourimetry method (Murphy and Riley, 1962).

2.6. Determination of soil particle size distributions

Soil particle size distributions (PSDs) were analysed by laser diffraction using a Longbench Mastersizer 2000 (Malvern Instruments, Malvern, England). The distributions of particles were defined as the percentages of clay (< 0.002 mm), fine silt (0.002–0.02 mm), coarse silt (0.02–0.05 mm), and sand (0.05–1 mm).

2.7. Data analysis

Linear mixed models (LMMs) were used to analyse the effect of natural revegetation and the size of soil aggregates on the accumulation of GRSP, SOC, oxidizable OC fractions, TN, and TP in soil aggregates. The LMMs included the "revegetation year" and the "the size of soil aggregates" as fixed effects, and the "sampling site" and the "soil particle size distribution" as random effects. All residuals were checked for normality and homogeneity of variance. If there was a significant effect, a comparison among means was conducted using Tukey's honest significant difference (HSD) test at the level of 0.05. Pearson correlation analysis was applied to investigate the relationships between GRSP concentration and the soil aggregate stability, the concentrations of SOC, oxidizable OC fractions, TN, and TP in each soil aggregate fraction. SPSS 20.0 (SPSS Inc., Chicago, IL, United States) was used for these statistical analyses. Variance partitioning test, which was conducted in the R program (version 3.0.2; http://www.r-project.org/) with vegan packages, was used to determine the effects of environmental factors on soil aggregation and recovery of soil nutrient. Soil physical factors included bulk density and particle size distribution. Soil chemical factors included SOC, TN, NO_3^--N, NH_4^+-N, TP, available P. Plant factors include aboveground biomass and root biomass. For response variables, soil aggregation included MWD and the proportion of each soil aggregate size, and nutrients in soil aggregates included GRSPt, GRSPe, SOC, oxidizable OC fractions, TN and TP in soil aggregates. Response variables were transformed by Hellinger transformation. The geographical distance variables were the coordinates transferred from the longitude and latitude by the order 'geoXY' in the package of 'SoDA' in R (https://CRAN.R-project.org/package=SoDA). GPS coordinate locations for all soil samplings were provided in Table S1. Adjusted R^2 in variation partitioning was used to determine the proportion of variation explained by factors, and the significance was tested by an ANOVA permutation at the 0.05 level. Figures were drawn using SigmaPlot 10.0 (Systat Software, San Jose, CA, United States).

3. Results

3.1. Soil physical and chemical properties

Soil SOC and TN concentrations significantly decreased within the first 17 years and significantly increased between 17 and 32 years (Table 2). However, soil nitrate nitrogen, TP and available phosphorus concentrations significantly increased within the first 17 years and significantly decreased between 17 and 37 years (Table 2). The proportion of clay and fine silt significantly decreased within the first 7 years, whereas the proportion of sand significantly decreased within the first 7 years (Table 3). The erodibility and particle fractal dimension significantly decreased within the first 7 years, and then remained relatively stable.

3.2. Aggregate distribution and stability

Microaggregates constituted the largest proportion of the soil aggregates (70.4–46.6%) followed by small macroaggregates (15.1–27.5%) (Fig. 1). The proportions of large and small macroaggregates significantly increased from 7.97% and 18.6% to 20.9% and 24.1%, respectively, between 22 and 32 years after natural revegetation, whereas the percentage of microaggregates significantly decreased from 70.4% to 46.6% between 17 and 32 years. The MWD significantly increased greater than three-fold between 22 and 32 years. The proportion of large macroaggregates and MWD at 32 years was significantly increased compared with the sloped farmland but significantly reduced compared with natural grassland.

3.3. GRSPt and GRSPe in soil aggregates

GRSPt concentrations in each soil aggregate significantly decreased within the first 12 years and significantly increased between 17 and 32 years (Fig. 2). GRSPt concentrations in each soil aggregate after 32 years of natural revegetation exceeded sloped farmland but were significantly reduced compared with natural grassland. GRSPe concentrations in macroaggregates and microaggregates significantly decreased within the first 7 years, remained stable between 7 and 22 years, and significantly increased between 22 and 32 years. Only the GRSPe concentration in microaggregates exceeded sloped cropland after 32 years of natural revegetation, whereas the GRSPe concentrations in other three fractions were significantly reduced compared with sloped farmland and natural grassland. The increase in GRSPt concentrations in each fraction after 32 years of natural revegetation decreased in the following order: large macroaggregates ($0.58\ g\ kg^{-1}$) > microaggregates ($0.51\ g\ kg^{-1}$) > silt + clay ($0.42\ g\ kg^{-1}$) > small macroaggregates ($0.20\ g\ kg^{-1}$). GRSPt and GRSPe concentrations in large and small macroaggregates were generally higher than it in microaggregates.

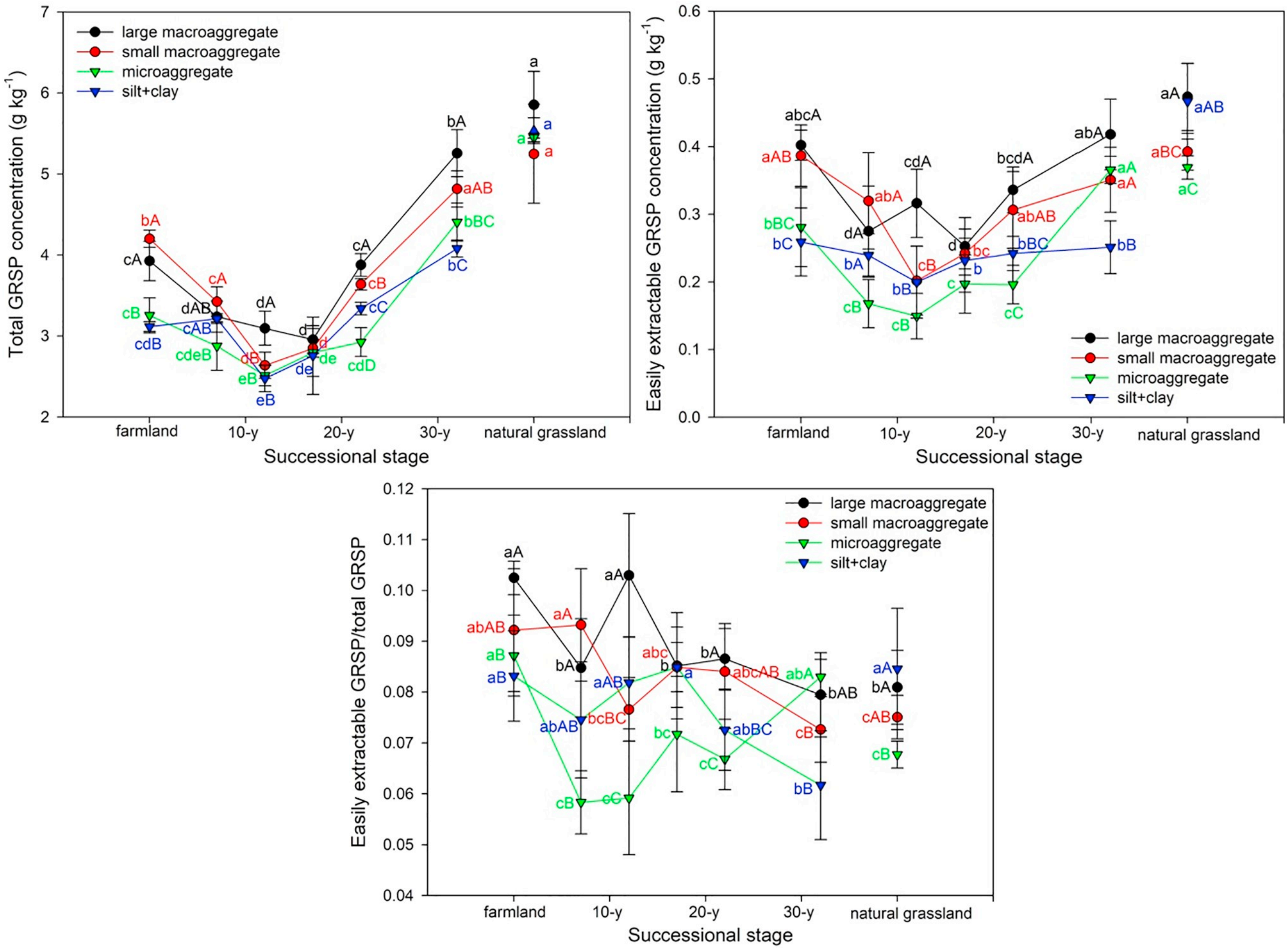

Fig. 2. GRSPt and GRSPe contents and GRSPe/GRSPt in soil aggregates following natural succession in the Loess Plateau, China. Error bars denote standard deviations (n = 3). Different capital letters indicate significant differences among different aggregate sizes in same study site. Different small letters indicate significant differences among study sites in same aggregate size.

GRSPe/GRSPt can reflect the differences in the potential accumulation of GRSP between soils (Jorge-Araujo et al., 2015). The reduction in GRSPe/GRSPt in each fraction within first 12 years occurred in the following order: silt + clay < large macroaggregates < small macroaggregates < microaggregates (Fig. 2). GRSPe/GRSPt in microaggregates significantly increased between 12 and 32 years, whereas GRSPe/GRSPt in the other three fractions dramatically decreased. The reduction in GRSPe/GRSPt in each fraction after 32 years of revegetation occurred in the following order: microaggregates < small macroaggregates < large macroaggregates < silt + clay. GRSPe/GRSPt in large and small macroaggregates at 32 years was slightly reduced compared with natural grassland. GRSPe/GRSPt at 32 years was significantly reduced in silt + clay but dramatically increased in microaggregates.

3.4. SOC, oxidizable OC fractions, TN and TP in soil aggregates

The concentrations of SOC and C_{f1} in each soil aggregate and C_{f2} in macroaggregates and microaggregates significantly decreased during the first 17 years of natural revegetation and then significantly increased over 17 to 32 years (Fig. 3). SOC, C_{f1} and C_{f2} concentrations in macroaggregates at 32 years were still significantly reduced compared with sloped farmland and natural grassland. In microaggregates and silt + clay, these concentrations were restored to the level of sloped farmland but remained significantly reduced compared with natural grassland. Thirty-two years of natural revegetation significantly deceased C_{f3} concentrations in large and small macroaggregates by 18.8% and 36.3%, respectively, but significantly increased C_{f4} concentrations in small macroaggregates and microaggregates by 100.7% and 420%, respectively. Lability of SOC in microaggregates significantly decreased within the first 17 years and then significantly increased between 17 and 32 years (Fig. 3). However, the lability of SOC in large and small macroaggregates significantly increased between 7 and 12 years and 0 and 7 years, respectively. The reductions in lability in macroaggregates and silt + clay within first 7 years were significantly reduced compared with that in microaggregates.

TN concentrations in each soil aggregate significantly decreased during the first 17 years and then significantly increased from 17 to 32 years (Fig. 4). TN concentrations in microaggregates at 32 years exceeded that in sloped cropland. However, in other fractions, TN concentrations remained significantly reduced compared with sloped cropland. In contrast to TN dynamics, TP concentrations in each soil aggregate dramatically decreased within the first 12 years, rapidly increased between 12 and 17 years, and ultimately reached the level of natural grassland. The increasing rate obviously reduced between 17 and 32 years (Fig. 4). After 32 years of natural revegetation, TN and TP concentrations in large and small macroaggregates were significantly increased compared with that in MIGA ($P < 0.05$).

3.5. Soil C/N, C/P, and N/P ratios in soil aggregates

Soil C/N ratios were generally highest in large macroaggregates during natural revegetation, ranging from 8.17 to 9.75. These ratios were the lowest in silt + clay, ranging from 4.11 to 7.43 (Table 3). In

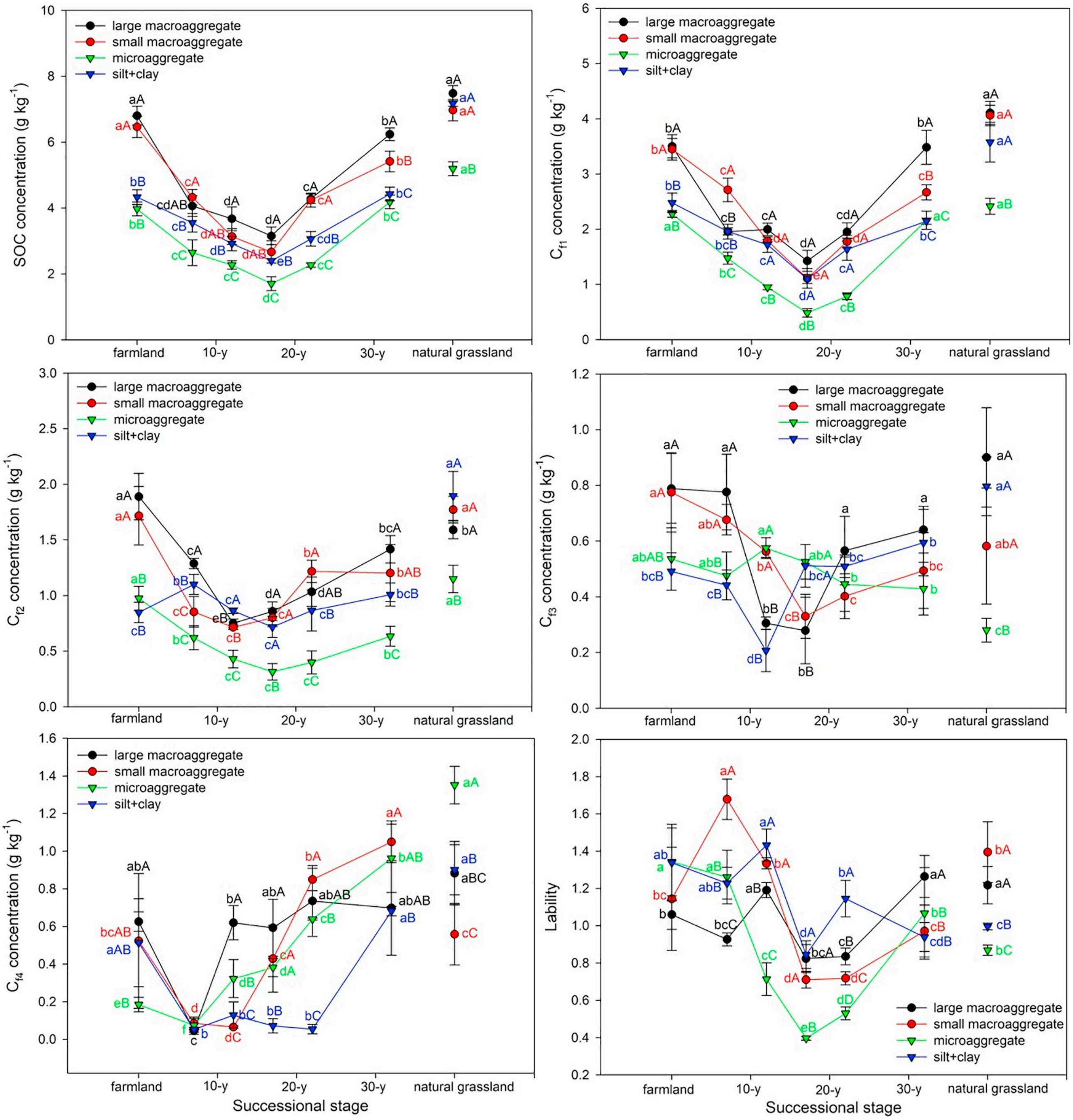

Fig. 3. SOC and oxidizable OC fractions contents and lability in soil aggregates following natural succession in the Loess Plateau, China. Error bars denote standard deviations (n = 3). Different capital letters indicate significant differences among different aggregate sizes in same study site. Different small letters indicate significant differences among study sites in same aggregate size.

natural grassland, soil C/N ratios were highest in small macroaggregates. Soil C/N ratios in small macroaggregates and microaggregates exhibited similar values and significantly increased between 17 years (8.49 and 8.33) and 32 years (9.04 and 9.05). After 32 years of natural revegetation, soil C/N ratios in macroaggregates and silt + clay reached the level of natural grassland. However, in microaggregates, these levels were significantly reduced compared with natural grassland. Soil C/P ratios in microaggregates (40.27–88.96) and silt + clay (50.62–89.95) were significantly reduced compared with large (68.92–133.61) and small (59.55–126.44) macroaggregates. Soil N/P ratios in small macroaggregates (4.84–9.85) were significantly reduced compared with large (7.33–14.49) and small (7.02–14.21) macroaggregates. Soil C/P ratios in each soil aggregate fractions and soil N/P ratios in macroaggregates and microaggregates significantly decreased between 12 and 17 years and dramatically increased between 17 and 32 years. However, these values remained significantly reduced compared with natural grassland (Table 4).

3.6. Relationship between GRSP and MWD, nutrients in soil aggregate

In large macroaggregates and microaggregates, GRSPt and GRSPe concentrations were linearly correlated with MWD (Table 5). In small macroaggregates, GRSPt concentration was linearly correlated with MWD. Soil GRSPt and GRSPe concentrations were linearly correlated with SOC, NLC and TN concentrations in macroaggregates and microaggregates (Tables 6 and 7). C_{f4} concentration was linearly correlated

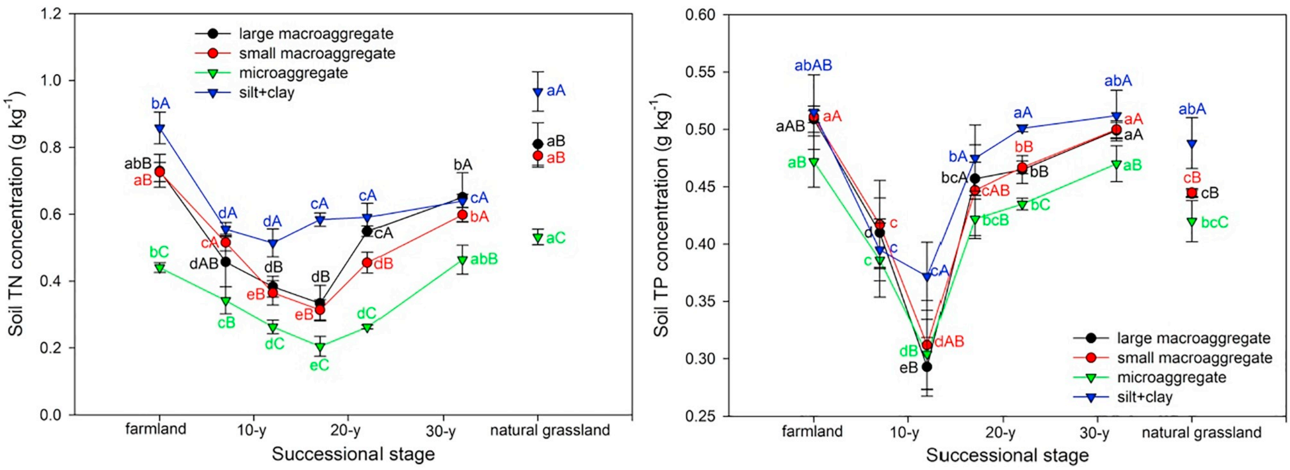

Fig. 4. Soil total nitrogen (TN) and total phosphorus (TP) contents in soil aggregates following natural succession in the Loess Plateau, China. Error bars denote standard deviations (n = 3). Different capital letters indicate significant differences among different aggregate sizes in same study site. Different small letters indicate significant differences among study sites in same aggregate size.

with GRSPe concentration in silt + clay. In silt + clay, GRSPt concentrations were also correlated with SOC and NLC concentrations.

4. Discussion

4.1. Effects of natural revegetation on nutrient allocation in soil aggregates and soil aggregate stability

The proportions of clay and fine silt, which significantly decreased within the first 7 years, were positively correlated with small macroaggregate (Table S3). The proportion of sand, which significantly decreased within the first 7 years, was negatively correlated with the proportion of small macroaggregate (Table S3). Numerous studies have reported that agricultural management, such as tillage, irrigation, and fertilization, can have a significant impact on soil particle size distribution in tillage layer (Are et al., 2018; Dong et al., 2018; Dong et al., 2017; Kisic et al., 2017; Ozgoz, 2009). The excessive agricultural activities in loess plateau might result in the significant variation of soil particle size distribution between farmland and the 7-year site. Clay can act as a cementing material that holds particles together in the aggregate (Emerson, 1977), so that increasing the clay content in the soil increases aggregate stability (Boix-Fayos et al., 2001; Lado et al., 2004; Mamedov et al., 2007; Mamedov et al., 2017). Previous study showed that the amount of large macro-aggregates (> 2000 mm) was positively correlated with clay content, and negatively with sand content (De Gryze et al., 2010). Additionally, the proportion of clay and fine silt were positively correlated with SOC concentration (Table S3). Therefore, the spatial variations of soil particle size distribution between farmland and the 7-year site and the rest sites, which was introduced by the approach of substituted space for time, should be taken into consideration when evaluating the restoration age effect on soil aggregation and SOC concentrations in soil aggregates in early years. In order

Table 4
Distribution of C/N, C/P, and N/P ratios in soil aggregates following natural revegetation.

Study sites	Large macroaggregate	Small macroaggregate	microaggregate	Silt + clay
C/N				
Farmland	9.23 ± 0.33aA	8.90 ± 0.24abcA	9.02 ± 0.20bA	5.05 ± 0.12dB
7-years	9.34 ± 0.94aA	8.40 ± 0.24cA	7.71 ± 0.46dB	6.39 ± 0.29bC
12-years	9.40 ± 0.37aA	8.61 ± 0.22bcB	8.66 ± 0.28bB	5.68 ± 0.10cC
17-years	9.41 ± 0.27aA	8.49 ± 0.32bcB	8.33 ± 0.17cAB	4.11 ± 0.03eC
22-years	8.17 ± 0.45bB	9.33 ± 0.21aA	8.64 ± 0.14bcB	5.18 ± 0.02cdC
32-years	9.75 ± 0.56aA	9.04 ± 0.21abA	9.05 ± 0.51bA	6.92 ± 0.10abB
NG	9.26 ± 0.23aB	8.99 ± 0.07abcB	9.77 ± 0.07aA	7.43 ± 0.38aC
C/P				
Farmland	133.61 ± 2.61bA	126.44 ± 5.06bA	84.13 ± 1.15bB	84.09 ± 2.57bcB
7-years	99.01 ± 3.56dA	104.08 ± 4.95cdA	68.30 ± 5.39dC	89.95 ± 4.09bB
12-years	125.27 ± 1.53cA	100.89 ± 6.05dB	75.00 ± 3.50cC	78.70 ± 3.26cC
17-years	68.92 ± 4.42fA	59.55 ± 2.24fB	40.27 ± 3.30fD	50.62 ± 2.16eC
22-years	92.19 ± 0.75eA	90.74 ± 3.81eA	52.12 ± 0.90eC	61.10 ± 4.11dB
32-years	125.03 ± 2.23cA	108.27 ± 4.70cB	88.96 ± 2.38bC	86.48 ± 3.73bC
NG	168.36 ± 8.27aA	156.67 ± 6.15aAB	123.57 ± 0.96aC	147.05 ± 5.19aB
N/P				
Farmland	14.49 ± 0.53bB	14.21 ± 0.44bB	9.33 ± 0.13bcC	16.66 ± 0.54bA
7-years	10.65 ± 0.78dC	12.39 ± 0.56cB	8.87 ± 0.73bcD	14.09 ± 0.58cA
12-years	13.34 ± 0.36cA	11.72 ± 0.42cB	8.67 ± 0.52cC	13.87 ± 0.80cA
17-years	7.33 ± 0.64eB	7.02 ± 0.13eB	4.84 ± 0.49eC	12.33 ± 0.44dA
22-years	11.31 ± 0.64dA	9.74 ± 0.58dB	6.03 ± 0.46dC	11.79 ± 0.77dA
32-years	12.85 ± 0.91cA	11.97 ± 0.29cA	9.85 ± 0.60bB	12.50 ± 0.35dA
NG	18.20 ± 0.88aAB	17.42 ± 0.64aB	12.65 ± 0.62aC	19.80 ± 0.91aA

NG: natural grassland; different capital letters indicate significant differences among different aggregate sizes in same study site; different small letters indicate significant differences among study sites in same aggregate size.

Table 5
The relationship between GRSP in soil aggregate fractions and MWD.

Aggregate	Equation	R^2	F	P
Large macroaggregate	MWD = 0.185GRSPt − 0.060	0.780	56.622	< 0.01
	MWD = 1.679GRSPe + 0.070	0.517	17.142	< 0.01
Small macroaggregate	MWD = 0.159GRSPt + 0.060	0.517	17.144	< 0.01
Microaggreggate	MWD = 0.227GRSPt − 0.081	0.759	50.384	< 0.01
	MWD = 1.657GRSPe − 0.255	0.644	28.972	< 0.01

Table 6
The relationship between GRSPt and nutrients in soil aggregate fractions.

Aggregate	Equation	R^2	F	P
Large macroaggregate	SOC = 1.282GRSPt − 0.069	0.564	20.672	< 0.01
	NLC = 0.555GRSPt + 0.303	0.458	13.537	< 0.01
	TN = 0.130GRSPt + 0.029	0.503	16.190	< 0.01
Small macroaggregate	SOC = 1.437GRSPt − 0.788	0.698	36.989	< 0.01
	NLC = 0.798GRSPt − 0.743	0.692	35.938	< 0.01
	TN = 0.150GRSPt − 0.044	0.655	30.326	< 0.01
Microaggreggate	SOC = 1.159GRSPt − 0.784	0.629	27.175	< 0.01
	NLC = 0.325GRSPt + 0.419	0.503	16.173	< 0.01
	TN = 0.115GRSPt − 0.030	0.559	20.279	< 0.01
Silt + clay	SOC = 0.908GRSPt + 0.577	0.406	10.942	< 0.01
	NLC = 0.650GRSPt − 0.446	0.691	35.808	< 0.01

to avoid this effect affecting the accuracy of statistical analysis, linear mixed models including the "sampling site" and the "soil particle size distribution" as random effects was applied to investigate the effect of natural revegetation and the size of soil aggregates on soil properties. Moreover, the soils in all sampling sites of this experiment are classified as silty loam texture according to USDA Soil Texture Classification system (Buol et al., 1997), indicating that no obvious spatial variations of soil texture between sampling sites existed. The recovery rate of vegetation with natural revegetation was highest within the first seven years (Table S2) (Sun et al., 2017), which largely decreased soil erodibility (Table 3) (C. Zhang et al., 2017), as an important regulating ecosystem function of vegetation is their potential to control soil erosion processes (Vannoppen et al., 2015; Wallace, 2007). The MWD and proportion of large macroaggregate were negatively correlated with erodibility (Table S3), indicating that soil aggregate stability is vital for reducing soil erosion.

SOC, TN and TP concentrations in soil aggregates significantly decreased over the first 17 years (Fig. 3, Table 2) due to the cessation of fertilization, which reduced organic matter input into soil (Zhang et al., 2016; Zhang et al., 2013). Microaggregates formation and small macroaggregates reduction occur simultaneously within the first 7 years after abandoning farmland. Aggregate stability, which was evaluated based on the mean weighted diameter (MWD), significantly decreased within the first 7 years. Previous studies reported that aggregate stability significantly increased after natural revegetation in the Loess Plateau and reached a steady state after six or seven years of recovery (An et al., 2013; Zhao et al., 2017). It is also reported that aggregate stability was enhanced during the early stage of vegetation succession but decreased in the middle and late stages (Cheng et al., 2015). Significantly more SOC and TN are lost in small macroaggregates relative to microaggregates within the first 7 years. Thus, the loss of SOC and TN in small macroaggregates causes the small macroaggregates to burst into microaggregates, resulting in reduced soil aggregate stability. In addition, significantly more NLC is lost in small macroaggregates relative to large macroaggregates and microaggregates within the first 7 years, indicating that small macroaggregates bursts to form microaggregates, significantly promoting the loss of stable carbon at the early stage of natural revegetation.

The vegetation biomass gradually increased at the middle and late stage of natural revegetation (Table S2) which contributed to the accumulation of SOC and TN by the decomposition of increased litter and through increased root exudates (Wei et al., 2017; Zhang et al., 2018). Sufficient soil C and N resources contribute to the accumulation of mucilage, which serves as an additional binding agent in the rhizosphere (S. Zhang et al., 2012; Zhu et al., 2017) by stimulating soil microbial growth (Wang et al., 2019), leading to increased aggregate water stability. In this study, the concentrations of SOC, TN, and TP increased with increasing aggregate size class because large aggregate size classes are composed of small aggregate size classes plus organic binding agents (Six et al., 2000). TN in soil is strongly related to SOC (Wang et al., 2018; Yao et al., 2019) given that microbes prefer to immobilize N rather than mineralize it as a soil carbon increases, which decreases net N mineralization rates (McLauchlan et al., 2006; C. Zhang et al., 2019b). After 32 years of natural revegetation, SOC and TN concentrations in microaggregates are restored to the level of sloped cropland; however, these concentrations in large and small macroaggregates remained significantly reduced compared with sloped cropland and natural grassland. Therefore, the process of carbon and nitrogen accumulation in macroaggregates along natural revegetation is important for restoration of SOC and TN levels (Y. Zhang et al., 2019). In addition, our results suggested that mid-term natural revegetation is important for soil TP recovery, and abandoning farmland in Loess Plateau is an effective method to restore soil TP concentrations to the level of natural grassland.

SOC fractions with different labilities differentially contributed to SOC restoration among soil aggregates following natural revegetation. In microaggregates, the most recalcitrant carbon fraction (C_{f4}) significantly increased between 7 and 32 years, and more labile carbon was lost compared with NLC within first 17 years. C_{f3} and C_{f4} are

Table 7
The relationship between GRSPe and nutrients in soil aggregate fractions.

Aggregate	Equation	R^2	F	P
Large macroaggregate	SOC = 15.231GRSPe − 3.373	0.643	28.829	< 0.01
	NLC = 7.446GRSPe − 0.114	0.668	32.134	< 0.01
	TN = 1.634GRSPe − 0.033	0.646	29.159	< 0.01
Small macroaggregate	SOC = 12.650GRSPe + 0.565	0.533	18.246	< 0.01
	NLC = 8.032GRSPe − 0.294	0.690	35.606	< 0.01
	TN = 1.362GRSPe + 0.086	0.529	17.993	< 0.01
Microaggreggate	SOC = 9.332GRSPe + 0.732	0.652	30.037	< 0.01
	NLC = 2.903GRSPe + 0.781	0.640	28.408	< 0.01
	TN = 0.892GRSPe + 0.128	0.537	18.580	< 0.01
Silt + clay	GRSPe = 7.288C_{f4} − 0.117	0.476	14.519	< 0.01

related to compounds of high chemical stability and are slowly altered by microbial activities (Chan et al., 2001). Persistent binding agents (humic compounds and polymers) are associated with microaggregation; thus, microaggregates contain old organic carbon and play an important role in long-term SOC sequestration and stabilization (Blanco-Canqui and Lal, 2004; Six et al., 2004). In large and small macroaggregates, NLC is more easily lost relative to labile carbon within the first 12 years, indicating the poor ability of macroaggregates in protecting stable carbon. Lower protective effects of biophysical and chemical processes were observed in macroaggregates compared with microaggregates (Gelaw et al., 2015). The increase concentrations of C_{f1} and C_{f2} in large and small macroaggregates between 22 and 32 years were considerably increased compared with microaggregates, consistent with the result of Deng et al. (2018). During the same period, large and small macroaggregates formation and microaggregates reduction occurred simultaneously. The C_{f1} and C_{f2} are the most readily oxidizable fractions and are mainly composed of polysaccharides, decaying young organic matter, fungal hyphae, and other microbial products, which are mostly associated with the formation of macroaggregates and the availability of nutrients (Maia et al., 2007). Given that temporary binding agents (plant roots, fungal hyphae, and bacterial cells) are mainly associated with macroaggregates, macroaggregates contain large amount of younger organic material (Blanco-Canqui and Lal, 2004; Six et al., 2004). The labile fractions of soil oxidizable OC served as an important binding agent for binding microaggregates together into macroaggregates given that more labile C is concentrated in macroaggregates compared with microaggregates (Deng et al., 2018; Six et al., 2000). Therefore, it is of great importance to facilitate the accumulation of labile C, which plays a vital role in maintaining soil aggregate stability. In addition, the lability of SOC in silt + clay, microaggregates and small macroaggregates deceased after 32 years of natural revegetation, whereas large macroaggregates significantly increased. These results confirmed that natural revegetation increased the stability of SOC in fractions < 2 mm, and large macroaggregates play an important role in labile carbon accumulation.

Soil C/N ratios in each soil aggregate ranged from 4.11 to 9.75 (< 10), indicating net N mineralization and low levels of organic matter are merged into the soil system (Yimer et al., 2007). Soil C/N ratios in small macroaggregates and microaggregates significantly increased between 17 and 32 years; thus, the rate of SOC accumulation is faster than TN in these two fractions. Soil C/P ratios in each soil aggregates ranged from 40.27 to 133.61 (< 200), indicating a net mineralization of nutrients (Paul, 2007). In addition, increased net mineralization was observed in microaggregates compared with large and small macroaggregates. Some believe that the soil N/P ratio is indicative of nutrient limitation (Gusewell, 2004), and higher limitation of N was generally observed in microaggregates compared with small and large macroaggregates. In addition, soil C/P ratios in each soil aggregate fraction and soil N/P ratios in macroaggregates and microaggregates significantly increased between 17 and 32 years. Thus, the immobilization of nutrients was promoted, and the limitation of N was relieved at the late stage of natural revegetation.

4.2. Effect of natural restoration on aggregate-associated GRSP and soil aggregate stability

The net primary production (NPP) in the ecosystem determines the upper bound of C available for glomalin production and the turnover of AM hyphae (Treseder and Cross, 2006; Treseder and Turner, 2007). A previous study reported that glomalin storage was greatest in an Alaskan boreal ecosystem where net primary production (NPP) was highest (Treseder, 2004). In this study, the NPP gradually increased at the middle and late stages of vegetation succession (Feng et al., 2016; Wei et al., 2017) given that plant biomass and vegetation coverage significantly increased between 17 and 32 years (Table S2). Thus, the turnover of AM hyphae was likely promoted, and increased C sources were provided by plants for glomalin production, contributing to the accumulation of GRSPt and GRSPe in soil aggregates between 17 and 32 years. Increased GRSPt and GRSPe concentrations were observed in large macroaggregates compared with small macroaggregates and microaggregates, which is consistent with previous studies (Fokom et al., 2012; Zhang et al., 2014; Zhu et al., 2019). Similar to SOC, GRSP stabilizes soil aggregates through a “gluing” action (Rillig, 2004; Spohn and Giani, 2010; Wright and Upadhyaya, 1998). Thus, large macroaggregates has the largest capacity for GRSP accumulation, and GRSP is highly correlated with aggregate stability (Deng et al., 2018; Yao et al., 2019; Zhu et al., 2017). In this study, GRSP concentrations in large and small macroaggregates were linearly correlated with MWD (Table 5), and the processes of rapid accumulation of GRSP (22 to 32 years) occurred simultaneously with LAGA and SMGA formation, MIGA reduction, and rapid increase in MWD (22 to 32 years) (Fig. 2), confirming that soil GRSP promoted the process of microaggregates binding to form macroaggregates, leading to increased soil aggregate stability.

Both GRSP production and degradation are processes taking place in soils; thus, a correlative relationship exists between cumulative production of protein and recently produced protein in the same land use type (Emran et al., 2012; Jorge-Araujo et al., 2015). GRSPe is considered recently produced fungal protein and relatively more labile, while GRSPt is the sum of both recent and old fungal protein production and more recalcitrance, and is expected to remain in the soil for a longer period of time (Koide and Peoples, 2013; Rillig, 2004; Wright and Upadhyaya, 1998). Therefore, differences between GRSPe and GRSPt can reflect differences in degradation rates between soils, and GRSPe/GRSPt can reflect the differences in the potential accumulation of GRSP between soils (Jorge-Araujo et al., 2015). Natural restoration significantly decreased GRSPe/GRSPt in large and small macroaggregates, but GRSPe/GRSPt in microaggregates was significantly increased between 7 and 32 years to levels that even slightly exceeded that in large and small macroaggregates at 32 years (Fig. 2). This result indicates that the potential of GRSP accumulation in large and small macroaggregates decreased during natural restoration. However, GRSP accumulation increased in microaggregates. Thus, the differences in GRSPt and GRSPe concentrations between large and small macroaggregates and microaggregates decreased during natural restoration, and GRSPt and GRSPe concentrations increased to the largest levels in microaggregates. Therefore, microaggregates play an important role in the recovery of soil GRSP content. Moreover, our model forecasted that GRSPt and GRSPe levels in large and small macroaggregates and microaggregates could be restored to the level of natural grassland within 40 years after abandoning farmland, indicating that abandoning farmland is an effective method of soil GRSP restoration.

GRSP plays a vital role in promoting SOC and TN accumulation. C and N levels in glomalin accounted for 4–5% of soil C and 5% of soil N, which even exceeds the contribution of soil microbial biomass (Sousa et al., 2012; Wang et al., 2017). In various ecosystems, GRSP content is significantly correlated with SOC and TN levels (Fokom et al., 2012; Vasconcellos et al., 2016; Xiao et al., 2019). In this study, GRSPt and GRSPe levels in each soil aggregate fraction were positively correlated with SOC and TN, confirming that GRSPt and GRSPe contributes the accumulation of SOC and TN in soil aggregates during natural restoration. Particularly, soil GRSPt and GRSPe concentrations in each aggregate fraction were positively correlated with NLC concentrations. GRSPt concentrations in large macroaggregates, microaggregates and silt + clay and GRSPe concentrations in microaggregates and silt + clay were linearly correlated with C_{f4} concentrations. GRSP has a turnover time of many years and is known to slowly turn over into a recalcitrant SOM fraction (Rillig, 2004; Treseder and Turner, 2007). GRSP facilitates SOC and contributes indirectly to the recalcitrant structure of the long-lasting organic C stored in soils (J. Zhang et al., 2017). The low degradation rates of GRSP in microaggregates and silt + clay contributed to the accumulation of the recalcitrant fraction of GRSP, which further confirmed that microaggregates promoted accumulation of recalcitrant carbon and increased SOC stability largely through its ability to retain GRSP.

The approach of substituted space for time has been applied to investigate dynamics of natural vegetation succession (Feldpausch et al., 2010; Holtkamp et al., 2011; Kuramae et al., 2010; Lozano et al., 2014). However, this approach is difficult to avoid the risk of pseudo replication, especially the influence from the sampling distance (Ramette and Tiedje, 2007;). Therefore, variance partitioning test was applied to identify the contributions of geographic distance between sampling sites of our experiment to soil aggregation and nutrients in soil aggregates, and the results showed that sampling distance played a non-significant role in determining the soil aggregation and recovery of soil nutrient (Fig. S2).

5. Conclusions

Soil aggregate stability and GRSP, SOC, labile C (C_{f1} and C_{f2}), TN and TP levels in each soil aggregate fraction significantly increased at the late stage of natural restoration, and abandoning farmland is an effective method of soil aggregate and nutrient restoration. Small macroaggregates burst to form microaggregates, largely leading to the loss of stable carbon at the early stage of natural revegetation, and the N limitation was relieved at the late stage of natural revegetation. Natural revegetation increased the stability of SOC in aggregates < 2 mm. Large macroaggregates play an important role in labile carbon accumulation, which is important for maintaining soil aggregate stability. The largest amount of carbon and nitrogen was restored in macroaggregates after 32 years of natural revegetation. Large macroaggregates contain the largest amount of GRSPt and GRSPe, but the largest amount of GRSPt and GRSPe increased in microaggregates after 32 years of natural revegetation. The accumulation and redistribution of GRSP in macroaggregates and microaggregates contributed to the increased soil aggregation. GRSP facilitates the accumulation of SOC and TN and contributes to the recalcitrant carbon reserved in soils. Microaggregates promoted accumulation of recalcitrant carbon and increased SOC stability largely through its ability to retain GRSP. Overall, the results suggest that 32 years of natural revegetation promoted the recovery of soil carbon and nitrogen and increased SOC and soil aggregate stability, which is largely via accumulation and redistribution of GRSP in macroaggregates and microaggregates.

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Declaration of competing interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors 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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2019.113921.

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