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Long-term manuring facilitates glomalin-related soil proteins accumulation by chemical composition shifts and macro-aggregation formation

Hongbo Yang [a,c], Qiong Xiao [a], Yaping Huang [a], Zejiang Cai [a,b], Dongchu Li [a,b], Lei Wu [a], Jeroen Meersmans [c], Gilles Colinet [c], Wenju Zhang [a,*]

[a] Key Laboratory of Arable Land Quality Monitoring and Evaluation, Ministry of Agriculture and Rural Affairs/State Key Laboratory of Efficient Utilization of Arid and Semi-Arid Arable Land in Northern China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China

[b] Qiyang Farmland Ecosystem National Observation and Research Station, Institute of Agricultural Resources and Regional Planning (CAAS), Beijing 100081, China

[c] TERRA Research Centre, Gembloux Agro-Bio Tech, University of Liège, 5030 Gembloux, Belgium

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ABSTRACT

Glomalin-related soil proteins (GRSP), derived from arbuscular mycorrhizal fungi (AMF), contributes significantly to soil stability and carbon sequestration. However, the responses of GRSP accumulation and associated AMF community and diversity to long-term fertilization regimes remain unclear. Here, we investigated the dynamics of GRSP contents, AMF biomass and diversity based on a 29-year fertilization experiment (including control, mineral fertilization, manuring and straw returning treatments). Results showed that GRSP contents increased over years across fertilization treatments. Compared with no fertilization, long-term manuring and straw returning significantly increased bulk soil GRSP by 100% and 80%, respectively, and altered the chemical composition of GRSP by increasing the recalcitrant (aromatic) C proportion. The proportion of aromatic C in GRSP was positively correlated with AMF biomass and diversity (Shannon and Chao1), indicating that the chemical composition of GRSP could be regulated by AMF community and diversity. Moreover, manuring facilitated the formation of macro-aggregates (>250 μm), thus increasing the physical protection of GRSP. The structural equation modeling further demonstrated that GRSP content was positively regulated by soil macro-aggregates, AMF biomass and diversity and their linkage with GRSP chemical composition. Collectively, long-term manuring could facilitate GRSP accumulation by shifts in AMF-mediated GRSP chemical composition (aromatic C) along with enhanced protection of macro-aggregates. This study highlights a feasible way forward for soil quality improvement and carbon sequestration for sustainable agriculture.

1. Introduction

Glomalin-related soil proteins (GRSP), as metabolites from arbuscular mycorrhizal fungi (AMF) assimilation (Wright et al., 1998; Driver et al., 2005), contributes to soil C sequestration and aggregate stability. GRSP is a mixture of compounds that contains humic, lipid, inorganic materials, etc. (Gillespie et al., 2011). It is estimated that the organic C in GRSP accounts for 4–15% of SOC (Rillig et al., 2001; Singh et al., 2016; Wang et al., 2020a), and its residence time in soil is around 35 years (Harner et al., 2004). Moreover, GRSP, by virtue of its cohesiveness and hydrophobicity, can enhance soil water retention and permeability, improve soil stability by promoting the bonding of soil mineral particles, thus reducing the risk of soil erosion and debris flows (Wright and Upadhyaya, 1996). To maintain the human demand for food production, amounts of fertilizers are used in agricultural practices. Fertilization significantly affects GRSP in the soil (Cissé et al., 2021a; Cissé et al., 2021b). However, the underlying mechanism of GRSP accumulation in response to fertilization remain limited. Therefore, studying the dynamics and controlling factors of GRSP accumulation under various fertilization managements is of significant importance for C sequestration and food stability.

Fertilization greatly alters the community composition and diversity of AMF (Xiao et al., 2019; Wang et al., 2022), which might had predominant effects on GRSP accumulation (Bedini et al., 2007; Holátko

* Correspondence to: Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, 12 South Road, Zhongguancun, Haidian District, Beijing 100081, China.

E-mail address: zhangwenju01@caas.cn (W. Zhang).

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et al., 2021). Large numbers of fertilizer application studies have been performed to analyze the impacts of nutrients on AMF. However, there is no consensus on the response of AMF to fertilization, with positive (Porras-Alfaro et al., 2007; Zheng et al., 2014) and negative (Zhu et al., 2016; Williams et al., 2017; Luo et al., 2021) effects being observed. For example, Mineral fertilization (e.g., N, P) causes soil acidification, thus decreasing AMF colonization and diversity (Liu et al., 2021b). Manure application decreases AMF richness and diversity (Garo et al., 2022), possibly due to reduced allocation of plant C to AMF (Johnson et al., 2013). However, some studies have found the opposite. N, P and organic fertilizer addition have beneficial effects on AMF biomass and diversity by increasing nutrient availability (Lee et al., 2008; Dia et al., 2013; Camenzind et al., 2016). These contradictions indicate that our understanding of the effects of fertilization on AMF and GRSP content is still unclear and needs further research. In addition, differences in chemical composition of GRSP can also affect its accumulation in soil due to the different decomposition rates of composition. The main chemical composition of similar humic substances can be obtained by Fourier transform infrared reflection (FTIR) and/or nuclear magnetic resonance (NMR), such as aromatic C, aliphatic C, polysaccharides and hydrocarbons in GRSP (Guo et al., 2022; Tivet et al., 2013; Wang et al., 2020b; Zhang et al., 2022). The aromatic C contained in GRSP is responsible for its stable character. (Schindler et al., 2007). However, studies on how fertilization affects GRSP composition through altering AMF diversity are still lacking. Additionally, the chemical composition of GRSP could potentially be modified through the manipulation of soil physicochemical properties (e.g., pH) (Zhong et al., 2017). Therefore, it is crucial to reveal the mechanisms of changes in GRSP composition and its linkage with AMF biomass and diversity under different fertilization.

In addition, fertilization regimes could influence GRSP through the promotion of stable soil aggregates, which can protect GRSP from decomposition (Sekaran et al., 2020). Based on the hierarchical model of aggregate formation concept, primary mineral particles are combined with bacteria, fungi, GRSP and plant residues to form microaggregates (<250 μm), which then become macroaggregates (>250 μm) through binder, such as roots and AMF hyphae (Tisdall and Oades, 1982; Rillig and Mummey, 2006). Consequently, GRSP is crucial for the formation of aggregates, and aggregation can likewise protect GRSP from degradation. However, the results presented by multiple studies considering the impact of fertilization on aggregate stability are inconsistent. For instance, Bottinelli et al. (2017) found that manure application increases the formation of large aggregates and aggregate stability (e.g., mean weight diameter (MWD)), whereas Guo et al. (2019) showed different results. In addition, the regulation mechanisms of GRSP accumulation by changes in aggregate stability after long-term fertilization are still lacking. Hence, further research on GRSP distribution among different aggregate size fractions is required to shed light on how its accumulation in aggregates responds to long-term fertilization regimes.

In this study, the aim was to clarify the response of GRSP content and its chemical composition to long-term fertilization regimes (29-year). We hypothesized that long-term manuring could facilitate GRSP accumulation by enhancing its recalcitrance of GRSP due to increased AMF biomass and diversity, and promoting the formation of macro-aggregates.

2. Materials and methods

2.1. Site description and soil sampling

A long-term field experiment was established on the basis of a cropland soil located in Qiyang County, Hunan Province, China (26°45′N, 111°52′E) in 1990. The site has a subtropical climate with mean annual temperature and mean annual precipitation of 18.1 °C and 1431 mm, respectively. The parent material of the soil is quaternary red clay, and classified as Ferralic Cambisol according to the FAO classification. The soil texture was loamy clay (i.e. clay 43.9%; silt 31.9%; sand 24.2%), with soil pH of 5.70, and SOC 8.58 g kg^{-1}, total nitrogen (TN) 1.07 g kg^{-1}, and total phosphorus (TP) 0.45 g kg^{-1}. The main cropping system is winter wheat-summer maize rotations after the experiment was launched. The experiment was a completely randomized design with two repetitions, each with two sub-replicates. To ensure sample adequacy, we selected three replicates for measurement. The treatments included: no fertilizer (CK); mineral fertilizer (NPK, mineral N, P, K fertilizer); manure (M, pig manure; NPKM, NPK plus pig manure; NPKMR, NPKM plus wheat-soybean-sweet potato rotation of three crops a year); Straw returning (NPKS, NPK plus straw); Fallow (F, natural succession). The fertilizer application schedule is shown in Table S1. Soil samples (0–20 cm in depth) were selected after crop harvest in September of 1990, 2006, 2012, and air-dried (Zhang et al., 2009; Xiao et al., 2021). Part of the fresh soil in 2019 was stored in a − 80 °C refrigerator.

2.2. Determination of soil physicochemical properties

The pH of the soil was tested at a soil-to-water ratio of 1:2.5. An elemental analyzer (EA3000, Milan, Italy) was used to measure the contents of SOC and TN. Nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) content were measured by continuous flow analyzer (San^{++} system, Netherlands). Soil available phosphorus (AP) was tested by molybdenum-antimony resistance colorimetric method. The soil properties are shown in Table 1.

2.3. Determination of soil water-stable aggregates

According to Cambardella and Elliott (1993), soil aggregates were tested using the wet sieve method, considering four particle size classes (i.e.<53 μm, 53–250 μm, 250–2000 μm, and >2000 μm), and using a grain-size analyzer (Damon, Shanghai, China) (Table S3). The MWD was calculated to assess the aggregates stability for each treatment (Zhang and Horn, 2001):

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

Table 1
Soil properties subjected to 29-year various fertilization (CK, no fertilizer; NPK, mineral nitrogen, phosphorus, potassium fertilizer; M, pig manure; NPKM, NPK plus pig manure; NPKS, NPK plus Straw; NPKMR, NPKM plus wheat-soybean/sweet potato rotation; F, Fallow, natural succession).

Treatments	pH	SOC	TN	AN	AP
		(g C kg^{-1})	(g N kg^{-1})	(mg N kg^{-1})	(mg P kg^{-1})
CK	5.33 ± 0.06c	6.77 ± 0.25c	1.21 ± 0.02d	10.21 ± 0.46d	1.93 ± 0.26e
NPK	4.41 ± 0.01d	9.99 ± 0.12b	1.50 ± 0.02c	6.63 ± 0.56e	43.34 ± 2.466d
NPKS	4.50 ± 0.04d	9.55 ± 0.31b	1.46 ± 0.04c	7.54 ± 0.60de	56.43 ± 4.25c
M	6.26 ± 0.01b	14.07 ± 0.33a	2.01 ± 0.07a	38.40 ± 0.18a	141.63 ± 2.99b
NPKM	6.12 ± 0.07b	13.55 ± 0.20a	1.77 ± 0.02b	30.02 ± 0.66b	147.94 ± 1.53b
NPKMR	5.83 ± 0.04c	14.26 ± 0.50a	1.99 ± 0.02ab	28.95 ± 2.04b	167.57 ± 1.82a
F	6.52 ± 0.08a	13.57 ± 1.51a	1.46 ± 0.18c	24.40 ± 1.01c	7.03 ± 0.88e

Note: SOC, soil organic carbon; TN, total nitrogen; AN, available nitrogen; AP, available phosphorus; Values represents the means with standard errors (n = 3). Different letters indicate significant differences among various fertilization for each parameter using Duncan's test ($p < 0.05$)

where m_i is the weight percentage of aggregate still on the ith sieve. r_i is the aperture size (mm) of the ith mesh. n value refers to the fractions of soil aggregate size that represent 2.00 mm.

2.4. Determination of AMF biomass and diversity

AMF biomass was characterized by C16:1ω5 phospholipid fatty acids (PLFAs) (Olsson, 1999). PLFAs were extracted using a slightly modified version of the Bligh-Dyer method (Tian et al., 2022). Briefly, 2 g of the freeze-dried fresh soil samples were placed in a Teflon centrifuge tube, after which the extracting solution (citric acid buffer solution, V(chloroform): V(methanol): V(citric acid) = 1:2:0.8) and the unesterified internal standard (C19: 0.1 ng μL^{-1}) were added.

AMF diversity was analyzed by high-throughput sequencing. From around 0.5 g of soil stored at − 80 ℃. The soil DNA genome was extracted by using the FastDNA™ SPIN Kit for Soil (MP Biomedicals). After extraction, nested PCR was used to amplify the target fragment. AML1F (5′-ATCAACTTTCGATGGTAGGATAGA-3′)-AML2R (5′GAACCC AAACACTTTGGTTTCC-3′) primers were used for the first round of PCR, and subsequently, AMV4.5NF (5′-AAGCTCGTAGTTGAATTTCG-3′)-AMDGR (5′-CCCAACTATCCCTATTAATCAT-3′) primers were used for the second round of PCR (Van Geel et al., 2014). The amplicon sequencing was performed by the Magigene platform (http://cloud.magigene.com). AMF α-diversity was calculated by the Shannon-Wiener index and Chao1 index.

2.5. GRSP determination and purification

GRSP was determined by a method provided by Wright et al. (1998). Briefly, total GRSP was extracted by using 2 mL of 50 mmol L^{-1} sodium citrate (pH=8.0) for 0.25 g of air-dried soil. The extractions were autoclaved for 1 h at 121 ℃ and centrifuged for 6 min at 10,000 ×*g*. This process was repeated four times on each sample, after which the supernatants were combined and centrifuged once more before quantification. 300 μL of the clear supernatant and 700 μL of dH_2O were drawn up into the sampling tank, and then 5 mL Coomassie bright blue (CBB) solution was added. After 2–3 min of color reaction (Liu et al., 2021a; Liu et al., 2021b), the optical density (OD) value of the GRSP was measured at 595 nm with a microplate reader (Biotek Synergy H1, USA) using bovine serum albumin (BSA) as the standard. All the collected supernatant was precipitated by titrating with hydrochloric acid (1 mol L^{-1}), cooling in an ice bath for 1 h, and then centrifuging at 10, 000 ×*g* for 6 min. The resulting sediment was solubilized in 0.1 M sodium hydroxide (NaOH) and dialyzed in dH_2O for 60 h (dialysis bag, molecular weight cut off=10,000 Da, USA). Following dialysis, the dialysate was centrifuged for 6 min at 10,000 ×*g* to eliminate insoluble residue, the supernatant was freeze-dried with a freeze-dryer (FD-1A-50, Beijing BoYiKang Laboratory Instrument Co., Ltd., China).

2.6. GRSP functional groups determination

The functional groups of GRSP were determined by the KBr pellet method (Schindler et al., 2007; Guo et al., 2022). Analysis method and instrument parameters were as follows: The KBr was dried in a drying oven at 60 ℃ for 6 h to ensure no residual water vapor, then ground and passed through a 100-mesh sieve. Subsequently, 1 mg of freeze-dried GRSP sample and 200 mg of KBr (≥99%) were weighed, respectively, both components were carefully placed into an agate mortar and ground in a 1:200 ratio to ensure proper mixing. After achieving a homogeneous mixture, the resulting blend was pressed into pellets. These pellets were scanned by infrared spectrometer (Brukervertex70, Germany). The resolution of Vertex70 was 4 cm^{-1}, the sample scan time was 32 s, the background scan time was 32 s, and the wavenumber range was 400 cm^{-1} to 4000 cm^{-1}. Baseline and ambient corrections (i.e.H_2O, CO_2) were applied to every obtained spectra. We found four functional groups by wavenumber matching, which belong to four chemical compositions: polysaccharides, aliphatic C, aromatic C, hydrocarbons. To estimate the proportion of functional groups that correspond to each peak in the spectrum, the area of each peak was estimated by integration to obtain semi-quantitative data for functional groups (Wang et al., 2014). It is demonstrated how functional features and peak wavenumbers correspond. The matching of functional characteristics and peak wavenumbers are shown in Table S2.

2.7. Statistical analysis

Statistical analyses were conducted after all data had been examined for homogeneity and normal distribution (K-S test). A one-way ANOVA was used with IBM SPSS 19.0 statistical software to evaluate the differences (Duncan's test) in soil nutrients, soil aggregates, AMF, GRSP, and its chemical compositions among fertilization treatments. The correlation between GRSP content and nutrients, AMF and MWD were assessed using the Pearson's correlation coefficient test. The partial least squares path modelling (PLSPM) was applied to identify the regulatory pathways of factors on GRSP, we substituted independent variables with the first component PC1 by principal component analysis (PCA) (Table S4). Variance partitioning analysis (VPA), random forest (RF) and PLSPM were all performed in R 4.0.5.

3. Results

3.1. Soil properties, GRSP contents and chemical composition

The soil physicochemical properties showed great difference across fertilization treatments (Table 1). More specifically, pH value was significantly higher in the M, NPKM and F treatments relative to CK, whereas this was significantly lower in the NPK, NPKS treatments. SOC, TN, AN and AP contents under M, NPKM and NPKMR were higher than that of CK and NPK treatments. However, SOC, TN contents were significantly higher under NPK and NPKS treatments than that under CK treatment. N accumulation showed a trend of increasing over time (Fig. S1).

The GRSP accumulated in all fertilizer and fallow treatments over time from 1990 to 2019, with values increasing from 1.9 mg g^{-1} in 1990 to 3.2–4.7 mg g^{-1} in 2019, whereas under the CK treatment this remained constant. Long-term fertilization significantly altered GRSP contents ($p < 0.05$, Fig. 1b). Compared with CK treatments, NPK, straw returning (NPKS) and manuring (M, NPKM, NPKMR) increased GRSP content by 55%, 80% and 100%, respectively. When considering manure application, diversified cropping (NPKMR) facilitated significantly higher GRSP accumulation in bulk soil than in wheat-maize rotation (NPKM). Fertilization also strongly affected the content of GRSP across aggregate size fractions ($p < 0.05$, Fig. 1b). GRSP mainly accumulated in the 250–2000 μm particle size fractions. Compared with CK treatment, NPK and NPKS treatments increased GRSP only significantly in aggregate size fractions larger than 53 μm (1.0–1.6 mg g^{-1} soil), whereas NPKM and NPKMR treatments increased GRSP across all particle sizes (1.3–2.6 mg g^{-1} soil).

Manuring (M, NPKM, NPKMR) increased the absorbance of each chemical composition compared to CK and NPK (Fig. 2a). According to peak area, the relative proportion of aromatic C under manuring and straw returning was 21%−73% higher than that of no/mineral fertilization ($p < 0.05$, Fig. 2b). NPKMR treatment resulted in a relative chemical composition of GRSP in which the aromatic C was 43% higher, but the polysaccharides were 12% lower than in the NPKM treatment. Finally, when considering the fallow treatment it is notable that the aromatic C was higher and polysaccharide was lower than all other treatments.

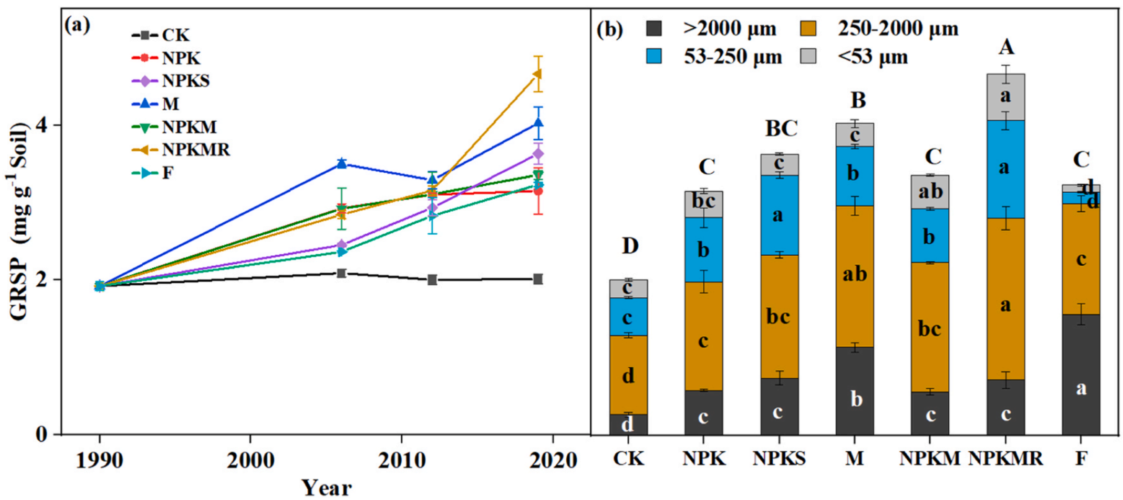

Fig. 1. Temporal glomalin-related soil protein (GRSP) trends between 1990 and 2019 (a). GRSP accumulation in bulk soil and aggregates (b) (i.e., >2000 μm, 2000–250 μm, 250–53 μm, <53 μm) under various fertilization treatments (CK, no fertilizer; NPK, mineral nitrogen, phosphorus, potassium fertilizer; M, pig manure; NPKM, NPK plus pig manure; NPKS, NPK plus Straw; NPKMR, NPKM plus wheat-soybean/sweet potato rotation; F, Fallow). Data represents the means with standard errors (n = 3). Different lowercase and uppercase letters respectively indicate significant differences in aggregates and the bulk soil under different fertilization treatments (Duncan's test, $p < 0.05$).

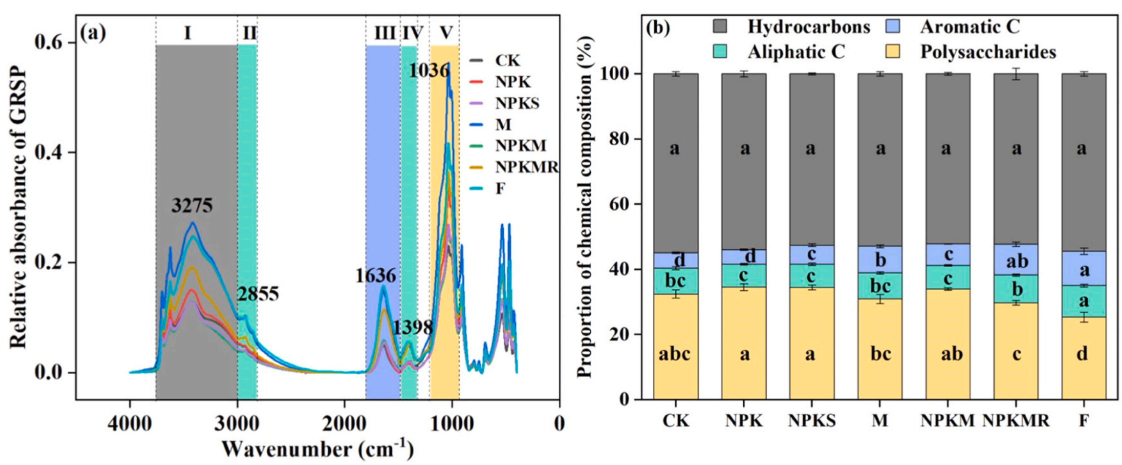

Fig. 2. Fourier transform infrared (FTIR) spectra (a) and relative percentage of compositional traits of glomalin-related soil protein (GRSP) (b) in soils subject to 29-year various fertilization treatments (CK, no fertilizer; NPK, mineral nitrogen, phosphorus, potassium fertilizer; M, pig manure; NPKM, NPK plus pig manure; NPKS, NPK plus Straw; NPKMR, NPKM plus wheat-soybean/sweet potato rotation; F, Fallow). Data represents the means with standard errors (n = 3). Different letters indicate significant differences in each compositional trait under the various fertilization treatments.

3.2. AMF community composition and diversity and their relationship with GRSP

According to the results of the non-metric multidimensional scaling (NMDS) analysis, mineral fertilization (NPK), manuring (M, NPKM, NPKMR) and straw returning (NPKS) greatly altered the AMF community and diversity compared to no fertilization (CK) as well as fallow (F) (Fig. 3a). The AMF biomass (PLFA 16:1ω5) and total PLFA was significantly higher under manuring (M, NPKMR) treatments compared to the CK treatment. However, there was no significant difference in AMF and PLFA between NPK, NPKS versus CK (Fig. 3b, S3). In addition, manuring (M, NPKM, NPKMR) and straw returning (NPKS) resulted in a significantly higher AMF alpha diversity index (Shannon and Chao1) compared with no and mineral fertilization (CK, NPK) (Fig. 3c-d). The heatmap results showed that AMF biomass was positively correlated with GRSP in bulk soil as well as GRSP in large aggregates (>250 μm), and positively correlated with aromatic C composition and negatively correlated with polysaccharides. The AMF alpha diversity index (both Shannon and Chao1) showed a significant positive correlation with aromatic C ($p < 0.05$, Fig. 3e).

3.3. The relationship between MWD, SOC and GRSP

Fertilization had a great effect on aggregates stability. Compared with no and mineral fertilizer treatments (with a MWD around 1.3 mm), manuring (M) and fallow (F) treatments had a significantly higher MWD resulting in a value of 1.9 mm and 1.7 mm, respectively ($p < 0.05$; Fig. S4a), whereas NPKS, NPKM and NPKMR treatments had no significant impact on MWD. In addition, the relationship between GRSP in bulk soil and MWD was weak ($p > 0.05$; Fig. S4b), while MWD was positively correlated with the GRSP in 250–2000 μm and > 2000 μm particle size fractions ($p < 0.01$; Fig. 4a). SOC content was positively correlated with GRSP ($p < 0.01$; Fig. 4b).

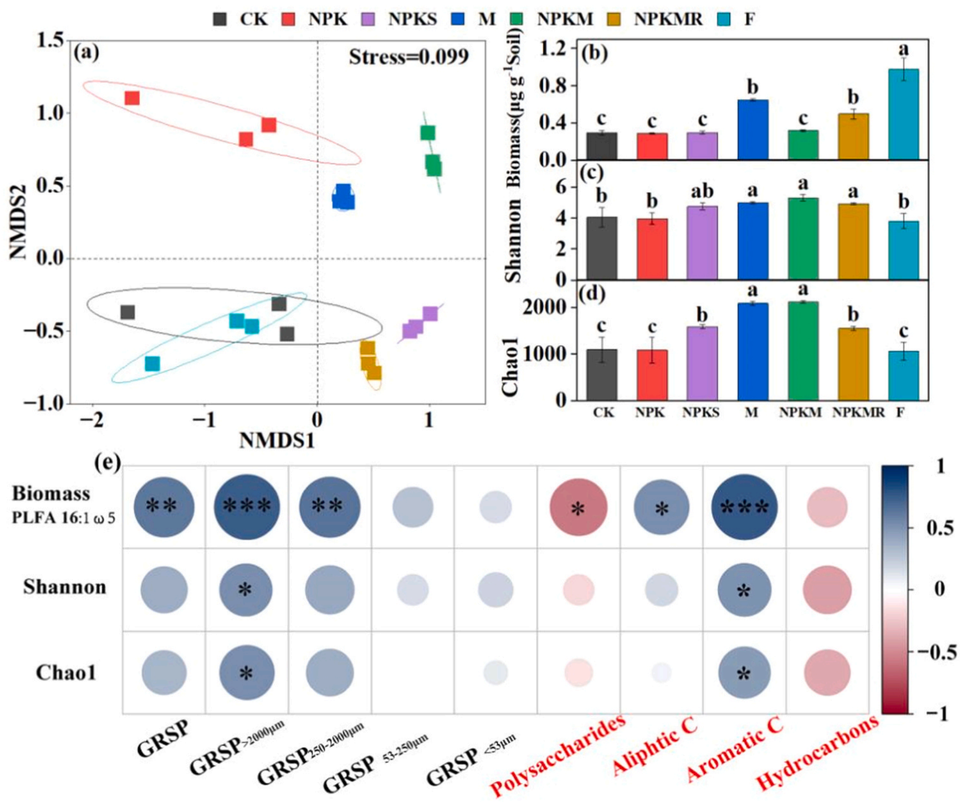

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plots based on operational taxonomic units (OTUs) (a), biomass (b), Shannon index (c) and Chao 1 index (d) of arbuscular mycorrhizal fungi (AMF) under various fertilization treatments (CK, no fertilizer; NPK, mineral nitrogen, phosphorus, potassium fertilizer; M, pig manure; NPKM, NPK plus pig manure; NPKS, NPK plus Straw; NPKMR, NPKM plus wheat-soybean/sweet potato rotation; F, Fallow), and their relationships with glomalin-related soil protein (GRSP) contents (in bulk soil and different aggregates) and compositional traits (e). Data represents the means with standard errors (n = 3). Different letters indicate significant differences between fertilization treatments (Duncan's test, $p < 0.05$).

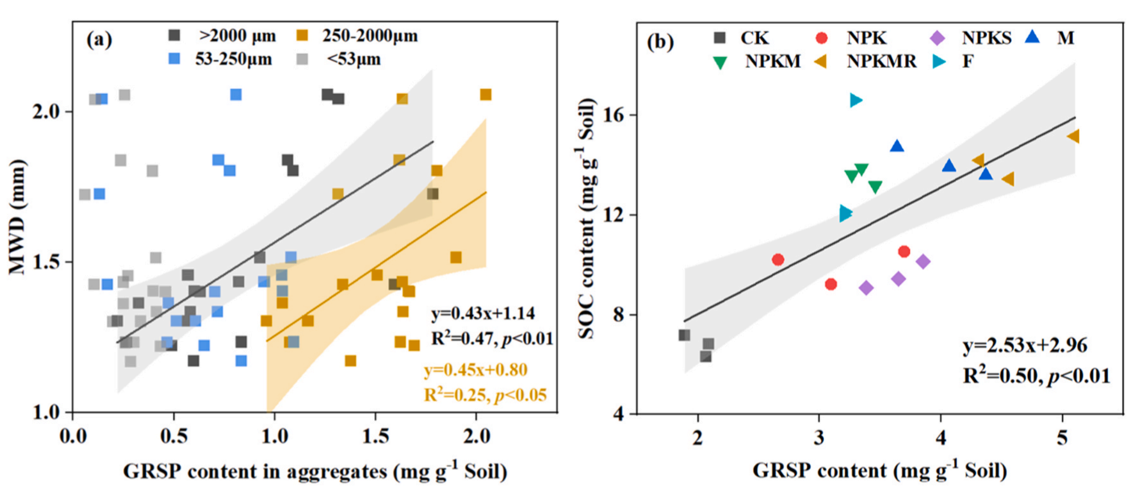

Fig. 4. The relationship between glomalin-related soil protein (GRSP) in aggregates and mean wight diameter (MWD) (a), and the relationships between glomalin-related soil protein (GRSP) in bulk soil and soil organic carbon (SOC) (b). (CK, no fertilizer; NPK, mineral nitrogen, phosphorus, potassium fertilizer; M, pig manure; NPKM, NPK plus pig manure; NPKS, NPK plus Straw; NPKMR, NPKM plus wheat-soybean/sweet potato rotation; F, Fallow).

3.4. Effect of fertilization on GRSP via regulating AMF and aggregate stability

The partial least squares path modelling (PLSPM) showed that pH had a significant positive effect on nutrients (pc (path coefficients)= 0.82, $p < 0.001$), but is not directly related to AMF and aggregate stability. However, nutrition significantly impacted AMF in a positive direction (pc=0.76, $p < 0.01$). In turn, AMF was positively correlated with soil aggregate stability and GRSP composition (pc=0.70, $p < 0.01$; pc=0.68, $p < 0.05$). GRSP accumulation was positively linked with soil aggregate stability and GRSP composition (pc=0.87, $p < 0.001$; pc=0.44, $p < 0.05$) (Fig. 5a). In addition, our analysis suggests that the GRSP composition had the greatest positive effect on GRSP accumulation (total standardized effects = 0.87), followed by AMF (total standardized effects = 0.60) (Fig. 5b). Therefore, soil aggregate stability and GRSP composition were the main controlling factors of the GRSP

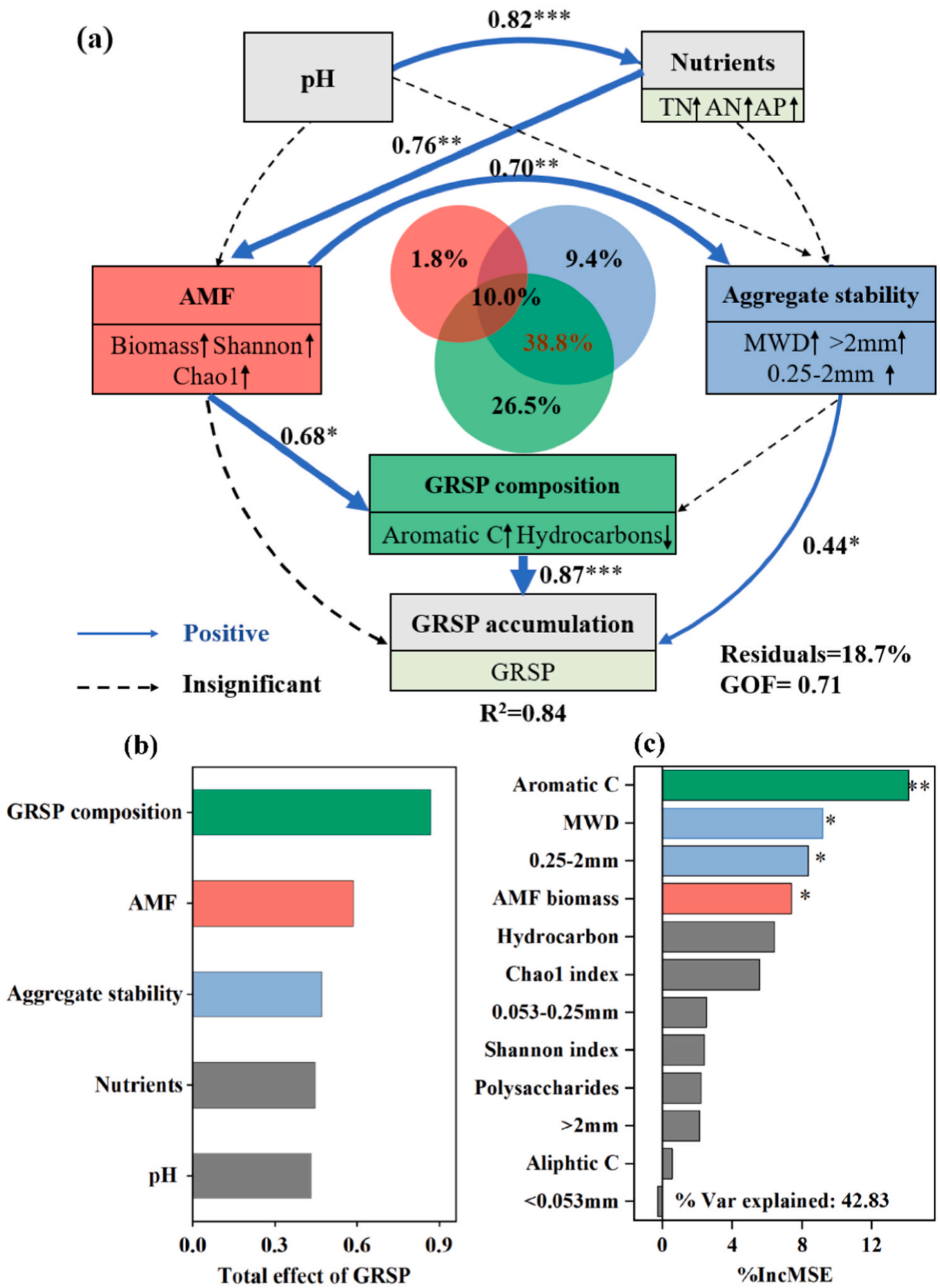

Fig. 5. Partial least squares path modelling (PLS-PM) displaying the effects of abiotic and biotic factors on glomalin-related soil protein (GRSP) accumulation (a), and the standardized effects on GRSP accumulation derived from PLS-PM (b). Variance partitioning analysis (VPA) and random forest to distinguish the relative importance of aggregate stability, GRSP chemical composition and AMF (c). Blue and black arrows represent positive and negative effects, significant and insignificant correlations are denoted by continuous and dashed arrows, respectively. The thickness of the arrow is positively correlated to the strength of the relationship. Two-layer rectangle boxes denote the first component from the PCA performed for AMF, soil aggregate stability and GRSP composition. The up arrow "↑" denotes a positive correlation between the variables and the first component of PCA, the opposite "↓" is negative.

accumulation process in the studied cropland ecosystems. These results were consistent with variance partitioning analysis (VPA). Random forest analysis (RF) showed that the proportion of aromatic C in GRSP dominated the accumulation of GRSP, followed by the protective effect of aggregates (MWD and 0.25–2 mm) (Fig. 5c).

4. Discussion

Our study elucidates the response of GRSP accumulation to long-term fertilization regimes (Fig. 6). GRSP content increased over time across fertilization, suggesting long-term fertilization is advantageous for the accumulation of GRSP (Guo et al., 2019). However, there was obviously divergences in the increasing trends in response to 29-year fertilization regimes (Fig. 1). Long-term manure and straw returning significantly promoted GRSP accumulation, confirming our hypothesis (Fig. 1a). which agreed with most previous studies (Zhang et al., 2014; Bertagnoli et al., 2020; Choudhary et al., 2021; Wang et al., 2022). The application of manure and straw into agricultural production strategies might hold significant value in achieving soil stability and sustainable development. In this study, structural equation modeling showed that

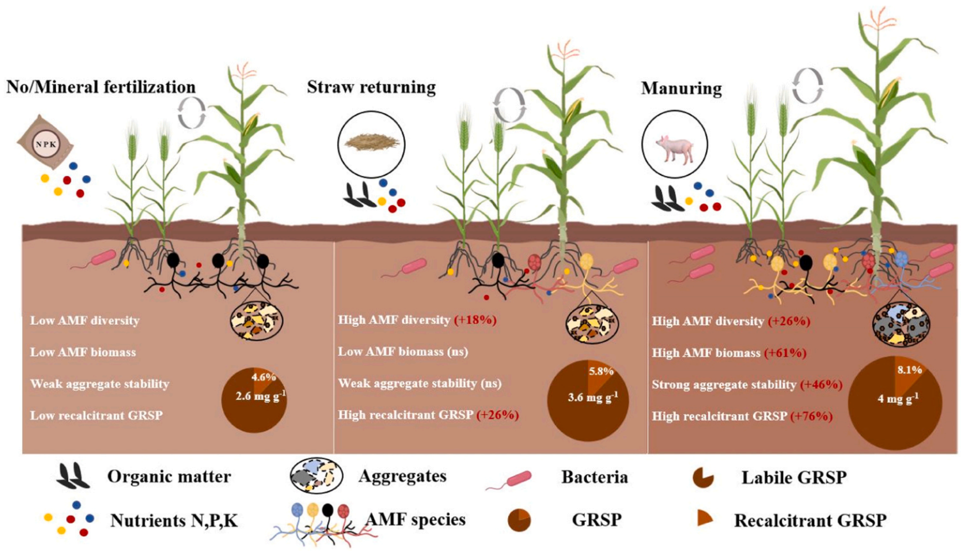

Fig. 6. Conceptual figure showing differences in glomalin-related soil protein (GRSP) accumulation under 29-year various fertilization treatments. ns indicates no significant difference. The size of the pie chart represents the GRSP content.

the accumulation of GRSP could be attributed to the synchronized regulation by (1) the production by AMF biomass and diversity, (2) the stabilization via soil aggregates protection and recalcitrance of GRSP.

4.1. Production mechanism of GRSP

The positive relationship between GRSP content and AMF diversity and biomass indicated that long-term fertilization regimes can directly or indirectly affect GRSP accumulation via regulated AMF diversity and/or biomass (Fig. 3c-e, Agnihotri et al., 2022). Manuring and fallow increased GRSP through AMF biomass by following pathways (Fig. 3b). First, Manuring led to an improvement in soil pH and nutrient levels (e.g., TN, AN, AP) (Table S1), alleviating competition for nutrients between host and AMF (Johnson, 2010). Second, manure application can decrease soil bulk density and increase soil porosity (Ozlu et al., 2019), providing AMF with a sufficient supply of oxygen and water (Wang et al., 1993). This reduces the obstruction of AMF hyphal elongation (Ma et al., 2006), promoting mycorrhizal development (Delavaux et al., 2017; Šarapatka et al., 2019). Third, AMF biomass was positively correlated with the GRSP content in bulk soil and in macroaggregates (>250 μm) ($p < 0.01$; Fig. 3e). This indicates that manuring-induced increases in AMF biomass directly enhanced the GRSP content in macroaggregates. Because soil aggregates provide a suitable habitat for microorganisms (Gupta and Germida, 2015; Trivedi et al., 2017). Malusà et al. (2016) showed that manure can promote the growth and metabolism of multiple AMF species, which has also been supported by more recent studies (Baltruschat et al., 2019; Song et al., 2015; Huo et al., 2022; Liu et al., 2022). In this study, the experimental location is situated in a subtropical region with significant soil acidification (Table S1; Zhang et al., 2009) and a serious imbalance of soil nutrients. Improving the living environment (N availability, pH) through organic fertilization can mitigate microbial nitrogen limitation, stimulating AMF species interactions (Yu et al., 2013), which results in the activation of previously inactive AMF species and contributes to their growth (Aggarwal et al., 2011). Low nutrients (i.e. N, P) under fallow treatment caused nutrient competition among AMF species and other microbes, resulting in low AMF diversity. However, fallow treatment had higher AMF biomass and total PLFA compared to fertilization, probably because as undisturbed natural succession progresses, the soil develops and becomes richer in organic matter (Table 1). The latter provides a favorable environment for growth of AMF, leading to higher biomass, meanwhile, hypha has a good environment to grow without disturbance.

Notably, straw returning and mineral fertilizer with manure had no significant effect on AMF biomass and total PLFA (Fig. 3). The explanation for these results is that straw returning significantly decreased soil pH (Table 1), while AMF prefer to grow in neutral or alkaline soils (Toljander et al., 2008). Therefore, low pH counteracts the positive regulatory effect of nutrients on AMF. Mineral fertilizer with manure increased P availability (Table 1), which could possibly suppress AMF growth (Lin et al., 2012). In addition, the content of added N in this experiment is equal between manure with/without mineral fertilizer, which may result in non-significant differences. Therefore, soil pH, N, P availability and their interactions could impact the AMF growth (Qin et al., 2020; Williams et al., 2017; Luo et al., 2021).

4.2. Stabilization mechanism of GRSP

The peak intensity of each functional group and fingerprints of GRSP altered under different fertilization treatments (Fig. 2a), suggesting that fertilization could change the chemical composition of GRSP. Specifically, Manure and fallow increased the proportion of aromatic C, but had no significant effect on hydrocarbons, enhancing recalcitrance of GRSP by altering its chemical composition (Fig. 2b). Organic matter from manure and straw decomposed to release mineral forms of nitrogen and phosphorus, which become available for plant and AMF uptake/utilization, hereby increasing AMF biomass. Meanwhile, an increase in soil pH following manuring and fallow can strongly alter the AMF community and their interactions (Wang et al., 2014). Furthermore, organic fertilizer application may activate dormant AMF species in the rhizosphere (Zhu et al., 2016). These might influence GRSP chemical composition. Therefore, soil nutrient levels and pH could potentially be significant factors influencing the chemical composition of GRSP (Zhong et al., 2017).

Another key factor is the soil aggregate protection mechanism (Chen et al., 2019). Fertilization regimes affect the redistribution of GRSP in aggregates. In our study, manuring increased MWD values (Fig. S4a) and the mass percentage of the large aggregate size fractions (Table S2). These results are supported by other findings (Van-Camp et al., 2004; Duan et al., 2021). Manure application reduces macroaggregate breakdown by improving hydrophobicity and the interparticle cohesiveness of aggregates. Generally, aggregate stability increases or decreases synergistically with GRSP content (Liu et al., 2021a). However, our study found that the relationship between MWD and GRSP in bulk soil was not significant (Fig. S4b). The reason might be that the method of sweet potato harvesting and tillage systems interfered with the aggregate–GRSP relationship. By investigating GRSP contents in aggregates, we observed a strong correlation between MWD and GRSP in both the > 2000 μm and 250–2000 μm particle size fractions (Fig. 4a), indicating that the formation of large aggregates facilitated the accumulation of GRSP in soil. The winding effect of hyphae and the bonding capacity of GRSP synergistically promote the transformation of small aggregates into large aggregates, thereby increasing the stability of aggregates (Bedini et al., 2009; Kohler et al., 2017; Ji et al., 2019). In turn, large aggregates protect GRSP from decomposition. Moreover, large aggregates provide a stable environment for the formation of micro-aggregates, increasing the ability to absorb or surround more unprotected organic matter (e.g., GRSP) (Liu et al., 2020).

In summary, shifts in the AMF community and diversity directly and indirectly mediated the effects of fertilizers on GRSP accumulation, via two paths (Fig. 5a): (1) Changes in pH and/or nutrients regulated the recalcitrance of GRSP by increasing the AMF biomass and diversity, thus affecting the accumulation of GRSP. (2) AMF regulated the stability of aggregates, thereby increasing the physical protection of GRSP. Therefore, GRSP composition (e.g., aromatic C) had a huge overall effect on GRSP accumulation. VPA and RF modelling (Fig. 5c) together supported the reliability of structural equation modelling, and concluded that the stabilization mechanisms of GRSP dominate its accumulation.

4.3. Implications

Previous studies have indicated that AMF diversity associated with GRSP accumulation will be influenced by plant diversity through natural succession (Wang et al., 2022; Li et al., 2023). In this study, we demonstrated that legume cropping considering a wheat, soybean and sweet potato rotation (NPKMR) was more beneficial for the accumulation of GRSP than a classical wheat-maize rotation (NPKM). The possible explanations were that (1) different AMF species and populations have been detected in legume and non-legume roots (Scheublin et al., 2004), the nitrogen fixation of rhizobium provides more favorable conditions for AMF growth and multiplication under legumes compared to non-legumes (Xavier and Germida, 2003), and as a consequence can increase GRSP accumulation. (2) Low C:N ratios and labile available substances, such as sugars and amino acids, found in leguminous crops and their root exudates can encourage AMF activity (Dinesh et al., 2004). (3) Legume cropping made the soil AMF community richer (Guzman et al., 2021). Therefore, legume cropping may be a sustainable way to increase sequestration and stability of soil C in cropland soils and could be considered as an interesting strategy to combat climate change. However, there are still some limitations in this paper, and further research is needed to investigate the differences in various crop rotations under mineral amendments.

Long-term manuring and straw returning significantly increased SOC (Table 1; Fig. 4b), although their extent of increase differed, indicating that the contribution of straw and/or root exudates and microbial necromass to SOC may be less than that of manure. An explanation is that pH under manuring is higher than that of straw returning (Table 1), increased pH in acid soil increased bacterial. Faster turnover of bacteria can promote accumulation of microbial necromass, which is beneficial to SOC accumulation (Prommer et al., 2020). In addition, the relationships of GRSP and SOC with soil physicochemical traits are not fully consistent. The factors in controlling GRSP and SOC formation might be different and need further research. Therefore, in future research, we can pay more attention to the impact of different carbon source inputs on the recalcitrance of GRSP and SOC.

5. Conclusion

Manuring and straw returning strongly promoted GRSP accumulation, mainly attributed to changes in AMF biomass, diversity, proportion of recalcitrant (aromatic) C in GRSP and macro-aggregates. Manuring and straw returning increased the AMF diversity through improving nutrients and/or alleviating acidification, therefore making its metabolites (GRSP) more difficult to decompose. In addition, manuring also increased the physical protection of GRSP through the formation of macro-aggregates. In summary, the application of organic fertilizer could promote the growth of AMF as well as aggregate stability, and finally facilitate GRSP accumulation. Our findings contribute to clarifying the process of GRSP accumulation under long-term fertilization regimes.

Declaration of Competing Interest

The authors declared that they have no conflicts of interest to this work submitted.

Data Availability

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2023.105904.

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