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Long-term fertilization-induced increases in glomalin-related soil protein depend on phosphorus input and aggregate stability across climatic zones

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF)-derived glomalin-related soil protein (GRSP) contributes to soil carbon sequestration and structural stability. However, how fertilization-induced changes in GRSP differ across climatic zones and management practices remains poorly understood. Here, we investigated the effects of climate, soil properties, and AMF on total GRSP (T-GRSP) based on 14 long-term managed fields (no fertilization, CK; chemical fertilization, CF; organic fertilization, OF) across eastern China, spanning the mid-temperate, warm-temperate zones, and subtropics. Results showed that T-GRSP ranged from 1.0 to 5.1 mg g^{-1}, with a nonlinear latitudinal pattern (mid-temperate zone > subtropics > warm-temperate zone). T-GRSP were significantly 46 % higher under OF compared to CK, whereas CF had no significant effect in temperate zones. Fertilization-induced increases in T-GRSP (ΔGRSP) declined from subtropics (CF: +35.1 %; OF: +80.4 %) to warm-temperate (CF: +13.5 %; OF: +66.6 %) to mid-temperate (OF: +26.7 %), indicating greater effects in warmer and wetter zones. Mixed-effects models revealed zone-specific drivers of ΔGRSP: P input (> 200 kg P_2O_5 ha^{-1}) dominated in mid-temperate zone, AMF biomass in warm-temperate zone, and aggregate stability in subtropics. These differences reflect climatic modulation of nutrient availability and soil structural constraints. Overall, GRSP responses to fertilization are jointly shaped by climate, nutrients, soil structure. Region-specific management strategies are recommended: enhancing aggregate stability in subtropical soils, and optimizing P input and organic amendments in colder zones. These findings offer valuable guidance for context-specific soil management in agricultural systems.

1. Introduction

Glomalin-related soil protein (GRSP) is a heat-stable glycoprotein primarily derived from arbuscular mycorrhizal fungi (AMF) in association with plant roots (Wright and Upadhyaya, 1998). GRSP plays a significant role in soil structure and carbon stability (Driver et al., 2005; Rillig, 2004). Specifically, GRSP, as a “binding agent” could bind with soil particles to form stable structures, promoting soil aggregation, increasing soil porosity and water retention capacity (Wright and Upadhyaya, 1996). GRSP, with aromatic carbon structure (Schindler et al., 2007; Yang et al., 2024), can remain in the soil for 35 years (Harner et al., 2004). However, GRSP can be threatened by intensive agricultural management and environmental stress. Practices such as fertilization (Cissé et al., 2021a; Guo et al., 2019) and cropping systems (Sekaran et al., 2020), can disrupt AMF hyphal networks, thereby impacting the production and storage of GRSP in soils. Moreover, changes in soil nutrients, soil structure, and temperature driven by fertilization or climate variation, may further alter GRSP. These effects might be significantly different across regions. Therefore, understanding the spatial distribution of GRSP in cropland soils and its response to agricultural practices is essential for optimizing the management regimes and maintaining agricultural functionality.

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The spatial variability of GRSP likely stems from the complex interactions between agricultural practices and local soil conditions (e.g., baseline fertility, texture). Previous studies have identified several major factors influencing soil GRSP, including soil nutrients (e.g., N and P), clay content, pH, and crop types (Chen et al., 2023; Commatteo et al., 2023; Rillig, 2004; Treseder and Turner, 2007). Specifically, N, P fertilization and manure amendments enhance AMF activity compared to no fertilization in low nutrients soils, thereby stimulating GRSP production (Wu et al., 2012; Yang et al., 2024). However, such stimulatory effects diminish or reverse in fertile soils, where nutrient saturation may suppress AMF symbiosis (Alguacil et al., 2014; Wu et al., 2014). Notably, high clay content promotes GRSP stabilization through organo-mineral complexation (Cheshire et al., 2000; Demyan et al., 2012), particularly via Fe/Al-oxides acting as "molecular glues" between GRSP and SOC (Quiquampoix and Burns, 2007). This mineral-mediated protection reduces GRSP vulnerability to microbial decomposition. Additionally, soil pH can change the structure and function of AMF and other microbial communities in soil (Aliasgharzad et al., 2010). For instance, neutral to slightly acidic soils (pH 5.5–7.0) favor AMF proliferation and GRSP accumulation (Chen et al., 2023), likely by optimizing fungal enzymatic activity. Conversely, alkaline conditions (pH >8.0) disrupt AMF hyphal networks, yet paradoxically correlate with higher GRSP stocks in calcareous soils (Peng et al., 2015)—a phenomenon potentially linked to carbonate-induced GRSP sequestration. Leguminous intercropping systems may amplify GRSP accrual through rhizodeposition of AMF-stimulatory compounds (Sekaran et al., 2020), whereas cereal monocultures often deplete GRSP via aggregate disruption. Overall, it remains unclear how these factors interact across climatic zones, and whether their relative importance changes with temperature and soil properties. Addressing these knowledge gaps is crucial for understanding the mechanisms underlying the spatial variability of GRSP in cropland soils.

On the spatial scale, the above-mentioned factors may jointly regulate soil GRSP contents and its response to fertilization, but their relative importance varies with environmental heterogeneity. Climate parameters (e.g., temperature and precipitation), soil properties (e.g., pH, texture, and nutrients), and vegetation types determine both AMF community structure and GRSP stability. For example, soil GRSP is driven by rainfall in the warm temperate coastal regions of China, while in subtropics, soil pH and clay content are the key regulatory factors (Chen et al., 2023; Li et al., 2020). These differences suggest that GRSP are tightly coupled to the environmental controls on AMF activity, soil texture, and organic matter turnover (Singh et al.,2024; Yang et al., 2024). Notably, there are differences in the response of different GRSP components to climate (Fokom et al., 2012; Singh et al., 2016). Where in Mollisols, easily extractable GRSP (EE-GRSP) is more sensitive to climate (temperature and rainfall) changes compared to total extractable GRSP (T-GRSP) (Li et al., 2020). Despite these insights, the mechanistic understanding of how spatial heterogeneity in soil–climate interactions regulate GRSP formation, stabilization, and response to fertilization remains limited. In particular, it is still unclear which driver controls GRSP dynamics under different climatic zones. Addressing these gaps is essential for analyzing multidimensional factors such as fertilizer management, climate, and soil properties to gain a deeper understanding of the interaction mechanism of GRSP.

Previous studies characterized the distribution patterns and drivers of GRSP in natural ecosystems such as forests and grasslands (Li et al., 2015; Wang et al., 2018; Wu et al., 2012). In forest soils, vertical stratification of GRSP is pronounced, with concentrations declining significantly with soil depth, a pattern attributed to variations in mycorrhizal colonization rates, soil organic carbon dynamics (Wu et al., 2012). Similarly, in grasslands, plant diversity regulates GRSP levels through its effects on AMF community composition (Li et al., 2015). However, the mechanisms governing GRSP dynamics in intensively managed agricultural systems remain poorly understood.

Therefore, this study aimed to (i) investigate the response of GRSP to fertilization regimes (mineral vs. organic amendments) and (ii) identify the spatial drivers of GRSP responses to fertilization across climatic zones. We hypothesize that: (i) fertilization-induced GRSP increase exhibits climatic zonation, and (ii) the spatial variability of GRSP's fertilization effects is co-regulated by edaphic and microbial factors. Specifically, aggregate stability that physically protects GRSP from microbial degradation becomes more critical in warmer regions, whereas AMF activity and GRSP production play a greater role in cooler regions.

2. Materials and methods

2.1. Study sites and experimental design

This study was conducted using 14 long-term (>29 years) field experimental sites spanning the northern to southern regions of eastern China. These sites cover different climatic zones: middle temperate zone (HH, Heihe; HEB, Harbin; GZL, Gongzhuling; SY, Shenyang), warm temperate zone (HS, Hengshui; LY, Laiyang; ZZ, Zhengzhou; XZ, Xuzhou; MC, Mengcheng), subtropics (YJ, Yanjiang; JX, Jinxian; QY, Qiyang). The mean annual temperature (MAT) and the mean annual precipitation (MAP) in the sites ranged from 1.5 °C to 18.1 °C and 510–1537 mm, respectively. The study sites are charactered by eight different soil orders according to FAO soil taxonomy system. Each site has different fertilizer types, including CK (no fertilizer), CF (one or more chemical fertilizers of N, P and K), OF (organic fertilizers with/without CF). Nutrient inputs refer to the amount of nutrients (N, P, K) supplied by each fertilization treatment. The chemical fertilizers for N, P, and K were urea ($CO(NH_2)_2$), superphosphate ($Ca(H_2PO_4)_2$) and potassium chloride (KCl), respectively. The organic fertilizers were manure and/or straw residue. The field experiment was arranged in a completely randomized design with three replicates per treatment. These sites have different cropping systems, including three systems: continuous cropping (GZL, SY, JX), non-legume rotation (HS, LY, ZZ, XZ, QY) and legume rotation (HH; HEB, MC, YJ). More detailed information is shown in Table S1.

2.2. Soil sampling

Soil samples (0–20 cm) were collected from 14 experimental sites after crop harvest during July to October of 2019. A total of 240 soil samples under different fertilization regimes were sieved through a 2 mm mesh, and each sample was divided into two subsamples, one subsample was stored at −80 °C refrigerator for microbial parameters determination, and the other subsample was air-dried to determine soil properties. The soil pH was measured using a 1:2.5 ratio of soil to water. The elemental content of soil organic carbon (SOC) and total nitrogen (TN) was determined using elemental analyzer (EA3000, Milan, Italy). Soil total phosphorus (TP) and available phosphorus (AP) were analyzed via the molybdenum-antimony resistance colorimetric method. Clay content was determined using the hydrometer method as described by Gavlak et al. (2005).

2.3. Data source of glomalin-related soil proteins

The determination of GRSP followed the method described by Wright et al. (1998). EE-GRSP was extracted from 0.25 g of air-dried soil using a solution composed of 2 mL of 20 mmol L^{-1} sodium citrate (pH=8.0) at 121°C for 30 min and then centrifuged at 10,000 ×g for 6 min. T-GRSP was extracted from 0.25 g of air-dried soil using 2 mL of 50 mmol L^{-1} sodium citrate (pH=8.0) at 121°C for 60 min. This process was repeated four times for each sample. Subsequently, the supernatants were combined and subjected to an additional centrifugation step before quantification. For quantification, 300 μL of the clear supernatant and 700 μL of dH_2O were transferred into the sampling tank, followed by the addition of 5 mL Coomassie Brilliant Blue (CBB) solution. Following a 2–3 min color reaction period (Liu et al., 2021), the optical density (OD) was measured at 595 nm using a microplate reader (Biotek Synergy H1, USA), with bovine serum albumin (BSA) serving as standard. The fertilization effect of GRSP was calculated as GRSP change rate (1):

$$GRSP\ change\ rate(\Delta,\ \ \%) = \frac{GRSP_{fertilizer} - GRSP_{no\ fertilizer}}{GRSP_{no\ fertilizer}} \times 100 \tag{1}$$

where $GRSP_{fertilizer}$ is the content of GRSP under fertilization. $GRSP_{no\ fertilizer}$ is the content of GRSP under no fertilization.

Data on GRSP contents in forest and grassland ecosystems were collected from published studies through a targeted literature search using Web of Science. The search used keywords including “China” and “ecosystem” and “glomalin or GRSP”.

2.4. Determination of microbial biomass

Arbuscular mycorrhizal fungi (AMF) biomass was characterized using C16:1ω5 phospholipid fatty acids (PLFAs) as described by Olsson et al. (1999). PLFAs were extracted with a slightly adapted Bligh-Dyer method, as detailed in Tian et al. (2022). 2 g of freeze-dried fresh soil samples were placed in a Teflon centrifuge tube. An extracting solution containing a citric acid buffer (chloroform: methanol: citric acid = 1:2:0.8), and an unesterified internal standard (C19: 0.1 ng μL^{-1}) was then added to the sample.

2.5. Determination of soil aggregates and glomalin-related soil proteins in aggregates

The screening technology provided by Cambardella and Elliott (1993) was used to divide soil aggregates into four fractions (i.e. > 2000 μm, 250–2000 μm, 53–250 μm, and < 53 μm). Mean weight diameter (MWD) is an indicator characterizing aggregate stability (Kemper and Rosenau, 1986). The MWD was computed as follows (2):

$$MWD = \sum_{i=1}^{n} x_i \times m_i \tag{2}$$

Where n is the number of fractions, m_i is the mass percentage of aggregate that is still on the ith sieve, x_i refers to the mean diameter of particle size fraction.

To examine the distribution of GRSP among soil particle size fractions, GRSP was then extracted and quantified from each size fraction following the procedures described above. The resulting data are presented in Figure S1.

2.6. Statistical analysis

A mixed-effect model was used to analyze the relationship between GRSP fertilization effect (ΔGRSP) and driving factors, using Equation (3):

$$\Delta GRSP = \beta_0 + \beta_1 \times X + \pi + \varepsilon \tag{3}$$

where β is the estimated coefficient, π is the random effect factor, ε is the sampling error and X represent the potential driving factors (Peng and Chen, 2021).

The data were examined for homogeneity and normal distribution of variance. Differences (Duncan’s test) in GRSP content, edaphic, and microbial factors among fertilization treatments were analyzed using one-way ANOVA with IBM SPSS 19.0 software. Multi-ways ANOVA was used to analyze the combined effects of climate, fertilization, and planting on GRSP. pH was grouped into < 6, 6–8, and > 8 (Zhou et al., 2023). The following parameters also adopt a similar classification method: Clay (> 25 % and < 25 %) (He et al., 2021), N input (< 100, 100–250 and > 250 kg N ha^{-1} yr^{-1}) (Curtright and Tiemann, 2021). C input (<1500, 1500–3000, and > 3000 kg C ha^{-1} yr^{-1}), P input (< 100, 100–200, and > 200 kg P_2O_5 ha^{-1} yr^{-1}). The “lme4” package in R was used to fit a linear mixed-effects model for the GRSP fertilization effect (ΔGRSP), to assess its response to temperature, precipitation, soil properties, microbial factors, and fertilizer input (Bates et al., 2015). Cropping systems were included as a random effect in the model. As EE-GRSP was not significant different among climatic zones, no model was constructed for it. All-subsets regression was applied using the "leaps" package to evaluate the best combination of variables (Hofmann et al., 2020), serving the purposes of variable screening and model optimization. All statistical analyses were performed using R 4.3.0.

3. Results

3.1. Spatial and climatic variations of GRSP under fertilization regimes

The contents of soil GRSP were influenced by climate zones, fertilization regimes and cropping systems, with climate exerting the most significant effect (Table 1). Therefore, the study sites were grouped according to temperature zones to explore the spatial distribution patterns of GRSP. Across eastern China, GRSP displayed spatial variability (Fig. 1a). EE-GRSP ranged from 0.2 to 1.7 mg g^{-1} (Fig. 1b), and T-GRSP contents ranged from 1.0 to 5.1 mg g^{-1} (Fig. 1c). GRSP content was highest in the mid-temperate zone, followed by the subtropics, and lowest in the warm temperate zone (Fig. 1d).

Effects of fertilization on GRSP varied across climatic zones. Overall, the OF treatment significantly increased EE-GRSP by 47 % and T-GRSP by 46 % compared to the CK treatments, while CF treatment only increased T-GRSP by 14 % (Fig. 1a, e, f). In the warm temperate zone and subtropics, EE-GRSP and T-GRSP were higher under the OF treatment compared to the CK treatment (Fig. 1c). Conversely, in the middle temperate zone, the OF treatment significantly increased EE-GRSP, but had no significant effect on T-GRSP (Fig. 1d, f). The CF treatment also significantly enhanced EE-GRSP and T-GRSP content in the subtropics (Fig. 1d, f).

At the aggregate scale, the OF treatment significantly increased T-GRSP content in the > 53 μm particle size in the warm temperate and subtropical zones compared to the CK treatments, while in the middle temperate, fertilization had little effect on the GRSP content across different particle sizes (Fig. S1). Moreover, In the subtropical and warm temperate zones, the contributions of EE-GRSP and T-GRSP to SOC were significantly higher than in the mid-temperate zone (Fig. 2a, b).

3.2. Response of fertilization induced GRSP change to climate and crop management

The response of fertilization induced GRSP change (ΔGRSP) were influenced by fertilization types, temperature zones, crop management, soil properties, and nutrient inputs (Fig. 3). ΔEE-GRSP and ΔT-GRSP were higher under the OF treatment than under the CF treatment (Fig. 3a, b). ΔT-GRSP showed a trend across climate zones and crop management, with more precisely: mid-temperate zone (OF: +26.7 %)

Table 1
Multi-way analysis of variance (MANOVA) of GRSP contents and AMF biomass under different climate zones, fertilization and cropping system (type II tests). MAT: mean annual temperature; MAP: mean annual precipitation; T-GRSP: Total glomalin-related soil proteins; EE-GRSP: easily extractable glomalin-related soil proteins; AMF: arbuscular mycorrhizal fungi.

	T-GRSP		EE-GRSP		AMF	
	F value	p	F value	p	F value	p
MAT	275.4	< 0.001	0.1	0.71	46.6	< 0.001
MAP	326.6	< 0.001	10.1	< 0.01	2.8	0.10
Fertilization types	58.1	< 0.001	32.8	< 0.001	25.2	< 0.001
Cropping system	28.5	< 0.001	12.7	< 0.001	12.7	< 0.001
MAT: MAP	14.3	< 0.001	104.0	< 0.001	0.3	0.60

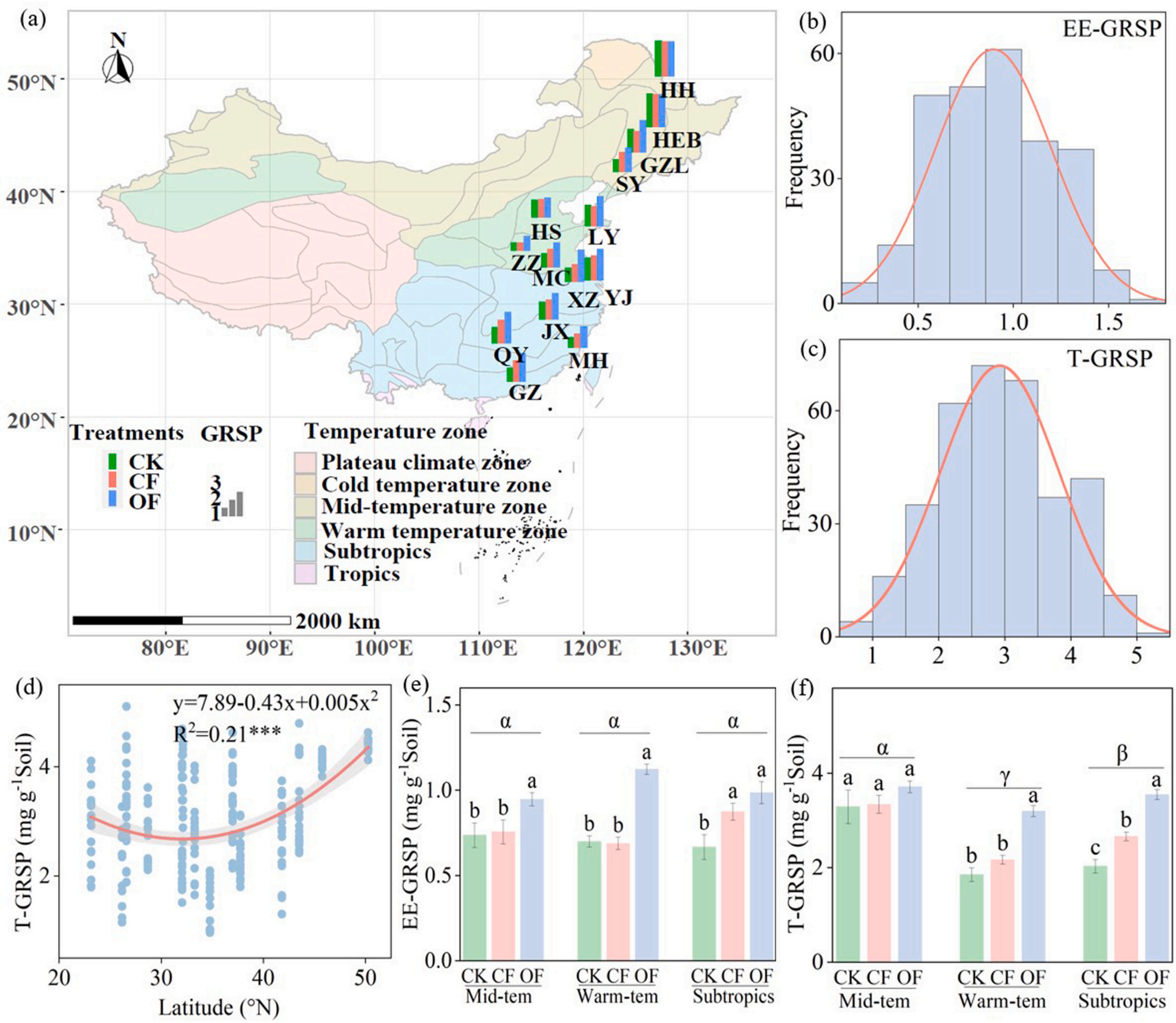

Fig. 1. Contents of T-GRSP in 14 long-term field experiment sites across eastern China (a). Frequency distribution of EE-GRSP (b) and T-GRSP (c). Relationship between GRSP and latitude under long-term fertilization regimes (d), and differences of EE-GRSP (e) and T-GRSP (f) contents under climatic zones and fertilization regimes. Different English letters and Greek letters represent significant differences under different fertilization regimes and climatic zones (Duncan's test, $p < 0.05$). HH, Heihe. HEB, Harbin. SY, Shenyang. GZL, Gongzhuling. SY, Shenyang. HS, Hengshui. LY, Laiyang. ZZ, Zhengzhou. XZ, Xuzhou. MC, Mengcheng. YJ, Yanjiang. JX, Jinxian. QY, Qiyang. MH, Minhou. GZ, Guangzhou. Mid-tem: middle temperate zone; Warm-tem: warm temperate zone. CK: no fertilizer; CF: chemical fertilizer; OF: organic fertilizer. T-GRSP: Total glomalin-related soil proteins; EE-GRSP: easily extractable glomalin-related soil proteins.

< warm temperate zone (CF: +13.5 %; OF: +66.6 %) < subtropics (CF: +35.1 %; OF: +80.4 %) (Fig. 3, S2a), and continuous cropping ≥ non-leguminous rotation > leguminous rotation (Fig. 3, S2b).

Arbuscular mycorrhizal fungi (AMF) biomass was highest in the warm temperate zone compared with the mid-temperate zone and subtropics (Fig. S3a). Mean weight diameter (MWD) increased with temperature following the order mid-temperate zone < warm temperate zone < subtropics) (Fig. S3b). pH and clay content also regulated ΔGRSP, with higher fertilization effect observed at pH 6–8 and clay content < 25 %. Nutrient inputs further impacted ΔGRSP: the largest increases occurred when C input was 1500–3000 kg C ha^{-1} yr^{-1}, N input was 250 kg N ha^{-1} yr^{-1}, and P input was > 200 kg P_2O_5 ha^{-1} yr^{-1}. Notably, the fertilization effect of AMF (ΔAMF) was consistent with that of GRSP under temperature zones, cropping systems, and fertilization types (Fig. 3).

3.3. *Associations of GRSP fractions with climatic, edaphic factors and nutrient inputs*

The contents of T-GRSP showed a positive correlation with AMF, PLFA, SOC, TN, and clay content, while exhibiting a unimodal relationship with pH and AP (Fig. S4). The fertilization effect of T-GRSP (ΔT-GRSP) exhibited close relationships with climatic, edaphic, and nutrients, and demonstrated variations across different climatic zones. In the mid- and warm-temperate zones, ΔT-GRSP showed positive correlations with MAT, MAP, nutrients change rates (ΔSOC, ΔTN, ΔAP), and nutrient inputs (N, P). In contrast, clay content was significantly negatively correlated with ΔT-GRSP, but no significant relationships were observed in the subtropics. MWD was positively correlated with T-GRSP in the subtropics but negatively correlated in the mid-temperate zone (Fig. 4).

The contents of EE-GRSP exhibited a positive correlation with AMF, total PLFA, and nutrients (SOC, TN, AP) across three climatic zones.

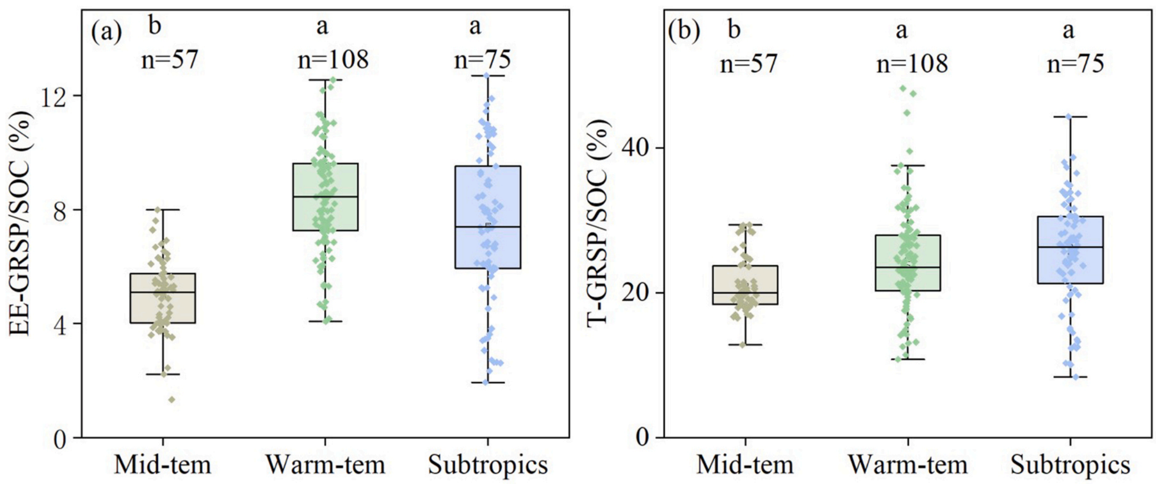

Fig. 2. Proportions of EE-GRSP (a) and T-GRSP (b) to SOC under different climate zones. Mid-tem: middle temperate zone; Warm-tem: warm temperate zone; GRSP: glomalin-related soil proteins; SOC: soil organic carbon. Different English letters represent significant differences under different climate zones (Duncan's test, $p < 0.05$).

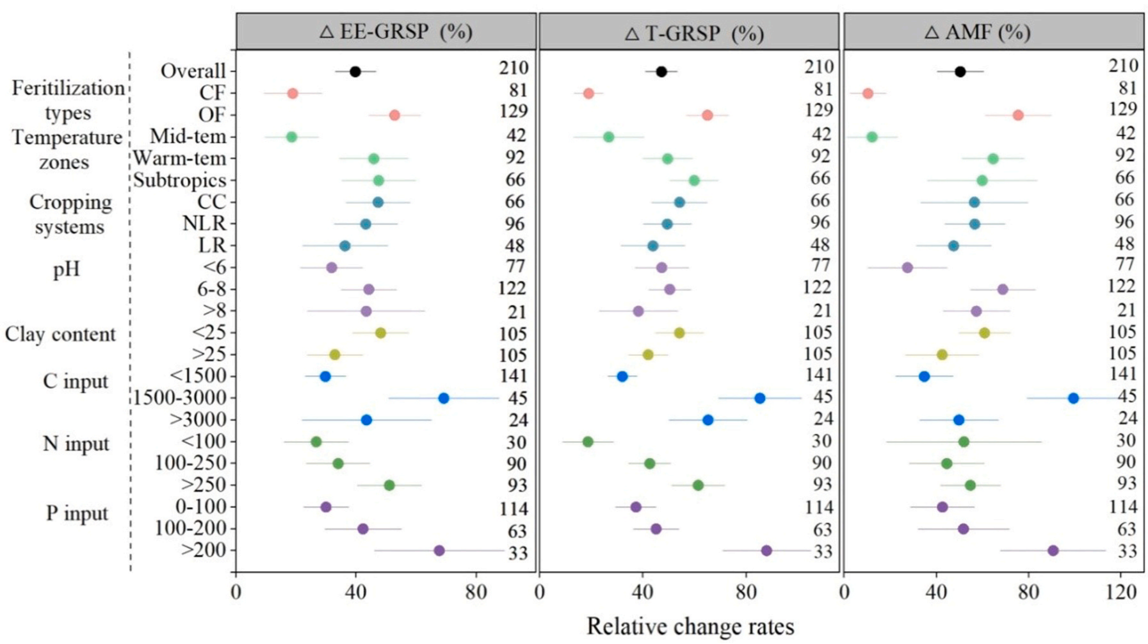

Fig. 3. Fertilization effects (△, %, change rates) on EE-GRSP (a), T-GRSP (b) and AMF (c) across climate, soil properties and management measures. The circles with error bars represent the mean values ± 95 % confidence interval (CI). EE-GRSP: easily extractable glomalin-related soil proteins; T-GRSP: Total glomalin-related soil proteins; AMF: arbuscular mycorrhizal fungi; CF: chemical fertilizer; OF: organic fertilizer; Mid-tem: middle temperate zone; Warm-tem: warm temperate zone; CC: continuous cropping; NLR: non-legume rotation; LR: legume rotation.

However, EE-GRSP significantly decreased with increasing clay content (Fig. S5). ΔEE-GRSP also varied among climatic zones: in the mid- and warm-temperate zones. ΔGRSP had a positive correlation with MAT, nutrients (ΔSOC, ΔTN, ΔAP), and C, N input, but no significant relationships were observed in the subtropics. The increase of clay content reduced the fertilization effect of GRSP (Fig. 4).

3.4. Main controlling factors driving soil GRSP changes under fertilization in climatic zones

Having established correlations between GRSP fractions and environmental factors, we next identified the primary drivers of the fertilization effect on GRSP using regression models. Linear mixed-effects models were employed to account for fertilization effects while minimizing interference from cropping systems. Meanwhile, all-subsets regression was used to screen the best-fit models (Fig. S6), and variance inflation factors (VIF) were calculated to test collinearity among variables (Table S2). Variables with VIF < 10 were retained as covariates, resulting in seven or eight variables for each climatic zone: mid-temperate (ΔTN, ΔAP, clay, MWD, P, N input, ΔAMF), warm-temperate (ΔTN, ΔAP, clay, MWD, C, P, N input, ΔAMF) and subtropics (ΔTN, ΔAP, MWD, C, P, N input, ΔAMF) (Fig. 5).

We found that climate, edaphic properties, fertilization and microbes jointly regulated GRSP contents (Fig. 5a). However, the dominant drivers regulating GRSP varied across climatic zones. In the mid-temperate zone, fertilization inputs were the major determinant, contributing 63 % to the variation (Fig. 5b). In the warm-temperate zone, microbial factors were most influential, accounting for 58 % of

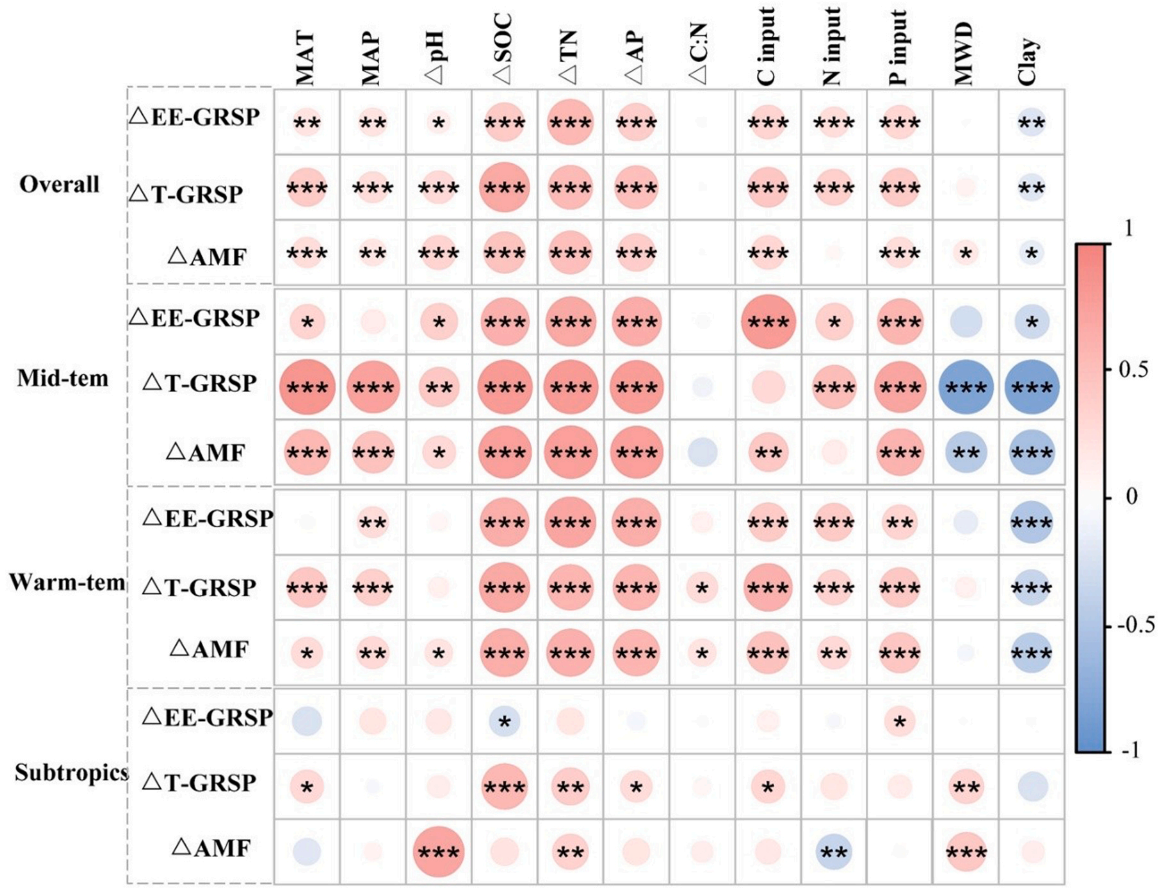

Fig. 4. Heatmap showing Pearson correlations between the fertilization effects (△, %, change rates) on GRSP fractions, AMF with climate, soil properties and management measures. EE-GRSP: easily extractable glomalin-related soil proteins; T-GRSP: Total glomalin-related soil proteins; AMF: arbuscular mycorrhizal fungi; MAT: mean annual temperature; MAP: mean annual precipitation; TN: total nitrogen; SOC: soil organic carbon; AP: available phosphorus; MWD: mean weight diameter.

the explained variance (Fig. 5c). In the subtropics, edaphic properties dominated, explaining 53 % of the variability (Fig. 5d). Further analysis indicated that P input, ΔAMF, and MWD were the key factors driving GRSP increasing in the mid-temperate, warm-temperate and subtropical zones, respectively.

4. Discussion

Based on soil samples under different fertilization regimes across all long-term experimental sites, we evaluated the levels and main drivers of GRSP in cropland across eastern China using multiple statistical methods. Our results showed lower levels of GRSP in cropland relative to forests and grassland ecosystems (Table S3). This phenomenon might be that agricultural management practices (fertilization, tillage, cropping) disturb GRSP production (Fokom et al., 2012). Although fertilization increased the content of GRSP (Fig. 1a, f), these practices were insufficient to compensate for the decomposition of GRSP. Furthermore, climate also had a significant impact on GRSP content (Fig. 1), and the differences among temperate zones may be attributed to specific factors. Therefore, this study elucidated the GRSP fertilization effects in different climatic zones and their primary driving factors.

4.1. Distribution characteristics of GRSP and its response to fertilization

GRSP is initially produced as EE-GRSP by AMF and accumulates in the soil (Rosier et al., 2006; Wu et al., 2014). Therefore, EE-GRSP levels can reflect the potential of GRSP production. In this study, the level of EE-GRSP ranged from 0.02 to 1.8 mg g^{-1} (Fig. 1b). Organic fertilization significantly increased the content of EE-GRSP in different temperate zones, while chemical fertilization had a significant effect only in the subtropics (Fig. 1e), likely due to nitrogen deficiency and nutrient imbalance in the subtropical soil types (Gispert et al., 2013; Zhang et al., 2015). Fertilization, especially balanced NPK inputs and organic amendments, may alleviate these stresses and promote AMF colonization and EE-GRSP production (Luo et al., 2021). T-GRSP content ranged from 1.0 to 5.1 mg g^{-1} (Fig. 1f), and its variation among sites suggests a complex interplay between climate, soil, and management practices. Notably, GRSP decreased and then increased with latitude (Fig. 1d), possibly due to a combination of climatic and edaphic factors. Several mechanisms may explain this trend: (i) higher temperatures accelerate GRSP decomposition, although this effect may be offset in subtropical regions by acidic soils and Fe/Al oxides that stabilize GRSP (Wang et al., 2014); (ii) lower clay content in warm-temperate soils limited mineral-associated protection of GRSP compared to other regions (Helassa et al., 2011); (iii) although AMF biomass was higher in warm-temperate zone than in other zones (Fig. S4a), EE-GRSP did not increase accordingly (Fig. 1e); (iv) the alkaline nature of warm-temperate soils may increase the solubility and reduce stabilization of GRSP, which is an alkaline-soluble protein. (Vodnik et al., 2008). Organic fertilization significantly enhanced GRSP content across different climate zones and cropping systems (Fig. 1f), with more pronounced fertilization effects (ΔGRSP) in warm-temperate and subtropical zones (Fig. S2a), supporting our first hypothesis. This phenomenon may be due to the fact that under low-temperature conditions, microbial decomposition of GRSP was limited, resulting in slower turnover rates and higher accumulation levels (Woignier et al., 2014). Additionally, the

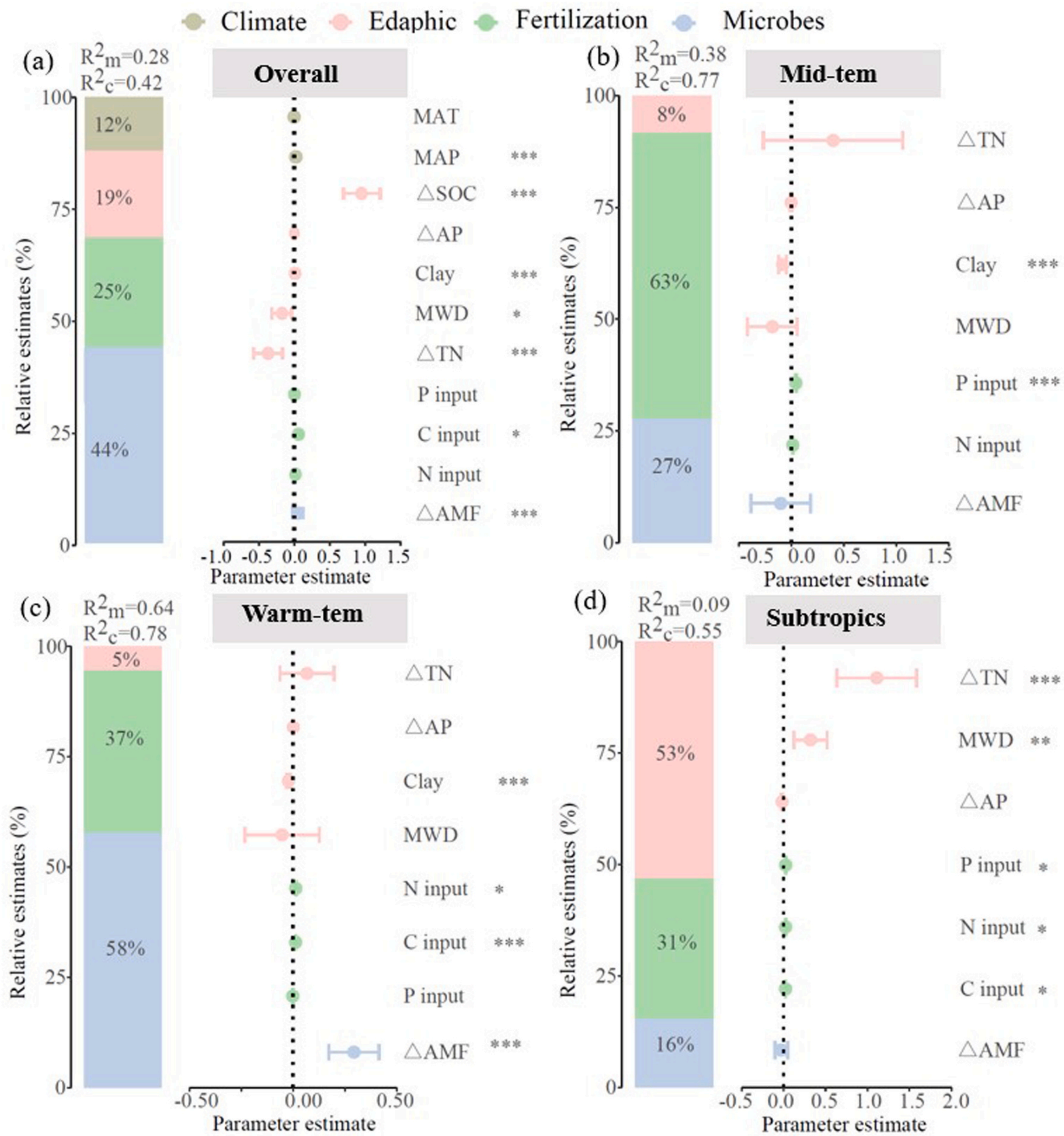

Fig. 5. Relative importance of climate variables, edaphic properties, microbes, and fertilizer application rates to the fertilization effects on T-GRSP (△, %, change rates), and the parameter estimates for each factor represent the percentage of explained variation to the fertilization effects on T-GRSP. The circles with error bars represent the mean values ± 95 % confidence interval (CI). T-GRSP: Total glomalin-related soil proteins; AMF: arbuscular mycorrhizal fungi; MAT: mean annual temperature; MAP: mean annual precipitation; TN: total nitrogen; SOC: soil organic carbon; AP: available phosphorus; MWD: mean weight diameter. Mid-tem: middle temperate zone; Warm-tem: warm temperate zone.

higher organic matter content in this region led microbes (r-strategy) to preferentially utilize readily decomposable organic matter (Blagodatskaya et al., 2004; Salome et al., 2010). However, the metabolic activity of AMF is higher in regions with higher temperature (Xiao et al., 2021), and nutrients addition may be more conducive to the growth of AMF, thereby promoting GRSP production and its contribution to SOC (Fig. 2). Interestingly, the increase in GRSP (ΔGRSP) through fertilization under leguminous crop rotation was lower than that under continuous cropping and non-leguminous crop rotation (Fig. 3, S2b), possibly because non-legumes and legumes contain distinct AMF communities (Scheublin et al., 2004), meanwhile, the nitrogen fixation of legumes reduces the dependence on AMF for fertilization. Similarly, Singh et al. (2018) reported that incorporating legumes, such as with chickpeas, increased root deposition, thereby stimulating the growth of AMF and GRSP production. Therefore, soil GRSP could be optimized by diversifying rotations and applying organic fertilizer in warmer regions.

4.2. *Influencing factors of GRSP fertilization effect in cropland*

In agricultural ecosystems, since the inclusion of artificial management practices, the factors influencing soil GRSP may differ compared to those in other ecosystems. Some studies indicated that factors such as AMF species and abundance, vegetation types, climate, and soil physicochemical properties may influence GRSP content to varying degrees (Rillig, 2004; Wang et al., 2018). Our study demonstrated that the fertilization-induced changes in GRSP are governed by different sets of factors across climatic zones, highlighting the importance of context-specific management strategies. It was noteworthy that MAT was negatively correlated with GRSP (Fig. S4a), while positively correlated with ΔGRSP (Fig. 4a), indicating that fertilization can alleviate the negative effect of temperature on soil GRSP. Because the increase in temperature accelerates GRSP decomposition by microorganisms (Hu et al., 2024). Organic fertilization promotes GRSP production by enhancing AMF metabolic activity, and simultaneously provides C, N sources for saprophytic microorganisms, reducing GRSP decomposition. AMF (PLFA16:1ω5) exhibits a positive correlation with GRSP in all regions, consistent with previous studies (Agnihotri et al., 2022; Li et al., 2020; Wang et al., 2022). T-GRSP and EE-GRSP are significantly positively correlated with total PLFA (Fig. S4d). These findings suggest that GRSP production is not exclusively determined by AMF abundance, but may also be influenced by other non-AMF fungal groups (Adam et al., 2011). Other microorganisms (like Saprophytic fungi) may also affect GRSP. As GRSP serves as an energy source for microbial growth and dispersal, the changes in GRSP content in soil can be reasonably explained by the contemporaneous AMF and total PLFA. This also explains why indicators such as SOC, TN, and AP were significantly positively correlated with GRSP (Fig. S4e-g), as nutrient addition in low-nutrient environments such as agricultural fields promoted AMF growth and dispersal, thereby facilitating GRSP accumulation (Bonser et al., 1996; Liu et al., 2008). Additionally, the relationship between pH and GRSP varies in different temperature zones. In warm-temperate zones, pH was negatively correlated with GRSP, while in subtropical regions, it was positively correlated (Fig. S4i). The reason may be that pH regulates AMF growth. AMF generally grow and disperse best under slightly acidic to neutral conditions. In warm-temperate regions of China, soil pH is > 7, while in subtropical regions, it is < 7. Therefore, higher pH levels are unfavorable for AMF growth, thereby reducing GRSP production (Chen et al., 2023; Peng et al., 2015; Zhang et al., 2017). Another significant factor is soil clay content. Our results indicated a close relationship between clay and GRSP in mid-temperate zones (Fig. S4h). This positive correlation had also been found in other ecosystems (Rillig and Steinberg, 2002; Treseder and Turner, 2007). GRSP, like general soil organic matter, can be stabilized by forming associations with clay minerals, which helps its long-term storage in soil (Woignier et al., 2014). Accordingly, one would expect a significant correlation between clay content and GRSP. However, studies in temperate forest soils of France found no significant relationship, possibly because the variations in vegetation type, particularly tree species and their AMF associations, exerted stronger control over GRSP distribution than the mineral protection provided by clay (Cissé et al., 2023). Therefore, the accumulation of GRSP in agricultural ecosystems is the result of the combined effects of multiple factors.

The fertilization effects of GRSP varied significantly with above related factors across different temperature zones (Fig. 4). In the middle and warm temperate zones, nutrients (C, N, P) input, soil nutrients (ΔSOC, ΔTN, ΔAP) were significantly positively correlated with ΔGRSP, while in subtropics, it shows a weak correlation (Fig. 4). It suggested that different factors control the response degree of GRSP to fertilization. The linear mixed-effects model revealed that fertilization inputs were the predominant drivers of GRSP response in middle temperate zone, explaining 63 % of the total variation (Fig. 5b). Because in this region, P removal by crops is relatively high, total and available soil P contents become key factors influencing GRSP under fertilization. P inputs showed significant positive effects, consistent with previous studies emphasizing the importance of P in soil organic matter dynamics (Hou et al., 2020; Cissé et al., 2021a). Soil P is often a limiting nutrient for microbes and plants in croplands with low P content (Hou et al., 2020). P availability affects AMF colonization and hyphal growth (Zhang et al., 2017), directly influencing GRSP secretion. P addition not only supports AMF but also influences saprophytic microbial communities. In addition, sufficient P supports saprophytic microbial growth, reducing the need for microbes to decompose existing soil organic matter and thereby indirectly contributing to GRSP accumulation. This is supported by our observation that total PLFA and GRSP were positively correlated (Fig. S4d). However, in soils with high available P, P application may not have a significant effect on GRSP content or even have a negative impact (Cissé et al., 2021a; Yang et al., 2023). Because AMF may shift life-history traits (Horsch et al., 2023), which could affect GRSP production. These findings highlight that P is a central, yet often overlooked, regulator of GRSP accumulation and soil organic matter cycling in agricultural ecosystems.

In the warm temperate zone, microbial factors, especially ΔAMF, were the dominant contributors (58 %), with ΔAMF emerging as the significantly positive predictor of GRSP (Fig. 5c). This suggests that in warmer climates, the capacity of AMF to mediate nutrients responses becomes more central, possibly due to enhanced microbial activity and mycorrhizal interactions (Wang et al., 2022). By contrast, in the subtropics, edaphic variables explained the majority of the variation (53 %) (Fig. 5d). The significance of ΔTN and MWD as positive predictors indicated that soil structural and nutrient conditions are more limiting for GRSP formation in highly weathered, acidic subtropical soils. In this region, the faster turnover of soil organic matter may lead to higher decomposition rates of GRSP, thus requiring greater physical protection (e.g., within soil aggregates) to prevent its breakdown (Cissé et al., 2021b). Additionally, the weaker correlations between AMF and GRSP (compared to mid-/warm-temperate zones) suggested that soil GRSP in this zone mainly relies on the physical protection of aggregates rather than the microbial pathway of AMF-GRSP (Fig. 4). To verify the conjecture, we measured the GRSP content in aggregates and found that the GRSP content in > 53 μm particle sizes and aggregate stability increased significantly under fertilization (Fig. S1, S3b). Notably, the contribution of GRSP to SOC showed climate-dependent patterns, with significantly stronger promotion of SOC accumulation in subtropics (Fig. 2, S7). This phenomenon may be attributed to two synergistic mechanisms: (i) The recalcitrant aromatic structure of GRSP, enhanced by elevated AMF metabolic activity under subtropics, conferring chemical stability to SOC (Yang et al., 2024); (ii) The adhesive properties of GRSP facilitate macroaggregate formation, physically protecting labile C from decomposition (Zhao et al., 2025). Overall, these findings highlighted that GRSP responses to fertilization are not uniform in climate gradients but are instead shaped by interactions among soil properties, microbes, and nutrient management. Thus, strategies to enhance GRSP accumulation in cropland should be adjusted accordingly: optimizing nutrient inputs in cooler climates, promoting AMF activity in warm temperate zones, and improving soil structural conditions in subtropical zones (Fig. 6).

4.3. Limitations and implications of GRSP accumulation

While this study offered important insights into the climate-dependent responses of GRSP to long-term fertilization, there are some implications regarding its generalizability and applicability to broader research contexts: (i) investigating the diversity and symbiotic specificity of AMF rather than just biomass may provide key insights into the ecological mechanisms governing GRSP accumulation at the spatial scale. (ii) The study is categorized by broad climatic zones (mid-temperate, warm-temperate, subtropics), which may overlook site-level variations such as topography, rainfall intensity, and land-use history. (iii) The research is based on long-term endpoints but does not explore seasonal or annual dynamics of GRSP in response to fertilization.

In the future, we can further research in the following aspects: (i) quantitative assessment of GRSP influencing factors across larger geographical entities. Statistical analysis methods such as regression analysis or principal component analysis (PCA) will be used to quantify the influence extent of factors on GRSP spatial variations. The dominant factors and their mechanisms will be further explained by constructing models to predict the relationship between GRSP content and factors like climatic conditions (e.g., temperature, precipitation), soil properties (e.g., texture, SOC), and land use practices (e.g., crop types, fertilization). (ii) Long-term monitoring and dynamic changes. Integrating long-term experimental platforms to collect and analyze soil samples from

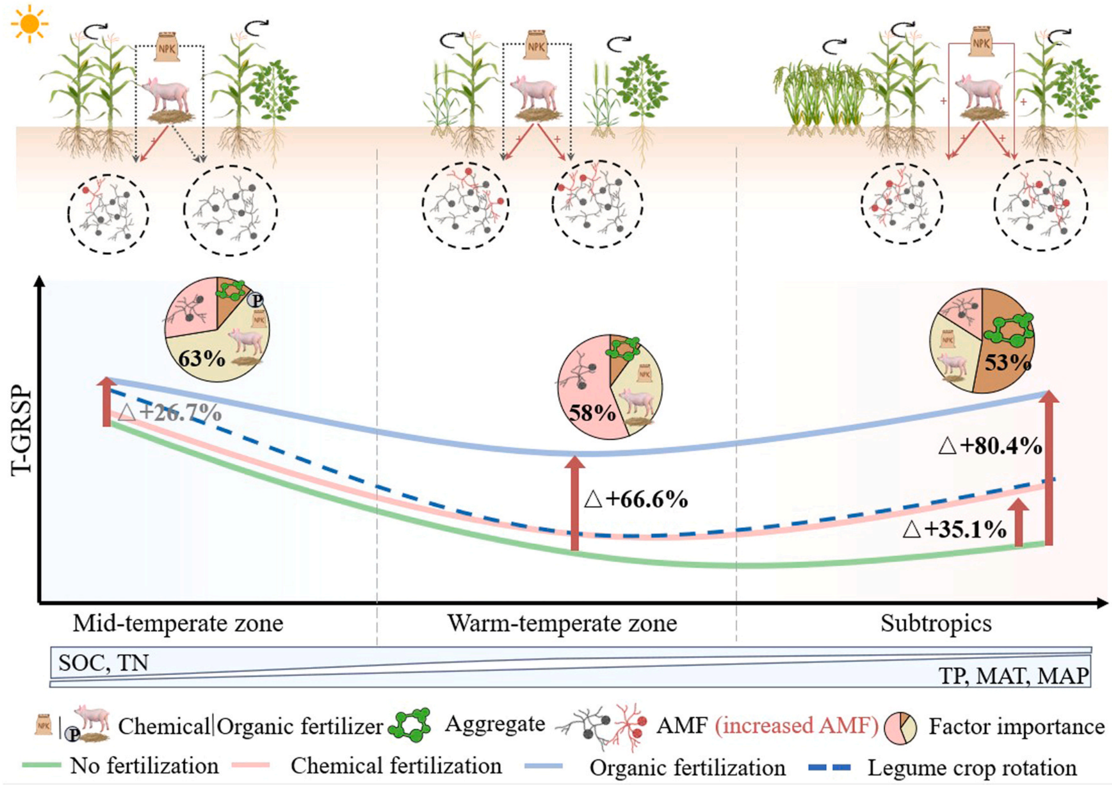

Fig. 6. Fertilization effects on GRSP accumulation across climate zones. GRSP: glomalin-related soil proteins.

different locations annually, and documenting GRSP content changes over time. This approach can help reveal the seasonal, annual, and long-term trends of GRSP, thus better understanding its dynamic characteristics. (iii) Evaluation of ecosystems functionality. To assess the impact of GRSP on soil moisture retention, nutrient cycling, crop growth and yield, as well as regulation of SOC storage and greenhouse gas emissions. This evaluation can provide scientific basis for ecosystem management and policy formulation.

5. Conclusion

This study assessed the spatial patterns and underlying drivers of GRSP accumulation under long-term fertilization across eastern China. Organic fertilization significantly enhanced EE-GRSP and T-GRSP contents, with the strongest responses observed in warmer and wetter regions. Subtropical soils exhibited the greatest enhancement, while the drivers of fertilization effects of GRSP varied among climatic zones: P input in mid-temperate zone, AMF biomass in warm temperate zone, and soil aggregate stability in subtropics. Legume rotation also reduced the dependence of GRSP accumulation on fertilization, highlighting its potential as a sustainable practice. Our findings highlight the need to adapt fertilization and cropping strategies to local climate and soil conditions to optimize soil structure and carbon sequestration. Future studies integrating AMF community composition and seasonal dynamics will further deepen our understanding of GRSP as a key indicator of soil health and sustainability.

CRediT authorship contribution statement

Guanmo Li: Software, Methodology, Investigation, Data curation. **Hongbo Yang:** Writing – original draft, Visualization, Validation, Conceptualization. **Qiong Xiao:** Supervision, Investigation, Formal analysis, Conceptualization. **Dong Wu:** Resources, Investigation, Data curation. **Minggang Xu:** Visualization, Validation, Supervision, Resources, Conceptualization. **Gilles Colinet:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Wenju Zhang:** Writing – review & editing, Resources, Project administration, Funding acquisition, Data curation. **Yaping Huang:** Writing – review & editing, Supervision, Conceptualization. **Xin Li:** Supervision, Software, Investigation, Data curation. **Jeroen Meersmans:** Writing – review & editing, Visualization, Validation, Supervision.

Declaration of Competing Interest

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

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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.2025.106950.

Data availability

Data will be made available on request.

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