No-tillage facilitates soil organic carbon sequestration by enhancing arbuscular mycorrhizal fungi-related soil proteins accumulation and aggregation

Hongbo Yang [a,c], Guangshuai Wang [b], Jun Wang [a], Qiong Xiao [a], Zhongyang Li [b], Caroline De Clerck [c], 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] *National Agro-ecological System Observation and Research Station of Shangqiu, Institute of Farmland Irrigation, Chinese Academy of Agricultural Sciences, Xinxiang, 453002, China*

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

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ABSTRACT

No-tillage is known to optimize soil structure and enhance soil organic carbon (SOC) stocks in cropland. However, the exact mechanisms driving the accumulation of SOC are still unclear, especially concerning the regulation of arbuscular mycorrhizal fungi (AMF) communities and diversity in SOC sequestration. Here, this study aims to elucidate the intricate relationship between AMF community, glomalin-related soil proteins (GRSP), and SOC within bulk soil and aggregates across four tillage treatments (i.e. FA, fallow; RT, rotary tillage; DT, deep tillage; NT, no-tillage) based on a 7-year tillage experiment. Results showed that the contents of SOC and GRSP were significantly higher by 1.14–1.46 mg/g and 0.43–0.72 mg/g in the bulk soil under NT relative to RT and DT, respectively. The contribution of GRSP-C to SOC under NT was also higher than RT and DT, especially in > 53 μm particle size. Additionally, NT increased AMF diversity and the abundance of *glomerales* and *diversisporales*, all showing a strong positive correlation with GRSP ($p < 0.05$), indicating their potential regulatory role in GRSP production. The positive correlations between GRSP and the mass percentage of the > 53 μm particle size fraction ($R^2 = 0.74$; $p < 0.01$) and MWD ($R^2 = 0.63$; $p < 0.01$) suggested that no-tillage may drive large aggregates (>53 μm) formation and enhance aggregate stability through GRSP levels. Overall, increased AMF diversity and keystone taxa abundance at the order level via no-tillage promoted SOC accumulation through the production of GRSP and the protection of large aggregates. This study highlights that no-tillage is an effective and sustainable soil management strategy for enhancing soil quality in agricultural ecosystems.

1. Introduction

Soil organic carbon (SOC) is the largest carbon (C) reservoir in the terrestrial ecosystems (Schlesinger, 1990). Even relatively small changes in SOC directly impact atmospheric CO_2 concentrations and the global C cycle (Sakschewski et al., 2016). Hence, in theory, improving SOC storage can be an effective measure to combat climate change. Notably, human activities like fertilization and tillage significantly impact SOC storage in cropland soil (Mazzoncini et al., 2011; Smith et al., 2005). Conservation tillage, especially no-tillage, is recognized for reducing soil disturbance and erosion, maintaining soil structure, and enhancing the accumulation of SOC in the upper 20 cm depth (Choudhary et al., 2013). Compared to conventional tillage, no-tillage promotes SOC accumulation in topsoil (0–20 cm) through physical protection of large aggregates (Six et al., 2000) and association with soil minerals (Kan et al., 2022). No-tillage also maintains stable soil environment and microbial activity (Jiang et al., 2011), thereby impacting the distribution and stability of SOC in aggregates (Kan et al., 2022). Consequently, it is crucial to elucidate how tillage practices influence SOC dynamics at the aggregate scale.

A key factor affecting SOC is glomalin-related soil proteins (GRSP), mainly derived from arbuscular mycorrhizal fungi (AMF) (Rillig, 2004; Wright et al., 1998). GRSP can enhance SOC stability due to aromatic C structure (Rillig et al., 2001; Yang et al., 2024). Meanwhile, the adhesive properties of GRSP also promote the formation and stability of large aggregates (Wright and Upadhyaya, 1998), thereby providing physical protection to SOC (Guo et al., 2020). Previous studies have explored the impacts of tillage practices on GRSP accumulation, but the findings have been inconsistent (Agnihotri et al., 2022; Helgason et al., 2010). For example, Wright et al. (2007) reported significantly higher GRSP content in untilled bulk soils and large aggregates compared to tilled soils in Ultisols, while Curaqueo et al. (2011) found no increase in GRSP content after ten years of no-tillage compared to conventional tillage in a thermic Entic Haploxeroll (sandy clay alluvial soil). These contradictions may arise from differences in soil types and climate effects, causing changes in the complexity and diversity of AMF network (Yang et al., 2022), as well as their interaction with the surrounding soil environment (pH and nutrients) (Avio et al., 2013; Zhang et al., 2017). Further research on the effect of tillage on GRSP is needed. Furthermore, different aggregate sizes and pores provide distinct habitats for microorganisms. This can lead to varied distributions of AMF within the soil microstructure (Lehmann et al., 2017), thereby influencing GRSP levels. Therefore, deeper understanding of the distribution of GRSP within aggregates is crucial for the accumulation of SOC under tillage practices.

In addition, compositions of AMF communities significantly influence the decomposition, resynthesis, transformation, and stabilization of SOC (Wu et al., 2023). The role of AMF in promoting SOC sequestration within aggregates shows highly variable results, with positive (Frey, 2019) and negative effects (Cheng et al., 2012) reported. Differences in life-history strategies between *glomerales* and *diversisporales* within AMF are notable, with *glomerales* orders demonstrating a higher *r*-strategy, and *diversisporales* orders leaning towards a more *K*-strategy (Hart and Reader, 2002; Sýkorová et al., 2007). Promoting specific AMF orders may enhance C sequestration strategies. It is noteworthy that the impacts of conventional tillage on AMF communities and diversity are inconsistent (Brito et al., 2012), possibly due to varying responses of AMF taxonomic groups to tillage practices concerning their activities and functions (Kabir, 2005). Further study is needed to understand these dynamics. Given the interrelation between the communities and diversity of AMF and GRSP accumulation (Yang et al., 2024), elucidating how different tillage practices affect AMF diversity and keystone taxa richness is crucial for deciphering their roles in regulating SOC storage.

The fluvo-aquic soil (Fluvents, USDA), formed by the alluvial sediments from the Yellow River in China, faces issues such as light texture, poor soil structure, low SOC content, and high pH (He et al., 2021). Traditional tillage practices exacerbate soil erosion and C loss in this region, making it a focal area for conservation tillage research. As such the objectives of this study was to explore the response of SOC, GRSP accumulation and shifts in AMF communities to tillage practices. Our hypotheses were that (i) no-tillage would increase the content of SOC and GRSP in topsoil (0–20 cm), along with the contribution of GRSP to SOC within large aggregates; (ii) no-tillage may promote an increase in AMF diversity, biomass and the abundance of taxa (*glomerales* and/or *diversisporales*) contributing to GRSP production.

2. Materials and methods

2.1. Site description

A long-term field experiment was launched on the basis of a cropland soil in Shangqiu city, Henan Province, China (34°31′13″ N, 115°35′30″ E) in October 2012. The site is located in a sub-humid, warm temperate monsoon climate. The annual agrometeorological data show averages of 708 mm for precipitation, 13.9 °C for temperature, and 1735 mm for evapotranspiration. The parent material of the soil is alluvial sediments from the Yellow River and the soil texture is fluvo-aquic soils (i.e. clay

14.3 %, silt 40.8 %, sand 44.9 %, classified as Fluvents in the USDA taxonomy). The soil in top 30 cm layer contains 5.35 g kg^{-1} SOC, 0.86 g kg^{-1} total nitrogen (TN), 157 mg kg^{-1} available nitrogen (AN) and 11 mg kg^{-1} available phosphorus (AP) and 8.7 soil pH. High groundwater table and salinity, combined with low annual precipitation and high evaporation rates, cause upward salt migration and carbonate accumulation, raising soil pH. The main cropping system is winter wheat-summer maize rotations. Winter wheat is sown in mid-to-late October with a seeding rate of approximately 225 kg ha^{-1}; summer maize was planted in early June with a seeding rate of 37.5 kg ha^{-1}, resulting in a density of 75,000 plants ha^{-1} when five leaves have been formed.

2.2. Experimental design

The experiment included four treatments: rotary tillage to a depth of 10–15 cm (RT), deep tillage to a depth of 30–35 cm (DT), no-tillage (NT), and fallow (FA, undisturbed soil). For ease of mechanical operations, the three replicates of the tillage treatments are combined into one continuous plot. The plot area for the RT, DT and NT treatments is 12 m × 75 m. The FA treatment is located in a nearby plot that had been cropland before 2007, and has remained in natural restoration of weeds and/or small shrubs since the experiment was established, with no fertilization, planting and tillage (Fig. S1). Maize was continuously planted after the previous crop in the RT, DT, and NT plots. The straw residue from both wheat and maize has been returned to the field for full coverage. Compound fertilizer has been used, with 375 kg ha^{-1} (N:P$_2$O$_5$: K$_2$O=25:18:7) applied before planting, and 375 kg ha^{-1} (30:5:5) applied 35–45 days after planting. For winter wheat, 375 kg ha^{-1} (25:18:7) is applied before planting, followed by 375 kg ha^{-1} (30:5:5) during the regreening stage (135–150 days after planting).

2.3. Soil sampling and soil physicochemical properties analysis

To ensure comparability and consistency with other studies and to account for the majority of impacts from both different tillage depths, soil samples from the top 20 cm were collected using a soil auger with a 38 mm diameter after crop harvest in October 2019. Each treatment included three replicates, with each replicate consisting of a composite sample from four points (Fig. S1). One portion of the soil was air-dried, while the other fresh soil was stored in a −80 °C refrigerator. The physicochemical properties of air-dried soil were determined. SOC in bulk soil and aggregates were determined using the sulfuric acid––potassium dichromate oxidation method, SOC content was corrected by factor of 1.3 (Walkley and Black, 1934). Determination of TN in bulk soil was made using the Kjeldahl method for nitrogen digestion and distillation. TP and AP in bulk soil were measured using molybdenum-antimony resistance colorimetric method. The soil pH was measured at a soil-to-water ratio of 1:2.5 (FE30, Mettler Toledo).

2.4. GRSP determination

The method from Wright et al. (1998) was used to determine GRSP. In brief, for every 0.25 g of air-dried bulk soil/aggregates, 2 mL of 50 mmol/L sodium citrate (pH=8.0) was used to extract the total GRSP. The extracts underwent a 1-hour autoclaving process at 121℃ and a 6-min 10,000 × g centrifugation. Each sample experienced this procedure four times, following which the supernatants were mixed and centrifuged once more in order to prepare them for measurement. Using bovine serum albumin (BSA) as the standard, the optical density (OD) value of the GRSP was measured at 595 nm using a microplate reader (Biotek Synergy H1, USA) after two to three minutes of color reaction (Liu et al., 2021).

The functional groups of GRSP were measured using the KBr pellet method (Schindler et al., 2007). We calculated the proportions of these functional groups semi-quantitatively using the peak area integration

method. For detailed steps of GRSP determination and purification, as well as the testing procedures and the corresponding peak wave numbers of the functional groups, please refer to Yang et al. (2024).

2.5. AMF biomass and diversity measurements

AMF biomass was quantified using C16:1ω5 phospholipid fatty acids (PLFAs) (Olsson, 1999). 2 g of the freeze-dried fresh bulk soil samples were used to measure PLFA. Detailed determination procedures were described in Yang et al. (2024). The AMF community was measured in 0.5 g of soil stored at − 80℃. Soil DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals). Nested PCR was employed to amplify the target fragment. For the first round of PCR, AML1F (5′-ATCAACTTTCGATGGTAGGATAGA-3′)-AML2R (5′GAACCCA AACACTTTGGTTTCC-3′) primers were utilized, while AMV4.5NF (5′-AAGCTCGTAGTTGAATTTCG-3′)-AMDGR (5′CCCAACTATCCCTATTAA TCAT-3′) primers were used for the second round of PCR (Van Geel et al., 2014). AMF α-diversity (including the Shannon-Wiener index and Chao1 index) was calculated using the Magigene platform (http://cloud.magig ene.com).

2.6. Determination of soil water-stable aggregates

The fractionation technique suggested by Cambardella and Elliott (1993) was used to separate the soil aggregates into four particle size classes (i.e. < 53 μm, 53–250 μm, 250–2000 μm, and > 2000 μm). Mean weight diameter (MWD) can be used to characterize the stability of aggregates. The MWD was calculated as:

$$MWD = \sum_{i=1}^{n} x_i \times m_i \qquad (1)$$

Where m_i represents the mass percentage of aggregate remaining on the ith sieve, x_i is the mean diameter of particle size fraction and n is the number of fractions.

2.7. Statistical analysis

The data were examined for homogeneity and normality using Kolmogorov-Smirnov test. Duncan's test was used to evaluate the effect of tillage practices on the dependent variables SOC, GRSP, AMF, MWD. The relationships between GRSP content and SOC, keystone species of AMF, and MWD were evaluated using Pearson's correlation coefficient. Analysis of Similarities (ANOSIM) was performed to evaluate the significance of differences in the composition of AMF communities. Random forest (RF) was used to analyze the importance of different species on GRSP accumulation. RF, non-metric multidimensional scaling (NMDS), and chord diagram were all performed using the statistical software R v.4.3.0.

3. Results

3.1. Soil properties

The different tillage practices exhibited significant differences in physicochemical properties ($p < 0.05$, Table 1). The pH value was significantly lower in the NT treatment compared with the DT, RT, and FA treatments. TP and AP showed no significant differences among the RT, DT, and NT treatments. TN is significantly higher under the NT treatment than under the DT and FA treatments. However, tillage practices (RT, DT, NT) resulted in higher TP, AP contents compared to the FA treatment, with increases of 26–38 %, and 270–331 %, respectively.

3.2. SOC and GRSP content in aggregates and their relationship

Tillage strongly affected the content of SOC in bulk soil and

Table 1

Soil physicochemical properties under tillage practices (FA: fallow; RT: rotary tillage; DT: deep tillage; NT: no-tillage).

Treatments	pH	TN	TP	AP
		(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})
FA	8.18 ± 0.06 a	1.07 ± 0.13b	0.77 ± 0.04b	2.50 ± 0.60b
RT	7.88 ± 0.11b	1.21 ± 0.03 ab	1.06 ± 0.04 a	9.24 ± 1.50 a
DT	7.80 ± 0.02b	1.10 ± 0.06b	0.97 ± 0.05 a	9.61 ± 0.16 a
NT	7.44 ± 0.07c	1.32 ± 0.00 a	1.02 ± 0.05 a	10.77 ± 1.06 a

Notes: TN, total nitrogen; TP: total phosphorus; AP, available phosphorus; Values represents the means with standard errors (n = 3). Different letters indicate significant differences among tillage regimes for each parameter using Duncan's test (p < 0.05).

aggregates ($p < 0.05$, Fig. 1a-c). In the bulk soil, the SOC content was significantly higher in the NT treatment than in the DT, RT, and FA treatments, i.e. 1.14, 1.46, and 2.24 mg/g, respectively ($p < 0.05$, Fig. 1a). In aggregates, SOC contents in > 53 μm particle size were 0.64, 1.00 mg/g higher in the NT treatment than in the RT and DT treatments, respectively ($p < 0.05$, Fig. 1b). However, SOC contents in < 53 μm particle size were lower in the NT treatment than in the RT and DT treatments ($p < 0.05$, Fig. 1c). NT treatment significantly increased the SOC content in > 53 μm particle size, but had no significant effect on SOC content in < 53 μm particle size as compared to the FA treatment. (Fig. 1b-c).

GRSP content in bulk soil was significantly higher in the NT treatment as compared to the RT, DT, and FA treatments, i.e. 0.43, 0.72, and 0.54 mg/g respectively ($p < 0.05$, Fig. 1d). Additionally, in aggregates, the GRSP content in > 53 μm particle size was significantly higher under the NT treatment than that under the RT and DT treatments ($p < 0.05$, Fig. 1e). However, GRSP in < 53 μm particle size was higher under the RT and DT treatments than that under the NT treatment ($p < 0.05$, Fig. 1f). Compared with the FA treatment, GRSP was significantly lower in the DT treatment and higher in the NT treatment ($p < 0.05$, Fig. 1e). In addition, there was no significant influence of tillage practices on the concentration of C in GRSP, so calculating the average resulted in a GRSP-C content of 31 % (Fig. S2). The ratio of GRSP-C in SOC under the NT treatment was significantly higher than that under the RT and DT treatments in bulk soil and > 53 μm particle size ($p < 0.05$, Fig. g, h). However, there was no significant difference in < 53 μm particle between NT and other treatments (RT, DT, FA) (Fig. 1i).

Regression analysis showed that SOC content in bulk soil increased linearly with an increase in GRSP content ($p < 0.05$, Fig. 1j). Specifically, GRSP had a positive relationship with SOC in > 53 μm particle size fraction ($p < 0.01$, Fig. 1k), but a negative correlation with SOC in < 53 μm particle size fraction ($p < 0.05$, Fig. 1l).

Our analysis identified four functional groups: polysaccharides, aliphatic C, aromatic C, and hydrocarbons (Fig. 2a). Tillage also changed GRSP functional groups. Under the NT treatment, the proportion of polysaccharides was significantly higher, while hydrocarbons were lower compared to the DT and/or FA treatments (p < 0.05, Fig. 2b). There was no significant difference in the proportion of aromatic and aliphatic C between NT, DT, and RT treatments. However, the FA treatment significantly increased the aromatic C proportion as compared to the DT, RT, and NT treatments (p < 0.05, Fig. 2b).

3.3. AMF community composition, diversity, keystone taxa abundances and their relationship with GRSP

NMDS analysis showed that the DT, RT, and NT treatments significantly altered the AMF community structure compared to the FA treatment ($p < 0.05$, Fig. 3a). However, the changes in AMF communities among RT, DT, and NT treatments were not significant (Fig. 3a). There was no significant difference in the biomass of AMF (PLFA 16:1ω5) among different tillage treatments (Fig. 3b), but AMF diversity,
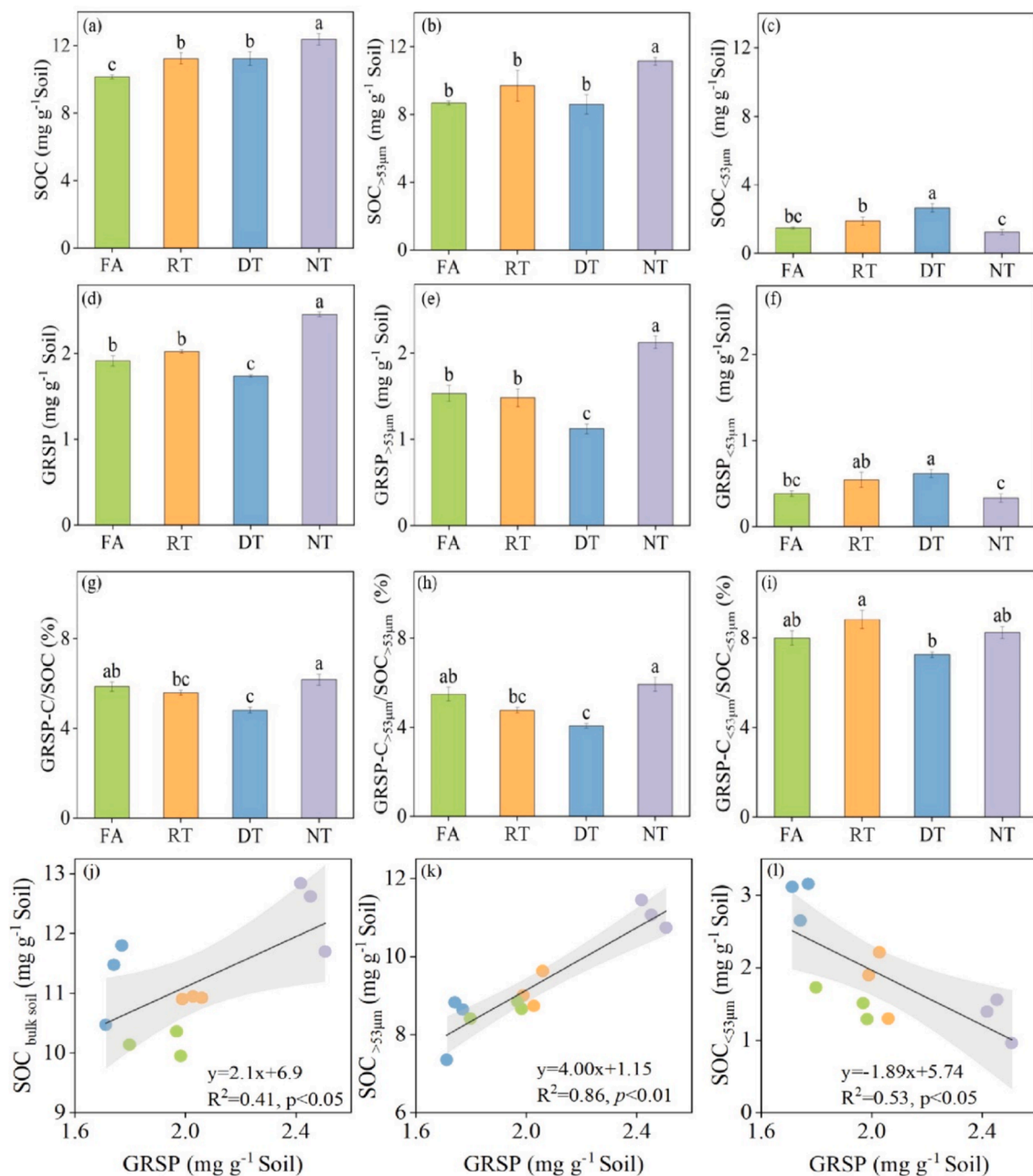
Fig. 1. The concentrations of SOC (a), SOC $_{>53\mu m}$ (b), SOC $_{<53\mu m}$ (c), GRSP in bulk soil (d), GRSP $_{>53\mu m}$ (e), GRSP$_{<53\mu m}$ (f), contribution of GRSP-C in bulk soil to SOC (g), contribution of GRSP-C $_{>53\mu m}$ to SOC$_{>53\mu m}$ (h), contribution of GRSP-C$_{<53\mu m}$ to SOC$_{<53\mu m}$ (i), and the relationship between SOC in bulk soil (j), >53 μm (k), <53 μm (l) and GRSP under tillage practices (FA: fallow; RT: rotary tillage; DT: deep tillage; NT: no-tillage). SOC: soil organic C; GRSP-C: C contents in glomalin-related soil proteins. Data represents the means with standard errors (n = 3). Different lowercase letters indicate significant differences under different tillage treatments (Duncan's test, $p < 0.05$).

especially chao1 index, was significantly higher in the NT than in the RT and DT treatments ($p < 0.05$, Fig. 3d). The Venn diagram showed the number of AMF species at the order level under different tillage practices.

This study selected the top 10 species under order level based on their relative abundance. The chord diagram showed that *Glomerales* and Others dominate in the AMF community, followed by *Diversisporales* and *Spizellomycetales* species (Fig. 3e). We also observed differences in relative abundance under different tillage practices. Specifically, compared to the RT and DT treatments, the relative abundance of *Glomerales* and *Diversisporales* increased under the NT treatment by 81–134 %, 226–380 %, repectively, and found that *Glomerales* exhibited the highest and statistically significant importance on GRSP, followed by

Collembola, Diversisporales, Mortierellales (Fig. 3f). Among them, the abundances of *Glomerales* and *Diversisporales* showed significant positive correlations with GRSP ($p < 0.05$, Fig. 3g, h, S3).

3.4. Aggregate fractions, stability and their relationship with GRSP

When comparing the different treatments, the NT and FA treatments resulted in a significant increase in the mass percentage of > 2000 μm particle size fraction relative to the RT and DT treatments, and a noticeable decrease in the mass proportion of < 53 μm particle size fraction under the NT treatment ($p < 0.05$, Fig. 4a). Compared to RT treatment, NT and FA treatments exhibited higher MWD values, whereas MWD value was significantly lower under the DT treatment ($p < 0.05$,
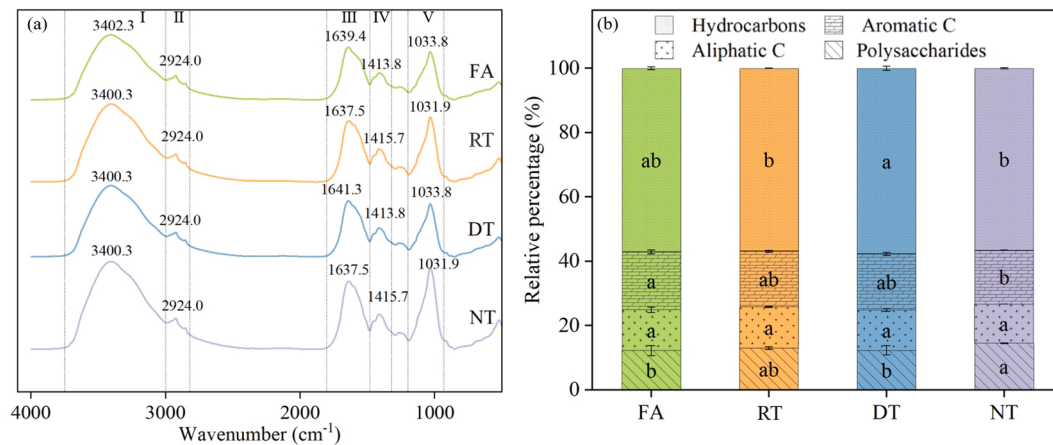
Fig. 2. Fourier transform infrared (FTIR) spectra (a) and relative percentage of compositional traits of glomalin-related soil protein (GRSP) (b) under different tillage practices (FA: fallow; RT: rotary tillage; DT: deep tillage; NT: no-tillage). Roman numerals from I to V represent hydrocarbons, aliphatic C_1, aromatic C, aliphatic C_2 and polysaccharides of GRSP components, respectively. Different lowercase letters indicate significant differences under different tillage treatments (Duncan's test, $p < 0.05$).

Fig. 4c). GRSP was significantly positively correlated with MWD value and mass percentage of > 53 μm aggregates ($p < 0.01$, Fig. 4b, d).

4. Discussion

4.1. Effects of tillage practices on GRSP and SOC sequestration

We found that long-term (7-year) no-tillage significantly increased SOC and GRSP contents compared to the FA treatment (12-year) and conventional tillage (RT, DT) practices (Fig. 1a, d), which is in line with our first hypothesis (Fig. 5). Similarly, no-tillage also enhanced SOC and GRSP within in > 53 μm aggregates compared to the FA treatment and conventional tillage (Fig. 1b, e), indicating that changes in SOC and GRSP contents were primarily driven by alterations in large aggregates. These results are consistent with previous research findings (Singh et al., 2016; Wright et al., 2007; Zhang et al., 2012). Soil aggregates are considered as one of the primary mechanisms governing the sequestration of SOC (Six et al., 2004). Reducing soil disturbance helps large aggregates formation and physically enclose free C, such as polysaccharides (Fig. 2), inducing an increase in labile C by minimizing contact with decomposing microbes, and as such facilitates SOC accumulation (Chenu et al., 2019; Six et al., 2000). However, conventional tillage practices, like DT or RT treatments, resulted in higher SOC concentrations in < 53 μm particle size fractions (Fig. 1c, f). It might be that tillage disrupted large aggregate, leading to an increase in the proportion of small aggregates, and a small part of the exposed free C was adsorbed onto the surface of mineral particles by cation bridging (Franzluebbers et al., 1996), resulting in a longer residence time for SOC (Rabbi et al., 2014). GRSP and SOC contents under the FA treatment were lower than under the NT treatment but higher than under the DT treatment in large aggregates (>53 μm) (Fig. 1b, e), while no significant difference was observed in small aggregates (<53 μm) (Fig. 1c, f). This discrepancy might be attributed to external nutrient (fertilization) and organic matter (straw returning) input under the NT treatment in field soils, increasing the activity of roots and AMF, thereby facilitating GRSP and SOC accumulation in large aggregates (Yang et al., 2024). However, DT greatly damaged the fungal hyphae. This damage offsets any positive effects of the nutrients on AMF, thus inhibiting GRSP production. Notably, the positive correlation between GRSP and SOC in bulk soil and > 53 μm particle size suggested that GRSP and SOC have a similar accumulation pattern (Fig. 1j, k). Because GRSP with aromatic structure can be used as a recalcitrant component in the SOC pool. In addition, according to the hierarchical model, both GRSP and SOC were binding agents that firmly hold primary particles together to form

microaggregates, which amalgamate to form large aggregates (Oades, 1984; Wright et al., 1998). In turn, large aggregates can provide physical protection to slow down the decomposition of organic matter.

Our study found that no-tillage increased GRSP-C/SOC in bulk soil which was mainly reflected in > 53 μm particle size, implying a faster accumulation rate of GRSP relative to SOC in > 53 μm particle size (Fig. 1g, h). The possible reason is that no-tillage preserves the structure and integrity of the soil, which supports the hyphal networks and activity of AMF (Kabir, 2005). This preservation likely enhances the production and accumulation of GRSP. However, SOC might be more influenced by diverse microbial communities (composed by microorganisms as saprophytic fungi and bacteria), whose growth rates is less influenced by tillage practices (Van Groenigen et al., 2010). This was also supported by our results of microbial total phospholipid fatty acid (PLFA) biomass under different tillage practices (Fig. S5). In < 53 μm particle sizes, the contribution of GRSP-C to SOC remained unaffected by different tillage treatments (Fig. 1i), indicating the stability in the organo-mineral complexes formed by organic matter and clay, which are less susceptible to microbial attack (Nyamadzawo et al., 2009).

Previous studies have highlighted the positive linear correlation between GRSP and SOC, which in turn suggested similar relationships with various soil physicochemical properties (e.g. TN, TP) (Singh et al., 2016). Our study found that no-tillage reduced soil pH by 0.36 to 0.74 units (Table 1), which was negatively correlated with SOC and GRSP content (Fig. S4). These results may be due to low precipitation (708 mm) and high evaporation (1735 mm) amounts at the study site by which salts migrate upwards with the water, causing carbonates to accumulate near the soil surface, and creating an alkaline soil environment. Typically, the AMF sporulation is inhibited in alkaline soil (Isobe et al., 2007). No-tillage benefits the secretion of organic acids by roots and other microorganisms (Dakora and Phillips, 2002), improving the adaptation (pH ranging from 4.5 to 7.5) of AMF to the soil environment by lowering pH (Bücking and Kafle, 2015), thereby favoring the production of GRSP. Tillage practices had no significant effect on nutrient levels (TP, AP) (Table 1), likely due to consistent fertilization amounts. Thus, pH, TN, AP, and related factors may directly or indirectly affect residue and root inputs, as well as the activity of AMF, ultimately influencing GRSP and SOC accumulation.

4.2. Effects of tillage practices on AMF community, diversity and soil aggregation

Our results showed no significant differences in AMF biomass among various tillage systems (Fig. 3b), which contrasted with the findings of
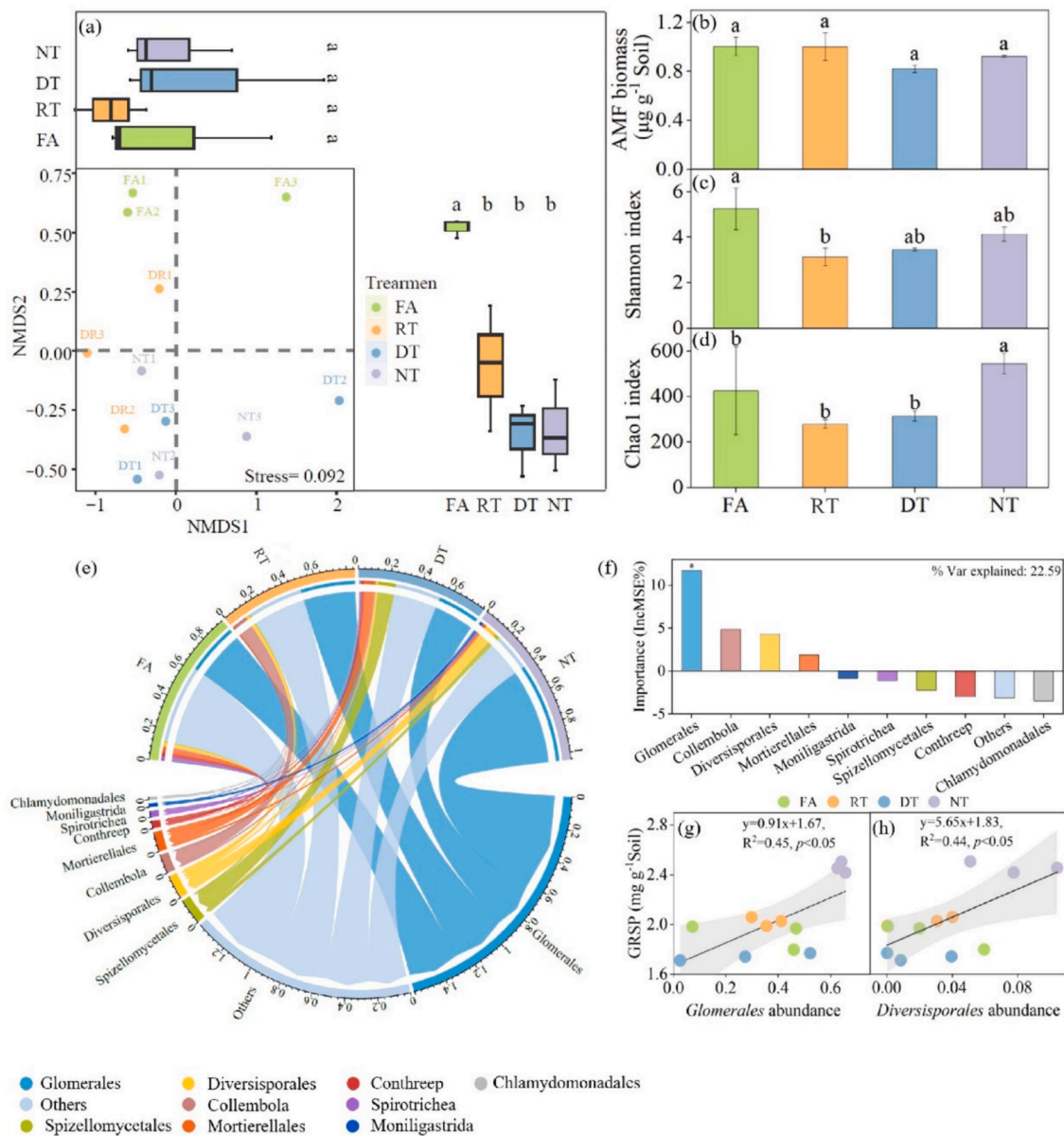
Fig. 3. Ordination plots for non-metric multidimensional scaling (NMDS) based on operational taxonomic units (OTUs), box plots represent the differences between different tillage practices (a). The biomass (b), Shannon index (c) and Chao 1 index (d) of arbuscular mycorrhizal fungi (AMF) under tillage practices. Chord diagram illustrated the distribution of arbuscular mycorrhizal fungi (AMF) order level of each tillage practices (e). The bar chart displayed the relative importance of different taxa at the order level to glomalin related soil proteins (GRSP) (f). The correlation between GRSP and the abundances of keystone taxa (*Glomerales* (g), *Diversisporales* (h)), respectively. Different lowercase letters indicate significant differences under tillage practices (FA: fallow; RT: rotary tillage; DT: deep tillage; NT: no-tillage). Data represents the means with standard errors (n = 3). Different lowercase letters indicate significant differences under different tillage treatments (Duncan's test, $p < 0.05$).

Agnihotri et al. (2022), who reported that no-till strongly increased AMF biomass. This observation might be attributed to the crop rotation or the application of organic matter that help maintain the biomass of AMF, possibly making it less susceptible to significant impacts from tillage practices (Thomopoulos et al., 2023). Therefore, further research and investigation will be needed to more fully explain the relationship between AMF biomass and GRSP. However, we discovered that tillage altered AMF community composition (Fig. 3a). Compared to conventional tillage (DT, RT), no-tillage increased AMF diversity, particularly the Chao1 index (Fig. 3d), which was positively correlated with GRSP (Fig. S4). This result is consistent with other studies (Brito et al., 2012; de Pontes et al., 2017), which found that reduced or minimal soil disturbance promoted AMF growth and reproduction, fostering a richer community (Kabir, 2005). The reason may be that soil pH positively

affects AMF spore germination and hyphal growth, impacting the diversity and structure of AMF communities (Sun et al., 2016). In fact, the soils are alkaline in this study, and some species could have disappeared due to the alkaline environment (Xu et al., 2016). Therefore, reducing pH through no-tillage can effectively maintain or increase AMF diversity and GRSP content. We further screened the taxa at order levels, revealing that no-tillage increased the abundance of *Glomerales* and *Diversisporales* species in AMF (Fig. 3e). This phenomenon could be explained by the fact that *Glomerales* might thrive in a relatively undisturbed soil environment, appearing more apt to form a closer symbiotic relationship with plant roots (Banerjee et al., 2019). Additionally, *Glomerales* and *Diversisporales* were more important orders involved in GRSP accumulation (Fig. 3f), showing a positive correlation with GRSP (Fig. 3g, h). This indicated that the greater presence of *Glomerales* and
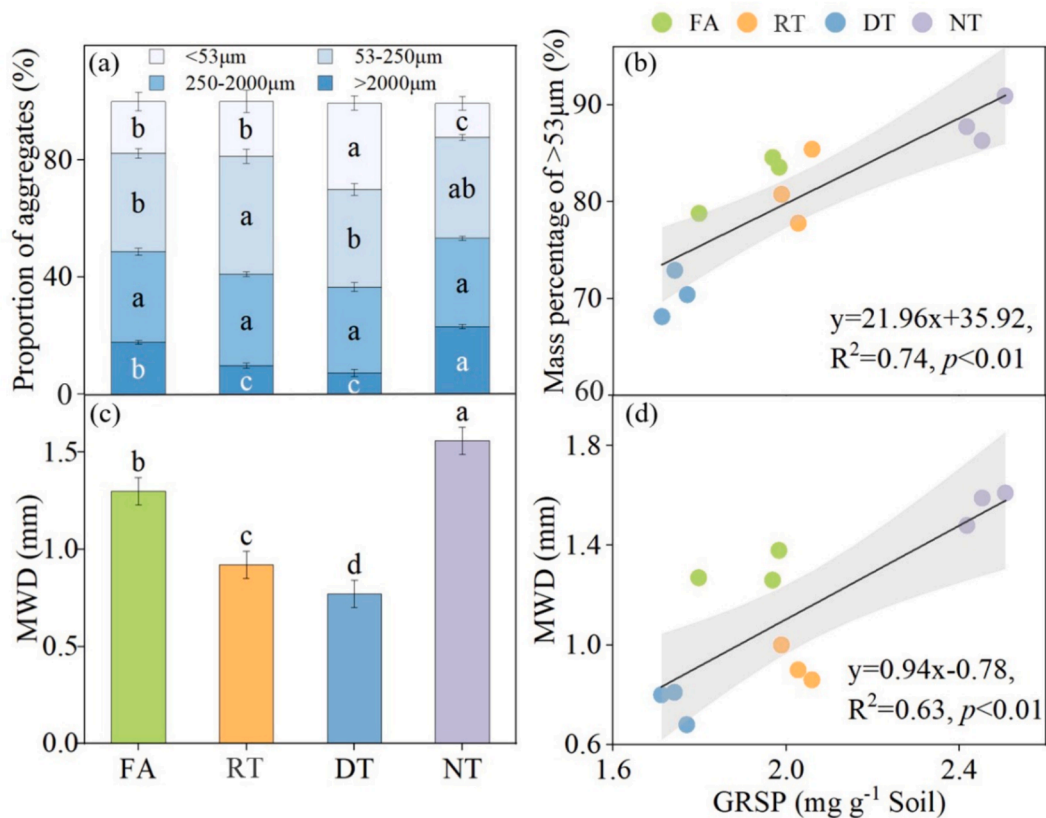
Fig. 4. The bar chart showed the mass percentage of aggregates (a) and the mean weight diameter (MWD) values (b) under tillage practices (FA: fallow; RT: rotary tillage; DT: deep tillage; NT: no-tillage). The correlation between glomalin related soil proteins (GRSP) and the mean weight diameter (MWD) (a), mass percentage of > 53 μm particle size fraction. Data represents the means with standard errors (n = 3). Different lowercase letters indicate significant differences under different tillage treatments (Duncan's test, $p < 0.05$).

Diversisporales under no-tillage practices might explain the higher soil content in GRSP, which confirms our second hypothesis (Fig. 5). Some studies have also supported this view. For example, Magurno et al. (2019) identified specific gene sequences in AMF species, such as those in the genus Glomus, that are responsible for GRSP production. Furthermore, Wang et al. (2023) demonstrated that GRSP is positively correlated with the *Glomeraceae* family, highlighting their crucial role in GRSP accumulation. Overall, the positive impact of no-tillage on GRSP accumulation primarily stems from the diversity and keystone species abundance rather than biomass of AMF (Fig. 5).

The stability of soil aggregates influences its capacity to withstand external environmental changes and maintain a stable state (Oades, 1984). In this study, tillage practices significantly affect the stability of soil aggregates (MWD) (Fig. 4c). No-tillage showed an increase in the percentage of large aggregates (>250 μm) and a decrease in the percentage of small aggregates (<53 μm) (Fig. 4a), suggesting no-tillage induced the transfer of small aggregates to large aggregates. This shift can be attributed to reduced mechanical disturbance, increased available organic substrates, and improved microenvironment under no-tillage, favoring the formation of fungal hyphal networks (Strickland and Rousk, 2010). These networks intertwined soil particles, facilitating the formation and stability of macroaggregates (Peng et al., 2013). Furthermore, SOC and GRSP act as binding agents in stabilizing soil aggregates (Bronick and Lal, 2005; Six et al., 2004). Therefore, the increase in AMF and GRSP in undisturbed soil may be the result of soil aggregate stability mediated by traditional agricultural practices. However, contrary to prior research, our study did not observe a significant positive relationship between aggregate stability (MWD) and SOC (Fig. S4). This being said, some other studies support our findings (Spohn and Giani, 2010; Zhang et al., 2012). Correspondingly, a strong

positive correlation was found between GRSP and aggregate stability (MWD) and the mass percentage of large aggregates ($p < 0.01$, Fig. 4b, d), indicating that GRSP could be a robust predictor of aggregate stability, while the influence of exogenous organic matter input often weakens the relationship between SOC and aggregates.

4.3. Implications of tillage practices on soil structure and quality

By demonstrating the role of tillage practices in modulating AMF diversity and GRSP accumulation, our research highlights a critical pathway through which agricultural practices can influence soil structure and quality. This study is important in its detailed examination of the synergistic effects of reduced physical disturbance on both microbial diversity and GRSP dynamics. Therefore, strategic tillage practices (no-tillage and conservation tillage) can enhance SOC sequestration, thereby contributing to agricultural sustainability and resilience.

In the future, we need to highlight the synergistic impact of tillage practices with other agricultural management practices to improve our understanding of SOC sequestration and agricultural sustainability. Some studies have suggested that tillage practices may not significantly alter microbial abundance, diversity, and SOC levels (Yin et al., 2017), possibly because other factors such as cover crop rotations potentially weaken tillage effects (Somenahally et al., 2018). In addition, Angers et al. (1997) indicated that in the surface soil (0–10 cm depth), C and N contents were higher under no-tillage compared to moldboard plowing, whereas the reverse was true at deeper levels (>20 cm depth). Because the AMF biomass and community composition in the subsoil (20–40 cm) differ significantly from those in the topsoil (0–20 cm) (Luo et al., 2021; Oehl et al., 2005) due to limitations in oxygen and/or nutrients (Zhang et al., 2018). Therefore, we need to explore the differences in SOC
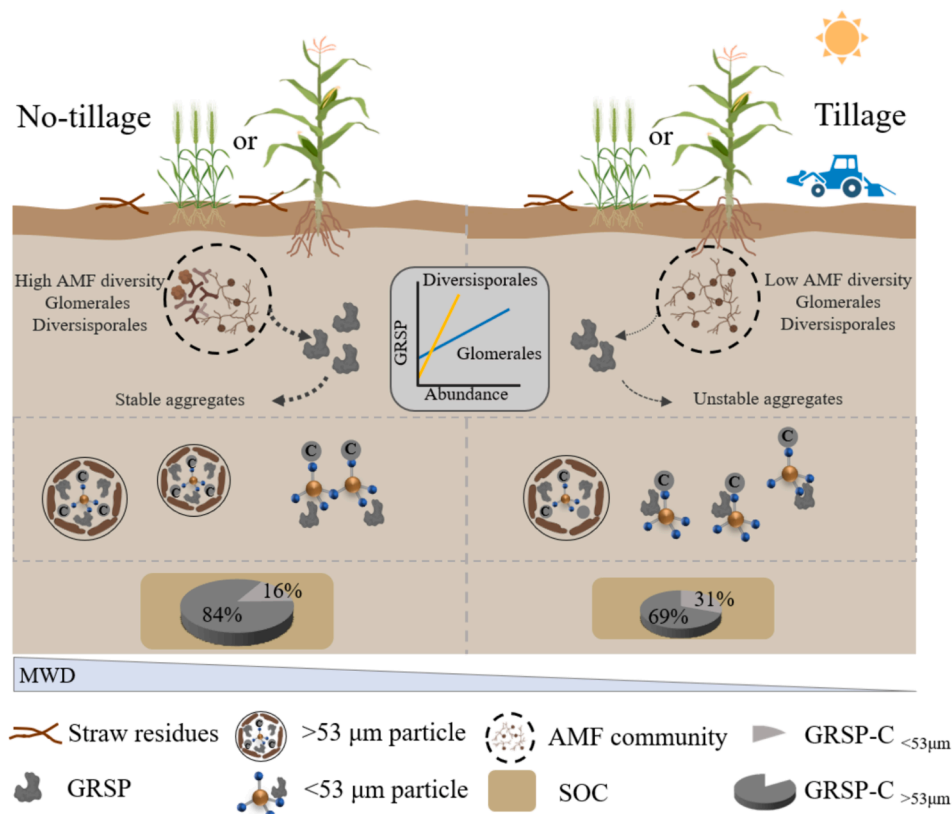
Fig. 5. Schematic illustration of the impact of tillage on glomalin related soil proteins (GRSP) and soil organic carbon (SOC) in aggregates through the regulation of arbuscular mycorrhizal fungi (AMF) community and diversity.

sequestration at different depths.

In addition, given the mechanisms by which AMF regulates SOC accumulation may be different in various study sites. Future research should build on these findings by exploring large-scale and long-term effects of different tillage practices on AMF diversity and SOC sequestration across various soil types and climatic conditions. It is crucial to investigate the specific roles of different AMF taxa in GRSP production and SOC stabilization, as well as the potential interactions between AMF and other soil microorganisms under varying tillage practices. By addressing these areas, we can develop more effective strategies for optimizing SOC storage and improving soil health in agricultural landscapes.

5. Conclusion

Our study revealed that AMF-mediated soil C sequestration mechanisms under different tillage practices. No-tillage significantly enhances the accumulation of SOC and GRSP, as well as the contribution of GRSP to SOC, especially in > 53 μm particle size. This effect is primarily attributed to the influence of *glomerales* and *diversisporales* of AMF and aggregate stability. Specifically, (i) no-tillage increased AMF diversity and keystone orders abundance of *glomerales* and *diversisporales*, which can regulate the production of GRSP(C). (ii) No-tillage also increases the physical protection of SOC through GRSP-regulated large aggregates formation and soil stability. Overall, no-tillage promotes GRSP accumulation along with large aggregates formation by enhancing AMF diversity, thus showing potential for enhancing SOC sequestration and soil stability, warranting further investigation into its long-term implications for soil health and agricultural ecosystem sustainability.

CRediT authorship contribution statement

Hongbo Yang: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Guangshuai Wang:** Supervision, Methodology, Data curation. **Jun Wang:** Writing – review & editing, Validation, Supervision. **Qiong Xiao:** Writing – review & editing, Validation, Supervision. **Zhongyang Li:** Supervision, Project administration, Methodology, Data curation. **Caroline De Clerck:** Writing – review & editing, Supervision, Conceptualization. **Jeroen Meersmans:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Gilles Colinet:** Writing – review & editing, Validation, Supervision, Conceptualization. **Wenju Zhang:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2024.108323.

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