

Plants and sludge synergistically improve soil structure and immobilize heavy metal in rare earth tailings soil

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Abstract

Aims Restoring soil structure through stable aggregate formation is essential for reclaiming soils on abandoned rare earth element (REE) mine sites. Sludge amendment may improve soil aggregation, but its use is constrained by potential heavy metal (HM) risks. This study examined whether plant–sludge co-application can balance soil structure improvement with HM risk mitigation. **Methods** A pot experiment was conducted to evaluate the effects of sludge amendment and seven plant configurations on soil aggregation, aggregate-associated binding agents, functional groups, enzyme activities and HM availability in a REE mine soil. **Results** Sludge amendment and plant cultivation jointly promoted macroaggregate formation and aggregate stability. Among all treatments, sludge combined with *Heptapleurum arboricola* (S-H) was most effective, increasing water-stable macroaggregates by 30.72% and reducing aggregate destruction by 82.70% compared with the unamended control. High-aggregation treatments showed greater accumulation of soil organic matter, polysaccharides and glomalin-related soil protein, together with enrichment of O–H functional groups. Mantel tests and random forest analysis indicated that carbon fractions were the dominant drivers of aggregate stability, followed by organic binding agents and enzyme activities. Although sludge amendment alone increased total and DTPA-extractable Cu, Zn and Ni, plant cultivation reduced metal availability and alleviated the associated risk. Comprehensive evaluation identified S-H as the optimal treatment. **Conclusion** Co-applying sludge with suitable plant species can enhance soil aggregation while mitigating HM risks, providing a feasible strategy for REE mine soil restoration.

1. Introduction

The intensive, long-term exploitation of rare earth resources has resulted in vast tracts of derelict mine lands, characterized by severe ecological degradation (Zhou et al. 2025; Luo et al. 2025). The mining process, which involves stripping topsoil and vegetation, creates soils with a hostile suite of characteristics, such as severe structural degradation, nutrient deficiencies, low water-holding capacity, acidic conditions, and an accumulation of potentially toxic metals (Han et al. 2025; Liu et al. 2025; Peco et al. 2021). These challenging conditions, coupled with low organic matter content and weak microbial activity, severely impede natural revegetation and hinder spontaneous ecological succession (Dong et al. 2025; Han et al. 2025). Among these multifaceted challenges, the degradation of soil structure is a primary barrier to land reclamation. An unstable soil structure increases susceptibility to erosion, restricts root penetration, and impairs the soil's capacity to retain water and nutrients, thus preventing the re-establishment of a healthy ecosystem (Bronick and Lal 2005; Rabot et al. 2018).

Soil aggregates are the fundamental units of soil structure, and their formation and stabilization are governed by coupled physical, chemical and biological processes (Bronick and Lal 2005; Six et al. 2004). At the biological level, plant roots can physically enmesh soil particles and promote macroaggregate formation through root penetration, root turnover and rhizosphere interactions (Gyssels et al. 2005; Oades 1993). At the chemical and biochemical levels, organic binding agents, including polysaccharides, humic substances, microbial products and root derived exudates, act as cementing materials that enhance particle adhesion and aggregate stability (Haichar et al. 2014; Lehmann et al. 2017). Recent work on reclaimed mining soils further indicates that aggregate recovery is closely linked to organic carbon restoration, mineral associated protection and microbial activity during reclamation processes (Liu et al. 2025). However, soils on abandoned REE mine sites usually suffer from severe losses of fine particles and shortages of organic cementing materials, which greatly constrain aggregate formation and lead to low macroaggregate proportions and poor aggregate stability. Therefore, introducing external binding materials remains an important strategy for improving aggregation in degraded mine soil (Amézketa 1999; Bronick and Lal 2005).

Among current approaches for improving degraded mine soils, organic amendments have received considerable attention because they can replenish organic matter, supply nutrients and improve particle cementation (Ma et al. 2024; Rodríguez-Berbel et al. 2022). Sewage sludge, which is rich in organic matter, nitrogen, phosphorus and colloidal components, has been regarded as a promising amendment with circular resource potential (Balkrishna et al. 2025; Kacprzak et al. 2017; Kominko et al. 2024). Recent studies have shown that sludge or sludge derived materials can increase soil organic carbon, enhance labile and mineral associated carbon fractions, stimulate microbial processes and improve soil physical functions (Buss et al. 2025; Simões-Mota et al. 2024). These effects are particularly important in mine soils where natural aggregation processes are strongly limited. For REE mine substrates, our previous incubation experiment also indicated that wall-breaking sludge could improve aggregate stability while maintaining relatively low heavy metal (HM) availability under an optimized sludge composition and incubation condition (Dong et al. 2025).

However, sewage sludge utilization also entails potential ecological risks. Because sludge may contain elevated concentrations of HM, soluble salts, antibiotic resistance determinants and emerging organic contaminants, excessive or poorly controlled application may cause secondary pollution and increase ecological risk (Mokni-Tlili et al. 2024; Schaap et al. 2025; Uddin et al. 2025). This issue is particularly important in mining soils that already have high background metal concentrations, where sludge inputs may further influence metal mobility, transformation and bioavailability. Organic matter can either immobilize metals through particulate organic matter, mineral associations and complexation or enhance dissolved organic matter bound metal mobility depending on amendment type, pH and metal loading (Li et al. 2024). Plant establishment may partly mitigate these risks. Plants can reduce metal bioavailability through uptake, root induced immobilization and rhizosphere regulation, while root activities can alter soil pH, organic ligand production and microbial processes that control metal behavior (Kafle et al. 2022; Zhao et al. 2022). Recent field-based phytoremediation studies in mine soil also emphasize that revegetation strategies should be evaluated jointly in terms of plant establishment, metal stabilization and ecosystem recovery (Díaz et al. 2025).

Despite these advances, most studies have focused on either the improvement of soil fertility by sewage sludge or the growth response of plants, whereas the mechanisms of aggregate formation and stabilization under combined sludge-plant interactions remain insufficiently understood, particularly in soils on abandoned REE mine sites. Different planting configurations, such as monoculture and mixed planting, may influence aggregation through differences in root architecture, rooting depth, rhizodeposition and spatial complementarity. Root traits are increasingly recognized as important drivers of plant effects on soil physical properties and ecosystem functioning (Freschet et al. 2021), but diversity effects are context dependent and may vary under nutrient limitation or metal stress (Gould et al. 2016). Previous studies have also reported that landscape or ornamental plants can participate in metal uptake and phytostabilization under sludge amended or contaminated conditions (Wu et al. 2021). However, their roles in regulating soil aggregation and metal occurrence under sewage sludge amendment remain unclear.

In this study, three plant species which perform excellent sludge adaptability and HM absorption capacity, including the tree *Neolamarckia cadamba* (Roxb.) Bosser, the shrub *Heptapleurum arboricola* Hayata, and the herb *Alocasia × mortfontanensis* André were selected as representative restoration plants (Wu et al. 2021). A controlled experiment was conducted to assess the combined effects of sewage sludge application and different planting configurations on soil aggregation and metal contamination in a REE mine soil. The study is expected to provide a scientific basis for designing multi-objective mine soil restoration plans that synergistically address soil aggregation, plant functional traits, and HM risk management.

2. Materials and Methods

2.1 Substrates

Mine soil was collected from an abandoned rare REE mine site in Meizhou City, Guangdong Province, China. Surface soil (0–20 cm) was collected, air-dried, homogenized and sieved to <3 mm before use. Municipal sewage sludge was obtained from Lvyou Sludge Treatment Plant, Qingyuan, China. The sludge amended substrate used in the pot experiment followed the optimal condition identified in our previous incubation experiment, in which treatment with 20% wall-breaking mixed sludge addition showed the highest comprehensive suitability for improving soil aggregation while maintaining low HM availability (Dong et al. 2025). The basic properties of mine soil and sludge are shown in Tables S1 and S2. Three plant species with contrasting growth forms were selected: *N. cadamba* (N), *H. arboricola* (H) and *A. × mortfontanensis* (A). Uniform seedlings were selected before transplanting to reduce the initial differences in plant size.

2.2 Experiment design and process

A pot experiment with 14 treatments was conducted as designing in Table 1. Two substrate types were established: mine soil without sludge addition (M) and sludge amended mine soil (S). The substrate in S treatment is a mixture of mine soil and wall-breaking sludge at the rate (V:V) of 4:1. Each pot contained 2.4 kg of substrate and was treated as one experimental replicate. Each treatment had at least four replicates. Pots were randomly arranged and repositioned every 20 d to minimize environmental heterogeneity. Soil moisture was maintained by daily watering with 50–100 mL water per pot according to plant growth and substrate moisture conditions.

Table 1 Experimental design of substrate types and planting patterns.

Treatment	Cultivation substrate	Planting species
M	Mine soil without sludge addition	No planting
M-N		<i>N. Cadamba</i>
M-H		<i>H. arboricola</i>
M-A		<i>A. × mortfontanensis</i>
M-N×H		<i>N. cadamba</i> mixing <i>H. arboricola</i>
M-N×A		<i>N. cadamba</i> mixing <i>A. × mortfontanensis</i>
M-H×A		<i>H. arboricola</i> mixing <i>A. × mortfontanensis</i>
S	Mine soil with sludge addition	No planting
S-N		<i>N. Cadamba</i>
S-H		<i>H. arboricola</i>
S-A		<i>Alocasia amazonica</i>
S-N×H		<i>N. cadamba</i> mixing <i>H. arboricola</i>
S-N×A		<i>N. cadamba</i> mixing <i>A. × mortfontanensis</i>
S-H×A		<i>H. arboricola</i> mixing <i>A. × mortfontanensis</i>

M, mine soil without sludge addition; S, sludge-amended mine soil; N, *Neolamarckia cadamba* (Roxb.) Bosser; H, *Heptapleurum arboricola* Hayata; A, *Alocasia × mortfontanensis* André. Paired species codes, such as N×H, N×A and H×A, indicate mixed planting of two species.

After 200 d, plants were harvested and separated into shoots and roots. Roots were washed with deionized water and stored for root morphological analysis. Then, Roots and shoots were heated at 105 °C for 30 min and then oven-dried at 65 °C to constant weight, respectively. Soil samples were collected after removing visible roots and plant residues. Fresh subsamples were stored at –20°C for enzyme analysis, and the remaining soil was air-dried for aggregate, organic matter, carbon fraction, FTIR and HM analyses.

2.3 Soil aggregate distribution and stability analysis

Air-dried soil samples were used to determine mechanically stable aggregates and water-stable aggregates following dry-sieving and wet-sieving procedures according to Dong et al. (2025). Aggregates >0.25 mm obtained by dry sieving were defined as mechanically stable macroaggregates (MSMA). Aggregates >0.25 mm obtained by wet sieving were defined as water-stable macroaggregates (WSMA). Aggregate stability was evaluated using mean

weight diameter (MWD), geometric mean diameter (GMD), fractal dimension (Dm) and percentage of aggregate destruction (PAD).

2.4 Soil organic matter, polysaccharides, and GRSP analyses

Soil organic matter (SOM) was determined using the dichromate oxidation method. Sample mass was adjusted according to the expected organic matter content of each substrate. Soil polysaccharides (SP) were determined using the phenol-sulfuric acid colorimetric method, which is widely used for quantifying sugars and polysaccharides (Dubois et al. 1956). Briefly, 0.1 g soil was extracted with deionized water, reacted with 5% phenol and concentrated sulfuric acid, heated in a boiling water bath, cooled, centrifuged and measured calorimetrically at 485 nm.

Easily extractable glomalin-related soil protein (E-GRSP) and total glomalin-related soil protein (T-GRSP) were extracted using sodium citrate buffer and quantified using the Bradford protein assay (Bradford 1976; Wright and Nichols 2002; Wright and Upadhyaya 1996). For E-GRSP, 0.25 g air-dried soil was extracted with 20 mmol L⁻¹ sodium citrate buffer at pH 7.0 by autoclaving at 121 °C for 30 min. For T-GRSP, repeated extraction was performed with 50 mmol L⁻¹ sodium citrate buffer at pH 8.0 until the supernatant became nearly colorless. The protein concentration of the extracts was determined at 595 nm using bovine serum albumin as the standard.

2.5 Fourier transform infrared spectroscopy analysis

Fourier transform infrared spectroscopy (FTIR) was used to characterize aggregate-associated functional groups. Air-dried aggregate samples were ground and passed through a 100-mesh sieve. Approximately 1 mg sample was mixed with 100 mg spectroscopic-grade KBr, ground thoroughly and pressed into a transparent pellet under 10 MPa for 30 s. FTIR spectra were recorded using a Vertex 70 Fourier transform infrared spectrometer (Bruker, Germany). Spectra were collected in the range of 4000–400 cm⁻¹ at a resolution of 4 cm⁻¹ with 32 scans. Spectra were baseline-corrected and normalized before comparison. Characteristic regions, including O-H stretching, carbonyl/aromatic groups, carboxylate/aliphatic groups, Si-O-Si stretching and fingerprint regions, were integrated to compare differences among aggregate-stability groups.

2.6 DTPA-extractable HM analysis and ecological risk assessment

Soil DTPA-extractable Cu, Zn and Ni were determined to evaluate HM availability according to the DTPA extraction method of Lindsay and Norvell (1978). Approximately 10.0 g air-dried soil was extracted with 20 mL DTPA solution containing 0.005 mol L⁻¹ DTPA, 0.01 mol L⁻¹ CaCl₂ and 0.1 mol L⁻¹ triethanolamine at pH 7.3. The suspension was shaken at 25 °C for 2 h and filtered. DTPA-Cu, DTPA-Zn and DTPA-Ni were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Leeman Prodigy 7, USA).

2.7 Statistical analysis

All data were processed using Microsoft Excel, SPSS 24.0, Origin 2026, R 4.5.0 and MATLAB 2022b. Data normality and homogeneity of variance were tested using the Shapiro-Wilk test and Levene test, respectively. One-way ANOVA followed by Duncan multiple range test was used to compare differences among treatments at $P < 0.05$. Two-way ANOVA was used to evaluate the effects of sludge application (S), planting pattern (P) and their interaction (S × P) on aggregate stability indices, carbon fractions and DTPA-extractable HM.

Pearson correlation analysis was used to evaluate relationships among aggregate stability indices. Mantel tests were conducted to assess the associations between aggregate composition and stability indices and different

explanatory variable groups using R and EcoAmp v0.17 (Mantel 1967). The explanatory variables were grouped as follows: root morphological traits (RL, RS, RV and RD), carbon fractions (SOM, LFOC, POC and MAOC), organic binding agents (SP, T-GRSP and E-GRSP), enzyme activities (BG, NAG, CBH and PPO), soil aggregate composition (MSMA, WSMA), and soil aggregate stability (MWD, GMD, Dm and PAD).

Random forest analysis was used to quantify the relative contribution of each explanatory variable group to aggregate stability, following the random forest algorithm of Breiman (2001). Among the 48 observations, 40 were used as the training set and eight as the testing set. The model was fitted with 100 decision trees. The increase in mean squared error (IncMSE) was used to evaluate variable importance, and the out-of-bag coefficient of determination (OOB R²) was used to assess model performance. Because root morphological traits were unavailable for the unplanted M and S treatments, these two treatments were excluded from Mantel tests and random forest analysis. Thus, these analyses were performed using the 12 planted treatments with four replicates each (n = 48).

Two membership function evaluations were performed. First, aggregate-stability membership function synthetic values (MFSV) were calculated using MSMA, WSMA, MWD, GMD, Dm and PAD to classify treatments into aggregate-stability groups. MSMA, WSMA, MWD and GMD were positively normalized, whereas Dm and PAD were reverse-normalized because lower values indicate higher aggregate stability. Treatments ranked 1-3, 7-9 and 12-14 were classified as high, intermediate, and low aggregation groups, respectively. Second, a comprehensive suitability evaluation was conducted by further including DTPA-HM. DTPA-HM represented the integrated membership value of DTPA-Cu, DTPA-Zn and DTPA-Ni and was reverse-normalized because lower HM availability indicates higher suitability. Higher comprehensive MFSV values indicated greater suitability for improving soil aggregate stability while controlling HM availability.

3. Results

3.1 Effects of sludge application and plant cultivation on soil aggregate distribution and stability

Compared with the control (M), plant cultivation and sludge application altered the distribution of both mechanically stable and water-stable aggregates (Fig. 1). Dry-sieving results showed that planted or sludge-amended treatments decreased the proportion of <0.25 mm mechanically stable aggregates from 17.63% in M to 8.91%–13.98% in other treatments, while the proportion of mechanically stable macroaggregates (>0.25 mm) from 82.38% to a range of 86.02%–91.10% (Fig. 1a). The highest proportion of >2 mm mechanically stable aggregates occurred in S-H×A (46.28%). A more marked response was observed for wet-sieving results (Fig. 1b; Table 2). Relative to the M (67.31%), all treatments substantially increased the proportion of water-stable macroaggregates (WSMA) to between 72.25% and 87.99%. The highest value was observed in the S-H treatment (87.99%), followed by S-H×A, S-N×A, M-H, and S-N×H. These results suggest that plant cultivation and sludge application promote macroaggregate formation, especially water-stable ones, though the response varied with the sludge amendment and planting strategy.

Aggregate stability indices further confirmed these changes (Table 2). Compared with the M, the other treatments generally showed increased MWD and GMD generally increased, whereas Dm and PAD decreased. The S-H showed the highest MWD (1.22 mm) and GMD (0.90 mm) and the lowest Dm (2.03) and PAD (3.33%). The S-H treatment increased WSMA, MWD, and GMD by 30.72%, 41.86%, and 76.47% relative to the M, while simultaneously decreasing Dm and PAD by 16.80% and 82.70%, respectively. According to a two-way ANOVA, both sludge application and plant

cultivation significantly affected all aggregate stability indices, and their interaction term was also significant for WSMA, MWD, and GMD.

Table 2 The changes of aggregate stability parameters.

Treatments	WSMA(%)	MWD (mm)	GMD (mm)	Dm	PAD (%)
M	67.31±1.26g	0.86±0.02g	0.51±0.01fg	2.44±0.02a	19.25±2.53a
M-N	80.40±1.37cde	0.99±0.03ef	0.64±0.02ef	2.28±0.03bcd	8.50±0.87defgh
M-H	85.07±0.81ab	1.06±0.07cde	0.72±0.06cde	2.27±0.03bcd	9.88±1.26cdefg
M-A	79.63±0.79de	1.02±0.03de	0.67±0.03de	2.32±0.02bc	12.25±2.926bcd
M-N×H	72.25±0.88f	0.92±0.01fg	0.57±0.01fg	2.37±0.01ab	16.75±1.11ab
M-N×A	76.95±2.31e	1.00±0.01ef	0.64±0.02ef	2.31±0.03bc	14.67±4.33abc
M-H×A	81.84±0.99bcd	1.09±0.06bcd	0.75±0.05cd	2.28±0.03bcd	11.33±1.86bcde
S	78.95±1.39de	1.07±0.02cde	0.71±0.02cde	2.28±0.03bcd	10.25±0.75cdef
S-N	83.64±1.08bc	1.18±0.01ab	0.85±0.01ab	2.20±0.04de	7.25±1.44defgh
S-H	87.99±1.01a	1.22±0.03a	0.90±0.03a	2.03±0.06f	3.33±0.67h
S-A	82.45±0.37bcd	1.16±0.03abc	0.78±0.03bc	2.18±0.02de	8.00±1.23defgh
S-N×H	84.52±0.73ab	1.11±0.01cd	0.79±0.02c	2.17±0.02de	11.00±1.00bcdef
S-N×A	85.19±1.44ab	1.11±0.04bcd	0.78±0.03bc	2.18±0.07de	6.00±2.04efgh
S-H×A	85.59±1.21ab	1.12±0.01bc	0.79±0.01bc	2.15±0.02e	5.00±1.29fgh
Source of Variation					
S	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
P	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
S×P	0.034*	<0.001***	<0.001***	0.568 ^{NS}	0.182 ^{NS}

WSMA, water-stable macroaggregates. MWD, mean weight diameter. GMD, geometric mean diameter. Dm, fractal dimension. PAD, percentage of aggregation destruction. Values are mean ± SE (n = 4). Means with different lowercase letters are significantly different (Duncan test, P < 0.05). In the two-way ANOVA, “S” represents sludge application, “P” represents plant cultivation, “NS” indicates no significant effect, “*” indicates significance at P < 0.05, “**” indicates significance at P < 0.01, and “***” indicates significance at P < 0.001.

To further explore the aggregation mechanism, all treatments were sorted and grouped based on membership function evaluation using the following parameters: MSMA, WSMA, MWD, GMD, Dm, and PAD (Table S3). The S-H treatment exhibited the highest membership function synthetic value (MFSV = 0.99), followed by S-H×A and S-N. In contrast, M, M-N×H, and M-A had the lowest MFSV values. Treatments ranked 1–3, 7–9, and 12–14 were classified into high, intermediate, and low aggregation groups, respectively.

3.2 Changes in organic binding agents and functional group composition among different aggregation groups

The high aggregation group contained the highest contents of SOM, polysaccharides, T-GRSP, and E-GRSP within both the bulk soil and all aggregate fractions, followed by the intermediate and low aggregation groups (Fig. 2). Compared to the low and intermediate groups, the high aggregation group contained, on average, 3.07 and 2.28 times more SOM, 3.92 and 2.60 times more polysaccharides, and 2.50 and 1.77 times more T-GRSP, respectively. The difference was even more pronounced for E-GRSP, which was 4.26- and 3.22-fold higher in the high aggregation group. These findings indicate that a high level of soil aggregation is characterized by a greater accumulation of organic binding agents.

To explore the chemical basis of the aggregation differences among different treatments, this study used FTIR spectroscopy to analyze the functional groups within aggregates (Fig. 3; Table 3). Within the MSMA fraction, high aggregation group was enriched in O–H groups (integrated area of 68.37), while low aggregation group was comparatively enriched in C=O/C=C, COO⁻/CH₂, and Si–O–Si functional groups. A different pattern emerged in the more stable WSMA fraction: high aggregation group dominated the O–H, Si–O–Si, and fingerprint regions (115.28, 193.02, and 80.46, respectively), whereas intermediate aggregation group was highest in C=O/C=C and COO⁻/CH₂. Collectively, these distinct chemical signatures demonstrate that the stability groups differ not just in the quantity of their binding agents, but also in their fundamental chemical composition.

Table 3 Integrated areas of characteristic FTIR regions of organic matter within macroaggregates.

Treatments		$\nu(\text{O-H})$ (3697-3000 cm ⁻¹)	$\nu(\text{C=O})/\nu(\text{C=C})$ (1800-1500 cm ⁻¹)	$\nu(\text{COO}^-)/\delta(\text{CH}_2)$ (1500-1300 cm ⁻¹)	$\nu(\text{Si-O-Si})$ (1200-800 cm ⁻¹)	$\delta(\text{Si-O})/\nu(\text{M-O})$ (800-400 cm ⁻¹)
MSMA	High-AS	68.36679	4.56053	4.03544	78.07498	41.60211
	Intermediate-AS	47.79827	3.72977	3.699221	111.06558	24.39478
	Low-AS	32.83137	10.88025	13.24258	131.65184	32.9561
WSMA	High-AS	115.27909	3.43166	1.71634	193.02358	80.4575
	Intermediate-AS	71.70205	15.95804	18.49475	157.81328	48.01408
	Low-AS	32.32193	7.45524	9.91699	147.20443	41.14775

Values represent relative absorbance intensity.

3.3 Relationships between aggregate stability, soil biochemical properties, and root morphology

To identify the primary drivers of aggregate stability, this study performed Mantel tests and random forest analysis, grouping potential explanatory variables into four categories: root morphological traits (Table S6), carbon fractions (Table S4), organic binding agents (Fig. 2), and soil enzyme activities (Fig. S1). The Mantel tests revealed that carbon fractions and organic binding agents were strongly associated with aggregate stability (Mantel's $r \geq 0.3$, $p < 0.001$), while enzyme activities showed a significant but moderate association (Mantel's $r = 0.2\text{--}0.3$, $p < 0.001$) (Fig. 4a). In contrast, root morphological traits exhibited the weakest correlations. The random forest model confirmed these findings, with carbon fractions making the highest predictive contribution to aggregate stability (%IncMSE = 71.2%), followed by organic binding agents (45.5%) and enzyme activities (42.8%). Root traits had the lowest contribution (15.4%). Together, these analyses consistently identify carbon fractions and organic binding agents as the dominant driver of aggregate stability in the system of synergistic application of plants and sludge.

3.4 Changes in soil HM contents

The safety of the sludge application was also evaluated. Although the HM content of the sludge was below the regulatory limits for soil utilization, concentrations of Cu, Zn, and Ni were notably higher than those of other HM (Fig. S2). Consequently, this study focused on the dynamics of these three metals. Sludge application significantly increased both the total and DTPA-extractable concentrations of Cu, Zn, and Ni in the soil. Conversely, plant cultivation mitigated these effects by reducing metal concentrations and availability in the sludge-amended soils (Table 4). Specifically, compared to the sludge-only treatment (S), the presence of plants led to significant reductions in DTPA-extractable Cu (29.14% to 52.49%), Zn (18.13% to 50.96%), and Ni (15.14% to 59.15%). Furthermore, the S-N×H significantly decreased the total concentrations of Cu, Zn, and Ni by 41.04%, 42.65%, and 48.89%, respectively. A two-way ANOVA confirmed that sludge application, plant cultivation, and their interaction all had significant effects on the concentrations of the three DTPA-extractable metals, as well as the total concentrations of Cu and Zn.

Table 4 Effects of sewage sludge addition and plant cultivation on total and DTPA-extractable Cu, Zn, and Ni concentrations in soil.

Treatment	Cu content (mg kg ⁻¹)		Zn content (mg kg ⁻¹)		Ni content (mg kg ⁻¹)	
Total	DTPA	Total	DTPA	Total	DTPA	
M	2.72±0.49d	0.58±0.06c	71.11±11.31bc	0.35±0.05d	2.96±0.33cd	0.02±0.01e
M-N	1.11±0.10d	0.56±0.05c	44.68±3.69de	0.37±0.06d	1.78±0.35d	0.01±0.01e
M-H	0.76±0.11d	0.54±0.01c	45.76±5.46cde	0.24±0.04d	1.68±0.42d	0.00±0.00e
M-A	0.75±0.16d	0.52±0.01c	53.74±4.07bcde	0.23±0.03d	2.10±0.26d	0.00±0.00e
M-N×H	1.89±0.14d	0.55±0.01c	59.71±3.31bcde	0.39±0.06d	2.50±0.04cd	0.00±0.00e
M-N×A	1.02±0.13d	0.52±0.03c	42.36±4.01de	0.30±0.06d	2.06±0.27d	0.00±0.00e
M-H×A	1.11±0.07d	0.55±0.03c	51.78±7.31bcde	0.25±0.04d	2.02±0.27d	0.01±0.01e
S	42.28±3.74a	24.50±1.84a	109.56±18.35a	7.28±0.58a	8.53±1.23a	2.84±0.32a
S-N	34.92±3.95ab	13.36±2.84b	73.97±2.28b	3.57±0.67bc	6.88±1.69ab	1.16±0.05d
S-H	37.79±4.11ab	12.70±3.04b	74.22±8.56b	4.75±1.00bc	5.21±1.47bc	1.82±0.43c
S-A	24.25±3.81c	17.36±1.95b	52.94±4.52bcde	5.96±0.80b	6.39±0.94ab	2.15±0.28bc
S-N×H	32.56±3.00bc	14.40±1.49b	62.83±11.73bcd	4.38±0.45bc	4.36±1.15bcd	1.67±0.20cd
S-N×A	24.39±3.36c	11.64±1.33b	33.27±4.68e	3.58±0.42c	6.91±1.37ab	1.19±0.13d
S-H×A	25.86±4.54c	16.54±1.39b	51.69±6.58bcde	5.85±0.44b	6.43±0.76ab	2.41±0.21ab
Source of Variation						
S	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***
P	0.004**	<0.001***	<0.001***	0.002**	0.014*	0.002**
S×P	0.025*	<0.001***	0.033*	0.003**	0.255 ^{NS}	0.003**

Values represent mean $\pm$ SE ($n = 4$). Different lowercase letters indicate significant differences among treatments (Duncan's test, $P < 0.05$). In the two-way ANOVA, "S" represents sludge application, "P" represents plant cultivation, and "S $\times$ P" represents their interaction. "NS" indicates no significant effect, while "*", "**", and "***" indicate significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

3.5 Comprehensive suitability evaluation of sludge–plant combinations

A membership function analysis was conducted to evaluate the suitability of various sludge–plant combinations for enhancing soil aggregate stability while minimizing heavy metal availability (Table 5). For this analysis, indices for aggregate stability (MSMA, WSMA, MWD, GMD) were positively normalized, whereas those related to particle size and metal availability (Dm, PAD, DTPA-HM) were negatively normalized. DTPA-HM represents the integrated availability of Cu, Zn, and Ni. The S-H treatment achieved the highest membership function suitability value (MFSV) of 0.9157, followed by S-H $\times$ A (0.7442) and S-N (0.7250). In contrast, the M, M-N $\times$ H, and S treatments performed poorly. For instance, although the M treatment posed low heavy metal availability, it was ineffective at improving aggregate stability. Conversely, the S treatment improved some aggregate indices but led to high heavy metal availability. Overall, this analysis identifies S-H as the optimal sludge–plant combination, with S-H $\times$ A and S-N also demonstrating high suitability.

Table 5 Comprehensive suitability evaluation of sludge–plant combinations based on aggregate stability and DTPA-extractable heavy metal.

Treatment	Mean subordinate function						MFSV		Rank
MSMA	WSMA	MWD	GMD	Dm	PAD	DTPA-HM			
M	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.14	14
M-N	0.65	0.63	0.36	0.33	0.39	0.68	0.99	0.58	9
M-H	0.58	0.86	0.56	0.54	0.41	0.59	1.00	0.57	10
M-A	0.56	0.60	0.44	0.41	0.29	0.44	1.00	0.61	8
M-N $\times$ H	0.42	0.24	0.17	0.15	0.17	0.16	0.99	0.33	13
M-N $\times$ A	1.00	0.47	0.39	0.33	0.32	0.29	1.00	0.54	11
M-H $\times$ A	0.81	0.70	0.64	0.62	0.39	0.50	1.00	0.66	5
S	0.50	0.56	0.58	0.51	0.39	0.57	0.00	0.44	12
S-N	0.70	0.79	0.89	0.87	0.59	0.75	0.49	0.72	3
S-H	0.96	1.00	1.00	1.00	1.00	1.00	0.45	0.92	1
S-A	0.74	0.73	0.83	0.69	0.63	0.71	0.27	0.66	6
S-N $\times$ H	0.63	0.83	0.69	0.72	0.66	0.52	0.42	0.64	7
S-N $\times$ A	0.61	0.86	0.69	0.69	0.63	0.83	0.54	0.70	4
S-H $\times$ A	0.99	0.88	0.72	0.72	0.71	0.90	0.29	0.74	2

4. Discussion

The restoration of soil structure is a foundational step in the ecological recovery of degraded mine soils. The application of sewage sludge is a widely adopted strategy for this purpose, as it enriches the soil with essential organic matter and nutrients (Uddin et al. 2025). However, this approach carries the inherent risk of introducing contaminants, such as HM, thereby posing potential environmental hazards (Kacprzak et al. 2017; Mokni-Tlili et al. 2024; Uddin et al. 2025). The study here revealed a promising synergy: the combined application of sludge and plants significantly enhanced soil aggregate stability. Crucially, phytoremediation also reduced the bioavailability of sludge-derived Cu, Zn, and Ni, thus mitigating the environmental risks associated with the amendment. Accordingly, this discussion will delve into the synergistic effects of this combined treatment on soil aggregation, the underlying mechanisms, and the overall suitability of this integrated approach for safe mine soil restoration.

4.1 Effects of sludge amendment and plant cultivation on soil aggregation

The application of sludge-based amendments to improve soil structure has been widely reported in degraded and reclaimed soils. Recent evidence from long-term sludge application and global organic-amendment syntheses shows that organic inputs can increase macroaggregate formation, improve MWD and GMD, and promote aggregate-associated organic carbon accumulation (Ma et al. 2024; Simões-Mota et al. 2024). Consistent with these studies, the present study showed that sludge amendment significantly improved aggregate stability in rare-earth mine soil. The increases in WSMA, MWD and GMD, together with the decreases in Dm and PAD, indicate that sludge amendment promoted the formation of larger and more stable aggregates. These results agree with the general interpretation that higher WSMA, MWD and GMD values reflect greater aggregate stability, whereas higher Dm and PAD values indicate greater aggregate dispersion and destruction (Bronick and Lal 2005; Rabot et al. 2018; Zhu et al. 2017).

While sludge amendment was a primary driver, results in the present study underscore that the trajectory of soil structure improvement was significantly modulated by plant cultivation. The synergistic effect of sludge and vegetation is highlighted by the superior performance of specific treatments. Notably, the S-H treatment (sludge + *H. arboricola*) exhibited the highest aggregate stability, achieving the greatest MWD and GMD and the lowest PAD. This observation was corroborated by the Membership Function Synthetic Value (MFSV) analysis, which ranked S-H as the most effective treatment. This finding strongly suggests that the beneficial impact of sludge on soil aggregation is not universal but is instead closely coupled with the functional traits of the established plant species, echoing recent work emphasizing that vegetation is a pivotal mediator of structural recovery in reclaimed soils (Liu et al. 2025; Zhou et al. 2025).

The diverse effects among plant treatments likely stem from distinct belowground strategies associated with different plant functional types. This experiment included herbaceous (*A. × mortfontanensis*), shrub (*H. arboricola*), and tree (*N. cadamba*) species, each with unique potential contributions to soil aggregation. Herbaceous species, with their typically rapid root turnover and extensive fine-root systems, may promote aggregation through continuous rhizodeposition. In contrast, shrubs can create dense, localized rhizosphere zones, while trees may foster long-term stability via deeper, more persistent root networks. As the influence of root traits on soil physical properties and ecosystem functioning becomes increasingly recognized (Freschet et al. 2021), it is plausible that the variation observed in the present study reflects these functional-type-specific belowground strategies more than simple species identity.

Intriguingly, and contrary to the common expectation that greater plant diversity enhances soil function (Gould et al. 2016), the mixed-planting treatment here did not consistently outperform the best-performing monoculture. The S-H treatment, a monoculture, surpassed most mixed-species plots in improving aggregate stability. While

complementary root traits in diverse plant communities can foster aggregation, this positive diversity effect may be attenuated or even negated under the severe environmental filters of a mining site. In such environments, factors like nutrient limitation, HM stress, and intense interspecific competition may mean that the adaptive advantages of a single, well-suited species outweigh the benefits of richness (Lin et al. 2025). This suggests that during the initial stages of sludge-assisted mine reclamation, the strategic selection of a highly adapted dominant plant species can be more critical than simply maximizing plant diversity.

4.2 Organic binding agents, functional groups and roots processes explained changes in soil aggregation

The formation of stable aggregates is generally driven by the combined effects of organic matter, colloidal particles, roots, fungal hyphae and microbial binding agents (Baumert et al. 2021). The SOM is regarded as a primary cementing substance for macroaggregate formation, and the role of organic binding agents in aggregate stabilization has long been established (Bronick and Lal 2005). Consistent with this mechanism, sludge amendment and plant cultivation in the present study altered the distribution of SOM, polysaccharides, T-GRSP, and E-GRSP among aggregate fractions. These substances act as binding agents and, promoting the association of mineral particles into stable aggregates.

This interpretation is further supported by recent studies on organic amendments and sludge-amended soils. Simões-Mota et al. (2024) reported that long-term sewage sludge addition increased labile carbon fractions and enhanced carbon accumulation in mineral-associated fractions. Ma et al. (2024) further showed, through a global meta-analysis, that organic amendments increased macroaggregate proportions and aggregate stability, with amendment quality and initial soil properties being important controls. At the aggregate level, Lin et al. (2026) demonstrated that organic amendment combinations influenced light-fraction organic matter and molecular diversity within macroaggregates. Together with our results, these findings suggested that sludge-derived organic inputs can improve aggregate stability by enhancing the biochemical foundation for soil particle cementation.

The GRSP may have been another important contributor to aggregate stabilization. Arbuscular mycorrhizal fungi and GRSP are closely associated with soil aggregate stability and carbon cycling (Cargill et al. 2025; Wright and Nichols 2002; Wright and Upadhyaya 1996). Recent studies have also highlighted the role of GRSP in aggregate formation and metal-contaminated soil remediation (Zhou et al. 2023). In the present study, changes in T-GRSP and E-GRSP across aggregate fractions suggest that microbial-derived binding agents likely participated in aggregate formation. This is especially relevant for mine soils, which typically exhibit weak biological activity, whereas external organic inputs may stimulate microbial and rhizosphere processes. Therefore, the improvement in aggregate stability was likely driven by the combined effects of sludge-derived organic matter, microbial binding agents and organic-mineral associations.

The FTIR results further indicated that aggregate stabilization was linked to changes in aggregate-associated functional groups. Variations in O-H, C=O/C=C, COO⁻, Si-O-Si, and metal-oxygen regions suggest that sludge amendment and plant cultivation altered the composition of organic functional groups and mineral-related bonds. Hydroxyl and carboxyl groups can form organic-inorganic complexes with multivalent cations on mineral surfaces, thereby strengthening soil particle cementation and aggregate stability (Kögel-Knabner and Amelung 2021; Zhu et al. 2024). Recent geochemical modelling also indicates that organic matter can strongly regulate HM binding and release by shifting metals between dissolved organic matter, particulate organic matter and mineral-associated phases (Li et al. 2024). Therefore, the FTIR results suggest that sludge-induced aggregate stabilization was not only physical aggregation but also organic-mineral interactions and biochemical cementation.

Interestingly, root physical traits, including root length, root surface area, and root volume, were not strongly correlated with aggregate stability indices in this study. This does not imply that roots played no role in aggregate formation. Rather, it suggests that root morphology alone was not the most direct controlling factor under the present experimental conditions. Roots can influence aggregation not only through physical enmeshment but also via root exudates, mucilage, rhizosphere microbial stimulation, mycorrhizal colonization, and extracellular polymeric substances (Haichar et al. 2014; Lehmann et al. 2017; Oades 1993). These rhizosphere processes were not directly measured in this study; therefore, root physical traits alone cannot fully capture belowground plant effects.

The weak relationship between root physical traits and aggregate stability may also reflect the coexistence of herbaceous, shrub, and tree species, as well as the comparison between monoculture and mixed planting. In mixed systems, root interactions may involve both complementarity and competition. A treatment with greater total root length may not necessarily produce more effective binding agents if root exudation, microbial stimulation, or mycorrhizal colonization is limited. Conversely, a plant species with moderate root biomass may still promote aggregation if it stimulates GRSP production, microbial polysaccharides, or organic–mineral complexation. Therefore, future studies should integrate root morphology with measurements of root exudates, rhizosphere microbial communities, and mycorrhizal colonization to further elucidate the belowground mechanisms of plant-mediated aggregation.

4.3 Suitability of sludge-assisted plant restoration for safely improving mine soil aggregation

The application of sewage sludge is a well-established method for enhancing soil fertility and promoting plant growth in degraded ecosystems. However, this practice is not without risks, as sludge can introduce a suite of contaminants, including HM, antibiotic resistance genes, and other emerging pollutants, with the specific risks depending on the sludge source, treatment, and application rate (Balkrishna et al. 2025; Eid et al. 2026; Mokni-Tlili et al. 2024; Nunes et al. 2021; Schaap et al. 2025; Uddin et al. 2025). Consequently, the benefits of sludge for soil aggregation must be weighed against the risks of metal mobilization. In the present study, the sludge-only treatment exhibited the highest concentrations of these metals, confirming that sludge application alone expands the pool of potentially bioavailable metals. Despite this, sludge amendment also significantly enhanced the biomass and root development of all three tested species: *N. cadamba*, *H. arboricola*, and *A. amazonica* (Tables S5 and S6). Crucially, the presence of these plants actively mitigated the risks; plant extraction reduced the ecological risk indices for Cu, Zn, and Ni (Tables S7–S10), demonstrating that vegetation can help immobilize or remove a portion of the bioavailable metals introduced by the sludge.

The choice of plant functional type further influences metal availability. Herbaceous species, shrubs, and trees exhibit distinct differences in growth rates, biomass allocation, root architecture, and metal uptake strategies. For instance, fast-growing herbaceous plants can rapidly sequester metals in the short term, while shrubs and trees, with their extensive and persistent root systems, can offer long-term stabilization through greater belowground carbon inputs. Several recent phytoremediation studies advocate for balancing plant establishment, metal stabilization, and soil function recovery, rather than focusing solely on metal uptake (Díaz et al. 2025; Kafle et al. 2022; Zhao et al. 2022). Our results reflect this complexity: sludge amendment significantly boosted the biomass and root development of *N. cadamba*, while *H. arboricola* and *A. amazonica* showed varied responses in monoculture versus mixed plantings. These species-specific responses highlight how different plant functional types can modulate metal availability through both direct uptake and indirect rhizosphere modifications (Wu et al. 2021; Zhao et al. 2022).

Synthesizing these findings, the S-H treatment emerged as the most effective, demonstrating a favorable balance between enhanced aggregate stability and controlled metal availability. It was followed, in order of suitability, by S-H×A, S-N, and S-N×A. This ranking suggests that *H. arboricola* is particularly adept at simultaneously promoting soil structure and regulating metal risks under sludge amendment. However, these results also underscore the inherent trade-offs in selecting a vegetation strategy. Some configurations may excel at improving aggregation but leave metal availability relatively high, while others might prioritize metal immobilization at the expense of slower structural recovery. Therefore, the optimal strategy must be tailored to specific restoration goals, whether the priority is rapid structural improvement, minimizing metal bioavailability, ensuring robust plant establishment, or achieving long-term ecological stability.

5. Conclusion

This study demonstrates that the combined application of sewage sludge and plants is an effective strategy for concurrently improving soil aggregate stability and mitigating HM bioavailability in soils on abandoned REE mine sites. The co-application of sludge with *H. arboricola* (S-H) yielded the most substantial improvements in soil structure. The analysis here revealed this effect was primarily driven by the enrichment of soil organic carbon fractions and associated binding agents, particularly soil polysaccharides and glomalin-related soil protein (GRSP). While sludge amendment introduced a significant load of Cu, Zn, and Ni, the presence of vegetation successfully counteracted this risk, reducing their total and DTPA-extractable concentrations substantially in amended soils. Consequently, the S-H treatment emerged as the optimal strategy, striking the most effective balance between enhancing soil aggregation and controlling HM risks. These findings provide strong evidence that sludge-assisted phytoremediation offers a practical and beneficial pathway for the ecological restoration of REE mine soil, highlighting a synergistic approach to waste recycling and land reclamation.

Declarations

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Competing interests

The authors declare no competing interests.

Author contributions

Jue Huang performed the experiment, collected and analyzed the data, prepared the figures and tables, and wrote the first draft of the manuscript. Xiaoquan Dong performed the experiment, collected and analyzed the data, and revised the manuscript. Xuqin Ran, Hanrong Liang, Yongjian Lu and Heyan Xing assisted with sample collection, laboratory analysis and data processing. Shuchai Zeng contributed to manuscript revision. Daoming Wu conceived

and supervised the study, contributed to experimental design and data interpretation, acquired funding, and revised the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

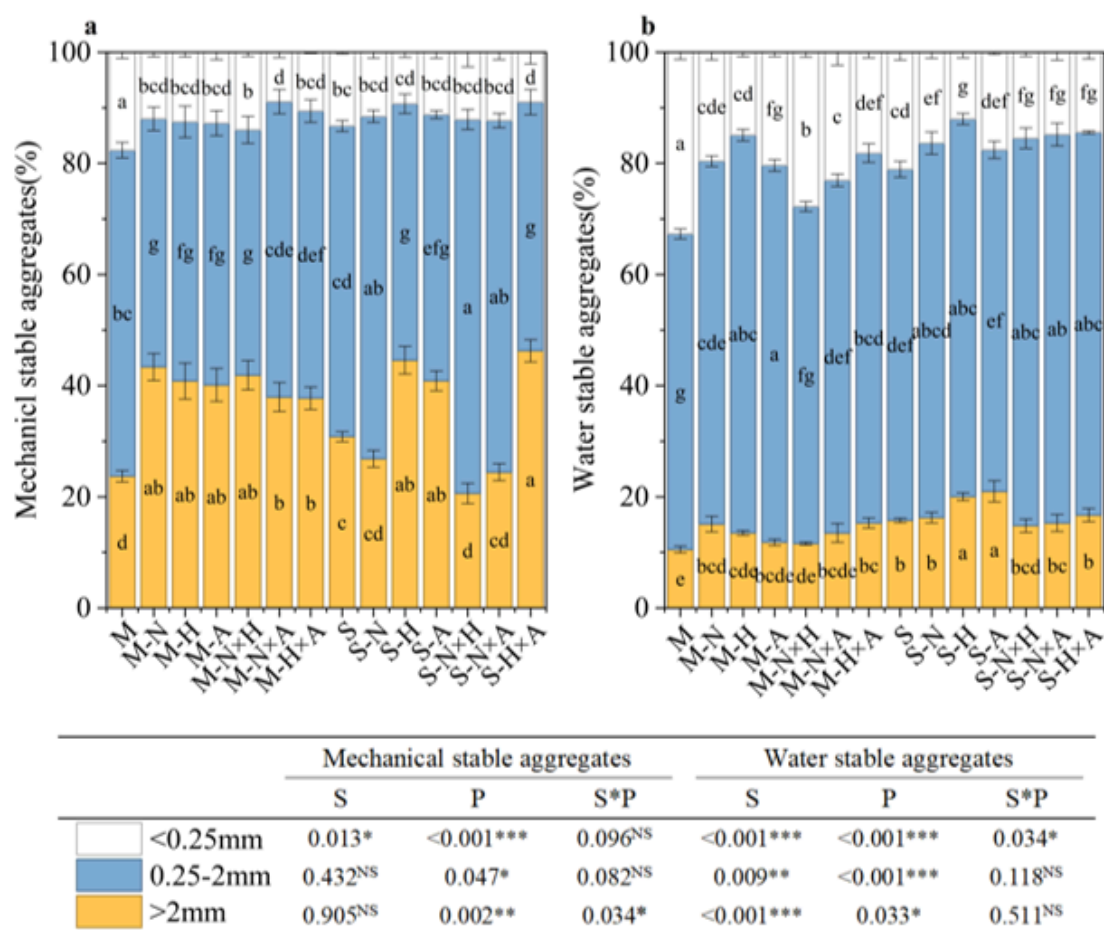


Figure 1

Aggregate size distribution under different treatments. (a) Mechanical-stable aggregates; (b) Water-stable aggregates. Values are presented as mean $\pm$ SE ($n = 4$). Different lowercase letters indicate significant differences among treatments (Duncan's test, $P < 0.05$). In the two-way ANOVA, "S" represents sludge application, "P"

represents plant cultivation, “NS” indicates no significant effect, “*” indicates significance at $P < 0.05$, “**” indicates significance at $P < 0.01$, and “***” indicates significance at $P < 0.001$.

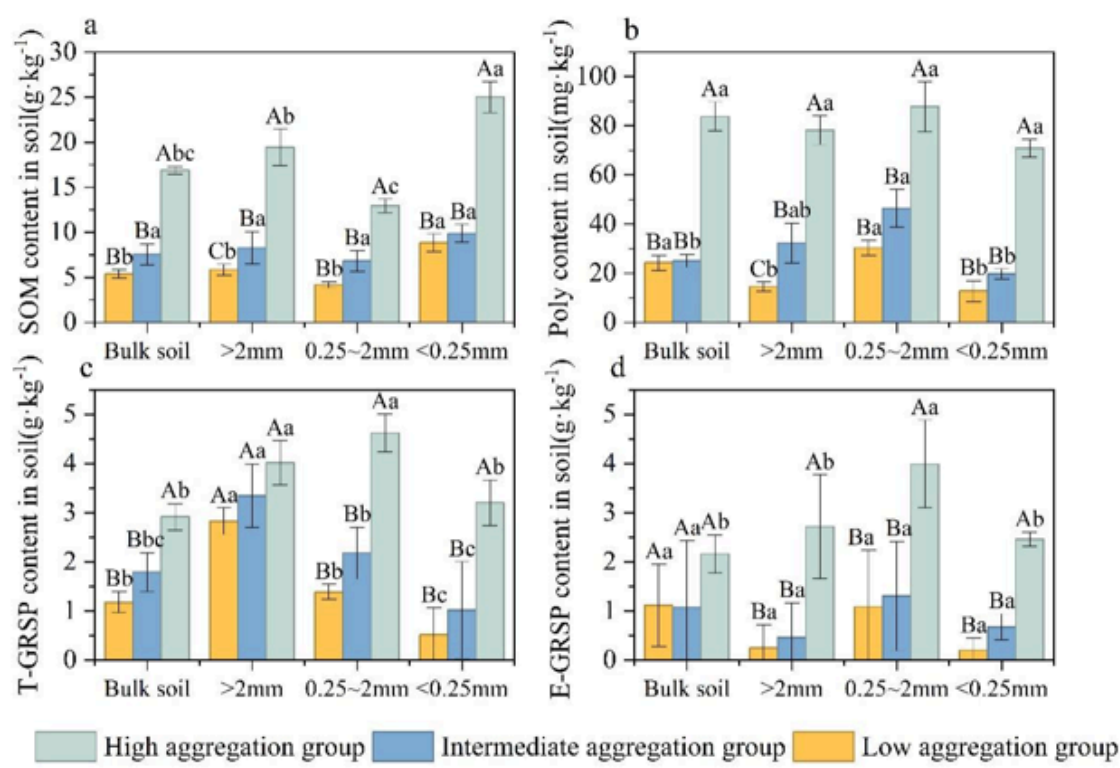


Figure 2

Contents of SOM, soil polysaccharides, T-GRSP, and E-GRSP in bulk soil and aggregate fractions among aggregation groups. (a) SOM; (b) soil polysaccharides; (c) T-GRSP; (d) E-GRSP. Values are means ± SD (n = 4). Different uppercase letters indicate significant differences among aggregation groups within the same particle-size fraction, and different lowercase letters indicate significant differences among aggregate fractions within the same aggregation group according to Duncan’s test at $P < 0.05$.

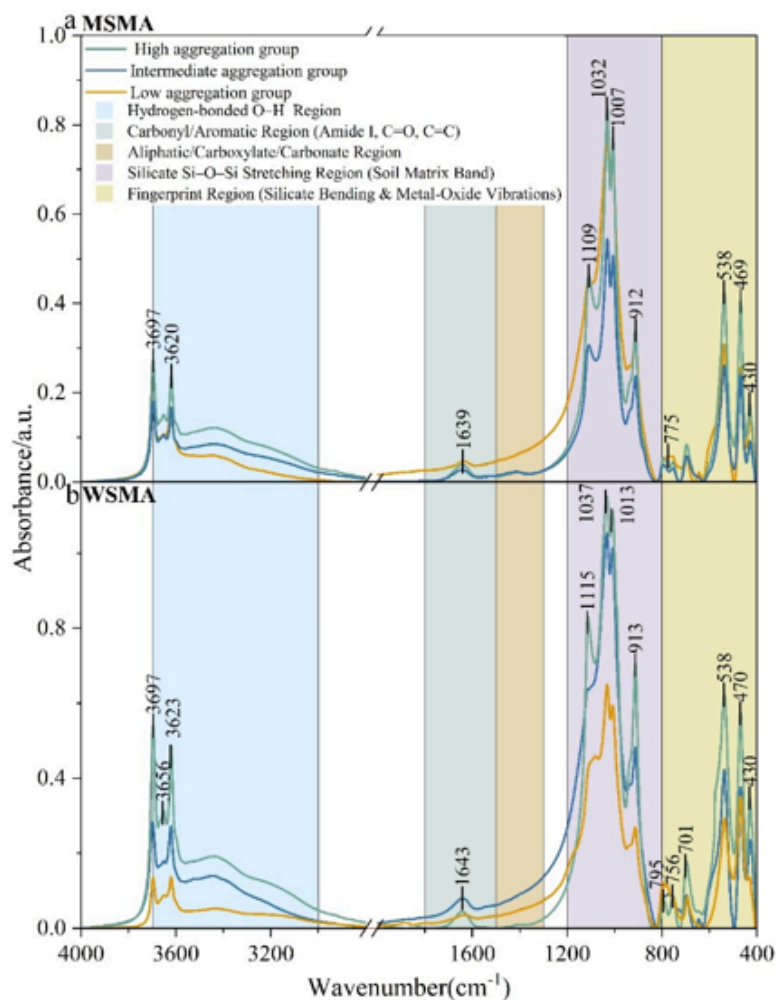


Figure 3

FTIR spectra of organic matter within macroaggregates across aggregation groups for mechanically stable macroaggregates (MSMA, a) and water-stable macroaggregates (WSMA, b).

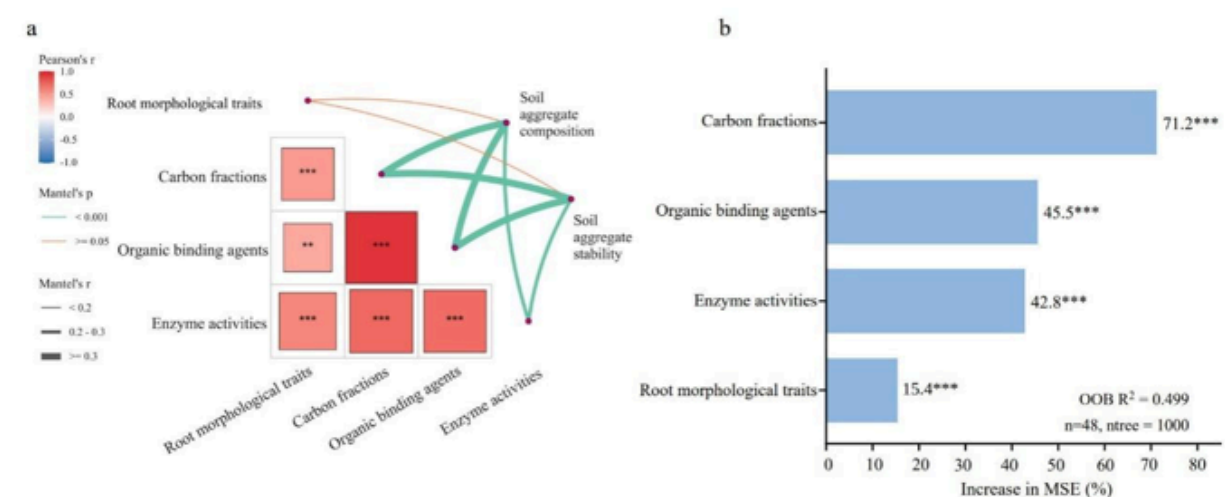


Figure 4

Relationships between soil aggregation and potential driving factors. (a) Mantel test showing the relationships among root morphological traits, carbon fractions, organic binding agents, enzyme activities, soil aggregate composition, and soil aggregate stability. (b) Random forest analysis identifying the relative importance of different variable groups in explaining soil aggregate stability, expressed as the percentage increase in mean squared error (MSE). Asterisks indicate significance levels: * $p < 0.01$ and ** $p < 0.001$. OOB R^2 , out-of-bag coefficient of determination.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarydata.docx](#)