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Soil aggregate structure, stability, and stoichiometric characteristics in a smelter-impacted soil under phytoremediation

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Smelter-impacted soils often result in soil degradation and the destruction of the soil structure. Although soil aggregate typically plays a crucial role in soil structure, the influence of phytoremediation on soil aggregate structure stability and stoichiometric characteristics remains unclear. To study the influence of phytoremediation on soil aggregate structure, stability and stoichiometric characteristics, a 3-year *in situ* experiment was conducted. After hydroxyapatite was applied, *Elsholtzia splendens*, *Sedum plumbizincicola*, and *Pennisetum* sp. were planted in a smelter-impacted soil. After 3 years, the soil aggregate structure, stability, and stoichiometric of chemical elements were analyzed. The results showed that the three phytoremediation treatments increased the content of >0.25 mm mechanically-stable ($DR_{0.25}$) and water-stable ($WR_{0.25}$) aggregates by 6.6%–10.4% and 13.3%–17.5%, respectively. Aggregate mean weight diameter (MWD), geometric mean diameter, and aggregate stability rate (AR, %) were significantly increased, and the soil mechanically stable aggregate fractal dimension (D) was significantly reduced after the 3-year remediation. Soil total nitrogen and phosphorus in aggregates with different particle sizes were significantly increased by 11.4%–46.4% and 107%–236% after different plant treatments. For the stoichiometric characteristics of the aggregates, the combined remediation only significantly reduced the value of N:P and C:P in different particle size aggregates and had no significant effect on the C:N in all particle size aggregates. Meanwhile, the combined remediation of hydroxyapatite and *Elsholtzia splendens*, *Sedum plumbizincicola*, and *Pennisetum* sp. in heavy metal heavily contaminated soil could reduce the availability of Cu and Cd by 54.1%–72.3% and 20.3%–47.2% during the 3 years, respectively. In summary, this combined remediation method can be used for the remediation of farmland that is contaminated by heavy metals.

KEYWORDS

vegetation restoration, aggregate structure, aggregate stability, stoichiometric characteristics, contamination

1 Introduction

Metal pollutants in the environment mainly come from anthropogenic industrial and agricultural activities, and they can enter soil system in many different ways, such as atmospheric sedimentation, waste water irrigation, and slag leaching. Excessive accumulation of metals in the soil not only reduces soil quality, microbial activity, and crop productivity but also threatens ecological security and human health (Taghipour et al., 2011; Dankoub et al., 2012; Azizi et al., 2022). During the past few decades, more cost-effective and environmentally friendly techniques (e.g., bioremediation and integrated remediation) have been developed to reduce the mobility and bioavailability of metals in agricultural soils (Rzab et al., 2021). *In situ* remediation technology using combined plant and chemical additives has been proven to be one of the cheapest and most effective methods for remediating soils that are contaminated with metals (Liu et al., 2020; Li et al., 2021).

The selection of plants is very important in the process of phytoremediation. *Elsholtzia splendens* is widely distributed in the middle and lower reaches of the Yangtze River in China and in copper mine areas such as Zhuji City, Zhejiang Province (Yang et al., 2015). In recent years, *Elsholtzia splendens* has been shown to have high tolerance and strong accumulation ability to Cu, and can be applied to remediate Cu contaminated soil (Zhang et al., 2019). *Sedum plumbizincicola* has a high tolerance to Cd and does not show toxic symptoms when the content of Cd in the aboveground is as high as 587 mg/kg (Wu et al., 2012a). Therefore, it has been widely used in the remediation of Cd contaminated farmland (Zou et al., 2021). *Pennisetum* sp. has the characteristics of fast growth, strong stress resistance, and large biomass and has the potential in the remediation of heavy metal-contaminated soil (Lei et al., 2019). However, the low soil pH and high metal toxicity may impede the growth of plants, which reduces the remediation effect (Pardo et al., 2011). Evidence has been found that some chemical materials such as biosorbents or limestone can adsorb and stabilize soil Cu and Cd (Huang et al., 2015; Chen et al., 2020; Pennanen et al., 2020). A large number of studies have shown that phosphorus containing materials (e.g., apatite, potassium dihydrogen phosphate, superphosphate, and hydroxyapatite) can effectively reduce the activities of metals, such as Pb, Cd, and Cu in soil and wastewater (Valipour et al., 2016). Among them, hydroxyapatite has been widely used to remediate metal-contaminated soil and sediments owing to its strong adsorption and fixation ability for metals (Guo et al., 2018; Xu et al., 2019). However, most of the studies about hydroxyapatite only focus on its effect on metal activity and

bioavailability, and only a few studies have paid attention to its potential effect on the soil aggregate structure and the influence of on soil stoichiometric characteristics.

The purpose of phytoremediation is not only to remove metals from soil but also to improve soil quality including soil physical structure, chemical properties, and biological properties (Qin et al., 2021). Soil aggregate is an important indicator of soil structure. At the same time, aggregate stability plays an important role in maintaining soil structure. Moreover, soil aggregate is the storage and transformation place of soil nutrients, such as carbon, nitrogen, and phosphorus. In addition, soil microorganisms accumulated in aggregate play an important role in regulating plant growth, reducing hydraulic erosion, and improving soil fertility (Ayoubi et al., 2012; Ayoubi et al., 2020). Different particle size aggregates have different abilities to fix and transform nutrients, which finally affects the geochemical cycle of soil nutrients (Gelaw et al., 2013). Meanwhile, different plants have different effects on soil aggregates. However, in the process of phytoremediation, there are few reports on the impact of phytoremediation model on soil aggregates.

Soil stoichiometric characteristics are the theoretical basis for analyzing the balance relationship between multiple elements, especially structural elements (C), restrictive elements (N and P), and the interaction of ecosystem. At the same time, stoichiometric characteristics are important indicators in plants, litter, soil and microorganism carbon and nitrogen nutrient cycling, nutrient limitation, carbon fixation and farmland vegetation restoration (An, 2012; Quan et al., 2015; Jia et al., 2018). Although there are many studies on the formation mechanism, stability, and nutrient retention of aggregate under different vegetation conditions, there are relatively few studies on the eco-stoichiometric characteristics of carbon, nitrogen, and phosphorus in soil aggregate in the process of phytoremediation (Erktan et al., 2016).

The objectives of this study were to (1) evaluate the changes of soil aggregate composition and structure after combined remediation, (2) analyze soil aggregate stability after combined remediation, (3) measure the stoichiometric characteristics of soil aggregate, and (4) explore the relationship between the combined remediation and the stoichiometric characteristics of nutrients in soil aggregate.

2 Materials and methods

2.1 Study site

The study site is located in Chenjia village group, Baili village, Binjiang Township, Guixi City, Jiangxi Province, China. This

area is close to a large Cu smelter in Guixi City. Due to long-term diversion irrigation discharged by the local Cu smelter and atmospheric sedimentation, the farmland in this area is seriously contaminated by metals. The main pollutants are Cu and Cd (Zhou et al., 2015). Because of the heavy degree of contamination, most of the farmland in this area has been abandoned for many years. In addition, this area is located in a typical acid rain area in south China, the pH of precipitation rainwater is 3.62 ~ 4.98. Moreover, the annual deposition fluxes of Cu, Cd, Pb, Zn, and Cr in this area are 638, 6.56, 70.0, 225, and 22.7 mg m⁻²·a⁻¹, respectively, which exacerbates the degree of soil contamination (Tao et al., 2014). The soil in the experimental area is sandy loam. Soil Cu and Cd concentrations are 632 and 0.41 mg kg⁻¹, respectively. Soil pH is 4.35 (acidic), and soil organic carbon (SOC) and cation exchange capacity (CEC) are 28.5 and 8.31 cmol kg⁻¹, respectively. Total N and total P in the soil are 1.11 and 0.19 g kg⁻¹, respectively.

2.2 Hydroxyapatite and plant species

Hydroxyapatite (purity >96.0%, pH 7.71, particle size 3 μm, specific surface area 45.7 m² g⁻¹, Ca:P 1.72, Cd and Cu concentrations 3.83 × 10⁻² and 5.85 mg kg⁻¹) was purchased from Emperor Nano Material Co. Ltd. (Nanjing, China). Three phytoextractors were selected: a Cu-tolerant plant (*Elsholtzia splendens*) (Wang et al., 2005), high biomass plant (*Pennisetum* sp.) (Xu et al., 2014), and a Cd hyperaccumulator (*Sedum plumbizincicola*) (Wu et al., 2012b). All of the plants that were used in the experiment were grown from seed in pots in a greenhouse.

2.3 Plot design

There were four treatments in the field experiment (CK: untreated soil; ME: hydroxyapatite + *Elsholtzia splendens*; MS: hydroxyapatite + *Sedum plumbizincicola*; MP: hydroxyapatite + *Pennisetum* sp.), each treatment had three repetitions, a total of 12 plots, and the plot area was 4 m² (2 m × 2 m). To prevent interaction between adjacent plots, the plots were separated by cement plates. The buried depth of cement plates was 0.3 m underground and 0.2 m above the ground. Meanwhile, 1% hydroxyapatite (based on the 0- to 17-cm soil weight) and 834 kg ha⁻¹ fertilizer (the content of N, 15%; P₂O₅, 15%; and K₂O, 15%) were applied into every plot on 23 December 2012, and the hydroxyapatite and fertilizer fully mixed into the soil by plowing. *Elsholtzia splendens* and *Sedum plumbizincicola* (planting spacing 0.2 m × 0.2 m), *Pennisetum* sp. (planting spacing 0.5 m × 0.5 m) were planted on 26 April each year (2013, 2014, and 2015), while the hydroxyapatite was applied once in 2012 but fertilizer was added into the soil each year for the next 3 years.

2.4 Sample collection

Elsholtzia splendens and *Pennisetum* sp. were harvested in the middle of December each year, *Sedum plumbizincicola* was harvested in early August each year. Soil samples were collected from each plot from an area of 20 cm × 20 cm × 17 cm in 2015, three samples were taken from each plot and then mixed together to form a mixed sample. These samples were air dried and sieved using a 5 mm sieve. The resulting samples were used for the analysis of soil aggregates. Surface soil (0–17 cm) was collected every year and passed through a 10-mesh sieve to determine the basic properties, and to assess the Cu and Cd concentrations.

2.5 Soil and plant chemical and metal analysis

Soil pH was measured by a pH electrode in a suspension of distilled water at a liquid to solid ratio of 2.5 (E-201-C, Shanghai Truelab Instrument Company, China) (Xu et al., 2016). SOC was measured by digesting soil with K₂Cr₂O₇ and concentrated H₂SO₄ at 170°C–180°C, and then titrating with FeSO₄ (Mikhailova et al., 2003). Total soil N was determined by the semimicro Kjeldahl method on a sample predigested with perchloric acid (HClO₄) and hydrofluoric acid (HF) (Zhang and Sheng-Xiu, 2005). Total soil P was determined by acidic molybdate-ascorbic acid blue color method after the soil digestion with nitric acid/perchloric acid mixture (4:1) (Olson et al., 1982). Total Cu and Cd were measured by a flame or graphite furnace atomic absorption spectrophotometer (Hitachi Model Z-2000, Japan) after digestion with mixed nitric acid (HNO₃), hydrofluoric acid (HF), and perchloric acid (HClO₄) (5:10:5) on a hot plate (120°C–240°C). Available Cu and Cd were measured by extracting soil samples with 0.01 M CaCl₂ at a 1:5 ratio and then shaking for 2 h at room temperature (25°C) (Walker et al., 2003). The suspension was centrifuged for 10 min, decanted and filtered through a 0.45-μm filter, acidified (with concentrated HNO₃), and stored in a polyethylene container at 4°C until analysis.

Plant samples (0.25 g) were digested using a mixture of 4 ml HClO₄ and 6 ml HNO₃ on an electric heating plate. The metal contents were measured by atomic absorption spectroscopy.

Blanks, replicate samples, and a certified reference material (GBW07401, provided by the Institute of Geophysical and Geochemical Exploration, Langfang, Hebei Province, China) were used to ensure the reliability of the experimental data.

2.6 Aggregate analysis

Mechanically stable aggregates: The mechanically stable aggregate was measured by the dry sieve method (Yan et al.,

2020), the aggregate was divided into six size fractions: >5 mm, 2–5 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm, and <0.25 mm.

Water-stable aggregates: The water-stable aggregate was measured by Wang’s method (Wang et al., 2020)— 25 g soil samples of each particle size according to the proportion of dry screening method was placed on a 5-mm soil sieve, the soil samples were soaked in distilled water for 10 min, and then passed the soil samples through 2, 1, 0.5, 0.25, and 0.053 mm soil sieves in turn. The aggregate was separated by moving the sieve 3 cm up and down, repeating 50 times (within 2 min), washing the soil particles that were left on each sieve into the aluminum box, drying at 50°C, and weighing. Water-stable aggregates were divided into six size fractions: >5 mm, 2–5 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm, and <0.25 mm. The percentage of >0.25 mm mechanically stable aggregates ($DR_{0.25}$) and >0.25 mm water-stable aggregates ($WR_{0.25}$) were calculated based on the results. $DR_{0.25}$ was >0.25 mm mechanically stable aggregate percentage (%), and $WR_{0.25}$ was >0.25 mm water-stable aggregate percentage (%). The mean weight diameter (MWD, mm), geometric mean diameter (GMD, mm), and fractal dimension (D) of aggregates were calculated by Zhao’s method (Zhao et al., 2012):

$$MWD = \sum_{i=1}^n XiWi \tag{1}$$

$$GMD = e^{\frac{\sum_{i=1}^n Wi \ln Xi}{\sum_{i=1}^n Wi}} \tag{2}$$

X_i is the average soil aggregate diameter of any grade, which is numerically equal to the average value of two adjacent sieve holes, for >5 mm aggregates, the upper limit of aggregate diameter is 10 mm; W_i is the percentage of aggregates corresponding to X_i .

$$D = 3 - \lg \frac{M(r < xi)}{M} / \lg \frac{Xi}{Xmax} \tag{3}$$

X_i is the average soil aggregate diameter of any grade, which is numerically equal to the average value of two adjacent sieve holes, for >5 mm aggregates, the upper limit of aggregate diameter is 10 mm; X_{max} is the average particle size of the maximum particle size, mm; $M(r < X_i)$ is the weight of the aggregate with particle size less than X_i ; and M is the total weight of the aggregates.

2.7 Statistical analysis

SPSS20.0 (IBM SPSS, Somers, NY, United States) was used for one-way ANOVA and correlation analysis (Pearson index was used for size), Duncan new repolarization difference method was used for difference significance analysis, and the significance level was $p < 0.05$. All of the graphics were plotted by Sigmaplot 12.5.

3 Results and discussion

3.1 Soil chemical characteristics and metal availability

The pH of CK slightly decreased from 4.24 in the first year to 4.20 in the third year (Table 1). With hydroxyapatite application, the pH was significantly improved by 21.5%–25.7%, 21.3%–23.9%, and 20.7%–25.2% in 2013, 2014, and 2015, respectively. The study area was an acid rain settlement region, where H^+ from the rainwater reduced soil pH over time (Tao et al., 2014). Differences in plant species did not significantly change soil pH (Table 1). Meanwhile, the roots may have secreted weak organic acid ions, sugars, amino acids, vitamins, and inorganic ions (HCO_3^- , OH^- , and H^+), all of which might alter soil pH (Bedolla-rivera et al., 2020). Untreated soil had the highest $CaCl_2$ extractability (Cu 100 and Cd 0.15 mg kg⁻¹ in 2015) (Table 1). Hydroxyapatite significantly decreased extractable Cu and Cd by 54.1%–73.5% and 20.1%–51.2%. The results showed that bioavailability of Cu and Cd in the CK were higher than that in hydroxyapatite treated soils, which might be mainly due to the lower pH in the CK.

3.2 Plant biomass and metal concentrations in plants

Although the three phytoextractors could grow normally after the application of hydroxyapatite, there was no plant growth in CK treatment without micron hydroxyapatite. There were significant differences in the biomass of the three plants, the largest biomass was found in *Pennisetum* sp. The biomass of *Elsholtzia splendens* was smaller than that of *Pennisetum* sp. but significantly higher than that of *Sedum plumbizincicola* (Table 2). *Sedum plumbizincicola* showed the highest absorption capacity for Cu and Cd in the enrichment capacity for metals (Figure 1).

As an effective and widely used remediation measure of soil heavy metal contamination, phytoremediation is considered to be an effective method to reduce the total amount of soil heavy metals over time (Ceschin et al., 2019). Thus, removal efficiency should always be used to evaluate the remediation capability during the phytoremediation, which can be calculated using tissue concentration and biomass produced (Mamirova et al., 2020). Therefore, the removal efficiency of different plants was not only related to the concentration of metals in plants but also depended on the biomass of plants. After considering biomass and Cu and Cd concentrations, *Pennisetum* sp. showed the greatest Cu and Cd removal efficiency, with 3-year cumulative amounts of 8.67×10^3 and 121 g hm⁻² for Cu and Cd, respectively (Table 2). Although the

TABLE 1 Soil chemical and metal availability characteristics.

Time	Treatment	pH	SOC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (g kg ⁻¹)	T-Cu (mg kg ⁻¹)	T-Cd (mg kg ⁻¹)	CaCl ₂ -Cu (mg kg ⁻¹)	CaCl ₂ -Cd (mg kg ⁻¹)
2013	CK	4.24 ± 0.21b	16.19 ± 0.19a	1.11 ± 0.04a	0.19 ± 0.01b	666 ± 16.3a	0.412 ± 2.44×10 ⁻² a	81.6 ± 25.9a	0.125 ± 1.85×10 ⁻² a
	ME	5.33 ± 0.05a	17.77 ± 0.40a	1.32 ± 0.09a	0.65 ± 0.03a	618 ± 13.4a	0.390 ± 9.13×10 ⁻³ a	23.2 ± 5.44b	0.076 ± 1.33×10 ⁻² b
	MS	5.19 ± 0.10a	17.97 ± 0.51a	1.26 ± 0.10a	0.78 ± 0.07a	633 ± 19.3a	0.377 ± 1.53×10 ⁻² a	22.8 ± 4.28b	0.066 ± 1.62×10 ⁻² b
2014	MP	5.15 ± 0.14a	17.96 ± 1.39a	1.27 ± 0.17a	0.71 ± 0.20a	657 ± 15.3a	0.380 ± 1.69×10 ⁻² a	23.4 ± 5.11b	0.073 ± 9.32×10 ⁻³ b
	CK	4.23 ± 0.11b	16.07 ± 0.17b	1.06 ± 0.03a	0.19 ± 0.04b	674 ± 12.5a	0.394 ± 1.42×10 ⁻² a	94.2 ± 36.3a	0.132 ± 2.83×10 ⁻² a
	ME	5.24 ± 0.26a	18.38 ± 0.73a	1.33 ± 0.11a	0.52 ± 0.04a	621 ± 16.9a	0.372 ± 8.11×10 ⁻³ ab	34.5 ± 13.2b	0.073 ± 1.73×10 ⁻² b
	MS	5.13 ± 0.20a	18.40 ± 0.24a	1.25 ± 0.12a	0.68 ± 0.06a	639 ± 29.0a	0.359 ± 2.56×10 ⁻² b	26.0 ± 13.5b	0.068 ± 2.15×10 ⁻² b
2015	MP	5.14 ± 0.25a	18.82 ± 0.55a	1.29 ± 0.23a	0.63 ± 0.24a	650 ± 25.2a	0.352 ± 3.46×10 ⁻² b	25.0 ± 3.30b	0.075 ± 1.13×10 ⁻² b
	CK	4.20 ± 0.28b	16.49 ± 0.45b	1.08 ± 0.06b	0.19 ± 0.02b	668 ± 11.7a	0.406 ± 1.54×10 ⁻² a	100.0 ± 21.1a	0.148 ± 1.75×10 ⁻² a
	ME	5.26 ± 0.17a	19.05 ± 0.59a	1.46 ± 0.44a	0.46 ± 0.03 ab	616 ± 8.67b	0.364 ± 5.26×10 ⁻³ b	45.9 ± 6.66b	0.118 ± 7.47×10 ⁻³ ab
	MS	5.13 ± 0.16a	18.62 ± 1.08a	1.28 ± 0.08 ab	0.62 ± 0.10a	630 ± 8.53b	0.350 ± 1.20×10 ⁻² b	43.5 ± 3.50b	0.090 ± 2.11×10 ⁻² b
	MP	5.07 ± 0.10a	19.52 ± 0.48a	1.35 ± 0.28 ab	0.55 ± 0.14a	649 ± 13.8 ab	0.350 ± 2.27×10 ⁻² b	44.3 ± 9.81b	0.089 ± 2.79×10 ⁻² b

CK, untreated soil; ME, hydroxyapatite + *Elsholtzia splendens*; MS, hydroxyapatite + *Sedum plumbizincicola*; MP, hydroxyapatite + *Pennisetum* sp.; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; T-Cu, total Cu concentration; T-Cd, total Cd concentration. Different lowercase letters indicate significant differences between treatments in the same year ($n = 3, p < 0.05$).

TABLE 2 Shoot biomass and Cu and Cd accumulation in each plant during phytoextraction.

Treatment	Shoot biomass (t hm ⁻²)			Metal accumulation (g hm ⁻²)					
				Cu			Cd		
	2013	2014	2015	2013	2014	2015	2013	2014	2015
CK	--	--	--	--	--	--	--	--	--
MW	10.1 ± 4.91bc	8.55 ± 1.52bc	5.20 ± 0.560c	236 ± 148c	285 ± 20.5b	224 ± 93.2b	10.4 ± 3.96b	10.3 ± 4.27b	6.50 ± 2.23c
ME	15.1 ± 4.17 ab	12.6 ± 1.38b	14.4 ± 4.22b	2.74×10 ³ ± 437a	2.54×10 ³ ± 759a	2.93×10 ³ ± 1.28 × 103a	39.2 ± 15.0a	32.1 ± 8.59a	37.6 ± 8.49b
MS	2.25 ± 0.365c	2.10 ± 0.210c	2.70 ± 0.468c	1.03×10 ³ ± 266c	910 ± 92.8b	1.28×10 ³ ± 395ab	29.8 ± 3.94ab	29.5 ± 1.10a	38.1 ± 5.15ab
MP	22.3 ± 3.36a	29.2 ± 6.10a	37.7 ± 4.14a	1.88×10 ³ ± 353b	2.98×10 ³ ± 949a	3.81×10 ³ ± 1.40 × 103a	29.1 ± 4.46 ab	39.8 ± 8.97a	52.0 ± 3.94a

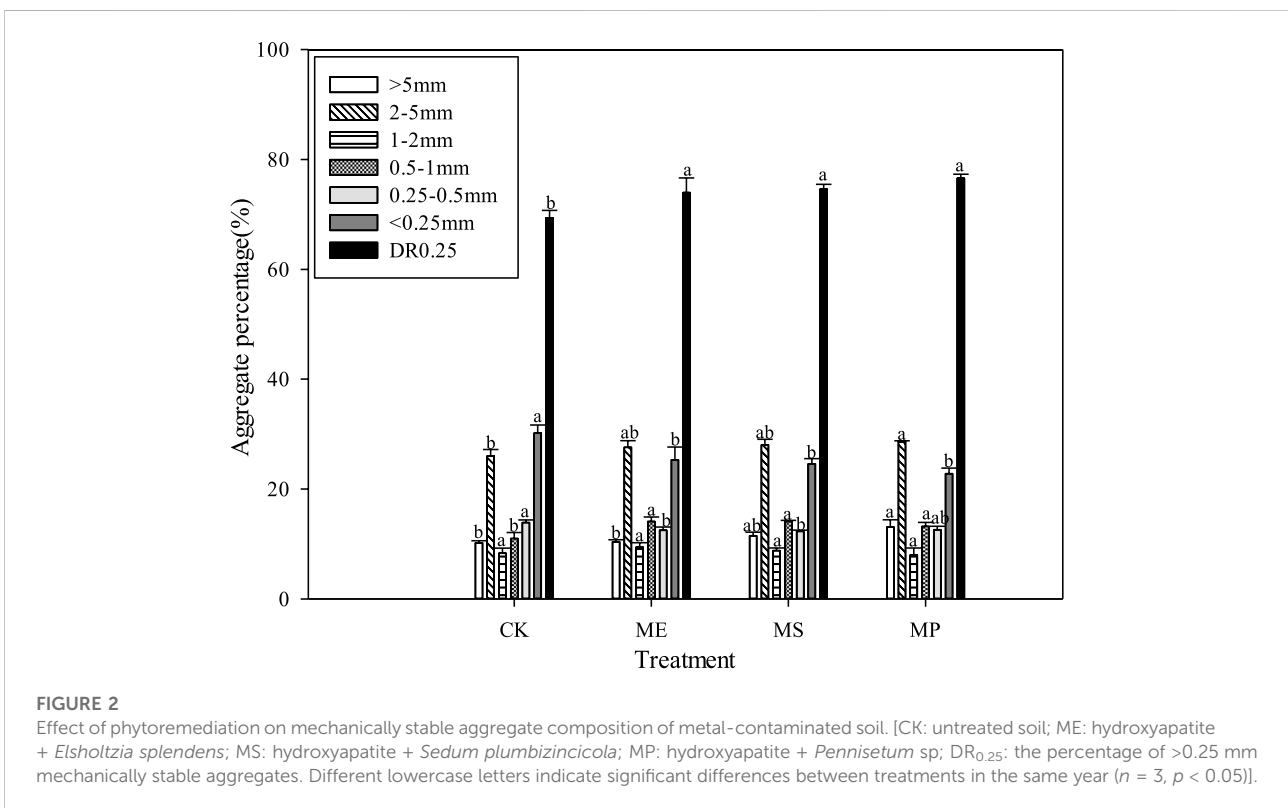
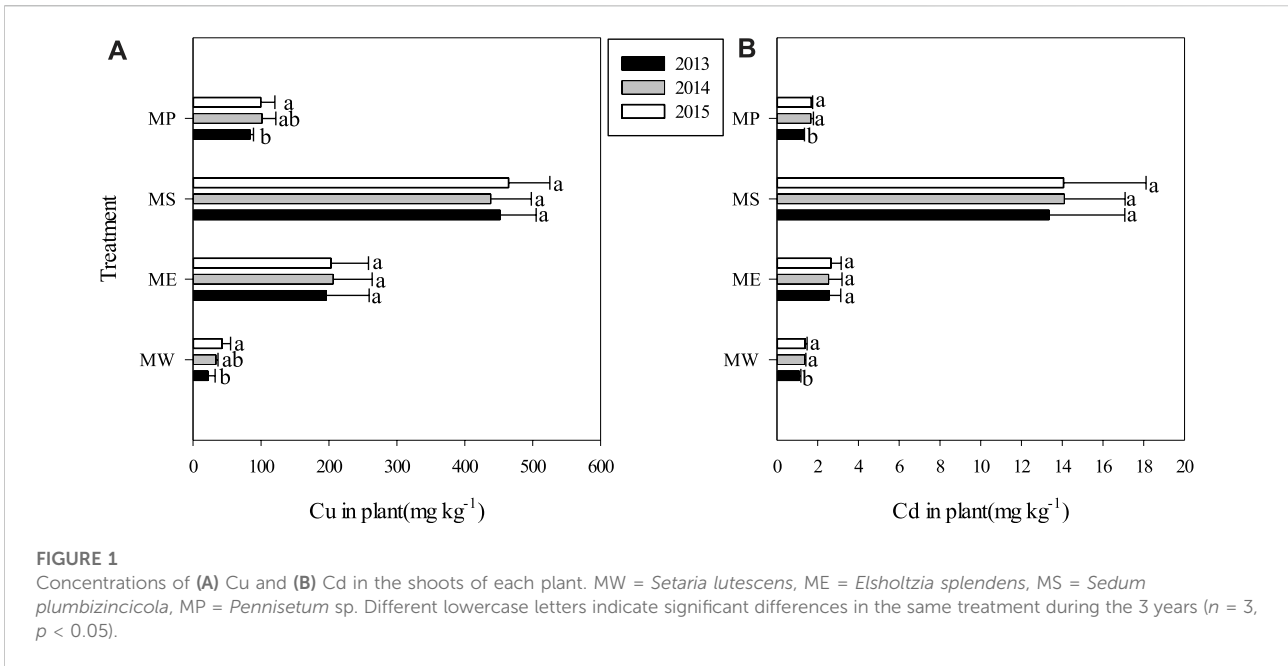
CK, untreated soil, MW, hydroxyapatite + *Setaria lutescens*, ME, hydroxyapatite + *Elsholtzia splendens*, MS, hydroxyapatite + *Sedum plumbizincicola*, MP, hydroxyapatite + *Pennisetum* sp. Different lowercase letters indicate significant differences between treatments in the same year ($n = 3, p < 0.05$). — indicates no plant growth.

concentrations of Cu and Cd in *Elsholtzia splendens* and *Sedum plumbizincicola* were quite different, they had similar removal efficiency of Cu and Cd after integrating the biomass. According to our study results and the living habits of different plants, we suggest that the intercropping of *Sedum plumbizincicola* and *Pennisetum* sp. or *Elsholtzia splendens* aided by amendments could be an efficient phytoremediation method. However, this assumption needs to be verified by further field experiments.

3.3 Changes in the soil aggregate structure

In 2015, the percentage of >0.25 mm mechanically-stable aggregates (DR_{0.25}) in the soil was 69.4%–76.6% (Figure 2), which

was lower than that in many reports in this area (84.6–90.2%) (Zheng et al., 2015a). This indicates that the soil physical structure in this area was poor, which might be related to the serious soil pollution in this area. After 3 years of phytoremediation, the soil DR_{0.25} was significantly increased. Moreover, the three phytoremediation modes mainly increased the percentage of >5 mm, 2–5 mm and 0.5–1 mm aggregates in mechanically stable aggregate, especially > 2 mm aggregates. Among the three plants, *Pennisetum* sp. had the greatest improvement on soil macroaggregates, especially for aggregates >5 mm and 2–5 mm, which were significantly increased by 29.0% and 10.1% compared with CK treatment, respectively. However, there was no significant difference among the three plant treatments in the impact on aggregates of 1–2 mm, 0.5–1 mm, and 0.25–0.5 mm, which indicates that



Pennisetum sp. had the greatest improvement on macroaggregates in the process of phytoremediation during the past 3 years and it could best improve the physical

structure of soil. This might be due to the large biomass of *Pennisetum* sp., the higher vegetation coverage could return more litter, the input of more exogenous organic matter increased the

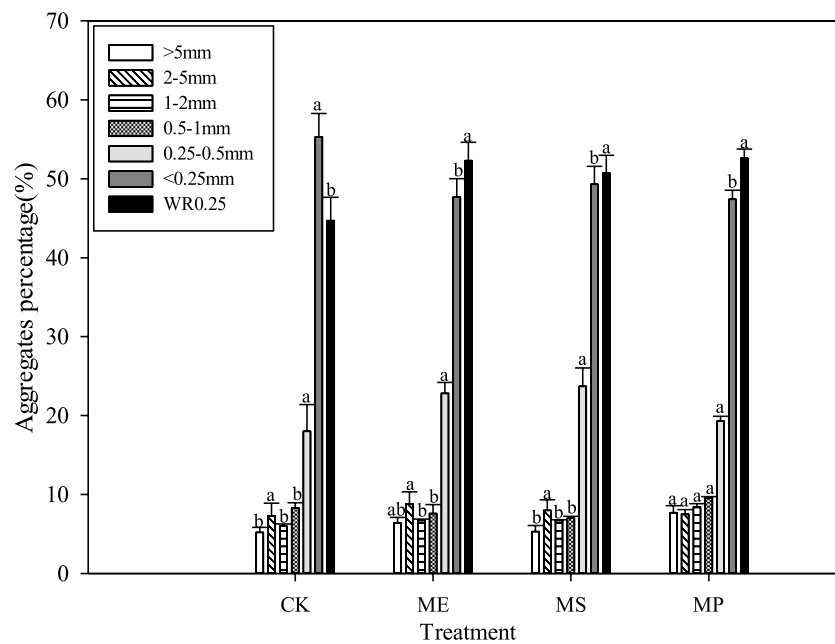


FIGURE 3 Effect of phytoremediation on water stability aggregates composition of metal-contaminated soil. [CK: untreated soil; ME: hydroxyapatite + *Elsholtzia splendens*; MS: hydroxyapatite + *Sedum plumbizincicola*; MP: hydroxyapatite + *Pennisetum* sp. Different lowercase letters indicate significant differences between treatments in the same year ($n = 3, p < 0.05$).].

content of organic matter in the soil, and the small aggregates were bonded and aggregated into larger aggregates through organic matter cement (Zeng et al., 2018).

In terms of the composition of soil water-stable aggregate, the percentage of >0.25 mm water-stable aggregate ($WR_{0.25}$) in each treatment was 44.7%–52.6% (Figure 3). After 3 years of phytoremediation, the percentage of soil water-stable aggregate could be significantly increased. However, similar to the mechanically-stable aggregate, there was no significant difference among the three plant treatments. By analyzing the particle size composition of water-stable aggregate, it was found that the increased part was mainly concentrated in the > 5-mm aggregate. Meanwhile, the increasing range in the other particle size compositions was not significant. Even the 0.5 to 1-mm aggregate in *Elsholtzia splendens* and *Sedum plumbizincicola* treatments had a significant downward trend which was the same as the research results of Zheng et al. (2015a) who found that vegetation restoration could mainly increase the percentage of the > 5-mm aggregate but had little effect on the percentage of other particle size aggregates.

The correlation between mechanical stability aggregates ($DR_{0.25}$), water stability aggregates ($WR_{0.25}$), and soil organic matter showed that $DR_{0.25}$ and $WR_{0.25}$ were significantly positively correlated with soil organic matter content, and the correlation coefficients R^2 were 0.829** and 0.741** ($p <$

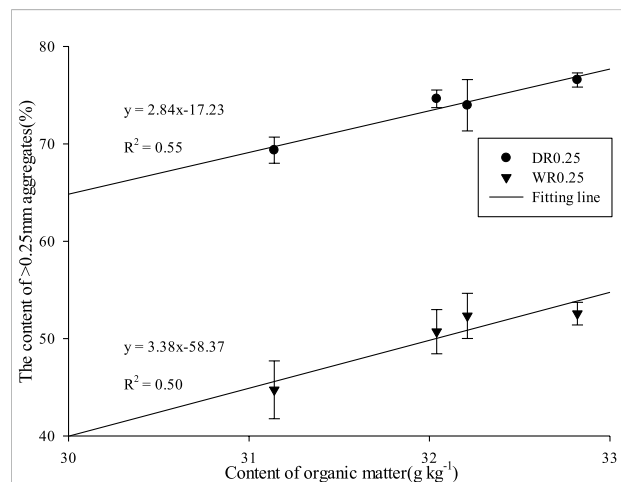


FIGURE 4 Fitting of >0.25 mm aggregates percentage ($DR_{0.25}$, $WR_{0.25}$) and organic matter content. $DR_{0.25}$: the percentage of >0.25 mm mechanically stable aggregates, $WR_{0.25}$: >0.25 mm water-stable aggregates.

0.01), respectively. To obtain the relationship between soil organic matter and >0.25 mm mechanically stable aggregate and water-stable aggregate, we further fitted them linearly with the content of soil organic matter (Figure 4). The result

TABLE 3 Effects of phytoremediation on MWD and GMD of metal-contaminated soil.

Treatment	Mean weight diameter (MWD) (mm)		Geometry weight diameter (GMD) (mm)	
	Mechanically stable aggregates	Water-stable soil aggregates	Mechanically stable aggregates	Water-stable soil aggregates
CK	1.98 ± 0.037c	0.95 ± 0.094b	0.79 ± 0.026c	0.32 ± 0.020b
ME	2.08 ± 0.061bc	1.10 ± 0.073 ab	0.91 ± 0.053b	0.38 ± 0.022a
MS	2.16 ± 0.035ab	1.00 ± 0.092ab	0.95 ± 0.024ab	0.35 ± 0.021ab
MP	2.30 ± 0.073a	1.18 ± 0.065a	1.02 ± 0.029a	0.40 ± 0.17a

CK, untreated soil; ME, hydroxyapatite + *Elsholtzia splendens*; MS, hydroxyapatite + *Sedum plumbizincicola*; MP, hydroxyapatite + *Pennisetum* sp. Different lowercase letters indicate significant differences between treatments in the same year ($n = 3$, $p < 0.05$).

shows that there was a linear relationship between $DR_{0.25}$, $WR_{0.25}$, and soil organic matter, which is consistent with the research results of Deng (Lei et al., 2018). Through the results of this correlation analysis, we infer that the main reason for the increase of the percentage of >0.25 mm mechanically stable aggregate and water-stable aggregate might be the increase in soil organic matter content. Increase of organic carbon concentration promoted the cementation process of aggregate and accelerated the transformation from small aggregates to large aggregates during the phytoremediation. This change played an important role in improving the soil structure and promoting the quality recovery of the metal-contaminated soil.

3.4 Effect of phytoremediation on the stability of soil aggregates

3.4.1 Aggregate size

In this study, the MWD and GMD of mechanically stable aggregate and water-stable aggregate were significantly increased after 3 years of phytoremediation (Table 3). The MWD and GMD values of the two types of aggregate were the highest in the treatment of *Pennisetum* sp. In terms of MWD, the treatment of *Pennisetum* sp. improved the mechanical stability aggregate and water stability aggregate by 16.2% and 24.2% compared with CK treatment, while in terms of GMD, the mechanical stability aggregate and water stability aggregate were increased by 29.1% and 25.0%, respectively. This indicates that the phytoremediation by *Pennisetum* sp. could improve the stability of soil aggregates, which was conducive to the improvement of soil physical structure. At the same time, it was found that the MWD and GMD of mechanically stable aggregate in each treatment were greater than those of water-stable aggregates, which indicated that mechanically stable aggregate was the main aggregate type in this soil. This result is consistent with other regional studies on the stability of soil aggregates.

Correlation analysis showed that the percentage of large aggregate was significantly correlated with the index of aggregate stability, which indicates that the stability of soil aggregate was mainly determined by the percentage of large aggregates (Table 4). The percentage of soil aggregate with each particle size was significantly correlated with the index of aggregate stability, and the positive and negative correlation was bounded by a particle size of 2 mm. This result proves that phytoremediation promoted the formation of large aggregate from fine aggregate and micro-aggregate, resulting in the strengthening of the aggregate's stability. This was different from the findings of Jiang et al. (2016), who found that the positive and negative correlation between MWD and GMD values and aggregate percentage of each particle size was bounded by 1 mm. This might be related to the high percentage of large aggregates and strong stability of aggregates in calcareous soil. In addition, compared with aggregate with particle size <0.25 mm, > 0.25 mm aggregate was more relevant to MWD and GMD (Table 5). Moreover, the percentage of > 0.25 mm particle size aggregate was significantly higher than that of <0.25 mm particle size aggregate. This shows that in the process of transformation from micro-aggregates to large aggregates, 0.25 mm is a critical value (Florio et al., 2019).

3.4.2 Stability rate and fractal dimension of aggregates

This study found that the soil aggregate stability rates (AR) of phytoremediation treatments were significantly increased by 5.4%–9.7% compared with the CK treatment (Table 5). In terms of fractal dimension (D), 3 years of phytoremediation could significantly reduce the fractal dimension of mechanically-stable aggregate, with a reduction range of 1.9%–3.8% (Table 5). This result shows that the particle size composition of soil aggregate was more uniform and the soil physical structure had been improved after 3 years of phytoremediation.

It was found that $DR_{0.25}$ and $WR_{0.25}$ had a very significant negative correlation with fractal dimension (D), while $WR_{0.25}$ had a significant positive correlation with aggregate stability rate

TABLE 4 Correlation between mechanical stability and water stability aggregates percentage and MWD, GMD, AR, and D.

Percentage of >0.25 mm soil aggregates	MWD	GMD	AR	D
DR _{0.25}	0.857**	0.966**	0.466	-0.926**
WR _{0.25}	0.657*	0.857**	0.893**	-0.582*

DR_{0.25}, >0.25 mm mechanically stable aggregates; WR_{0.25}, >0.25 mm water-stable aggregates; MWD, the mean weight diameter; GMD, geometric mean diameter; AR, aggregates stability rate; D, fractal dimension.

TABLE 5 Effects of phytoremediation on the stability rate and fractal dimension of soil aggregates polluted by metals.

Treatment	Stability of soil aggregates (AR, %)	Fractal dimension (D)	
		Mechanically stable soil aggregates	Water-stable soil aggregates
CK	64.48 ± 1.66b	3.15 ± 0.010a	3.54 ± 0.056a
ME	70.74 ± 2.03a	3.09 ± 0.021b	3.47 ± 0.036a
MS	67.96 ± 2.28 ab	3.07 ± 0.014b	3.52 ± 0.048a
MP	68.65 ± 1.08 ab	3.03 ± 0.015c	3.44 ± 0.026a

CK, untreated spoil; ME, hydroxyapatite + *Elsholtzia splendens*; MS, hydroxyapatite + *Sedum plumbizincicola*; MP, hydroxyapatite + *Pennisetum* sp. Different lowercase letters indicate significant differences between treatments in the same year ($n = 3, p < 0.05$).

(AR,%) (Table 4), which shows that WR_{0.25} made a greater contribution to improving the aggregate stability rate. In this study, we found that the increase of organic matter content under the condition of phytoremediation played an important role in the formation and stability of water-stable aggregate. This is also consistent with the conclusion that additional organic carbon could significantly improve the percentage, average mass diameter, and stability of soil water-stable aggregates (Dong et al., 2014). Micro-aggregates in the soil form water-stable large aggregate through the adhesion and cementation of organic matter (Rabbi et al., 2019; Hong et al.2020). Therefore, increasing the content of soil organic matter is an effective way to improve the stability of aggregates and prevent soil erosion.

A large number of studies have confirmed that with the increase of phytoremediation years, the vegetation coverage gradually increases, the aboveground biomass gradually increases, and the litter returns to the soil also increase (Catania et al., 2018; Lei et al., 2018). The decomposition of litter increases the content of soil organic matter, and the cementation of organic matter is strengthened, which enhances the agglomeration of the aggregates (Maietta et al., 2019). Vegetation restoration in the process of phytoremediation will increase the root biomass and root exudates, improve the soil structure, promote the formation and structural stability of soil aggregate, and improve the soil erosion resistance and soil erodibility decreased (Lxab et al., 2021).

3.5 Effect of phytoremediation on nutrient content of aggregates

In the process of phytoremediation, the law of organic carbon content in soil aggregates is as follows: when the particle size of soil aggregate was <1 mm, the content of organic carbon in aggregates increased with the increase of particle size; but when the particle size of aggregate was greater than 1 mm, the content of organic carbon in aggregates decreased with the increase of particle size (Figure 5). In all particle size aggregates, the organic carbon content of <0.25 mm aggregates was the lowest, which was 12.9–13.4 g kg⁻¹; and the organic carbon content of 0.5–1 mm aggregate was the highest, which was 23.0–25.4 g kg⁻¹. After 3 years of combined remediation with hydroxyapatite and different plants, the content of organic carbon in > 5 mm, 2–5 mm, and 1–2 mm aggregates were significantly increased by 6.3%–14.8%, 12.4%–25.5%, and 4.0%–12.2%, respectively. The pattern of the content of soil total nitrogen in soil aggregate was similar to the soil organic carbon. In all particle size aggregates, the soil total nitrogen content of <0.25 mm aggregate was the lowest, which was 0.85–1.02 g kg⁻¹; and the soil total nitrogen content of 0.5–1 mm aggregates was the highest, which was 1.45–1.91 g kg⁻¹. After 3 years of combined remediation with hydroxyapatite and different plants, the content of soil total nitrogen in > 5 mm, 2–5 mm aggregates were significantly increased by 16.9%–41.9% and 22.4%–46.4%, respectively. In contrast from soil organic carbon and total nitrogen, combined

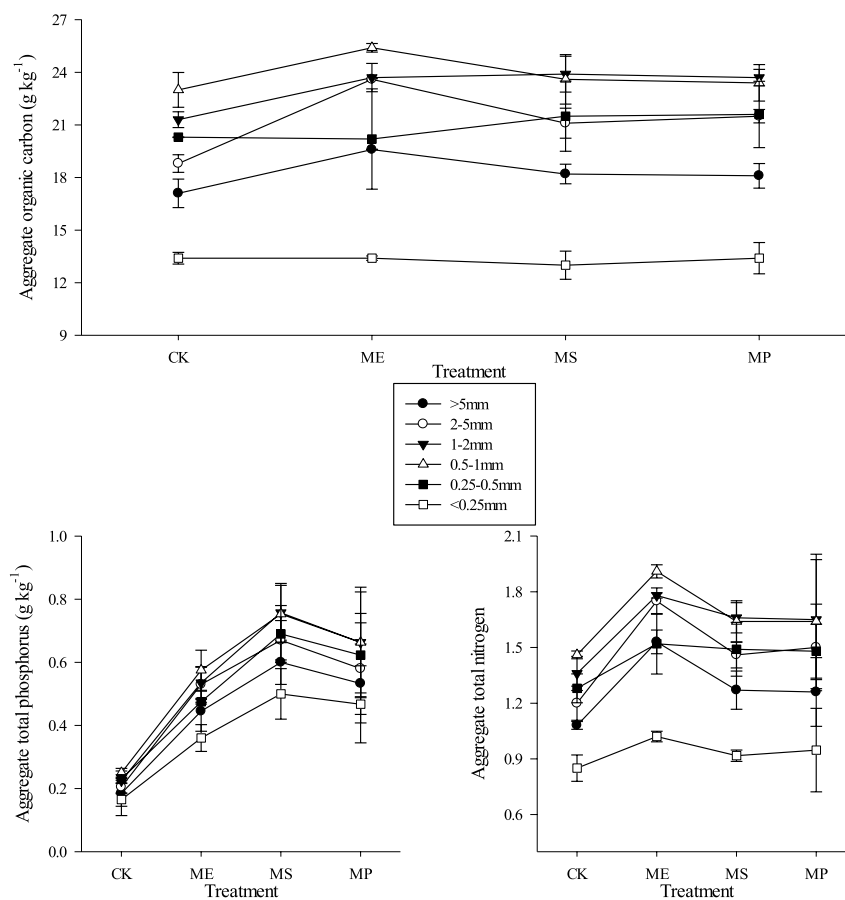


FIGURE 5 Distribution characteristics of soil organic carbon, total nitrogen, and total phosphorus concentration in soil aggregates relative to phytoremediation. (CK: untreated soil; ME: hydroxyapatite + *Elsholtzia splendens*; MS: hydroxyapatite + *Sedum plumbizincicola*; MP: hydroxyapatite + *Pennisetum* sp.).

remediation could significantly increase the content of phosphorus in all particle size aggregates. At the same time, the change of phosphorus content in aggregates with different particle size had no obvious pattern. These results are similar to other studies, which showed that the organic carbon and total nitrogen of soil aggregates with various particle size would increase after phytoremediation. This indicating that phytoremediation made soil aggregates reduce nutrient loss and led to a stronger ability to collect organic matter (Xue et al., 2008; Nguyen et al., 2016).

Moreover, it was found that the organic carbon content of large aggregate was higher than that of the whole soil in this study, which showed that large aggregates had a certain enrichment effect on organic carbon (Xiao et al., 2017). Phytoremediation had an important impact on the distribution of organic carbon in soil aggregates. In the process of phytoremediation, the increase of organic carbon reserved in large aggregates was significantly faster than that in micro-aggregates. This shows that the newly added carbon

might first appear in large aggregates, thus increasing the distribution proportion of organic carbon in large aggregates (GALE et al., 2000). The soil in this study was abandoned for a long time, the surface was bare, and the content of soil organic carbon was low. With the progress of phytoremediation, the content of soil organic carbon increased, which made the content of large aggregates increase faster, and improved the particle size and stability of aggregates (GALE et al., 2000).

In this study, it was found that the distribution of nitrogen in aggregates was similar to that of organic carbon. The results of this study were similar to the results of previous studies on the distribution of carbon and nitrogen in different forest vegetation types (Liu et al., 2019). In the process of vegetation change from grass-shrubwood-arbor forest, with the improvement of soil organic carbon content, other nutrients in the soil had also been improved, and there was a good positive correlation between soil organic carbon, total nitrogen, alkali-hydro nitrogen, and carbon nitrogen ratio (Hongkai et al., 2012).

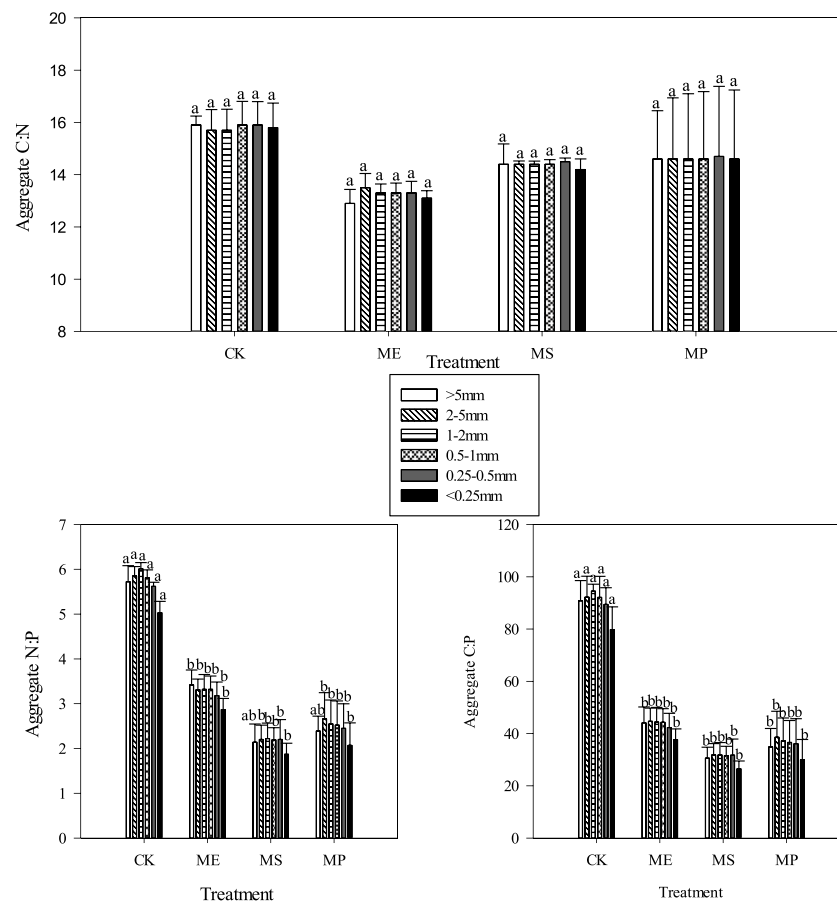


FIGURE 6
Stoichiometric characteristics of soil aggregates relative to phytoremediation [CK: untreated soil; ME: hydroxyapatite + *Elsholtzia splendens*; MS: hydroxyapatite + *Sedum plumbizincicola*; MP: hydroxyapatite + *Pennisetum sp.* Different lowercase letters indicate significant differences between treatments in the same year ($n = 3, p < 0.05$).

Phosphorus in soil mainly comes from the weathering of geological rocks. The phosphorus content of southern red soil is mainly related to the soil forming process, weathering characteristics, and soil erosion related factors (Turner et al., 2007). At the same time, it is also affected by the decomposition of animal and plant residues (Hou et al., 2015). The strong weathering and leaching in low latitudes caused the low content of total phosphorus in the soil. In addition, the study area had suffered from serious soil erosion, the phosphorus had already been leached out, and the content of total phosphorus was very low. In this study, hydroxyapatite was added to the soil, hydroxyapatite could dissolve in the soil, which improves the content of phosphorus in the aggregate (Cui et al., 2017). At the same time, it was found that the decomposition of surface litter improved the accumulation of soil organic phosphorus, which improves the total storage of soil phosphorus. Therefore, the surface litter thickens and the total phosphorus content in mineralized soil

aggregate increased through microbial decomposition over time (Tian et al., 2013).

3.6 Effect of phytoremediation on stoichiometric characteristics of soil aggregates

Figure 6 shows the effect of phytoremediation on stoichiometric characteristics of soil aggregate. The variation range of C:N of aggregates with each particle size was 12.9–15.9 after the phytoremediation. There was no significant difference in C:N of all different particle size aggregates during different plant treatments. Meanwhile, the change of C:N with different particle size was also not significant. The variation range of C:P and N:P of each particle size aggregates were 1.87–6.01 and 26.5–94.5, respectively. The same as the C:N, there was no significant difference in C:P and N:P among different particle sizes aggregates. However, in contrast to the C:N,

the combined remediation of hydroxyapatite and phytoremediation significantly reduced the value of C:P and N:P in different particle size aggregates.

Soil stoichiometry was the balance coupling mechanism between soil organic carbon, nitrogen, and phosphorus. The balance coupling between the input/output of soil carbon, nitrogen and phosphorus and its effectiveness determined the carbon cycle and carbon fixation efficiency of the ecosystem (Quanchao et al., 2016). In this study, the low value of C:N in aggregates indicated that the “sink” ability of carbon and nitrogen was poor, which was not conducive to the sequestration of carbon and nitrogen nutrients in soil aggregates. The value of C:N in different particle size aggregates increased significantly with phytoremediation. This result could improve the solid stock of soil carbon and nitrogen and increase the “sink” function of soil aggregates carbon and nitrogen, which is of great significance to improve soil fertility and even global climate change.

In this study, hydroxyapatite and phytoremediation significantly reduced the value of C:P in different particle size aggregates. This might be mainly related to the dissolution of added hydroxyapatite and the release of a large amount of phosphorus. The reduction of C:P could made microorganisms release phosphorus in mineralizing soil organic matter, which promotes the absorption and utilization of phosphorus by plants. This is of great significance because it can help to alleviate the widespread problem of phosphorus deficiency in southern China.

Hydroxyapatite and phytoremediation significantly reduced the value of N:P in different particle size aggregates. The reason for this phenomenon is similar to that of C: P. In CK treatment, the N:P level of the soil aggregates was higher than the average level of subtropical soil N:P (Tian et al., 2013). Previous studies showed that the subtropical area was generally limited by P element. In the process of phytoremediation, the value of N:P in eroded red soil aggregates remained high, and the soil aggregates were obviously limited by the P element (Fan et al., 2015). However, in this study, the addition of hydroxyapatite significantly changed this phenomenon, the value of N:P was reduced to a low level, which is of great significance to solve the problem of soil phosphorus deficiency.

4 Conclusion

Applying hydroxyapatite to metal heavily contaminated soil combined with phytoremediation for 3 years can increase the content of mechanically-stable aggregates ($DR_{0.25}$) and water-stable aggregates ($WR_{0.25}$) by 6.6%–10.4% and 13.3%–17.5%, respectively. After 3 years of combined restoration of vegetation and hydroxyapatite, the mechanical stability and water stability aggregates mean weight diameter (MWD) are significantly improved by 5.1%–16.2% and 5.3%–24.2%, and the geometric average diameter (GMD) is increased by 15.2%–29.1% and

9.4%–25.0%. At the same time, it can significantly reduce the fractal dimension of soil mechanical stability aggregates by 1.9%–3.8%. Soil organic carbon, total nitrogen, and total phosphorus in aggregates with different particle sizes were significantly increased by different plant treatments. However, for the stoichiometric characteristics of aggregates, the combined remediation only significantly reduces the value of N:P and C:P in different particle size aggregates, and has no significant effect on the C:N. By applying hydroxyapatite in metal heavily contaminated soil and reducing the toxicity of metals, planting *Elsholtzia splendens*, *Sedum plumbizincicola*, and *Pennisetum* sp. can improve soil structure, alleviate soil phosphorus deficiency, and improve soil quality. Therefore, it is suitable to be popularized in the field remediation practice of metal heavily contaminated soil.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LX conducted all the experiments and wrote the manuscript. XX revise the language of the manuscript. JB conducted part of the experiments. DL conducted part of the experiments.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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