**Optimizing the formulation of engineering slag using modified organic materials and microbial inoculants**

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# Abstract

As China’s urbanization grows by leaps and bounds, engineering slag has turn into one of the broadest forms of solid wastes. The purpose of this research was to utilize engineering slag as a new planting substrate component to improve the soil environment in mine sites and address the growing shortage of land resources. The substrate optimal formulation was filtrated utilizing an orthogonal experiment involving four factors: slow-release fertilizer (SRF), microbial inoculants (MI), water-retaining polymer (WRP), and soil-to-slag ratio (SS). The results presented that SRF significantly increased the nutrients availability of the slag substrate. The addition of MI induced changes in the physicochemical properties of the substrate, ultimately affecting plant germination. Furthermore, at a concentration of 0.8% WRP had a significant effect on the physical properties and soil dehydrogenase (S-DH). However, 1% WRP was most favorable for plant growth. Exogenous soil significantly improved SOC content and Soil alkaline phosphatase (S-ALP) activity when applied at a dosage of 500 g. There existed significant correlations between soil properties and plant indicators. Afterwards we comprehensively analyzed the effects of 20 parameters, and from an economic perspective, the optimal parameters were as follows: SRF content of 1 g kg-1, MI content of 90 mL kg-1, WRP concentration of 1.0%, and SS of 30:70. Additionally, amplicon sequencing showed a positive impact on soil microbial community diversity due to the treatment. The results of this research will establish a theoretical foundation on combining microbial inoculants with external soil spray seeding techniques in mine sites reclamation.

# Keywords: Engineering slag, Planting substrate, Microbial inoculants, Microbial community, Mine sites reclamation

# Introduction

As economy develops and the acceleration of the urbanization process, engineering slag production reached 3 billion tons in 2020 and continues to increase annually (Duan et al., 2008). However, the resource utilization rate is less than 10% (Akhtar & Ksarmah, 2018; Ren, 2019). The engineering slag, randomly piled up, pollutes the water and soil (Kalbe et al., 2008; Lee et al., 2008), worsening plant roots development conditions and leading to serious soil erosion (Sun et al., 2017). This has become an important environmental issue to be solved in the process of urban development in China (Chen et al., 2020). The steep slope has unstable angles, thus inviting a persistent hazard of landslips and rock-falls (Gao et al., 2007; Milgrom, 2008). At the same time, the slag will also occupy land resources, which may not be immediately noticeable but further aggravate the issues of limited land resources in China due to a growing population. Therefore, recycling engineering slag is necessary to improve the soil environment and vegetation restoration.

Slag resource utilization, such as adding soil amendments, can advance the physicochemical properties of slag, making it a substrate conducive to plant growth (Guo et al., 2013; Zhang, 2013). The use of engineering slag as a plant growth substrate can reduce the need for stacking, relieve land pressure, eliminate safety hazards associated with stacking, and reduce soil erosion (Deng et al., 2016). However, the related research on the resource utilization of waste slag needs to be further explored.

Slag is blocky, unrestricted draining, and lacks topsoil and biological activity. Consequently, the primary limitations to vegetation establishment on slag are nutrient availability and low moisture holding capacity (Rowe et al., 2006; Salgueiro et al., 2020; Williamson et al., 2011). Treatments involving fertilizers, microbial inoculants, or water-holding polymers are promising approaches to enhance vegetation resilience on land, particularly for areas of low moisture insufficient moisture and nutrient availability (Caravaca et al., 2003; Sarvas et al., 2007). Soil nutrients not only meet the immediate growth needs of plants but also provide a medium term and beyond supply sufficient to maintain the pedosphere (Williamson et al., 2011). Slow-release fertilizer (SRF) slowly releases nutrients over an extended period (Zheng et al., 2016). Guan et al. (2014) reported that attapulgite-coated fertilizer not only exhibits excellent adsorption performance but also shows slow-release behavior for soil mineral N and available P. This property allows for better coordination between nutrient availability and plant demands. A recent review shows that a new type slow-release fertilizer, utilized biochar as a coating material (BSRFs), can actively improve soil water retention and soil fertility (Wang C. et al., 2022). Furthermore, in tests conducted in karst mountainous areas, it was found that BSRFs change the soil microbiome structure and functioning (Yan et al., 2021). Microbial inoculants can ameliorate soil fertility and plant growth (Singh et al., 2016). Deng et al. (2021) and Yin et al. (2016) found that microbial inoculants significantly enhanced soil aggregate stability, increased organic matter content, improved available nutrient levels, and raised soil enzyme activity in structurally poor soil. Meanwhile, it also significantly influenced the relative abundance of soil bacteria. Water-retaining polymers are widely applied in the ecological restoration of rocky soil (Didar et al., 2022). Previous studies have shown that aqua-dispersing-nano-binder (ADNB) and super absorbent polymer (SAP) can improve seed germination rate. This improvement is achieved by increasing soil strength, available water, field capacity, enzyme activity, SOC, and other factors (Huang et al., 2020a; Yang et al., 2014). However, research on the mixing of materials with slag has been rarely reported.

The purpose of this study is to explore the effects of attapulgite-based slow-release fertilizer, microbial inoculants, water-retaining polymer, and guest soil on slag planting substrate properties and optimize the dosage of each component. We specifically explored the improvement effects of these four ameliorants on the following aspects: (1) pore structure, water retaining capacity, and available nutrient of the soil; (2) nutrient conversion capacity and microbial metabolic activity; (3) plant growth; and (4) soil microbiome characteristics. This study aimed to determine the optimal formula for a planting substrate, providing theoretical support for the resource utilization of engineering slag.

# Materials and methods

## 2.1 Chemicals and plant materials

The components of the planting substrates include engineering slag, soil, slow-release fertilizer, microbial inoculants, and water-retaining polymer. The particle size of the engineering slag was less than 5mm, and it was collected from Mozidong limestone mine (106°92′E 29°24′N) in Banan District, Chongqing. China. The soil, primarily composed of organic matter such as peat and coconut bran in a 1:1 ratio, was purchased from " Huayuanmei Electronic Technology Company" in Sichuan, China. The slow-release fertilizer, provided by Chengdu University of Technology, contains 35% humic acid (HA), 20% attapulgite and 40% organic matter, which slowly release nutrients to sustain medium and long-term plant growth. The microbial inoculants, a mixture of three types of growth-promoting *Bacillus* strains isolated and purified (Li et al., 2021) from alpine meadow soil by a scientific research team (OD600=0.8), have the ability to promote phosphorus and potassium solubilization in minerals, while producing 3-indoleacetic acid to facilitate plant growth. The water-retaining polymer, independently developed by the research team, mainly consists of polymer materials and inorganic additives, commonly known as double - poly material, and has been obtained the Chinese invention patent. The grass plant, ryegrass (*Lolium perenne* L*.*), was selected because it is a pioneer specie for ecological restoration of rock slopes, with developed root systems and tolerance to barrenness (Liu & Zhang, 2014). *Lolium perenne* L*.* seeds were purchased from Yunlanmei Flowers Co. in Jiangxi, China.

## 2.2 Experimental design

The substrate optimal formulation was screened adopting an orthogonal experiment including four factors: slow-release fertilizer (SRF) content (1, 3, 5, and 0 g kg−1), microbial inoculants (MI) content (45, 90, 135, and 0 mL kg−1), water-retaining polymer (WRP) content (0.8, 1, 1.2, and 0%), and soil (g)-to-slag (g) ratio (SS) (500:500, 300:700, 100:900, 0:1000) (Table S1). The orthogonal test produced 16 different component proportions; the control group was treated with 100% slag. Three parallel tests were implemented for each group (Du et al., 2020; Li et al., 2017), resulting in a total of 51 groups (Table S2).

We conducted the experiment in a greenhouse with an average humidity of 50% and 25°C temperature for 75 days. On April 10, 2023, *Lolium perenne* L*.* seeds were manually sown in flowerpots with a height of 12cm and a diameter of 18.5cm at a rate of 0.5g kg-1 substrate. Seeds were sterilized with 10% H2O2 before sowing. To ensure the normal state of growth of *Lolium perenne* L*.*, irrigation should be sustained at 88 g per (80% of the field water capacity of the 100% slag group) No fertilizers were applied during the growth of the plants.

## 2.3 Analysis of soil physicochemical properties and enzymatic activity

Soil physical properties, i.e., bulk density, soil nature, saturated moisture content, total porosity, and capillary porosity, were obtained applying the cutting - ring method (Li et al., 2007). soil chemical properties, i.e., NH4+-N, NO3--N, soil organic carbon (SOC), available phosphorus (P), and available kalium (K), were measured using the methods mentioned in the other study (Han et al., 2023). pH and electrical conductivity (EC) were tested by the potentiometric method, where the ratio of soil to water is 2.5:1 (Ma et al., 2023). Soil enzymatic activities were measured using enzyme linked immunosorbent assay kits manufactured by Keming Biological Technology Co., Ltd. in Suzhou, China.

## 2.4 Determination of plant growth

The germination rate can be calculated by the formula: (the number of germinated seeds/the number of tested seeds) ×100%. Remove the plants root soil and rinse with water when they are harvested, firstly dried at 105 °C in the lab oven for 20 min (Du et al., 2020) and subsequently dried at 60 °C for 24 h. Measuring plant height and root length with a scale, while biomass (dry weight) was measured using an analytical balance to assess the growth conditions of *Lolium perenne* L*.*.

## 2.5 Obtaining the optimal ratio

Twenty indicators (bulk density, EC, natural moisture content, saturated water content, capillary porosity, total porosity, pH, SOC, NH4+-N, NO3--N, available K, available P, germination rate, plant height, root length, biomass, S-SC, S-ALP, S-CAT, and S-DH) were obtained in the above experimental method. The optimal ratio should be the result of comprehensive consideration of these parameters. Therefore, we referenced to principal component analysis (PCA), which adopts the concept of dimensionality reduction to make comprehensive indicators represent various types of information in each variable. Therefore, these indicators can be selected to represent the principal components (Yang et al., 2020).

The total analysis results of the variance involved the eigenvalue (ei, i is the ith eigenvalue), variance contribution rate (λi), and cumulative variane contribution rate. Generally, eigenvalues greater than 1 were considered as principal components. Using λi as a weight and combining it with Eq. (1), we calculate the final score Y for different formulation treatments. At the same time, the scores of each planting substrate are ranked, and the optimal planting substrate is selected based on the ranking. The scoring expression of the principal component Yi is given by Eq. (2), where Zxj represents the standardized values of the twenty parameters (xj is the jth indicator from top to bottom in tables), and Uij is the correlation coefficient between the primary variable and the principal component (Zhao et al., 2022).

(1) (2)

(3)

## 2.6 Microbial diversity analysis

Microbiological analysis was executed for the fresh soil samples gathered from treatments with different formulation when the experiment finished. Fresh soil samples were collected weighing 5.0 g each, placed in an incubator with ice and transferred to the analytical laboratory, and subsequently stored at -80℃. The samples were unfrozen overnight at 4 °C and then sieved (Ø 2 mm), with special care taken to remove animals, seeds, roots, and plant materials. DNA extraction and high-throughput sequencing were implemented following a previously mentioned method (Benjamini & Hochberg, 1995). The extracted sequences were compared with reference sequences from the SILVA database (Desantis et al., 2006). The subsequent analysis was conducted on the online Personal Cloud platform (http://www. genescloud.cn/home).

## 2.7 Data analysis

Statistical software SPSS 23.0 (IBM, USA) and Excel 2016 (Microsoft, USA) software were used to optimize and process the data, performed analysis of variance (ANOVA), followed by a correlation analysis to illustrate the relationship among these 20 indicators. The data were analyzed using the ROUND function in Excel and were presented in the form of the mean ± standard deviation. Differences were regarded significant at P < 0.05 and highly significant at P < 0.01. Additionally, each experimental group was repeated three times to meet the requirements for ANOVA.

# Results and discussion

## 3.1 Physical properties of soil

Bulk density is the elementary physical properties indices of soil (Wang et al., 2019). Compared to the control group, all of the experimental groups showed significant improvement in soil bulk density (P < 0.05). The bulk density reduced to 0.992 g cm-3 when the ratio of soil to slag is 30:70 (Fig. 1a), illustrating a significant effect of SS in decreasing the bulk density of the substances (P < 0.01). Additionally, the physical properties of the substrates were effectively ameliorated by increasing the amount of soil to 500 g, which is line with the finding of a predecessors' research (Chenot Lescure et al., 2022). However, the general minimum standards is determined to be 0.9 g cm-3 in the slope areas(Du et al., 2020), when reconstructing slag vegetative substrate for areas with steep rock slopes for stability consideration. Therefore, more SS is not necessarily better.

Both capillary porosity and total porosity showed an opposite trend to bulk density (P < 0.01). When the concentration of WRP was 0.8%, the total porosity reached up to 45.83%, and the capillary porosity can reach 34.58%. These results may be attributed to WRP, which enhances the interparticle bonding, generates a large number of adhesive bodies, and transforms medium-sized pores to minute pores, improving soil agglomeration (Zhu et al., 2020). As shown in Fig. 1, an increase in the application of MI resulted in higher capillary porosity and total porosity (P < 0.05). This result may indicate that carbon dioxide release through microbial respiration deforms the soil structure, leading to an increase in porosity, which is likely to facilitate root soil aeration and water molecule movement (Helliwell et al., 2014). However, it is important to maintain the SRF within an appropriate range. Similar to WRP, increasing excessively the dosage of SRF led to a decrease in both total porosity and soil natural moisture content. It is possible that the HA present in SRF effectively improved the soil physical properties. Li et al. (2020) also found that adding the minimum amount of 3 g kg-1 of humic acid had the most desirable result on improving soil porosity. Electrical conductivity (EC) general serves as an important comprehensive indicator of changes in soil analysis (Ss et al., 2015; Stadler et al., 2015). When the SS was 50:50, the EC value of soil was significantly far more than in the other treatment groups.

## 3.2 Chemical properties of soil

The results of pH, SOC, NH4+-N, NO3--N, available K, and available P are presented in Table S4. In most cases, the addition of exogenous materials to the original slag highly improved chemical properties (P < 0.05). Soil pH is often used as a fundamental indicator for soil remediation (Du et al., 2020). The addition of SRF significantly reduced PH (Fig. 2a). However, there was no significant difference among the SRF treatment levels. This may be attributed to the narrow HA application amount range (Ampong et al., 2022). Furthermore, pH decreased as the soil ratio increased (P < 0.01), possibly due to the near-neutral nature of the exogenous soil. Compared to other factors, SS had a more pronounced impact. Specifically, adding 500 g of soil obviously increased the values of SOC and available nutrients (NO3--N, available K, available P) of the planting substrates (P < 0.01). Similarly, SRF significantly increased the available nutrients (NH4+-N, NO3--N, available K, available P) in the soil. This finding may be attributed to the presence of HA, which can accelerate the release of nutrient elements and increase the availability of soil nutrients (Li et al., 2020; Maji et al., 2017). The potassium solubilizing and phosphorus solubilizing microorganisms presented in MI can promote the ripening of the slag and significantly increase the values of available K and available P (P < 0.05). All these factors are conducive to the reconstruction of slag planting substrate.

## 3.3 Soil enzyme activity

S-SC serves as an essential parameter of soil fertility, which is associated with the soil ecosystem carbon cycle (Wang et al., 2020). The WRP with a concentration of 0.8% (C1) significantly increased soil S-SC activity (Fig. 3a), which is keeping with the result of an earlier research (Ma et al., 2020), where water-retaining materials increased both sucrase and catalase activities under microorganism treatments. The S-SC activity was significantly enhanced by increasing the dose of MI (P < 0.05), possibly related to biological metabolic activity (Jing et al., 2022; Wang H. et al., 2022).Other factors had a smaller impact on S-ALP compared to SS (Fig. 3b). This aligns with the change in available P content in the soil (Fig. 2f) and confirms the significant role of S-ALP in promoting organic phosphate mineralization and increasing available P content in soil (Li et al., 2021; Xu et al., 2022). The addition of SRF, MI, and WRF had significant effects on S-CAT (P < 0.05). However, in the absence of SRF application, S-DH was actually significantly higher than in other levels. This may be attributed to attapulgite adsorbing enzyme molecules present in soil, generating an isolated effect on the enzymatic reaction binding sites, thereby inhibiting soil enzyme activity (Wang et al., 2023). S-DH can be considered as a susceptive marker of soil microbial active quantity and metabolic activity (Garcia et al., 1997; Luo et al., 2015). Moisture directly influences soil microbial composition and metabolic activity (Brockett et al., 2012). The soil water regime significantly influenced the activity of S-DH (Siebielec et al., 2020). This study showed that 1.2% (C3) WRP significantly increased S-DH activity, which aligns with a previously reported study (Rossato et al., 2011).

## 3.4 Plant germination and growth

The related results of plant growth in each combined treatment are visually presented in Table S6. In comparison to the 100% slag group, germination rates were significantly higher in N2, N5, N14, and N1 treatments, with increases of 37%, 27%, 26%, and 21%, respectively (P < 0.01). In the case of surface soil on a slope, plants growth and development require a certain void structure and permeability. However, the excessive use of polymer adhesive can cause soil to harden, which in turn leads to poor soil aeration (Huang et al., 2020b). This condition is not conducive to seed germination (Bu et al., 2020). The germination rate initially increased and subsequently decreased with increasing WRP concentration (Fig. 4a). In addition, the germination rate showed the similar trend under MI treatment. This phenomenon may result from the excessive addition of *Bacillus*, leading to increased phenol and other harmful substances that suppress germination (Xiao et al., 2020). Analyzing the plant height, we found that N2, N5, N6, N8, and N12 performed better than the control group (P < 0 .01) (Fig. 4b). An increase in SRF was favorable for plant height (P < 0.05), with the optimal result observed with a medium amount of SRF (3 g).

As shown in Table 6, the plant root length in both N1 (9.40 cm) and N2 (8.80 cm) was observably higher values than that in the 100% slag group (4.54 cm). Similar conditions were reflected for germination rates, with the highest value found in N2 (91.47%). As shown in Fig. 4, reducing slag content to 500 g and using moderate or even small amounts of exogenous amendments significantly beneficial to plant height and root length. promoted plant growth (root length, plant height, biomass) indicates a suitable soil condition and adequate nutrients for *Lolium perenne* L. great development. These results may arise for the reason that exogenous amendments can reduce cracks in slag structure, increase soil compressive strength and cohesion, improve crack filling ability, and provide essential plant macro and micronutrient elements (He et al., 2022; Reddy & Revathi, 2019; Xie et al., 2011). However, when the dosage of exogenous materials is excessively high, it can result in significant substrates loosening and high concentrations of microelements, which, in turn, may delay or hinder plant growth (Nan et al., 2016; Passioura, 2002; Veen et al., 1992). It is a remarkable fact that assessing the growth of annual *Lolium perenne* L*.* based solely on biomass may not always be accurate. Previous research has established that *Lolium perenne* L*.* is a 3 - leaves plant, meaning that at any given time, there are typically only 3 green leaves, the senescence of first leave is often accompanied by the initiation of a fresh leaf (Fulkerson & Donaghy, 2001; Robson, 1973). Due to the different substrate conditions in each pot, the growth rate and tillering stage of *Lolium perenne* L*.* differed among treatments, potentially resulting in smaller biomass in some treatment groups.

## 3.5 Correlation analysis of planting substrate evaluation indexes

To more accurately describe the internal mechanism of slag improvement and assess the optimal planting substrate proportion comprehensively, this study conducted Spearman correlation analysis on soil physicochemical properties and plant indicators*.* As shown in Fig. 5, indicating certain relationships between different indicators. Bulk density presented an observably inverse correlation with most of the indices and biomass. The natural moisture content reflected a positive correlation with the germination rate and root length (p < 0.05, p < 0.01). Saturated water content was positively connected with plant height and root length (p < 0.05, p < 0.01). Both capillary porosity and total porosity exhibited a positive correlation with root length, whereas pH presented a evidently negative connection with root length. NH4+-N existed a highly positive relation with biomass. NO3--N revealed a positive connection with plant height, biomass, as well as germination rate, but extremely with root length. Moreover, EC, available K, available P, S-ALP and S-CAT activities were obviously positive related with root length, while available P and S-DH activity exhibited a positive relation with biomass. and S-DH activity was positively connected with root length. In summary, the decrease in bulk density and pH of the planting substrates led to an increase in capillary porosity, total porosity, saturated moisture content, and soil fertility, thereby promoting the growth of *Lolium perenne* L*..*

**3.6 Screening and optimization of substrates proportion**

After conducting a correlation analysis of *Lolium perenne* L*.* indicators, it is evident that significant correlations exist in the physicochemical properties and certain plant growth indicators. To transform various indicators into comprehensive variables for analysis, this study used principal component analysis to derive the final scores for each experimental group. Simultaneously, combined with the price of exogenous materials, the most suitable planting substrate is selected. The Table 1 indicated that the cumulative variance contribution rate of the five factors is 83.573%, exceeding 80%. Hence, these five factors can be considered as principal component.

The principal component comprehensive score was calculated on 16 planting substrates, and as an example, the first principal component formula of N1 planting substrate is:

Y1= -0.322 \* Zx1 + 0.296 \* Zx2 + 0.293 \* Zx3 + 0.309 \* Zx4 + 0.167 \* Zx5 + 0.253 \* Zx6 + -0.266 \* Zx7 + 0.266 \* Zx8 + 0.116 \* Zx9 + -0.125 \* Zx10 + 0.299 \* Zx11 + 0.26 \* Zx12 + -0.17 \* Zx13 + 0.291 \*Zx14 + 0.214 \* Zx15 + 0.118 \* Zx16 + 0.017 \* Zx17 + -0.03 \* Zx18 + 0.17 \* Zx19 + -0.065 \* Zx20. The formulas for the other four principal components and so on are calculated similarly. Subsequently, the total score for the N1 planting substrate is determined to be 2.203 using Eq (1). Following the same method, comprehensive scores of all 16 groups of planting substrate formulations can be obtained (Table S9).

The planting substrate groups were ranked from highest to lowest final score as follows: N1> N7> N12> N14> N2. Therefore, considering cost-saving factors, we have selected N2 as our final choice. Based on the comprehensive analysis, the optimal ratios for the slag planting substrates were determined as follows: a SRF dosage of 1 g kg-1, a MI dosage of 90 mL kg-1, and a WRP concentration of 1%, a SS of 30:70.

**3.7 Soil** **microbial diversity**

Soil microorganism diversity, activity, and biomass serve as parameters of measuring soil fertility and productivity. In reality, good soil quality, characterized by abundant and diverse microbial community and high activity levels, is a precondition for plant growth (Dincă et al., 2022). Soil amendments not only improve soil pore structure and serve as a supplier of nutrients, but they can also intensely influence the soil microbial (Pérez-Piqueres et al., 2006). For the purpose of analyzing response of microflora to SRF, MI, WRP, and SS, we determine the operational taxonomic units (OTUs) and diversity indices for different treatment through sequence analysis. A total of 917,597 high-quality sequences (Table S10) were counted into 14,238 OTUs be integrated from these sequences by cluster analysis. Additionally, Good' s coverage were all greater than 99% (Table S11) and the rarefaction curves (Fig. 6a), showing the actual condition of the soil microbial community in all treatments. There exists positive correlation between the Chao 1 index and microbial richness, both the Simpson index and Shannon index values positively reflect microbial community diversity (Li et al., 2023). The Chao 1 index of N1, N2, N7, N12, and N14 were obviously higher than that the 100% slag group (N18), indicating that five formulations calculated by the principal component analysis impacted positively on microbial richness in soil (Table S11). The Shannon index and Simpson index (N8, N16) were higher than those of the other groups, which may be attributed to a delayed microbial response, significantly alleviating intra- and interspecific competitions. Therefore, this process can induce microbial community to exhibit unusually high species diversity in oligotrophic environments (Yang et al., 2021).

Proteobacteria were the dominant phyla in the soil (Fierer et al., 2007) , with Gammaproteobacteria and Alphaproteobacteria as the main classes in treatments (Fig. 6b). This could be attributed to the adaptability of Proteobacteria in extreme environments, such as alkaline soils with limited nutrients (Ke et al., 2021). According to the flower plot (Fig. 6c), the distribution of OTUs in the slag dealt with different doses of amendments combinations can be reflected. Soil substrates samples under each treatment included 36 mutual OTUs, whereas the quantity of different OTUs presented in the six treatments we focused on were as follows: 996 (N1), 914 (N2), 921 (N7), 1148 (N12), 1214 (N14), and 532 (N18). This indicated that these amendments had an obvious impact on the microbial community diversity. Additionally, on premise of the genus level, the resulting heat map is shown (Fig. 6d). In N10, *Terrimonas* is the dominant genus, whereas *Pseudoxanthomonas* is the major genus in N3. The microbial community composition was various among different formulations, revealing that bacteria may adapt to different soil environment by adjusting their abundance.(Niu et al., 2020).

**4. Conclusions**

In this research, we explored the impact of modified organic materials and microbial inoculants (SRF, MI, WRP, SS) on physicochemical properties, enzyme activity and microbial diversity of the soil and plant growth. The results shown that slag, when used as a component in conjunction with SRF, MI, and WRP, effectively improved the physicochemical properties of the slag with poor vegetative property, promoted plant development and enriched the microbial community. Therefore, the slag can be used as a planting substrate for resource utilization. The optimal composition ratio for the slag planting substrate is as follows: 1 g kg-1 of SRF content, 90 mL kg-1 of MI content, 1.0% WRP concentration, and a SS ratio of 30:70. The results could establish a theoretical foundation and a reference for soil amelioration and vegetation restoration in limestone mine sites.

# Author contribution statement

**Yuxin Zou**: Writing original draft, Conducting experiments, Software. **Qi Li**: Supervision, Resource Data formal analysis. **Ningfei Lei & Xiangjun Pei**: Resource, Supervision, Writing Reviewing. **Peng Du**: Conducting experiments, Data curation. **Rui Lei**: Methodology. **Xiaochao Zhang**: Investigation. **Weibiao Zhang**: Project administration.**Xiaowei Li:** Funding acquisition.

# Declaration of competing Interest

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

# Data availability

Data will be made available on request.

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