



Shahrood University of
Technology



Iranian Society of
Mining Engineering
(IRSE)

Interactions Between Cultivated Grasses And Culturable Microbial Populations During The Phytoremediation Of Mine Tailings

Snežana Brajević¹, Aleksandar Simić², Vera Karličić², Nikola Milanović¹, Monika Stojanova³, Blažo Lalević², Željko Dželetović¹

1. Institute for the Application of Nuclear Energy-INEP, University of Belgrade, Belgrade, Serbia

2. Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

3. Association for Scientific Research, Educational and Cultural Activities, Open Science, Ohrid, North Macedonia

Article Info

Received 12 October 2025

Received in Revised form 27
December 2025

Accepted 26 February 2026

Published online 26 February 2026

DOI: [10.22044/jme.2026.16918.3338](https://doi.org/10.22044/jme.2026.16918.3338)

Keywords

Culturable microbial populations

Grasses

Fertilization

Flotation tailing

Irrigation

Abstract

Permanent mining generates substantial amounts of flotation tailings with highly unfavourable physical and chemical properties, often devoid of vegetation. Their stabilization relies on phytoremediation, particularly through the establishment of grass cover. Successful revegetation requires sufficient nutrient availability and the activity of soil microorganisms that transform nutrients into plant-accessible forms. However, the interactions between plants, nutrients, and microflora during this process remain poorly understood. This study aimed to investigate the temporal dynamics and interrelationships within the plant–nutrient–microorganism system during the revegetation of flotation waste using four grass species—tall fescue, red fescue, meadow fescue, and perennial ryegrass. Plants were grown under controlled conditions on flotation tailings with different fertilizer treatments: organic (NPK 4:4:4) and mineral (NPK 20:20:20) fertilizers at varying concentrations (1% and 2% O; 0.25% and 0.5% M) and irrigation levels (50% and 75% of field water capacity). Microbial diversity (culturable bacteria, ammonifiers, fungi, and actinomycetes) was used as an indicator of remediation efficiency. Organic fertilization had the most pronounced effect, improving plant height, biomass yield, and microbial activity, particularly in tall fescue. Bacteria and ammonifiers responded positively to mineral fertilization under higher irrigation in red fescue and to organo-mineral treatment under lower irrigation in perennial ryegrass. The highest abundance of actinomycetes occurred under reduced irrigation in red fescue and perennial ryegrass. Overall, perennial ryegrass demonstrated the strongest correlation between cultivation conditions, microbial activity, and phytoremediation potential, highlighting its suitability for the ecological rehabilitation of flotation tailings.

1. Introduction

Mining is essential for producing raw materials for various industries and has a significant impact on social, industrial, and economic growth [1]. However, these benefits are often outweighed by negative effects on environmental quality, including deforestation, land use change, chemical pollution, dust emissions, and topsoil displacement [2]. One of the most hazardous consequences of mining activities is the formation of flotation tailings on surrounding soils. Flotation tailings contain hazardous elements that can accumulate in living organisms and pose a high risk to human

health, environmental quality [3], the food chain, and biodiversity [4]. Many conventional methods, such as ion exchange, chemical precipitation, coagulation, or electrochemical removal [5], have been intended to reduce hazardous elements; however, they have several disadvantages, including high energy consumption and the generation of secondary pollution, which limit their application [6].

Since the late decades of the 20th century, extensive remediation programs have been implemented to reduce contamination of tailings

 Corresponding author: snezana.brajevic@inep.co.rs (S. Brajevic)

[7]. These techniques include the introduction of plants [8] and/or microbes to reduce pollution [9] and improve environmental quality. Plants decrease the amount of water seeping into the soil, alleviating soil erosion and decreasing the spread of potentially toxic metals to other sites [10]. Due to their robust fibrous root systems and rapid growth, grasses from the Poaceae family can be effectively used for stabilization [11]. *Festuca pratensis* and *Festuca arundinacea* are suitable candidates for phytoremediation due to their low environmental requirements and tolerance to high levels of heavy metals in the substrate [12]. Additionally, *Lolium* species are considered suitable for revegetation and remediation because of their rapid root and shoot growth and resistance to metals [13]. However, approaches for estimating microbial diversity are often limited by several factors. There are flaws in the methods used to measure microbial diversity; due to sample variability and uneven microbe distribution, findings are often insufficient [14]. While rRNA analysis identifies the most active bacterial populations at a given time [15], it has limitations in identifying some of the most prevalent bacterial genera [16]. The phospholipid fatty acid (PLFA) method does not provide the taxonomic resolution that some other methods offer [17]. Nevertheless, Verma et al. [18] suggest that the use of agar media is the most effective way to detect a large number of microorganisms, which have a significant impact on processes in flotation tailings [19].

It is well known that the presence of plants in soil positively influences the activity of soil microorganisms, and that interactions between plants and microorganisms are highly complex [20]. They support each other in restoring soil structure and bioogenicity. Plants release exudates—mixtures of bioactive compounds—that serve as nutrient sources for indigenous microflora in the rhizosphere, thereby increasing microbial activity. Microorganisms help plants cope with metal toxicity and promote their growth [21]. The microbial component of flotation tailings, polluted and other unfavorable substrates, is a valuable source of microbes adapted to specific, harsh conditions, exhibiting high metal tolerance and the ability for bioaccumulation, precipitation, transformation, and detoxification of pollutants [22]. Additionally, Qian et al. [23] have shown that phytostabilization of polluted areas can be supported by various soil amendments and/or practices, such as fertilization, inoculation with biostimulants, and irrigation, which promote plant establishment and growth under adverse

conditions. The application of biostimulants capable of tolerating high levels of heavy metals potentially stimulates plant growth [22] by mechanisms that increase the bioavailability of plant nutrients, produce phytohormones, and mitigate the effects of abiotic and biotic stressors [22, 21].

Our previous study showed growth-promoting effects of organic chicken manure on tall fescue and meadow fescue [24]. In addition, the application of organic amendments such as compost or manure improves plant growth, stimulates microbial activity, and increases the success rate of phytostabilization [25]. According to Mohammad et al. [26] and Ventorino et al. [27] the application of organic fertilizers such as compost has a positive impact on the physico-chemical properties and microbial activity of degraded soils. In cadmium-contaminated soils, Liu et al. [28] identified the integrated application of organic and mineral fertilizers as the most advantageous strategy for perennial ryegrass cultivation, as organic amendments, despite their lower nutrient bioavailability, substantially ameliorate substrate properties through organic matter enrichment. Although drought-tolerant species are preferred in phytostabilization, early irrigation is often essential to ensure seedling establishment, especially when seeds are sown directly into mine tailings. The use of drip irrigation for a period of 3–6 months, or until plants are fully established, has proven successful in promoting revegetation of mine tailings [29].

The tripartite system of plants, microorganisms, and agrotechnical measures generates interactions that are difficult to predict, even in conventionally fertile substrates such as soil, and its effects are especially unpredictable in substrates lacking classical fertility and microbiological activity. The global expansion of technogenic soils increasingly necessitates sustainable management approaches focused on renovation and the reactivation of biological processes. Flotation tailings from mining operations cover extensive areas near ore-processing facilities, making it essential to identify rapid and cost-effective methods for their revitalization. Sowing perennial grasses, supplemented with nutrients and water, along with microbial activity within the substrate, may form pioneer communities that initiate the successional transformation of tailings into a revitalized system.

There is a lack of studies on the effects of revegetation and agricultural activities on microbial richness in flotation tailings ecosystems, especially in lightly or heavily polluted

environments. Consequently, additional data are needed, particularly for planning and evaluating the efficiency of phytostabilization of contaminated flotation tailings. The aim of this work was therefore to extend our knowledge of the effects of fertilization and irrigation on the prevalence of culturable microbial populations during the phytostabilization of flotation tailings.

2. Material and methods

In this study, seeds of tall fescue (*Festuca arundinacea* Schreb.), red fescue (*Festuca rubra* L.), meadow fescue (*Festuca pratensis* L.), and perennial ryegrass (*Lolium perenne* L.) from the Institute for Forage Crops in Kruševac, Serbia, were used.

The trial was established in October 2021 in the greenhouse of the Faculty of Agriculture, University of Belgrade. Plastic pots with a diameter of 10 cm were used for the experiment. Samples of flotation tailings from a Pb, Cu, and Zn mine were used as the substrate.

Fresh flotation tailings were collected from a Pb, Zn and Cu mine in Central Serbia, 44.11°N

20,49°E (Figure. 1). Flotation tailings are characterised by a poor sandy structure with high contents of Pb, Ni, Zn, Cu and Cd and a low content of nutrients [30]. According to Radulović et al. [31], the most abundant minerals in the tailings are quartz and aluminosilicates with sphalerite identified as the most abundant non-ferrous metal mineral. The substrate used in the experiment was air-dried under natural conditions, sieved through a 2 mm mesh and manually homogenised to achieve the highest possible uniformity. Moisture content was maintained at the desired level based on pot weight measurements taken every 3–4 days. The weight of the growing medium in the pots was 1.3 kg. The sowing rate for red fescue was 0.53 g per pot, and for tall fescue, meadow fescue and perennial ryegrass, 0.67 g per pot. Two types of fertilizers were applied: organic (NPK 4:4:4) and mineral (NPK 20:20:20) in different doses. Two irrigation rates were used in this trial: a higher rate (up to 75% of field water capacity- FWC) and a lower rate (up to 50% of FWC). The treatments were as follows (Table 1):



Figure 1. Flotation tailing dump in central Serbia

Table 1. Treatments

| Description |
|---|
| C – control (flotation tailings) |
| O – organic fertilizer (2%) |
| O1 – organic fertilizer (2%) + irrigation dose 75% of field water capacity (FWC) |
| O2 – organic fertilizer (2%) + irrigation dose 50% of FWC |
| M – mineral NPK fertilizer (0.5%) |
| M1 – mineral fertilizer (0.5%) + irrigation dose 75% of FWC |
| M2 – mineral fertilizer (0.5%) + irrigation dose 50% of FWC |
| OM – organic fertilizer (1%) + mineral fertilizer (0.25%) |
| OM1 – organic fertilizer (1%) + mineral fertilizer (0.25%) + irrigation dose 75% of FWC |
| OM2 – organic fertilizer (1%) + mineral fertilizer (0.25%) + irrigation dose 50% of FWC |

Fertilizer application rates in the experiment were calculated by converting the recommended field rates per hectare to the corresponding amounts per pot, based on the substrate mass.

The treatments were conducted in three replicates, and the trial lasted 16 weeks. At the end of the experiment, plants were harvested, and plant height and biomass were measured. Additionally, at the end of the incubation period, the diversity of the culturable microbial population was determined using the agar plate method. The total number of bacteria was determined using 0.1×TSA (Torlak, Serbia), the number of ammonifiers on nutrient agar (Torlak, Serbia), the number of fungi on rose bengal-streptomycin agar [32], and the number of actinomycetes on starch-ammonia agar. All culture media were sterilized in an autoclave at 121°C (Panasonic MLS-3781L, Italy) for 20 minutes. Bacteria and actinomycetes were incubated in an incubator (Binder, Germany) at 30°C for 7 and 12 days, respectively, while fungi were incubated at 25°C for 5 days. The number of cultivable microbes was expressed as CFU (colony-forming units) per

gram of dry sample. The samples were dried in an oven (Binder, Germany) at 105°C for 2 hours.

Statistical analysis

Statistical analyses of the experimental data were performed using STATISTICA version 10 (StatSoft, Tulsa, OK, USA). The pot experiment was set up in a completely randomised design with three replicates. A one-way analysis of variance (ANOVA) was used to determine significant differences between treatments and comparisons of means were performed using the LSD test at the 5% significance level. Relationships between the analysed parameters were assessed with Pearson correlation coefficients ($p \leq 0.05$). Principal component analysis (PCA) was performed to assess the effects of treatments on plant growth and microbial activity.

3. Results

Our results show that the analysed plant species have no possibility to grow without fertilization or irrigation, i.e. on substrates prepared as in treatments C, O, M and OM (Table 2).

Table 2. Effect of fertilization treatments on plant height and aboveground biomass yield of the studied grass species

| | Tall fescue | | | Meadow fescue | | | Red fescue | | | Perennial ryegrass | | |
|-------|-------------|-------------------|-----------------|---------------|-------------------|-----------------|-------------|-------------------|-----------------|--------------------|-------------------|-----------------|
| | Height (cm) | Fresh biomass (g) | Dry biomass (g) | Height (cm) | Fresh biomass (g) | Dry biomass (g) | Height (cm) | Fresh biomass (g) | Dry biomass (g) | Height (cm) | Fresh biomass (g) | Dry biomass (g) |
| C | Øf | Øc | Ød | Øf | Øe | Øe | Ød | Øe | Ød | Øe | Øe | Ød |
| O | Øf | Øc | Ød | Øf | Øe | Øe | Ød | Øe | Ød | Øe | Øe | Ød |
| O1 | 21.0b | 2.6a | 0.32c | 15.3a | 1.26bc | 0.13c | 14.0a | 1.08a | 0.35a | 15.3b | 1.88b | 0.20c |
| O2 | 22.0a | 3.2a | 0.4b | 13.0c | 1.07c | 0.11cd | 12.0b | 0.49c | 0.23b | 16.0a | 2.1a | 0.28a |
| M | Øf | Øc | Ød | Øf | Øe | Øe | Øc | Øe | Ød | Øe | Øe | Ød |
| M1 | 12.3d | 1.1bc | 0.65a | 12.0d | 0.59d | 0.0d | 12.0b | 0.67b | 0.28ab | 11.7d | 1.44c | 0.19c |
| M2 | 13.7c | 0.97bc | 0.25c | 11.0e | 0.50d | 0.08cd | 11.3c | 0.66b | 0.29ab | 11.7d | 1.19d | 0.18c |
| OM | Øf | Øc | Ød | Øe | Øe | Øe | Ød | Øe | Ød | Øe | Øe | Ød |
| OM1 | 10.3e | 0.96bc | 0.25c | 13.3c | 1.67a | 0.21b | 11.3c | 0.23d | 0.11c | 13.0e | 1.79b | 0.20c |
| OM2 | 10.0e | 1.1bc | 0.25c | 14.7b | 1.4b | 0.33a | 11.3c | 0.23d | 0.12c | 13.0e | 1.81b | 0.25b |
| ANOVA | | | | | | | | | | | | |
| LSD | * | * | * | * | * | * | * | * | * | * | * | * |

Treatment means were compared using the LSD test at a 5% significance level (*). Means with the same letter are not significantly different, whereas means with different letters indicate a significant difference ($p \leq 0.05$). As shown in Table 2, all treatments had a significant influence on plant height and biomass. The best results were observed in treatments with organic fertilizer.

The best results for plant height and biomass were achieved with treatments O1 and O2, especially for tall fescue (Table 2). Tall fescue's fresh biomass and height were significantly lower in the M and particularly the OM treatments at both irrigation levels than in O1 and O2. For tall fescue dry biomass, the highest production was measured in the M1 treatment followed by O2, while other

treatments showed significantly lower biomass production compared to M1. In red fescue, treatment O1 yielded the best results for plant height (14.0 cm) and fresh biomass (1.08 g). Meadow fescue achieved the best results for plant height and fresh biomass in the OM treatments. Among treatments with lower irrigation doses, OM2 gave the highest plant height of 14.7 cm and the highest dry biomass yield of 0.33 g per pot. Dry biomass was highest in the O1 and O2 treatments for all plant species, except for meadow fescue, where the highest dry biomass yield of 0.33 g per pot was measured in OM2. Dry biomass varied less compared with fresh biomass and plant height. According to the ANOVA results, all treatments had a significant effect on plant growth (Table 2).

The pot experiment was followed by microbiological analyses using culture-dependent methods, and the results showed that planting increased microbial presence in the substrates.

Microbial analyses of the control sample, where plants were sown on pure flotation residues, showed low microbial activity (Figures 1 and 2).

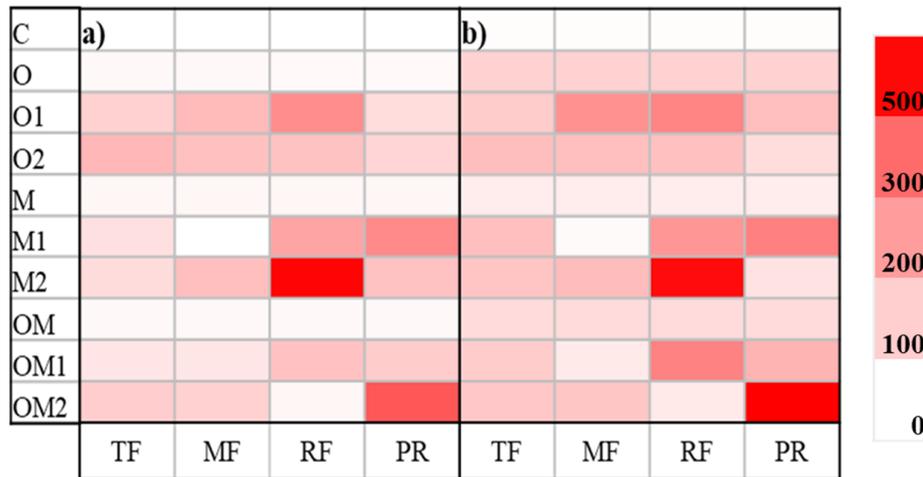


Figure 1. Number of: a) culturable bacteria (10^4 CFU/g) and b) ammonifiers (10^4 CFU/g)
Legend: O-organic fertilizer; M-mineral fertilizer; OM-combination of organic and mineral fertilizer; 1-higher irrigation dose-75% FWC; 2-lower irrigation dose-50% FWC; TF-tall fescue, MF-meadow fescue, RF-red fescue, PR-perennial ryegrass

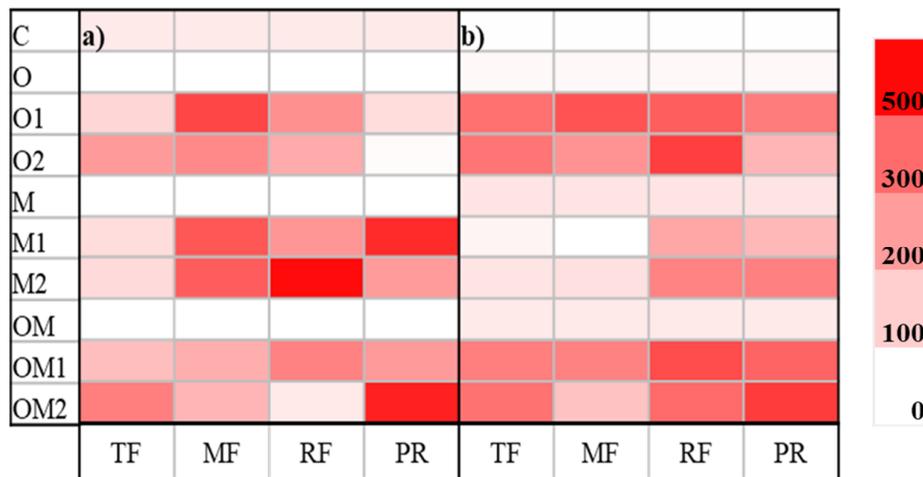


Figure 2. Number of: a) culturable fungi (10^2 CFU/g) and b) culturable actinomycetes (10^2 CFU/g)
Legend: C-pure flotation tailing; O-organic fertilizer; M-mineral fertilizer; OM-combination of organic and mineral fertilizer; 1-higher irrigation dose-75% FWC; 2-lower irrigation dose-50% FWC; TF-tall fescue, MF-meadow fescue, RF-red fescue, PR-perennial ryegrass

However, organic fertilization significantly increases the population of culturable bacteria in substrates (Figure 1). In the case of tall fescue, treatments O2 (149.65×10^4 CFU/g) and OM2 (106.64×10^4 CFU/g) showed the highest bacterial abundance, while meadow fescue exhibited similar results in the O1, O2 and M2 treatments (142.95×10^4 , CFU/g, 132.31×10^4 CFU/g and 133.80×10^4 CFU/g). Interestingly, in the M1

treatment analyses showed an absence of bacteria, ammonifiers, and actinomycetes.

Red fescue microbial communities responded differently to the applied treatments, with the M2 treatment being the most stimulating for the establishment of culturable bacteria, ammonifiers, and fungi. The highest numbers of culturable bacteria, ammonifiers, fungi and actinomycetes in rhizosphere of perennial ryegrass were reported in the OM2 treatment (341.19×10^4 CFU/g,

514.48x10⁴ CFU/g, 4.50x10⁴ CFU/g and 3.96x10⁴ CFU/g, respectively).

In most cases, the M treatment had a stimulating effect on microbial populations compared to the control (Figures 1 and 2). The use of mineral fertilizer has various effects on microbial abundance in the plant rhizosphere, ranging from insignificant in tall fescue to considerable in red fescue. However, mineral fertilization had a non-significant effect on microbial abundance in the rhizosphere of meadow fescue, especially at low

irrigation rates. Compared to mineral fertilizers, the use of organo-mineral fertilizers led to a significant increase in the prevalence of all bacterial groups in most treatments.

Our results show that there are no significant differences in the number of culturable bacteria and ammonifiers in most treatments. In general, their abundance was very low in all treatments, which may be related to the lack of organic matter in the spoil heaps and the stress conditions (Figure 1).

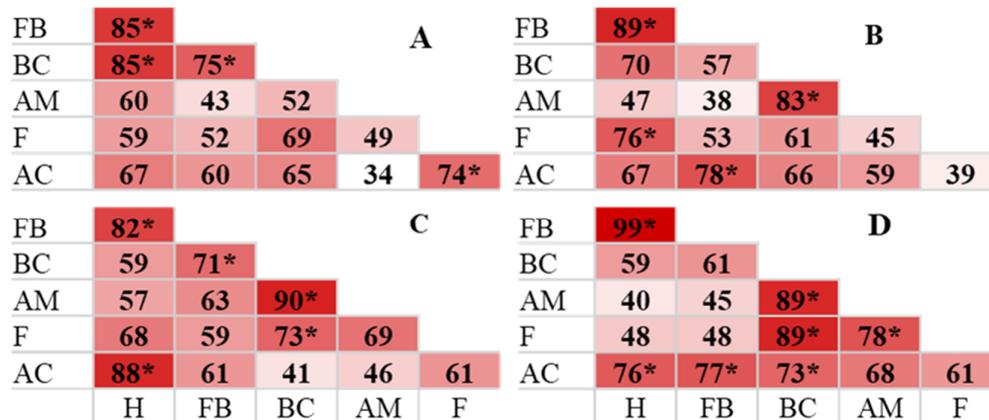


Figure 3. Correlation between plant characteristics and number of culturable microorganisms: A) tall fescue; B) meadow fescue; C) red fescue; D) perennial ryegrass

Legend: H—plant height; FB—fresh plant biomass; BC—culturable bacteria; AM—ammonifiers; F—fungi; AC—actinomycetes.

***Strong correlation $p < 0.05$; [33].**

It can be seen that the plant height of tall fescue correlates very strongly (over 85%) with both fresh plant biomass and the number of culturable bacteria (Figure 3.A). A very strong correlation was observed between plant height and fresh biomass of meadow fescue, and a strong correlation was found between the number of culturable bacteria and culturable ammonifiers (Figure 3.B). A similar observation was made between the height of red fescue and the number of culturable actinomycetes, as well as between the number of culturable bacteria and culturable ammonifiers (Figure 3.C). Figure 3.D shows a very strong correlation between plant height and the biomass of perennial ryegrass, as well as between the number of culturable bacteria and culturable fungi and ammonifiers.

Principal components analysis (PCA) clearly showed that the type of fertilizer applied had a dominant effect on the differences between treatments. Organic (O) and combined organo-mineral (OM) fertilization were positioned in the positive part of the first principal component (PC1), indicating a significant impact on the

examined parameters compared to the control (C) and mineral fertilization (M), which are grouped in the negative part of the same axis. This arrangement suggests that the organic component plays a key role in plant growth and microbial abundance on flotation tailings.

The second principal component (PC2) separated the samples primarily by irrigation dose. The effect of irrigation was more pronounced with organic and combined treatments, indicating a synergistic effect of increased water and organic matter availability on the measured parameters. In the control and mineral treatments, however, the irrigation doses did not significantly change the position of the samples in the PCA space, suggesting that in the absence of the organic component, water has a limited effect on the investigated parameters (Figure 4).

The results indicate that the type of fertilizer is the main source of variability in the data, while irrigation is a modulating factor whose influence depends on the presence of organic matter.

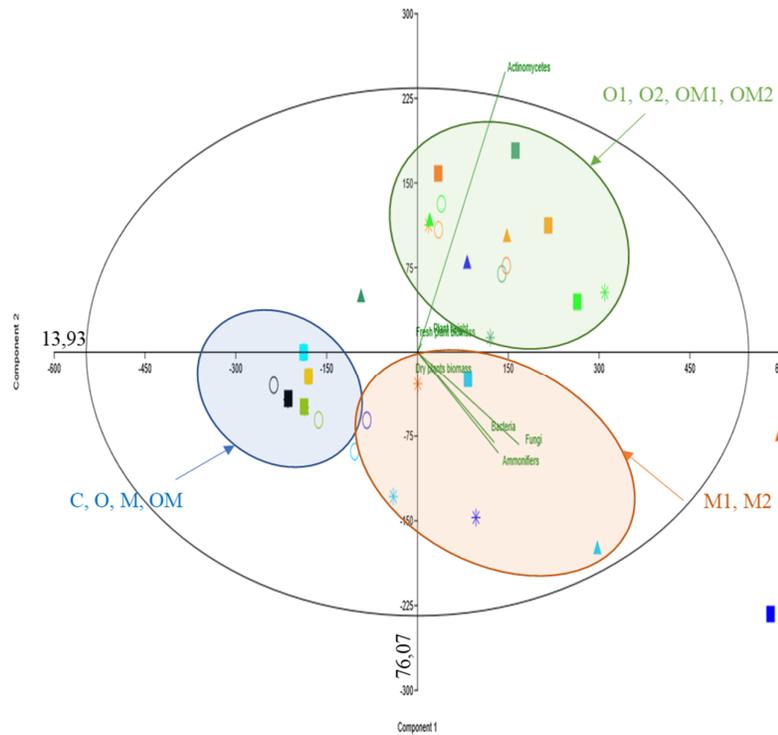


Figure 4. PCA diagram illustrating impact of different fertilization treatments on plant biomass yield and number of culturable microorganisms

Legend: ■—red fescue; ▲—perennial ryegrass; ○—tall fescue; *—meadow fescue

4. Discussion

Effective management of tailings presents a significant challenge for the mining sector [34] due to high levels of trace metals [35]. Błonska et al. [36] found that plant species can successfully colonize mining areas; some are adapted to harsh environmental conditions and can be used in the restoration process. Additionally, flotation tailing-associated microbes play a crucial role in nutrient cycling and ecosystem recovery [37]. Lin et al. [38] observed that the stability of flotation tailings correlates with an increase in bacterial abundance.

Several studies have examined the effects of fertilization on soil microbial abundance, but the results have been inconsistent, ranging from positive and neutral to negative effects [39,40,41]. Sun et al. [39] reported that the abundance of soil bacteria increased after the application of manure, while it decreased with mineral fertilizers. In contrast, Guo et al. [40] found that the number of bacteria was not affected by manure application, and Grzyb et al. [41] described the stimulation of some bacterial populations by mineral fertilizers.

All fertilization doses and irrigation regime treatments had a significant effect on plant growth

(Table 2). Similarly, Źurek and Martyniak [42] used flotation tailings as a growth substrate and found that tall fescue had greater root length, plant height and seed germination than meadow fescue and perennial ryegrass. Sarathchandra et al. [43] reported the significant increase in perennial ryegrass in iron ore mine tailings after the addition of favourable additives. Xue et al. [44] analysed parameters of tall fescue planted on copper tailings and found a significant increase in plant biomass, but the values were significantly lower compared to our study. Mark Vidler [45] also suggested that the physical, chemical, and geochemical properties of mining areas may be important factors in plant sensitivity.

As shown in Figures 1 and 2, low culturable microbial abundance was detected in flotation tailings. The probable reason for these results lies in the limitations of culture-dependent methods, which provide only partial insight into microbial diversity. Similarly, analyses of microbial activity in tailings from the Singrauli coalfields conducted by Pandley et al. [46] confirmed low microbial abundance. The low microbial richness in flotation tailings can be attributed to several factors, such as mining activities, lack of structure, low water-

holding capacity, low organic matter content, and the presence of metal(oid)s [47,22].

Furthermore, insufficient moisture in the substrate leads to diminished microbial activity [48], reduced microbial biomass, and impaired nutrient cycling [49]. At minimal substrate humidity, microbial taxa cease reproduction. According to Metze et al. [50], only 4% of microbial communities reproduce during drought conditions. Ait-Al-Mokhar et al. [51] demonstrated that drought may affect root microbes, while Santos-Medellín et al. [52] reported long-term effects of drought on microbial composition. Low moisture promotes the growth of actinomycetes due to the low availability of iron [53]. Actinomycetes were more prevalent than fungi in most of our treatments, particularly in O treatments. Finally, shifts in microbial composition and plant metabolism alter the chemical properties of litter, the chemistry of organic carbon, and the cycling of this element [54].

Organic fertilization showed a stimulatory effect on the number of culturable microbial populations. This agrees with Liu et al. [55], who concluded that organic amendments lead to an increase in the prevalence of beneficial microbes. Wang et al. [56] also found that organic fertilizers, unlike NPK fertilizers, stimulate microbial activity in some soils. Interestingly, the increase in microbial richness is more pronounced in the prevalence of fungi and actinomycetes. In contrast, Zhang et al. [57] reported that the use of organic fertilizers stimulates bacterial populations, but an inhibits actinomycetes. Our findings demonstrated that the application of fertilizers resulted in a decrease in fungal abundance. Semenov et al. [58] claim that using organic fertilizers lowers the number and diversity of fungal species. Vegetation is a crucial driver of fungal community shifts, as previously confirmed by Liu et al. [59].

Although chemical fertilization is one of the fundamental crop treatments [60], prolonged use of these amendments results in the loss of soil quality, and shifts in plant development and microbial composition [61]. Although mineral fertilizers may negatively impact microbial diversity and soil health [62], some authors argue that they promote microbial activity and nutrient cycling [63]. Our findings revealed an increase in culturable microbial populations depending on plant species. According to Bo et al. [64], environmental factors, plant species and agricultural practices significantly impact microorganisms in the rhizosphere. Similarly, the application of organo-mineral fertilizers significantly improved

culturable microbial populations. Uddin et al. [65] reported that organo-mineral fertilizers improved nutrient cycling, plant growth and soil health, while Bo et al. [66] showed that the combination of organic and mineral fertilizers increased enzymatic and bacterial activity in the soil. However, Malal et al. [67] indicate limited information about the impact of organic and mineral fertilizers on culturable microbial communities under stress conditions.

In most samples, a higher number of culturable microorganisms was found in low irrigation rate treatments. Our results are in agreement with Bai et al. [68] who showed that moderate irrigation can promote soil microbial population composition. Muhammad et al. [69] showed that a low irrigation rate stimulated enzyme activity, nutrient content and bacterial diversity in the soil.

In our study, perennial ryegrass exhibited the strongest relationship with microbial parameters compared to the other plants investigated (Figure 3). Li et al. [70] found a correlation between perennial ryegrass and bacterial diversity in soils contaminated with cadmium and pyrene, while Zhang et al. [71] observed a correlation between perennial ryegrass and nitrogen-fixing bacteria in Tungfram tailings, which is essential for effective tailings remediation.

Through the interactions observed between perennial grasses cultivated on flotation tailings and microorganisms under various fertilization and irrigation regimes, a complex response was identified, characterized by the intertwined influence of abiotic components on living organisms and vice versa. This produced a range of possibilities enabling selection under different environmental conditions, nutrient availability, and water sources. Accordingly, grass species were ranked by their nutrient and water requirements as follows: PR > TF > MF > RF. As a general recommendation for enhanced microbiological activity and successful grass growth and development, a lower irrigation rate (50% of field water capacity) and fertilization treatments in the order O > OM > M > C were identified as most favorable.

5. Conclusions

The results confirm that tailings are an unfavourable substrate for plant growth; however, with appropriate management measures, they can be rapidly and cost-effectively restored to biological functioning. The pot experiment showed that, within a short period, a grass cover comprising

four grass species can be successfully established under 50% water-holding capacity, combined with the application of organic or organomineral fertilizers.

Our findings revealed an increase in culturable microbial populations depending on the plant species. The most pronounced increase in microbial abundance was observed in perennial ryegrass (*Lolium perenne*), indicating its high sensitivity to management inputs and its potential role in stimulating the biological recovery of degraded substrates. Nevertheless, tall fescue (*Festuca arundinacea*) exhibited the most favourable agronomic parameters, including aboveground biomass, yield, and plant height, confirming its suitability as the leading candidate for long-term stabilization and revegetation of mine tailings.

The implications of our results suggest that sustainable recovery of mine tailings requires investment in organic fertilizers, as they positively affect both microbial activity and plant performance when irrigation is provided. The seeds of the grass species investigated are commercially available, allowing for easy establishment of vegetation cover; however, all findings should be validated under field conditions. Given the limitations of experiments conducted under controlled conditions, future research should focus on assessing sustainability under outdoor conditions, as well as the long-term persistence and stability of vegetation cover formed by each grass species.

Acknowledgement

This study was partially supported by the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia under Nos. 451-03-33/2026-03/ 200019 and 451-03-34/2026-03/200116.

Conflicts of interest

The authors have no conflicts of interest that could be declared relevant to the content of this article.

References

- [1]. Pouresmaieli, M., Ataei, M., & Qarahasanlou, A.N. (2023). A scientometrics view on sustainable development in surface mining: Everything from the beginning. *Resources policy*, 82, 103410.
- [2]. Sonter, L.J., Moran, C.J., Barrett, D.J., & Soares-Filho, B.S. (2014). Processes of land use change in

mining regions. *Journal of Cleaner Production*, 84, 494–501.

- [3]. Cacciuttolo, C., Cano, D., & Custodio, M. (2023). Socio-Environmental risks linked with mine tailings chemical composition: Promoting Responsible and Safe Mine Tailings Management Considering Copper and Gold Mining Experiences from Chile and Peru. *Toxics* 11(5), 462.
- [4]. Pouresmaieli, M., Ataei, M., Forouzandeh, P., Azizollahi, P., & Mahmoudifard, M. (2022). Recent progress on sustainable phytoremediation of heavy metals from soil. *Journal of Environmental Chemical Engineering*, 10(5), 108482.
- [5]. Ghotbi-Ravandi, A.A., Pouresmaieli, M., Noorbakhsh, N., Khayati, N., Ekrami, E., & Zarezadeh, S. (2025). Innovative biosorption techniques for heavy metal removal using microalgae: A comprehensive review. *Plant, Algae and Environment*, 9(2), 71-112.
- [6]. Razzak, S.A., Faruque, M.O., Alsheikh, Z., Alsheikhmohamad, L., Alkuroud, D., Alfayez, A., Hossain, S.M.Z., & Hossain, M.M. (2022). A Comprehensive review on conventional and biological-driven heavy metals removal from industrial wastewater. *Environmental Advances*, 7, 100168.
- [7]. Sarathchandra, S.S., Rengel, Z., & Solaiman, Z.M. (2023). A review on remediation of iron ore mine tailings via organic amendments coupled with phytoremediation. *Plants*, 12, 1871.
- [8]. Salehi, S., Pouresmaieli, M., & Qarahasanlou, A.N. (2025). A sustainable way to prevent oral diseases caused by heavy metals with phytoremediation. *Case Studies in Chemical and Environmental Engineering*, 11, 101106.
- [9]. Martínez-Sánchez, M. J., García-Lorenzo, M. L., Pérez-Sirvent, C., & Bech, J. (2012). Trace element accumulation in plants from an aridic area affected by mining activities. *Journal of Geochemical Exploration*, 123, 8-12
- [10]. Bakshe, P., & Jugade, R. (2023). Phytostabilization and rhizofiltration of toxic heavy metals by heavy metal accumulator plants for sustainable management of contaminated industrial sites: A comprehensive review. *Journal of Hazardous Materials Advances*, 10, 100293.
- [11]. Pires-Lira, M. F., Castro, E. M., Lira, J. M., Oliveira, C., Pereira, F. J., & Pereira, M. P. (2020). Potential of *Panicum aquaticum* Poir. (Poaceae) for the phytoremediation of aquatic environments contaminated by lead. *Ecotoxicology and Environment Safety* 193, 110336.
- [12]. Nefed'eva, E.E., Sevriukova, G. A., Zheltobryukhov, V. F., Gracheva, N. V., & Abdulabbas, A. Y. A. (2020). Assortment of herbaceous plants for remediation of soils contaminated with oil products and heavy metals. In *IOP Conference Series: Earth and Environmental Science*, 421(6) IOP Publishing.

- [13]. Sladkovska, T., Wolski, K., Bujak, H., Radkowski, A., & Sobol, Ł. (2022). A review of research on the use of selected grass species in removal of heavy metals. *Agronomy*, 12, 2587.
- [14]. Salmonova, H., & Bunešova, V. (2017). Methods of studying diversity of bacterial communities: a review. *Scienia Agriculturae Bohemica*, 48, 154-165.
- [15]. De Souza, P.A., Ramos, J.N., Vasconcellos, L., Costa, L.V., Forsythe, S.J., & Brandão, M.L.L. (2025). Application and limitations of 16S rRNA gene sequencing for identifying WHO priority pathogenic Gram-negative bacilli. *Infection and Drug Resistance*, 18, 6353-6375.
- [16]. Ramos, J.N., Costa, V.L., Vieira, V.V., & Brandão, M.L.L. (2025). Challenges in the identification of environmental bacterial isolates from a pharmaceutical industry facility by 16S rRNA gene sequences. *DNA*, 5(3), 33.
- [17]. Balsler, T.C., Liang, C., & Gutknecht, J.L.M. (2019). Linking microbial community analysis and ecosystem studies: A rapid lipid analysis protocol for high throughput. *Soil Ecology Letters*, 1, 22-32.
- [18]. Verma, P., Pandey, V., Seleyi, S.C., Alagarsamy, A., & Dharani, G. (2024). Exploring the hidden treasures: Deep-sea bacterial community structure in the Bay of Bengal and their metabolic profile. *Frontiers in Marine Science*, 10, 1308953.
- [19]. Wang, X., Meng, D., Li, J., Lu, Z., Zhangm Z., Zhang, C., Song, S., Peng, Y., & Xia, L. (2023). Composition and dynamics of bacterial communities during flotation in coal preparation plant. *Journal of Cleaner production*, 385, 13569.
- [20]. Zhang, Y., Wang, X., & Hongbing, J. (2020). Co-remediation of Pb Contaminated Soils by Heat Modified Sawdust and *Festuca arundinacea*. *Scientific Reports*, 10, 4663.
- [21]. Zhao, Y., Yao, J., Li, H., Sunahara, G., Li, M., Tang, C., Duran, R., Ma, B., Liu, H., Feng, L., Zhu, J., & Wu, J. (2024). Effects of three plant growth-promoting bacterial symbiosis with ryegrass for remediation of Cd, Pb and Zn soil in a mining area. *Journal of Environmental Management*, 353, 120167.
- [22]. Jiménez-Venegas, J., Zamora-Levia, L., Univaso, L., Soto, J., Tapia, Y., & Paneque, M. (2024). Profile of bacterial communities in copper mine tailings revealed through high-throughput sequencing. *Microorganisms*, 12, 1820.
- [23]. Qian, L., Lin, H., Li, B., & Dong, Y. (2023). Physicochemical characteristics and microbial communities of rhizosphere in complex amendment-assisted soilless revegetation of gold mine tailings. *Chemosphere*, 320, 138052.
- [24]. Brajević, S., Simić, A., Andrejić, G., & Dželetović, Ž. (2023). Phytoremediation abilities of species from the *Festuca* genus. "The environment - research, charge, administration 15-16 june 2023" *Book of proceedings*; 49-53.
- [25]. Visconti, D., Caporale, A. G., Pontoni, L., Ventrino, V., Fagnano, M., Adamo, P., Pepe, O., Woo, S. L., & Fiorentino, N. (2020). Securing of an industrial soil using turfgrass assisted by biostimulants and compost amendment. *Agronomy*, 10, 1310.
- [26]. Mohammad, S.J., Ling, Y.E., Halim, K.A., Sani, B.S., & Abdullahi, N.I. (2025). Heavy metal pollution and transformation in soil: A comprehensive review of natural bioremediation strategies. *Journal of Umm-Al-Qura University for Applied Sciences*, 11, 528-544.
- [27]. Ventrino, V., Faraco V., Romano, I., & Pepe, O. (2018). Responses of bacterial community structure and diversity to soil eco-friendly bioremediation treatments of two multi-contaminated fields. *Italian Journal of Agronomy*, 13(1), 53-58.
- [28]. Liu, M., Li, Y., Che, Y., Deng, S., & Xiao, Y. (2017). Effects of different fertilizers on growth and nutrient uptake of *Lolium multiflorum* grown in Cd-contaminated soils. *Environmental Science and Pollution Research*, 24(29), 23363-23370.
- [29]. Williams, D.J., & Currey, N.A. (2002). Engineering closure of an open pit gold operation in a semi-arid climate. *International Journal of Mining, Reclamation and Environment*, 16, 270-288.
- [30]. Andrejić G., Brajević S., Simić A., Dželetović Ž., Aleksić U., & Sokolović D. (2022): Accumulation of heavy metals in root and shoot of red fescue grown at the flotation tailings dump. Book of proceedings XI International Symposium on Agricultural Sciences AgroReS 2022, Trebinje 26-28 May, 2022, 64-72.
- [31]. Radulović, D.S., Stojanović, J., Bogdanović, G., Jovanović, V., Todorović, D., Ivošević, B., & Simić, V. (2025). Study of mineralogical and physicochemical properties of samples of flotation tailings "Rudnik" mine. In: Štirbanović Z and Trumić M (Eds), *XVI International Mineral Processing and Recycling Conference, IMPRC* (28-30 May 2025, Belgrade, Serbia), University of Belgrade, Technical Faculty, Bor, 575-580 (ISBN [978-86-6305-158-4](#)).
- [32]. Pepper, I. L., Gerba, C. P., & Brendecke, J. W. (1995). *Environmental microbiology: a laboratory manual*. Academic Press.
- [33]. Marjanović, M., Mihailović, I., & Spasić, K. (2016). Statistics in Economics and Business with a Collection of Solved Problems. Textbook; College of Applied Business Studies in Leskovac.
- [34]. Cotrina-Teatino, M.A., Marquina-Araujo, J.J., Avalos-Murga, J.A., & Carrion-Villacorta, F.L. (2025). Flotation of mine tailings: A bibliometric analysis and systematic literature review. *Journal of Environmental Chemical Engineering*, 13(2), 116136.
- [35]. Vujović, N., Alivojvodić, V., Radovanović, D., Štulović, M., Sokić, M., & Kokalj, F. (2025). Towards

circularity in Serbian mining: unlocking the potential of flotation tailings and fly ash. *Minerals*, 15, 254.

[36]. Błńska, A., Kompała-Bąbal, A., Sierka, E., Bierzal, W., Magurno, F., Besenyi, L., Ryś, K., & Woźniak, G. (2019). Diversity of vegetation dominated by selected grass species on coal-mine spoil heaps in terms of reclamation of post-industrial areas. *Journal of Ecological Engineering*, 20(2), 209-217.

[37]. Van-Dongen, A., Samad, A., Heshka, N.E., Rathie, K., Martineau, C., Bruant, G., & Derenhardt, D.A. (2021). Deep look into the microbiology and chemistry of froth treatment tailings: a review. *Microorganisms*, 9(5), 1091.

[38]. Lin, H., Jiang, X., Li, B., Dong, Y., & Qian, L. (2021). An efficient means of improving physicochemical properties and reshaping microbial communities of high-salty gold mine tailings. *Ecotoxicology and Environmental Safety*, 207, 111246.

[39]. Sun, R.B., Zhang, X.X., Guo, X.S., Wang, D.Z., & Chu, H.Y. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry*, 88, 9–18.

[40]. Guo, Z.B., Wan, S.X., Hua, K.K., Yin, Y., Chu, H.Y., Wang, D.Z., & Guo, X.S. (2020). Fertilization regime has a greater effect on soil microbial community structure than crop rotation and growth stage in an agroecosystem. *Applied Soil Ecology*, 149, 103510.

[41]. Grzyb, A., Wolna-Maruwka, A., & Niewiadomska, A. (2020). Environmental factors affecting the mineralization of crop residues. *Agronomy*, 10, 1951.

[42]. Żurek, G., & Martyniak, D. (2025). Studies on grass germination and growth on post-flotation sediments. *Sustainability*, 17, 3438.

[43]. Sarathchandra, S.S., Rengel, Z., & Solaiman, Z.M. (2024). Metal uptake from iron ore mine tailings by perennial ryegrass (*Lolium perenne* L.) is higher after wheat straw than wheat straw biochar amendment. *Plant Soil*, 502(1), 481-496.

[44]. Xue, J., Wang, W., He, M., You, J., & Han, H. (2022). Study on the effect of the copper tailing substrate with different treatments on the growth of tall fescue (*Festuca arundinacea*). *Sustainability*, 14, 15387.

[45]. Vidler, A.M. (2022). Water retention properties of engineered soils for mine rehabilitation. PhD thesis. Faculty of Engineering and Built Environment, University of Newcastle, Australia.

[46]. Pandey, P., Verma, M. K., Mukhopadhyay, R., & De, N. (2016). Biological properties of selected overburdens of Singrauli coalfields. *Nature Environment and Pollution Technology*, 15, 853–858.

[47]. Chung, A.P., Coimbra, C., Farias, P., Francisco R., Branco, R., Simao, F.V., Gomes, E., Pereira, A., Fiuza, A., Mortensen, M.S., Sorensen, S.J., & Morais, P.V.

(2019). Tailings microbial community profile and prediction of its functionality in basins of tungsten mine. *Scientific Reports*, 9, 19596.

[48]. Naylor, D., DeGraaf, S., Purdom, E., & Coleman-Derr, D. (2017). Drought and host selection influence bacterial community dynamics in the grass root microbiome. *International Society for Microbial Ecology Journal*, 11, 2691-2704.

[49]. De Silva, S., Kariyawasam Hetti Gamage, L., & Thapa, V.R. (2025). Impact of drought on soil microbial communities. *Microorganisms*, 13(7), 1625.

[50]. Metze, D., Schneckner, J., Canarini, A., Fuchslueger, L., Koch, B.J., Stone, B.W., Hungate, B.A., Hausmann, B., Schmidt, H., Schaumberger, A., Bahn, M., Kaiser, C., & Richter, A. (2023). Microbial growth under drought is confined to district taxa and modified by potential future climate conditions. *Nature Communications*, 14, 5895.

[51]. Ait-El-Mokhtar, M., Meddich, A., & Baslam, M. (2023). Plant-microbiome interactions under drought-insights from the molecular machinist's toolbox. *Frontiers in Sustainable Food Systems*, 7, 1253735.

[52]. Santos-Medellín, C., Edwards, J., Liechty, Z., Nguyen, B., & Sundaresan, V. (2017). Drought stress results in a compartment-specific restructuring of the rice root associated microbiomes. *MBio*, 8, 1-15.

[53]. Xu, L., Dong, Z., Chiniquy, D., Pierroz, G., Deng, S., Gao, C., Diamond, S., Simmons, T., Wipf, H.M.L., Caddel, D., Varoquaux, N., Madera, M.A., Hutmacher, R., Deutchshauer, A., Dahlberg, J., Guerinot, M.L., Purdom, E., Banfield, J.F., Taylor, J.W., Lemaux, P.G., & Coleman-Derr, D. (2021). Genome resolved metagenomics reveals role of iron metabolism in drought-induced rhizosphere microbiome dynamics. *Nature Communications*, 12, 553.

[54]. Malik, A.A., & Bouskill, N.J. (2022). Drought impacts on microbial trait distribution and feedback to soil carbon cycling. *Functional Ecology*, 36, 1442-1456.

[55]. Liu, Y., Lan, X., Hou, H., Ji, J., Liu, X., & Lv, Z. (2024). Multifaceted Ability of Organic Fertilizers to Improve Crop Productivity and Abiotic Stress Tolerance: Review and Perspectives. *Agronomy*, 14, 1141.

[56]. Wang, W., Xue, J., Zhang, L., He, M., & You, J. (2024). Extraction of heavy metals from copper tailings by ryegrass (*Lolium perenne* L.) with the assistance of degradable chelating agents. *Scientific Reports*, 14, 7663.

[57]. Zhang, Q. C., Shamsi, I.H., Xu, D.-T., Wang, G.-H., Lin, X.-Y., Jilani, G., Hussain, N., & Chaudhry, A.N. (2012). Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Applied Soil Ecology*, 57, 1-8.

[58]. Semenov, M. V., Krasnov, G. S., Semenov, V. M., & van Bruggen, A. (2022). Mineral and organic

fertilizers distinctly affect fungal communities in the crop rhizosphere. *Journal of Fungi*, 8(3), 251.

[59]. Liu, H., Yao, J., Liu, B., Li, M., Liu, J., Jiang, S., Yu, W., Zhao, Y., & Duran, R. (2023). Active tailings disturb the surrounding vegetation soil fungal community: Diversity, assembly process and co-occurrence patterns. *Science of the Total Environment*, 865, 161133.

[60]. Xing, Y., Xie, Y., & Wang, X. (2025). Enhancing soil health through balanced fertilization: a pathway to sustainable agriculture and food security. *Frontiers in Microbiology*, 16, 1536524.

[61]. Hartmann, M., & Six, J. (2023). Soil structure and microbiome functions in agroecosystems. *Nature Reviews Earth & Environment*, 4, 4-18.

[62]. Castellano-Hinojosa, A., Strauss, S.L., González-López, J., & Bedmar, E.J. (2021). Changes in the diversity and predicted functional composition of the bulk and rhizosphere soil bacterial microbiomes of tomato and common bean after inorganic N-fertilization. *Rhizosphere*, 18, 100362.

[63]. Chen, X., Ma, X., Liu, Z., Gu, H., Fang, H., Shen, Z, Zhang, H., Wan, S., Li, W., Hao, X., Clarke, N.J., & Liu, J. (2025). Organic fertilizers increase microbial community diversity and stability down the transformation process of nutrient cycling. *Environmental Microbiome*, 20, 130.

[64]. Bo, H., Li, Z., Jin, D., Xu, M., & Zhang, Q. (2023). Fertilizer management methods affects bacterial community structure and diversity in the maize rhizosphere soil of a coal mine reclamation area. *Annals of Microbiology*, 73, 24.

[65]. Uddin, Md.K., Saha, B.K., Wong, V.N.L., & Patti, A.F. (2025). Organo-mineral fertilizer to sustain soil health and crop yield for reducing environmental

impact: A comprehensive review. *European Journal of Agronomy*, 162, 127433.

[66]. Bo, H., Li, Z., Wang, W., Zhang, R., Wang, H., Jin, D., Xu, M., & Zhang, Q. (2024). Combining organic and inorganic fertilization enhances soil enzyme activity, the bacterial community, and molecular ecological network complexity in coal mine reclamation areas. *Agronomy*, 14, 1427.

[67]. Malal, H., Garcia, J.A., Marrs, A., Ait Hamza, M., Emerson, C., Nocco, M., Lakhtar, H., & Lazcano, C. (2025). Organic and inorganic fertilizers modulate the response of the soil microbiome to salinity stress. *Frontiers in Microbiology*, 16, 1551586.

[68]. Bai, H., He, S., Qin, T., Yan, D., Weng, B., Zhao, X., Li, X., Bai, Y., & Ma, J. (2019). Influences of irrigation amount on the rhizospheric microorganism composition and carbon dioxide flux of maize crops. *Geoderma*, 343, 1-9

[69]. Muhammad, I., Yang, L., Ahmad, S., Zeeshan, M., Farooq, S., Ali, I., Khan, A., & Zhou, X. B. (2022). Irrigation and nitrogen fertilization alter soil bacterial communities, soil enzyme activities and nutrient availability in maize crop. *Frontiers in Microbiology*, 13, 833758

[70]. Li, G., Wang, Z., Lv, Y., Jia, S., Chen, F., Liu, Y., & Huang, L. (2021). Effect of culturing ryegrass (*Lolium perenne* L.) on Cd and pyrene removal and bacteria variations in co-contaminated soil, *Environmental Technology and Innovation*, 24, 101963.

[71]. Zheng, X., Li, Q., Peng, Y., Wang, Z., & Chen, M. (2024). Phytoremediation of tungsten tailings under conditions of adding clean soil: microbiological research by metagenomic analysis. *Sustainability*, 16, 5715.