

## Review

# Responses of Natural Microorganisms to Land Reclamation and Applications of Functional Microorganisms in Bioremediation of Coal Mining Area

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**Abstract:** Extensive coal mining causes significant ecological and environmental impacts on the local ecosystem, especially on the terrestrial ecosystem. Mining activities induce the degradation of topsoil physico-chemical characteristics and the succession of soil microbial communities. The soil microbial community is sensitive to soil disturbance and restoration practices, being significant in soil reconstruction and land restoration. Microbes could be effective instruments to restore or reclaim disturbed terrestrial ecosystems and indispensable, unambiguous, indicators to assess reclaimed soils. In the present review, we aimed to provide insight into the effects of mining and subsequent land reclamation on soil microorganisms and the importance and application of microorganisms in the reclamation process. We address changes in the diversity and structure of the soil microbial community after reclamation and discuss the main driving factors of the community. We hypothesize that there is a discernible pattern or regularity in the variation of microbial community composition during the process of restoration succession. By employing the life strategy concept, the study attempts to identify and understand how microbial communities evolve during land reclamation. Land reclamation could improve the nutrients in the soil while increasing the proportion of saprotrophic microorganisms. In community succession, vegetation, soil properties, and reclamation time are key determining factors. Whereas bacteria, fungi, and archaea showed different responses to these factors, as they responded differently to varied soil environments, nutrition, and plants, and occupied different biological niches. Finally, we describe the applications of microorganisms as land reclamation monitors or promoters. This knowledge and understanding can provide comprehensive insight into the soil health condition and strong support for forecasting and decision-making in mine land restoration.

**Keywords:** soil microbial community; coal mining area; land reclamation; response; application



**Citation:** Mao, Z.; Harris, J.; Zhang, Z. Responses of Natural Microorganisms to Land Reclamation and Applications of Functional Microorganisms in Bioremediation of Coal Mining Area. *Diversity* **2024**, *16*, 86. <https://doi.org/10.3390/d16020086>

Academic Editors: Xin-Cun Wang and Ipek Kurtboke

Received: 7 December 2023

Revised: 21 January 2024

Accepted: 22 January 2024

Published: 28 January 2024



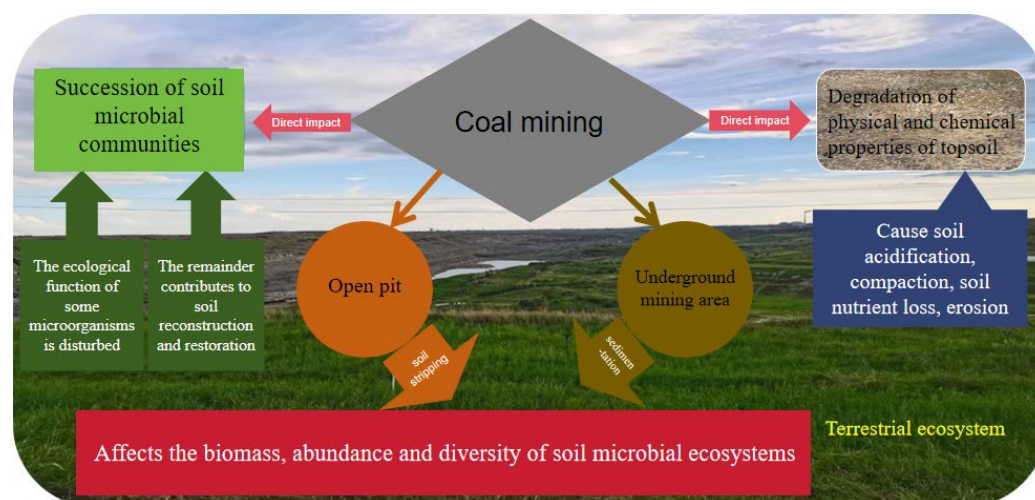
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## 1. Introduction

The mining of coal can be traced back thousands of years. Its use fueled the Industrial Revolution when it was widely used to power steam engines and generate electricity. In 2022, global coal supplies went to new highs of about 8582 Mt, which was about a quarter of the world's primary energy (<https://www.iea.org/reports/coal-2023> (accessed on 22 January 2024)). In many regions, coal will continue to be a major contributor to production as a reliable energy source for years to come, even despite the increasing popularity of renewable energy sources, and its impact on global warming.

Exhaustive exploitation destroys landscapes, hydrology, and habitats, threatens endangered species, and causes loss of biodiversity [1]. Mining activities also bring surface collapse, land subsidence, and serious pollution of air, soil, and water [2,3], and even affect the ecosystem function and services [4,5]. However, mining can also create more complex

habitats with the potential for high conservation value. These secondary habitats have the potential to replace rapidly disappearing natural wetlands, which further complicates conservation efforts [6]. In addition, coal mining activities also have profound effects on soil characteristics and the microbial community. During surface mining, all the surface layers (topsoil, subsoil, and overburden rock) are removed. Then the soil horizons are frequently inverted or mixed, which will reduce nutrient content, alter the availability of nutrients, and consequently affect microbial ecosystems [7]. The microbial biomass, richness, and diversity of soil from post-mined sites are significantly lower than undisturbed soils. The composition of the microbial community of post-mined sites was also significantly different from the undisturbed sites [8]. As for underground mining, land subsidence induced alteration of soil structure, loss of surface soil water and nutrients, and ultimately soil quality degradation. Without active restoration, the affected systems do not recover by “self-healing” after two years of land subsidence [9]. Coal mining can have significant impacts on various groups of microorganisms in the soil. The disturbance caused by mining activities can alter the physico-chemical properties of the soil, affecting microbial communities. Almost all soil microorganisms are affected during the mining process, including bacteria, fungi, and archaea. Although coal mining disturbs the soil microbial community and corresponding ecological functions, the remaining microbes make an essential contribution to soil reconstruction and land restoration (Figure 1). Bacteria are important in the decomposition of organic matter and the cycling of nutrients; fungi are involved in the breakdown of macromolecules and symbiosis with other species; and archaea are crucial in the nitrogen cycle. Understanding the precise function of the soil microbial community, including its response to land restoration, is the next important step in the application of microorganisms to promote ecological restoration in post-mining areas.



**Figure 1.** Impacts of coal mining on terrestrial ecosystems.

There has been much work carried out establishing the conceptual framework of ecosystem restoration, providing insight into the process and mechanisms at play, and forming the basis for developing guidance and practice [10–12].

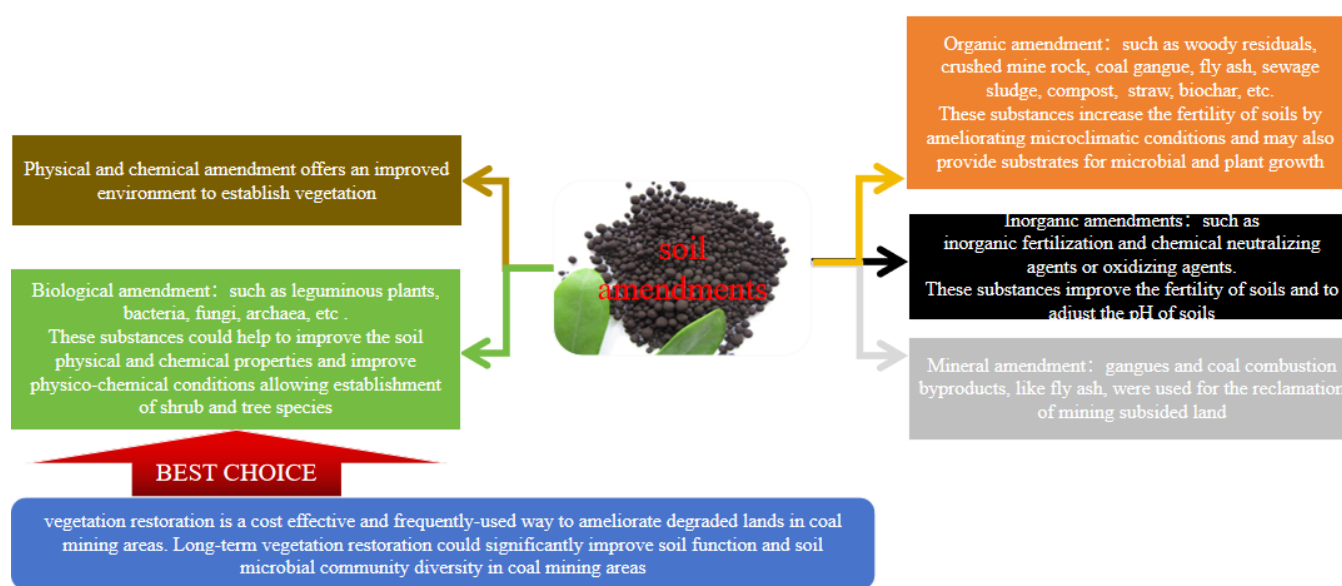
In general, soil quality could be improved by physical, chemical, or biological amendment. Some soil amendments and mechanical actions are usually applied to cross the abiotic barriers by improving soil structure, moisture, and chemical properties. These measures are also helpful in crossing biotic barriers. In consideration of the reclamation cost and resource recycling, nontoxic industrial by-products, domestic wastes, or the admixture of them may be used in mine soil reclamation, such as woody residues, crushed mine rock, coal gangue, fly ash, sewage sludge, compost, straw, biochar [13–17]. These substances are rich in organic matter and nutrition which increase the fertility of soils by

ameliorating microclimatic conditions and may also provide substrates for microbial and plant growth [18].

Besides organic amendments, inorganic or mineral amendments, such as inorganic fertilization and chemical neutralizing agents or oxidizing agents, have also been applied to improve the fertility of soils and to adjust the pH of soils [19,20]. By metal-binding and acid-neutralizing capacity, drinking water treatment residuals showed the potential to effectively treat AMD-impacted soils [21]. Gangues and coal combustion byproducts, like fly ash, have been used for the reclamation of mining subsided land in some areas. However, it is controversial as gangues and fly ash may contain high amounts of trace elements and may threaten the surrounding environment [22].

The physical and chemical amendment offers an improved environment to establish vegetation. In addition, biological amendment could help to improve the soil's physical and chemical properties. Leguminous plants show great potential for fertility enhancement, nutrient accumulation, and accumulation of C and N during the early stages of reclamation, which suggests that legumes can be used as initial colonizers to restore coal mine spoils [23], or as “nurse plants” to facilitate the stabilization of soil, and improve physico-chemical conditions allowing establishment of shrub and tree species [24]. Overall, vegetation restoration is a cost-effective and frequently used way to ameliorate degraded lands in coal mining areas. Many groups have shown that long-term vegetation restoration could significantly improve soil function and soil microbial community diversity in coal mining areas [25,26].

Successful mine land reclamation relies not only on the establishment of vegetation but also on the regeneration of soil microbial communities [27], thus, soil reclamation projects often combine physical, chemical, or biological amendment to shorten the succession time and to improve the stability of soil microbial ecosystem (Figure 2).

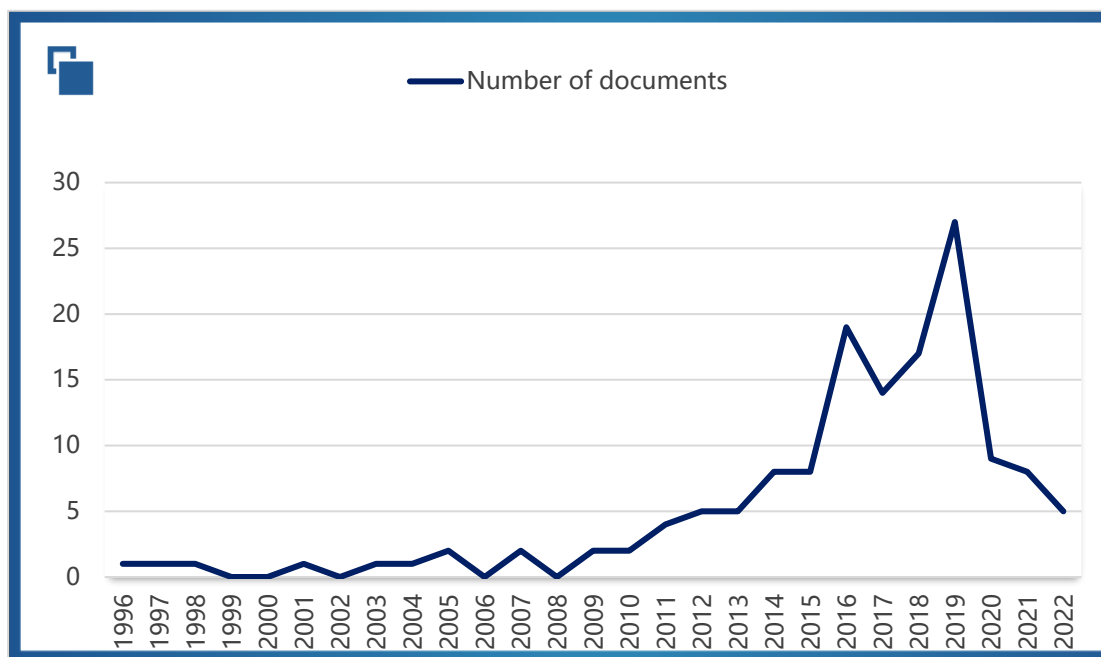


**Figure 2.** Effects of soil amendments on soil.

Soil microorganisms were considered as the first responders upon land disruption by coal mining and played an important role in land reclamation. In the present review, we aimed to provide insight into the effects of mining and subsequent land reclamation on soil microorganisms and the importance and application of microorganisms in the reclamation process, thus helping to give decision-making in the reclamation process.

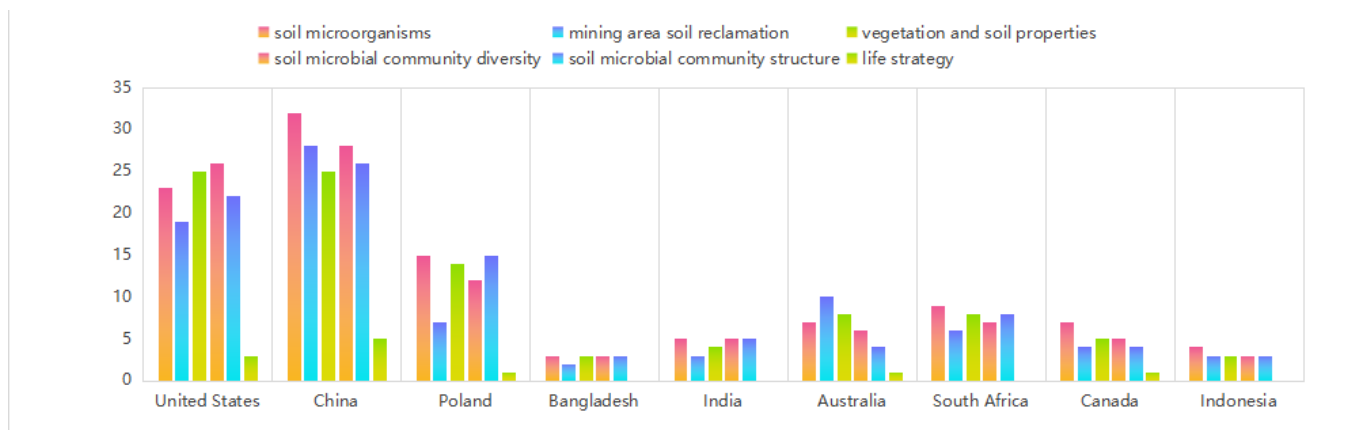
## 2. Literature Review

We reviewed and collected the literature on soil microorganisms after land reclamation in mining areas in the past 20 years (2000–2022) and drew a discounted graph of the number of literature changes over time (Figure 3), which directly and vividly showed us that more and more attention has been paid to the ecological restoration of land in mining areas in recent years.



**Figure 3.** The responses and applications of soil microorganisms to land reclamation in mining areas in recent 20 years.

We utilized multiple academic databases, including but not limited to Web of Science, PubMed, Scopus, and Google Scholar. This diverse database selection aimed to ensure comprehensive coverage of relevant studies across different disciplines (search terms: “soil microorganisms”, “mining area soil reclamation”, “vegetation and soil properties”, “soil microbial community diversity”, “soil microbial community structure”, “life strategy”, “response to land reclamation”). The articles were filtered according to the following criteria: (i) the relationship between coal mining and topsoil physicochemical characteristics and soil microbial community succession; (ii) Response of microorganisms to coal mine land reclamation and its application in reclamation. Articles were excluded if they did not pertain directly to soil microorganisms in reclaimed mining areas. Relevant data from each included article were systematically extracted. This encompassed information on the publication year, study location (country), research topics, methodologies employed, key findings, and any notable conclusions or recommendations. We summarized the distribution of literature by country and research topic (Figure 4). The United States and China had the most articles under the above keywords, which also correlates with the number of coal mines in these two countries and the degree of ecological importance attached to mining areas. In all the articles, in addition to the keywords “soil microorganisms” and “land reclamation”, “vegetation and soil properties” appeared frequently too, indicating that vegetation, soil, and soil microorganisms have received significant attention as key components of mine ecosystems during the process of mine restoration.



**Figure 4.** Distribution of literature by country and research topic.

### 3. Responses of Natural Microorganisms to Land Reclamation

Traditional reclamation strategies of soils degraded by coal mining aim for the establishment of vegetation, as vegetation contributes significantly to controlling soil erosion and evapotranspiration, restoring soil fertility and microbial activity [28]. Whereas, coal exploitation, especially surface mining, induces surface soil loss and therefore of soil nutrients, which is not suitable for highly productive vegetation establishment, but may provide appropriate conditions for pioneer and early successional species. Therefore, the re-establishment of the soil ecosystem is an essential precondition for ecosystem reclamation. Since soil microorganisms are sensitive to land disruption by mining and play an important ecosystem function in the soil, recovery of the soil microbial community is very important for the successful reclamation of the mining area [29–31]. Although soil microorganisms could respond to reclamation measures (such as physical, chemical, and biological amendment) and agronomic treatments (such as selected herbicide, mulching, succession planting, and green manure) [28,32,33], recovery of soil microbial community to “normal” or non-mined analogue sites require years or decades [29,34,35]. During the restoration process, soil microorganisms showed different responses to reclamation approaches and played different roles in the reclamation (Table S1). On account of their ecological functions in biogeochemical cycles, energy, and nutrient transfer, and their important roles in land reclamation, understanding how microbial communities respond to reclamation measures can provide insights into the potential management of ecosystem recovery.

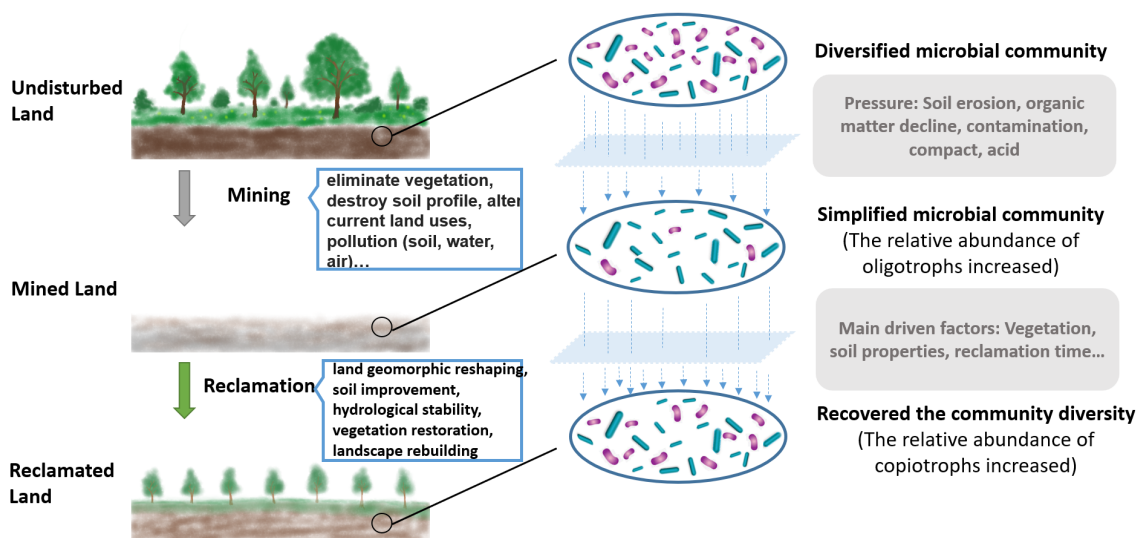
#### 3.1. Biodiversity Changes of Microbial Community after Land Reclamation

In many ecosystems, biodiversity, including that of soil microbial communities, is positively correlated with ecosystem responses for restoration compared to degraded sites [36]. Microbial diversity usually refers to the number of individuals that are assigned to different taxa and their distribution among taxa, including richness and evenness [37]. The measurement of soil microbial diversity is dominated by high-throughput sequencing and bioinformatics analysis. Operational taxonomic units (OTUs) are used to represent a taxonomic unit of a microbial species or genus depending on the sequence similarity threshold [38]. Microbial diversity can be decomposed into mathematical metrics (alpha diversity, beta diversity, or gamma diversity) based on the number of OTUs observed and the distance between taxa [39].

Microbial diversity drives multiple ecosystem functions and services in terrestrial ecosystems [40]. High microbial diversity promotes soil ecosystem functioning, while low microbial diversity hinders multiple above- and belowground ecosystem functions [41]. Numerous factors are known to affect soil microbial diversity, such as climate, soil factors, vegetation, land use, and disturbance (Figure 5). Coal mining directly impacts the immediate area and results in soil acidification, compaction, soil nutrition loss, and erosion, reducing the biomass, richness, and diversity of soil microbial ecosystems [8,42]. How-



ever, soil fungal and bacterial communities responded differently to opencast coal mining disturbance, that is, the fungal community was more stable than the bacterial community, showing more biomarkers and a more intricate ecological network despite having a smaller species diversity [43]. In underground mining areas, subsidence could also decrease the soil microbial richness and diversity by altering soil nutrition [9].



**Figure 5.** Responses of soil microbial community to reclamation.

Soil microbial diversity responds to most of the reclamation measures, including vegetation and fertilization [44]. Generally, vegetation will help the re-establishment of the soil microbial community, and vice-versa. Many factors, such as plant species type, plant species richness, and plant competition will affect the soil microbial diversity. Vegetation could significantly improve the abundance (ACE and Chao1) and diversity (Shannon and Simpson) of the bacterial community in reclaimed soils [25,34]. The soil nutrition (soil organic matter, total nitrogen, and phosphorus) also has positive relationships with ACE, Chao1, and Shannon indices [29]. Under different vegetative regimes, the bacterial community in pasture-reclaimed soils was richer and more diverse than wood-reclaimed soils [45]. In another research about the effect of different trees on abandoned coal land, all plantations except Mongolian pine improved soil microbial biomass to varying degrees compared with the abandoned land [46]. In addition, fertilization can boost soil-available nutrients including nitrogen, phosphorus, potassium, and soil organic matter, which can lead to an increase in the diversity of soil bacterial communities [44,47]. Both chemical and organic fertilizers could increase the bacterial diversity in reclaimed soils and shorten the process of reclamation [48]. As organic fertilizers could improve soil nutrition and the growth of plants and microbiology, the application of organic fertilizers could promote bacterial diversity in reclamation soils [47].

Along the chronosequence of reclamation, compared to the undisturbed site bacterial diversity significantly declined in younger reclaimed sites (1, 8-year-old), this reduction might be attributed to the disturbance caused by mining activities, leading to altered soil physico-chemical properties and a less stable microbial community. Then bacterial diversity increased in older reclaimed sites (15, 20-year-old), suggesting that a gradual adaptation of bacterial communities to the reclamation process, possibly driven by the establishment of vegetation and the accumulation of organic matter over time, was similar to pre-disturbance levels nearly 20 years after reclamation [29]. This indicates a successful restoration trajectory where the microbial communities have adapted and stabilized to resemble those of undisturbed ecosystems. It has the same trend in both 0–20 cm and 40–60 cm soil layers, bacterial and fungal diversity were higher in the soil after long-term

vegetation restoration than the short-term restoration [26]. This might be attributed to the accumulation of leaf litter and root exudation with the plant growth.

Soil bacterial, archaeal, and fungal communities showed variable responses to different reclamation scenarios, with bacteria and archaea appearing to be less sensitive than fungi to reclaimed treatments due to the vulnerability of hyphae to compaction and shear forces [33,46]. Afforestation could increase both fungal and bacterial biomass, however, fungal biomass approximately increased two times higher than bacterial, possibly due to conditions favourable to mycorrhizae, which drove the ecosystem toward stability [46]. Between vegetated and non-vegetated soil, there were significant differences in the  $\beta$ -diversity values of bacterial and fungal communities, with the changes being more noticeable for bacterial populations [49]. Fungal diversity significantly changed in response to reclamation vegetation and time, while bacterial and archaeal diversities were less influenced by reclamation [33]. Bacteria, archaea, and fungi play different ecological roles in reclamation and display different patterns along the reclaimed succession. Fungal diversity was sensitive to reclaimed scenarios in most cases; archaeal diversity was only sensitive in the initial reclaimed phase; bacteria made a great contribution to ecological stability during the initial restoration period [50]. Bacterial succession displayed a similar pattern to plant succession, while fungal communities showed a dissimilar pattern [33]. In habitats with various land-use types, there were differences in the patterns of bacterial and fungal communities. There were differences in the main fungal phyla among the three habitats (wetland, farmland, and grassland) but no differences in the major bacterial phyla [51].

Coal mining has not only impacted soil structure and nutrient partitioning but has also led to changes in soil microbial communities. Mine land reclamation not only improved soil physicochemical properties, but also led to changes in microbial communities, promoting microbial abundance and diversity. Because bacterial, archaeal, and fungal communities respond differently to varied soil environments, nutrition, and plants, and occupy different biological niches, there are significant variances in how different microorganisms correspond to different reclamation approaches.

### 3.2. Changes of Microbial Community Structure after Land Reclamation

Soil microbial community structure was related to soil properties, environmental factors, and land use [52,53]. Among these, soil properties contributed the most to soil microbial distribution, however other environmental factors showed less impact [53]. Soil properties, precipitation, and temperature represent the most critical drivers of soil microbial community distribution at a continental scale [54]. Land use (including vegetation cover and agricultural activities) could also strongly impact the microbial distribution, but this influence was more local [52,53].

Coal mining induced the alteration in soil properties, plant-derived exudates, and biomass, which influenced soil microbial community structure and composition and ecological niche partitioning of microorganisms. The impaired microbial community structure may have negative impacts on microbes drove soil biological processes, such as nutrient cycling and ecosystem sustainability [55]. If the change in the soil microbial community takes its course, the structure of the soil microbial community cannot recover in a short time. The recovering time depends on many factors including the extent of land damage, the existence of propagule, the growing cycle of plants, climate, etc. In mining subsidence of the aeolian sand area, it will take at least 2 years for the soil microbial community to recover [9]. However, it will take at least 30–40 years to recover into the forest on coal mining heaps [56]; and 54 years into a complex forest ecosystem on the originally barren spoil heaps of the temperate zone [57]. Therefore, some moderate artificial restoration would help to accelerate the succession process.

The structure and composition of microbial communities respond to reclamation treatments and reclaimed time [34,50,58]. The bacterial genera were similar between reclaimed soils and reference soils [26,45]. However, the ratio of dominant microbial communities in the soil of reclaimed soil successively changed [30]. We attempt to identify the variation

regularity in restoration succession by copiotrophy–oligotrophy continuum [59]. Based on this life strategy concept, oligotrophic (*K*-strategists) and copiotrophic (*r*-strategists) microorganisms are distinguishable by their growth kinetics and substrate affinity. Generally speaking, oligotrophs are slow-growing microbes that are dominant in nutrient-poor environments, whereas copiotrophs are those that have high growth rates under nutrient-rich conditions [60]. The mining disturbance induces soil nutrition loss, so before reclamation oligotrophs were abundant in the degraded soils. After reclamation, copiotrophs increased with the improvement of soil nutrition. Some research could support the hypothesis.

Although soil microbial community composition changed in the different mining areas, in many reclaimed soils, the relative abundance of some copiotrophic microorganisms, such as *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, increased, and the relative abundance of oligotrophs, such as *Actinobacteria* and *Chloroflexi* decreased [29,45,47]. *Firmicutes* are considered copiotrophs since they respond positively to carbon and nitrogen amendments [61,62]. *Firmicutes* could also degrade lignocellulosic biomass efficiently [62]. The reclaimed soils with more carbon, nitrogen, and lignocellulosic biomass are advantageous to the growth of *Firmicutes*. A significant increase of *Firmicutes* was shown in post-mined soils undergoing reclamation for 18 or 20 years after mining [47]. There's still controversy about the life strategy of *Proteobacteria*. However, in many research,  $\alpha$ -*Proteobacteria*,  $\beta$ -*Proteobacteria*, and  $\gamma$ -*Proteobacteria* could be categorized into copiotrophic groups, and the  $\delta$ -*Proteobacteria* could be assigned into oligotrophic groups [63–65]. *Proteobacteria* abundance correlated positively with soil organic carbon and total nitrogen [66]. *Proteobacteria* are the most omnipresent group in soils which were abundant in soils before and after reclamation. The relative abundance of *Proteobacteria* was significantly correlated with the reclamation period and increased in older rehabilitated sites [25,29]. These results indicated that the ratio of *K*-strategists to *r*-strategists would increase with the reclamation stage, which was consistent with the trends in soil microbial communities during secondary succession [67].

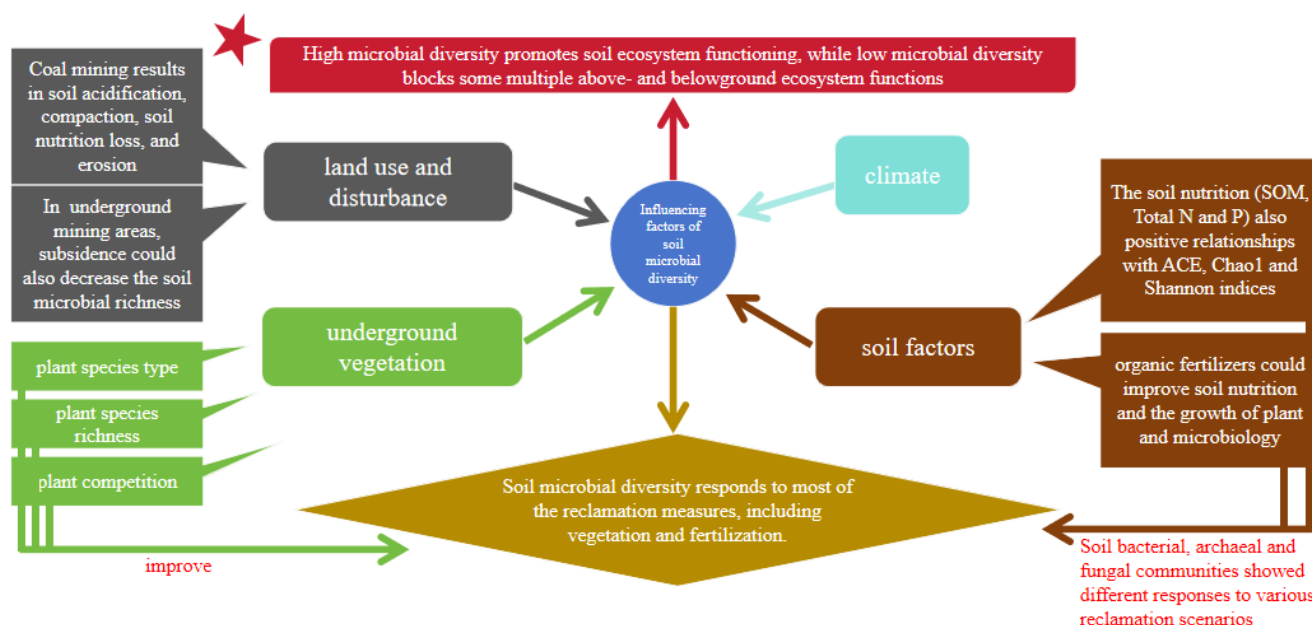
Mining activities can lead to a decline in soil nutrients, which can increase the proportion of microorganisms adapted to oligotrophic environments in the soil. Land reclamation, on the other hand, will result in a significant improvement in the nutrient-poor environment while increasing the proportion of copiotrophic microorganisms. Although there are still some problems with applying the oligotrophic-copiotrophic theory to describe microorganisms on the phyla level, this theory provides insights into the ecological mechanism of microbial community response to land reclamation in the mining area. Future research is needed to broaden our knowledge of the ecological role of soil microbes at finer taxonomic resolution and the soil nutrition variation in the reclamation succession to provide explanations for the community response of copiotrophic- and oligotrophic-associated microorganisms to reclamation measures.

### 3.3. Main Driven Factors of the Microbial Community in Reclamation

Vegetation, soil properties, and reclamation time are key determining factors of soil microbial community succession and ecological function development (Figure 6). Among these, the primary driver of soil bacterial, archaeal, and fungal community dynamics was vegetation [33,45,46,58]. Plant density and plant species were associated significantly with microbial community composition [34,46]. Dominant plants not only directly affect the soil microbial communities [68] but also indirectly influence these microbial communities by impacting the soil microflora and soil properties, such as pH; C, N, and P contents; and aggregate structure [46,69]. Besides, vegetation composition strongly influences the successional trajectories of soil microbial communities in the mining area. Research conducted in open-pit mines in western China, for instance, revealed that the soils re-vegetated by *Astragalus laxmannii*, *Halogeton arachnoideus*, and *Artemisia desertorum* exhibited a higher diversity of bacterial species than the bare land; conversely, the soils re-vegetated by *A. desertorum* and *A. laxmannii* demonstrated a significantly higher diversity of fungal species than the bare land [49]. In many countries, to reach the vegetation cover determined by regulation in the shortest time, alien and fast-growing herbs and shrubs with low diversity were



often adopted in the reclamation [70]. The aggressive competition caused native species to be suppressed and may prevent natural succession by inhibiting tree growth. The underground plant competition also affected soil bacterial communities [71]. There is some evidence that sowing seeds for ecological restoration decreased the diversity of fungi and bacteria by decreasing plant diversity [72]. Therefore, to create a stable healthy ecosystem with higher diversity, mixing planting or seeding with native species has been increasing in mine land reclamation [69].

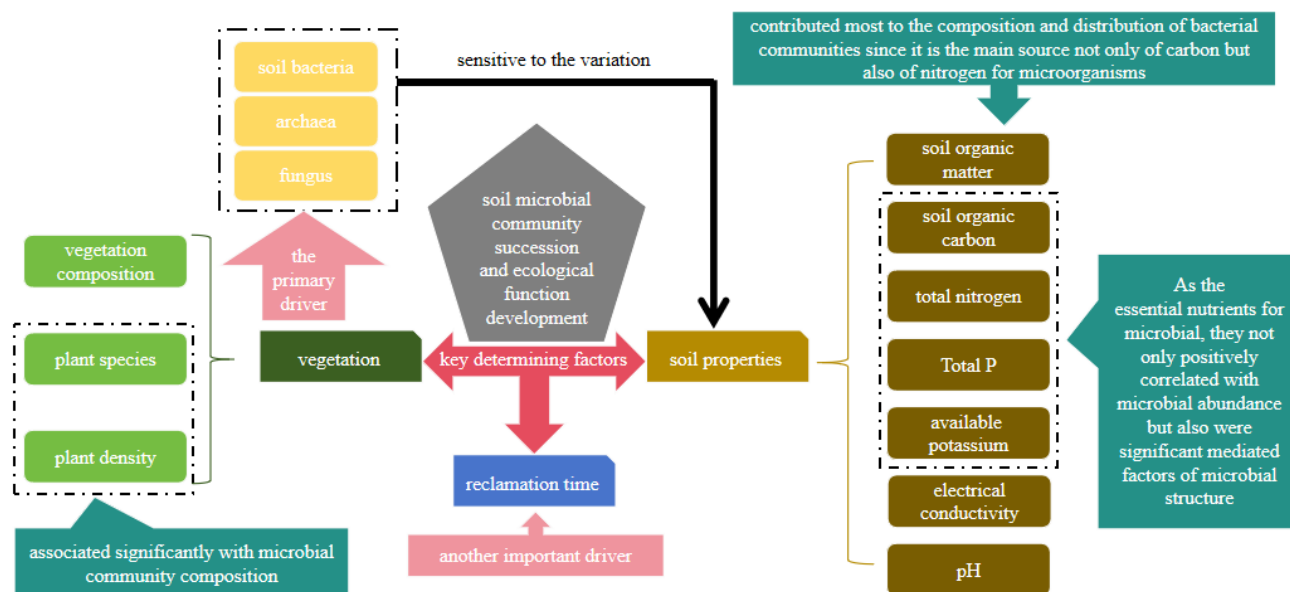


**Figure 6.** Biodiversity Changes of microbial community after land reclamation.

The composition of soil bacterial, archaeal, and fungal communities was sensitive to the variation of soil physicochemical properties in reclamation progress. Many reclamation measures, such as fertilization and vegetation, could improve the soil properties in the mining area, and then influence the soil microbial composition. Soil organic matter, soil organic carbon, total nitrogen, total phosphorus, available potassium, electrical conductivity, and pH were important factors shaping the underlying microbial communities of mining areas [26,29,34,46]. Soil organic matter contributed most to the composition and distribution of bacterial communities since it is the main source not only of carbon but also of nitrogen for microorganisms [26]. As the essential nutrients for microbial, soil organic carbon, total nitrogen, total phosphorus, and available potassium not only positively correlated with microbial abundance but also were significant mediated factors of microbial structure [50].

Time is another important driver of bacterial community dynamics in land reclamation of the mining area [34,35]. During the succession in a reclaimed mine land, vegetation succession, and soil properties variation were developing toward being healthier and more stable. Using an artificial reclamation approach, vegetation and soil nutrition could be restored in a few years, whereas the underground microbial community was still very difficult to recover. Especially for the community structure, even sometimes richness and diversity values exceeded reference, the bacterial community was still not structurally recovered [45] taking decades to recover the microbial community structure [29,34].

Reclamation time is the main driving force to recover microbial community and vegetation, and measures to improve soil properties could accelerate successional processes. The primary drivers of the bacterial, archaeal, and fungal communities vary due to their distinct responses to environmental factors in the soil (Figure 7).



**Figure 7.** Main driven factors of the microbial community in reclamation.

#### 4. Applications of Functional Microorganisms on Bioremediation

##### 4.1. Application of Microbial Inoculation on Bioremediation in the Coal Mining Area

Microbial inoculation is an approach showing some promise in restoring disturbed terrestrial ecosystems. Bacteria and fungi inoculation has been shown to increase soil fertility, ameliorate soil structure, suppress soil pathogens, and improve plant growth [73]. Mycorrhizal fungi could also significantly enhance plant facilitative interactions mainly through increasing plant biomass and nutrient content [74]. Arbuscular mycorrhizal fungi (AMF) play a crucial role in nutrient cycling and soil amelioration and are keystone organisms with myriads of ecosystem roles [75]. AMF is a type of mycorrhiza that can form a symbiotic association with legume roots, and early to mid-succession species. AMF could improve soil aggregation and plant nutrition; increase soil organic matter and soil water relation, enhance plants' abiotic stress tolerance and plant resistance; raise soil microbial diversity and abundance; boost plant productivity; regulate nutrient cycling; and drive succession and influence plant community structure [75]. However, in degraded soils, the AMF abundance, diversity, and infectivity fall to a low level [75]. Introducing AMF to degraded soil will help to improve soil properties and vegetation growth to contribute to forming a stable ecological system. In coal mining subsidence soils, AMF inoculation promotes plant acquisition of soil resources, especially nitrogen, and then mitigates soil erosion and desertification [76,77]. Mycorrhizal fungi also affect the function of ecosystems, local biodiversity, and plant growth. Plant symbiosis has an array of effects that range from mutualism to parasitism. The majority of trees symbiose with ectomycorrhizal fungi [78]. The role of ectomycorrhizae in tree and shrub establishment and growth is well known. Ectomycorrhizal fungi composition is a powerful bio-indicator of the underlying factors that drive tree growth and/or the idea that variations in the Ectomycorrhizal fungi communities of forests contribute to variations in tree growth [79].

Bacterial inoculation is another way to promote plant growth and be used in land restoration. Some specific genera, such as *Rhizobia* and *Azospirillum*, are often selected as inoculants applied in the restoration with AMF or solely [80]. In some mining areas, sewage sludge as a utilizable waste was applied to the reclamation of abandoned opencast mining areas [14]. Sewage sludge could act as an organic fertilizer and soil conditioner by providing large amounts of organic matter and available plant nutrients to microorganisms and plants. By itself, sewage sludge is a potent microorganism resource that contains various active microorganisms and plays a positive role in increasing the total microorganism population. Even so, the application of sewage sludge also holds environmental risks as it may contain

heavy metals, persistent organic pollutants, and pathogenic microorganisms. Therefore, before any microbial inoculation is applied to restoration, rigorous evaluation is required to guarantee ecological security. So Singh proposed that a successful restoration of disturbed ecosystems using microbes should combine all the knowledge about microbiology, ecology, biochemical mechanisms, and field engineering [81].

Recent research indicated that the use of local soil, but not commercial AMF inoculum, increased native and non-native grass growth at a mine restoration site [82]. There is increasing evidence that the use of local soil as a propagule source can not only aid vegetation establishment but also control assembly and below-ground network connectivity [83–86].

Various methods involving microorganisms are employed in the context of land reclamation to enhance soil fertility, structure, and overall ecosystem recoveries such as biofertilizers, biostimulation, bioaugmentation, and coating seeds with beneficial microorganisms. Biofertilizers are formulations containing beneficial microorganisms, such as nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, and mycorrhizal fungi, that enhance nutrient availability to plants. Biofertilizers are applied directly to the soil or seed, promoting plant-microbe interactions that improve nutrient uptake. Biofertilizers combined with effluent treatment plant sludge play an important role in the remediation of coal mine spoil dumps by improving the soil microbial community and promoting biomass (aboveground biomass and belowground biomass) production [87]. Biostimulation involves enhancing the activity of indigenous microorganisms in the soil by providing them with necessary nutrients, organic matter, or other stimulants. Organic amendments, such as compost or plant residues, can be added to the soil to stimulate microbial activity and accelerate natural reclamation processes. Fertilization can be viewed as a biostimulation action by adding nutrients to the soil, thereby stimulating the multiplication of soil microorganisms, and promoting self-restoration. Bioaugmentation involves introducing specific strains of microorganisms into the soil to enhance specific functions. In contaminated soils, bioaugmentation can be applied to introduce microorganisms capable of degrading pollutants, contributing to soil remediation. This technology is mainly used in the remediation of polycyclic aromatic hydrocarbon (PAH) contaminated sites in some coal mining areas [88]. Seeds are coated with beneficial microorganisms, including plant growth-promoting bacteria or mycorrhizal fungi, to enhance germination, seedling growth, and nutrient uptake. The coated seeds are sown directly into the reclaimed area, and the microorganisms assist in establishing a symbiotic relationship with the emerging plants. It was found that inoculating seeds of *A. saman* and *P. falcataria* with three native AM fungi (*Rhizophagus clarus*, *Gigaspora decipiens*, and *Scutellospora* sp.) could improve stem diameter, shoot nitrogen content, shoot phosphorus content, shoot dry weight, and survival rate, and thus promoted reforestation of post-opencast coal mine field [89]. These methods showcase the versatility of microorganisms in promoting soil health and ecological restoration. It's essential to tailor the chosen method to the specific needs and conditions of the reclamation site for optimal effectiveness.

#### 4.2. Applications of Microorganisms in Assessing Reclaimed Soils

It's important to find suitable criteria to evaluate the success of the land reclamation of the mining area. Success criteria need to be based on the specific restoration goals related to the nature of the systems, the factors of degradation, and the restoration approaches required to achieve restoration of different attributes [11]. Traditional assessment systems focus on soil physicochemical properties and vegetation characteristics [90]. In recent years, soil microbial community assessment gradually become an indispensable indicator to assess the quality of mine soil in the restoration process, as soil microbes can provide a more precise representation of the immediate recovery of degraded land and serve as an early indicator of successful restoration [32,91,92]. Measurement of the soil microbial community indicated that the restoration target of the community was effective in the management interventions, and manipulation of the community helped increase the recovery rate of the degraded soils [92]. These microbial-related indicators often include

microbial diversity, microbial community structure, richness, soil enzyme activities, and diversity [50,55,93]. Generally, higher soil microbial diversity and soil enzyme activities indicated the higher functional plasticity or stability of the soil ecosystem [55]. In addition to this, indicators such as microbial biomass, microbial respiration, rhizosphere microbiome microbial extra-cellular polymerase, etc. have been used to the success of land reclamation [45]. An increase in microbial biomass signifies improved soil health and microbial activity, reflecting positive responses to reclamation efforts [6]. An increase in microbial respiration indicates enhanced microbial metabolic activity, which is a positive indicator of soil fertility and nutrient cycling [94]. A diverse and well-functioning rhizosphere microbiome contributes to plant health, nutrient cycling, and overall ecosystem stability [71]. Increased microbial extracellular enzyme activity indicates enhanced decomposition of organic matter and nutrient cycling in reclaimed soils [39]. Monitoring shifts in microbial community structure helps gauge the recovery of native microbial populations and their functional roles in reclaimed ecosystems [55]. An increase in soil organic carbon indicates improved soil fertility, as it is a key component for microbial growth and nutrient availability [92]. Nowadays, metagenomics serves as a powerful tool in assessing microbial diversity and provides in-depth information about the gene function which would help comprehend the variety of ecological functions in the reclamation process [95]. The biological information combined with physical and chemical properties could provide comprehensive insight into the soil health condition and strong support for forecasting and decision-making in mine land restoration.

Microorganisms, as an important part of soil ecosystems, their community structure composition, diversity, and physiological changes can be used as important indicators for the evaluation of reclaimed soils. The comprehensive use of these indicators can reflect the degree of soil ecosystem health, thus providing a comprehensive assessment of the success of land reclamation in mining areas.

#### 4.3. Applications of Microorganisms to Remediate Pollution in Mining Areas

Acid mine drainage (AMD) brings great environmental problems in the mining area, because of its acidity, high concentrations of  $\text{SO}_4^{2-}$  and heavy metals. It pollutes water and soil and damages essential ecosystem services [96]. When AMD was mixed with the original soil, microbial communities were formed, mediating rapid oxidative precipitation of iron in AMD [97]. As an adaptive strategy for soil microbial survival in a very acidic environment, microbial processes including lipopolysaccharide and peptidoglycan formation, translation, and repair, were improved, and the expression of nitrogen-fixing genes in soil was high [98]. Physicochemical treatment methods are common methods to remediate AMD pollution, including the application of alkaline chemicals to precipitate metals or using adsorption, ion exchange, and membrane technology to remove it from the environment [99]. In recent years, bioremediation has received more concern due to its economic and environment-friendly character. Bioremediation could be accomplished in two ways: one is to utilize a specialized microbial consortium to remove pollutants; the other is to add nutrients to enhance the activity of indigenous microorganisms [94]. At the phylum level, *Proteobacteria*, *Nitrospira*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, *Aquificae*, and *Candidatus* represent the primary bacterial lineages detected in the AMD environments [100]. The indicator genus in samples with  $\text{pH} < 3$  was *Sulfobacillus*, whereas bacteria associated with *Acidobacteriaceae* predominated in samples with  $\text{pH} 3 < \text{pH} < 3.5$  [101]. Some of them are sulfur and iron-oxidizers genera which could reduce ferric iron when growing on elemental sulfur as an energy source [102]. These members are often selected for AMD treatment to precipitate metal, raise pH, and reduce  $\text{SO}_4^{2-}$  and metal concentrations. The iron and sulfate-reducing bacterial consortium was also applied in enhanced bioremediation of acid mine drainage impacted soil of the mining area [94]. The development of omics approaches, and molecular technology provides novel tools to explore the ecology, evolution, and mechanisms of microbial communities acting in bioremediation and significant clues for biotechnological applications of AMD microorganisms [96,100].

## 5. Conclusions

Land reclamation, as an effective method to countering the negative impacts of coal mining, not only affects the aboveground vegetation, but also brings great influence on the underground microbial community, and vice versa, the soil microbial community makes a great contribution to soil reconstruction and land reclamation. The recovery of microbial communities is primarily influenced by vegetation, soil characteristics, and age of reclamation. Various restoration techniques will have varying effects on soil microbial communities. As a process that reinforces itself, soil microorganisms themselves can promote the restoration of the mining ecosystem and serve as evaluation markers for land reclamation in mining areas.

Understanding how microbial communities respond to reclamation measures can provide insights into the potential for ecosystems to recover, and how successful management interventions have been. It will be helpful to expand the application of microbes in land reclamation. Especially with the development of new sequencing and analysis technology, people will have a further understanding of the response of microorganisms to land restoration, and microorganisms will play a more important role in land reclamation in coal mining areas.

Future research could investigate the fine-scale dynamics of microbial communities during different stages of land reclamation. Investigating temporal shifts, key microbial players, and their functional roles at a finer resolution will enhance our ability to predict and manage reclamation outcomes. Utilizing functional metagenomics could uncover the specific genetic potential and metabolic pathways of microbial communities involved in soil reconstruction. This knowledge will inform targeted interventions, allowing for the manipulation of microbial functions to optimize reclamation success.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16020086/s1>, Table S1: Responses of microorganisms to land reclamation.

**Author Contributions:** Conceptualization, Z.M. and J.H.; methodology, Z.M.; software, Z.M.; validation, Z.M. and Z.Z.; formal analysis, Z.Z. and Z.M.; investigation, Z.M.; resources, Z.M. and J.H.; data curation, Z.M. and Z.Z.; writing—original draft preparation, Z.M. and J.H.; writing—review and editing, J.H. and Z.M.; visualization, Z.M. and Z.Z.; supervision, J.H. and Z.M.; project administration, Z.M.; funding acquisition, Z.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Natural Science Foundation of China (grant number 52004274) and the Major National Science and Technology projects of China (2020YFC1806500).

**Institutional Review Board Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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