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Mycorrhizal Fungi Reclamation Promotes Stoichiometric Homeostasis of Re-Vegetation Types and Affects Soil Bacterial Function in Mining Subsidence of Northern Loess Plateau

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Abstract: Re-vegetation types and mycorrhizal fungi reclamation play a vital role in the improvement of soil quality in the mining subsidence of the northern Loess Plateau. However, the effects of re-vegetation types and mycorrhizal fungi reclamation on plant stoichiometric homeostasis, soil bacterial communities and functional characteristics are still not understood well but are vital for mining green construction. Based on the fact that mycorrhizal fungi reclamation has been implemented for more than 10 years (inoculation with arbuscular mycorrhizal fungi (AMF) and control), we examined five re-vegetation types with different C:N:P stoichiometry in the roots, leaves and calculated homeostasis. Meanwhile, second-generation sequencing technology was used to measure soil bacterial communities and functional characteristics to further reveal the relationships between soil factors and bacteria that drive plant stoichiometry and homeostasis in the biological reclamation area of coal mining subsidence. Our results indicated that plant N:P ratio in the leaves of all re-vegetation types was less than 14, with the highest ratio observed in *A. fruticosa* (nitrogen-fixing plants), showing that re-vegetation growth was limited by the availability of nitrogen. Only leaves in AMF-inoculated plants were categorized as 'homeostatic', while inoculation with AMF in both leaves and roots could alleviate nitrogen restriction and improve ecological stoichiometric homeostasis. The dominant phylum was *Proteobacteria*, followed by *Actinobacteria*, *Acidobacteria*, accounting for 69.92%–73.22% of all bacterial species and 82% with *Chloroflexi*. Soil copiotrophic community (*Proteobacteria*) in the AMF inoculation area was higher than those in the control area under all re-vegetation types, while the oligotrophic community (*Acidobacteria*) was lower than the control. Further analysis showed that soil TP, SOC, C:N and HD played vital roles in shifting the soil bacteria community. Soil stoichiometry and AMF affect microbial composition. These results indicated that the re-vegetation types and mycorrhizal fungi reclamation could shift bacterial homogeneity. Hence, our results expound that mycorrhizal fungi reclamation could optimize the ecological strategies of reclaimed vegetation, alleviate N-limitations in plants, improve endogenous stability and promote the ecological function of soil bacteria, which provided theoretical bases for further understanding and application of green restoration and sustainable development in the mining subsidence of the northern Loess Plateau.

Keywords: mycorrhizal fungi reclamation; re-vegetation types; ecological stoichiometry; soil bacteria; mining subsidence

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1. Introduction

China's energy endowment structure is dominated by coal and makes great contributions to economic development [1]. However, coal mining is also associated with a series

of problems, e.g., waste production, vegetation destruction and soil degradation [2] in the mining subsidence areas [3,4]. Mining and land reclamation, especially restoration by artificial vegetation, have significantly altered the soil physicochemical properties and played a key role in restoring the ecosystems in ecologically sensitive regions [5,6]. In recent years, microbial reclamation technology, especially restoration by arbuscular mycorrhizal fungi (AMF), has been developed, which can not only accelerate the recovery of the greenery but also promote the rapid absorption of nutrients and improve the resistance of plants [7,8]. Although the ecological effects of successful mine reclamation can be evaluated and estimated through many models [9,10], soil availability and microbial activity are important components of ecosystem restoration after mining reclamation [11]. Meanwhile, understanding microbial communities and their functions in soil bioremediation is important for detailed ecological functions of mine reclamation [12]. Mine soil after reclamation determines the direction and sustainable development of land utilization after reclamation, and further understanding of soil change characteristics during reclamation and restoration is of great significance for guiding mine ecological construction.

Ecological stoichiometry is a key tool for determining the interaction and coordination of multiple elements in the ecosystem, which is of great significance to predicting the ecological recovery potential and understanding the functions and dynamics of fragile ecosystems after reclamation in subsidence areas. [13]. Leaves and roots are vital organs for nutrient storage. Nutrient content necessarily reflects its distribution in plants under varying environmental conditions [14]. The ratios of C:N and C:P in the leaves can indicate the characteristics of plant nutrient utilization. Therefore, investigating the ecological stoichiometry and limiting characteristics of leaves is a simple and effective method for detailing nutrient cycling and balance in coal mining subsidence areas [15] and also an effective way to explore the response of vegetation restoration to reclamation patterns. Furthermore, AMF influences the ratio of plant N:P by shifting plant N and P assimilation, which further affects vegetation stoichiometric homeostasis [16]. Additionally, AMF can also affect the N:P homeostasis of dominant plants in response to changes in soil nitrogen and phosphorus availability [17]. To date, C:N and N:P imbalance of mycorrhizal fungi reclamation re-vegetation types and its impacts on microbial metabolism and activities are still unclear. To accommodate the imbalance, microorganisms may adjust microbial activity to maximize the mobilization of abundant elements and substrates, improving the efficiency of element utilization [18]. Hence, understanding ecological stoichiometry and homeostasis in plant leaves and roots under different vegetation and mycorrhizal fungi treatment and revealing the mitigation effect of mycorrhizal reclamation on nutrient restriction is of great ecological significance for the promotion of green mine construction in the mining subsidence of the northern Loess Plateau.

Soil bacteria, as important participants in nutrient cycling and soil fertility changes in terrestrial ecosystems, could respond to soil physiological and biochemical processes and are closely related to changes in soil nutrient availability [19]. Soil microorganisms are very sensitive to environmental changes, so the characteristics of soil microbial community structure can be used as a key index to reflect soil quality changes during vegetation regeneration and succession. Several studies reported that vegetation types dramatically affect the soil microbial biomass and community, and these effects are the result of a complex process that is simultaneously regulated by many biotic and abiotic factors [20]. During the restoration process, the differences in diversity between plant and microbial communities indicate a lack of common environmental control factors and direct functional connections between plants and soil microorganisms [21]. In addition, previous studies have shown that AMF can influence the wider soil community by altering bacterial community composition [22]. The influence of AMF extends far beyond single pairwise interactions between AMF and other microbes, as their activity also alters many physicochemical characteristics of the soil environment [23]. In addition, the relationship between soil C:N:P stoichiometry and soil bacteria indicated that soil microorganisms were largely controlled by soil nutrient stoichiometry [24]. Particularly, the C:N:P ratio of soil is an important factor driving micro-

bial composition, playing a crucial role in soil–plant interactions and terrestrial nutrient constraints [25]. We propose assessing the biological composition of microbial communities in conjunction with mycorrhizal bioreclamation in the context of ecological stoichiometry and homeostasis to gain insight into the constraints of biogeochemical cycles. Therefore, it is necessary to elucidate the relationship between soil bacterial diversity and function, soil nutrient restriction and mycorrhizal biological reclamation, which is of great significance for the sustainable development of mine ecological reclamation and ecological restoration.

Here, based on the fact that mycorrhizal biological reclamation has been implemented in the Shendong mining area for more than 10 years with both AMF inoculation and control areas, we examined five re-vegetations with differing C:N:P stoichiometries of leaves, roots and calculated homeostasis. We analyzed soil bacterial communities and functional characteristics to further reveal the coupling relationships between soil factors and bacteria that drive ecological stoichiometry and stoichiometric homeostasis in the biological reclamation area of coal mining subsidence. Specifically, we hypothesized that (1) in the barren reclamation area of the coal mine, different re-vegetation types are limited by nutrients, but mycorrhizal fungi have certain alleviating effects and can improve stoichiometric homeostasis after reclamation, and (2) re-vegetation type and mycorrhizal reclamation can drive soil bacterial community composition and network characteristics and promote soil bacterial function through the mitigation of nutrient restriction and ecological stoichiometry homeostasis.

2. Materials and Methods

2.1. Study Location and Sampling Design

This research area was located in the mycorrhizal reclamation area (38°50′–39°47′ N, 109°13′–110°67′ E) of Daliuta Coal Mine in Shenmu county, northern Loess Plateau (Jizi Bay in Yellow River basin). The area is the transition zone from the Loess Plateau area to the Ordos Plateau sandstorm area, with an altitude of 738 to 1448 m. The annual average temperature is 8.7 °C, the extreme minimum temperature is −29 °C, and the extreme maximum temperature is 41.2 °C. The annual average frost-free period is 130 days, and the annual average precipitation is 405.6 mm. According to the IUSS (FAO) soil classification system, the soil in this area is *Arenosols* with poor fertilizer-retaining ability. After coal mining, the area experienced a series of subsidence events, exacerbating vegetation degradation and soil impoverishment. Since 2012, our research group has conducted land reclamation and mycorrhizal ecological restoration in the area, with five types of artificial shrub forest vegetation. The re-vegetation types were *Amorpha fruticosa* (AF), *Hippophae rhamnoides* (HR), *Xanthoceras sorbifolium* (XS), *Cerasus humilis* (CH) and *Cerasus szechuanica* (CS). Each vegetation type was provided with inoculation zone (FM) and control zone (CK), and the FM was planted in 2012 and simultaneously inoculated with AMF (*Funneliformis mosseae*). Mycorrhizal fungi were isolated and purified from the study area, identified as *Funneliformis mosseae* and cultured to obtain microbial inoculants, with inoculation amount of 100 g/seedling) [26]. These sample collections still had a high infection rate of plants with AMF [27].

According to on-site investigation, five types of vegetation were selected for the study in August 2021, including the inoculation area and the control area. The location and sampling design of the study area are shown in Figure 1. Three replicates were set for each vegetation type and mycorrhizal reclamation, and three random quadrats were set for each replicate (each 20 × 20 m). The sampling depth of soil was 0~20 cm, seven samples were collected in each quadrate according to 'S' with soil drill, and one sample was obtained after mixing. Plant leaves and roots were collected at the same time in each 20 × 20 m quadrat. The collected soil samples were divided into three parts: one part was naturally air-dried for basic physicochemical properties; one copy was stored at 4 °C for conventional biological traits; and the third sample was stored at −80 °C for second-generation biosequencing.

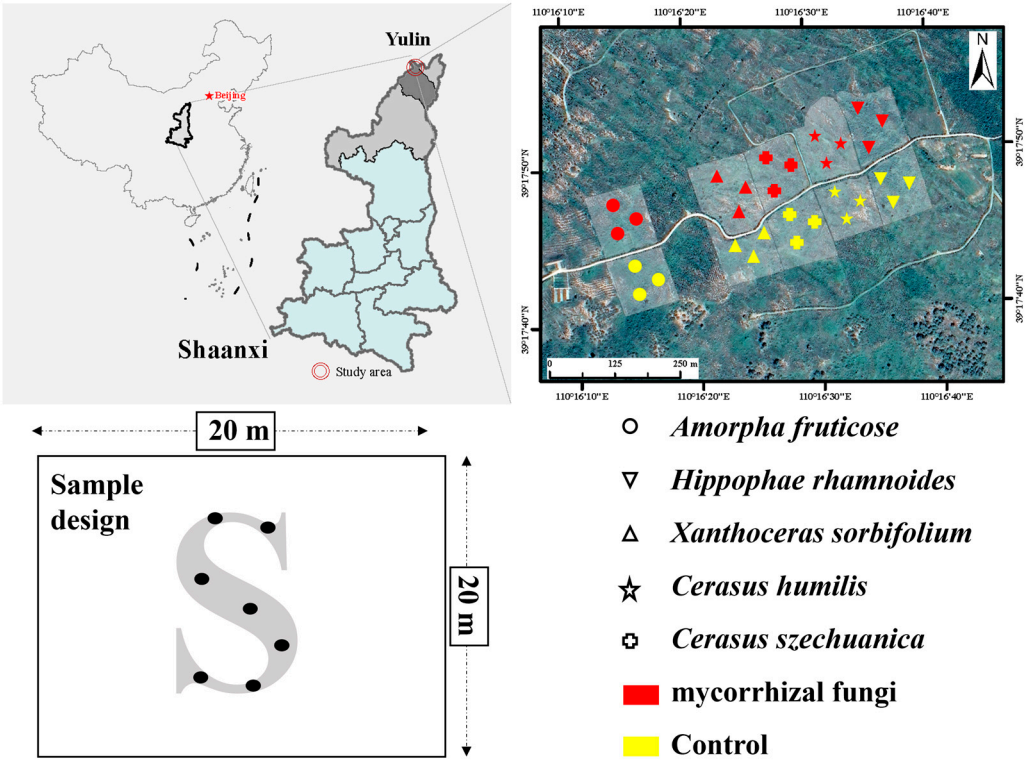


Figure 1. Study area and sample design. Sampling points with different shapes represent different revegetation types. The color of different shape sampling points indicates different microbial treatments: red indicates areas of inoculated arbuscular mycorrhizal fungi (FM), and yellow indicates the control area (CK); the same below.

2.2. Determination of Soil Physicochemical Properties

Soil pH was determined using a glass electrode in a 1:2.5 (w/v) soil:water suspension. Soil total P ($g \cdot kg^{-1}$) was acidified with H_2SO_4 and $HClO_4$ and measured using coupled plasma optical emission spectrometer (ICP-OES, Optima 5300DV, Perkinelmer Co. Ltd., Waltham, MA, USA). The soil and plant C ($g \cdot kg^{-1}$) were determined by using dichromate oxidation, and total nitrogen (TN $g \cdot kg^{-1}$) contents were determined using the Kjeldahl method [28]. Soil easily extractable glomalin (EEG) was extracted from citric acid solution and disodium hydrogen phosphate solution and measured using spectrophotometer [29]. The rate of mycorrhizal colonization (MC) was measured by staining with 0.05% Brilliant blue dyeing, and the hyphae density (HD) was measured using vacuum pump microporous filtration membrane method [30].

2.3. Soil Microbial Sequencing

Cryopreserved soil samples were sent to Majorbio BioPharm Technology Co., Ltd. (Shanghai, China) for Illumina Second generation sequencing using HiSeq 2500 platform (Illimina, Illumina cBot, San Diego, CA, USA). All soil microbial DNA was extracted by using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's instruction. After genomic DNA extraction was completed, the extracted genomic DNA was detected using 1% agarose gel electrophoresis. The 16S rRNA genes were amplified using specific primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with the barcode [31]. The PCR products of the same sample were mixed and detected using 2% agarose gel electrophoresis. The PCR products were recovered by using AxyPrepDNA gel recovery kit (AXYGEN) and elution by Tris-HCl; 2% agarose electrophoresis detection. The PCR products were quantified using the QuantiFluor™-ST Blue fluorescence quantification system (Promega) in reference to the initial quantitative results of electrophoresis and then mixed proportionally according

to the sequencing volume requirements of each sample. The library was sequenced on an Illumina HiSeq2500 platform, and 250 bp paired-end reads were generated.

2.4. Biological Information and Data Analysis

All paired-end (PE) reads sequenced with Illumina were first spliced according to overlap, and the sequence quality was controlled and filtered using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/, accessed on 20 July 2023). OTU cluster analysis and species taxonomic analysis were performed after samples were distinguished (clustering method: USEARCH7-uparse algorithm; OTU sequence similarity: 0.97; species classification database: silva128/16s_bacteria). Multiple diversity index analysis could be conducted based on OTU cluster analysis. The OTU was analyzed using various diversity indices, and the sequencing depth could be detected. Based on taxonomic information, statistical analysis of community structure could be performed at various taxonomic levels. On the basis of the above analysis, a series of in-depth statistical and visual analyses and Functional Annotation of Prokaryotic Taxa (FAPROTAX) such as multivariate analysis and difference significance test could be carried out on the community composition and phylogenetic information of diverse samples (Majorbio BioPharm cloud platform, https://cloud.majorbio.com/page/tools/, accessed on 20 July 2023).

Ecological stoichiometric internal stability is one that could be quantitatively calculated [32] and is expressed as the relationship between the stoichiometric characteristics of organisms and environmental stoichiometric characteristics as $\ln(y) = \ln c + 1/H \times \ln(x)$, where x refers to the supply of nutrients in the environment, and y refers to the amount of elements in the organism. The index was interpreted into five types: $1/H \geq 1$, not homeostatic; $1/H > 0.75$, 'plastic'; $0.5 < 1/H < 0.75$, 'weakly plastic'; $0.25 < 1/H < 0.5$, 'weakly homeostatic'; and $0 < 1/H < 0.25$, 'homeostatic' [33].

All data were collated and analyzed using SPSS22.00 (two-factor analysis of variance) compared with the difference between the mean values of soil physicochemical properties and microbial diversity index, and the significance was determined using LSD test at $p < 0.05$ level and 95% confidence level (means $\pm$ standard). Redundancy analysis (RDA) was used to investigate the effects of soil environmental factors on bacterial communities using R 4.3.0 ("vegan" package). The figures of bacterial relative abundance were generated with an origin of 2021, and co-occurrence patterns were generated using Gephi 0.9.2.

3. Results

3.1. Ecological Stoichiometry in Root and Leaf under Re-Vegetation Types and Mycorrhizal Fungi Reclamation

Different C:N:P ratios were showed in leaves and roots under re-vegetation types (Tables 1 and 2). The average C:N, C:P and N:P ratios were 17.53, 180 and 10.60, respectively, in leaves while the average C:N, C:P and N:P ratios in roots were 42.42, 268.10 and 8.05, respectively (Tables 1 and 2). The C:N ratios in leaves and roots under all re-vegetation types in FM were significantly lower than those in CK treatments. AF-FM had the lowest C:N ratio in leaves and roots ($p < 0.05$). The C:P ratios in leaves in the CH-FM and CH-CK were significantly higher than those in the other re-vegetation types ($p < 0.05$), and the C:P ratios in roots in CS-CK were significantly higher than those in the other re-vegetation types ($p < 0.05$). Meanwhile, the N:P ratios in leaves in all re-vegetation types were less than 14 but were the highest in AF-FM in leaves and roots.

Ecological stoichiometric homeostasis was significantly different under the five re-vegetation types and mycorrhizal fungi reclamation (Figure 2). In terms of N content, the leaves in FM and CK were considered as 'homeostatic' and 'weakly homeostatic', with 1/H values of 0.1385 and 0.3119 ($R^2 = 0.2316$ and $R^2 = 0.5935$), respectively. However, the roots in FM and CK were categorized as 'weakly plastic' and 'plastic', with 1/H values of 0.6970 and 0.8810 ($R^2 = 0.4577$ and $R^2 = 0.9192$), respectively. Interestingly, ecological stoichiometric homeostasis could be improved in leaves and roots that were inoculated with AMF.

Table 1. Ecological stoichiometry of leaves under different AMF treatments and re-vegetation types.

	C:N		C:P		N:P	
	FM	**CK**	**FM**	**CK**	**FM**	**CK**
AF	12.01 ± 0.29 f	12.73 ± 0.09 f	157.93 ± 4.01 c	159.66 ± 5.44 c	13.15 ± 0.12 a	12.54 ± 0.28 ab
HR	13.38 ± 0.40 ef	14.50 ± 0.45 e	160.73 ± 3.64 c	160.71 ± 5.04 c	12.02 ± 0.43 b	11.10 ± 0.38 c
XS	18.65 ± 1.11 cd	21.96 ± 1.27 b	178.94 ± 4.99 b	185.03 ± 7.34 b	9.60 ± 0.35 e	8.44 ± 0.38 f
CH	20.02 ± 1.06 c	24.88 ± 0.68 a	213.96 ± 13.28 a	215.28 ± 18.27 a	10.69 ± 0.21 cd	8.70 ± 0.95 f
CS	17.80 ± 0.95 d	19.49 ± 0.52 c	179.51 ± 4.60 b	188.24 ± 1.62 b	10.10 ± 0.28 de	9.66 ± 0.29 e
	P(M) *, P(V) NS, P(V*M) *		P(M)NS, P(V) NS, P(F*V) *		P(M) NS, P(V) NS, P(M*V) NS	

Note: AF, *Amorpha fruticosa*; HR, *Hippophae rhamnoides*; XS, *Xanthoceras sorbifolium*; CH, *Cerasus humilis*; CS, *Cerasus szechuanica*; C:N, the ratio of leaf C:N; C:P, the ratio of leaf C:P; N:P, the ratio of leaf N:P; Each vegetation type was provided with inoculation zone (FM) and control zone (CK); different letters indicate significant differences among re-vegetation type and arbuscular mycorrhizal fungi; P(M): difference among arbuscular mycorrhizal fungi; P(V): difference among re-vegetation types; P(M*V): interaction of arbuscular mycorrhizal fungi and re-vegetation type. * ($p < 0.05$). NS: no significance; the same below.

Table 2. Ecological stoichiometry of root under different AMF treatments and re-vegetation types.

	C:N		C:P		N:P	
	FM	**CK**	**FM**	**CK**	**FM**	**CK**
AF	18.73 ± 0.56 f	19.88 ± 0.56 ef	274.88 ± 10.46 ab	256.83 ± 12.16 bc	14.68 ± 0.31 a	12.98 ± 0.52 b
HR	24.03 ± 0.72 de	26.78 ± 2.73 cd	270.50 ± 8.34 ab	285.33 ± 27.22 a	11.26 ± 0.14 c	10.67 ± 0.12 d
XS	31.56 ± 1.74 bc	33.20 ± 2.58 b	232.43 ± 11.06 d	238.54 ± 12.31 cd	7.37 ± 0.41 e	6.87 ± 0.72 e
CH	66.26 ± 5.84 a	68.66 ± 5.22 a	277.15 ± 9.13 ab	271.62 ± 10.89 ab	4.20 ± 0.25 f	3.99 ± 0.13 f
CS	65.15 ± 1.06 a	69.90 ± 2.66 a	281.08 ± 6.97 ab	292.62 ± 7.65 a	4.31 ± 0.08 f	4.16 ± 0.13 f
	P(M) *, P(V) NS, P(M*V) *		P(F)NS, P(V) NS, P(M*V) *		P(M) NS, P(V) NS, P(M*V) NS	

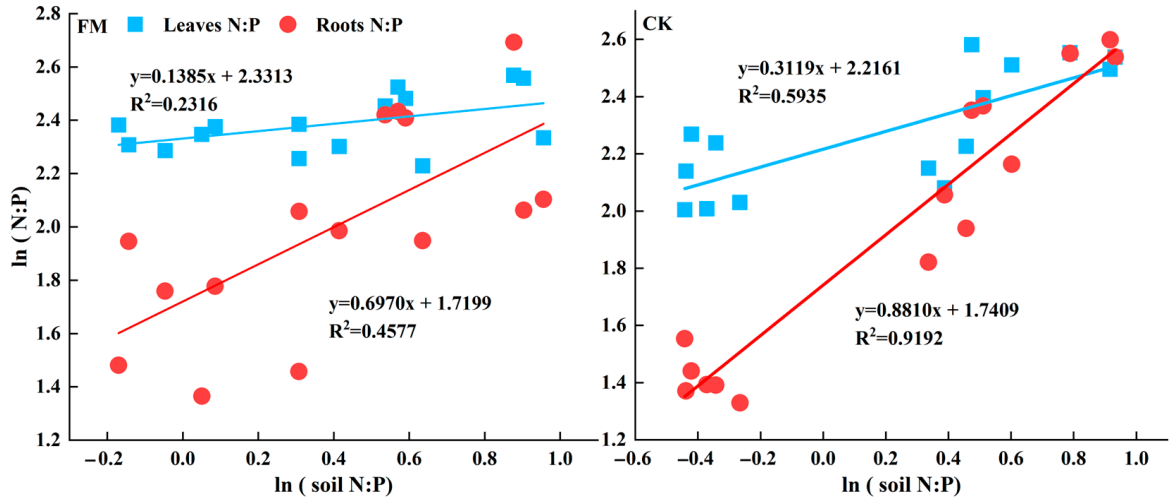


Figure 2. Ecological stoichiometric homeostasis under inoculation with arbuscular mycorrhizal fungi and control.

3.2. Soil Alpha Diversity Index and Community Composition under Re-Vegetation Types and Mycorrhizal Fungi Reclamation

Soil bacterial community diversity index was affected by re-vegetation types, AMF inoculation and their interactions (Table 3). The highest and lowest diversity (Shannon index) was observed in the HR-FM and CS-CK re-vegetation types, respectively. Simpson diversity index was the highest in CS-FM and lowest in HR-CK. The ACE index was similar

to other indices in all re-vegetation types. Based on sequences analysis, bacteria in the ten treatments could be classified into 42 phyla, 101 classes, 227 orders, 377 families and 432 genera. The composition of soil bacterial communities is shown in Figures 3 and 4. According to the relative abundance of each phylum, 10 dominant phylum groups were revealed in the soils under the three re-vegetation types (Figure 3). The dominant phylum was *Proteobacteria* (24.88%–31.50%), followed by *Actinobacteria* (17.68%–23.30%), *Chloroflexi* (9.38%–12.12), *Acidobacteria* (19.24%–26.11%), *Bacteroidetes* (3.90%–6.61%), *Gemmatimonadetes* (3.10%–4.34%), *Nitrospirae* (0.93%–2.41%), *Saccharibacteria* (0.64%–1.81%), *Firmicutes* (0.63%–1.93%) and others (4.40%–5.58%). The relative abundance of *Proteobacteria*, *Acidobacteria* and *Actinobacteria* was 69.92%–73.22% among all bacterial species, with an even higher percentage of around 82% when *Chloroflexi* was included. *Proteobacteria* in HR-FM and AF-FM had a higher relative abundance (31.38% and 31.50%, respectively) than that in other re-vegetation types. However, *Acidobacteria* in CK had a higher relative abundance compared to that in all re-vegetation types with AMF.

Table 3. Soil bacterial diversity index under different AMF treatments and re-vegetation types.

	Shannon		**Simpson**		**ACE**	
	FM	**CK**	**FM**	**CK**	**FM**	**CK**
AF	1.95 ± 0.02 adc	1.93 ± 0.04 bc	0.202 ± 0.013 ab	0.193 ± 0.002 ab	35.61 ± 5.19 a	35.43 ± 2.82 a
HR	1.99 ± 0.02 a	1.94 ± 0.01 abc	0.197 ± 0.002 ab	0.186 ± 0.004 b	38.49 ± 4.05 a	34.19 ± 1.05 a
XS	1.98 ± 0.03 ab	1.96 ± 0.00 ab	0.191 ± 0.007 b	0.189 ± 0.002 b	37.08 ± 2.00 a	34.63 ± 0.77 a
CH	1.95 ± 0.01 adc	1.94 ± 0.03 abc	0.198 ± 0.004 ab	0.197 ± 0.008 ab	35.77 ± 1.01 a	34.59 ± 2.09 a
CS	1.96 ± 0.02 ab	1.90 ± 0.07 c	0.210 ± 0.022 a	0.197 ± 0.003 ab	36.72 ± 0.73 a	35.73 ± 3.54 a
	P(M) *, P(V) NS, P(M*V) *		P(M)NS, P(V) NS, P(M*V) *		P(M) NS, P(V) NS, P(M*V) NS	

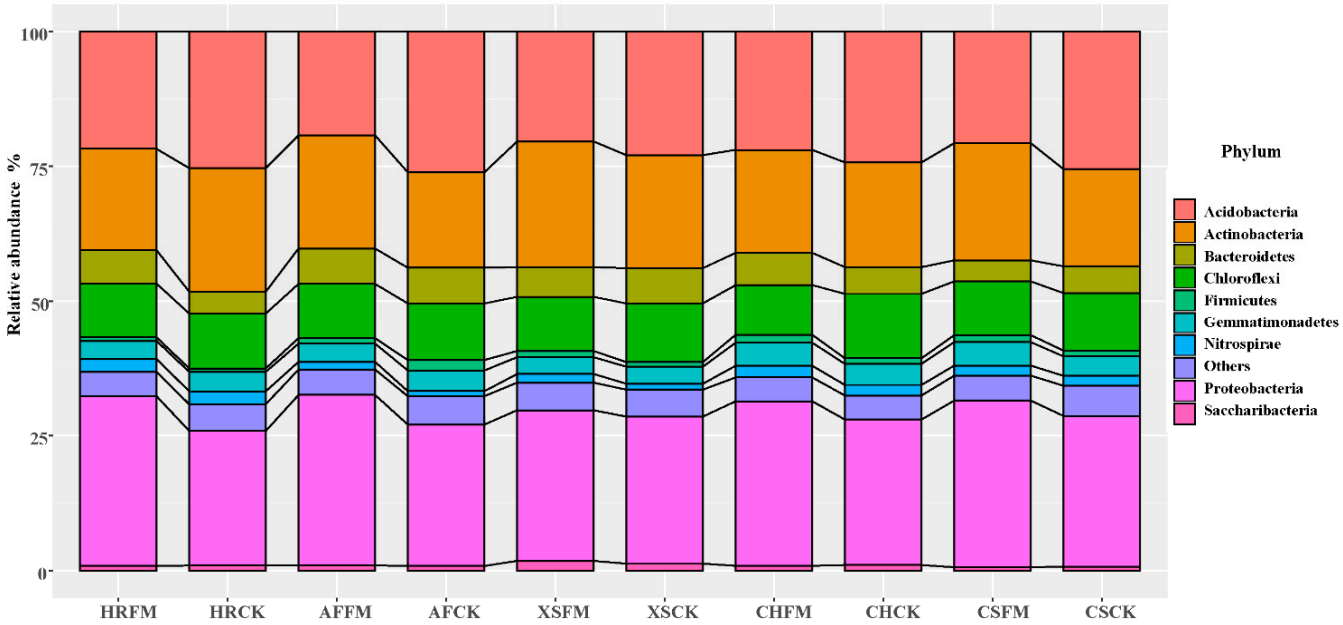


Figure 3. Soil bacterial community compositions under inoculation arbuscular mycorrhizal fungi and control. Note: AF, *Amorpha fruticosa*; HR, *Hippophae rhamnoides*; XS, *Xanthoceras sorbifolium*; CH, *Cerasus humilis*; CS, *Cerasus szechuanic*. Each vegetation type was provided with inoculation zone (FM) and control zone (CK); the same below.

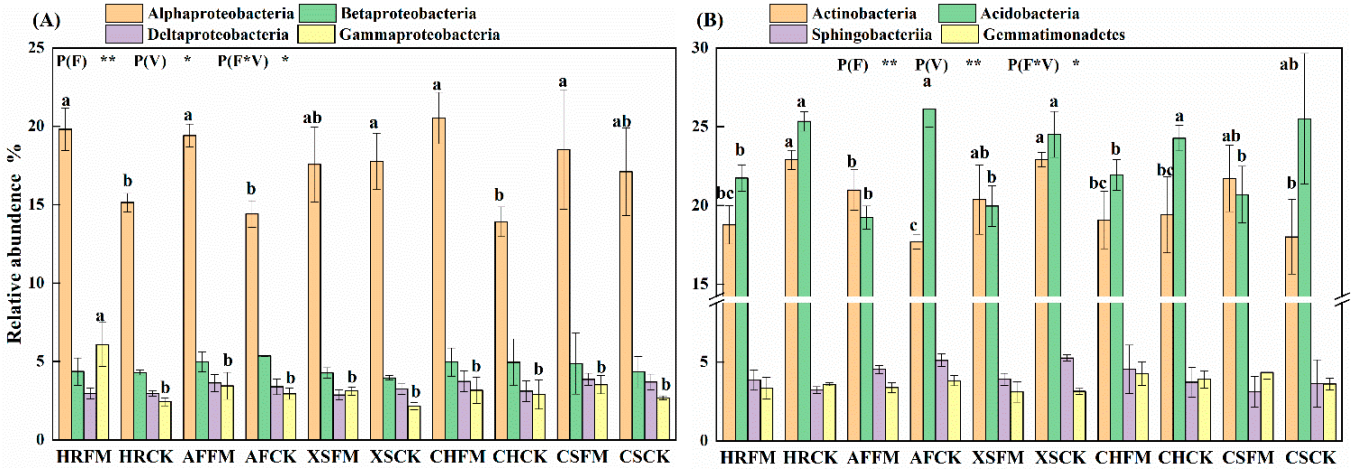


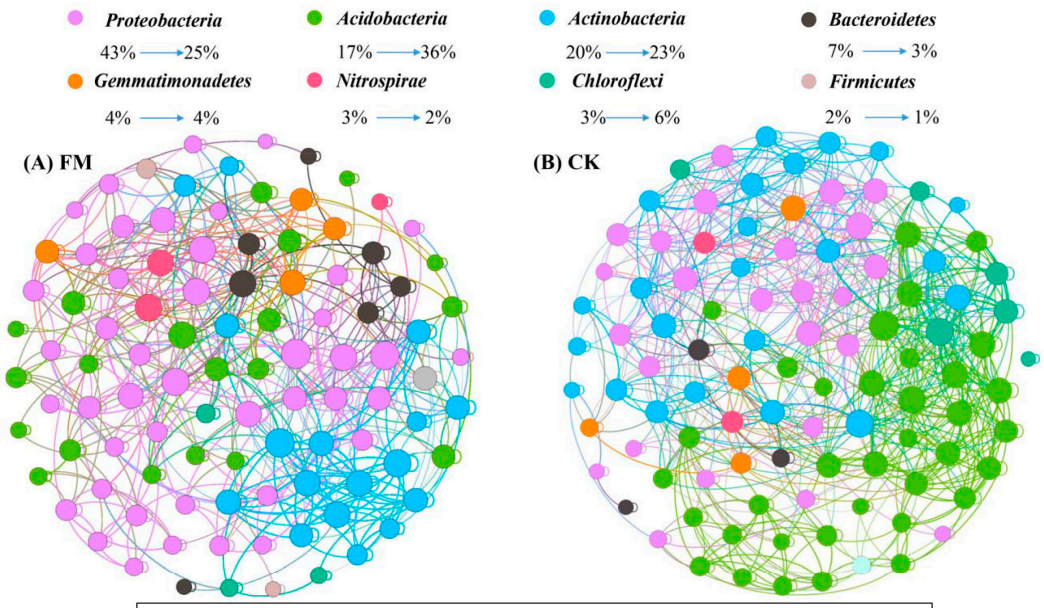
Figure 4. Soil bacterial community compositions at the class level. Note: different letters indicate significant differences among re-vegetation type and arbuscular mycorrhizal fungi. (**A**) *Ptoteobacteria* (Alpha-, Beta-, Delta-, Gamma); (**B**) Actinobacteria, Acidobacteria, Sphingobacteria, Gemmatimonadetes. ** ($p < 0.01$). * ($p < 0.05$).

The relative abundances of the top 8 classes were different among different re-vegetation types and mycorrhizal fungi reclamation (Figure 4A,B). The dominant abundant classes were *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Actinobacteria*, *Acidobacteria*, *Sphingobacteria* and *Gemmatimonadetcs* in all soils. *Alphaproteobacteria* were higher in the FM than in the CK, with relative abundances ranging from 14 to 21%. Furthermore, the relative abundances of *Acidobacteria* showed distinctly different responses in different re-vegetation types: the relative abundances in the FM were lower than those in the CK under all re-vegetation types.

3.3. Network Analysis and Functional Prediction of Soil Bacterial Communities

Further molecular ecological network analysis revealed the molecular ecological network structure of the bacterial community was significantly different among re-vegetation types and mycorrhizal fungi reclamation (Figure 5). Co-occurrence network analysis showed that the bacterial interactions were weaker during CK than FM. The relationship of Proteobacteria with other species increased in FM compared to CK, and the links had minimum connection points in Firmicutes compared with other species. For instance, the ratio of positive relationships among Proteobacteria, Bacteroidetes and other species was increased by 25%–43% and 3%–7%, respectively. These results indicated that Proteobacteria had a more predatory and symbiotic relationship under mycorrhizal fungi reclamation.

Furthermore, FAPROTAX is a functional prediction tool based on the 16S rRNA gene sequence to predict functional types in microbial communities under different re-vegetation types and mycorrhizal fungi reclamation (Figure 6). The results also showed that there were more abundant groups capable of chemoheterotrophy, nitrification, aerobic_chemoheterotrophy, aerobic_nitrite_oxidation and ureolysis than other functional communities. Interestingly, some functional communities that could promote soil nutrients and organic carbon, such as hydrocarbon_degradation, cellulolysis and aromatic_hydrocarbon_degradation, were higher in the FM than CK treatment. In addition, animal_parasites_or_symbiontss or human pathogens in CK were significantly higher than those in FM, even twice more than those in FM.



	Positive links	Negative links	Node	Degree Distribution	Size Distribution
FM	488	84	100	15.68	0.789
CK	735	49	100	11.44	0.546

Figure 5. Co-occurrence networks of bacteria and eukaryotic microbial communities in epiphytic biofilms.

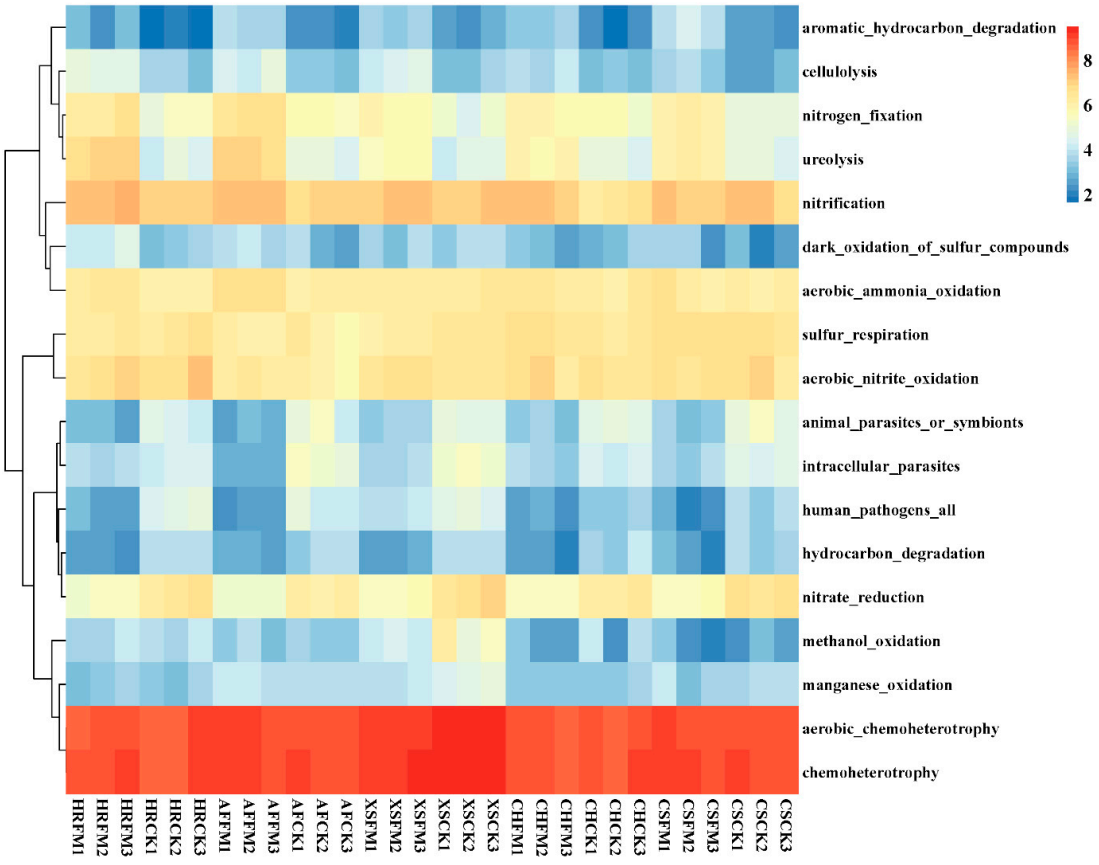


Figure 6. Prediction of soil bacterial function under re-vegetation types and mycorrhizal fungi reclamation.

3.4. Relationships among Soil Bacteria, Physicochemical Properties, Re-Vegetation Types and Mycorrhizal Fungi Reclamation

Redundancy analysis (RDA) showed that soil bacterial community and soil physical and chemical properties differed among re-vegetation types and mycorrhizal fungi reclamation (Figure 7, Table S1). Clustering analysis showed that the bacterial community characteristics were similar in AF-FM and AF-CK. The bacterial community in XS-CK was clearly distinct from that in other re-vegetation types. Furthermore, the responses of soil properties to the soil bacterial community and re-vegetation types were further illustrated in the RDA plot (Figure 7). The two axes explained 40.64% of the total variation in the soil bacteria community, with the first axis accounting for 30.69%. Among all of the soil properties, TP, SOC, C:N and HD represented 20.3, 18.6, 10.2 and 6.5% of the variation, indicating that these soil properties play a key role in shifting the soil bacteria community.

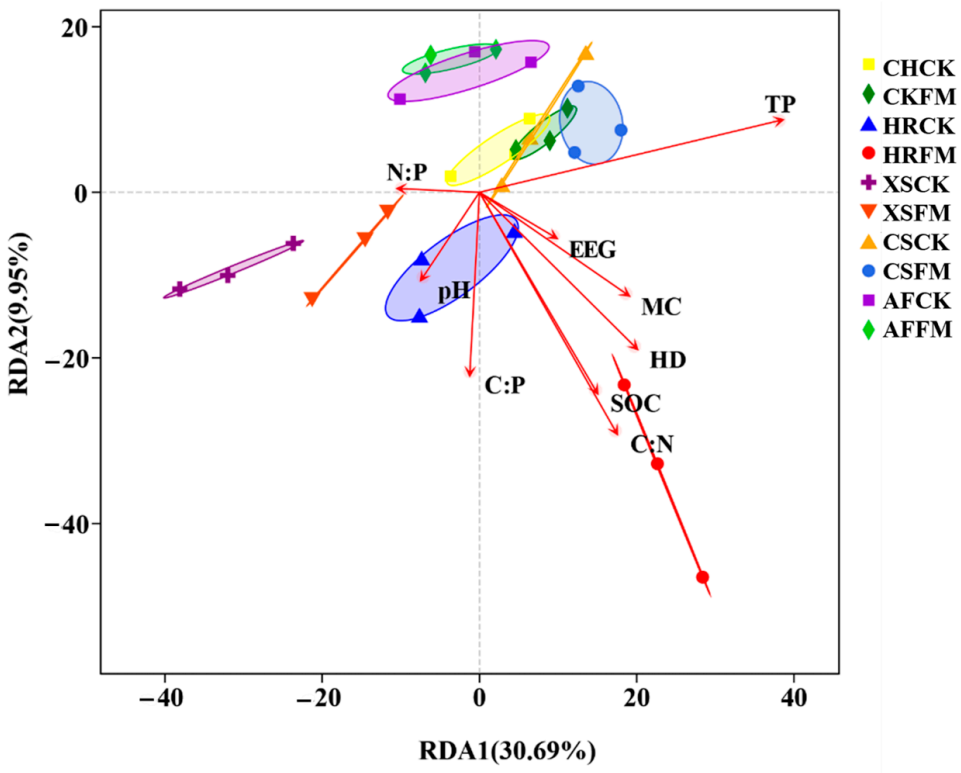


Figure 7. Redundancy analysis of soil properties and bacteria.

To verify this pattern of re-vegetation types and mycorrhizal fungi reclamation and identify environmental drivers in the areas, a Mantel test was carried out to explore the relationships between microbial communities and environmental factors (Figure 8). The results showed that bacterial community composition was significantly ($p < 0.05$) correlated with multiple environmental variables. The re-vegetation types were more strongly associated with environmental factors than mycorrhizal fungi reclamation. Soil bacterial function had significant effects on soil variables ($p < 0.05$). Meanwhile, soil variables had a close relationship with AMF and re-vegetation types. These results indicated that bacterial function had significant effects on SOC, TN, C:N and EEG ($p < 0.05$). Soil SOC, TN, TP and EEG concentrations were significantly influenced by re-vegetation types and mycorrhizal fungi reclamation.

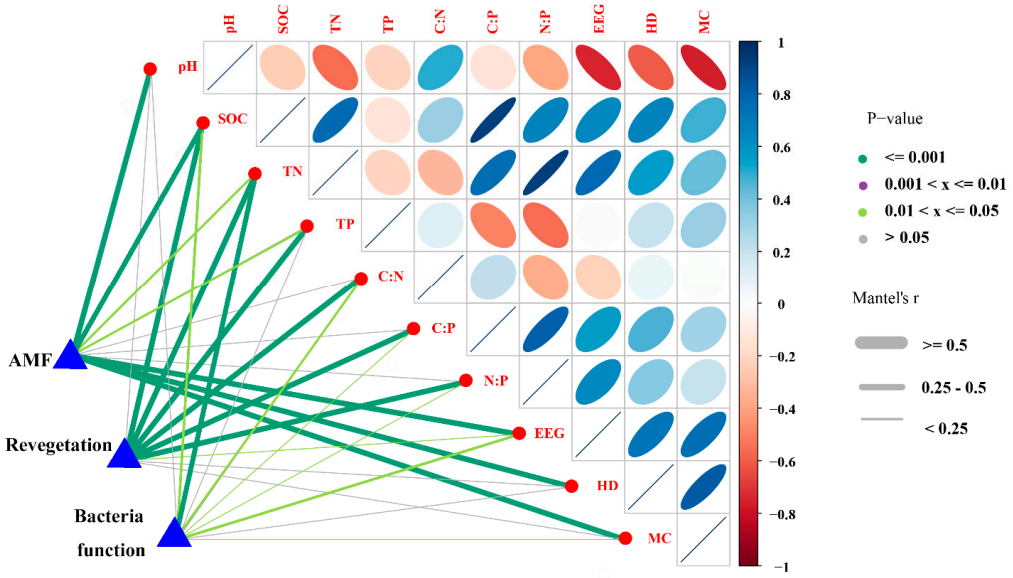


Figure 8. Environmental drivers of the bacterioplankton community using Mantel test (**bottom-left**). Edge width corresponds to correlation coefficient, and edge color indicates statistical significance. Pairwise correlations of environmental attributes are shown (**upper-right**) with color gradient representing correlation value and cross mark indicating no significant difference.

4. Discussion

4.1. Effects of AMF and Re-Vegetation Types on Plant Stoichiometry and Homeostasis

Ecological stoichiometry can reflect the balance of nutrient elements under ecological interactions, which are crucial for biogeochemical cycles in ecosystems [34], especially for the sustainable development of mining subsidence areas following vegetation restoration in northwest China [35]. Previous studies showed that the leaf of plants is most sensitive to environmental changes, while ecological stoichiometry in leaves can reflect nutrient accumulation and limitation in the ecosystem [36]. In addition, the ratio of C to N or P in a plant can directly indicate the plant utilization efficiency of N or P and is negatively associated with plant growth rate [37]. The leaf stoichiometry ratio was usually applied to identify nutrient restriction during plant growth [38]. Previous studies consider that there was a certain 'break point' in leaves, that is, phosphorus restriction (N:P > 16) or nitrogen restriction (N:P < 14). This theory defined the plant nitrogen and phosphorus ratio as a key indicator of nutrient restriction and was widely used in most terrestrial ecosystems [38–40]. In this study, the N/P ratio in leaves gradually ranged from 8.44 to 13.15, and all leaves' N:P were <14 in all re-vegetation types, indicating that plant growth was N-limited under vegetation restoration. Generally, higher N concentrations and N:P improve litter decomposition rates and nutrient release, which can enhance soil nutrient availability and alleviate plant nutrient limitation [41]. As shown in Tables 1 and 2, N limitation was alleviated after mycorrhizal fungi reclamation, which was consistent with our first hypothesis. In addition, the ratios of C:N and C:P in leaves ranged from 12.01 to 24.88 and 157.93 to 215.28, respectively, and were both the highest in CH-CK and lowest in AF-FM. A previous study showed that a higher plant C:N ratio enables survival through higher N utilization efficiency (survival priority strategy) under severe N-limited conditions, while a lower C:N ratio means that the plant sustains rapid growth through favorable competition under a suitable environment [42], indicating that AF (Leguminosae) had the highest growth rates. Consequently, the relatively lower leaf C:N ratio in this region, using AF (or with AMF), might indicate that N utilization is more efficient to ensure durable growth in current soil conditions.

Stoichiometric homeostasis (1/H) is one of the core concepts of ecological stoichiometric research that refers to the ability of organisms to keep their own chemical composition relatively stable in the face of external changes [43]. In this study, (1/H) N:P ranged from

0.1385 to 0.8810 across all re-vegetation types (Figure 2). Based on the homeostatic patterns, the leaves in FM and CK were categorized as 'homeostatic' and 'weakly homeostatic'. However, the roots in FM and CK were categorized as 'weakly plastic' and 'plastic', respectively, indicating that the needle N concentration in the FM treatment had stable nitrogen homeostasis. Mariotte et al. [44] also suggested that high stoichiometric N:P effectiveness coupled with a strong ecological function with AMF helps vegetation reduce environmental stresses. According to previous research, plants with a stable stoichiometric steady state have higher nutrient regulation efficiency when soil nutrients cannot meet their growth needs, and they have an obligation to regulate nutrient composition to store nutrients [45]. Therefore, AF is a fast-growing species in the coal mining reclamation area, and the rapid growth in biomass and volume must be supported by a stable nitrogen supply. Relatively stable stoichiometric balance is one of the adaptation strategies for plants to cope with heterogeneous habitats [46], which could be acquired by regulating nutrient absorption from leaves. To adapt to such C:N imbalance, microorganisms may adjust the production of biological activity to maximize the mobilization of limiting element-rich substrates and/or their element utilization efficiency [18]. Hence, AMF expand the nutrient resource foraging range of legumes (HR) under nutrient-deficient conditions. Compared to re-vegetation types without AMF, re-vegetation types with AMF are more efficient in consuming excessive N when this soil nutrient is mitigatory [47]. This ecological strategy allows plant N/P to change with soil N/P, thereby improving and promoting the quality of mining ecological restoration.

4.2. Effects of AMF and Re-Vegetation Types on Soil Bacterial Community Composition and Function

Although the vital role of AMF in a variety of plant species and other individual guilds of the soil microbial community has already been illustrated [23,48], the effect of mycorrhizal biological reclamation on the soil bacteria and function has not yet been thoroughly examined under the reclamation area of coal mining subsidence. Our results showed that the soil bacterial communities under all re-vegetation types mainly consisted of *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Choroflexi* (Figure 3). Furthermore, *Proteobacteria* was the most abundant phylum in the soil, a result that is also in line with past studies [49,50]. However, there was no difference in the population of dominant microorganisms under different re-vegetation types and reclamation modes, but there was a significant difference in relative abundance, especially for FM and CK. These results indicated that AMF influence the relative abundance of these groups (Figure 3). *Proteobacteria* and *Acidobacteria* accounted for 30.39%, 26.59% and 20.80%, 24.83%, indicating that AMF increased the relative abundance of *Proteobacteria* and decreased the relative abundance of *Acidobacteria*. *Proteobacteria* is a copiotrophic community [51], and *Acidobacteria* is an oligotrophic community with rapid propagation in a nutrient-insufficient environment [52]. In other words, the copiotrophic communities in the FM area were higher than those in the CK area under the reclamation area of coal mining subsidence, while the oligotrophic communities were the opposite, which is consistent with previous studies showing that bacteria are more affected by abiotic variables [53] and less affected by biotic interactions compared to eukaryotes. Furthermore, compositions of *Proteobacteria* (Alpha-, Beta-, etc.) have been considered as positive-response bacteria to AMF by utilizing AMF exudates [54]. This could explain the observations that the relative abundance of *Alphaproteobacteria* was increased under the FM treatment, which is similar to the decrease in the *Betaproteobacterial* family due to AMF inoculation [55].

In addition, different factors can trigger changes in the microbial community composition. According to all of the soil properties, TP, SOC, C:N and HD represented 20.3, 18.6, 10.2 and 6.5% of the variation, indicating that these properties played a key role in shifting the soil bacteria community. Soil stoichiometry tended to impact soil bacteria composition, with Ren et al. [56] showing that the ratio of soil C:N was the vital determinant of the soil microbial community. Moreover, in the present study, we showed that bacterial diversity in re-vegetation types and mycorrhizal fungi reclamation soil was mainly due to changes

in the relative abundance of specific bacterial taxa. The relative abundance of *Proteobacteria*, *Acidobacteria* and *Actinobacteria* accounted for 69.92%–73.22% of all bacterial organisms (Figure 3). The relationship between these bacteria and soil C and N cycling has been reviewed by Fierer et al. [57]. For example, the net rate of carbon mineralization in soil could be predicted by changes in gate abundance, especially *Proteobacteria*, *Acidobacteria* and *Bacteroidetes* [58]. In addition, some microbial communities are influenced by more soil factors and soil stoichiometry. Therefore, the overall change in microbial community composition may be affected by soil ecostoichiometry [59]. For specific functional soil bacteria, mycorrhizal fungi affect the element cycles by regulating microbial community composition, which further proves that ecological stoichiometry is closely associated with the composition of the soil bacterial community following re-vegetation types and mycorrhizal fungi reclamation in the coal mining subsidence area.

5. Conclusions

Here, we demonstrated for the first time the detailed relationship among soil bacterial diversity and function, soil nutrient restriction and mycorrhizal biological reclamation in coal mining subsidence. All re-vegetation types investigated in this study were N-limited, but AF (or with AMF) can alleviate nitrogen restriction and promote plant growth by regulating C:N reduction. Only leaves in FM were 'homeostatic', and both leaves and roots inoculated with AMF can alleviate nitrogen restriction and improve ecological stoichiometric homeostasis. The bacterial communities under all re-vegetation types mainly consisted of *Proteobacteria*, *Acidobacteria*, *Actinobacteria* and *Choroflexi*, while the copiotrophic groups (*Proteobacteria*) in the FM area were higher than those in the CK area under all re-vegetation types. Soil TP, SOC, C:N and HD played a key role in shifting the soil bacteria community. These observations indicate that AMF affects the element cycles by regulating microbial community composition, which further proves that ecological stoichiometry is closely associated with the soil bacterial function and is conducive to sustainable development by mycorrhizal fungi reclamation in coal mining subsidence in the Loess Plateau terraces.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f14091720/s1, Table S1: Soil physical and chemical properties under mycorrhizal fungi reclamation and re-vegetation types.

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