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Improving Effects of Afforestation with Different Forest Types on Soil Nutrients and Bacterial Community in Barren Hills of North China

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Abstract: Afforestation can improve soil nutrient content and microbial community structure, increase soil carbon sequestration, and reduce greenhouse gas emissions. However, at present, there is a lack of research on the low hills and mountainous areas in North China. In order to scientifically evaluate the effect of afforestation recovery with different forest types on the improvement of the soil ecological system, the Fanggan ecological restoration in North China was taken as the research sample, and the coniferous forests, mixed coniferous and broad-leaved forest quadrats and broad-leaved forests, as well as the contrast of barren hills bushes were set to achieve the research goals. Research results of different forest types on soil nutrient and bacterial community in the Fanggan ecological restoration area have shown that afforestation with broad-leaved forests most obviously improved the nutrition properties and bacterial community of soil. (1) Broad-leaved forest afforestation obviously improved water retention and ammonia nitrogen content but reduced the content of available phosphorus and nitrate nitrogen of surface soil. It also increased available phosphorus, ammonia nitrogen, and nitrate nitrogen content of lower soil. (2) Broad-leaved forest afforestation significantly increased α-diversity of the bacterial community in surface soil, but only enhanced the Chao1 and ACE indices of lower soil. In addition, afforestation has also significantly changed the structure of soil bacterial community and β-diversity index. (3) Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia accounted for the highest proportion of soil bacterial community. Proteobacteria and Verrucomicrobia occupied higher proportion in broad-leaved forests than in other forest types, while the proportion of Acidobacteria and Actinobacteria was the opposite. (4) Afforestation decreased cooperation and increased competition among bacteria of surface soil as well as increased coexistence and rejection among subsoil bacteria. (5) pH, ammonia nitrogen, organic carbon, and available phosphorus have exhibited a significant impact on the structure of bacterial community in the surface soil, while the bacterial community structure of the lower soil was mainly affected by pH and available phosphorus. Results have fully demonstrated the positive effects of broad-leaved forest on the restoration of soil nutrients and microbial community structure. Meanwhile, the important combinations of soil physical and chemical factors affecting soil bacterial community structure were also explored. The results can provide scientific basis for revealing the mechanism of soil organic matter, nutrient and ecological function restoration by artificial afforestation, and also offer theoretical support and practical reference for the restoration of artificial afforestation in the hilly and mountainous areas of North China.

Keywords: vegetation restoration; soil nutrition; soil bacteria community; diversity; high-throughput sequencing
1. Introduction

There exists a series of ecological functions of forest ecosystems such as water and soil conservation, greenhouse effect mitigation, plant growth promoting and so on. It also plays a crucial role in coordinating resources, environment, and social development [1,2]. Soil is the largest and most active carbon pool in terrestrial ecosystems, about twice as much as atmospheric carbon pool and 2–3 times as much as plant carbon pool [3]. Historically, terrestrial C pools, have been largely depleted by anthropogenic activities such as deforestation, tillage and overgrazing [4,5]. Nutrient deficiency and improper fertilization lead to decomposition of soil organic matter, increased greenhouse gas emissions and further soil degradation during forest or grassland extraction [6]. Afforestation can promote the assimilation of atmospheric carbon dioxide into organic carbon and sequestration in soil organic carbon pool, which can greatly reduce the emission of greenhouse gas [7], so it plays a very positive role in restoring soil organic matter reserves and alleviating the greenhouse effect. It will also directly affect all soil ecological functions.

Vegetation restoration includes active restoration and passive restoration, in which ecosystem functions were reshaped. Active restoration aims to recuperate or improve the state of the ecosystem by the active intervention of humans, while passive restoration is the natural restoration of ecosystem on the premise of reducing or eliminating anthropogenic effects [8]. Soil quality is an important component of ecosystem restoration due to its physical, chemical, and biological (nutritional) supporting for the settlement and growth of plants [9]. Changes in plant community composition and structure alter soil physicochemical properties [10], improving soil nutrients and microbial structure through litter and roots [11,12]. Zhang et al. shows that Organic C as well as total N and P accumulated in soil along with secondary succession in the three quarries, which were positively correlated with woody species richness [13]. Previous studies have shown that vegetation restoration could improve soil nutrient quality, accelerate soil nutrient cycling [14], and trigger higher demand for soil nutrients in vegetation communities. Even though these individual soil properties can be considered as soil quality indicators, the impacts of vegetation restoration on soil quality cannot be assessed using individual soil parameters as they are interdependent and unlikely to thoroughly reflect these complex ecosystems [15].

Soil microorganisms are key regulators in biogeochemical cycle and display high structural, genetic, and functional diversity [16]. For example, soil bacteria, including cellulose-degrading bacteria, nitrogen-fixing bacteria, and phosphorus-soluble bacteria, could regulate soil element cycle and plant nutrition in the ecosystem [17]. Bacteria are particularly sensitive to environmental varies, and their activities, community structure, and functions may alter with the changes of plant community structure and soil properties during vegetation restoration [18]. Afforestation changed the soil chemical properties, thus indirectly affecting the soil bacterial composition rather than richness. Grassland restoration altered soil chemical properties and, thus, regulated the composition and ecological function of soil bacterial community [19]. Soil pH, C content, N content, and C/N ratio were also important determinants of microbial community structure [20]. The stability of vegetation community was highly positively correlated with the abundance and composition of bacteria. It has been reported that the composition of soil bacterial community varies with plant community and recovery age. For example, acid bacteria are more prevalent under birch trees, while Firmicutes and proteobacteria are more prevalent under young pine forests.

Although vegetation restoration can improve soil properties and microbial communities [21], the effects of afforestation from barren and degraded mountains in North China with different forest types on soil nutrient properties and biological communities still remain to be further studied. Moreover, most of the research areas are national or local guided and planned afforestation restoration, and the research on vegetation restoration of villages in the Fanggan ecological restoration area, where villagers spontaneously plant trees, is very rare. Therefore, it is vital to assess the impacts of vegetation restoration on soil quality during soil restoration processes in the Fanggan ecological restoration. Among
them, molecular biological methods, especially the extraction and sequence analysis of soil microbial DNA, have become difficult to achieve this goal. Soil DNA extraction is very difficult due to low total nitrogen and phosphorus content and high humus content. Zong Meijuan et al. invented high salt buffer lysis extraction method and achieved good DNA extraction effect [22]. In recent years, with the development of DNA extraction and sequencing technology, high-throughput sequencing technology has been widely used in soil microbial sequencing and diverse new molecules.

After nearly 50 years of artificial afforestation with villagers spontaneously planting 3,000,000 trees, Fanggan ecological restoration area has developed into a basically perfect artificial forest community with the coexistence of coniferous forests, mixed forests, and broad-leaved forest as well as complete vertical structure including arbor, shrub, and herb layer. At present, all kinds of stands have maintained well growth and development state. However, the different ways of nutrients input into soil ecosystem of different forest types during the process of development has brought differences in soil ecosystem biological structure and nutrient cycle and, thus, feedback to the growth and development of the plant community. Zhou Juan et al. studied the diversity of culturable bacteria under different vegetation in the Fanggan ecological restoration area, which laid a foundation for the study of soil microbial community in this area [23]. In the years of afforestation and restoration practice in Fanggan ecological restoration area, there still exits a lack of research on the difference of vegetation-soil feedback brought by different forest types and the evaluation of the most suitable afforestation and restoration mode for the actual situation of the Fanggan ecological restoration area.

The purpose of this paper is to study the improvement of soil nutrients and bacterial community in different afforestation including coniferous forests, mixed forests, and broad-leaved forests comparing with the barren mountain shrub without afforestation and restoration, so as to reflect restoration mechanism of soil nutrients and microbial community structures in different stands. It would also provide scientific reference for evaluation of afforestation restoration effect in the Fanggan ecological restoration area and effective guidance of the maintenance and management of existing stands.

2. Materials and Methods

2.1. Study Sites

The Fanggan ecological restoration area, located in the north of the central mountain area of the Shandong Province, is a monsoon-hilly landform and a has a warm temperate continental monsoon climate with distinct seasons and the rain heat over the same period. Light, heat, water, and other conditions are superior, the annual average temperature is about 12.4 °C, the average temperature in January is 1.8–3.8 °C, extreme low temperature could reach −22.5 °C. The annual average precipitation is more than 830 mm, mainly distributed in July, August, and September. In the past 40 years, local villagers have moved about 20,000,000 m³ of earth and rock on the basis of the original vegetation and geomorphology of the barren mountain shrubs, and have built 135 hm² of mountain shelter forest, including coniferous forests (Pinus tabuliformis, P. densiflora), coniferous and broadleaf mixed forests, and broad-leaved forests. So far, the area has developed into an ecological recovery area with a forest coverage rate up to 90% and perfect vertical community structure of tree–shrub–herb layer. The arbor layer of the vegetation community is mainly composed of P. densiflora, P. tabuliformis, Populus davidiana, Quercus acutissima, Robinia pseudoacacia, and Diospyros lotus. The shrub layer is mainly composed of Vitex negundo var. Heterophylla, Ziziphus jujuba var. Spinosa, Grewia biloba var. parviflora, and seedlings of the arbor layer. The herb community is mainly composed of Gramineae, Cyperaceae, and Asteraceae (Supplementary Figure S1).

2.2. Plot Design, Vegetation Survey and Soil Sampling

After reviewing literatures and data of local ecological restoration areas and conducting field investigation and research on the restoration areas, it was found that the
restoration forests were mainly distributed within the range of 300–750 m above sea level. So, the community plots were set at high canopy cover region of the altitude between 300 m and 750 m of the ecological restoration area, including *Vitex negundo* var. *heterophylla* shrub (S), coniferous pine forests (CF), coniferous and broad-leaved mixed forests (including *Pinus sp.*-*Populus davidiana*, *Pinus sp.*-*R. pseudoacacia*, *Pinus sp.*-*Q. acutissima* mixed forest) (MF), and deciduous broad-leaved forest (including *R. pseudoacacia*, *Populus davidiana* and *Q. acutissima* forests) (BF). In order to reduce the influence of topographic factors on the research results, the quadrat of each forest type should contain consistent amplitude of topographic variation as far as possible. The geographic coordinates, elevation, aspect, and slope position were recorded. The area of each quadrat was 10 m × 10 m, and each individual tree was checked. The shrub quadrats with the area of 5 m × 5 m have been set at four corners and center of each quadrat. The name, base diameter, height, and abundance of each species were recorded. A 1 m × 1 m herb quadrat was set in the center of each shrub quadrat. The name, coverage, abundance, and height of each species were recorded. The details of the quadrat are shown in Table 1. Soil sampling was carried out according to the five-point sampling method, with sampling points located at the four corners and the center of each quadrat. Soil samples from each quadrat were fully mixed and divided into two parts. One was stored in liquid nitrogen for molecular biological sequencing analysis, and the other was brought back to the laboratory for soil physical and chemical properties analysis as soon as possible after collection.

Table 1. Community information of shrub and afforestation forests.

| Quadrat       | Elevation (m) | Longitude (°) | Latitude (°) | Slope (%) | Aspect (°) | Coverage (%) | Dominant Species                                      |
|---------------|---------------|---------------|---------------|-----------|------------|--------------|--------------------------------------------------------|
| Shrub (S)     | 400           | 117.55062     | 36.45683      | 6.2       | S189       | 65%          | *Vitex negundo* var. *heterophylla*                    |
|               | 381           | 117.43693     | 36.40997      | 21.1      | SE131      | 95%          | *V. negundo* var. *heterophylla*                       |
|               | 301           | 117.56891     | 36.48426      | 2.3       | W270       | 70%          | *V. negundo* var. *heterophylla*                       |
| Coniferous Forests (CF) | 758         | 117.42622     | 36.42089      | 28.7      | E81        | 45%          | *P. tabuliformis*, *P. densiflora*                     |
|               | 484           | 117.45266     | 36.45675      | 16.7      | W269       | 60%          | *Pinus tabuliformis*, *P. densiflora*                  |
|               | 458           | 117.56334     | 36.42401      | 38.8      | NE47       | 60%          | *P. tabuliformis*, *P. densiflora*                     |
| Mixed Forest (MF) | 526         | 117.44411     | 36.42148      | 13.5      | NW303      | 60%          | *P. densiflora*, *Populus davidiana*                   |
|               | 469           | 117.45251     | 36.45733      | 19.8      | W256       | 40%          | *P. densiflora*, *Quercus acutissima*                  |
|               | 448           | 117.45253     | 36.45747      | 22.8      | N18        | 70%          | *Pinus densiflora*, *Robinia pseudocacia*              |
| Broad-leaved Forest (BF) | 542         | 117.44382     | 36.42024      | 16        | W248       | 80%          | *R. pseudoacacia*, *Q. acutissima*, *D. lotus*, *Salix matsudana*, *P. davidiana* |
|               | 540           | 117.43137     | 36.42351      | 15.8      | NE28       | 55%          | *R. pseudoacacia*, *Q. acutissima*, *D. lotus*, *Salix matsudana* |
|               | 447           | 117.48876     | 36.42349      | 2.5       | N338       | 70%          | *P. davidiana*                                         |
|               | 396           | 117.44651     | 36.44243      | 8.2       | E69        | 85%          | *R. pseudoacacia*                                      |
|               | 390           | 117.44973     | 36.456        | 1.4       | NW327      | 85%          | *Populus davidiana*, *Pterocarya stenoptera*, *Diospyros lotus*, *Amygdalus persica* |
|               | 388           | 117.43705     | 36.41067      | 25.4      | E82        | 95%          | *R. pseudoacacia*, *Koelreuteria paniculata*            |
|               | 380           | 117.46176     | 36.42259      | 22.3      | E111       | 75%          | *R. pseudoacacia*, *Q. acutissima*, *Platycladus orientalis* |
|               | 339           | 117.44869     | 36.4427       | 3.3       | S167       | 60%          | *R. pseudoacacia*, *Q. acutissima*, *Platycladus orientalis* |
|               | 324           | 117.44866     | 36.44234      | 6.4       | SE142      | 80%          | *Q. acutissima*                                        |
|               | 317           | 117.4665      | 36.46934      | 12.7      | NW297      | 70%          | *Populus davidiana*                                    |

2.3. Soil Properties Assessment

Soil moisture and dry matter content were determined by gravimetric method [24], pH was determined by potentiometric method, organic carbon content was determined by potassium dichromate oxidation-spectrophotometry [25], and available phosphorus was determined by molybdenum-antimony anti-color spectrophotometry [26]. Ammonia nitrogen and nitrite nitrogen were determined by potassium chloride extraction-spectrophotometry [27], and nitrate nitrogen was determined by ultraviolet spectrophotometry [28].
2.4. Soil Bacterial Community Analysis

Soil bacterial DNA was extracted by TGuide S96 magnetic bead method and 16S rDNA fragments of DNA were amplified by primers 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTTYGMTTTRAGTTT-3′). After PCR detection, the sequence was sequenced on the machine. Double-ended primers of the original sequence were removed and sequences of low quality and length that did not meet the standard were filtered. After obtaining high-quality sequences, OUT division was performed at 97% similarity level and OTUs was used as the basic operation unit for subsequent community analysis. α diversity index includes Chao index, Ace index, Shannon index, and Simpson index, calculated respectively. The difference between groups was analyzed by T statistics. β diversity is analyzed by PcoA principal coordinate based on Euclidean distance. Variance analysis was used to analyze the differences in community species composition at phylum and genus levels. At the same time, spearman rank correlation coefficient was used to show the coexistence network between genera at the genus level, and the significant difference levels were all defined as \( p < 0.05 \). The interpretation of soil physical and chemical properties to bacterial community composition was completed by RDA analysis. All the graphs in this paper are completed by ggplot2 program package of R language except the co-occurrence network graph which is implemented by Python.

\[
\text{chao1} = S_{\text{obs}} + \frac{F_1^2}{2F_2} \tag{1}
\]

where \( F_1 \) and \( F_2 \) are the count of singletons and doubletons, respectively.

\[
S_{\text{ace}} = S_{\text{abund}} + \frac{S_{\text{rare}}}{C_{\text{ace}}} + \frac{F_1}{|C_{\text{ace}}|} \gamma_{\text{ace}}^2 \tag{2}
\]

where \( S_{\text{abund}} \) is the number of abundant OTUs (with more than rare threshold individuals) when all samples are pooled, \( S_{\text{rare}} \) is the number of rare OTUs (with less than or equal to rare threshold individuals) when all samples are pooled, \( C_{\text{ace}} \) is the sample abundance coverage estimator, \( F_1 \) is the frequency of singletons, and \( \gamma_{\text{ace}}^2 \) is the estimated coefficient of variation for rare OTUs.

\[
\gamma_{\text{ace}}^2 = \max \left[ \frac{S_{\text{rare}}}{C_{\text{ace}}} \sum_{i=1}^{10} \frac{(i-1)F_i}{(N_{\text{rare}})(N_{\text{rare}} - 1)} - 1, 0 \right] \tag{3}
\]

\[
H = -\sum_{i=1}^{s} P_i \log_2 P_i \tag{4}
\]

where \( s \) is the number of OTUs and \( P_i \) is the proportion of the community represented by OUT \( i \)

\[
\text{Simpson's index} = 1 - \sum P_i^2 \tag{5}
\]

where \( p_i \) is the same as which in Shannon-winner index.

3. Result

3.1. Vegetation Community Structure

The DBH of coniferous forests, mixed forests, and broad-leaved forests increased successively but there was no significant difference among different forest types. Shannon-winner index, Simpson index diversity index, and Pielou evenness index of coniferous forests and mixed forests were significantly higher than those of broad-leaved forests (Figure 1).
Figure 1. DBH and diversity of vegetation community. (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forests and S represents shrub).

3.2. Soil Physicochemical Properties

In the surface soil, planting broad-leaved forest significantly reduced the content of dry matter, available phosphorus, and nitrate nitrogen ($p < 0.05$). It has also enhanced the content of ammonia nitrogen as well as pH ($p < 0.05$). The highest content of organic carbon occurred in the coniferous forests ($p < 0.05$) and all the planted forest have significantly enhanced organic carbon content compared with that of shrub soil ($p < 0.05$). The change trend of soil nutrient in lower soil was mostly consistent with that of surface soil except the completely opposite trend of available phosphorus and nitrate nitrogen, all the variation was not significant (Table 2).

Table 2. Soil nutrient contents of shrub and different forest types. (BF represents broad-leaved forests, MF represents mixed forests, CF represents coniferous forests and S represents shrub, Data marked with different letters within the same row at each nutrition parameter represent significant difference among different afforestation types at the level of 0.05 level).

| Surface | Dry Matter | Available Phosphorus (g·kg⁻¹) | Organic Carbon (mg·kg⁻¹) | NH₄⁺-N (mg·kg⁻¹) | NO₃⁻-N (mg·kg⁻¹) | NO₂⁻-N (mg·kg⁻¹) | pH |
|---------|------------|--------------------------------|-------------------------|------------------|------------------|------------------|----|
| S       | 0.987 ± 0.002a | 8.341 ± 1.069a  | 2.641 ± 0.688c | 11.637 ± 1.212b  | 7.614 ± 0.934b  | 4.628 ± 3.044a  | 5.01 ± 0.157ab |
| CF      | 0.982 ± 0.005a | 3.273 ± 1.627b  | 4.032 ± 0.757a | 11.551 ± 0.703b  | 8.443 ± 2.375a  | 4.221 ± 0.919a  | 4.693 ± 0.257b |
| MF      | 0.983 ± 0.003a | 3.923 ± 2.370b  | 3.943 ± 0.576b | 12.692 ± 0.514b  | 7.240 ± 1.709b  | 4.561 ± 1.276a  | 4.727 ± 0.340b |
| BF      | 0.978 ± 0.009b | 2.614 ± 1.535c  | 3.496 ± 1.065b | 14.121 ± 4.760a  | 7.304 ± 1.648b  | 5.274 ± 0.492a  |    |

| Lower Dry matter | Available Phosphorus (g·kg⁻¹) | Organic Carbon (mg·kg⁻¹) | NH₄⁺-N (mg·kg⁻¹) | NO₃⁻-N (mg·kg⁻¹) | NO₂⁻-N (mg·kg⁻¹) | pH |
|------------------|------------------------------|-------------------------|------------------|------------------|------------------|----|
| S                | 0.988 ± 0.001a  | 2.523 ± 1.887b  | 1.539 ± 0.779b | 8.986 ± 1.076b  | 5.655 ± 1.747b  | 2.874 ± 0.464b | 5.017 ± 0.068a |
| CF               | 0.986 ± 0.003a  | 3.005 ± 2.564b  | 2.568 ± 0.680a | 11.970 ± 3.779a | 7.101 ± 1.729a | 3.683 ± 0.252a | 4.767 ± 0.328a |
| MF               | 0.986 ± 0.003a  | 1.903 ± 0.662c  | 1.778 ± 0.505b | 10.633 ± 1.320a | 5.767 ± 0.363b | 3.292 ± 0.800a | 4.837 ± 0.188a |
| BF               | 0.984 ± 0.005a  | 3.294 ± 1.314a  | 2.060 ± 0.789ab | 11.658 ± 3.992a | 7.136 ± 2.916a | 3.928 ± 2.020a | 5.285 ± 0.416a |

3.3. Soil Microbial Diversity and Composition

3.3.1. α-Diversity

The four α-diversity indices include Chao, ACE, Shannon-winner, and Simpson and decreased after the afforestation of forests while they increased after planting mixed forest. However, the two artificial forests mentioned above have not affected the indices significantly. The indices only increased significantly in broad-leaved planted forests ($p < 0.05$). In the lower soil, only planting broad-leaved forests could significantly increase Chao and Ace.
indices ($p < 0.05$), while all afforestation types have shown no significant effect on Shannon and Simpson index (Figure 2a–d).

Figure 2. (a). Changes of bacterial community chao index in surface (left) and lower (right) soil after afforestation (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forests, S represents shrub). (b). Changes of bacterial community Ace index in surface (left) and lower (right) soil after afforestation (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forests, S represents shrub). (c). Changes of bacterial community Simpson index in surface (left) and lower (right) soil after afforestation (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub). (d). Changes of bacterial community Shannon index in surface (left) and lower (right) soil after afforestation (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub. Bar marked with different letters above represent significant difference of each diversity indices among different afforestation types at the level of 0.05 level).

3.3.2. $\beta$-Diversity

The PCoA axis 1 and 2 have explained a total of 50.98% of the community variation in surface soil, of which axis 1 explained 39.14% and axis 2 explained 11.84%. According
to the location distribution of each point, the bacterial community of shrub have shown to be significantly separated from the restoration forests on axis 2. The value of the shrub on the axis 2 was relatively low while that of the artificial restoration forest was relatively high. The results have shown that the β diversity of soil bacterial community has been significantly changed after planting artificial forest. The location of broad-leaved forests on axis 1 was significantly separated from the other two artificial stands, indicating that there were significant differences in soil bacterial community structure between broad-leaved forest and the other two stands. However, there have not existed significant difference in the bacterial community structure between coniferous forests and mixed forests (Figure 3, left).

Figure 3. β-diversity of shrub and different afforestation forests in the surface and lower soil (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub. The left figure represents surface soil layer and the right figure represents the lower layer).

The first two axes have totally explained 45.16% of the variation of bacterial community in the lower soil, of which the axis 1 explained 35.42% and axis 2 explained 9.74%. The significant location separation of shrub and artificial restoration forests on axis 2 indicated that the structure of the lower soil bacterial community has been significantly changed by afforestation restoration. However, there has been no obvious separation between different restoration forest types on axis 1 and 2, indicating no significant difference in community structure between them (Figure 3, right).

3.3.3. Community Structure

Afforestation have enhanced the OTUs abundance of soil bacteria, especially for broad-leaved forests, which have increased the OTUs abundance of surface and lower soil by 27.2% and 15.7%, respectively. Planting coniferous forests and mixed forests had little improvement effect on OTUs abundance, which increased by 1.8% and 4% in surface and lower soil of coniferous forests, as well as 6.1% and 3.5% in mixed forests (Figure 4).

The proportion of common OTUs in each stand has shown to be the highest, with 1234 OTUs being detected in the surface layer and 1404 OTUs in the lower layer. In broad-leaved forests, 145 endemic OTUs were detected in the surface layer and 53 in the lower layer. In coniferous forests and mixed forests, no endemic OTUs belonging to the very forests were detected, indicating that planting broad-leaved forests has most effectively changed the OTUs of the soil bacterial community (Figure 5).
For the phyla of abundance higher than 1%, Proteobacteria, Acidobacteria, Actinobacteria, and Verrucomicrobia occupied the highest proportion in each stand. Anova analysis has shown that the abundance of Proteobacteria varied significantly between broad-leaved forests and shrub and also between mixed forest and shrub ($p < 0.01$). Planting broad-leaved trees, including broad-leaved forests and mixed forests, has significantly enhanced the abundance of Proteobacteria. The abundance of Acidobacteria in the soil of coniferous forests and mixed forests was higher than that in broad-leaved forest and shrub, but the difference was not significant. Afforestation with broad-leaved forests ($p < 0.01$) and mixed forests ($p < 0.05$) both have exhibited significant reduction effect on the abundance of Actinomycetes compared with that in shrub, but the abundance of actinomycetes was not significantly changed by planting coniferous forest. The abundance of Verrucomicrobia was higher in broad-leaved forest than in other stands and shrubs, but the difference was not significant. In addition, there existed significant differences among the afforestation forests, including Chloroflexi and Planctomycetes, among which the abundance of Planctomycetes in shrubs was significantly higher than that of several planted restoration forests (Figure 6). The variation characteristics of Proteobacteria, Acidobacteria, Actinobacteria, and Chloroflexi in each stand in the lower layer of soil were consistent with those of the upper layer, but the differences among stands were no longer significant (Figure 7).

For the surface soil, afforestation has reduced the relative proportion of all genera with relative high abundance due to the increase in the number of genera of soil bacteria after afforestation, thus resulting in a decrease of the relative abundance. Afforestation has decreased the relative abundance of Bryobacter, an important bacterial genus of decomposing organic matter and using carbon sources, but only significantly reduced in the broad-leaved forests. The abundance of Acidiphilium have been significantly decreased in mixed forests and broadleaved forests. The same variation has also occurred in Conexibacter. Afforestation
has declined the abundance of most genera, including *Jatrophihabitans*, *Methylobacterium*, *Singulisphaera*, and *Thermosporothrix*. Only the abundance of *Inquilinus* has increased after afforestation, with a more significant difference in coniferous forests and mixed forests than in shrub (Figure 8).

For the lower soil, although the abundance of the main genera has decreased after afforestation compared with the shrubs, the number of genera with significant differences was obviously less than that of the surface soil. Only the abundance of *Massilia*, *Modestobacter*, *Steptomyces*, and *Virgibacillus* was significantly lower in afforestation forests than in shrubs (Figure 9).

**Figure 6.** Changes of soil bacterial abundance in different vegetation types at phylum level (surface soil) (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub. Bar marked with different letters above represent significant difference of relative abundance of corresponding phylum among different afforestation types at the level of 0.05 level).

**Figure 7.** Changes of soil bacterial abundance in different vegetation types at phylum level (lower soil) (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub).
Figure 8. Changes of soil bacterial abundance in different vegetation types at genera level (surface soil) (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub. Bar marked with different letters above represent significant difference of relative abundance of corresponding genera among different afforestation types at the level of 0.05 level).

The cluster heat map by the abundance of phylum of surface soil bacteria shows that the soil bacterial community structure clustered into different groups after afforestation, which indicated that afforestation changed the structure of the bacterial community in the surface soil. The abundance of Firmicutes, Actinobacteria, and Planctomycetes, which had higher abundance in shrubs, decreased significantly after afforestation. The abundance of Proteobacteria increased significantly in the planted forest. No obvious clustering relationship between different restoration stands has been presented (Figure 10, left).

Figure 9. Changes of soil bacterial abundance in different vegetation types at genera level (lower soil) (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub. Bar marked with different letters above represent significant difference of relative abundance of corresponding genera among different afforestation types at the level of 0.05 level).

The bacterial phyla in lower soil of shrub have shown aggregation in the clustering relationship but did not form an independent cluster as in the surface soil, indicating that afforestation has not significantly changed the abundance of the bacteria community in the lower soil. No obvious clustering relationship among different restoration stands in terms of horizontal abundance has been exhibited (Figure 10, right).
Figure 10. Clustering heat map of soil bacteria in different vegetation types at the phylum level. (BF represents broad-leaved forests, CF represents coniferous forests, MF represents mixed forest, S represents shrub. The left figure represents surface soil layer and the right figure represents the lower layer).

3.4. Soil Microbial Network

It has been shown from Supplementary Materials Figures S1–S4 a soil bacterial network in total stands, shrubs, coniferous forests, and mixed forests, respectively, while no network with $r > 0.7$ and $p < 0.05$ has been formed for the broad-leaved forests. This may be because the change of soil bacterial structure in broad-leaved forests results in the decline of the strength and significance of coexistence and exclusive relationship. The size of the point in the figure represents the relative abundance of the genus in the stand, the thickness of the line represents the correlation coefficient between the two connected genera. The red line represents positive correlation, while green line represents negative correlation. It can be seen that there existed a strong and significant correlation among a large number of genera in the shrub, among which most have shown a positive correlation, indicating a tendency of coexistence. However, in the coniferous forests and mixed forests, the overall correlation intensity decreased but the number of genera with negative correlation significantly increased. For the lower soil (Supplementary Materials Figures S5–S8), the coexistence and exclusion between genus in coniferous and mixed forests was more obvious than in the shrub, which was different from that in the upper soil.

3.5. Relationship between Soil Properties and Bacterial Communities

For the surface soil, physical and chemical properties have explained 26.06% of the variation in bacteria community structure, in which axis 1 explained 18.01% and axis 2 explained 8.05%. An obvious separation of coordinate positions between shrub and plantation forests on axis 2 has been shown, indicating that afforestation restoration has caused the changes in the bacterial community structure of the surface soil. There was also obvious separation of coordinate positions between different restoration forest types on axis 1, especially between coniferous forests and the other two kinds of forests. Compared with the shrub, the coordinate positions of plantations forest tend to increase the aspect of the nutrient content such as organic carbon and available phosphorus, and also to decrease the aspect of the ammonia nitrogen content. Among the physical and chemical factors, pH (positively) and organic carbon (negative) have shown to be the most correlated with axis 1. The pH is positively correlated with Bryobacter, while organic carbon content is strongly positively correlated with Candidatus. The available p and ammonia nitrogen have exhibited the strongest correlation with axis 2 and the content of ammonia nitrogen was positively correlated with the abundance of Sphingomonas and Gemmatimonas (Figure 11, left).

For the lower soil, The RDA axis 1 has explained 13.34% of the variation and the axis 2 explained 6.62%, totally 19.96%. The position and separation trend of RDA coordinates among different stands were basically consistent with that in the surface soil, indicating
that afforestation has significantly changed the bacterial community structure in the lower soil. Among soil physical and chemical factors, pH has shown the highest correlation with axis 1 and the strongest positive correlation with the abundance of *Paraburkholeria*, as well as the strongest negative correlation with the abundance of *Ellin*. The highest correlation is between available P and axis 2 as well as between available P and the abundance of *Sphingomonas* (Figure 11, right).

![Figure 11. RDA analysis of soil nutrient effects on bacterial community after afforestation. The left figure represents surface soil layer and the right figure represents the lower layer.](image)

4. Discussion

4.1. Effects of Afforestation with Different Forest Types on Soil Nutrients

During the process of vegetation restoration, different forest types have exerted different effects on soil nutrients due to different nutrient input into soil from litter and fine roots. It has been proved that soil erosion and irrational tillage are the main reasons for the depletion of soil organic carbon (SOC) pool [5,29,30]. Previous studies have shown that the change of vegetation is an important factor affecting the inventory and accumulation of SOC, thus, to a certain extent, reducing greenhouse gas emissions [31–33]. For example, the organic carbon content of white pine forests in the Mediterranean has increased significantly after afforestation [34], which is consistent with the results of our study, which showed that planting coniferous forests significantly improved SOC content. Therefore, afforestation restoration over the past 50 years has increased the sequestration of soil organic carbon and played a positive role in reducing greenhouse gas emissions of the Fanggan ecological restoration area. Yang et al. have pointed out that artificial restoration on the basis of primary forest being felled may lead to changes of soil phosphorus content [35]. Different forest restoration pathways regulated soil phosphorus cycling by changing vegetation community composition, thus affecting soil physical and chemical properties and litter yield [36]. Relevant studies have shown that the accumulation of organic carbon will increase the availability of Phosphorus [37], but this is inconsistent with our research results, which have shown a differentiation rule of organic carbon and available phosphorus contents in different forest types. The decrease of available phosphorus after afforestation might be due to the higher absorption of litter in the forests. The content of soil nitrogen is decided to a large extent by the strength of soil nitrogen fixation, nitrogen mineralization, nitrification, and denitrification process. This study has suggested that planting broad-leaved forests significantly improved the of soil ammonia nitrogen content but decreased nitrate nitrogen content of surface soil. This may be because the permeability of broad-leaved forests was better than that of coniferous forests and the activity strength of nitrogen fixation process was higher than mixed forests and coniferous forests. Afforestation have shown to reduce nitrate nitrogen content with the strongest effect in broad-leaved forests, probably because broadleaved forests had developed soil roots and could absorb more nitrate nitrogen.
4.2. Effects of Different Forest Types Restoration on Soil Bacterial Community Structure

The results have shown that restoration with broad-leaved forests significantly enhanced $\alpha$ diversity indices of soil bacteria of the surface soil but only chao1 and ACE indices of the lower soil. Studies have shown that afforestation species are correlated with soil microbial community structure [38]. Vegetation and soil properties could also synergistically regulate soil microbial community. The composition and diversity of soil bacterial community have proved to be mainly dependent on plant biomass and soil nutrients [39], which is consistent with this paper. Broadleaved forest could significantly improve soil nutrient quality and could provide diverse and sufficient substrates for soil bacteria to decompose and utilize. Similarly, this could also be explained by the fact that the litter of coniferous forests is difficult to decompose and has poor soil nutrients. Relevant studies have shown that changes in soil nitrogen components after afforestation are the main cause of variation of soil microbial community structure [38], which could well explain the significant changes of soil nitrogen content in artificial forests, especially in broad-leaved forests. After afforestation, the abundance in the main phylum of soil bacteria has strongly changed the $\alpha$ and $\beta$ diversity of the soil bacterial community. This study has shown that Proteobacteria, Acidobacteria, and Actinobacteria were the main phyla of the soil bacteria community, which was consistent with the results of many studies concerned with forest soil bacteria. The abundance of Proteobacteria has increased significantly after afforestation, of the most obvious effect in broad-leaved forests. The abundance of Acidobacteria and Actinobacteria has decreased after afforestation, and the effect was still the most significant in broad-leaved forests. The changes could be explained by the life cycle strategy of bacteria. Proteobacteria are mostly eutrophic bacteria growing and reproducing well in nutrient-rich soil of broad-leaved forests [40]. However, most species of Acidobacteria [41–43] and Actinomycota [44] are oligotrophic bacteria and are more likely to occur in nutrient-poor soil of shrub and coniferous forests.

4.3. Changes of Intergeneric Association of Soil Bacterial Communities during Afforestation Restoration

There are often up to tens of thousands of microbial species per gram of soil. The co-occurrence networks of different taxa could show how microbes are interconnected at this taxonomic level and could help to understand the complexity of changes in microbial communities in response to environmental factors and the impact of microbial–microbial associations on ecosystem function [45,46]. The co-occurrence network diagram could reflect the cooperation and competition relationship between microorganisms in terms of the number and size of nodes, as well as the thickness and color of the connecting lines [47]. On the surface soil, afforestation has reduced the number of strongly correlated genera of the soil bacterial community but has increased the number and intensity of negatively correlated genera in coniferous forests and mixed forests. The relationship between bacterial genera in broad-leaved forest has shown to be weak, which indicated that planting broad-leaved forests has increased competition and decreased cooperation between bacterial genera of surface soil. However, the intergeneric coexistence in broad-leaved forests is already weak. This may be related to the relatively poor soil nutrients in coniferous forests and mixed forests as well as relatively rich soil nutrients in broad-leaved forests. In addition, the diversity of different forest types could reasonably explain the coexistence of soil bacteria in different stands. However, in the lower soil, the relationship of coexistence and rejection between genera of bacterial community has shown to be more obvious after artificial afforestation, which was different from that in the surface soil.

4.4. Interpretation of Soil Nutrients Characteristics on the Change of Bacterial Community in Different Forest Types

RDA analysis has shown that afforestation significantly changed the composition and structure of soil bacterial community, which strongly responded to the changes of soil nutrients. The value of the soil bacterial community on RDA axis 2 in plantation
forests has presented significantly higher than that in shrub. The strongest correlation with axis 2 was available phosphorus and ammonia nitrogen, consistent with that afforestation significantly changed the content of soil ammonia nitrogen and available phosphorus. The values on RDA axis 1 of coniferous forests were significantly different from other plantations, which was consistent with the physicochemical properties of the coniferous forest with the lowest pH and the highest organic carbon content. Relevant studies have pointed out that soil NH$_4^+$-N, DOCN and available phosphorus mainly drive the changes in the bacterial community [48], which can be inferred from the fact that N is one of the most important nutrients for life and explained by the fact that NH$_4^+$-N is one of the easiest nitrogen sources for microorganisms [49]. In addition, soil pH has also shown to be regarded as an effective predictor of bacterial community composition [21]. These were all consistent with our research conclusions.

5. Conclusions

The aim of this important work is to better understand the mechanisms of soil organic matter, nutrients, and functional recovery. The research results have clearly shown that afforestation significantly improved soil nutrients and changed soil bacterial community structure in the Fanggan ecological restoration area. The response of surface soil to different afforestation types has presented more positively than that of the lower soil. Among the artificial forests, broad-leaved forests have exerted the most obvious effects on soil nutrients and microbial community. The content of available P, ammonia nitrogen, and the water retention capacity of the soil surface layer have to be enhanced after planting broad-leaved forests with the opposite tendency of nitrate N content. The number of total and endemic OTUs has shown to be the highest in the broad-leaved forests, and α diversity indices are also significantly higher compared with shrub. Afforestation has weakened the overall correlation between soil surface bacterial communities. After planting mixed forests and coniferous forests, the cooperation between soil surface bacterial communities have been reduced and competition have been strengthened. Soil pH, content of soil organic carbon, available phosphorus and ammonia nitrogen have been proved to be the best indicators of explaining the changes of soil bacterial community after afforestation. In conclusion, afforestation’s improvement of soil nutrient content and structure and its regulation of microbial community structure will play a positive role in mitigating greenhouse gas emissions, reversing land degradation and restoring soil ecological functions. The research results can provide scientific support for improving the nutrient and microbial functions of the soil ecosystem in the process of afforestation and restoration of barren mountains in North China, and can also provide scientific management measures and guidance for promoting the coordinated and healthy development of artificial forest and soil ecosystem.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/su14031202/s1, Figure S1: Topographic map of Fanggan ecological restoration area; Supplement Figures S2–S5: Network of surface soil bacteria in different vegetation types at genera level (Figure S2: total; Figure S3: shrub; Figure S4: coniferous forest; Figure S5: mixed forests); Supplement Figures S6–S9: Network of lower soil bacteria in different vegetation types at genera level (Figure S6: total; Figure S7: shrub; Figure S8: coniferous forests; Figure S9: mixed forests).

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