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Afforestation Enhances Potential Bacterial Metabolic Function without Concurrent Soil Carbon: A Case Study of Mu Us Sandy Land

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Abstract: Elucidating the impact of afforestation on soil bacterial community composition and its potential function in afforestation is imperative for comprehending the biochemical processes of land use change. This study employed high-throughput genomic sequencing to determine the bacterial phylogenetic assembly and assess functional groups following afforestation encompassing shrubland and woodland. Compared with non-afforested cropland, the soil organic carbon (SOC) remained unchanged, but significant alterations were observed in the bacterial composition and potential functions under afforestation. Afforestation enhanced bacterial diversity and even shifted the bacteria from the r- to K-strategy, as indicated by higher oligotroph/copiotroph ratios. Soil properties explained 66.45% and 68.9% of the total variation in bacterial community composition at the phylum level and the functional group. A 60.44% decrease in soil water content, a 3.82% increase in pH, a 7.5% increase in bulk density, and a 66.8% decrease in available phosphorus (AP) were the main soil factors affecting both bacterial community composition and functional traits in afforestation. In particular, lower available nutrients, AP, and nitrate nitrogen in afforestation drive the bacterial life history strategies. We conclude that changes in bacterial metabolic functions due to reduced soil available nutrients from dryland afforestation might be the main driver for microbial-inhibited SOC accumulation. These results could provide strong microbiological evidence to help further evaluate the importance of dryland afforestation.

Keywords: soil organic carbon; community composition; life history strategy; bacterial potential functions

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1. Introduction

Approximately 41% of the Earth's land surface is classified as drylands, with desertification affecting about 25% of these regions [1]. China's desertification-prone region covers an area of more than 1.2 million km^2, and the government has implemented several ecological restoration projects, such as the Great Green Wall Programme and Returning Farmland to Forests/Grassland Programme, to address desertification [2]. These projects have converted large areas of cropland to afforestation land, thereby changing vegetation type and soil properties. The changed habitat characteristics affect the soil microbial community composition [3]. Soil microbial communities regulate nutrient cycling, energy flow, and the ecosystem's response to anthropogenic disturbance and climate change [4]. Comprehending the variances in soil microbial communities among different land-use types is essential for advancing our understanding of ecosystem functions and processes. In addition, microorganisms are a prime bioindicator of the impact of environmental perturbations on soil quality due to their strong genetic adaptability, enabling them to swiftly

respond to environmental fluctuations [5]. Therefore, monitoring and assessing the soil of different land use types should also focus on soil microorganisms [6].

Bacterial diversity and composition are of great attention, as they are usually more abundant than archaea and fungi [7]. Numerous studies have demonstrated that alterations in land use exert substantial effects on the diversity and composition of bacterial communities [8]. While the majority of studies indicate that dryland afforestation enhances bacterial diversity [9–12], afforestation efforts have also been associated with declines in bacterial diversity [13,14]. Focusing only on changes in bacterial diversity can mask subtle differences between communities and individual populations [15]. There have been studies showing that afforestation alters the composition of soil bacteria rather than their diversity [16,17]. The response of the soil bacterial community composition to various land use types is characterised by considerable variability, owing to their high sensitivity to habitat alterations and the intricate nature of community composition [18]. Research findings indicate that the soil bacterial structure following afforestation (forest and shrub planting) is similar to that of the original soil [19]. The dominant soil bacterial populations from adjacent non-afforested and forest planting have high similarity at the genus level [11]. In contrast, many studies in arid regions have reported substantial alterations in soil bacterial composition after land-use change and could not be recovered in the long term [20,21]. For example, research conducted in the Loess Plateau showed that afforestation made the bacterial compositions shift from Proteobacteria to Actinobacteria [16] or from Actinobacteria to Proteobacteria [9,22]. In addition, differences in the soil bacterial community composition between shrubland and woodland were much smaller than between shrubland and cropland [23]. These uncertainties imply that an in-depth understanding of the shifts in soil microbial communities across various land use types and the main factors controlling them is crucial for preserving ecosystem functions and facilitating carbon cycling in drylands.

Soil bacteria are pivotal in the terrestrial carbon cycle, exerting significant control over both above- and below-ground carbon dynamics [24]. This balance can be subject to change in response to changes in the environment. Microbial composition and functions are influenced by trade-offs among the bacterial growth rate, resource utilisation efficiency, and stress tolerance [25]. Recent research suggests that life history strategies can be determined to meet this challenge of predicting alterations in soil carbon [26]. Metabolic functions significantly contribute to the decomposition of litter and the circulation of other macroelements [27]. Bacterial metabolic investments for resource acquisition may reduce cell growth efficiency [28]. Afforestation significantly influences bacterial life strategies and metabolic activities [14]. Blending life strategies and functional features could unveil connections between soil microbiome and carbon accumulation post-afforestation.

Under land use change scenarios in the Mu Us desert dryland, field surveys were carried out to further explore the impacts of afforestation on soil bacterial communities. We postulate that afforestation in the arid northern drylands diminishes soil nutrients and moisture, which may be unfavourable for bacterial viability due to limited soil moisture and nutrient availability. This leads to a shift from the copiotrophic to oligotrophic bacteria life history strategy and changes their functioning. Hence, this study aimed to (1) discover the changing patterns of soil bacterial community composition throughout land use change in dryland; (2) investigate the changing characteristics of the life history strategy and functional groups across different land use types; and (3) identify factors influencing bacterial composition and functions with the possible consequences of such changes.

2. Material and Methods

2.1. Study Site and Soil Sampling

The survey was carried out on Mu Us Sandy Land in the Ordos afforestation area, Northeastern China (38.18–39.55° N, 107.51–108.50° E). This region falls under an arid to semi-arid continental monsoon climate, and the average annual temperature is 6–8 °C. The annual precipitation reaches around 360 mm, with more than 60% of precipitation occurring during July and September. Soil types in this area encompass kastanozem and

grassland aeolian sandy soil. We selected three sites, including Site1, Site2, and Site3 (Figure 1b), with three land use types (cropland, shrubland, and woodland) at each site (Figure 1c). To ensure comparability, the three land use types were contiguous at each site. The cropland was reclaimed in the 1970s, and the main crop for the last decade has been *Zea mays* L., grown under traditional farming practices, which was irrigated using sprinkler irrigation and an inorganic NPK fertiliser. The woodlands were planted with *Pinus sylvestris*, *Populus simonii*, and *Ulmus pumila* L., and the shrublands were planted with *Artemisia ordosica Krasch* and *Caragana intermedia*, as detailed in Table S1. Based on the nearby farmers and support from the literature, the planting age of shrubland and woodland was about 20–30 years [29]. At the end of May 2021, three 1 m × 1 m squares were drawn within each land use type from each site. After removing the surface litter layer, three soil samples from each square were collected from the 0 to 10 cm depth, combined and sieved through a 2 mm sieve. A portion of the composite soil was promptly frozen in liquid nitrogen and transported back to the laboratory. The samples were then stored at −80 °C until DNA extraction. A portion of the remaining soil sample was used for the determination of soil water content (SWC), microbial biomass carbon (MBC), ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N), and the other part was air-dried for the determination of soil pH, sand content, soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), and available phosphorus (AP). Additionally, three soil cores measuring 100 cm^{-3} each were collected from every square to assess soil bulk density (BD).

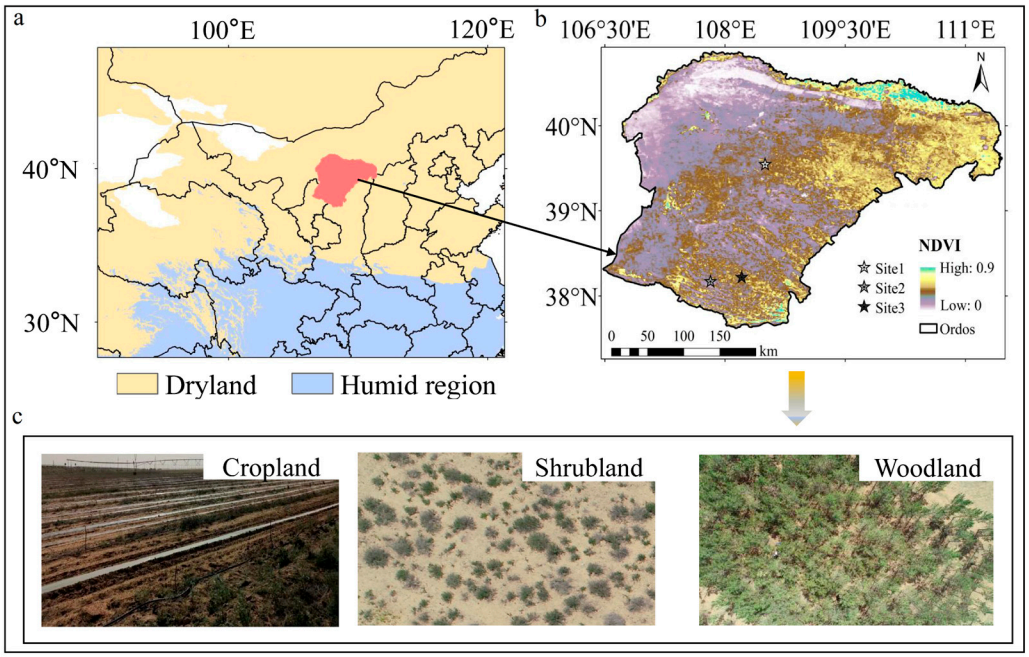

Figure 1. Location of the study area and sampling sites, (**a**) the climate zone of Ordos, (**b**) three sampling sites located in Mu Us Sandy Land, and (**c**) three land use types for each sampling site.

2.2. Soil Physicochemical Properties Analysis

SWC and BD were assessed via the gravimetric method. MBC was determined using the fumigation–extraction method [30]. NH_4^+-N and NO_3^--N were extracted with a 2 mol L^{-1} HCl solution and quantified utilising an auto-flow injection system (Foss Tecator, Höganäs, Sweden). Soil pH was determined using a glass electrode with a soil–water ratio of 1:2.5. TN was measured using the Kjeldahi digestion procedure [31]. TP and AP were assessed using the molybdate colorimetric method [32] and the Olsen method [33], respectively. SOC was determined by the Walkley and Black dichromate oxidation method [34].

2.3. DNA Extraction, PCR Amplification, and Illumina Sequencing

DNA extraction was performed from 0.5 g freeze-dried soil samples using the FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The quality of the extracted DNA was assessed using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), and its integrity was confirmed via agarose gel electrophoresis. Polymerase chain reaction (PCR) amplification targeting the V4–V5 region was conducted within the qualified sample detection area using specific primer pairs. Standard bacterial genomic DNA (*Escherichia coli* genome DNA) served as a positive control, and three replicate experiments were established. Specific tag sequences compatible with the Illumina platform were added to the end of the library with high-fidelity PCR using primers with index sequences. The amplified products were detected by agarose gel electrophoresis, and the amplified products were purified using AgencourtAMpure XP (Beckman Coulter, Brea, CA, USA) magnetic beads to obtain the original library from a sample. Upon initial quantification of the results obtained via agarose gel electrophoresis, the library concentrations of samples pre-labelled with their respective indexes were suitably diluted. The PCR reaction mixture contained 4 μL of a 5× reaction buffer, 2.4 μL dNTP (2.5 mM), 0.8 μL NGMPCRFN5XX (10 μM), 2 μL NGMPCRRN7XX (4 μM), 0.2 μL Herculase® II Fusion DNA polymerase, 2 μL diluted PCR product and 10.6 μL ddH_2O. The libraries were precisely quantified using the Invitrogen Qubit3.0 Spectrophotometer (Thermo Fisher Scientific, USA). Subsequently, the samples were blended following sequencing throughput necessities specific to each sample. Mixed libraries were examined for the size of sequenced insert fragments by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) to determine the size of sequenced insert fragments. This examination ensured the absence of non-specific amplifications within the 120–200 bp range and quantified the concentration of sequenced libraries. The NovaSeq 6000 platform by Illumina, San Diego, CA, USA, employing the SP-Xp (PE250) double-ended sequencing strategy conducted Illumina MiSeq sequencing (Genesky Biotechnologies Inc., Shanghai, China). The sequencing data underwent bioinformatics analysis conducted with QIIME 2 (version 2018.11). Because of the need to construct libraries and sequencing, the raw sequencing data contained some artificial additives, such as adapter sequences, primers, and so on. After removing the possible adapter sequences and primers using the cut adapt plugin, the quality of the raw data was counted. The DADA2 plugin performed the quality filtering, noise reduction, splicing, and de-chimerisation of the data and generated an amplicon sequence variant (ASV) abundance table. Each ASV representative sequence was aligned with the database, thus completing the taxonomic annotation of ASVs.

2.4. Statistical Analyses

The diversity plugin was utilised within QIIME2 to assess alpha diversity metrics, encompassing the Chao1, Shannon index, and Simpson index. The ASVs representing bacterial similarity between samples were then analysed utilising the Bray–Curtis distance matrix of similarity (ANOSIM) to delineate overall differences in the composition. A visual representation of these results was achieved via non-metric multidimensional scaling (NMDS). Furthermore, to discern variations in the relative abundance of bacterial groups among different land use types, the linear discriminant analysis effect size (LEfSe) was applied. To retain as many meaningful species taxa as possible, relative abundances were removed from the analysis of rare taxa with <0.0005 [35]. The criterion of significance at the categorical level was LDA > 3, $p < 0.05$. The constructed co-occurrence network utilised the relative abundance of bacterial ASVs as weights, operating at the species level. ASVs occurring in at least 60% of the samples were selected in each ecosystem. Pearson correlation coefficients between species were calculated, correlated ASVs with $p < 0.05$, and the top 200 ASVs were selected in descending order to construct the association network [36]. Network analyses were performed at the level of each of the 3 ecosystem bacterial phyla. The network properties were computed in Gephi v 0.9.2. Bacterial life strategies were categorised according to phylum distinctions and community-level attributes [37]. PICRUSt2

prediction is based on the measured bacterial ASVs and compares the GreenGenus 16S rRNA full-length sequence database [38].

Linear mixed models were employed to assess the impact of various land use types on diverse soil properties and microbial diversity. Land use types, including cropland, shrubland, and woodland, were treated as fixed factors while sampling locations (Site1, Site2, and Site3) were considered random factors. If significant differences existed, the variance between treatments was analysed using Tukey's test ($p < 0.05$). Before conducting linear mixed-effects model analysis, the normality and homogeneity of variance were assessed for all data using Shapiro–Wilk and Levene's tests, respectively. In cases where the data did not meet the assumptions of normality and homogeneity of variance, log transformation was applied before analysis. The impacts of soil factors on bacterial phylum levels and functional groups were assessed using transformation-based redundancy analysis (tb-RDA). The analyses mentioned above were conducted utilising R software (v.4.0.2, R Core Team, Vienna, Austria).

3. Results

3.1. Soil Properties and Microbial Biomass in Different Land Use Types

For various land use types, significant differences ($p < 0.05$) were observed in SWC, pH, and BD, while the sand content remained relatively constant (Table 1). Both shrubland and woodland SWC exhibited significantly lower levels than cropland, while soil pH and BD were significantly higher than cropland ($p < 0.05$, Table 1). Compared with cropland, shrubland and woodland displayed significant reductions in TP, NO_3^--N, and AP content ($p < 0.05$), while the alterations in TN and NH_4^+-N were not significant (Table 1).

Table 1. Changes in soil properties in different land use types.

Soil Properties	Cropland	Shrubland	Woodland	*p*
pH	8.52 ± 0.21 b	8.86 ± 0.15 a	8.83 ± 0.16 a	<0.001
SWC (%)	4.55 ± 1.29 a	1.53 ± 0.8 b	2.07 ± 0.75 b	<0.001
Sand	0.85 ± 0.04 a	0.87 ± 0.08 a	0.87 ± 0.05 a	0.474
BD (g cm^{-3})	1.4 ± 0.13 b	1.54 ± 0.07 a	1.49 ± 0.1 a	<0.01
TN (g kg^{-1})	0.83 ± 0.18 a	0.54 ± 0.35 a	0.81 ± 0.53 a	0.285
TP (g kg^{-1})	0.38 ± 0.18 a	0.25 ± 0.05 b	0.24 ± 0.04 b	<0.05
NO_3^--N (mg kg^{-1})	40.01 ± 31.34 a	3.46 ± 2.98 b	4.01 ± 2.73 b	<0.001
NH_4^+-N (mg kg^{-1})	6.69 ± 1.57 a	5.42 ± 0.96 a	5.54 ± 0.95 a	0.054
AP (mg kg^{-1})	5.09 ± 2.64 a	1.67 ± 0.63 b	1.69 ± 0.75 b	<0.001

Note: SWC, BD, TN, TP, NO_3^--N, NH_4^+-N, and AP represent soil water content, bulk density, total nitrogen, total phosphorus, nitrate nitrogen, ammonium nitrogen, and available phosphorus, respectively. Values are the means ± SE ($n = 9$). Lowercase letters indicate significant differences within different land use types ($p < 0.05$) and *p* indicates significant differences between land use types.

3.2. Soil Bacterial Diversity and Community Composition in Different Land Use Types

Compared to cropland, woodland significantly increased soil bacterial richness (Chao1) and the Shannon index ($p < 0.05$, Figure 2a,b), while the Simpson index significantly decreased ($p < 0.05$, Figure 2c). In shrubland, bacterial alpha diversity (richness and Shannon index) also surpassed cropland, albeit insignificantly ($p > 0.05$, Figure 2a,b). There were no significant differences between SOC, DOC, and MBC for different land use types (Figure 2d–f).

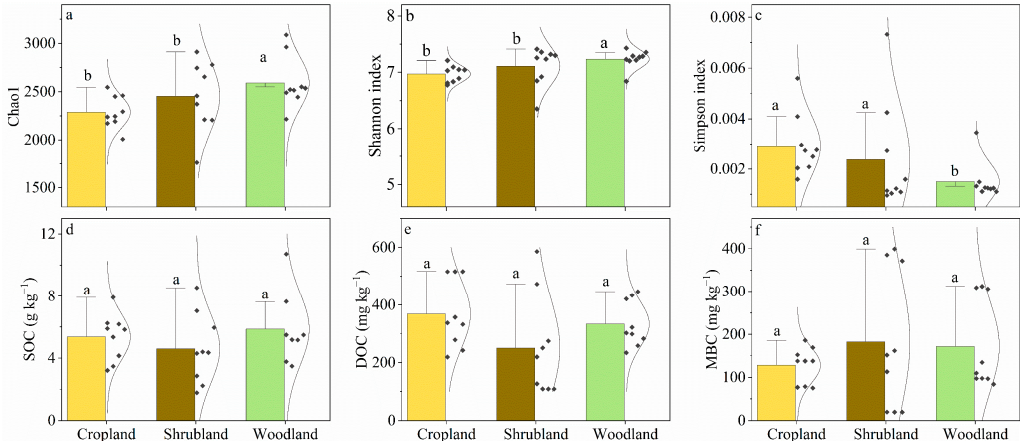

Figure 2. Differences in bacterial (**a**) richness, (**b**) Shannon index, (**c**) Simpson index, (**d**) soil organic carbon (SOC), (**e**) dissolved organic carbon (DOC), and (**f**) microbial biomass carbon (MBC) across the land use types (n = 9). Different letters indicate significant differences between different land use types ($p < 0.05$).

ANOSIM analysis revealed significant divergence in bacterial structures across cropland, shrubland, and woodland, as evidenced by R > 0, indicating greater inter-group disparities than intra-group variances ($p < 0.01$, Figure 3a,b). The relative abundance of predominant bacterial phylum was Proteobacteria (23.6%), Acidobacteria (18.71%), Bacteroidetes (9.6%), Actinobacteria (17.85%), and Chloroflexi (6.82%) (Figure 3c). The relative abundance of Proteobacteria and Bacteroidetes was highest in the cropland (Figure 3c). The relative abundance of acidobacteria and actinobacteria in shrubland and woodland showed a consistent pattern, both surpassing the levels observed in cropland (Figure 3c). Unlike woodland, Chloroflexi exhibited higher relative abundance in shrubland compared to cropland (Figure 3c).

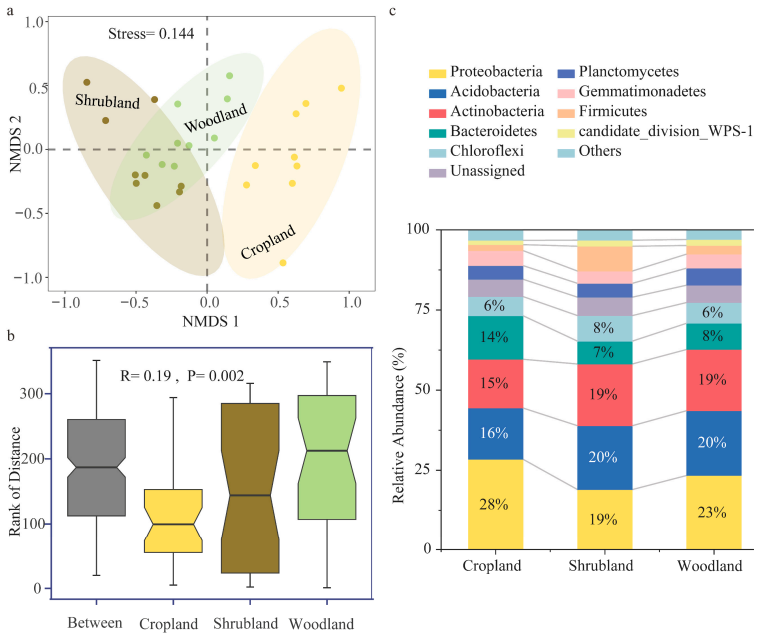

Figure 3. Bacterial (**a**) non-metric multidimensional scaling (NMDS) ordination, (**b**) analysis of similarity (ANOSIM), and (**c**) relative abundance (%) at the phylum level in different land use types (n = 9). R indicates the relative variability between samples, ranging from [−1 to 1]; R = 0: no distinction between the groups; and R > 0: the difference between the groups surpasses the difference within the groups. $p < 0.05$ indicates significant differences.

To elucidate the factors influencing the varied impacts of land use changes on bacterial structure, we conducted LEfSe analysis to identify significant enrichments of bacterial taxa at various taxonomic levels across different land-use types (Figure 4). The relative abundance of taxa included Gammaproteobacteria, Oxalobacteraceae, Xanthomonadales, Hyphomicrobiaceae, Rhodospirillaceae, Massilia within Proteobacteria, and Cytophagia, Cytophagales, Cytophagaceae, Chryseolinea, and Rufibacter within Bacteroidetes, which were enriched in cropland (Figure 4). Actinobacteria exhibited enrichment in shrubland and woodland, including taxa such as Solirubrobacterales, Aciditerrimonas, Rubrobacter, Rubrobacterales, and Rubrobacteraceae in shrubland, and Acidimicrobiales, Pseudonocardia, Conexibacteraceae, and Conexibacter in woodland (Figure 4). Moreover, Acidobacterium_sp._Ac_12_G8, belonging to Acidobacteria, showed higher relative abundance in shrubland (Figure 4).

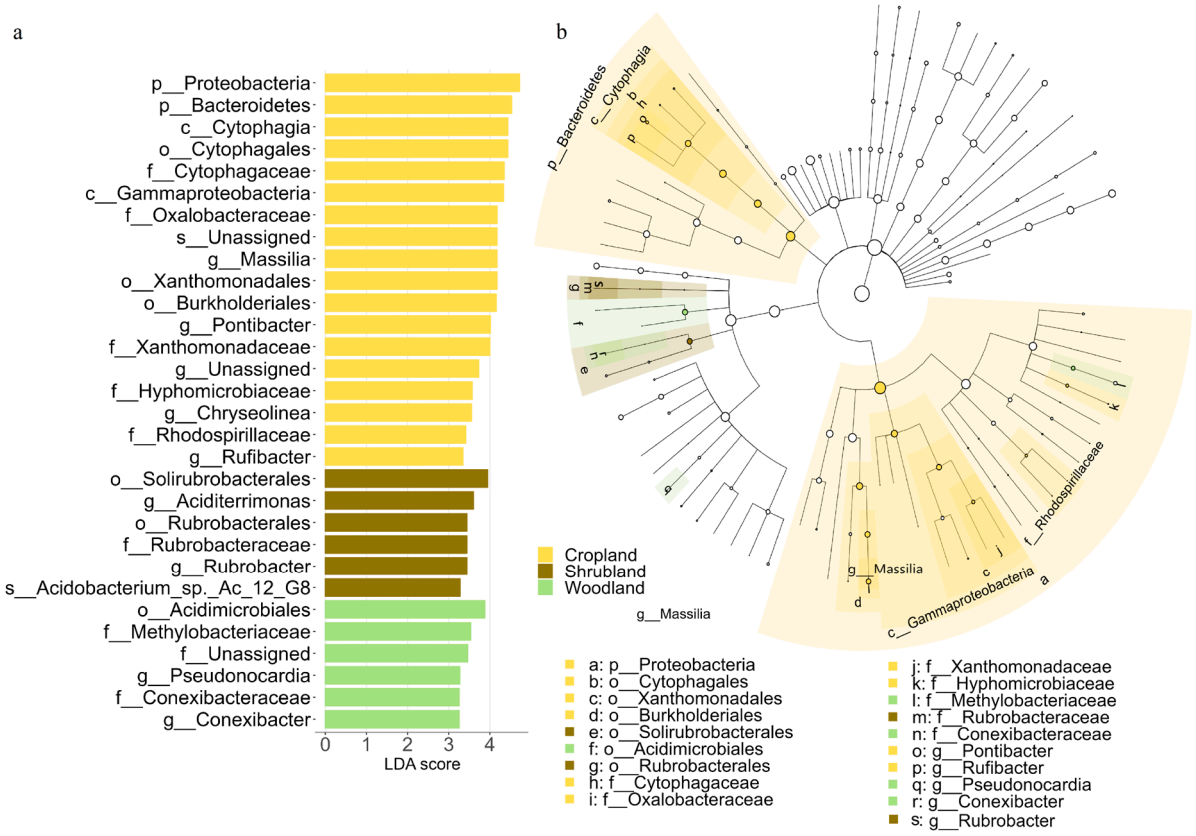

Figure 4. Bacterial (**a**) taxa with significant differences and (**b**) cladogram in different land use types (n = 9). The circles in the cladogram represent taxa from the phylum to genus, and coloured cycles represent taxa with significant differences among land use. The level of significance is $p < 0.05$.

Co-occurrence networks facilitate the examination of interaction dynamics among bacteria at the ASV level across cropland, shrubland, and woodland environments. The bacterial co-occurrence networks in cropland, shrubland, and woodland comprised 665, 716, and 643 nodes, respectively, with corresponding link counts of 1885, 3262, and 2716 (Figure 5). Significantly, the soil bacterial co-occurrence networks exhibited higher link densities in the shrubland and woodland compared to the cropland (Figure 5). Moreover, the node counts were notably greater in the shrubland than in both the cropland and woodland (Figure 5). The modularity indices in cropland, shrubland, and woodland soils were 0.942, 0.891, and 0.841, respectively (Figure 5). Due to an increase in oligotrophic bacteria and a decrease in copiotrophic bacteria in shrubland and cropland, the ratio of oligotrophic/copiotrophic bacteria was higher than in cropland (Figure S1a). Additionally, the relative abundance of bacterial rrn operon copies in woodland was lower (Figure S1b). Correlation analysis

revealed a significant negative correlation between bacterial rrn operon copies and the oligotroph/copiotroph ratio ($p < 0.001$, Figure S1c). There were four categories in the soil potential function according to the PICRUSt2, with results indicating significant differences in bacterial functional characteristics under different land-use patterns (Figure S2). The relative abundance of the potential metabolism function in afforestation (shrubland and woodland) was significantly higher than in cropland (Figure S2a). Specifically, the increase in the relative abundance of the potential metabolism function in shrubland and woodland was mainly associated with amino acids, carbohydrates, terpenoids and polyketides, glycan, and xenobiotic metabolism (Figure S2b). Afforestation significantly reduced the abundance of cellular processes (Figure S2a), particularly those associated with cell growth, death and cell motility (Figure S2b).

Land use types	Nodes	Links	Average degree	Betweenness centralization	Modularity
Cropland	665	1885	5.669	0.057	0.942
Shrubland	716	3262	9.112	0.051	0.891
Woodland	643	2716	8.448	0.038	0.841

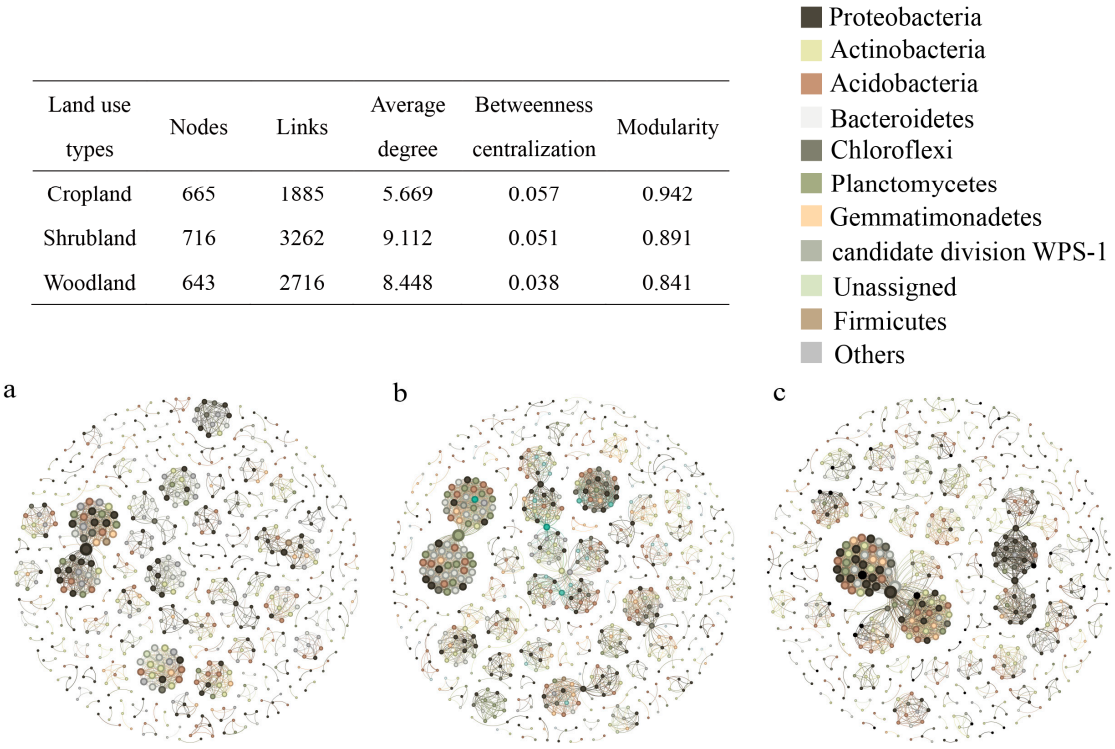

Figure 5. Soil bacterial co-occurrence networks in (**a**) cropland, (**b**) shrubland, and (**c**) woodland and the table of network characteristics for different land use types ($n = 9$). The nodes in the co-occurrence network are coloured at the bacterial phylum level, with larger nodes indicating more links assigned to the node. Nodes represent amplicon sequence variant (ASV), and links indicate significant co-occurrence ($p < 0.05$). The nodes in the co-occurrence network are coloured with a bacterial gate level, where larger nodes indicate more edges assigned to the node. The average degree reflects the average number of links for all nodes, and betweenness centralisation measures the potential impact of a given node on the connectivity of other nodes. Modularity quantifies the difference between the links of the modules and the expected links of random networks with the same number of edges.

3.3. Soil Factors Affecting Bacterial Community Composition and Function

Given the significant changes in the soil bacterial community composition due to variations in land use types, further analysis was conducted to examine the primary soil factors shaping the alterations in community composition (Table 2 and Figure 6). As shown in Table 2, the bacterial richness and Shannon index were significantly positively correlated with SOC and MBC. Moreover, TN and DOC were significantly positive, while NO_3^--N was negatively correlated with bacterial richness ($p < 0.05$, Table 2). The bacterial oligotroph/copiotroph ratio significantly decreased with increasing SWC, NO_3^--N, NH_4^+-N, and AP, while it significantly increased with the increasing soil pH (Table 2).

Table 2. Correlation between environmental factors and soil bacterial richness, Shannon index, and oligotroph/copiotroph ratio.

Soil Factors	Chao 1	Shannon Index	Simpson Index	Oligotroph/ Copiotroph Ratio
pH	0.053	0.107	0.018	0.381 *
SWC	−0.170	−0.169	0.061	−0.411 *
Sand	−0.185	−0.309	0.332	−0.275
BD	−0.069	0.033	−0.100	0.220
SOC	0.519 *	0.414 *	−0.387 *	0.102
TN	0.485 *	0.373	−0.384 *	0.020
TP	−0.207	−0.216	0.015	−0.375
DOC	0.421*	0.314	−0.301	0.137
NO_3^--N	−0.412 *	−0.364	0.152	−0.483 *
NH_4^+-N	−0.064	−0.143	0.007	−0.381 *
AP	−0.255	−0.368	0.428 *	−0.488 **
MBC	0.636*	0.534 *	−0.481 *	0.325

Note: SWC, BD, SOC, TN, TP, DOC, NO_3^--N, NH_4^+-N, AP, and MBC represent soil water content, bulk density, soil organic carbon, total nitrogen, total phosphorus, dissolved organic carbon, nitrate nitrogen, ammonium nitrogen, available phosphorus, and microbial biomass carbon, respectively. Values are the means ± SE (n = 9). "*" and "**" represent $p < 0.05$ and $p < 0.01$, respectively.

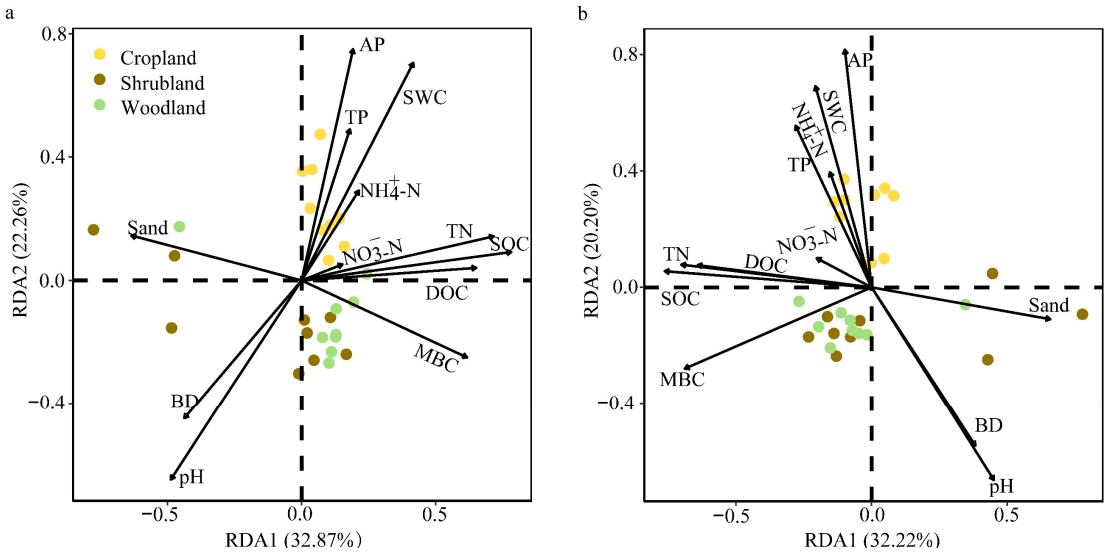

Figure 6. Redundancy analysis to show the correlation between the bacterial communities and soil properties under three land use types, including bacterial (**a**) phylum-level taxonomy and (**b**) function category (n = 9). SWC, BD, SOC, TN, TP, DOC, NO_3^--N, NH_4^+-N, AP, and MBC represent soil water content, bulk density, soil organic carbon, total nitrogen, total phosphorus, dissolved organic carbon, nitrate nitrogen, ammonium nitrogen, available phosphorus, and microbial biomass carbon, respectively.

tb-RDA accounted for 66.45% and 68.9% of the variance in bacterial phylum-level species and functional groups concerning soil factors, respectively (Figure 6). The first two canonical axes explained 32.87% and 22.26% of the relationship between bacterial phylum-level species and soil factors (Figure 6a), and similarly, they accounted for 32.22% and 22.20% of the association between bacterial phylum-level species and soil factors (Figure 6b). Additionally, the findings revealed the significant impacts of pH, SWC, BD, TP, and AP on the bacterial phylum-level community composition (Figure 6a), whereas the pH, sand content, BD, SOC, TN, DOC, NH_4^+-N and AP emerged as significant factors influencing bacterial functional groups ($p < 0.05$) (Figure 6b).

4. Discussion

4.1. Changes in Soil Properties in Different Land Use Types

Due to the limited soil moisture and nutrients in dryland, cropland usually requires irrigation, tillage, and fertilisation [39], as was the case in our study. Compared to undisturbed shrubland and woodland, agricultural practices greatly alter the physicochemical properties of cropland soil [40]. The increased available nutrients (NO_3^--N and AP) and SWC in cropland are mainly attributed to irrigation, fertilisation, and the nutrients input from crop residues [41,42]. Furthermore, the transpiration of shrubland and woodland in drylands exceeds the amount of water replenishment, which leads to a further reduction in soil moisture [43]. A study has shown that afforestation in alkaline soils leads to acidification [44], which is contrary to our result. From this discrepancy, it can be speculated that the vegetation in shrubland and woodland absorbs and utilises NO_3^--N, which significantly increases soil pH [45]. The conversion of cropland to afforestation in drylands means that seasonal crops are replaced with long-term vegetation growth [46]. This change reduces root development and soil disturbance, which, in turn, increases soil BD in shrubland and woodland [47]. Despite the long-term vegetation growth, the lack of increase in SOC in shrubland and woodland can be attributed to the slow process of plant residues and microorganisms fixing and stabilising across soil aggregates or minerals [48]. The planting age of shrubland and woodland ranges from 20 to 30 years, which was apparently not long enough to increase carbon accumulation.

4.2. The Response of Bacterial Diversity and Co-Occurrence Networks to Land Use Types

The increase in bacterial diversity attributed to woodland root exudates and apoplastic inputs providing a more diverse nutrient source for the bacterial microbial community [49] is further evidenced by the positive correlation between DOC and bacterial diversity. This finding is consistent with other increases in bacterial diversity associated with afforestation in drylands [11,12]. The co-occurrence network showed that afforestation had a lower degree of modularity, while the nodes and links were greater than cropland (Figure 5). A high degree of modularity indicates a strong ecological niche differentiation [50]. Higher available nutrients NO_3^--N and AP in cropland promote the differentiation of bacterial ecological niches [51]. Enhanced bacterial ecological niche specialisation in cropland may diminish microbial interactions [50], potentially accounting for the observed reduction in links. Crop cultivation in cropland could streamline the diversity of organic matter while amplifying the prevalence of specific organic compounds. Therefore, fewer bacterial taxa collaborate among organic compounds, leading to the segregation of modules [52]. Other studies have also reported a decreasing trend in the bacterial network complexity in cropland [53], whereby more anthropogenic disturbances disrupt the structure of tightly connected bacterial networks. Previous findings suggest that species in a module might share similarities and overlap in ecological niches [54,55]. Overlapping ecological niches induced complex interactions so that bacterial co-occurrence networks in shrubland and woodland have more nodes and links. Furthermore, bacterial co-occurrence networks in shrubland and woodland are complex (with high link counts and averaging) and are more resistant to environmental disturbances than cropland [56]. Microbial networks are not randomly assembled but rather aligned according to their ecological roles or functions [52]. Changes in the bacterial co-occurrence network may consequently influence its functional properties.

4.3. Changed Soil Properties in Shaping Bacterial Taxa-Specific

Actinobacteria and Acidobacteria are recognised as K-strategy oligotrophic bacteria, prioritising stress resistance overgrowth rates [57]. In shrubland and woodland ecosystems, the prevalences of Actinobacteria and Acidobacteria exceeded those observed in cropland, which is likely attributed to their significant involvement in soil organic matter regulation [58]. Actinobacteria and Acidobacteria are spore-forming bacteria that benefit from their spores and filaments to mitigate damage caused by drought, heat, and infertility [22].

Therefore, it can be inferred that the alteration in the relative abundance of Actinobacteria and Acidobacteria might stem from the decline in soil properties (SWC, NO_3^--N and AP) in shrubland and woodland. Many Proteobacteria and Bacteroidetes are considered r-strategy copiotrophic bacteria [59]. Cropland has higher levels of labile nutrients compared to shrubland and woodland, which favours the rapid growth of copiotrophs [60]. This is further supported by the negative relationship between labile nutrients (NO_3^--N and AP) and the oligotroph/copiotroph ratio (Table 2). Higher available nutrients NO_3^--N and AP in cropland favour the rapid growth of Proteobacteria and Bacteroidetes [60,61]. Earlier research indicates that an increase in readily decomposable soil organic matter triggers a swift proliferation of r-strategy (copiotroph) bacteria [62]. There were no significant differences between land use types in DOC (Table 1). Conversely, the lower SWC in shrubland and woodland limited the growth of Bacteroidetes [60]. The transition from cropland to afforestation and declining nutrient levels serve as the primary catalyst for the transition of bacteria from the r- to the K-strategy [63]. Metabolic variability is an essential characteristic of K-strategy bacteria, which may scavenge a diverse array of carbon substrates [21]. An increase in K-strategy bacteria stimulates the rate of microbial carbon mineralisation [64]. There were differences at the bacterial genus level, although shrubland and woodland were similar at the phylum level, and Rubrobacter was the dominant actinobacterial genus in shrubland, whereas Pseudonocardia and Conexibacter predominated in woodland (Figure 4). Typically, Rubrobacter is widespread in some extreme dry environments, where very low soil moisture in scrubland soils may be responsible for its accumulation [65]. The woodland root may provide a more favourable environment for the coexistence of Pseudonocardia and Conexibacter in the rhizosphere [66]. It is worth noting that Massilia enriched on cropland can help make soil phosphorus more available for uptake, thereby promoting plant growth [67]. Therefore, studying variances in bacterial compositions across distinct land use types at various taxonomic hierarchies has advanced our comprehension of afforestation's impacts on soil bacterial communities.

4.4. The Shift of Bacterial Potential Function to Land Use Change

Changes in the availability of soil nutrients could potentially trigger the metabolic activity of amino acids, carbohydrates, and other organic compounds [68]. Its function in metabolism helps the bacteria obtain energy, amino acids and carbohydrates from the soil to sustain their growth [69]. Increased amino acid metabolism in shrubland and woodland leads to increased secondary products [70]. Metabolism pathways associated with energy production included lipid, energy and carbohydrate metabolism [71]. Although lipid metabolism was unchanged and energy metabolism decreased by 0.06%, the 0.3% increase in carbohydrate metabolism in shrubland and woodland still increased energy production. Moreover, nucleotide pathways aid soil bacteria in adapting to environmental stress [72], and the diminished nucleotide metabolic functions in shrubland and woodland environments hinder bacterial growth (Figure S2a). In summary, the nutrient limitation under afforestation speeds up bacterial metabolism [73], implying that bacterial communities in afforested soils are more capable of degrading organic matter. Increased organic matter decomposition may also explain why apoplastic and root secretions increase in shrublands and woodlands but not SOC. Increased soil pH and decreases in SWC also trigger changes in the environmental adaptive capacity of bacteria [74]. Changes in the relative abundance of bacterial functional groups suggest that resource availability and microbial interactions are key to bacterial responses during land use conversion. Due to the limitations of the PICRUSt2 function prediction analysis, this paper only provides a preliminary prediction of the functions of the bacteria involved.

5. Conclusions

Afforestation resulted in increased bacterial diversity, more complex co-occurrence networks, and a shift in the life history strategy from r to K. However, there was no increase in soil organic carbon and microbial biomass. The predicted bacterial functions indicate

that the shrubland and woodland were enriched in amino acids, carbohydrates, xenobiotics, and glycan metabolism involved in organic matter degradation. Shifts in land-use types lead to changes in soil properties, which is the most important factor affecting bacterial composition and potential function. This study highlights the responsiveness of bacterial-specific species to cropland-to-forest conversion. The resulting potential functional changes may have implications for carbon dynamic processes in Mu Us Sandy Land. In the future, the function of bacterial degradation of various carbon fractions in response to afforestation and its contribution to the carbon cycle mechanism should be further investigated in combination with functional genomics and metabolomics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15050867/s1, Figure S1: The (a) oligotrophs/copiotrophs ratio and (b) rrn operon copy number in different land use types, and (c) the relationship between bacterial rrn operon copy number and oligotrophs/copiotrophs ratio Figure S2: (a) 4 key bacterial ecologically relevant functions and (b) their sub-functions based on PICRUSt2 prediction (n = 9); Table S1: Contextual characteristics of the study sites.

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