



Soil bacteria with distinct diversity and functions mediates the soil nutrients after introducing leguminous shrub in desert ecosystems

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ABSTRACT

Legume plants are pioneer species in desert ecosystem that can be used to reestablish degraded ecosystem functions and soil properties, and reshape underground microbial communities. However, the distribution in soil nutrients and bacterial communities after introducing leguminous shrubs have not been well described. Here, we quantified the variations in soil nutrients and bacterial communities in soil profiles (0–100 cm) across stand ages (5-year, 8-year, 15-year) and severe desertification (SD), and further investigated the bacterial contributions to soil nutrients during stand development. The total phosphorus (TP), total nitrogen (TN) and organic carbon (OC) contents significantly increased, whereas the available nitrogen (AN) and available phosphorus (AP) contents showed a downward trend across stand ages. Bacterial diversity increased after introducing leguminous shrubs. Vertical spatial variation of bacterial communities attenuated during stand development. Bacterial beta-diversity, specific microbial phyla (i.e., Proteobacteria and Firmicutes) and nitrification process were strongly associated with vertical spatial variation in soil nutrients. Combined analyses revealed that leguminous shrubs can ameliorate soil OC, TN and TP with increasing legumes growth time, but could still exhibit no facilitating effect on the improvement of soil available N and P even for legumes aged 15 years. Improving the bioavailability of nutrient elements and microbial function diversity could be targeted for further studies in arid barren sandy.

1. Introduction

Desert ecosystems are sensitive and vulnerable to environmental change and human disturbance, and are suffering from increasingly serious soil degradation and biodiversity loss (Bastin et al., 2017; Bu et al., 2020; Wang et al., 2015). Legume plants are thought to restore degraded environments; part of the N demand of regrowing and reestablishing vegetation can be satisfied by legumes, which have the capacity to fix atmospheric N₂ through interactions with rhizobia bacteria (Hu et al., 2016; Mahmud et al., 2020). Establishment of legume plantations in desert ecosystems is currently one of the most massively used reconstruction methods,

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as it not only reestablishes degraded ecological functions and soil characteristics, but also affects the underground microbial community (Hu et al., 2016). Thus, it is critical to understand the ecological importance of legumes to soil properties and microbial communities in desert ecosystems.

The ecological importance of pioneer legume plants can extend to their potential regulation effects on nutrients in degraded soil (Marquez and Allen, 1996; Padilla and Pugnaire, 2006; Roa-Fuentes et al., 2015). A previous study in 299 shrub sites have reported that legume shrubs were more plentiful and homeostatic in N compared to non-N₂-fixing shrubs (Guo et al., 2017). Litterfall and decomposition of these N-rich tissues are one of the major means for legumes to fertilize the around soil (Adams et al., 2016; Su and Zhao, 2003; Yang and Liu, 2019). In general, the contents of soil nutrients in superficial soils (i.e., below 20 cm) are higher than those in deep soils (Balesdent et al., 2018; Kang et al., 2021). Numerous studies have exclusively emphasized the significance of nutrients (i.e., OM, TN, AN, AP) in top soils (~20 cm) due to their predominant ecological importance, as they are responsible for supplying available nutrients (i.e., AN, AP) for plant roots uptake (Albuquerque et al., 2013; Nkebiwe et al., 2016; Zhang et al., 2016). In desert ecosystems, desertification soils were normally characterized by high permeability capacity and leaching capacity, leading to the enhanced migration and accumulation of nutrients (i.e., C, N and P) in deeper soils (D'Odorico et al., 2013; Tang et al., 2015). Despite this knowledge, our understanding of soil nutrient changes after introducing leguminous plants, and how soil nutrients, particularly in the deep soil profiles, will change with the increasing of leguminous plant ages, remains scant.

Soil microbiomes are strongly affected by vegetation cover (Fahey et al., 2020). The variability in the soil microbial communities has been reported to be significantly higher in shrub cover samples than in intershrub samples (Ben-David et al., 2011). This suggests that a living shrub is a major driving force behind the differentiation of microbial communities. Furthermore, Zuo et al. (2020) studied the factors affecting the soil microbial communities and metabolic diversity in arid desert ecosystems and found that they were generally best explained by the effect of plant cover. Generally, plant communities play an important role in reshaping soil micro-organism communities via root exudates and mucilage as well as litter supplementation and microclimate effects (Shi et al., 2011; Zak et al., 2003). Plants may directly change microbial communities, such as by producing allelochemicals that inhibit symbiosis (Bais et al., 2006), and indirectly reshape microbial communities through, for example, competition for nutrients (Zak et al., 2003). To date, numerous studies have widely emphasized the significance of microbial communities to the topsoil (0–20 cm), where the biological activity is the greatest (Delgado-Baquerizo et al., 2019; Fierer et al., 2003). However, most plant root systems in desert ecosystems are distributed in the subsoil (deeper than 20 cm) (Wu et al., 2016), which contains approximately 35% of the microbial biomass (Eilers et al., 2012). In particular, the enormous reservoirs of subsoil microbial communities have great potential value for soil structure formation and organic compound degradation (Fierer et al., 2003). Given this knowledge, we can use modern sequencing technology to more accurately assess the response of the microbial community and its potential ecological functions after introducing leguminous plants, which is helpful to improve our grasp of the ecological interactions occurring underground (Fahey et al., 2020).

The Mu Us Sandy Land of Northwest China, a typical temperate continental area, suffers from severe desertification. The loose sandy soil is prone to erosion by wind and forming movable sand dunes (Chen and Tang, 2005). Vegetation reconstruction has been accepted a main measure to combat desertification since the 1950 s (Lu et al., 2018). Among the species planted, a leguminous shrub, *Amorpha fruticosa* is widely used as the pioneer species in the Mu Us Desert with the characteristics of high adverse resistance and developed root systems (Liu et al., 2018; Saifuddin et al., 2015). Several studies have reported that the rhizosphere process and nitrogen fixation potential of legumes, which deepens the understanding of their ecological values (Bi et al., 2020; Xiao et al., 2020). Nevertheless, the spatial distribution in soil nutrients and bacterial communities after the establishment of leguminous shrub in barren sandy land remain largely unexplored.

Herein, we used three sites in different leguminous plant ages (5-year, 8-year, 15-year) and the severe desertification (SD) site to evaluate how the soil nutrients and bacterial communities change during stand development. The vertical spatial distribution of soil nutrients and bacterial communities were determined from soil depth 0–10 cm, 10–20 cm, 20–30 cm, 30–60 cm and 60–100 cm. The objectives of the study were to (1) investigate the variations in nutrients and bacterial communities in soil profiles (0–100 cm) after introducing leguminous shrub; (2) assess the contributions of bacterial community structure and eco- functions to soil nutrients.

2. Materials and methods

2.1. Study area

The study site (38°13'~39°27'N, 109°40'~110°54'E) is located in the Mu Us Desert in the southern Ordos Plateau, northwestern Shenmu County, Shaanxi. This area has a typical temperate continental climate. The average annual temperature at the site is 8.9 °C, with a substantial day-night temperature difference. The average annual rainfall is 440 mm, and the temporal and spatial distribution of precipitation is uneven, occurring mainly from July to September. The local soil type consists chiefly of loose sand with low soil fertility. At present, the main vegetation communities in this area are dominated by xerophytes, including *Artemisia desertorum*, *Caragana korshinskii*, *Salix psammophila*, *Sabina vulgaris* and *Hedysarum scoparium*. *Amorpha fruticosa*, a leguminous shrub, is widely used as the pioneer species to vegetation construction in degraded ecosystems and has been shown to hold tremendous potential for enhancing soil nutrients (Adams et al., 2016; Yang and Liu, 2019).

The space-for-time substitution method is an effective way, used in ecological studies of the process of ecological restoration (Johnson and Miyanishi, 2008; Walker et al., 2010). This method was adopted to examine the impact of legume plantations on soil nutrients and bacterial communities in desert lands. Three locations (in this case, *Amorpha fruticosa* plantation, AF) with different years (5, 8 and 15) were chosen as the experiential sites (10 m × 10 m) along a successional chronosequence with confirmed planting dates. An adjacent severe desertification (SD) site without plant growth was chosen as the control site.

2.2. Sample collection and analysis

In July 2018, soil samples were gathered from each soil depth (0–10, 10–20, 20–30, 30–60, 60–100 cm) with a stainless-steel auger 5 cm in diameter after removing the litter layer. Nine soil cores were collected per plot in an S pattern and blended into one sample, and a total of 60 composite samples were obtained (4 stages \times 5 soil depths \times 3 replications). We cleared obvious plant roots, litter, and rubble from each soil sample, mixed them well and partitioned them into two sections. One section was immediately stored at $-80\text{ }^{\circ}\text{C}$ for genomic DNA analysis, and the other section was dried for the soil property analysis.

The contents of soil nutrients, such as organic carbon (OC), total phosphorus (TP), total nitrogen (TN), available phosphorus (AP), available nitrogen (AN), pH, EC, Zn^{2+} and Ca^{2+} were measured as previously reported (Bao, 2020).

2.3. DNA extraction and high-throughput sequencing

Soil microbial DNA was collected from 0.5 g soil of the sample plots using the Fast DNA spin kit for soil (MP Biomedicals, Santa Ana, CA). The V4-V5 region was targeted using primer pair 515F (GTGCCAGCMGCCGCGGTAA) and 907R (CCGTCAATTCCTTTGAGTTT) for the bacterial 16S rRNA gene. The PCR amplicons were checked with 2% agarose gel electrophoresis and purified with AxyPrep DNA gel extraction kit (AXYGEN, New York, USA). Deep sequencing was obtained on the Illumina MiSeq (300-bp paired-end-reads) platform (Illumina Corporation, San Diego, USA) at Personal Biotechnology Co., Ltd. (Shanghai, PR China). All sequence data were uploaded to the NCBI Sequence Read Archive with the accession number PRJNA765466. The obtained sequences were filtered for quality, and chimeric sequences were checked and eliminated with the USEARCH tool (v5.2.236, <http://www.drive5.com/usearch/>) via Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0, <http://qiime.org/>) tool. The sequences were merged and assigned to operational taxonomic units (OTUs) at a 3% dissimilarity level, by using the UCLUST clustering method (Edgar, 2010). To guarantee the reliability, the OTUs with less than two sequences were discarded, and then the OTUs were classified within the Greengenes database release 13.8 (<http://greengenes.secondgenome.com/>) for bacteria.

2.4. Statistical analysis

The differences in soil nutrients were evaluated by one-way analysis of variance (ANOVA) and the least significant difference (LSD) test ($P < 0.05$) using Origin 2021 (OriginLab Corporation, Northampton, Massachusetts, USA). Circos Analysis about bacterial community composition distribution was performed using “statnet” and “circlize” packages in RStudio environment (version 1.3.1093). All samples in the OTUs abundance matrix are randomly resampled at the lowest sequencing depth level of 90%, so as to correct for diversity difference caused by sequencing depth. Subsequently, QIIME software was used to calculate the bacterial community ACE estimator and Shannon diversity index. Bacterial beta-diversity was characterized by using two axes of a nonmetric multidimensional scaling (NMDS) analysis of Bray–Curtis dissimilarities in the OTU community matrix. Similarity values among the samples were tested via the ANOSIM test. Redundancy analysis (RDA) using Monte Carlo permutation test (999 repetitions) was used to test the relationships among the soil environmental factors, bacterial community composition and putative functional groups of bacterial communities with the “vegan” package in RStudio environment (version 1.3.1093). Detrended correspondence analysis on the data was performed prior to the RDA to confirm that the lengths of gradient match a linear model.

The Functional Annotation of Prokaryotic Taxa (FAPROTAX) is a prospective tool for predicting putative functions in biogeochemical cycling based on 16S rRNA high-throughput sequencing (Louca et al., 2016). Briefly, FAPROTAX database was maps bacterial taxa to ecological functions using the published literature on culturable bacteria (Khan et al., 2019; Wei et al., 2018). The putative functional roles of bacterial communities were conducted by using the FAPROTAX 1.2.1 (complete package), includes a python script (‘collapse_table.py’, 2.7) for analyzing OUT tables classified previously.

The essence of ecosystem is multidimensional, and it was capable of providing multiple functions and services (multifunctionality) simultaneously, not just a single function trait (Delgado-Baquerizo et al., 2016). Accordingly, multiple nutrient cycling is the main drivers to the multifunctionality of ecosystem (Quero et al., 2013). Five measured nutrients were selected to quantify the multiple nutrient cycling, comprising organic carbon, total nitrogen, available nitrogen, total phosphorus and available phosphorus. We normalized and standardized the five nutrient properties by Z score in all samples. Subsequently, we averaged five ecosystem functions to obtain an index, which is defined as multiple nutrient cycling index (hereinafter called the N-index) following Wang et al., 2021. The index provides a perspicuous standard for different communities to maintain ecosystem multifunctionality (Jiao et al., 2018; Soliveres et al., 2016). Classification random forest (RF) analysis was applied to determine the predominant edaphic predictors of soil multiple nutrients cycling (Jiao et al., 2018; Wang et al., 2021). Microbial diversity (alpha- and beta- diversity) indices, the dominant bacterial phyla and their putative ecological functions served as predictors for the soil multiple nutrient cycling index and five measure nutrient properties in the RF analysis. These models were established with the “randomForest” package in RStudio environment (version 1.3.1093). Significance of each predictor to the selected variables was estimated with the “rfPermute” package. The lm function in the “relaimpo” package was adapted to evaluate the RF analysis outcome.

3. Results

3.1. Impact of legume introduction on soil properties

After the establishment of leguminous plantations, the soil nutrients varied in certain patterns along the chronosequence (Fig. 1).

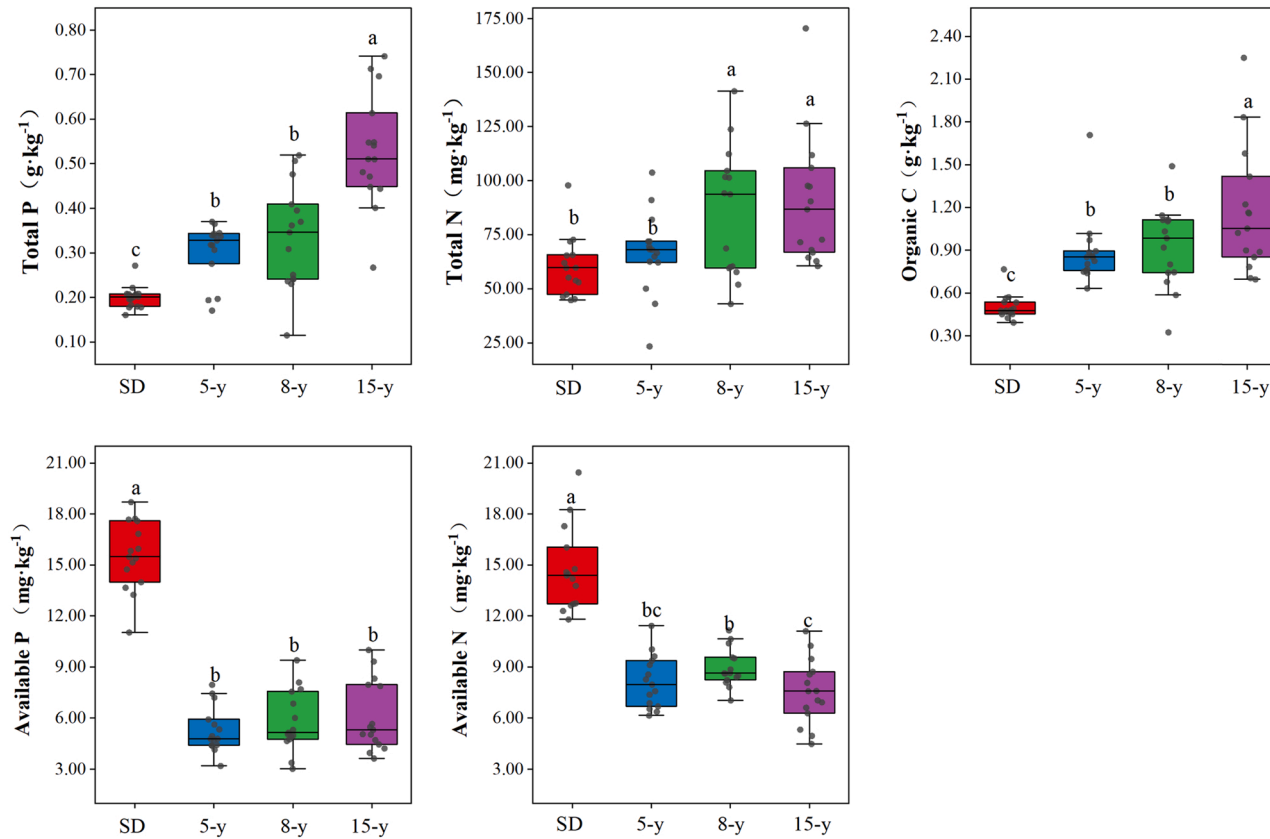


Fig. 1. Variation in soil nutrients across stand ages. Significant differences among stand ages were based on a one-way ANOVA followed by an LSD test. Different lowercase letters indicate significant differences at the 0.05 level ($P < 0.05$). SD: severe desertification; y: year; Total P: total phosphorus; Total N: total nitrogen; Organic C: organic carbon.

The total phosphorus (TP), total nitrogen (TN) and organic carbon (OC) contents significantly increased with increasing soil age, while both the available phosphorus (AP) and available nitrogen (AN) contents significantly decreased.

For the soil profiles (0–100 cm), we estimated the differences in all soil nutrients between the SD and AF soils (Table S1, Fig. S1). As soil depth increased, the TN and AN contents dramatically decreased in both soils. However, the TP and AP contents did not change among the different soil layers. The OC content did not change significantly among soil layers in SD, but it began to decrease gradually after establishing legume shrub in degraded sandy land.

3.2. Dynamic changes in soil bacterial communities

Across all samples, we obtained a total of 810180 high-quality sequences, which were identified into 9451 OTUs. These OTUs were divided to 28 phyla, 80 classes, 109 orders, 180 families, and 274 genera. The dominant phylum (> 1% of total community) was Actinobacteria (48.76% on average), followed by Proteobacteria (30.90%), Acidobacteria (6.00%), Chloroflexi (5.59%), Gemmatimonadetes (2.86%), and Firmicutes (1.71%) (Fig. 2). The alpha-diversity levels of soil bacteria were higher in the AF soils than in the SD soil (Fig. 3a, b). In the SD soil, alpha-diversity decreased with increasing soil depth, but there was no change in alpha-diversity with depth in the AF soils (Fig. S2). Least-squares linear regression analysis confirmed the significance of these trends. The non-metric multidimensional scaling (NMDS) analysis showed that the soil samples of SD and different plantation ages formed distinct clusters in the ordination space, with notable differences according to ANOSIM (Fig. 3c). Additionally, we observed significant difference in bacterial communities among soil depths. Furthermore, we assessed the differences in beta-diversity among stand ages according to the Bray-Curtis distance (Fig. 3d). The bacterial communities after introducing legumes exhibited lower beta-diversity, indicating their higher similarity.

We compared the vertical spatial variation in bacterial communities with increasing soil depth between the SD and AF soils

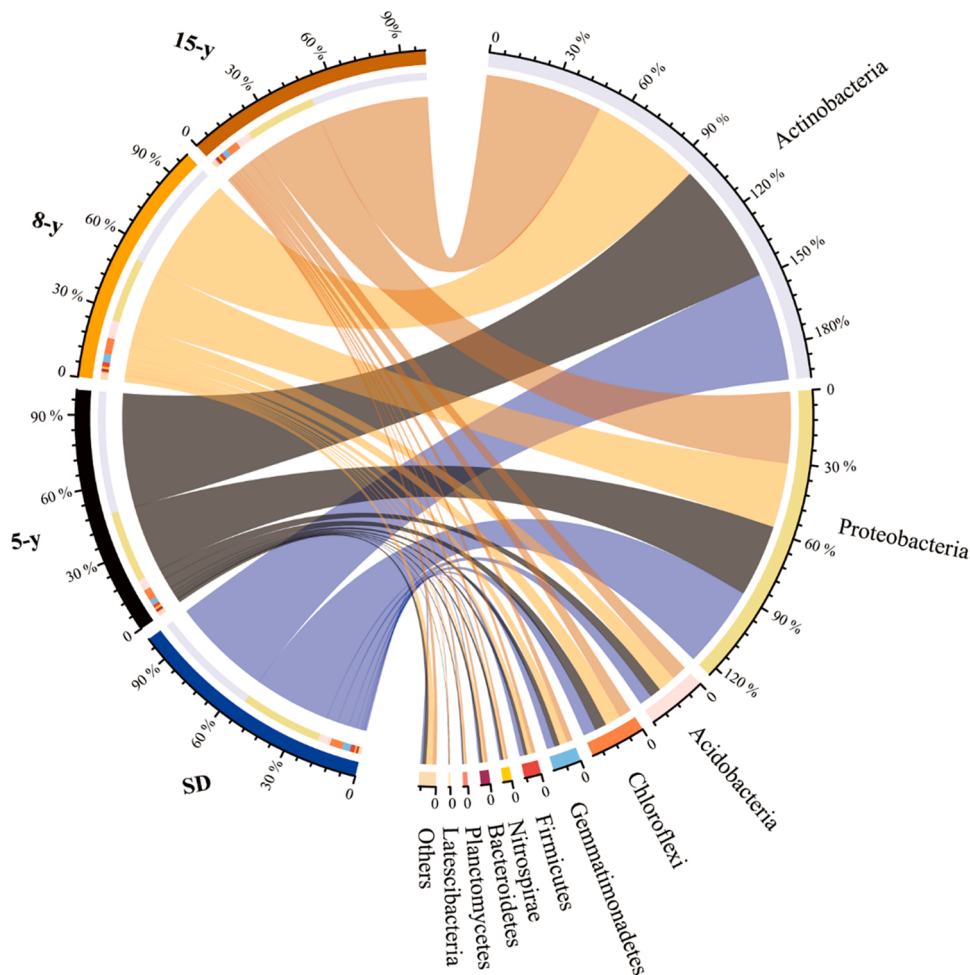


Fig. 2. Circos plots showing the bacterial community composition across stand ages. Categories of bacteria at the phylum level are shown along the right side of plot, and the length of bars on the outer-ring represents the percentage. Colors of the bars correspond to each site. The samples across stand ages are on the left of this ring. SD: severe desertification; y: year.

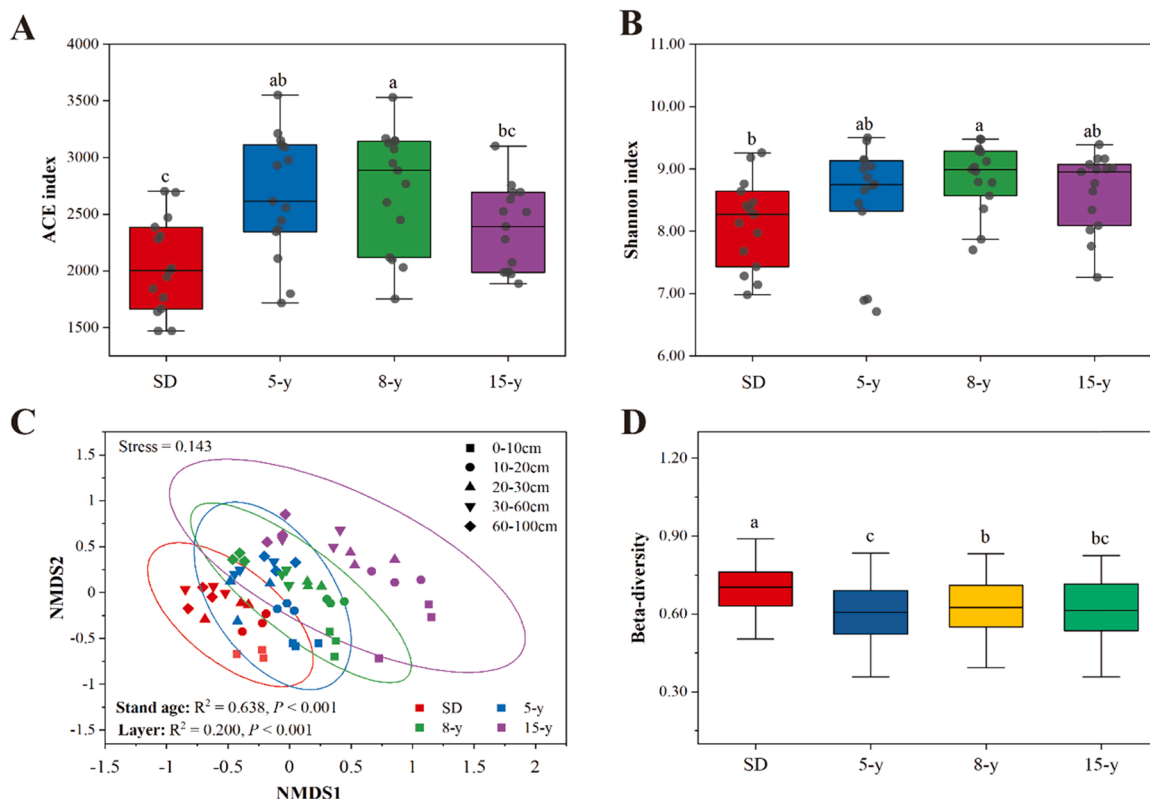


Fig. 3. General patterns of soil microbial diversity during stand development. (a, b) Variation in the alpha-diversity indices of bacteria across stand ages. Significant differences were based on a one-way ANOVA followed by an LSD test. (c) NMDS showed the variation in the microbial community for soil bacteria. 95% confidence ellipses were shown around the samples grouped based on stand ages. Similarity values among the samples of stand ages and soil depth were examined via ANOSIM test ($P < 0.001$). (d) Differences in beta-diversity among stand ages were estimated based on a Bray-Curtis distance matrix. Significant differences among stand ages were based on a one-way ANOVA followed by an LSD test. Different lowercase letters indicate significant difference at the 0.05 level ($P < 0.05$). SD: severe desertification; y: year.

(Fig. S3). The vertical spatial decay relationship (VDR) slope of the soil bacterial community was the steepest in the 15-year site, despite the similarity between the communities in the 5-year and 8-year sites and all higher than those in SD soil. These results indicate that legume introduction in the degraded sandy land decreased the vertical spatial variation in bacterial communities.

3.3. Variation in putative ecological functions of the soil bacterial communities

To better understand the functional ecology of the bacterial community in this environment, we observed the functional information inferred and assembled from 16S rRNA gene sequences by the FAPROTAX database (Fig. 4). A total of 61 function types were associated with the bacterial communities after legume introduction. Among them, we found no P-related functions, so we concentrated only on C- and N-related processes. We noticed that legume plantations extremely increased nitrogen fixation and denitrification processes. The predicted aerobic chemoheterotrophic process decreased from the SD site to 8-y site, and then, increased from 8-y site to 15-y site, hydrocarbon degradation process exhibited the opposite trend. Interestingly, the nitrification process fluctuated.

Among the different soil layers, the predicted ecological functions had no obvious change in the SD soil (Fig. S4). After legume introduction, nitrogen fixation and aerobic chemoheterotrophic processes significantly decreased with soil depth, while the nitrification process showed a clear increase.

3.4. Relationship between soil properties and bacterial communities

Redundancy analysis (RDA) was used to confirm the effects of legume introduction on soil bacterial communities and bacterial putative functions within the context of the six measured soil environmental factors. The results showed that pH ($P < 0.001$) was the dominant factor on the bacterial community composition, while Ca^{2+} ($P < 0.001$) had the highest environmental effect on the bacterial ecological functions. The RDA derived from the relative abundance of the dominant bacterial phyla (Fig. 5a) showed that Actinobacteria was positively correlated with environmental factors except pH. Acidobacteria, Chloroflexi and Gemmatimonadetes were all positively correlated with N:P and negatively correlated with EC, Ca^{2+} , C: N and pH. Moreover, pH had a significant positive

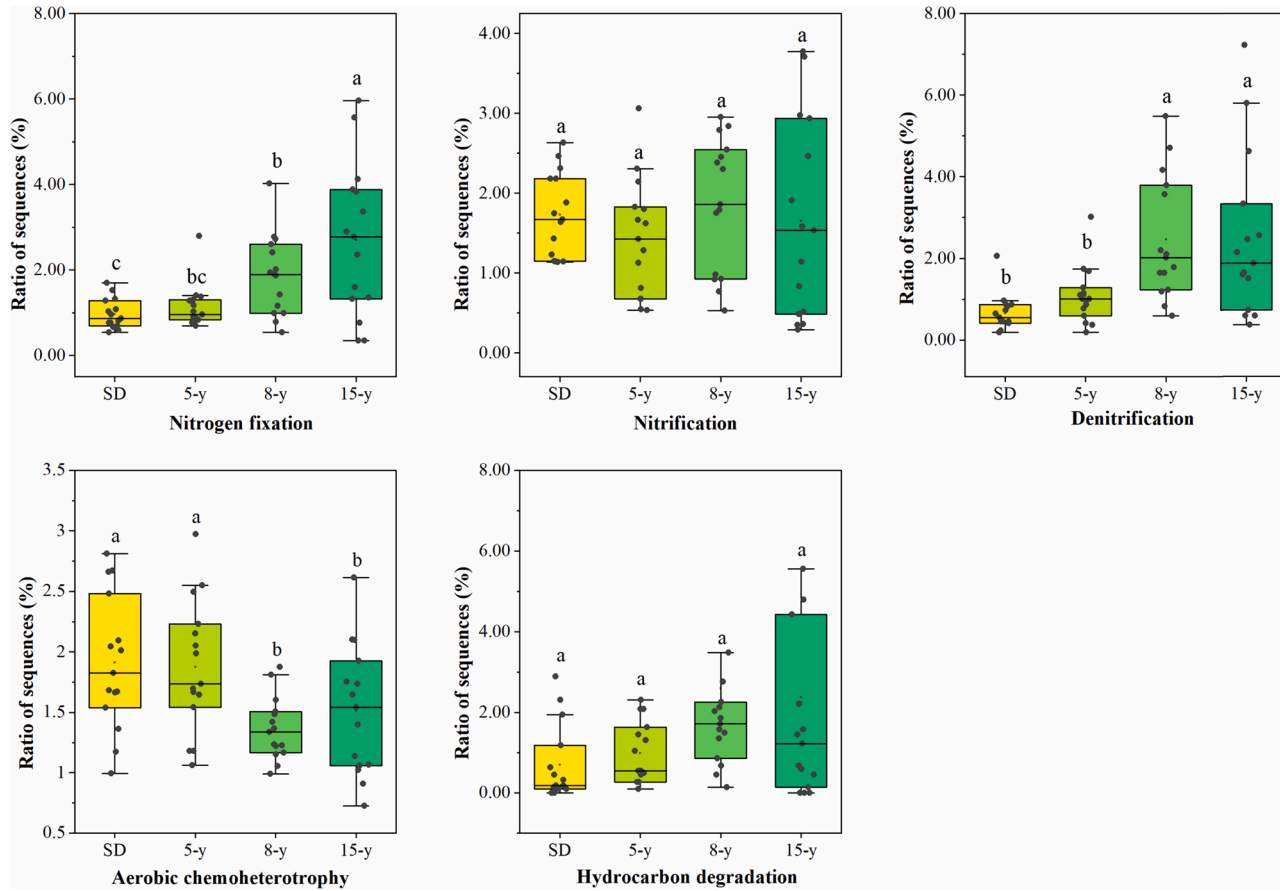


Fig. 4. Putative ecological functions during stand development are shown by the FAPROTAX database. Significant differences between stand ages were based on a one-way ANOVA followed by an LSD test. Different lowercase letters indicate significant differences at the 0.05 level ($P < 0.05$). SD: severe desertification; y: year.

effect on Firmicutes, while N: P had the opposite effect. The RDA derived from the putative functions (Fig. 5b) showed that the measured ecological functions were closely associated with soil properties, including pH and N: P.

3.5. Potential drivers of soil nutrients in afforested ecosystems

To untangle the potential drivers of changes in soil nutrients after the establishment of leguminous shrub, we determined the major microbe-related predictive factors of the soil multi-nutrient cycling index (N-index) via random forest (RF) analysis (Fig. 6). We found that the most significant variable for predicting the N-index was bacterial beta- diversity. Additionally, we assessed the ecological contributions of dominant microbial phyla and their putative functions to soil nutrient levels via RF analysis (Fig. 6). Obviously, not all microbial phyla had an equal influence on the nutrient variables. Proteobacteria and Firmicutes, for instance, were the most significant variables for predicting changes in several soil nutrients, including AP, AN, OC, and TN, which indicates that these phyla are critical for maintaining soil nutrient levels. The remaining critical variables for predicting soil nutrients were the Actinobacteria and Chloroflexi, for AP, OC, TN and AN; the Gemmatimonadetes, for AP, AN, OC and TP. In addition, the putative bacterial functions were also vital predictors, including aerobic chemoheterotrophy, for AN; nitrification, for OC and TN.

4. Discussion

4.1. Variation in soil properties after introducing leguminous shrubs in desert ecosystems

Divergent variation occurred in soil nutrient levels after legume introduction in desert ecosystem. The variations included increases in soil OC, TN and TP and decreases in AN and AP. Soil OC has a long retention time, from less than a year to several centuries or more, and responds differently to land-use change, especially after a transformation from sandy land to coppice (Deng et al., 2016; Hong et al., 2020). The introduction of a legume species broke the balance of soil OC and changed the way of soil C input, which is probably traceable in aboveground biomass accumulation (Yu et al., 2021; Zhu et al., 2017). Establishing legume plantations on barren sandy land reversed the lower status of the OC content (Fig. 1 and S1). However, this situation seemed to be limited mainly to the topsoil. The OC in the subsoils was restored much more slowly than that in the topsoil during stand development (Table S1), so it may take many years to replenish the soil OC lost from subsoils (Balesdent et al., 2018). Whether individually or in combination, soil N and P are the key limiting factors for primary productivity in most desert ecosystems (Wang et al., 2020). After the legumes established in the desert, TN and TP were reserved in the plant tissues, and their recycling through litter increased, in contrast, soil AN and AP were reduced (Fig. 1 and S1). The reason may be showed that establishing vegetation in nutrient-poor soils may meet some of the growth demand of fast-growing shrubs by allowing them to absorb available nutrients from the subsoil (Wu et al., 2009), this represents a potential nutrient-acquisition strategy that has been mostly been neglected in research on vegetation establishment.

In addition, most legumes have higher N contents in their tissues (seeds, stems, and leaves) than non-legumes (Barron et al., 2011). Not all legumes have the potential for biological N fixation; most legume species are facultative N-fixers, which means that they fix N only under specific environmental conditions (Heath and Tiffin, 2007; Menge et al., 2009). Thus, a critical element to consider, is that legumes play a dual role as both soil nutrients consumers and providers. In our study, *Amorpha fruticosa* may have played a consumer

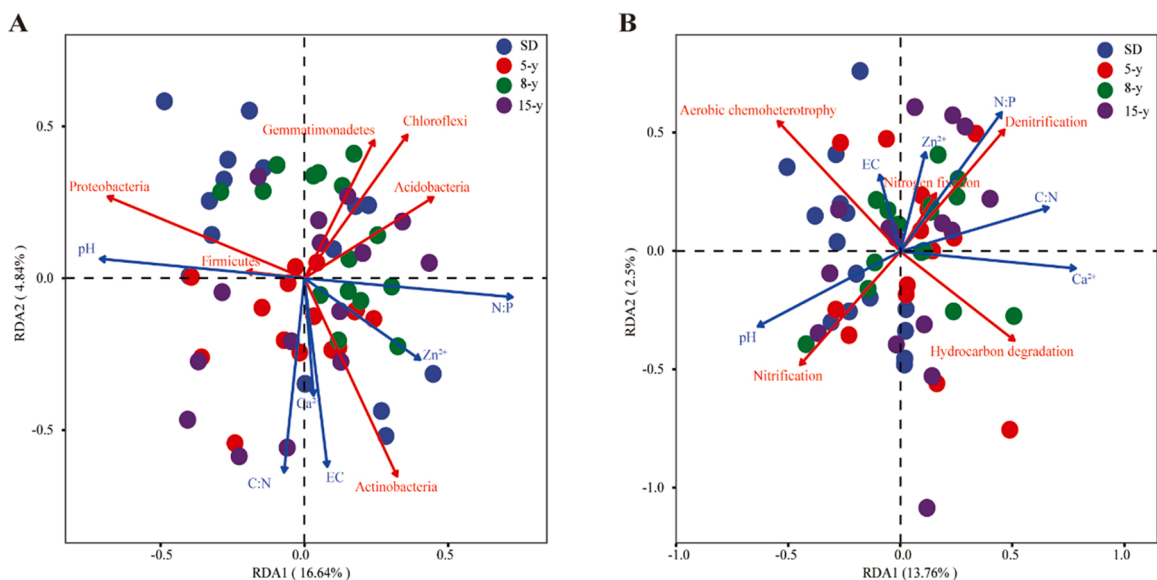


Fig. 5. Correlations of selected soil environmental factors and the relative abundance of bacterial phyla (a) or putative functional groups (b), as determined by redundancy analysis (RDA). SD: severe desertification; y: year.

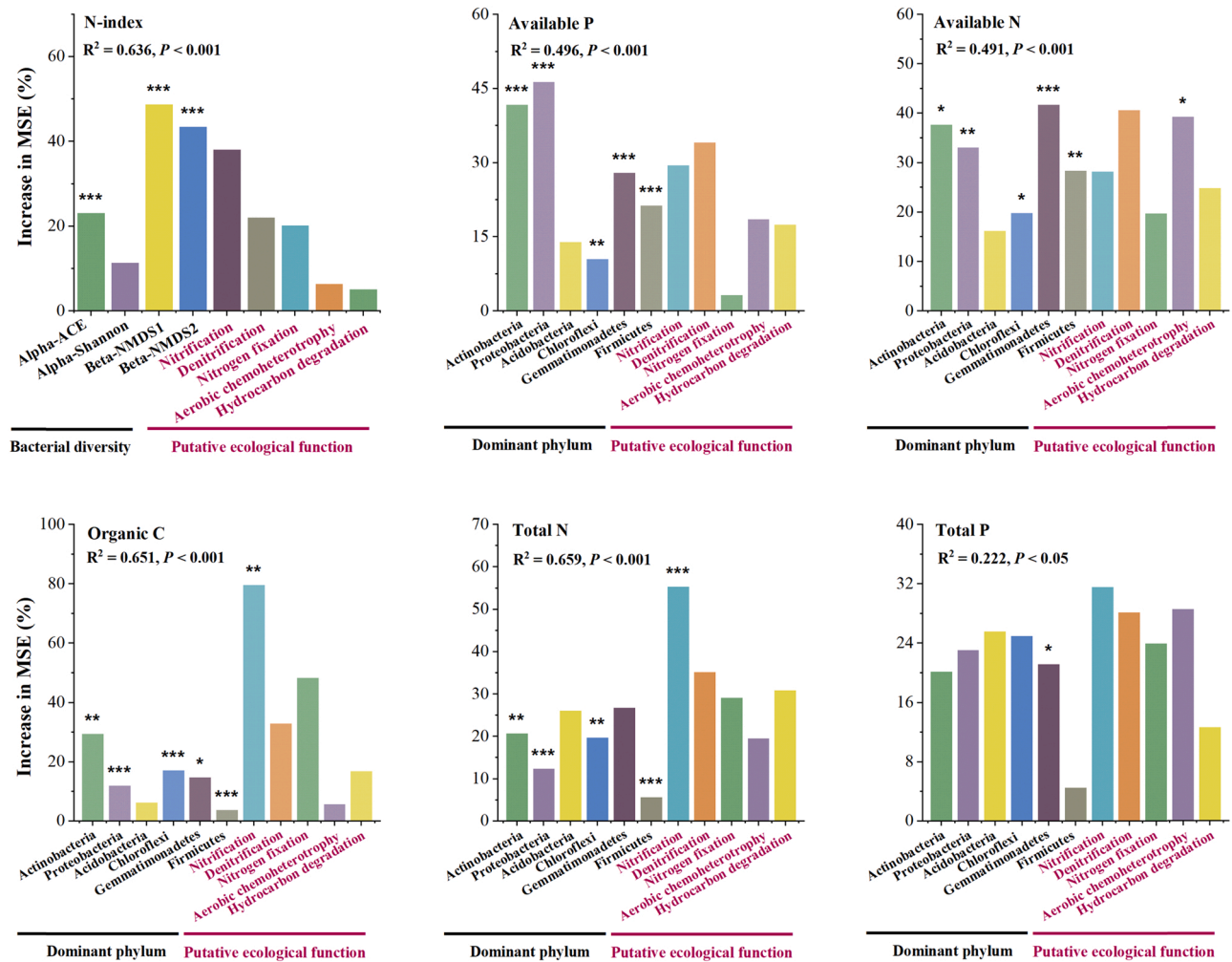


Fig. 6. Potential drivers of variation of soil nutrients during stand development. Random forest (RF) mean predictor importance (percentage increase in the MSE) of microbial diversity indices and putative functions as drivers for the N- index; the dominant phyla (> 1% of total community) and putative ecological functions as drivers for soil nutrients in the whole soil profiles (0–100 cm). The accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). The percentage increases in the MSE of the variables were used to estimate the importance of these predictors, and higher MSE% values indicate more important predictors. Significance levels are as follows: *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$. N- index, soil multi-nutrient cycling index; Total P: total phosphorus; Total N: total nitrogen; Organic C: organic carbon. MSE: mean squared error.

role in the degraded ecosystem; the plants first met their own needs with the available nutrient resources and then competed for soil resources with other plants and soil microorganisms (Li et al., 2021). More studies are needed to confirm this phenomenon. Simultaneously, N₂-fixing species have plentiful and strengthened roots (Schenk and Jackson, 2002), enabling them to utilize P situated below the root system. The roots of N₂-fixing species generally maintain higher phosphatase activity, which allows them to absorb more of the P in organic matter (Gillespie and Pope, 1990). However, in the present study, we observed no obvious change trend in the P content with soil depth in the soil profiles (Fig. S1). This may be because in addition to biological factors, abiotic factors such as precipitation, temperature, soil water content and pH also play significant roles in influencing desert soil properties.

4.2. Variation in soil microbiomes after introducing leguminous shrubs in desert ecosystems

Generally, the soil microbial community composition and structure reflect soil responses to frequent disturbances. Our results showed that legume-introduction increased the biodiversity of soil bacteria and reshaped their structure, highlighting the particular significance of vegetation cover. Stronger competitors may become dominant for long periods as the ecosystem matures, leading to a decline in bacterial species (i.e., ACE index) from 5- y to 15- y site (Fig. S2). Our results meet this assumption, as the alpha-diversity showed a downward trend as a whole, this is in line with the results of Liu et al. (2019) from soil bacterial communities in coniferous *Pinus tabulaeformis* Carr. forests.

The NMDS analysis clearly separated the bacterial community composition in coppice stands of differently aged legumes (Fig. 3c, d), indicating that establishing the leguminous plantations on nutrient-poor sandy land significantly impacts bacterial community development. Further analysis revealed that the vertical spatial variation in soil bacterial communities decreased during stand development (Fig. S3). With plant growth, the vegetation community can increase the available niches by homogenizing local nutrient resources and the microscales soil abiotic environment, resulting in less vertical spatial heterogeneity throughout the soil profiles (Leibold and McPeck, 2006; Shi et al., 2015). Furthermore, the dominant exhibited distinct responses due to the changes of soil nutrients. At the phylum level, we found that copiotrophic bacteria (i.e., Actinobacteria, and Proteobacteria) responded rapidly to legume introduction. Conversely, some oligotrophic bacteria, such as Acidobacteria declined. These observed changes were further confirmed by the findings of other studies (Goldfarb et al., 2011), suggesting that the utilization of carbon sources by microorganisms differs. Imported litter is utilized mainly by fast-growing copiotrophs and is exhausted before oligotrophs can assimilate it (Yu et al., 2020).

Interestingly, legume introduction notably influenced several N cycling processes (Table S3, Fig. 4), including nitrogen fixation, nitrification and denitrification. Legumes provide additional C resources for microorganisms, which increase the microbial N demand for growth owing to the change in the stoichiometric C and N ratios (Zinn et al., 2018). Therefore, the nitrogen fixation process was enhanced under legume colonization to provide more resources to the soil microorganisms. The nitrification process did not show an obvious upward or downward trend, but the denitrification process increased sharply. This may have been to the large amount of decomposable organic matter input to the soil by the plants, which created anaerobic soil conditions, and led to a strong denitrification process (Senga et al., 2015). To leverage plant C and N requirements, C transformation functions were promoted, including hydrocarbon degradation and aerobic chemoheterotrophic processes. The FAPROTAX database proposed some fascinating assumptions concerning the putative functional shifts in the microbial community (Fahey et al., 2020; Louca et al., 2016). However, we emphasize that FAPROTAX analysis is only a predictive tool, and the database may be limited to including information about culturable microorganisms with definite functions (Sansupa et al., 2021). Therefore, further studies are needed to confirm the variation of microbial functions through metagenomics and transcriptomics.

4.3. Main predictors of soil nutrients

The complex changes in soil nutrients can be partly interpreted by the soil microbiomes themselves, whose symbiosis modes and complex interactions drive the transformation and cycling of soil nutrients (Delgado-Baquerizo et al., 2016; Falkowski et al., 2008; Li et al., 2019). A previous study on the impacts of soil communities with different composition and diversity on eight ecosystem functions demonstrated that there was a positive relationship between the changes in ecosystem multifunctionality and soil biodiversity indicators (Wagg et al., 2014). Hence, in our research, we hypothesized that the microbial community would be involved in the cycling of multi-nutrient in soil. As expected, bacterial beta-diversity played a leading role in the cycling of soil multiple nutrients in underground soil after introducing legumes (Fig. 6).

Bacteria have been demonstrated to participate in various global biogeochemical cycling processes, such as carbon and hydrogen metabolism, nitrogen cycling and phosphorus cycling. In the present study, both copiotrophic (Actinobacteria, Proteobacteria and Firmicutes) and oligotrophic (Chloroflexi and Gemmatimonadetes) microbial groups played important roles in driving changes in nutrient levels (Fig. 6). It has been reported that copiotrophs are often associated with the degradation of recalcitrant compounds and are thus considered to be ecologically important in the turnover of organic matter in soil (Goldfarb et al., 2011). In contrast, Chloroflexi in soil are considered ubiquitous and physiologically active. The phylogenetic diversity and relative abundance of Chloroflexi in diverse habitats suggests their vital role in driving biogeochemical processes and diverse metabolic functions (Cole et al., 2013). Some members of Chloroflexi are aerobic thermophiles, that grow well at high temperatures, and promote organic matter decomposition (Yamada et al., 2005). Intriguingly, Only Gemmatimonadetes could predict TP in the present study. A previous study reported that Gemmatimonadetes are specifically adapted to arid environments (DeBruyn et al., 2011). In brief, our results revealed that bacterial taxa (at the phylum level) and diversity indices were of great importance for soil nutrients, this was particularly notable after the establishment of leguminous plantations in degraded ecosystem, where ecological function maintenance should be explicitly considered

in sustainability strategies aiming to increase soil available nutrient levels in order to better provide vital ecological services.

5. Conclusions

Overall, our study revealed the effects of conversion of severe desertification soil to *Amorpha fruticosa* coppice stands on soil habitats. The introduction of legumes had a negative impact on soil nutrients such as AP and AN. Soil bacterial alpha-diversity increased, whereas beta-diversity decreased after legume introduction. Legume introduction significantly influenced C and N cycling processes. Additionally, bacterial beta-diversity, specific microbial phyla (i.e., Proteobacteria and Firmicutes) and nitrification process were the best explanatory variables for the vertical spatial soil variation in nutrients. The response characteristics of soil nutrients and microorganisms to legume introduction could be significant for thoroughly describing the underground soil ecology.

6. Environment implications and significance

This study contributes to a quantitative understanding of the impacts on nutrients and bacterial communities in the soil profiles after the establishment of leguminous shrub in degraded ecosystems. Meanwhile, we determined the fundamental microorganism-related predictive factors for soil nutrients. Our results clearly indicated that vegetation construction strategies for deserts result in changes in soil nutrients and in the abundance, diversity and putative functions of bacteria. However, it should be noted that legume introduction had a negative effect on available nutrients. Why N-fixing legumes did not work a substantial role in these nitrogen-limited ecosystems despite the powerful biological advantage that they expected to provide? We deduced that, when legumes are alive, most of them play major roles as soil nutrient consumers; this role should be taken into consideration when legumes are used to restore degraded ecosystems. This information will be valuable to guide the selection of suitable habitats for legume planting in the future. Additionally, more sophisticated analyses within the selected sites are also needed, such as the link between available nutrient fixation and utilization efficiency in the field over a longer period of time.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.gecco.2021.e01841](https://doi.org/10.1016/j.gecco.2021.e01841).

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