Changes in rhizosphere bacterial and fungal community composition with vegetation restoration in planted forests

Article in Land Degradation and Development - January 2019
DOI: 10.1002/ldr.3275

7 authors, including:

Tr Lock
University of Missouri
6 PUBLICATIONS 12 CITATIONS

R.L. Kallenbach
University of Missouri
112 PUBLICATIONS 976 CITATIONS

All content following this page was uploaded by R.L. Kallenbach on 06 March 2019.
The user has requested enhancement of the downloaded file.
Changes in rhizosphere bacterial and fungal community composition with vegetation restoration in planted forests

Gui-yao Liu1,3,4 | Li-li Chen5 | Xin-rong Shi1,2 | Zhi-you Yuan1,2 | Lois Y. Yuan6 | T. Ryan Lock7 | Robert L. Kallenbach7

1 State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, CAS and MWR, Yangling, Shaanxi 712100, PR China
2 Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi 712100, PR China
3 Poyang Lake Eco-economy Research Center, Jiujiang University, Jiujiang 332005, PR China
4 University of Chinese Academy of Sciences, Beijing 100049, PR China
5 Yuxi Research Center for Eco-Environmental Sciences on Plateau Lakes, Yuxi Normal University, Yuxi, Yunnan 653100, PR China
6 University of Toronto, St George, Human Biology, 27 Kings College Circle, Toronto, ON M5S 1A1, Canada
7 University of Missouri, Division of Plant Sciences, 108 Waters Hall, Columbia, MO 65211, USA

Correspondence
Zhi-you Yuan, State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Yangling, Shaanxi 712100, PR China.
Email: zyyuan@ms.iswc.ac.cn

Abstract
Soil microbial communities affect nutrient cycling and ecosystem functioning. However, the variations in microbial diversity and community composition within degraded landscapes remain unclear. Using high-throughput sequencing of bacterial 16S ribosomal RNA genes and internal transcribed spacer fungal sequences, we investigated the rhizosphere microbial diversity and community of coniferous Pinus tabulaeformis Carr. forests in degraded lands across a chronosequence that spanned over 60 years (10, 25, 40, and 60 years since restoration, four forest stands). We found significant differences in soil bacterial and fungal communities among stand ages. Actinobacteria, Proteobacteria, and Acidobacteria dominated the rhizosphere, whereas Basidiomycota, Ascomycota, and Zygomycota prevailed as fungal components. With stand development, bacterial diversity decreased, but fungal diversity increased. Nonmetric multidimensional scaling analysis separated bacterial community clusters well by stands. Fungal community clusters of 25- and 60-year-old stands overlapped. The dominant bacteria Acidobacteria showed the highest relative abundance at the 40-year-old stands. Soil microbial communities correlated significantly with the macro-nutrients (soil organic carbon, total nitrogen, and total phosphorous). Additionally, the relative abundance of Acidobacteria at the phylum level correlated positively with soil total phosphorous; Deltaproteobacteria at the class level correlated positively with soil organic carbon and total nitrogen. Thus, restoring vegetation in degraded temperate forests enhanced some macronutrients and influenced microbial communities. Our results revealed that restoring vegetation in degraded lands decreased the diversity of bacterial communities over time. In contrast, the soil fungal diversity increased after restoration, and fungal communities in the 25- and 60-year-old forest stands overlapped on degraded soils.

KEYWORDS
16S ribosomal RNA and internal transcribed spacer sequences, ecological restoration, Illumina HiSeq sequencing, microbial communities, microbial diversity
1 | INTRODUCTION

Climatic and anthropogenic disturbances over time degrade lands of the Loess Plateau, China. Restoring these degraded landscapes requires a better understanding of the degradation mechanisms. Soil microorganisms remain key regulators in biogeochemical cycling and display high structural, genetic, and functional diversity (Bouffaud et al., 2012). Soil physicochemical characteristics govern the diversity and community structure of microorganisms (Albornoz et al., 2016) by affecting biological and ecological processes in the complicated plant rhizosphere. In general, soil microbial community structure and composition tend to reflect soil responses to disturbances (Tian et al., 2017).

Microbiologists that study microbial communities to assess ecosystem sustainability report limited evidence of the microbial community structure and composition during ecosystem restoration (Jiao, Chen, et al., 2016; Jiao, Liu, et al., 2016; Jiao et al., 2017; Martinez-Garcia, Richardson, Tylianakis, Peltzer, & Dickie, 2015). These ecosystem relationships receive more attention in plant studies. Various root exudates (i.e., compounds such as low-molecular weight sugar, amino acid, organic acid, growth regulators, and inhibitory compounds) function as feedback mechanisms and signaling compounds to impact microorganisms in the rhizosphere (Mestre, Rosa, Safar, Libkind, & Fontenla, 2011; Pires et al., 2012; Shi et al., 2012). As a result, rhizosphere-dwelling soil microorganisms exhibit a more complicated community structure and provide more complicated feedback to plants than those in bulk soil (Fan et al., 2017; Massaccesi et al., 2015).

In many parts of the world, land managers restored soil fertility and ecological functions with trees, shrubs, and grasses after exploitation farming (Li et al., 2018; Parrotta, 1992; Stone & Gibson, 1975). For several decades, revegetation occurred in China’s ecologically fragile Loess Plateau. Studies of these efforts primarily focused on soil physical and chemical properties, water conservation (Zeng et al., 2014), ecological stoichiometry characteristics (Jiao, Wen, An, & Yuan, 2013), element reserves (Ma, Teng, & Shangguan, 2014), ectomycorrhizal fungal communities (Zhang, Tang, Chen, & Zheng, 2010), and understory plant species diversity (Chen & Cao, 2014). Information about soil microbial community structure and/or composition in the rhizosphere of forests, especially along a successional gradient or in areas of ecosystem restoration, remains scarce.

*Pinus tabulaeformis*, a predominant pioneer tree species in afforestation areas of northwestern China (Yuan & Yue, 2012; Zhang, Zheng, & Shangguan, 2006), adapts to cold, drought, barren soil, and wind because of its ectomycorrhizal symbionts (Bahram, Koljalg, Kohout, Mirshahvaladi, & Tedersoo, 2013). After revegetation on this degraded site, root exudates accumulated and soil nutrient accumulation and consumption occurred. Gros, Monrozier, Bartoli, Chotte, and Faivre (2004) postulated that this process correlated with the accumulation of root exudates. The soil nutrient change with increased canopy closure inevitably influences microbial diversity and community composition within restored vegetation (Figure 1). Here, we analyzed the changes in bacterial and fungal community structure in relation to revegetated stand age and environmental factors. We obtained the soil samples of four different forest stands (10, 25, 40, and 60 years old) and used 16S ribosomal RNA (rRNA) and internal transcribed spacer (ITS) sequences for bacterial and fungal species, respectively. We aimed to (a) characterize the changes in bacterial and fungal diversity along a successional gradient after revegetation and (b) identify the changes in microbial community structure and their relationships with soil physicochemical properties. We hypothesized that microbial diversity increased along the successional gradient, but bacterial and fungal communities would change in different ways. In addition, we expected bacterial and fungal community structure to change with altered soil nutrients.

2 | MATERIALS AND METHODS

2.1 | Study area description and sampling protocol

The study sites were located south of Yan’An City in Shaanxi Province, China (109°16’25”-109°29’39” E, 36°9’51”-36°29’47”N). This hilly, eroded region lies in the centre of the Loess Plateau at an altitude of...
of 1.074 to 1.173 m. The area comprises a complex but fragile semi-arid forest and grass ecosystem. Mean annual precipitation is 560 mm and mean annual temperature is 9°C. The dry season includes winter, spring, and early summer, and most precipitation occurs in July and August (Chen et al., 2016). Authorities classify soils as calcic cambisols (Jiao et al., 2013).

We used space-for-time substitution (chronosequence) to evaluate the effect of revegetation on soil microbial diversity and community composition in the rhizosphere of *P. tabuliformis*. Chronosequence previously proved effective for assessing the changes in soil physico-chemical properties (An, Huang, & Zheng, 2009; Jangid, Whitman, Conndon, Turner, & Williams, 2013; Williams, Jangid, Shanmugam, & Whitman, 2013; Yuan & Chen, 2010; Yuan & Chen, 2012a) and microbial community composition (Albornoz et al., 2016; Li et al., 2018; Zhang, Liu, Xue, & Wang, 2015) in revegetation. We selected four different stands (10, 25, 40, and 60 years old) based on archives of the local forest service. The selected sites had similar elevations, slopes, and aspects. Cultivated crops grew here for many years before trees were planted. In July 2015, we established three 10 m × 10 m plots as replicates at all sites, yielding a total of 12 plots. The plots were more than 20 m away from the forest boundary and at least 500 m separated each plot.

We selected five individual trees at random in each plot to obtain rhizosphere soil samples. After removing the litter layer, soil cores were collected to a depth of 20 cm from the top soil layer. We extracted soil cores 50 cm from the tree stems using a soil corer with a 9-cm in diameter. For each tree, four soil cores were collected around the tree trunks, yielding a total of 20 soil cores from each plot. We mixed these cores immediately to obtain a composite sample. After gently crushing the whole soil and root system, we obtained the loosely held soil, (i.e., the bulk soil) by shaking. The remaining tightly held soil, (i.e., the rhizosphere soil) was also separated. We obtained rhizosphere soil samples by gently shaking the roots and catching the soil in polyethylene bags (Kidd, Prieto-Fernandez, Monterroso, & Acea, 2008). We then removed the visible roots, stones, and other foreign matter. Soil samples were immediately transported in an ice box to the laboratory. We stored approximately 10 g of each soil sample at −80°C for DNA analysis and air dried approximately 500 g to determine soil characteristics.

### 2.2 Soil characteristics measurements

We measured soil organic carbon concentration via the potassium dichromate oxidation method (Kalembasa & Jenkinson, 1973). We determined soil total nitrogen by using the semimicro Kjeldahl method and soil total phosphorus by using the molybdenum-antimony antiscophtomometric method following wet digestion with H2SO4 + HClO4 (Parkinson & Allen, 1975). We determined soil available nitrogen using microdiffusion after alkaline hydrolysis and soil available phosphorus using the NaHCO3 extraction method (Bao, 2005). We measured soil pH with a pH electrode in a 1:2.5 soil-water suspension (Bao, 2005). We measured soil total Mg and Ca using microwave digestion and inductively coupled plasma-mass spectrometry (Prodigy7, Leeman, USA), according to the manufacturer’s instructions.

### 2.3 DNA extraction and sequencing data analysis

We extracted total DNA from 0.25-g soil samples using a TIANamp Soil DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). The primers 515F and 907R (Table S1) were designed to amplify the hypervariable V4 and V5 region of the bacterial 16S rRNA gene (Jiao, Liu, et al., 2016). The primers ITS5-1737F and ITS2-2043R (Table S1) were designed to amplify the hypervariable ITS1 region in fungal DNA (Lu et al., 2013). We tagged the primers in every sample with unique barcodes (Table S1). An Illumina HiSeq 2500 platform sequenced the purified amplicons at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China), yielding 250-bp paired-end reads.

Sequences were qualitatively filtered and checked for chimeric reads using the Quantitative Insights Into Microbial Ecology (QIME) workflow (V1.7.0, http://qiime.org/index.html; Bokulich et al., 2013). Sequences with the same barcode were sorted into the same sample, and reads containing unresolved nucleotides or ambiguous bases were removed via the UCHIME algorithm (Haas et al., 2011). Paired-end reads were merged using the FLASH tool (V1.2.7; Magoc & Salzberg, 2011). A total of 619,327 bacterial 16S rRNA sequences (average 51,611 per sample) and 528,968 fungal ITS sequences (average 44,081 per sample) from the 12 rhizosphere soil samples were sequenced. The remaining high-quality sequences were clustered using UPARSE software to generate operational taxonomic units (OTUs) at 97% similarity level (Edgar, 2013). We used four indices to estimate the community diversity: (a) Shannon indices, expressing the relative evenness component and sensitivity to changes in the importance of the rare species in the sample, (b) Simpson indices, expressing the dominance component and sensitivity to changes in the most abundant species in the sample (Whittaker, 1972), (c) abundance-based coverage estimator (ACE), a coverage estimator of species richness, and (d) the Chao1, an estimator of true species diversity (Zitanova & Popova, 2018). We obtained rarefaction curves using QIIME (Version 1.7.0) and displayed using R software (Version 3.3.1). For each representative sequence, we used the SILVA (https://www.arb-silva.de/; Quast et al., 2013) and UNITE (Abarenkov et al., 2010) databases to annotate the taxonomic information of bacteria and fungi, respectively. We conducted multiple sequence alignment with MUSCLE software (Edgar, 2004) to determine the phylogenetic relationships between OTUs and the dominant species in all samples.

### 2.4 Data analysis

We used one-way analysis of variance to evaluate the significant effects of stand age on soil properties and microbial diversity. We considered p values <0.05 to represent significant differences. Pearson correlation analysis analyzed the relationships between soil properties and the relative abundances of microbes. We calculated the longest gradient (LG) before running a detrended correspondence analysis.
We used redundancy analysis (RDA) for bacterial communities (LG < 3.0) and canonical correspondence analysis (CCA, LG > 3) for fungal communities (Guo et al., 2015) to identify the relationship between microbial structure and variables of soil properties. Finally, we used the envfit analysis to test for significance between microbial community compositions and soil characteristics. RDA, CCA, and envfit analysis were conducted using R software (v3.3.1).

3 | RESULTS

3.1 | Soil characteristics of the rhizosphere

Forty-year-old stands contained more rhizosphere soil organic carbon than 10-, 25-, and 60-year-old stands. They also showed greater soil total nitrogen and phosphorus (1.17 and 1.10 g kg⁻¹, respectively) than the 10- (0.77 and 0.73 g kg⁻¹, respectively) and 60-year-old (0.59 and 0.84 g kg⁻¹, respectively) stands (Table 1). The 10-year-old stands contained more total Mg than the other stands.

3.2 | Bacterial and fungal alpha diversity with stand development

The rarefaction curves at 97% similarity tended to approach saturation with increasing sequence number and indicated that the majority of the microbial community was represented in the obtained sequences. The Shannon indices of alpha diversity generally decreased for bacterial and lower fungal diversity in the rhizosphere soil among the four stands. The fungal ACE index showed the lowest bacterial and the highest fungal diversity compared to the Shannon indices for fungi (Table 2). Ten-year-old stand showed greater bacterial and lower fungal diversity in the rhizosphere soil among the four stands. The opposite was true for the 60-year-old stand, which showed the lowest bacterial and the highest fungal diversity compared with the other three stands (Table 2). The bacterial ACE index generally decreased with stand age except that the 40-year-old stand showed a greater ACE score than the 25-year stand (Table 2). In contrast, the fungal ACE index generally increased as stands aged except that the 25-year-old stand showed a numerically greater ACE score than 10-year-old stand.

3.3 | Bacterial and fungal community structure with revegetation

The nonmetric multidimensional scaling (NMDS) analysis separated the bacterial OTUs of the four stands (Figure 2a), although one sample of the 60-year-old stands appeared to be an outlier (Pt602 compared with Pt601 and Pt603). Nonmetric multidimensional scaling 1 (NMDS1) separated the 10- and 25-year-old stands from the 60-year-old stands, and nonmetric multidimensional scaling 2 (NMDS2) separated the 10-year-old stands from the 25- and 40-year-old stands (Figure 2a).

The RDA assessed the relationships between soil characteristics and bacterial community structure in the rhizosphere soil. RDA revealed that soil organic carbon, total nitrogen, total phosphorus, and available phosphorus were significantly associated with bacterial community communities (p < 0.05). Bacteria in 10-year-old forests clustered in areas with high available phosphorus content whereas bacteria in 40-year-old forests clustered in areas with high organic carbon, total nitrogen, and total phosphorus content. For the RDA ordination plots, the first two axes of the RDA explained 13% and 11% of the variation in site-environment relationships. The bacterial RDA plots clearly separated the four forests across the studied successional gradient (Figure 3a).

For the fungal OTUs, NMDS1 separated the 10-year-old stands from the 40-year-old stands, and NMDS2 separated the 10-year-old stands from the 25-year-old stands (Figure 2b). On the NMDS plots, the sites of the 10- and 40-year-old stands formed two separate clusters, but sites of the 25- and 60-year-old stands overlapped to some degree with respect to fungal community structure (Figure 2b). CCA

### TABLE 1 Physicochemical properties of rhizosphere soil in Pinus tabulaeformis plantation forest

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Stand age, years</th>
<th>10</th>
<th>25</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>8.37 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.37 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.37 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.41 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOC, g kg⁻¹</td>
<td></td>
<td>17.32 ± 2.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.74 ± 2.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.46 ± 2.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.57 ± 1.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TN, g kg⁻¹</td>
<td></td>
<td>0.77 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.16 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.17 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TP, g kg⁻¹</td>
<td></td>
<td>0.73 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.84 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AN, mg kg⁻¹</td>
<td></td>
<td>24.04 ± 6.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.02 ± 5.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.47 ± 3.97&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>18.40 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AP, mg kg⁻¹</td>
<td></td>
<td>2.76 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.70 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.59 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SWC, %</td>
<td></td>
<td>5.52 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.63 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.32 ± 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.20 ± 0.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C/N</td>
<td></td>
<td>24.28 ± 9.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.99 ± 1.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.44 ± 3.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.67 ± 2.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg, g kg⁻¹</td>
<td></td>
<td>3.65 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.49 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.83 ± 0.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significant difference among stand age in each line denoted with a different letter (p < 0.05; least significant difference multiple comparison test).

Note. SOC: soil organic carbon; TN: total nitrogen; TP: total phosphorus; AN: available nitrogen; AP: available phosphorus; SWC: soil water content; C/N: ratio of total carbon to nitrogen.
TABLE 2  Microbial diversity in the rhizosphere soil of *Pinus tabulaeformis* plantation forest at 97% similarity

<table>
<thead>
<tr>
<th>Type of microorganism</th>
<th>Stand age, years</th>
<th>ACE</th>
<th>Chao1</th>
<th>Shannon</th>
<th>Simpson</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial community</td>
<td>10</td>
<td>3046.246 ± 55.436&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2997.958 ± 82.435</td>
<td>9.195 ± 0.190</td>
<td>0.996 ± 0.001</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>2778.696 ± 203.294&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2732.020 ± 268.394</td>
<td>9.175 ± 0.053</td>
<td>0.995 ± 0.001</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>2798.387 ± 213.893&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2755.529 ± 249.824</td>
<td>9.155 ± 0.046</td>
<td>0.995 ± 0.001</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>2608.029 ± 262.676&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2598.810 ± 319.046</td>
<td>9.067 ± 0.136</td>
<td>0.995 ± 0.001</td>
</tr>
<tr>
<td>Fungal community</td>
<td>10</td>
<td>664.555 ± 198.064</td>
<td>639.613 ± 205.702</td>
<td>4.499 ± 1.003</td>
<td>0.847 ± 0.105</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td>602.295 ± 299.283</td>
<td>583.803 ± 292.776</td>
<td>5.149 ± 1.739</td>
<td>0.880 ± 0.132</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>733.427 ± 103.698</td>
<td>721.185 ± 115.654</td>
<td>5.429 ± 0.531</td>
<td>0.907 ± 0.039</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>850.963 ± 153.651</td>
<td>828.063 ± 145.335</td>
<td>5.854 ± 0.406</td>
<td>0.945 ± 0.023</td>
</tr>
</tbody>
</table>

Significant differences in each column denoted with a different letter (*p* < 0.05; least significant difference test), lack of letter indicates no significant difference among stand ages.

Note. ACE: abundance-based coverage estimator.

FIGURE 2  Nonmetric multidimensional scaling analysis of bacterial and fungal communities in the rhizosphere soils of *Pinus tabulaeformis* forests. Pt101, Pt102, and Pt103 represent three replicates in a 10-year-old stand; Pt251, Pt252, and Pt253 represent three replicates in a 25-year-old stand; Pt401, Pt402, and Pt403 represent three replicates in a 40-year-old stand; and Pt601, Pt602, and Pt603 represent plots in a 60-year-old stand (The same below). Stress: <0.05 = excellent, <0.10 = good, <0.20 = usable, >0.20 = not acceptable (Clarke, 1993) [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 3  Ordination plots of the results from the redundancy analysis (RDA) and canonical correspondence analysis (CCA) of sampling sites based on bacterial OTUs (a) and fungal OTUs (b) in relation to soil physicochemical properties (blue arrows). SOC: soil organic carbon; pH: the pH of the soil; TN: total nitrogen; TP: total phosphorus; AN: available nitrogen; AP: available phosphorus. SWC: soil water content. (RDA was used when the length of gradient less than 3.0 or else CCA was used. The length of gradient of bacterial community = 0.61, the length of gradient of fungal community = 4.24) [Colour figure can be viewed at wileyonlinelibrary.com]
assessed the relationships between soil physicochemical properties and fungal communities in the rhizosphere soil (Figure 3b). The results showed that total phosphorus and total nitrogen were significantly associated with fungal communities ($p < 0.05$). The 40-year-old stands centered in areas with high total phosphorus content. The first two axes of the CCA explained 15% and 12% of variation in site-environment relationships. The fungal CCA plots separated the four stands across the successional gradient, and greatest between-stand variation occurred in the transition from 25- to 40-year-old stands.

### 3.4 Bacterial and fungal compositions with revegetation

The bacterial OTUs were assigned to two kingdoms (bacteria and archaea), 37 phyla, 74 classes, and 134 orders. The dominant phyla of bacteria were Actinobacteria (25.1%), Proteobacteria (24.5%), and Acidobacteria (22.4%; Figure 4a). The relative abundance of Actinobacteria decreased along the successional gradient during the first 40 years and then increased in the 60-year-old stands. In contrast, the relative abundance of Acidobacteria was significantly higher in the 40-year-old stands than in the other stands. The subdominant phyla were Planctomycetes (7.5%), Chloroflexi (6.3%), Gemmatimonadetes (5.0%), Verrucomicrobia (2.3%), Nitrospirae (2.9%), Bacteroidetes (1.1%), and Armatimonadetes (0.7%; Figure 4a). The relative abundance of Chloroflexi and Gemmatimonadetes increased whereas that of Bacteroidetes and Armatimonadetes decreased along the successional gradient (Figure 4a). The archaea included the phyla Thaumarchaeota (0.014–0.063%) and Euryarchaeota (0.006–0.017%).

To further explore the dominant bacteria (Actinobacteria, Proteobacteria, and Acidobacteria), we analyzed the dynamics of the major taxa along the 60-year successional gradient. Among the Actinobacteria phyla, Thermoleophilia, an unidentified class of Actinobacteria, Acidimicrobia, and MB-A2-108 were found in every sample. The relative abundance of Acidimicrobia and MB-A2-108 increased along the successional gradient, whereas that of an unidentified class of Actinobacteria decreased. Proteobacteria include Alpha-, Beta-, Gamma-, and Deltaproteobacteria. The relative abundance of Deltaproteobacteria increased with stand age up to 40 years and then was decreased in the 60-year-old stand (Figure S1a).

Almost all of the fungal OTUs (99.17%) were assigned to four phyla. The dominant phyla were Basidiomycota (72.7%) and Ascomycota (21.7%; Figure 4b). In addition, a few fungal OTUs were assigned to an unidentified phylum (2.4%), Zygomycota (2.4%), Chytridiomycota (0.2%), Glomeromycota (<0.1%), Rozellomycota (<0.1%), and others (0.5%; Figure 4b). The relative abundance of Basidiomycota generally decreased during succession with 60-year-old stands containing less than 10- and 25-year-old stands (Figure 4b). Among the Basidiomycota phylum, Agaricomycetes, Walleniomycetes, Tremellomycetes, and Microbotryomycetes were the most abundant classes. The relative abundance of Agaricomycetes decreased whereas that of Tremellomycetes increased along the successional gradient (Figure S1b). In contrast to Basidiomycota, the relative abundance of Ascomycota increased along the successional gradient with a significantly higher value in the 60-year-old stands than in the 10- and 25-year-old stands (Figure 4b). Saccharomycetes, Pezizomycetes, Sordariomycetes, Eurotiomycetes, Dothideomycetes, an unidentified class of Ascomycota, and Leotiomycetes were the most abundant classes of Ascomycota. The relative abundance of both Saccharomycetes and Sordariomycetes increased along the successional gradient, similar to the trend observed for Ascomycota relative abundance (Figure S1b).

### 4 DISCUSSION

Water-induced soil erosion resulting from unreasonable land use degraded land and affected vast areas in the Loess Plateau of China. Our results demonstrated that the successional stage of planted forest under a revegetation program significantly affected soil physicochemical properties and microbial communities. NMDS analysis clearly separated bacterial community composition among four different aged stands. However, fungi exhibited less distinct community composition with overlap between 25- and 60-year-old stands by this analysis.
Perhaps, this analysis reveals a summit of soil nutrient accumulation during revegetation between 25 and 60 years for fungi.

Microbial community composition correlated with soil characteristics, especially organic carbon, total nitrogen, and total phosphorus. Soil microorganisms remain key regulators in biogeochemical cycling and can transform soil nutrient elements (nonavailable nitrogen and phosphorus) into usable forms (available nitrogen and phosphorus). This attribute of microorganisms allows their establishment where nutrients are chemically bound; their important work is to make those nutrients available. Therefore, the total (not limited soil available) nitrogen and phosphorus contents affected microbial communities. Available nitrogen and phosphorus are usually limited in soil and microbial communities generally have rigid C:N:P ratios. In our study, available phosphorus, which showed low concentration limited microbial communities and thus no significant correlations were found between available nitrogen and microbial community. This result suggests that soil nutrient content is an important determinant of microbial community composition.

Our data suggest that the restoration of *P. tabulaeformis* forests in China affected rhizosphere microbial diversity and community composition. Rhizosphere soil organic carbon, total nitrogen, and total phosphorus content increased with stand age up to 40 years and then decreased in 60-year-old stands. This result suggests that soil nutrient accumulation peaked at approximately 40 years after revegetation and seems a reasonable explanation based on the changes in soil organic carbon and nitrogen after revegetation. The changes in soil phosphorus over time likely resulted from plant roots that reached deep soil layers and extracted phosphorus for plant growth. As leaves and other aerial plant parts senesced to the soil surface over time, topsoil phosphorus content increased. This finding differed from a previous forest revegetation study (Jia, Cao, Wang, & Wang, 2005) that showed greatest organic carbon and total nitrogen contents in 17-year-old stands. The discrepancy may stem from *P. tabulaeformis* being the dominant tree species in our study whereas Jia et al. (2005) worked with more even distributions of *P. tabulaeformis*, *Quercus liaotungensis*, and *Populus davidiana*. Wu et al. (2015) also observed a decline in soil nutrient concentration after a peak in *Pinus elliottii* and further supports that soil nutrients reach an upper asymptote at a certain stage during secondary succession. Interestingly, we found that both total nitrogen and total phosphorus increased from 10 to 40 years along the successional gradient, whereas available nitrogen and phosphorus decreased over time. This divergence may result from the alkaline soil environment limited phosphorus availability. Soil pH did not significantly change across the successional gradient, which agreed with a previous study assessing soil characteristics during natural succession on abandoned farmland in the Loess Plateau (Zhang et al., 2016). Together, these findings indicate that revegetation in temperate forests does not significantly alter soil pH in the Loess Plateau.

In our study, rhizosphere microbial richness and diversity differed among stand ages in *P. tabulaeformis* forest (Table 2). In general, bacterial richness and diversity decreased with stand development. Our findings aligned with a study in *P. elliottii* forests (Wu et al., 2015). However, another study of abandoned farmland on the Loess Plateau revealed that soil bacterial richness and diversity increased during natural succession (Zhang et al., 2016). These contrary results likely reflect the effects of root exudates from different vegetation types on bacterial growth (Yuan & Chen, 2012b). In contrast, fungal richness and diversity increased across the successional gradient in our study. This finding was consistent with a previous study in *P. tabulaeformis* forests on the Loess Plateau (Yu, Wang, & Tang, 2013) and suggests that successional stage may be a positive factor controlling the development of fungi in the rhizosphere of forest trees.

The results of bacterial ordination analysis agreed with other studies on succession in tropical forests of Costa Rica (Chaverri & Vilchez, 2006). Similar to studies by Blalid et al. (2012) and Zhang et al. (2016), our results also showed a clear separation of soil bacteria among successional stages and suggested that revegetation significantly impacts bacterial community development. Samples of 25- and 60-year-old stands did not show distinct clusters based on fungal community structure (Figure 2b), and these overlapping results were similar to studies from intermediate and late secondary succession stages of chalk grasslands (Kuramae, Gamper, van Veen, & Kowalchuk, 2011). These results may indicate that revegetation induced a peak soil nutrient accumulation period, which corresponded to soil physicochemical properties (Table 1).

The dominant phyla of bacteria in our study were *Actinobacteria*, *Proteobacteria*, and *Acidobacteria*. Our findings agreed with those on abandoned farmland in the same studied region (Zhang et al., 2016; Tian et al., 2017; Zhong, Yan, & Zhoupingle, 2015; Figure 4a). One previous study on rhizosphere microbial communities of *P. tabulaeformis* forest indicated that Proteobacteria, Acidobacteria, and Firmicutes dominated the soil, whereas *Actinobacteria* were detected in low abundance (Yu et al., 2013). This inconsistency might result because Yu et al. (2013) employed low-resolution profiling with nested PCR and other differences in lab techniques. Numerous studies reported that Acidobacteria prefer environments with low carbon, and they are considered an oligotrophic species (Chu, Neufeld, Walker, & Grogan, 2011; Goldfarb et al., 2011; Li, Rui, Mao, Yannarell, & Mackie, 2014). More recently, Zhang et al. (2016) reported greater relative abundance of Acidobacteria under very low soil organic carbon contents (2.66 g kg$^{-1}$). These conditions often prevail at the beginning of revegetation. After 30 years of revegetation, soil organic carbon content increased (peak 4.59 g kg$^{-1}$), and the relative abundance of Acidobacteria decreased, but the total carbon values remained low. In contrast, we observed the highest relative abundance of Acidobacteria (22.4% of total bacteria) in the 40-year-old stands with soil organic carbon contents ranging from 15.57 to 21.46 g kg$^{-1}$ during the 60-year succession (Table 1 and Figure 4a). This finding suggests that Acidobacteria thrive under both oligotrophic and copiotrophic conditions. Additionally, the relative abundances of Acidobacteria were positively correlated with total phosphorus content (Table 3). Previous studies showed that the dominant bacteria Proteobacteria and Acidobacteria are abundant across a wide range of soil pH (approximately 4 to 8.7 [Nie et al., 2012; Zhang et al., 2016]). In our study, soil pH after revegetation ranged little from 8.37 to 8.41, but this supports the idea that Proteobacteria and Acidobacteria thrive in high pH soil.
Ascomycota reportedly preferred arid and cool environments due to their evolutionary histories (Treseder et al., 2014). Furthermore, they dominated in Arctic soils (Chu et al., 2011). Basidiomycota with environment. Similarly, revegetation. We found that dominant fungal species in soils change to reveal that fungal community composition differed based on stage of succession of revegetated forests is characterized not only by changes in soil properties but also by changes in microbial community composition and diversity. Along a 60-year revegetation gradient of P. tabulaeformis forest in the Loess Plateau, bacterial diversity decreased and fungal diversity increased. The changes in microbial populations arose from environmental shifts in nutrient availability; shifts more suitable for fungi and less suitable for bacteria over time. Although fungal diversity increased with stand age, fungal community composition peaked in the 40-year-old stands.

### 5 | CONCLUSION

In this study, we found the relative abundance of Deltaproteobacteria increased with stand age up to 40 years, and its relative abundance was positively correlated with soil organic carbon and total nitrogen (Table 3). Compared with other stands, the 40-year-old stands had the highest soil organic carbon content. Therefore, copiotrophic groups or r-selected populations become the dominant species in such nutrient-rich conditions (Fierer, Bradford, & Jackson, 2007; Goldfarb et al., 2011). Thus, Proteobacteria were abundant in soils with high resource availability (Axelrood, Chow, Radomski, McDermott, & Davies, 2002; Kennedy, Gleeson, Connolly, & Clipson, 2005). The four classes within Proteobacteria, (i.e., Alpha-, Beta-, Gamma-, and Deltaproteobacteria) exhibit two different trophic mechanisms. Most of Beta-, Gamma-, and Deltaproteobacteria belong to copiotrophic groups, and most Alphaproteobacteria belong to oligotrophic groups (Goldfarb et al., 2011). As a result, no correlation existed between soil properties and the relative abundance of Proteobacteria. Within the bacterial phyla Acidobacteria, the unidentified class of Acidobacteria was prevalent and comprised the basis of Acidobacteria response (Figure S1a). Hence, the properties of Acidobacteria as a whole reflected that of the unidentified class of Acidobacteria.

As others reported across ecosystems such as pea fields (Xu, Ravnkov, Larsen, Nilsson, & Nicolaelsen, 2012), forests (Yu et al., 2013), and grasslands (Leff et al., 2015), the dominant phyla of fungi in our study were Basidiomycota and Ascomycota (Figure 4b). The relative abundance of Basidiomycota was higher than Ascomycota. The highest relative abundance difference was 65.6% in 25-year-old stands, and the lowest value was 27.2% in 60-year-old stands. These values reveal that fungal community composition differed based on stage of revegetation. We found that dominant fungal species in soils change with environment. Similarly, Zygomycota and Chytridiomycota dominated in Arctic soils (Chu et al., 2011). Basidiomycota and Ascomycota reportedly preferred arid and cool environments due to their evolutionary histories (Treseder et al., 2014). Furthermore, Zygomycota are commonly observed soil fungal species detected in both native and planted forests (He, Xu, & Hughes, 2005; Yu et al., 2013).

Table 3: Pearson correlation matrix describing the relationship among soil properties and relative abundances of dominant bacterial and fungal groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Actin</th>
<th>Prote</th>
<th>Acid</th>
<th>Basi</th>
<th>Asco</th>
<th>Delta</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>-0.279</td>
<td>0.338</td>
<td>0.230</td>
<td>0.264</td>
<td>-0.145</td>
<td>0.676*</td>
</tr>
<tr>
<td>SWC</td>
<td>0.059</td>
<td>0.111</td>
<td>-0.361</td>
<td>0.375</td>
<td>-0.357</td>
<td>-0.369</td>
</tr>
<tr>
<td>TN</td>
<td>-0.536</td>
<td>0.359</td>
<td>0.449</td>
<td>0.371</td>
<td>-0.427</td>
<td>0.680*</td>
</tr>
<tr>
<td>TP</td>
<td>-0.294</td>
<td>-0.149</td>
<td>0.601*</td>
<td>-0.278</td>
<td>0.250</td>
<td>0.461</td>
</tr>
<tr>
<td>AN</td>
<td>0.298</td>
<td>0.086</td>
<td>-0.023</td>
<td>-0.136</td>
<td>0.054</td>
<td>-0.207</td>
</tr>
<tr>
<td>AP</td>
<td>0.544</td>
<td>-0.275</td>
<td>-0.498</td>
<td>-0.153</td>
<td>0.234</td>
<td>-0.829**</td>
</tr>
<tr>
<td>Mg</td>
<td>0.279</td>
<td>0.089</td>
<td>-0.429</td>
<td>0.188</td>
<td>-0.049</td>
<td>-0.493</td>
</tr>
<tr>
<td>Ca</td>
<td>0.326</td>
<td>-0.331</td>
<td>-0.536</td>
<td>-0.009</td>
<td>0.241</td>
<td>0.395</td>
</tr>
<tr>
<td>C/N</td>
<td>0.477</td>
<td>-0.121</td>
<td>-0.481</td>
<td>-0.301</td>
<td>0.423</td>
<td>-0.343</td>
</tr>
</tbody>
</table>


*Correlation is significant at p < 0.05 (two tailed)
**Correlation is significant at p < 0.01 (two tailed). Bold font indicates a significant correlation (positive or negative)
These findings provide insight into how forest successional stages differentially affect rhizosphere bacterial and fungal community composition. These findings also improve our knowledge of soil microbial communities during forest revegetation on degraded soils.

ACKNOWLEDGMENTS
We thank Qiang Deng and Yunxia Wang for experimental assistance. This work was supported by the National Key Research and Development Program of China (Grant 2016YFA0600801), the National Natural Sciences Foundation of China (Grant 31570438), Hundred Talents Program of Shaanxi Province (Grant A289021701), Natural Science Basic Research Plan in Shaanxi Province of China (Grant 2018JZ3002), and Double First Class University Plan of NWSUAF (Grant Z102021829).

CONFLICT OF INTEREST
All authors declare no conflicts of interest either financial or personal.

ORCID
Gui-yao Liu https://orcid.org/0000-0002-9401-8820
Xin-rong Shi https://orcid.org/0000-0003-3963-9269
Zhi-you Yuan https://orcid.org/0000-0003-0925-3226
Lois Y. Yuan https://orcid.org/0000-0003-4875-199X

REFERENCES


**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.