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Natural revegetation of a semiarid habitat alters taxonomic and functional diversity of soil microbial communities

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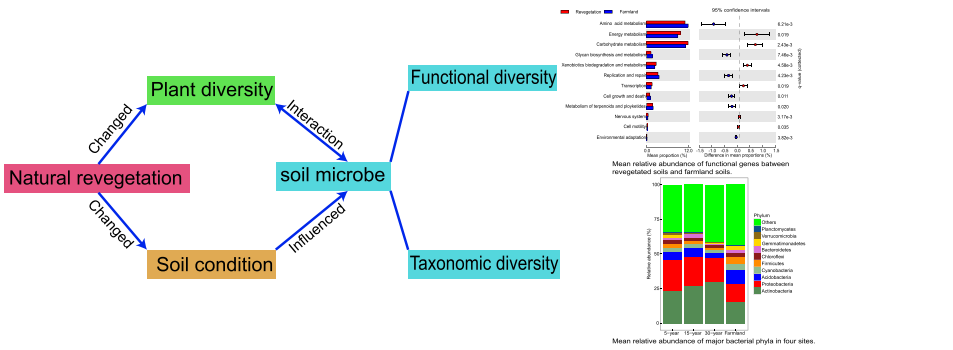
HIGHLIGHTS

- We analyzed the microbial taxonomic and functional diversity of revegetated soils.
- We used both 16S rRNA gene amplicon and shotgun metagenomic sequencing.
- The microbial taxonomic diversity increased with plant diversity.
- Soil organic matter was the best predictor of microbial community structure.
- Revegetation increased the potential of microbe-mediated nutrient cycling.

GRAPHICAL ABSTRACT



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ABSTRACT

Revegetation of degraded lands has a profound impact on the maintenance and stability of ecosystem processes. However, the impacts of this land use change on functional diversity of soil microbial communities are poorly understood. Here, using 16S rRNA gene amplicon and shotgun metagenomic sequencing, we compared the taxonomic and functional communities of soil microbiome, and analyzed the effects of plant diversity and soil chemical properties, in a chronosequence of restored ex-farmland that had been naturally revegetated to grassland over periods of 5, 15 and 30 years with adjacent farmland, on the Loess Plateau, China. We found that microbial taxonomic diversity was positively correlated with plant diversity and was higher in the revegetated sites. Functional diversity increased significantly in the oldest grassland. Actinobacteria, commonly considered a copiotrophic phylum, was more abundant in the revegetated sites, while Acidobacteria, an oligotrophic phylum, was more abundant in farmland. Furthermore, the structure of taxonomic and functional communities was significantly different between revegetated sites and farmland, and organic matter was the best environmental predictor in determining these microbial communities. Compared with the farmland, revegetation increased the proportion of genes associated with energy metabolism, carbohydrate metabolism and xenobiotics biodegradation and metabolism. Notably, the higher proportion of carbohydrate degradation gene subfamilies in the revegetated sites indicated higher levels of soil nutrient cycling. These results elucidate the significant shifts in belowground microbial taxonomic and functional diversity following vegetation restoration and have implications for ecological restoration programs in arid and semi-arid ecosystems.

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1. Introduction

Land degradation exerts a great impact on ecosystem services and threatens the survival of humans (Smith et al., 2016), especially in arid and semi-arid regions (Sun et al., 2015). Natural revegetation of degraded land is one of the most widely used restoration strategies, because the development of plant communities can effectively reduce soil erosion, improve biodiversity, and associated ecosystem services (Bullock et al., 2011; Liu et al., 2008). Yet, the restoration of ecosystem function is a long-term process that can vary with local climate and soil conditions (Munroe et al., 2013). Complex interactions between aboveground and belowground communities drive productivity and diversity in ecosystems and determine the successional establishment and development of biological communities (Kardol and Wardle, 2010; van der Putten et al., 2013). However, the majority of studies exploring ecosystem restoration focus on plants and little attention has been paid to soil microbial dynamics.

Plant-soil feedback processes play important roles in determining the structure and successional dynamics of both plant and microbial communities (Herrera Paredes et al., 2016). Plants can alter the abiotic soil environment, including pH, organic matter, carbon to nitrogen ratio and soil texture, through litter fall and root exudation, where such changes impact soil microbial diversity and community structure (Frouz et al., 2016; Schlatter et al., 2015). In turn, soil microbiome affect plant community composition and productivity through microbe-mediated organic matter decomposition and nutrient cycling and direct interactions with plants (van der Putten et al., 2016). In secondary succession of habitats, plant communities shift from fast to slow-growing species, with varying effects on plant diversity, community composition and biomass; there may also be changes in litter quality and quantity (Mahaming et al., 2009). Due to the strong interactions between plants, soil and microbes, successful habitat restoration should be concomitant with shifts in soil microbial communities.

Despite the importance of revegetation in ecological restoration, the impacts of revegetation processes on microbial taxonomic and functional diversity remain largely unknown. Previous studies have demonstrated that the composition of soil bacterial communities is affected by natural revegetation and its diversity increases with post-restoration time (Kuramae et al., 2010; Zhang et al., 2016), but they have mainly focused on taxonomic diversity of microbial communities that cannot accurately predict microbial functional characteristics owing to high functional redundancy (Allison and Martiny, 2008). It has been shown that better predictions of microbial responses to habitat change may be gained from functional genes rather than taxonomic traits (Burke et al., 2011). Therefore, assessment of changes in microbial gene functional diversity may better elucidate the impacts of revegetation on ecosystem restoration.

The Loess Plateau of China, characterized by extensive wind-blown sedimentary deposits, is one of the most eroded areas in the world due to its naturally erodible soil, complex terrain and frequent human disturbance (Fu et al., 2011). Various ecological construction strategies have been implemented since 1950s to reduce soil erosion and restore the environment, including the “Grain for Green” program in 1999, which aimed to convert farmland to forest, shrub and grassland (Deng et al., 2014). Recently, the plant communities (Kou et al., 2016; Sun et al., 2017) and corresponding belowground parameters, including soil chemical properties and microbial communities (Zhang et al., 2016) of naturally revegetated sites have been reported. However, these studies of the soil microbial communities were mainly based on amplicon sequencing that provided limited information about functional gene composition and diversity. Understanding the impacts of vegetation restoration on microbial taxonomic and functional communities is important for ecological monitoring and restoration assessment.

Here, we studied the soil microbial community at three naturally revegetated sites, which had been retired from agricultural use since the last 5, 15 and 30 years, and adjacent farmland on the Loess Plateau, China. By using Illumina HiSeq sequencing of 16S rRNA gene and metagenome, we aimed to (a) assess the influence of revegetation on the taxonomic and functional diversity of soil microbial communities and (b) determine the relationship between microbial and plant diversity and identify the major soil chemical properties that shape the structure of taxonomic and functional communities.

2. Materials and methods

2.1. Study site and soil sampling

Our study system is an active “Grain for Green” restoration site, located on the Loess Plateau, China (36°10′–36°17′ N, 106°21′–106°27′ E; altitude 1860–2000 m). The site is a semiarid habitat with temperate continental climate. The annual average precipitation is 424 mm, 60% of which occurs from July to September. The annual average temperature is 5 °C, ranging from a January minimum of −14 °C to a July maximum of 25 °C. Loessial soil (Calcaric Cambisols, FAO) and gray cinnamon soil (Haplic Greyxems, FAO) dominate. Since the 1980s, adjacent agricultural fields, which were similar in landscape position and cropping systems and had been cultivated for >30 years, were removed from production and fenced, to exclude livestock and anthropogenic disturbance, to allow natural revegetation. This has resulted in a series of secondary successional grasslands that differ in age.

In August 2014, we selected naturally revegetated grasslands that were 5-, 15- and 30-year old and adjacent, long-established cultivated farmland planted with corn. In each of the four study sites, three 1 × 1 m plots were arranged randomly within an area of 10 × 10 m. Within each plot, all plant species were identified and quantified (Sup. Table 1). We collected five topsoil cores (5 cm diameter × 10 cm depth) from each plots using an auger, which were pooled and transported to laboratory on ice. All soil samples were thoroughly homogenized and manually removed stones and roots. The single soil samples from each plot were then divided into two parts, where one was frozen at −80 °C for DNA extraction, and the remaining part was sieved (2 mm gauge) and air dried for soil chemical analyses. Soil organic matter (OM) content, total N (TN) content, available N (AN), available K (AK) and available P (AP) content and pH were determined as procedures previous described (Bao, 2000).

2.2. Soil DNA isolation, 16S rRNA gene sequencing and bioinformatic analysis

Total DNA was extracted from all soil samples using FastDNA® SPIN Kit (MP Biochemicals, Solon, OH, USA) with 500 mg of soil per sample following the manufacturer's recommendations. To obtain sufficient DNA quantity for sequencing and to ensure adequate representation of soil, five replicates were conducted and pooled for each sample. The V4–V5 region of bacterial 16S rRNA genes was PCR amplified using primer pair 515F (5′-GTG CCA GCM GCC GCG GTA A-3′) and 907R (5′-CCG TCA ATT CCT TTG AGT TT-3′) (Edwards et al., 2015), and all PCR amplifications were conducted in triplicate for each sample. Amplicon samples were sequenced on the paired 250-bp Illumina HiSeq 2500 platform (Illumina, Inc., CA, USA). Paired-end sequences were merged by FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/), then quality filtered with QIIME (Caporaso et al., 2010). After removed chimeric sequences (Edgar et al., 2011), the remaining sequences were assigned to OTUs at similarities of 97% using UPARSE (Edgar, 2013). Taxonomic information was annotated for a representative sequence of each OTU by RDP classifier at a confidence of 80% (Wang et al., 2007).

2.3. Shotgun metagenomic sequencing and bioinformatic analysis

1 μg of the above-mentioned DNA extracted from each soil sample was prepared for shotgun metagenomic sequencing. The DNA samples

were sequenced on the paired 150-bp Illumina HiSeq 2500 platform (Illumina, Inc., CA, USA). Raw reads were quality filtered and the valid high-quality reads were assembled using SOAP denovo (Luo et al., 2012). Functional genes were predicted using MetaGeneMark (Zhu et al., 2010), and the microbial profiles of taxonomy and function were generated by BLASTP through comparison with the NCBI nr, KEGG and CAZY database with $E \le 1 \times 10^{-5}$, respectively.

2.4. Data analysis

Data analyses were conducted with R (R Core Team, 2017). Normality of the distributions of the residuals of models was analyzed using Shapiro-Wilk test, and non-normally distributed data were log transformed. To estimate the taxonomic (OTUs and metagenomic species level) and functional (KEGG level 3) alpha diversity, the Shannon diversity index was calculated by 'diversity' in vegan R package. One-way ANOVA was used to test for the main effect of land use on microbial diversity, the relative abundance of microbial phyla and soil chemicals. Pairwise comparisons between means were conducted using Student's *t*-tests with Benjamini-Hochberg correction. The relationships between microbial and plant diversity, and the correlations between microbial taxonomic and functional diversity, groups and measured soil chemical properties were calculated using Pearson's correlation coefficient. Network analysis was constructed using igraph R package and visualized by Gephi (Ju et al., 2014).

Principal coordinate analysis (PCoA) of Bray-Curtis distances between taxonomic (OTUs and metagenomic species level) and functional (KEGG level 3) profiles of microbial communities at the study sites was based on the 'pcoa' function of the ape package in R. Permutational multivariate analyses of variance (PERMANOVA, 'adonis' in vegan R package) with 999 random permutations was used to test the effect of revegetation on the community variances and redundancy analysis (RDA, 'rda' in vegan R package) was performed to assess the correlation between structure of microbial communities and soil chemical variables. Forward selection with 999 permutation tests of these soil chemicals was determined by using the 'forward. sel' function of the packfor R package. In addition to R, the STAMP software (Parks et al., 2014) was also applied to test functional gene differences between the revegetated and farmland sites (confidence interval method) using Welch's *t*-test with Benjamini-Hochberg FDR correction.

3. Results

3.1. Microbial genetic abundance

We obtained 424,594 (79.68% of total sequences) high quality sequences from 16S rRNA gene sequencing for all soil samples, ranging from 18,325 to 59,590 sequences per sample, and an average 3280 OTUs were identified in each sample. These OTUs formed a network with 281 nodes and 1036 edges, and 23 modules were detected (Sup. Fig. 1a). All the detected OTUs were classified into 47 bacterial phyla. Among the identified phyla, the most abundant from the four land use sites were the Actinobacteria (29.37% on average), Proteobacteria (27.32%) and Acidobacteria (18.05%) (Sup. Fig. 2a). Less abundant phyla included the Chloroflexi (6.72%), Planctomycetes (5.04%), Gemmatimonadetes (4.50%), Bacteroidetes (2.38%), Nitrospirae (1.75%), Verrucomicrobia (1.55%) and Armatimonadetes (0.68%). Actinobacteria was more abundant in the revegetated sites than farmland ($P < 0.05$) and increased with restoration age. By contrast, Acidobacteria, Chloroflexi and Gemmatimonadetes were less abundant in the revegetated sites compared with farmland (Table 1, $P < 0.05$).

3.2. Microbial genomic abundance

Shotgun metagenomic sequencing resulted in 1040 million raw reads (80–92 million per sample) with a total of 156 Gb from the twelve samples (11.95–13.80 Gb per sample) and 99.1% of reads passed quality control for downstream analysis. The annotated sequences of metagenome were mainly including Bacteria (84.73% of total predicted microbes), Archaea (14.50%) and Eukarya (0.61%). Network analysis was conducted with metagenomic taxonomic profile at species level (Sup. Fig. 1b). The network was consisted of 126 nodes, 556 edges and 11 modules. Just like results obtained from bacterial amplicon sequencing, the most abundant bacterial phyla were the Actinobacteria (23.91% on average), Proteobacteria (18.56%) and Acidobacteria (6.24%) (Sup. Fig. 2b), then followed by the Cyanobacteria (3.35%), Firmicutes (2.74%), Chloroflexi (2.47%), Bacteroidetes (2.00%), Gemmatimonadetes (1.29%), Verrucomicrobia (0.78%) and Planctomycetes (0.64%). The relative abundance of Actinobacteria increased with restoration age, while the relative abundance of Proteobacteria, Cyanobacteria, Firmicutes, Chloroflexi and Gemmatimonadetes decreased with restoration age. Actinobacteria and Proteobacteria were more abundant in the revegetated sites; while the Acidobacteria, Firmicutes and Gemmatimonadetes were less abundant, compared with the farmland (Table 1, $P < 0.05$).

Table 1
Comparison of relative abundance of the bacteria phyla from 16S rRNA gene and shotgun metagenomic sequences between the revegetated soils (5-, 15- and 30-year old) and farmland soils.

Bacterial taxa	$F_{(3, 8)}$	*P*	Pairwise comparison
16S rRNA gene sequences			
Actinobacteria	46.42	<0.001	Revegetation > farmland
Acidobacteria	12.76	0.002	Revegetation < farmland
Chloroflexi	83.13	<0.001	Revegetation < farmland
Gemmatimonadetes	132.80	<0.001	Revegetation < farmland
Nitrospirae	5.26	0.027	–
Metagenomic sequences			
Actinobacteria	13.78	0.002	Revegetation > farmland
Proteobacteria	10.75	0.004	Revegetation > farmland
Acidobacteria	15.09	0.001	Revegetation < farmland
Bacteroidetes	7.49	0.010	–
Firmicutes	23.16	<0.001	Revegetation < farmland
Chloroflexi	9.56	0.005	–
Gemmatimonadetes	22.37	< 0.001	Revegetation < farmland

Only phyla with mean relative abundance > 1% and significant differences are shown ($P < 0.05$).

3.3. Microbial function potential

In order to reveal the influence of revegetation on microbial function, obtained sequences of metagenome were annotated to the KEGG database. Six functional categories, including cellular processes (0.67% of the total predicted genes), environmental information processing (2.92%), genetic information processing (5.54%), human diseases (1.45%), metabolism (15.59%) and organismal systems (0.59%) were determined finally. The KEGG level 2 functional profiles were compared between the revegetated and farmland soils using STAMP and we found that the significantly enriched function gene categories in revegetated soils were related to energy metabolism, carbohydrate metabolism, xenobiotics biodegradation and metabolism, transcription, nervous system and cell motility (Fig. 1, Sup. Fig. 3). Genes associated with amino acid metabolism, glycan biosynthesis and metabolism, replication and repair, cell growth and death, metabolism of terpenoids and polyketides and environmental adaptation were more abundant in farmland than the revegetated sites (Fig. 1, Sup. Fig. 3). The network calculated using KEGG level 3 functional profile is shown in Sup. Fig. 1c.

Considering the vital importance of OM degradation in vegetated soils, we focused our analysis on the responses of carbohydrate-active enzymes (CAZymes) coding sequences to natural revegetation. By annotating metagenomic sequences to the CAZy database, six enzyme categories were obtained, including glycoside hydrolases (1.18%, proportion of the total predicted genes), glycosyl transferases (0.98%), polysaccharide lyases (0.01%), carbohydrate esterases (0.18%), auxiliary

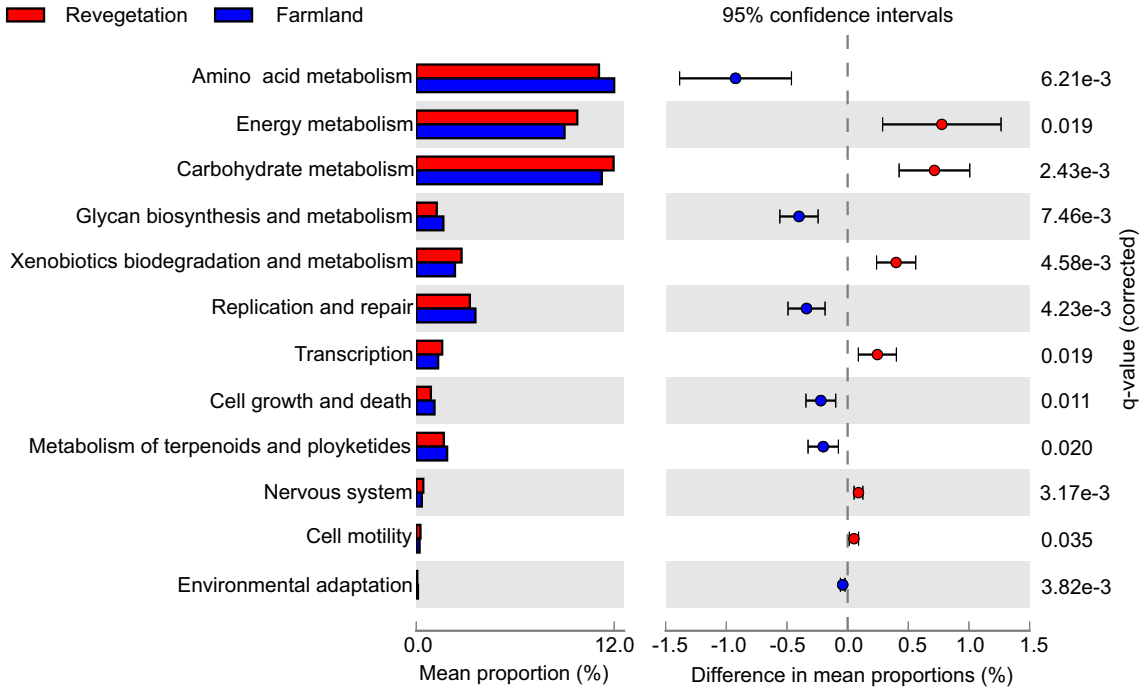

Fig. 1. STAMP analysis of relative abundance of functional gene categories at KEGG level 2 obtained from the revegetated soils (5-, 15- and 30-year old) versus farmland soils. *P*-values were corrected using Benjamini-Hochberg false discovery rate ($P < 0.05$).

activities (0.16%) and carbohydrate-binding modules (0.63%). When the CAZyme genes of the sites were taken together, revegetated soils were associated with significantly increased GH3, GH12, GH13, GH15, GH42, GH50, GH63, GH65, GH92 and GH103 enzyme subfamilies, whereas only two enzyme subfamilies (GH9 and GH94) were more abundant in farmland soils (Fig. 2). In addition, these genes showed a similar trend when the revegetated soils were compared with farmland soils, respectively (Sup. Fig. 4).

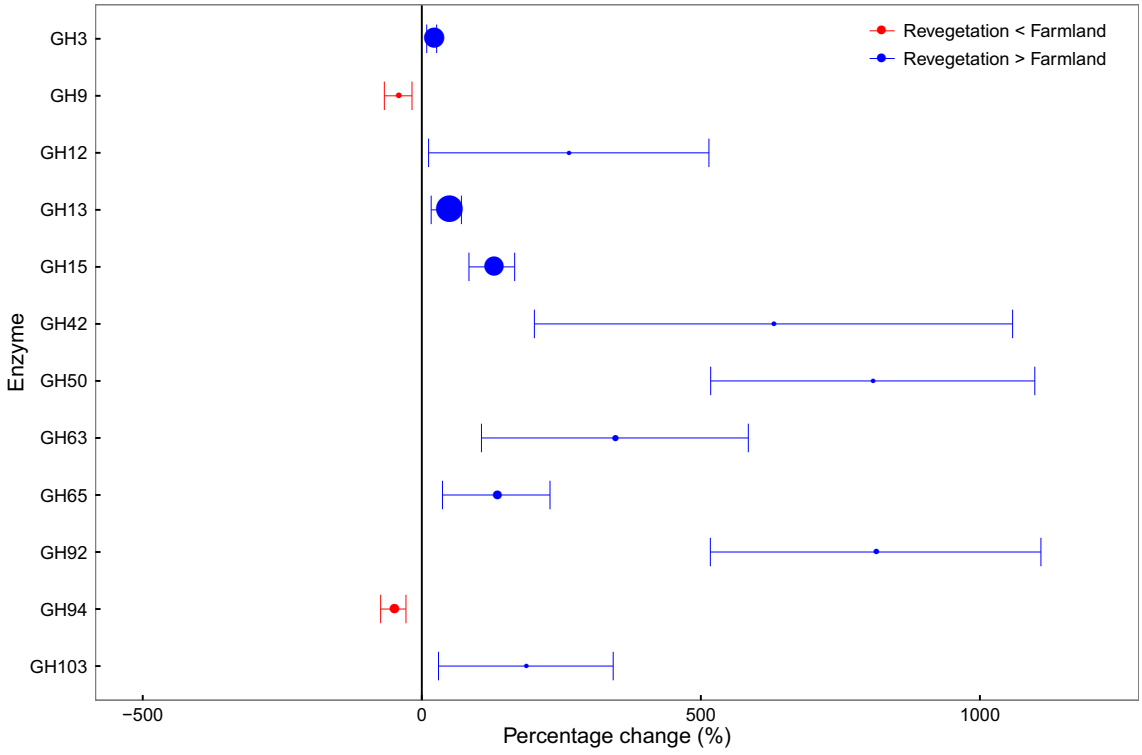

Fig. 2. Impact of revegetation on the relative abundance of polysaccharide-degrading gene subfamilies in soils. Points denote mean percent change in relative abundance of CAZymes between the revegetated soils (5-, 15- and 30-year old) and farmland soils. Symbol size is proportionate to the relative abundance of those gene subfamilies. *P*-values were corrected using Benjamini-Hochberg false discovery rate ($P < 0.05$).

3.4. Microbial taxonomic and functional community structure

Compared with farmland, we found that all three revegetated sites had lower bacterial taxonomic diversity, which increased with restoration age (Fig. 3a), but significantly higher microbial taxonomic diversity (Fig. 3b, calculated at metagenomic species level, $P < 0.05$). Microbial functional diversity was higher in the 30-year old restored grassland than farmland and the 5-year and 15-year restored grasslands (all $P < 0.05$; Fig. 3c, calculated at KEGG level 3). These alpha diversity indices of each site were also shown in Sup. Table 2. Linear regression analysis revealed that bacterial taxonomic diversity and microbial functional diversity were independent of plant diversity (Fig. 4a, c), whereas microbial taxonomic diversity significantly correlated with plant diversity (Fig. 4b).

PCoA showed that taxonomic and functional composition of the three revegetated sites tended to be distinct from those of farmland (Fig. 5). PERMANOVA indicated that soil microbial community structure differed significantly between revegetated soils and farmland soils for both taxonomy ($F = 6.903$, $P = 0.003$ for bacterial OTUs and $F = 9.368$, $P = 0.006$ for metagenome) and function ($F = 3.695$, $P = 0.012$).

3.5. Relationships between microbes and soil properties

We found that all the soil chemical variables varied among the land use sites ($P < 0.05$; Sup. Table 3). Soil OM, TN and AN content increased with restoration age, whereas pH decreased. The 30-year soils had significantly higher soil OM and TN and lower pH than 5-year, 15-year and farmland soils ($P < 0.05$). Taxonomic diversity calculated from sequences of bacterial amplicon and metagenome was not influenced by the soil chemical variables ($P > 0.05$), but functional diversity was correlated with soil pH ($r = -0.740$, $P < 0.05$), OM ($r = 0.764$, $P < 0.05$) and TN ($r = 0.674$, $P < 0.05$) (Sup. Table 4). We found that the major bacterial phyla and prevalent functional categories were also correlated with the soil chemical variables (Sup. Tables 5, 6).

RDA showed that soil OM, AN and AK were the most important soil variables influencing the structural variation in communities of bacterial OTUs, taxonomic and functional metagenomic sequences (explained for 58.6%, 71.0% and 68.4%, respectively) (Fig. 6). Among all the correlated soil chemical properties, OM explained the most proportion (24.4%, 34.2% and 37.9%) of the total variation in bacterial, microbial taxonomic and functional communities, respectively (Table 2).

4. Discussion

4.1. Taxonomic and functional diversity shifts in revegetated microbial communities

Revegetation is considered the principal land use change on the Loess Plateau of China (Deng et al., 2014), but its impact on the taxonomic and functional diversity of soil microbial communities is less studied in this region. The novelty of our study was that we applied Illumina HiSeq sequencing of 16S rRNA gene and metagenome to integrate taxonomical and functional information for a comprehensive understanding of the revegetation impact. We observed that structure of taxonomic and functional communities were significantly affected by the restoration of farmland to grassland, supporting descriptions of bacterial communities in other sites that used more limited PCR-based approaches (Lozano et al., 2014). We also found that while 16S rRNA gene diversity of bacterial communities increased with age of restored site, it was not correlated with plant diversity. Bacterial diversity could increase with the increase of source pools (mainly carbon) derived from vegetation restoration, whereas competition exclusion might result in hump-backed pattern of plant diversity during revegetation (Grime, 1973). The different models between bacteria and plant diversity suggesting that the driving factors for alpha diversity in belowground bacterial communities differed from drivers of aboveground plant alpha diversity (Prober et al., 2015).

We used shotgun metagenomic sequencing to characterize belowground microbial diversity, because this can better represent soil microbial diversity than bacterial diversity alone. We found that total microbial taxonomic diversity increased with plant diversity and was higher in all the revegetated sites compared with farmland. After the ex-farmland was fenced to encourage natural succession, various plant species colonized the bare soil, and it has been shown that greater plant diversity increases diversity of types of litter and root exudates entering the soil (Wardle, 2006). The associated increases in nutrients become available to microorganisms, leading to a positive plant-microbial diversity relationship (Lange et al., 2015). Farmland soils are assumed to be relatively heterogeneous (Moll et al., 2016), and agricultural practices can positively alter microbial diversity (de Carvalho et al., 2016). However, natural revegetation will increase spatial heterogeneity in soil conditions and soil microbes have a variety of responses to soil heterogeneity (Ettema and Wardle, 2002; Moll et al., 2016). Increased soil heterogeneity of revegetated soils could contribute to diverse of microbes, and this could result in higher microbe diversity than farmland.

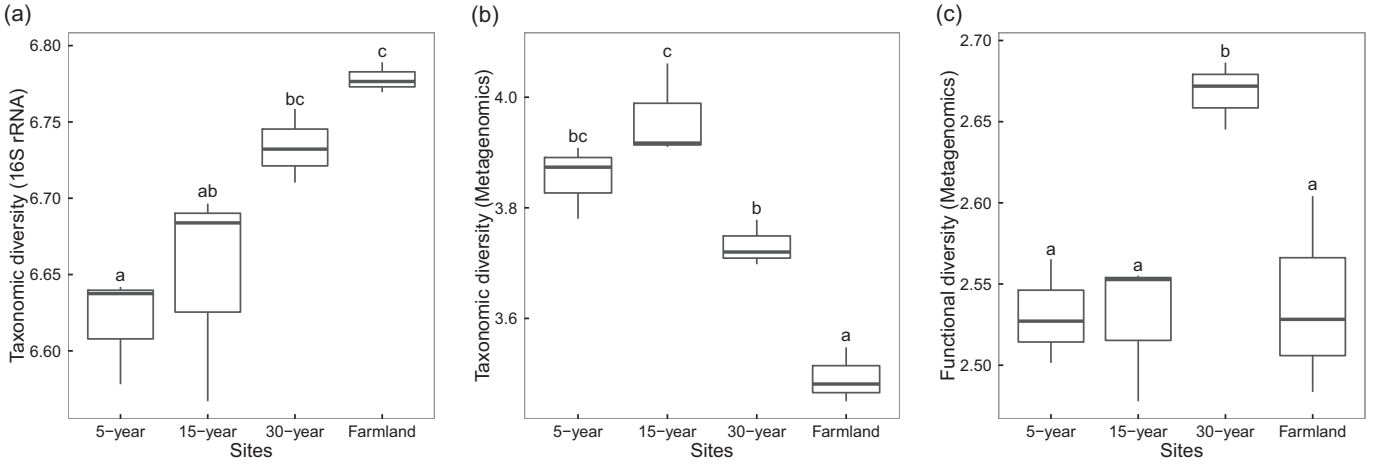

Fig. 3. Taxonomic (a, b) and functional (c) diversity of soil microbial communities in four land use sites. Different letters indicate significant differences between means of three replications ($P < 0.05$).

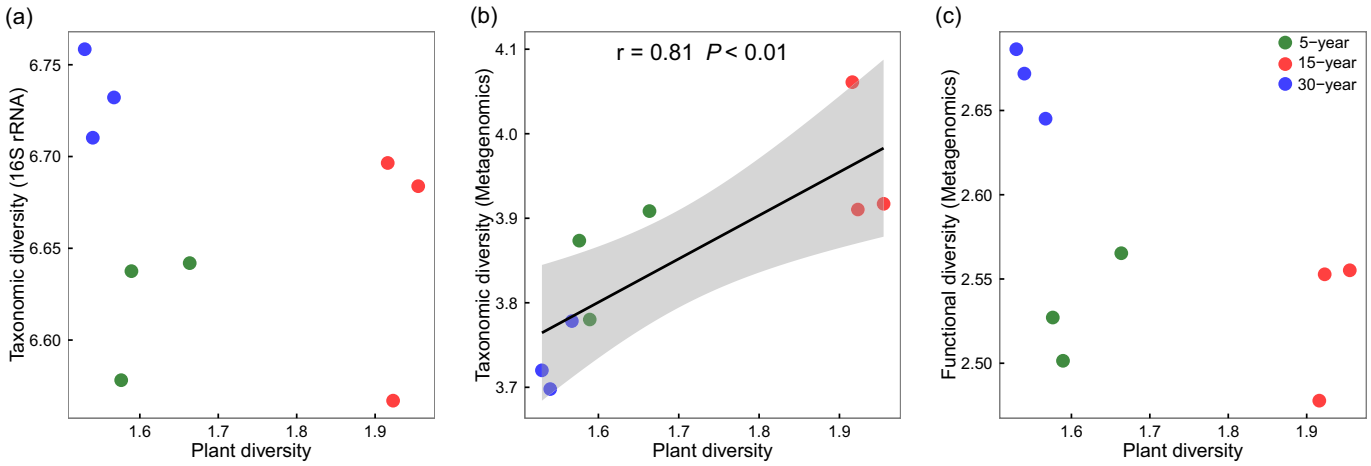

Fig. 4. Relationship between plant diversity and soil microbial taxonomic (a, b) and functional (c) diversity in three revegetated sites.

Revegetated soils have greater environmental heterogeneity than farmland soils, and therefore it was expected that the revegetated soils in our study would have higher microbial functional diversity, as has been previously reported (Mayfield et al., 2010). However, we found that although there was higher taxonomic diversity in the early and mid-successional restoration sites (5-year and 15-year), functional diversity was not different from farmland soil, suggesting there was higher functional redundancy in these two sites. The increase of microbial diversity could provide an adequate trait-redundancy guarantee for specific ecological functions to cope with marked changes in land use, including plant diversity and cover, in these initial and later successional stages (Griffiths and Philippot, 2013). Despite the apparent functional redundancy in the early and mid-successional restoration sites, we found 30-year revegetated sites had significantly higher functional diversity, which perhaps as a result of the adaptation of more diverse functional genes to increasingly heterogeneous soils and other environmental factors linked to secondary succession (Hooper et al., 2000). In general, we found that the taxonomic and functional diversity was higher at the long-term revegetated sites, and it is likely that functional performance is more stable and resilient to environmental perturbations.

4.2. Responses of microbial taxonomic and functional groups to revegetation

As with microbial community diversity, the relative abundance of dominant bacterial phyla was also influenced by revegetation. Phyla abundance varied among the revegetated soils, and there were differences in abundance between these and the farmland soils. Previous research has suggested that the nutrient conditions may enrich copiotrophic and oligotrophic microbial populations (Fierer et al., 2007), and that there are competitive interactions between copiotrophs and oligotrophs (Ramirez et al., 2012). Indeed, we found more abundant Actinobacteria and less abundant Acidobacteria in the restored sites compared with the farmland. Actinobacteria are ubiquitous and commonly be regarded as copiotrophic microbes that enriched in nutrition-rich soils, and play important roles in the soil carbon-cycle (Ramirez et al., 2012) and the gradual increase of Actinobacteria in the restored sites in our study is consistent with the accumulation of soil organ matter during plant secondary succession. In contrast, the farmland soil in our study contained more Acidobacteria, which are known to be oligotrophic and preferentially survive in conditions with low nutrient availability (Fierer et al., 2007). Members of the Proteobacteria are ecologically diverse and contain copiotrophic bacteria (Alphaproteobacteria and Betaproteobacteria) (Koyama et al., 2014) that we found to be more abundant in the revegetated soils. We also found that the farmland soil harbored higher relative abundance of Chloroflexi, Gemmatimonadetes and Firmicutes that are usually found in nutrient-limited and disturbed environments (Barnard et al., 2013; DeBruyn et al., 2011; Rodrigues et al., 2013).

Understanding functional group response to revegetation is essential for the accurate prediction of potential impacts of restoration programs on ecological processes. The difference in functional gene

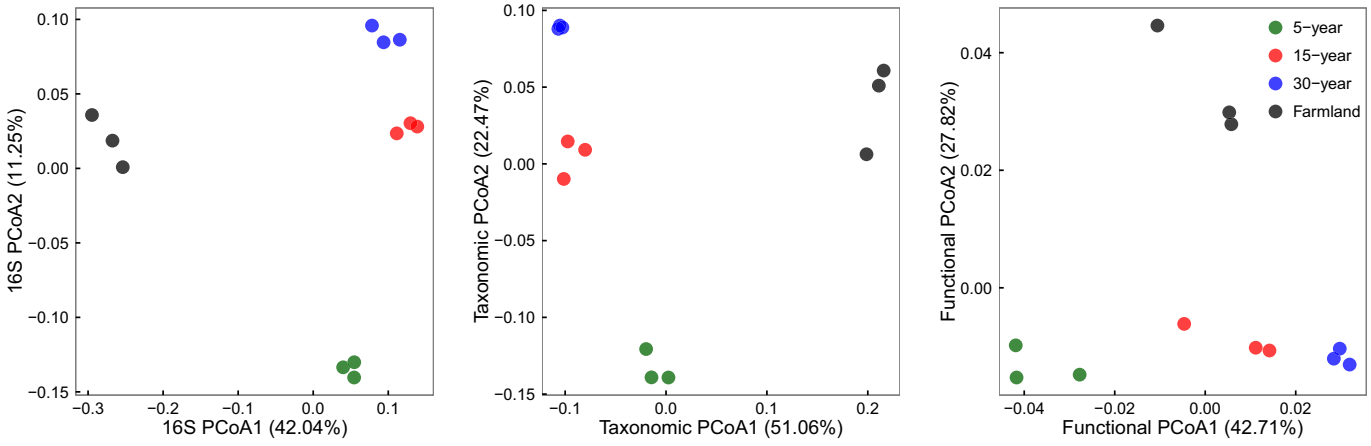

Fig. 5. PCoA plots of microbial community composition of (a) OTUs of 16S rRNA gene sequences, (b) taxonomic profile at species level, and (c) KEGG functional profile at level 3 of shotgun metagenomic sequences from soil samples of four land use sites.

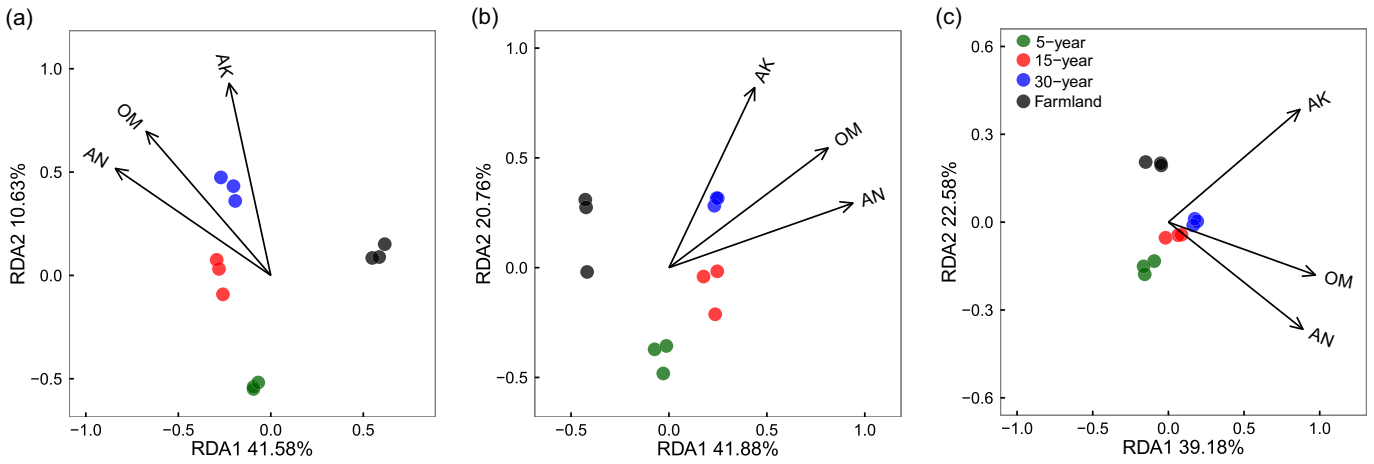

Fig. 6. RDA of microbial community composition of (a) OTUs of 16S rRNA gene sequences, (b) taxonomic profile at species level, and (c) KEGG functional profile at level 3 of shotgun metagenomic sequences from samples of four land use sites. Only significant ($P < 0.05$) soil chemical variables including OM (organic matter), AN (available N) and AK (available K) are shown.

abundances between the revegetated and farmland soils in our study were directly related to the contrasting land use types. For example, soil tillage and the lack of plant cover in crop seedling and harvest stages would lead to seasonal water stress for soil microbes, and that was confirmed by the higher relative abundance of genes responsible for amino acid metabolism in farmland soils (Fierer et al., 2012). Likewise, agricultural practices, including tillage, fertilization and the use of machinery for crop management would disturb soil microenvironment, and the genome stability of soil microbes can be threatened by environmental variations (Mendes et al., 2015). Therefore, farmland soil microbes could enrich more abundant genes associated with replication and repair, cell growth and death and environmental adaptation to cope with these disturbances as we found. Compared with farmland soils, gene sequences related to energy metabolism and carbohydrate metabolism were more abundant in revegetated soils. Restored habitats with higher nutrient availability are more favorable for microbial activities, and this can accelerate energy metabolism and nutrient cycling. Moreover, the plants in these sites create sources of organic carbon, and more enriched aromatic compounds associated with these plants may result in greater abundance of genes responsible for xenobiotic biodegradation and metabolism (Grandy and Neff, 2008).

We found that revegetation not only influenced functional group abundance, but also altered the capacity of microbial communities for biomass degradation. A detailed analysis of CAZYme profiles revealed that revegetation increased the relative abundance of carbohydrate-degrading genes, among which, the most abundant were the GH3, GH13 and GH15 subfamilies. The GH3 subfamily is responsible for many activities, for example, β glucosidase, xylan1, 4 β xylosidase, β glucosylceramidase and β *N* acetylhexosaminidase, and is mainly involved in the degradation of hemicellulose and modification of antibiotic molecules (Cardenas et al., 2015; Faure, 2002), while the GH13 subfamily is responsible for activities, such as, α amylase, pullulanase and cyclomaltodextrin glucanotransferase and the GH15 subfamily is responsible for glucoamylase, glucodextranase, α, α trehalase and dextran dextrinase activities (Cantarel et al., 2009). The higher abundance of these increased carbohydrate-degrading genes in the revegetated sites was probably due to the higher levels of plant biomass, and it is likely that there would be an associated, substantial alteration to carbon cycling at these sites.

Table 2
Community variances explained by soil chemical properties (RDA using forward selection followed by 999 permutation tests).

Variable	16S rRNA		Metagenomic taxonomic profile		Metagenomic functional profile	
	R^2	*P*	R^2	*P*	R^2	*P*
OM	**0.244**	**0.003**	**0.342**	**0.001**	**0.379**	**0.001**
AN	**0.164**	**0.012**	**0.180**	**0.003**	**0.099**	**0.049**
AK	**0.178**	**0.012**	**0.188**	**0.002**	**0.206**	**0.003**
Residuals	0.414		0.290		0.316	
Total	1.000		1.000		1.000	

OM, organic matter; AN, available N; and AK, available K. Bold values denote significant at $P < 0.05$.

4.3. Relationships between microbial communities and soil chemical variables

Soil characteristics are one of the most important factors shaping the soil microbial communities (Thomson et al., 2015). Previous studies have demonstrated the crucial role of soil chemical variables in shaping microbial communities during vegetation restoration. For example, Kuramae et al. (2010) reported that pH can drive the change of microbial successional trajectories in restored chalk grasslands and bacterial community composition of revegetated of post-mining soils were shown to respond to associated increases in soil organic matter, total nitrogen, total phosphorus and available potassium (Li et al., 2014).

The positive impact of revegetation on soil organic carbon sequestration is widely observed (Wang et al., 2011). Our study showed that soil OM and TN content at the revegetated sites increased with successional stage, a result that is consistent with previous work on a Minnesota sand plain (Knops and Tilman, 2000). We found that OM had a positive correlation with the relative abundance of Actinobacteria, but a negative correlation with Acidobacteria. The change of OM during revegetation may explain the difference in the abundance of these microbes, because OM is known to affect copiotrophs and oligotrophs (Fierer et al., 2007). Although there were not significant correlations between soil chemical properties and microbial taxonomic diversity, microbial functional diversity was found to be strongly correlated with pH, OM and TN. Moreover, microbial functional categories (including carbohydrate and energy metabolism, and biodegradation and metabolism of xenobiotics) showed correlations with soil OM and TN. These results indicate that the differences observed in microbial functional diversity and gene categories between the revegetated and farmland soils are likely to be due to the effects of soil properties, and the functional changes might influence the nutrient cycle processes.

The quantity and quality of soil OM are important in regulating soil microbial functional processes (Ding et al., 2015). In our study, there were changes in the richness and biochemical diversity of plant detritus in the restored sites, especially in OM, as a result of the colonization of new plant species and community succession, and these changes

appeared to shape the microbial taxonomic and functional communities. The RDA analysis revealed the significant effect of soil OM on community structure of 16S rRNA gene sequences and total microbial taxonomic and functional structure at our study sites. Many land use change studies have consistently demonstrated the key role of soil OM in structuring microbial communities, including revegetation (Smith et al., 2015) and deforestation (Navarrete et al., 2015; Tripathi et al., 2016). Fundamentally, revegetation significantly influenced the amount of soil OM, which in turn affected microbial diversity and community composition. Thus, soil OM could be a good predictor for microbial community structure change in revegetation restoration programs.

5. Conclusions

Our results suggest that after 30 years of succession, a revegetation restoration program on the Loess Plateau altered the taxonomic and functional structure of soil microbial communities, improved the total microbial taxonomic diversity and significantly increased the functional diversity. During the process of vegetation colonization and establishment, microbial taxonomic diversity increased with plant diversity, and it is likely that the accumulated soil nutrients derived from increased plant biomass stimulated nutrient cycling potential. We suggest that the altered microbial communities could have profound impacts on ecological processes. Our study provides a comprehensive understanding of the belowground microbial taxonomic and functional responses to revegetation restoration programs in semi-arid areas.

Conflict of interest

No conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.171.

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