Soil Biology & Biochemistry 97 (2016) 40–49

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau

Chao Zhang [a, *], Guobin Liu [a, b], Sha Xue [a], Guoliang Wang [a]

[a] State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, 712100, PR China
[b] Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, 712100, PR China

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form
19 February 2016
Accepted 19 February 2016
Available online 2 March 2016

Keywords:
Bacterial diversity
16S rRNA sequencing
Plant community
Secondary succession
Loess Plateau

ABSTRACT

The effects of natural succession on plant communities and soil variables have been established, but changes in microbial communities and their response to plants and soils have not been well characterized in secondary succession. We investigated the changes in soil properties and plant and soil microbial communities during the secondary succession on abandoned cropland in the Loess Plateau of China using high-throughput sequencing of the 16S rRNA gene. The study analyzed a chronosequence of farmland undergoing spontaneous succession after being abandoned for 0 (farmland), 5, 10, 15, 20 and 30 years(y). Plant community metrics including percent cover, and above/belowground biomass, first decreased in the initial stage (<10 y) and then increased during the succession. *Proteobacteria*, *Acidobacteria*, *and Actinobacteria* were the dominant phyla of soil bacteria across all succession. Bacterial communities transitioned from *Acidobacteria*-dominant to *Proteobacteria*-dominant communities during the 30 years of succession. Levels of soil organic carbon (C), total nitrogen (N), nitrate N and bacterial diversity were lower soon (<5 years) after abandonment compared to the farmland, but they could recover to farmland levels after 15–20 years and were much improved after continued succession. Plant and bacterial community diversities (Shannon index and species richness) changed along successional time, but they showed different patterns, suggesting an incongruous process between plant and microbial succession. Organic C, total N, available N, and available P contents were significantly correlated with the abundance of most bacterial groups and the Shannon index, indicating the dependence of bacterial community diversity on soil nutrient supply.

1. Introduction

Plant secondary succession without human disturbance is an effective way to improve soil conditions and restore degraded environments (Walker et al., 2007; An et al., 2009; Zhang et al., 2015). Several studies have suggested that plant secondary succession occurs in these extreme environments (Urbanová et al., 2011; Xu et al., 2012), however, our understanding of secondary processes in arid and semiarid ecosystems is still poor, especially in the more temperate regions (Abella, 2010) where species diversity and composition usually changed rapidly (Lozano et al., 2014). Plant growth during the establishment of vegetation community in degraded ecosystems is most commonly limited by the shortage of mineral nutrients. Specifically, since soil microorganisms transform organic substrates, release mineral elements, and they may strongly influence the growth of plants during secondary succession. Plants, in turn, import carbon (C) and nitrogen (N) to the soil subsystem in the form of litter and root exudates, and specifically select for heterotrophic microbial communities (Singh et al., 2004). Plant succession is essentially the interaction between aboveground plants and belowground microorganisms (Kardol et al., 2006; Kuramae et al., 2011).

A variety of culture based studies have examined shifts in the structure and activity of microbial communities in and across vegetated stages of secondary succession (Chan et al., 2008; Banning et al., 2011; Kuramae et al., 2011; Zhang et al., 2015). These studies have partly characterized microbial succession across successional time but were not able to fully and accurately identify

* Corresponding author. Northwest A&F University, Yangling, Shaanxi, 712100, China. Tel./fax: +86 29 87012872.
E-mail address: zhangchaolynn@163.com (C. Zhang).

http://dx.doi.org/10.1016/j.soilbio.2016.02.013
0038-0717/

specific functional microbial groups, because many microbes are not easily isolated from environmental soils by traditional approaches (Prosser, 2002; Bailly et al., 2007). The structure and diversity of belowground microbial communities during ecological restoration have thus remained essentially a gray box. Next-generation sequencing technologies have recently offered new opportunities for studying belowground communities by profiling microbial composition at the species level. Microbial community composition is affected by soil factors such as pH, C, N and phosphorus (P) contents (Koranda et al., 2011; Shen et al., 2013; Li et al., 2014a), plant factors such as vegetation type (Oh et al., 2012), plant–microbial interactions (Knelman et al., 2012), and land-use history and restoration time (Jangid et al., 2013). Plant community structure and soil variability are partly responsible for the changes to soil microbial communities (Mahnert et al., 2015), but the relationships among plants, soils and microorganisms remain unknown.

The Loess Plateau, a temperate semiarid area, in the upper and middle region of the Yellow River in China has an area of 6.2×10^5 km^2. The loessial soil is prone to erosion by wind and water, and this region has suffered serious soil erosion where most of the topsoil has been lost. Abandoning sloped farmland to allow secondary succession is a traditional practice widely used for preventing soil erosion and for the rehabilitation of ecological environments on the plateau. Many croplands with slopes >15° have been abandoned for natural recovery without anthropogenic interference. The effects of succession on soil physicochemical properties, microbial dynamics, and enzymatic activities have been reported (An et al., 2008; Wang et al., 2009; Liang et al., 2010; Zhang et al., 2015), but information on the relationship between soil variables and plant and soil microbial communities is still scarce. This information is essential for understanding the essence of secondary succession and for the appropriate management and conservation of the ecological environment.

The present study investigated the dynamics of soil properties, plant communities and the structure of soil bacterial communities at sites representing 30 years of secondary succession on abandoned cropland in the Loess Plateau using 16S rRNA high-throughput sequencing. Our objective was to (i) evaluate the patterns of change in soil properties, plant communities, and soil bacterial communities during secondary succession after agricultural abandonment, (ii) to determine if changes between the plant and microbial communities are congruous, and (iii) determine the response soil bacterial communities to plant community and soil properties along the chronosequence.

2. Material and methods

2.1. Study site and sampling

A field experiment was carried out in natural grassland ecosystems. These sites were in the Zhifanggou Ecological Restoration Watershed (109°16′E, 36°46′N), near the Ansai Soil and Water Conservation Research Station on the Loess Plateau, where the mean annual temperature is 8.8 °C, with a mean minimum temperature in January of −6.2 °C and a mean maximum temperature in August of 37.2 °C. The area has a temperate semiarid climate. Mean annual precipitation is 510 mm, with over 60% falling from July to September. The soil, derived from wind-blown deposits, is classified as a Huangmian soil (a Calcaric Cambisol in the FAO classification). This area has been used as an experimental field base by the Chinese Academy of Sciences to monitor vegetation restoration.

The substitution of space for time, a common method in ecosystem research, is an effective way to investigate the changes in soil conditions and plant communities during natural succession (Felske et al., 2000; Tscherko et al., 2004; An et al., 2009; Walker et al., 2010; Jangid et al., 2013; Williams et al., 2013). We used this method to study the effect on soil bacterial communities of abandoning cropland for natural recovery. Five sloped farmlands abandoned for 5, 10, 15, 20, and 30 years were chosen as experimental sites based on their well-dated successional chronosequence. These sites had similar slope gradients, slope aspects, elevations, and previous framing practices. The main crops grown in these farmlands before abandonment were millet (*Setaria italica*) and soybean (*Glycine max*) in rotation. An active sloped farmland growing millet and soybean was used as a reference. The crops were manually harvested, and the plots at this site were manually plowed to a depth of 20 cm each year after harvesting. The sloped farmland was fertilized each year with 6.0 t ha^{-1} goat manure, 60 kg ha^{-1} N and.45 kg ha^{-1} phosphorus pentoxide (P_2O_5). Properties of the experimental sites are presented in Table 1.

Three 20 m × 20 m plots were established at each site in September 2014. Six 1 m × 1 m quadrats were randomly selected in each plot for measuring the characteristics of the vegetation. Plant coverage, aboveground/belowground biomass, and maximum/mean height were separately measured for each species in each quadrat. The plant was dug up and the aboveground parts were clipped and dried to obtain the aboveground biomass, and the roots were washed with tap water and dried at 70 °C for 48 h to obtain belowground biomass. Shannon diversity index of plant community (H_{Plant}) was calculated using the equation by Tscherko et al. (2004), where P_i is the relative abundance of each species in total sum, and n is the number of species. The number of species was used to estimate the richness (S_{Plant}).

Soil samples were collected from the top 20 cm of the soil profiles by a stainless steel corer 5 cm in diameter after the litter horizons were removed. All selected sampling points were free of lichens, biological crusts, and/or any other vegetation within a radius of 0.75 m. Twelve soil cores were collected along an S-shaped pattern from each plot and then mixed to form one sample. Visible plant roots, stones, litter, and debris were removed from each soil sample, which was then divided into two subsamples. One subsample was immediately stored at −80 °C for DNA analysis, and the other sample was air-dried for physicochemical analysis.

2.2. Soil chemical parameters

Soil organic carbon (OC) content was determined using the Walkley-Black method (Nelson and Sommers, 1982), and total nitrogen (TN) content was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). Total phosphorus (TP) content was determined by melt-molybdenum, antimony, and scandium colorimetry, and available phosphorus (AP) was measured by the Olsen method (Olsen and Sommers, 1982). Ammonium nitrogen ($NH_4^+ - N$) and nitrate nitrogen ($NO_3^- - N$) were determined following extractions of fresh soil with 2 M KCl for 18 h and were analyzed colorimetrically on an Alpkem Autoanalyzer (OI Analytical, College Station, USA). Soil pH was determined by an automatic titrator (Metrohm 702, Swiss) in 1:2.5 soil: water suspensions.

2.3. DNA extractions and Illumina MiSeq high-throughput sequencing

DNA was extracted from 0.5 g of soil using a FastDNA spin kit for soil (MP Biomedicals, Cleveland, USA). The DNA extracts were diluted tenfold and assessed for quality and quantity using a spectrophotometer (NanoDrop ND-1000, NanoDrop Technologies, Wilmington, USA). The integrity of the DNA extracts was confirmed by 2% agarose gel electrophoresis. DNA was amplified by PCR in

Table 1
Geographical features and floristic composition of the sampling sites. All sites contained are loessial soil.

Abandoned site	Slope aspect	Slope gradient	Altitude (m)	Vegetation community	Minor species
Farmland	E15°N	25°	1274	*S. italic* + *G. max*	*S. collina*; *P. australis*
5-y	W10°N	20°	1303	*A. capillaries*	*H. altaicus*; *S. collina*., *P. annua*
10-y	E40°N	26°	1276	*A. capillaries* + *H. altaicus*	*L. davurica*, *P. bifurca*, *P. annua*, *C. squarrosa*
15-y	E25°N	28°	1307	*A. sacrorum* + *S. bungeana*	*L. davurica*, *P. annua*, *C. Squarrosa*, *V sepium*, *P. annua*, *S. collina*; *P. heterophylla*
20-y	E10°N	30°	1267	*S. bungeana* + *A. sacrorum*	*S. bungeana*, *H. altaicus*, *V. sepium*, *P. annua*, *L. davurica*
30-y	E16°N	30°	1246	*A. sacrorum*	*L. davurica*, *P. bifurca*, *O. bicolor*, *S. Collina*, *V. sepium*.

S. italic: Setaria italic; G. max: Glycine max; A. capillaries: Artemisia capillaries; H. altaicus: Heteropappus altaicus; A. sacrorum: Artemisia sacrorum; S. bungeana: Stipa bungeana; S. collina: Salsola collina; P. australi: Phragmites australis; L. davurica: Lespedeza davurica; P. bifurca: Potentilla bifurca; P. Annua: Poa annua; C. squarrosa: Cleistogenes squarrosa; V. sepium: Vicia sepium; P. heterophylla: Patrinia heterophylla; P. bifurca: Potentilla bifurca; O. bicolor: Oxytropis bicolor.

triplicate using primers for the 16S rRNA gene. The 520F (5′-AYTGGGYDTAAAGNG -3′) and 802R (5′-TACNVGGGTATCTAATCC-3′) primers (Mahnert et al., 2015) were designed to amplify the hypervariable V4 region of the 16S rRNA gene from the bacteria. The primers were tagged with unique barcodes for each sample. PCR reactions were performed in a volume of 25 μl containing 8.75 μl of sterile ultrapure water, 0.25 μl of polymerase at 5 U/μl (TransStart Fastpfu DNA Polymerase, TransGen), 2.00 μl of dNTPs (2.5 mM each), 5.0 μl of 5 × high enhancer, 5.0 μl of 5 × Ex Taq Buffer, 1 μl of each primer at 10 μM, and 2 μl of template DNA (0.2 ng/μl). The PCR procedure was: 98 °C for 30 s; 27 cycles of 98 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s; 72 °C for 5 min; and hold at 4 °C. Sterile ultrapure water was used as negative controls for detecting primer or DNA contamination. Successful PCR amplification was verified by 2% agarose gel electrophoresis. The triplicate amplicons were pooled and purified by gel extraction and quantified using Quant-iT™ PicoGreen kit (Invitrogen, Carlsbad, USA). The purified PCR amplicons were then mixed at equimolar ratios for sequencing analysis. Sequencing was conducted on the Illumina MiSeq platform (Illumina Corporation, San Diego, USA). Approximately 60,000 high-quality sequences per sample with average lengths of approximately 230 bp were produced.

2.4. Processing of sequencing data

The sequences were quality-filtered and chimera checked using the Quantitative Insights Into Microbial Ecology (QIIME) workflow (Caporaso et al., 2010a). In brief, sequences <150 bp and reads containing ambiguous bases or any unresolved nucleotides were removed. Sequences with the same barcode were sorted into the same sample (Caporaso et al., 2010a). Raw flowgrams (sff files) were filtered and noise and chimeras were identified by the MOTHUR program with the UCHIME algorithm (Schloss et al., 2009; Edgar et al., 2011). The remaining sequences were clustered by complete-linkage clustering using the UCLUST method (Edgar, 2010) and assigned to OTUs at similarities of 97%. Indices of community diversity Chao 1 estimator, abundance-based coverage estimator (ACE) were calculated, and rarefaction curves were obtained using MOTHUR (http://www.mothur.org/). Finally, the most abundant sequence in each OTU cluster was selected as a representative sequence and was aligned using PyNAST method (Caporaso et al., 2010b). The taxonomic identity of each phylotype was determined by the bacterial 16S rRNA Silva reference database (http://www.arb-silva.de) using the RDP naïve Bayesian classifier (Wang et al., 2007). The accuracy of the classifier results comparing the representative sequences of the dominant bacteria to the non-environmental sequences and non-metagenomes in the NCBI nucleotide database was assessed using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov) (Altschul et al., 1990).

2.5. Statistical analysis

A one-way analysis of variation (ANOVA) and a least significant difference (LSD) multiple comparison ($P < 0.05$) were used to assess the significant effects of successional age on the plant characteristics (coverage, above- and belowground biomasses, plant diversity, and species richness), soil properties, and microbial composition and diversity. Statistical analyses of the data were carried out using SPSS 11.5 for Windows (SPSS Inc, Chicago, USA). Principal coordinates analysis (PCoA) was used to evaluate the overall differences in the structures of the plant and microbial communities based on Bray-Curtis distances between successional stages. Shannon diversity index of bacterial community (H_{Bacteria}) was calculated using the equation same to the plant community, where P_i is the relative abundance of each operational taxonomic unit (OTU) in the total sum. The number of OTUs was used to estimate species richness (S_{Bacteria}). Pearson correlation analysis and redundancy analysis (RDA) using Monte Carlo permutation (999 repetitions) was used to test the relationships among the soil properties, plant communities and microbial groups. The data sets were analyzed prior to the RDA using detrended correspondence analyses to confirm that the gradient lengths fit a linear model. Analysis of PCoA, correlation, RDA was conducted by the R software package v.3.2.3.

3. Results

3.1. Vegetation characteristics

The crops on the farmland were harvested, so the plant characteristics were not assessed for this site (Table 2). Plant coverage and above- and belowground biomasses greatly increased within 5 years after the farmland was abandoned then considerably decreased between 5 and 10 years, and again increased with longer successional times. Plant coverage and above- and belowground biomasses were highest at the 30-y site. H_{Plant} and S_{Plant} increased in the first 15 years and then decreased with abandonment time, with a peak at the 15-y site.

The dominant species of the community varied along successional time (Table 3). *Artemisia capillaries* and *Heteropappus altaicus* dominated the community during the early successional stage (first 10 years). The coverage of *A. capillaries* and *H. altaicus* gradually decreased as the succession proceeded, and these species became the companion species at the 15-yr sites and had disappeared at the 30-y sites. Two other perennial species, *Artemisia sacrorum* and *Stipa bungeana*, emerged in the community at the 10-y sites, gradually became the dominant species at the 15-yr site, and co-dominated the plant community from 15 to 30 years. *A. sacrorum* was strongly dominant at the 30-y site, covering 38.9% of the area and accounting for more than half the total cover (57.0%). The plant community generally transitioned from dominance by *A. capillaries*

Table 2
Characteristics of the vegetation during the succession.

Abandoned site	Coverage (%)	Aboveground biomass (g cm^{-3})	Belowground biomass (g cm^{-3})	H_{Plant}	S_{Plant}
Farmland[a]	–	-	-	NA	NA
5-y	72.6 ± 2.8 a	202.6 ± 27.5 b	38.0 ± 3.7 d	2.02 ± 0.15 c	4 ± 2 c
10-y	33.0 ± 3.5 d	43.1 ± 4.3 e	17.3 ± 3.8 e	2.36 ± 0.10 b	9 ± 1 b
15-y	42.9 ± 2.2 c	88.5 ± 17.8 d	83.6 ± 11.5 c	2.51 ± 0.08 a	19 ± 2 a
20-y	47.6 ± 3.3 b	126.7 ± 10.4 c	188.3 ± 29.8 b	2.42 ± 0.14 ab	16 ± 2 ab
30-y	68.2 ± 4.0 a	362.9 ± 50.9 a	576.9 ± 130.5 a	2.28 ± 0.10 bc	11 ± 2 b
F	67.53	120.41	89.63	11.27	14.77
P	<0.001	<0.001	<0.001	0.014	0.008

[a] Coverage, aboveground biomass, and belowground biomass were not taken in to account because the farmland was harvested. H_{Plant}: Shannon diversity index of plant community. S_{Plant}: species richness of plant community. NA: not applicable. Values are means ± standard error (n = 3). Different letters indicate significant differences ($P < 0.05$) among soils for the individual variables based on a one-way ANOVA followed by an LSD test.

Table 3
Coverage of plant species at the different successional stages.

Coverage (%)	Farmland	5-y	10-y	15-y	20-y	30-y
Artemisia capillaries	–	45.2 ± 3.6 a	10.2 ± 1.7 b	2.4 ± 1.2 c	0.1 ± 0.1 d	–
Heteropappus altaicus	–	17.6 ± 2.0 a	15.8 ± 0.9 a	1.4 ± 0.7 ab	0.9 ± 0.3 b	–
Salsola collina	–	7.1 ± 0.7 a	0.6 ± 0.2 c	0.2 ± 0.1 c	1.6 ± 1.0 b	–
Phragmites australis	–	–	3.4 ± 0.4 b	6.7 ± 0.6 a	2.7 ± 0.1 b	0.9 ± 0.4 c
Lespedeza davurica	–	–	0.6 ± 0.1 a	0.8 ± 0.2 a	0.8 ± 0.2 a	–
Potentilla bifurca	–	–	–	3.7 ± 0.4 a	5.1 ± 0.5 a	2.9 ± 0.8 a
Poa annua	–	0.1 ± 0.1 c	0.2 ± 0.2 c	1.3 ± 0.3 ab	1.6 ± 0.2 a	0.9 ± 0.4 b
Cleistogenes squarrosa	–	–	–	2.4 ± 0.1 a	2.7 ± 0.2 a	2.4 ± 0.2 a
Artemisia sacrorum	–	–	1.7 ± 0.5 d	6.9 ± 2.1 c	15.4 ± 2.8 b	38.9 ± 4.1 a
Stipa bungeana	–	–	0.6 ± 0.1 c	4.6 ± 1.1 b	10.8 ± 2.4 a	6.5 ± 1.9 b
Vicia sepium	–	–	–	1.2 ± 0.2 a	–	–
Patrinia heterophylla	–	–	–	0.7 ± 0.2 a	0.7 ± 0.3 a	–
Oxytropis bicolor	–	–	1.2 ± 0.3 b	1.5 ± 0.2 b	0.7 ± 0.3 c	2.7 ± 0.2 a
Setaira viridis	–	–	–	2.4 ± 0.7 a	1.1 ± 0.1 b	2.7 ± 0.3 a
Ixeritis denticulatae	–	–	–	0.1 ± 0.1b	0.8 ± 0.2 b	3.6 ± 0.2 a
Potentilla chinensis	–	–	–	2.1 ± 0.6 a	1.7 ± 0.3 a	1.4 ± 0.2 a
Clematis fruticosa	–	–	0.9 ± 0.4 a	1.2 ± 0.3 a	–	–
Poa sphondylodes	–	–	–	0.7 ± 0.5 a	–	1.1 ± 0.4 a
Spiraea salicifolia	–	–	0.4 ± 0.2 b	0.2 ± 0.2 b	1.4 ± 0.3 a	–

Values are means ± standard error (n = 3). "–"indicates the absence of a plant species in a successional stage. Different letters within a row indicate significant differences ($P < 0.05$) among successional stages based on a one-way ANOVA, followed by an LSD test.

and *H. altaicus* to dominance by *A. sacrorum* and *S. bungeana* during the 30 years of succession. The plot of the PCoA (Fig. 1) suggested a clear difference in plant composition along coordinate 1 among the successional sites.

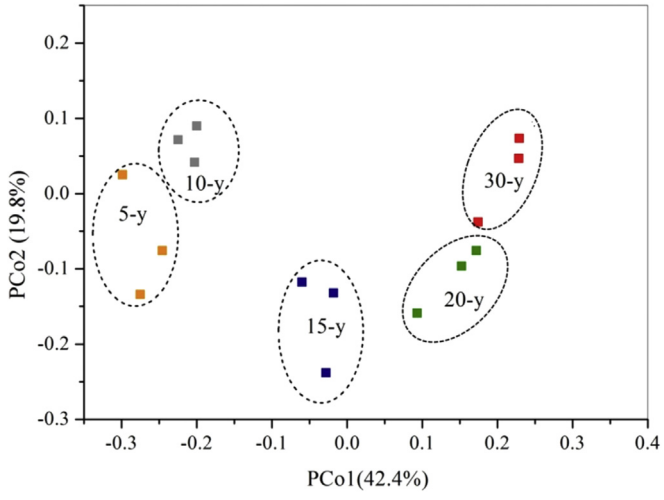

Fig. 1. Principal coordinates analysis (PCoA) of plant composition based on Bray-Curtis distances.

3.2. Soil properties

The contents of soil OC, TN, and NO_3^- – N at the 5-y site decreased dramatically compared to the sloped farmland but then increased significantly with time until reaching the levels of the farmland at the 15- or 20-y sites (no significant difference between these two sites) (Table 4). OC, TN, and NO_3^- – N contents were highest at the 30-y site, increasing by 35.4%, 9.7%, and 16.1%, respectively, compared to the farmland. NH_4^+ – N contents and pH did not differ significantly among the six sites. The content of AP significantly increased at the 5-y site compared to the farmland and subsequently gradually decreased with time. TP varied little along the succession, with a range of 0.54–0.57 mg kg^{-1}, although some differences were statistically significant. Soil bulk density tended to decrease as abandonment time increased.

3.3. Microbial diversity and composition

3.3.1. Diversity of bacterial communities

The OTUs were clustered at distances ≤0.03 (about 97% sequence similarity) for calculating the diversity indices. The ACE and Chao 1 estimators and the Shannon index indicated that the diversity of the soil bacterial community at the 5-y site decreased dramatically compared to the farmland, then increased significantly with time, and remained stable after 20 years (Table 5). The three indicators were significantly higher at the 30-y site than the farmland. This conclusion was supported by the rarefaction curve

Table 4
Soil physicochemical properties during the succession.

Abandoned sites	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Available P (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+ – N (mg kg^{-1})	pH	Bulk density (g cm^{-3})
Farmland	3.39 ± 0.09 c	0.41 ± 0.02 b	0.54 ± 0.00 bc	1.68 ± 0.11 b	4.72 ± 0.08 b	10.62 ± 0.72 a	8.47 ± 0.02 a	1.27 ± 0.01 a
5-y	2.66 ± 0.10 d	0.31 ± 0.01 d	0.57 ± 0.01 a	1.99 ± 0.07 a	3.67 ± 0.19 c	8.54 ± 1.16 a	8.53 ± 0.02 a	1.25 ± 0.01 ab
10-y	3.03 ± 0.03 d	0.32 ± 0.01 cd	0.56 ± 0.00 ab	1.78 ± 0.01 ab	3.80 ± 0.23 c	8.95 ± 0.31 a	8.68 ± 0.22 a	1.20 ± 0.00 abc
15-y	3.44 ± 0.02 c	0.34 ± 0.01 c	0.54 ± 0.01 bc	1.16 ± 0.12 c	4.13 ± 0.20 c	10.37 ± 1.02 a	8.68 ± 0.21 a	1.18 ± 0.04 bcd
20-y	4.08 ± 0.05 b	0.41 ± 0.00 b	0.55 ± 0.01 abc	0.73 ± 0.02 d	4.81 ± 0.05 b	10.60 ± 0.91 a	8.49 ± 0.00 a	1.15 ± 0.04 cd
30-y	4.59 ± 0.29 a	0.45 ± 0.00 a	0.54 ± 0.00 c	0.59 ± 0.03 e	5.48 ± 0.21 a	11.02 ± 1.20 a	8.54 ± 0.03 a	1.14 ± 0.02 d
F	27.93	31.49	3.66	62.50	15.86	1.18	0.58	5.09
P	<0.001	<0.001	0.031	<0.001	<0.000	NS	NS	0.01

Values are means ± standard error (n = 3). NS: not significant.
Different letters indicate significant differences ($P < 0.05$) among soils for the individual variables based on a one-way ANOVA followed by an LSD test.

Table 5
Diversity index of the bacterial communities during the succession.[a]

Abandoned sites	Chao 1 estimator	ACE estimator	$H_{Bacteria}$
Farmland	5726 ± 76 c	5812 ± 55 c	7.14 ± 0.02 c
5-y	5531 ± 39 d	5547 ± 75 d	6.99 ± 0.04 d
10-y	5815 ± 35 bc	5927 ± 54 bc	7.14 ± 0.02 c
15-y	6078 ± 187 b	6162 ± 177 ab	7.19 ± 0.05 b
20-y	6179 ± 35 ab	6242 ± 17 ab	7.25 ± 0.02 ab
30-y	6194 ± 17 a	6296 ± 30 a	7.29 ± 0.05 a
F	9.50	11.39	4.69
P	0.001	<0.01	0.013

[a] Calculations based on the OTUs at 97% sequence similarity. $H_{Bacteria}$: Shannon diversity index of bacterial community. Values are means ± standard error (n = 3). Different letters indicate significant differences ($P < 0.05$) among the soils for the individual variables based on a one-way ANOVA followed by an LSD test.

analysis (Supplementary Fig. S1). The total number of OTUs varied widely among the sites. All rarefaction curves tended to approach saturation at a similarity level of 97%, indicating that the volume of data for the sequenced reads was reasonable, and the discovery of a high number of reads made a small contribution to the total number of OTUs. The saturation at a similarity level of 97% suggested that these communities were adequately sampled.

We compared the Shannon diversity index and species richness for the aboveground vegetation community and the belowground soil bacterial community to gain further insight into the relative diversities of the microbial communities (Fig. 2). The changes of the diversity indices differed significantly between the two communities during the 30 year's succession. H_{Plant} and S_{Plant} increased in the first 15 years and then decreased drastically, with a peak at the 15-y site (Fig. 2a). In contrast, $H_{Bacteria}$ and $S_{Bacteria}$ increased as the abandonment time increased (Fig. 2b).

3.3.2. Compositions of bacterial communities

A total of 1 084 461 high-quality sequences remained from the complete data set after quality trimming and the removal of chimeras (average of 60,247 per sample), of which a total of 7153 OTUs were identified. The dominant phylum was *Proteobacteria* (21.3% on average), followed by *Acidobacteria* (17.0%), *Actinobacteria* (15.3%), *Bacteroidetes* (3.6%), *Verrucomicrobia* (3.6%), *Planctomycetes* (2.7%), and *Firmicutes* (1.6%) (Fig. 3). The relative abundance of *Proteobacteria* decreased at the 5-y site compared to the farmland and then increased with abandonment time. In contrast, the abundance of *Acidobacteria* increased at the 5-y site compared to the farmland and thereafter decreased with time.

We analyzed the dominant phyla further to explore the dynamics of the major microbial taxa along the natural succession. Among the *Proteobacteria* (Supplementary Fig. S2), *Alpha-*, *Beta-*, *Gamma-*, and *Deltaproteobacteria* were found in all samples (Fig. S2a). The members of *Alphaproteobacteria* dominated this

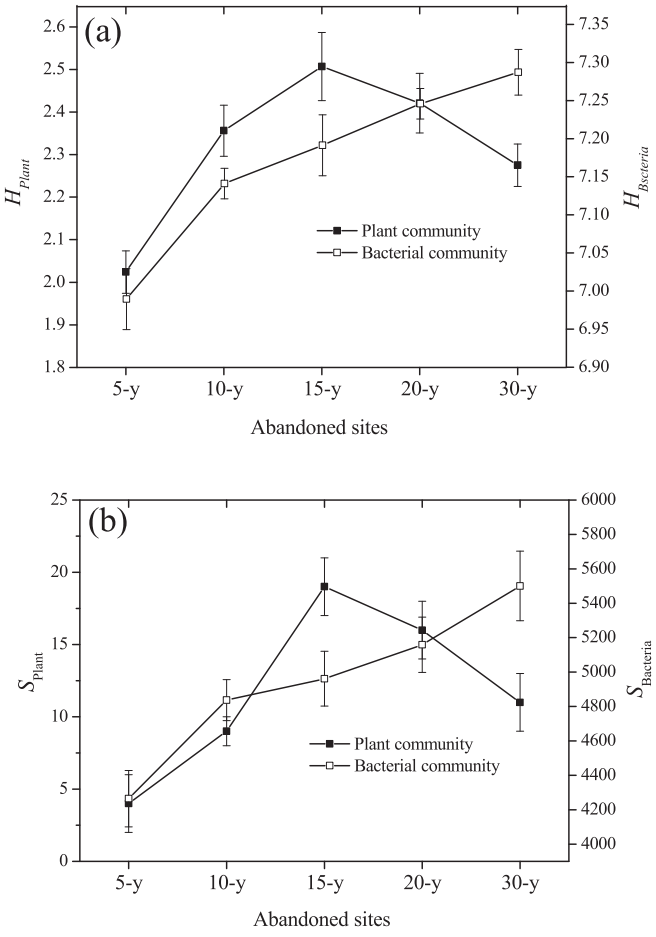

Fig. 2. Comparison of community diversities between plants and bacteria during the succession. (a) Shannon diversity index; (b) Species richness.

phylum, occupied 8.4% of all populations, decreased at the 5-y site compared to the farmland, and then increased with abandonment time. *Betaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria* comprised 4.3%, 1.8%, and 1.0% of the total populations, respectively. At the order level (Fig. S2b), *Rhizobiales* dominated the *Alphaproteobacteria* populations by an average of 5.6% and behaved similarly to *Alphaproteobacteria* along the succession. *Gp4* and *Gp6* were the dominant populations in the *Acidobacteria* phylum and increased in the first 5 or 10 years and subsequently decreased with time (Fig. S3a). *Actinomycetales* and *Sphingobacteriales* dominated the *Actinobacteria* and *Bacteroidetes* phyla, respectively (Fig. S3b, c). The plot of PCoA (Fig. 4) clearly identified variations in bacterial

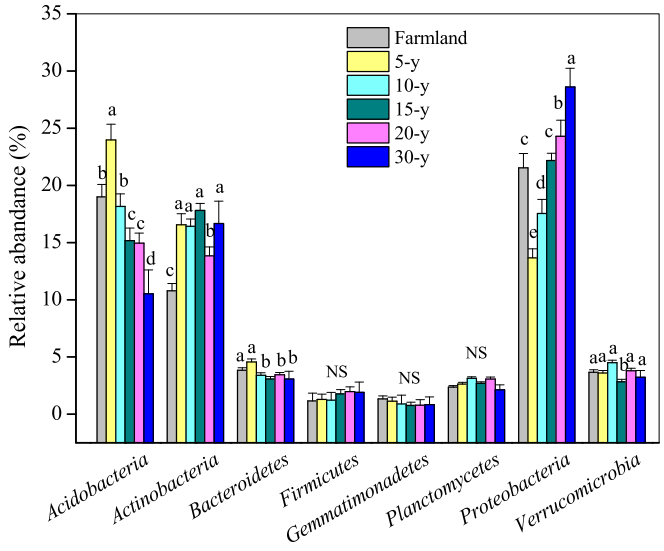

Fig. 3. Relative abundance of the soil bacterial communities at the phylum level. The data for the average relative abundances from three replicates were calculated as the ratio between the abundance of the sequence type and the total number of sequences. All calculations used normalized data. Values are means ± standard error (n = 3). Different letters indicate significant differences ($P < 0.05$) among the successional stages.

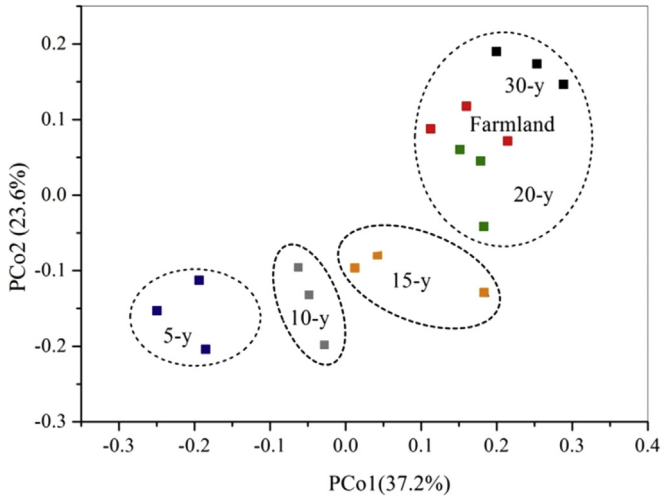

Fig. 4. Principal coordinates analysis (PCoA) of bacterial composition based on Bray-Curtis distances.

community composition among the sites, with the abundance of each group varying with successional time. The profiles of the bacterial communities at the 20- and 30-y sites and the farmland tended to group together and were clearly separated from those at the 5-, 10-, and 15-y sites, which were clearly separated from each other.

3.3.3. Relationships among plant communities, soil properties and bacterial communities

The ordination of PCoA (Figs. 1 and 4) showed clear differences along coordinate 1 in the compositions of the plant and bacterial communities among the successional stages. Total coverage, aboveground biomass, and coverage of dominant species (*A. sacrorum)* were significantly correlated with the relative abundance of the dominant groups (Supplementary Table S1), such as *Proteobacteria, Acidobacteria* and *Verrucomicrobia,* and with $H_{Bacteria}$, but not with H_{Plant}. Soil organic C, total N, and $NO_3^- - N$ contents were correlated positively with the abundance of *Proteobacteria* and *Verrucomicrobia* and with $H_{Bacteria}$ and negatively with the abundance of *Acidobacteria.* AP was correlated positively with the relative abundance of *Actinobacteria* and *Bacteroidetes*, and negatively with the relative abundance of *Proteobacteria.* Bulk density was correlated negatively with *Proteobacteria* and $H_{Bacteria}$. $NH_4^+ - N$ content was not significantly correlated with the abundance of most bacterial groups. The RDA confirmed the results of the correlation analysis and further identified the effect of the plants and soil on the bacterial communities at the order level (Fig. 5). The first two axes explained 71.2% of the total variance, indicating that OC, TN, $NO_3^- - N$, and AP were the most influential factors driving the changes in the composition and diversity of the bacterial communities.

4. Discussion

4.1. Plant characteristics and soil properties along the succession

Plant coverage and the above- and belowground biomasses dramatically increased in the first 5 years after farmland was abandoned for natural succession (Table 2). The annual species *A. capillaries* was a pioneer species that colonized the bare soil of an abandoned farmland and became the dominant population at the 5-y site due to its rapid growth. The higher adaptability to the environment and competitiveness for resources of *A. capillaries* negatively affected the growth of other species to some extent, so the diversity (Shannon index and species richness) at this stage was low. A five-year period of community development, though, is too short to enable plant species to colonize bare soil. More species had entered the ecosystem at the 10-y site, such as *Lespedeza davurica*, *Potentilla bifurca*, and *Poa annua,* and the diversity was higher at this stage than at the 5-y site. Above- and belowground biomasses remained low as the succession continued but significantly increased until peaking at the 30-y site. The high diversity at the 15-y site was consistent with the results by Wang et al. (2009), who observed the highest species richness at the mid-successional stage.

Natural recovery also had a large influence on the soil physicochemical properties. Not surprisingly, abandoning farmland can substantially decrease nutrient contents in soils due to the cessation of input from fertilizers, especially of OC, TN, $NO_3^- - N$, and AP (Table 4). As the vegetation biomass gradually increased, however, nutrients accumulated in the soil in part from the decomposition of litter and from root exudates. The concentrations of OC, TN, $NO_3^- - N$, and AP at the 15- and 20-y sites had reached the levels of the farmland and exceeded them at the 30-y site. This result indicated that soil nutrients could recover with abandonment time, despite the initial negative effect at the beginning of abandonment. Soil pH did not vary significantly along the succession, consistent with the findings by previous studies (An et al., 2008; Wang et al., 2010), suggesting that abandoning cropland for natural recovery had little effect on the pH of the soils on the Loess Plateau. The decreased bulk density indicated an increase in soil porosity with time.

4.2. Changes in microbial communities along the succession

4.2.1. Diversity of bacterial communities

Newly exposed areas were generally colonized by a few opportunistic species, followed by an increase in species richness associated with an increase in accumulated resources. Strong competitors may dominate at later stages as the ecosystem

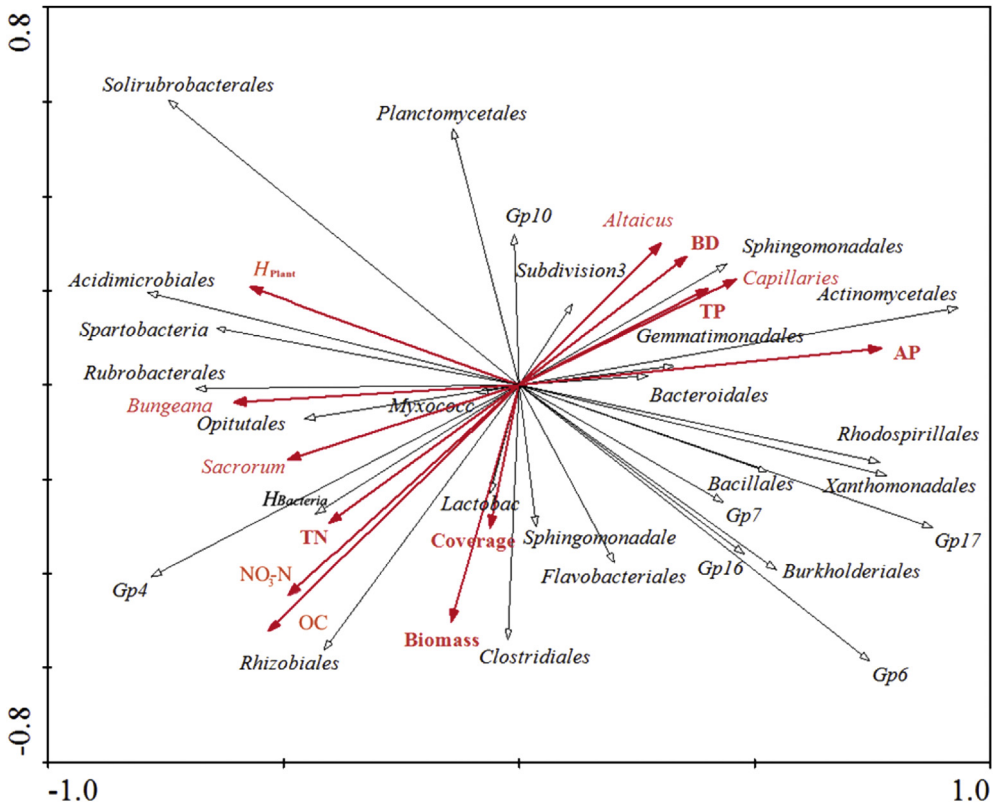

Fig. 5. Ordination plots of the results from the redundancy analysis to identify the relationships among the microbial populations (black arrows), plant characteristics and soil properties (red arrows). OC: organic C, TN: total N, TP: total P, AP: available P, BD: bulk density, Biomass: aboveground biomass, *Capillaries: Artemisia capillaries*, *Sacrorum*: *Artemisia Sacrorum*, *Altaicus*: *Heteropappus Altaicus*, *Bungeana*: *Stipa bungeana*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

matures, resulting in a decrease in species richness. Our results supported this premise that the highest H_{Plant} and S_{Plant} at the 15-y site were significantly higher than those at the 5–10 y and 20–30 y sites (Table 2). $H_{Bacteria}$ and $S_{Bacteria}$ were relatively high in the later stages (20- and 30-y sites), similar to the findings by Schütte et al. (2010) where the diversity of soil bacteria at the leading edge of an arctic glacier was generally high and increased significantly along the successional gradient. Blaalid et al. (2012) also found a higher root-associated fungal richness along a successional gradient of a glacier on the Hardangerjøkulen Plateau in Norway. Competition may be less important for limiting the richness of microorganisms compared to macroorganisms in a climax system, because more niches may be available due to the improvement in nutrient resources.

Agricultural practices, such as tillage, fertilization, and the use of machines for the management of crops, modify the physical and chemical properties of soil and consequently alter the diversity of soil microbial communities (Bissett et al., 2011; Suleiman et al., 2013). Reports of the effects of human practices on soil bacterial communities have been inconsistent (Hossain and Sugiyama, 2011; Lin et al., 2012). They suggested that microbial communities may be modified or reduced in soils exposed to frequent human disturbance. Human cultural activities, as observed in our experiment however, might increase the diversity of soil bacterial communities compared to the abandoned sites, with higher Chao 1 and ACE estimators and Shannon index in the farmland soil than at the 5-y site (Table 5). The abundant nutrient resources in the farmland may be able to support more microflora. Natural recovery without anthropogenic interference has positive impacts on soil systems, including the restoration of vegetation (Zhang, 2005), improved soil structure (An et al., 2008), increased mineralization of soil organic matter (Liang et al., 2010), and increase of soil biota (Jangid et al., 2013). The levels of soil nutrients will initially be low after the abandonment of farmland due to the cessation of fertilization, which will likely negatively affect microbial diversity, but the levels will increase as the natural succession proceeds due to the increase in aboveground vegetation.

Our results showed significant differences in the compositions of plant and soil bacterial communities among successional sites with time (Figs. 1 and 4), suggesting synchronous successional process in both plant and microbial communities. The diversity ($H_{Bacteria}$ and $S_{Bacteria}$) of microbial communities, however, interestingly changed differently from those of the plant communities (Fig. 2), and no significant relationship was observed between them (Table S1). This result was inconsistent with the finding by Lozano et al. (2014) who observed that the same pattern developed between above- and below-ground secondary successions during 84 years' natural restoration in an abandoned agricultural field in Span. This discrepancy might be due to differences in the time-scaling of studying and the environmental conditions in the two studies. Our results suggested a difference in patterns existed between above and belowground communities diversities during the secondary succession, even though the changes in aboveground plant composition over time were synchronous with the changes in the belowground bacterial community. Possible explanations are discussed in the Section 4.3.

4.2.2. Composition of bacterial communities

The PCoA of the abundances of bacterial groups identified large shifts in the compositions of the bacterial communities along the

chronosequence (Fig. 4), consistent with the results by Lozano et al. (2014) who reported a clear separation between the successional stages in the soil bacterial communities in abandoned arable field, in contrast to the findings by Kuramae et al. (2011) who reported a large overlap of microbial communities with no clear difference in a chronosequence of grasslands. Our results showed that the bacterial communities at the farmland and in the 20- and 30-y recovered soils were more similar to each other than to those at the sites rehabilitated for 5, 10, and 15 years, suggesting that farmland and older recovered soils harbored more similar bacterial assemblages.

The bacterial communities were mainly composed of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia*, *Planctomycetes*, and *Firmicutes* (Fig. 3). This phylum-level profile was similar to those in other soils and environments (Li et al., 2014a, b, c, 2015; Lin et al., 2014). *Proteobacteria* and *Acidobacteria* were the most abundant phyla regardless of successional age, generally consistent with other findings that soils often contain two ubiquitous and common bacterial groups (Kolton et al., 2011; Shen et al., 2013; Kim et al., 2014; Li et al., 2014b). Jangid et al. (2013) have recently reported an increasing number of *Proteobacteria* with increasing age in a vegetation succession along the Franz Josef chronosequence in New Zealand. Li et al. (2014c) reported similar findings for restored soils in a 1–20 year chronosequence after the halt of mining operations. These findings suggest that nutrient limitation, among other factors, favors bacteria belonging to *Proteobacteria*, which likely play a functional role for decades in the restoration of soil. Many soil *Proteobacteria* are copiotrophic and become abundant when labile substrates are available (Goldfarb et al., 2011; Li et al., 2014b), but *Acidobacteria* belong to oligotrophic groups and prefer nutrient-poor environments. The bacterial communities in our study generally changed from *Acidobacteria*-dominant to *Proteobacteria*-dominant during the 30 years of succession, suggesting that belowground communities transitioned from slow-growing oligotrophic groups to fast-growing copiotrophic groups.

4.3. Relationships among plant community, microbial succession and soil properties

The correlation analysis of and the RDA in our study identified the effects of plant and soil on bacterial diversity and community composition (Supplementary Table S1 and Fig. 5), which suggested that the variation in abundance and diversity of bacterial communities could be partially attributable to changes in the plant communities and soil properties.

4.3.1. Response of bacterial communities to plant succession

Plant coverage and biomass (expected 10-y), soil C and N contents, the abundance of *Proteobacteria* groups and H_{Bacteria} increased as both plant and microbial succession developed. Total coverage, biomasses, and the coverage of dominant plant species (*A. sacrorum*) were correlated positively with the abundance of *Proteobacteria*, and with H_{Bacteria} and negatively correlated with the abundance of *Acidobacteria*, suggesting that plants may have a large influence on the soil microbial community. Our correlation analysis suggests the possibility that plant effects on bacteria are likely mediated by changes in soil nutrient pools. This result was consistent with those by Tscherko et al. (2004) and Peiffer et al. (2013) who found that the identity of a plant species determined the type of microorganisms in the rhizosphere. H_{Plant}, however, was not correlated with the abundance of the dominant microbial groups or with H_{Bacteria}, suggesting that belowground diversity differed from aboveground diversity due to the different factors influencing the above- and belowground diversities. In our study, the 30-y site had the highest productivity (indicated by aboveground biomass), the 15-y site had the highest diversity and richness, and biomass and H_{Plant} were not significantly correlated. These results indicated that community diversity was not proportional to productivity. Competitive interactions among plants may drive community diversity and richness (Grime, 1973; Huston, 1979). Decreases in plant diversity and species richness in communities with mid-to high productivity may be due to increased competition and the exclusion of species with lower competitive capacities (Arroyo et al., 2015). The plant compositions (Table 3) supported the assumption that some minor species that occupied the early to middle stages disappeared in the late stages, such as *Salsola collina*, *Phragmites australis*, and *Vicia sepium*. H_{Plant} was also not significantly correlated with soil C and N contents, implying that aboveground diversity was probably not affected by soil nutrient conditions. Other competition resources (e.g. soil moisture and living space) may be the limiting factors, but this possibility requires further investigation. H_{Bacteria} was positively correlated with organic C and N contents, suggesting the crucial role of soil nutrient supply in the quantity and richness of microorganisms.

4.3.2. Response of bacterial communities to soil properties

The correlation analysis and the RDA indicated that the abundances of the dominant phyla (*Proteobacteria, Acidobacteria, and Verrucomicrobia*) were significantly correlated with the levels of soil nutrients. For example, OC, TN and $NO_3^- - N$ contents were significantly correlated with the abundances of *Rhizobiales, Xanthomoadales, Lactobacillales*, *Opitutales*, and *Sphingomonadales*. AP content was correlated with the abundances of *Sphingobacteriales, Gemmatimonadales* and *Bacteroidales*. These results supported the importance of soil C, N, and P fractions in the formation of bacterial communities.

In our study, *Proteobacteria* tended to significantly decrease in the first 5 years and then increase with abandonment time (Fig. 3), perhaps due to the C and N supplies. The abundances of these phyla have been positively correlated with pools of available soil C and N (Fierer et al., 2007; Nemergut et al., 2010; Goldfarb et al., 2011). The farmland soil was a C-rich environment due to the input from fertilizers, and ecological theory suggests that copiotrophic behavior or r-selected populations should be well adapted to such nutrient-rich conditions. The *Acidobacteria* in our study behaved oppositely to the copiotrophic *Proteobacteria*, with a higher abundance at the 5-y site compared to the farmland. A negative correlation with organic C, total N, and NO_3–N contents for *Acidobacteria* indicated that these bacteria belong to oligotrophic groups and prefer nutrient-poor environments (Jones et al., 2009; Bergmann et al., 2011). The cessation of fertilization when the farmland was abandoned may have severely restricted the supplies of C and N, creating a favorable environment for the growth of oligotrophic bacterial groups. As the succession proceeded, however, the increased aboveground biomass and coverage could lead to the accumulation of C and N from the decomposition of litter and from root exudates. The dynamics of the two predominant populations, *Proteobacteria* and *Acidobacteria*, thus tended to increase and decrease, respectively, after 5 years. *Alphaproteobacteria* was the largest sub-group of *Proteobacteria* at the various successional sites, with high proportions of populations of specific N-fixing *Rhizobiales* (Fig. S2). A positive correlation found between *Rhizobiales* and N contents indicated an important role for this N-cycling group of bacteria in this arid system (Erlacher et al., 2015). Additionally, *Xanthomonadales* were highly abundant *Proteobacteria* during the early stages of succession, and their abundance was negatively correlated with C and N contents (Fig. 5), indicating that they are oligotrophic and fast-growing bacteria, able to exploit early, transient niches.

Yuan et al. (2014) observed that soil $NH_4^+ - N$ was a dominant environmental factor that influenced bacterial communities to a depth of 5 cm along elevation gradients on the Tibetan Plateau. Yao et al. (2014) found that the rate of N addition and soil $NH_4^+ - N$ content positively affected microbial biomass and the relative abundances of the main bacterial groups occupying the soils covered by C3 grass species. The content of $NH_4^+ - N$ in our study, however, was not closely correlated with the relative abundances of the bacterial communities, but $NO_3^- - N$ had a significant influence, indicating that not all the N in the soil contributed to the variation of the bacterial communities. $NO_3^- - N$ may play an important role in shaping bacterial communities during natural recovery on the Loess Plateau. The abandonment of farmland for natural recovery did not increase the TP levels in a study of cropland soils on the plateau (Jiao et al., 2011), in agreement with the narrow range of TP (0.54–0.57 kg g^{-1}) within the 30 years of restoration in our study. This narrow range may have been responsible for the absence of a significant relationship between TP content and the abundance of specific bacterial groups. AP, however, was closely correlated with the bacterial communities, suggesting that P was an important element in the formation of soil bacterial communities.

Our results suggested that the abundance of the dominant group, *Proteobacteria*, was negatively correlated with soil bulk density, as reported by Li et al. (2002) who observed higher microbial populations in soils with low bulk densities. Agricultural practices, such as mechanical compaction, may accelerate increases in soil density and thus may indirectly increase soil resistance, and plant roots have difficulty penetrating compacted soil layers (Li and Zhou, 1994), and may decrease the number of large pores that serve as pathways for the drainage of water and exchange of gases, which would decrease nutrient uptake and subsequently negatively affect microbial activities. Plant colonization during secondary succession, however, can increase soil porosity due to a larger amount of roots and their deeper penetration into the soil, which would decrease the bulk density and promote the aeration in the soil.

5. Conclusions

Our results suggested that abandoned farmland on the Loess Plateau undergoes secondary succession both below- and aboveground, characterized by changes in plant composition, soil properties, and microbial community composition. Bacterial communities transitioned from *Acidobacteria*-dominant to *Proteobacteria*-dominant communities during the 30 years of succession. The levels of soil nutrients and bacterial diversity were lower compared to the farmland soon after abandonment, but they were able to recover to the farmland levels after 15–20 years and were much improved after further succession. The different patterns of diversities between the bacterial and plant communities suggested an incongruous process between the below- and aboveground communities during the succession. Soil nutrient supply, such as organic C, total N, available N, and AP, had crucial roles in shaping the composition and diversity of the bacterial communities.

Acknowledgments

This work was financially supported by the Fundamental Research Funds for the Central Universities (2452015088) and the National Natural Sciences Foundation of China (41401621).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.02.013

References

Abella, S., 2010. Disturbance and plant succession in the Mojave and Sonoran deserts of the American southwest. International Journal of Environmental Research and Public Health 7, 1248–1284.
Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. Journal of Molecular Biology 215, 403–410.
An, S.S., Huang, Y.M., Zheng, F.L., 2009. Evaluation of soil microbial indices along a revegetation chronosequence in grassland soils on the Loess Plateau, Northwest China. Applied Soil Ecology 41, 286–292.
An, S.S., Huang, Y.M., Zheng, F.L., Yang, J.G., 2008. Aggregate characteristics during natural revegetation on the Loess Plateau. Pedosphere 18, 809–816.
Arroyo, A.I., Pueyo, Y., Saiz, H., Alados, C.L., 2015. Plant–plant interactions as a mechanism structuring plant diversity in a Mediterranean semi-arid ecosystem. Ecology and Evolution 5 (22), 5305–5317.
Bailly, J., Fraissinet-Tachet, L., Verner, M.C., Debaud, J.C., Lemaire, M., Wesolowski-Louvel, M., Marmeisse, R., 2007. Soil eukaryotic functional diversity, a metatranscriptomic approach. ISME Journal 1, 632–642.
Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D.V., 2011. Soil microbial community successional patterns during forest ecosystem restoration. Applied and Environmental Microbiology 77, 6158–6164.
Bergmann, G.T., Bates, S.T., Eilers, K.G., Lauber, C.L., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biology & Biochemistry 43, 1450–1455.
Bissett, A., Richardson, A.E., Baker, G., Thrall, P.H., 2011. Long-term land use effects on soil microbial community structure and function. Applied Soil Ecology 51, 66–78.
Blaalid, R., Carlsen, T.O.R., Kumar, S., Halvorsen, R., Ugland, K.I., Fontana, G., Kauserud, H., 2012. Changes in the root-associated fungal communities along a primary succession gradient analysed by 454 pyrosequencing. Molecular Ecology 21, 1897–1908.
Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbial Properties, pp. 595–624. Agronomy Society of America, Agronomy Monograph 9, Madison, Wisconsin.
Caporaso, J.G., Bittinger, K., Bushman, F.D., DeSantis, T.Z., Andersen, G.L., Knight, R., 2010b. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26, 266–267.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010a. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.
Chan, C.O., Casper, P., Sha, L.Q., Feng, Z.L., Fu, Y., Yang, X.D., Ulrich, A., Zou, X.M., 2008. Vegetation cover of forest, shrub and pasture strongly influences soil bacterial community structure as revealed by 16S rRNA gene T-RFLP analysis. FEMS Microbiology Ecology 64, 449–458.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C.W., Grube, M., Berg, G., 2015. Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of Lobaria pulmonaria L. Frontiers in Microbiology 6.
Felske, A., Wolterink, A., Van Lis, R., De Vos, W.M., Akkermans, A.D.L., 2000. Response of a soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. Applied and Enviornmental Microbiology 66, 3998–4003.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Goldfarb, K.C., Karaoz, U., Hanson, C.A., Santee, C.A., Bradford, M.A., Treseder, K.K., Wallenstein, M.D., Brodie, E.L., 2011. Differential growth responses of soil bacterial taxa to carbon substrates of varying chemical recalcitrance. Frontiers in Microbiology 2, 94.
Grime, J.P., 1973. Competitive exclusion in herbaceous vegetation. Nature 242, 344–347.
Hossain, Z., Sugiyama, S., 2011. Geographical structure of soil microbial communities in northern Japan: effects of distance, land use type and soil properties. European Journal of Soil Biology 47, 88–97.
Huston, M., 1979. A general hypothesis of species diversity. American Naturalist 113, 81–101.
Jangid, K., Whitman, W.B., Condron, L.M., Turner, B.L., Williams, M.A., 2013. Soil bacterial community succession during long-term ecosystem development. Molecular Ecology 22, 3415–3424.
Jiao, F., Wen, Z.M., An, S.S., 2011. Changes in soil properties across a chronosequence of vegetation restoration on the Loess Plateau of China. Catena 86, 110–116.
Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME Journal 3, 442–453.
Kardol, P., Bezemer, T.M., van der Putten, W.H., 2006. Temporal variation in plant-soil feedback controls succession. Ecology Letters 9 (9), 1080–1088.
Kim, H.M., Jung, J.Y., Yergeau, E., Hwang, C.Y., Hinzman, L., Nam, S., Hong, S.G.,

Kim, O.S., Chun, J., Lee, Y.K., 2014. Bacterial community structure and soil properties of a subarctic tundra soil in council, Alaska. FEMS Microbiology Ecology 89, 465–475.
Knelman, J.E., Legg, T.M., O'Neill, S.P., Washenberger, C.L., González, A., Cleveland, C.C., Nemergut, D.R., 2012. Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. Soil Biology & Biochemistry 46, 172–180.
Kolton, M., Harel, Y.M., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. Applied and Environmental Microbiology 77, 4924–4930.
Koranda, M., Schnecker, J., Kaiser, C., Fuchslueger, L., Kitzler, B., Stange, C.F., Sessitsch, A., Sophie, Z.B., Richter, A., 2011. Microbial processes and community composition in the rhizosphere of European beech-the influence of plant C exudates. Soil Biology and Biochemistry 43, 551–558.
Kuramae, E., Gamper, H., Van Veen, J., Kowalchuk, G., 2011. Soil and plant factors driving the community of soil-borne microorganisms across chronosequences of secondary succession of chalk grasslands with a neutral pH. FEMS Microbiology Ecology 77, 285–294.
Li, C.H., Ma, B.L., Zhang, T.Q., 2002. Soil bulk density effects on microbial populations and enzyme activities during the growth of Maize (Zea Mays L) planted in large pots under field exposure. Canadian Journal of Soil Science 82 (2), 1024–1032.
Li, C.H., Yan, K., Tang, L.S., Jia, Z.J., Li, Y., 2014a. Change in deep soil microbial communities due to long-term fertilization. Soil Biology & Biochemistry 75, 264–272.
Li, C.H., Zhou, S., 1994. Soil bulk density effects on the seedling growth of maize. North China Agronomy 9, 22–28 (in Chinese).
Li, J., Hu, H.W., Ma, Y.B., Wang, J.T., Liu, Y.R., He, J.Z., 2015. Long-term nickel exposure altered the bacterial community composition but not diversity in two contrasting agricultural soils. Environmental Science and Pollution Research 22, 10496–10505.
Li, X.Z., Rui, J.P., Mao, Y.J., Yannarell, A., Mackie, R., 2014b. Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. Soil Biology & Biochemistry 68, 392–401.
Li, Y., Wen, H., Chen, L., Yin, T., 2014c. Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. PLoS One 9, e115024.
Liang, J., Wang, X.A., Yu, Z.D., Dong, Z.M., Wang, J.C., 2010. Effects of vegetation succession on soil fertility within farming-plantation ecotone in Ziwuling Mountains of the Loess Plateau in China. Agricultural Sciences in China 9, 1481–1491.
Lin, Y.T., Whitman, W.B., Coleman, D.C., Chiu, C.Y., 2012. Comparison of soil bacterial communities between coastal and inland forests in a subtropical area. Applied Soil Ecology 60, 49–55.
Lin, Y.T., Whitman, W.B., Coleman, D.C., Chen, T.H., Chiu, C.Y., 2014. Composition of bacterial communities in sand dunes of subtropical coastal forests. Biology and Fertility of Soils 50, 809–814.
Lozano, Y.M., Hortal, S., Armas, C., Pugnaire, F.I., 2014. Interactions among soil, plants, and microorganisms drive secondary succession in a dry environment. Soil Biology & Biochemistry 78, 298–306.
Mahnert, A., Moissl-Eichinger, C., Berg, G., 2015. Microbiome interplay: plants alter microbial abundance and diversity within the built environment. Frontiers in Microbiology 6, 887.
Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbial Properties, pp. 539–552. Agronomy Society of America, Agronomy Monograph 9, Madison, Wisconsin.
Nemergut, D.R., Cleveland, C.C., Wieder, W.R., Washenberger, C.L., Townsend, A.R., 2010. Plot-scale manipulations of organic matter inputs to soils correlate with shifts in microbial community composition in a lowland tropical rain forest. Soil Biology and Biochemistry 42, 2153–2160.
Oh, Y.M., Kim, M., Lee-Cruz, L., Lai-Hoe, A., Go, R., Ainuddin, N., Rahim, R.A., Shukor, N., Adams, J.M., 2012. Distinctive bacterial communities in the rhizoplane of four tropical tree species. Microbial Ecology 64, 1018–1027.
Olsen, S.R., Sommers, L.E., 1982. Phosphorous. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbial Properties, pp. 403–430. Agronomy Society of America, Agronomy Monograph 9, Madison, Wisconsin.
Peiffer, J.A., Spor, A., Koren, O., Zhao, J., Tringe, S.G., Dangl, J.L., Buckler, E.S., Ley, R.E., 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proceedings of the National Academy of Sciences 110, 6548–6553.
Prosser, J.I., 2002. Molecular and functional diversity in soil micro-organisms. Plant Soil 244, 9–17.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75, 7537–7541.
Schütte, U.M.E., Abdo, Z., Foster, J., Ravel, J., Bunge, J., Solheim, B., Forney, L.J., 2010. Bacterial diversity in a glacier foreland of the high Arctic. Molecular Ecology 19, 54–66.
Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., Chu, H., 2013. Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biology and Biochemistry 57, 204–211.
Singh, B.K., Millard, P., Whiteley, A.S., Murrell, J.C., 2004. Unravelling rhizosphere-microbial interactions: opportunities and limitations. Trends in Microbiology 12, 386–393.
Suleiman, A.K.A., Manoeli, L., Boldo, J.T., Pereira, M.G., Roesch, L.F.W., 2013. Shifts in soil bacterial community after eight years of land-use change. Systematic and Applied Microbiology 36, 137–144.
Tscherko, D., Ute, H., Marie-Claude, M., Ellen, K., 2004. Shifts in rhizosphere microbial communities and enzyme activity of Poa alpina across an alpine chronosequence. Soil Biology & Biochemistry 36, 1685–1698.
Urbanová, M., Kopecký, J., Valášková, V., Ságová-Marečková, M., Elhottová, D., Kyselková, M., Moënne-Loccoz, Y., Baldrian, P., 2011. Development of bacterial community during spontaneous succession on spoil heaps after brown coal mining. FEMS Microbiology Ecology 78, 59–69.
Walker, L.R., Walker, J., Hobbs, R.J., 2007. Linking Restoration and Ecological Succession. Springer, The Netherlands, pp. 5–21.
Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. Journal of Ecology 98, 725–736.
Wang, B., Liu, G.B., Xue, S., Zhu, B.B., 2010. Changes in soil physico-chemical and microbiological properties during natural succession on abandoned farmland in the Loess Plateau. Environmental Earth Sciences 62, 915–925.
Wang, G.L., Liu, G.B., Xu, M.X., 2009. Above and belowground dynamics of plant community succession following abandonment of farmland on the Loess Plateau, China. Plant Soil 316, 227–239.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73, 5261–5267.
Williams, M.A., Jangid, K., Shanmugam, S.G., Whitman, W.B., 2013. Bacterial communities in soil mimic patterns of vegetative succession and ecosystem climax but are resilient to change between seasons. Soil Biology & Biochemistry 57, 749–757.
Xu, L.h., Ravnskov, Sabine, Larsen, J., Nilsson, R.H., Nicolaisen, M., 2012. Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing. Soil Biology & Biochemistry 46, 26–32.
Yao, M.J., Rui, J.P., Li, J.B., Dai, Y.M., Bai, Y.F., Hedenec, P., Wang, J.M., Zhang, S.H., Pei, K.Q., Liu, C., Wang, Y.F., He, Z.L., Frouz, J., Li, X.Z., 2014. Rate-specific responses of prokaryotic diversity and structure to nitrogen deposition in the Leymus chinensis steppe. Soil Biology & Biochemistry 79, 81–90.
Yuan, Y.L., Si, G.C., Wang, J., Luo, T.X., Zhang, G.X., 2014. Bacterial community in alpine grasslands along an altitudinal gradient on the Tibetan Plateau. FEMS Microbiology Ecology 87, 121–132.
Zhang, C., Liu, G.B., Xue, S., Wang, G.L., 2015. Changes in rhizospheric microbial community structure and function during the natural recovery of abandoned cropland on the Loess Plateau, China. Ecological Engineering 75, 161–171.
Zhang, J.T., 2005. Succession analysis of plant communities in abandoned croplands in the eastern Loess Plateau of China. Journal of Arid Environments 63, 458–474.