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REGULAR ARTICLE

Linking soil bacterial and fungal communities to vegetation succession following agricultural abandonment

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Abstract
Background and aims Globally, the rate and extent of cropland abandonment increase greatly since 1950s. Knowledge of soil bacteria and fungi succession during long-term vegetation development is particularly limited for the abandoned croplands. In order to test the effects of agricultural abandonment on soil biota succession, we studied the soil bacterial and fungal composition and diversity in abandoned farmlands across a century of secondary vegetation successional gradient in China's Qinling Mountains.
Methods Using high-throughput sequencing technologies, the soil fungal and bacterial communities were studied in 22 abandoned farmlands, as well as 7 adjacent arable fields representing non-abandoned references.
Results The stand age, i.e., years since agricultural abandonment, affected the soil bacterial and fungal composition and explained 8.7 and 31.6% variations of bacterial and fungal communities (at order level), repectively. The Proteobacteria, dominated by chemoorganotrophic bacteria, kept its absolute dominance status (38.66% - 40.77%) constantly during succession even though the vegetation changed obviously from crop to grass, shrub, and forest. The relative abundances of Acidobacteria, Planctomycetes, Verrucomicrobia, Nitrospirae, and Spirochaetes increased significantly with stand age (i.e., years since abandonment), while the Firmicutes, Actinobacteria, Gemmatimonadetes, Cyanobacteria, and Armatimonadetes showed an opposite trend. A distinct shift in fungal communities from Ascomycota - dominant in young stands to Basidiomycota -dominant in older stands was observed, which could be attributed to the increase of vegetation coverage and soil moisture during succession. The soil bacterial richness and diversity increased logarithmically with increasing stand age and gradually reached equilibrium in late-successional stage. Soil fungal diversity tended to increase in the early successional stages and then followed by a decreasing trend. The soil pH was the most important environmental factor predicating the soil fungal α-diversity measurements.
Conclusions Both the soil bacterial and fungal communities displayed successional trends along with vegetation succession. The soil bacteria and fungi exhibited marked differences in successional pattern during secondary succession following agricultural abandonment.

Keywords Microbial biodiversity · Plant-soil interaction · Secondary succession · Community structure · Microbial ecology · Abandoned cropland

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Introduction

Globally, the rate and extent of cropland abandonment increase greatly since the 1950s due to rural depopulation, market incentives, and ecological restoration programs (Campbell et al. 2008; Cramera et al. 2008; Zhang et al. 2013a, 2013b). For example, the U.S. Conservation Reserve Program established by the Food Security Act in 1985 transferred almost 1.37×10^7 ha croplands to native grass or tree covers (Roberts and Lubowski 2007). In China, 9.26×10^6 ha croplands have been converted to forests or grasslands during 1999 to 2010 under the 'Grain-for-Green' program (Zhang et al. 2013b). After abandonment, the former croplands involves change from crops to secondary vegetations, accompanied by alterations of soil properties and biotic community composition (Awiti et al. 2008; Cramera et al. 2008). Thus, the abandoned croplands provide unique opportunities to test and develop ecological theories (Cramera et al. 2008), in particular for the succession or ecological restroration theories.

Understanding the changes in biotic community composition and diversity during ecosystem succession is a central theme in ecology (Connell and Slatyer 1977; Dini-Andreote et al. 2015). The succession processes, patterns, and mechanisms of macro-organism communities, especially for the plant, have been well demonstrated over several decades (Odum 1969; Chapin et al. 1994; Wardle et al. 2004). In contrast, the bacterial and fungal succession remains less understood mainly due to the complexity of microbes in ecosystems and the limitation of traditional culturing and isolating methods (Amann et al. 1995; Buee et al. 2009; Schmidt et al. 2014). The rapid development of high-throughput sequencing technologies during last decade enables ecologists to reveal the detailed information of microbial community in various ecosystems and fill the important knowledge gaps in ecological succession (Jangid et al. 2013; Schmidt et al. 2014; Zhang et al. 2016). Due to the soil bacteria and fungi are essential functional components in ecosystems and play vital roles in soil development and nutrient cycling (Buee et al. 2009; Liu et al. 2015), an increasing number of studies explored soil microbial community succession using high-throughput sequencing technologies (Cline and Zak 2015; Alfaro et al. 2017; Zhang et al. 2017). In these cases, bacterial and fungal succession dynamics in soils have been detected along environmental chronosequences, e.g., forelands of receding glacier, lava flows, wildfire disturbance, etc. (Ferrenberg et al. 2013; Cutler et al. 2014; Schmidt et al. 2014; Cline and Zak 2015; Zhang et al. 2016).

Soil microbial communities are linked to vegetation and soil properties during ecosystem succession (Kuramae et al. 2010; Cline and Zak 2015). For instance, the shifts in soil pH and nutrient availability during succession could directly drive changes in soil microbial composition and abundance, because the acidity is a particularly important environmental factor affecting soil microbial survival or growth, and the nutrients provide substrates and energy for soil microbes (Shen et al. 2014; Alfaro et al. 2017). Meanwhile, the changes in growth forms or functional types of plants during succession may indirectly affect the soil microbial communities by shaping environments and providing resources (Cline and Zak 2015; Alfaro et al. 2017). Generally, vegetation communities change obviously during succession and different succesional stages differ in the plant species composition and community structure (Zhang et al. 2010), thus, the soil microbial composition and diversity would be likely to change concomitantly with vegetation succesional stages if soil microbes possess ecological trade-offs analogous to plant (Cline and Zak 2015).

The investigations from receding glacier soils indicated that primary successional trajectories for bacteria and fungi may be greatly different (Brown and Jumpponen 2014). Brown and Jumpponen (2014) found that vegetation strongly impacted bacterial community but played only a very minor role in structuring the soil fungal communities in the receding glacier forefront chronosequence. Compared to soil fungi, many bacteria are better adapted to life in barren conditions (Schmidt et al. 2014). Bacteria exhibit an extremely wide variety of metabolic types, e.g., photoautotrophs, chemoautotrophs, and heterotrophs. Some bacteria can fix nitrogen and carbon from the atmosphere, whereas fungi are heterotrophs and in general none fungus can fix nitrogen (Duc et al. 2009; Schmidt et al. 2014). In the early stage of primary succession, fungi may not have plentiful available niches before there has been a build-up of organic matters (Schmidt et al. 2014). Fungi may be more dependent on pre-existing resources (e.g., nitrogen and organic compounds) than bacteria. Thus, bacteria and fungi can be differentially affected by changes in vegetation and soil

during primary succession (Alfaro et al. 2017). Generally, the environmental conditions and nutrient availability in the early stage of secondary succession are not as poor as that of primary succession. Secondary succession usually occurs on previously vegetated sites with preexisting soil (Horn 1974; Tilman 1987; Lorenz and Lal 2010). Consequently, the patterns of bacteria and fungi dynamics over secondary succesional age may differ from those of primary succession but we know very little about that. Whether the bacterial and fungal communities respond differentially to the changes in vegetation or soil during secondary succession following agricultural abandonment remains unknown. Also, the existence of successional process in both communities is not clear. Investigating the dynamics of bacterial and fungal communities during secondary succession of abandoned farmlands can fill these important knowledge gaps in ecological succession theory and provide essential information for managing the agriculture ecosystems.

In the present study, we studied the soil fungal and bacterial composition and diversity using high-throughput sequencing technologies across 22 abandoned farmlands in central China, ranging from 1 to 110 years since agricultural abandonment, as well as seven arable fields (adjacent to the abandoned farmlands) representing non-abandoned references or initial stage ecosystems during secondary succession. Since the selected plots were along a century of successional gradient, the vegetation varied markedly and may change the habitats (e.g., coverage, soil moisure, etc.) and the soil carbon or nutrients. Moreover, previous work conducted in this region found that the soil carbon and nitrogen, the most important resources for soil bacteria and fungi, increased significantly with years since abandonment (Zhang et al. 2013a). Thus, we hypothesized that the soil bacterial and fungal communities displayed obvious successional trends along with vegetation succession and the relative abundances of dominant bacterial and fungal groups changed with stand age (i.e., years since agricultural abandonment). Due to the bacteria and fungi may responded differentially to resource availability and soil chemistry (Schmidt et al. 2014; Alfaro et al. 2017), we hypothesized that the soil bacteria and fungi diversity showed different dynamic patterns during secondary vegetation succession following agricultural abandonment.

Materials and methods

Site description and sampling

The study sites are located in the Foping National Nature Reserve (FNNR, a member of UNESCO/MAB World Network of Biosphere Reserves), south aspect of Qinling Mountains, central China. This region is the transitional zone between subtropical humid zone and warm temperate zone and belongs to international biodiversity hotspots. Annual precipitation ranges from 950 to 1200 mm, with 66% falling from June to September. Yearly annual average temperature is 11. 8 °C, and the mean January and July temperatures are −0.3 °C and 21.9 °C, respectively. Frost free days average 220 per year. The soil type is yellow- brown soil developed from granite, and the natural vegetation is dominated by deciduous broad-leaved trees. (Zhang et al. 2010).

Before plot selection, we conducted a comprehensive survey of vegetation and soil to ensure the comparability (e.g., similar soil parent material, topography, and disturbances) among the selected plots. We also investigated the land-use type and history from documents, remote sensing images, and local residents (Zhang et al. 2013a). Historical documents showed that agriculture practices in the study region had been intensive in history, and the main crop was maize (*Zea mays*). Since the Guangxu period of Qing dynasty (1875–1908), lots of residents had emigrated from this area (Ren et al. 1998). In 1978, the FNNR was established for giant panda conservation, and in 1999, the "Grain-for-Green" project, also known as returning farmland to forests project, was started for vegetation recovery. Consequently, massive croplands were abandoned and natural plant communities grow well with little human activities, and it is easy to find abandoned croplands at different secondary succession stages. Previous work has showed that the natural successional pathway after agricultural abandonment in the study area follows grass, shrub, and secondary forest (Zhang et al. 2010) (Fig.1). A series of plots (29 total) located in a small watershed (107.81°-107.86°E, 33.53°-33.62°N) were selected, including 7 croplands, 8 grasslands, 5 shrublands, and 9 secondary forests (Fig. 1; Table S1). All plots were located within 5 km of the Yueba Research & Conservation Station of FNNR. The areas of various vegetation types (i.e., croplands, grasslands, shrublands, and secondary forests) in were different in the small watershed. Thus, we selected 5–9 typical plots for each successional stage. The stand age

(i.e., years since abandonment) and vegetation types of each plot were showed in Fig. 1 and Table S1. The abandoned irrigation ditches and cropland borders ensured that the sampling stands were abandoned croplands (Fig.1). The stand age was sought from landowners or estimated from the tree-rings of the oldest pioneer trees. There were no fertilization, insect pest control, cutting, and other human management and disturbance practices in all abandoned croplands. The croplands were planted with maize (*Z. mays*) when soils were sampled (Fig.1).

For vegetation survey, five 1 m × 1 m grass subplots, four 5 m × 5 m shrub subplots, and four 10 m × 10 m tree subplots were established randomly in each plot, while they were applicable. The total vegetation coverage, species name, coverage and numbers of each species, and diameters at breast height (DBH) of trees were recorded. Three soil sampling subplots (5 m × 5 m) were chosen randomly in each plot. Ten soil cores (100 cm^3) were randomly collected at a depth of 0–10 cm within each soil sampling subplot and then mixed to form one sample. Therefore, three mixed soil samples were taken within each plot and immediately put into the carry-on box filled with carbon dioxide ice (dry-ice). In order to reduce the sampling error, all the soil samples were taken at the same day in vegetation growing season (August 13th, 2016). The dry-ice boxes were transported to laboratory and the each soil samples were subdivided into two subsamples. One subsample was kept at −80 °C for DNA extractions, and the other subsample was used to measure physico-chemical properties. To measure bulk density, three additional intact soil cores were taken at a depth of 0–10 cm by core sampler (100 cm^3) in each plot.

Soil physico-chemical properties

Fresh soil samples were dried at 105 °C to constant weight for soil water content measurement, and the intact soil cores were also dried to constant weight for bulk density determination. The air-dried soil samples were used to determine other physico-chemical parameters. Soil total carbon (C) concentration was measured using multi N/C 3100 (Analytik Jena, Germany). Soil total nitrogen (N) concentration was determined using the Kjeldahl method. The total phosphorus (P) total potassium (K) concentrations were measured by molybdenum blue colorimetry and atomic absorption spectrophotometer, respectively. The available N (colorimetric method), available P (Mo-Sb colorimetric method), available K (atomic absorption spectrophotometer), and cation exchange capacity (CEC) (the ammonium saturation method) were determined for each soil sample. Soil pH value was measured in 1:2.5 soil-water slurry using a combination glass electrode.

DNA extractions and Illumina MiSeq high-throughput sequencing

Soil genomic DNA was extracted from 0.5 g fresh soil using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Inc., Norcross, USA) according to the manufacturer's instructions. The quality of the extracted DNA was assessed by agarose gel electrophoresis and the unqualified samples were extracted over again until all the samples passed the quality control. A total of 87 DNA samples were obtained from the 87 mixed soil samples. We pooled the three DNA samples from the same plot. Thus, total of 29 DNA samples were used in the following experiments.

Primer set 515F (5′- GTGCCAGCMGCCGCGG-3′) and 806R (5′- GGACTACHVGGGTWTCTAAT-3′) was used to amplify the bacterial V4 region of 16SrRNA genes, and primer set ITS1F (5′- CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′- GCTGCGTTCTTCATCGATGC-3′) was used to amplify the Internal Transcribed Spacer 1(ITS1) of fungi. Sequencing libraries were constructed using the Illumina® TruSeq® Nano DNA Library Prep kits (Illumina, USA) following the manufacturer's instruction. After assessing the library quality and quantity (Agilent Bioanalyzer 2100 system and Qubit®2.0 Fluorometer), sequencing was conducted on the Illumina MiSeq platform.

Processing of sequencing data

The raw sequence data were assigned to each sample according to the unique barcodes, and then the barcodes and the primer sequences were removed. All of the sequences have been deposited in the NCBI Sequence Read Archive SRR5856495 and SRR5856482. The paired-end reads were merged using FLASH (V1.2.7, http://ccb.jhu. edu/software/FLASH/). The sequences were quality-filtered using the QIIME (Caporaso et al. 2010), and the chimeras were checked by UCHIME and then removed (Edgar et al. 2011). The qualified sequences were clustered into OTUs (i.e., Operational

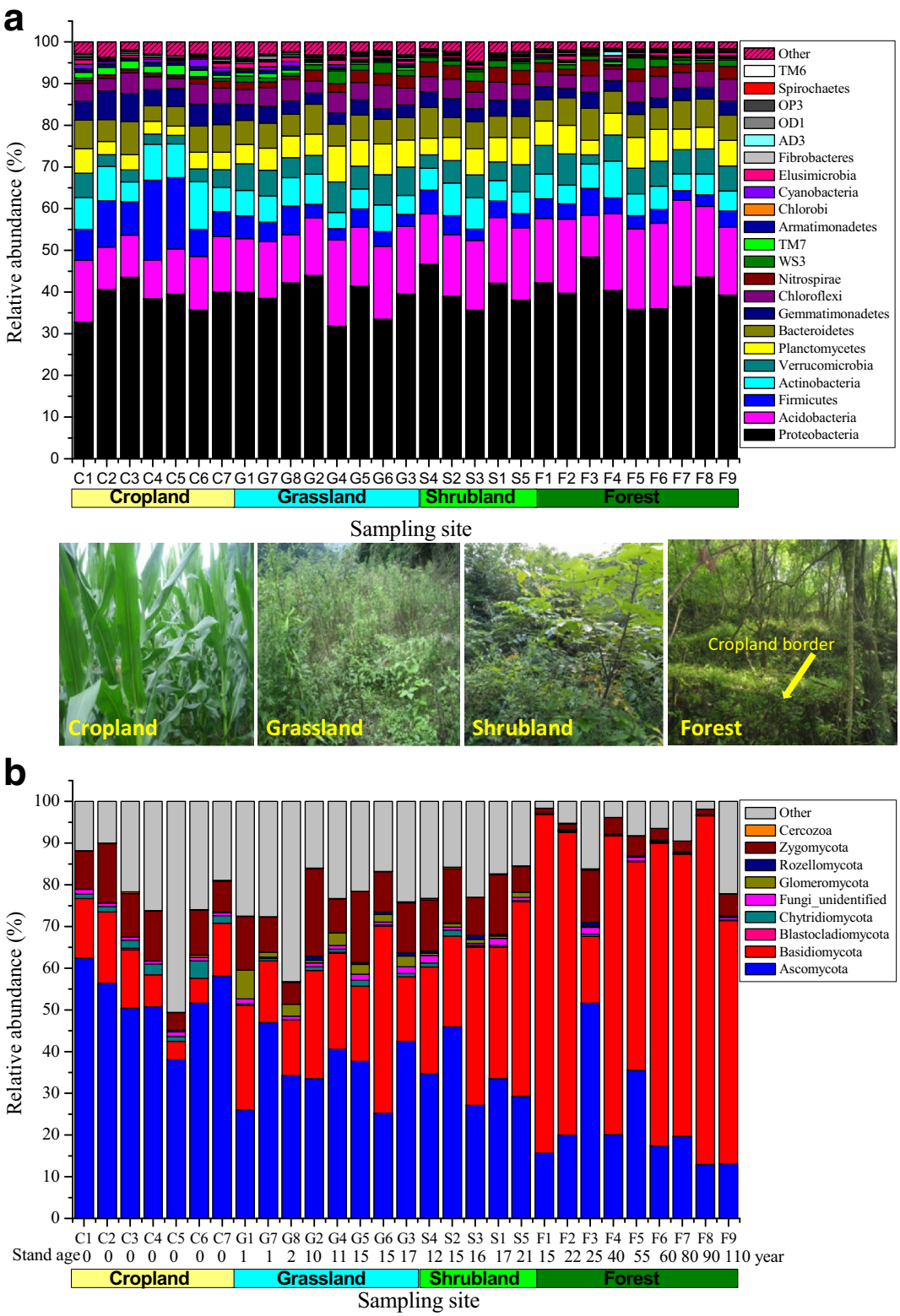

Fig. 1 Relative abundances of the dominant soil bacterial (**a**) and fungal (**b**) phyla at 29 sampling sites along the secondary successional gradient

taxonomic units) at a similarity of 97% using the UPARSE pipeline (Edgar 2013), and OTU taxonomic identity was determined using Qiime based on the database Silva (http://www.arb-silva.de) and Unite (ITS, http://unite.ut.ee/index.php). To ensure equal sampling across all stands, bacterial and fungal sequences were respectively rarefied to 34,086 and 35,054 sequences per stand, according to the stand that yielded the least number of sequences (Table 2) (Lauber et al. 2013; Cline and Zak 2015). The α-diversity measurements of soil bacteria and fungi, including the number of observed OTUs, Chao1 richness, Shannon's diversity index, and Faith's phylogenetic diverstiy (an indicator of the overall phylogenetic diversity across all taxonomic levels, PD) were calculated for each stand (Dini-Andreote et al. 2014). We used the alpha_diversity.py of QIIME (http://qiime.org/scripts/alpha _ diversity.html) to calculate the α-diversity measurements following its instructions. More detailed information about the α-diversity measurements could be gained from the package skbio.diversity.alpha (http://scikit-bio.org/docs/latest/generated/skbio. diversity.alpha.html). To assess the phylogenetic β-diversity, the weighted Unifrac and unweighted Unifrac distances were estimated using QIIME.

Statistical analyses

The effect of successional stage on the relative abundance of the dominant soil bacterial and fungal phylum, and α-diversity measurements of soil bacteria and fungi was determined using ANOVAs. The dissimilarities in total bacterial and fungal OTU community composition between successional stages were tested by Analysis of Similarities (ANOSIM) (R Development Core Team 2013). Principal coordinate analysis (PCoA) of unweighted and weighted UniFrac distances, and UPGMA (Unweighted Pair-group Method with Arithmetic Mean) cluster analysis based on weighted UniFrac distances were performed to compare the microbial communities from different samples.

We used the partial RDA (redundancy analysis) to test the successional effect or the effect of stand age by models constraining the space with time. The space variables were the coordinates transferred from the longitude and latitude by the order 'geoXY' in the package of 'SoDA' in R (R Development Core Team 2013). The stand age was the explanatory variable when run the partial RDA in Canoco (Version 5.0 for Windows, Ter Braak and Smilauer 2012). The percentage of variations in bacteria or fungi communities (order level) explained by stand age were calculated.

The RDA was carried out to study the relationships between environmental factors and the 25 most abundant bacterial or fungal orders (Canoco 5.0, Ter Braak and Smilauer 2012). We first analyzed the percentage of variation in community data (order level) explained by each environmental variable (Canoco 5.0). The significant environmental variables were selected based on the results of RDA, and then the Pearson correlation analysis was performed and the highly correlated environmental variables were identified. One of the two environmental variables was removed when their correlation coefficient was greater than 0.7 (Table S2). Finally, we selected 3–5 significant environmental variables to carry out the RDA and plot the results.

To further examine the changes in soil bacterial and fungal community variables (i.e., the abundances of soil bacterial and fungal phyla and the α-diversity measurements) and the environmental variables, simple linear and nonlinear regression was used to analyze the relationships of the studied variables with stand age (SPSS 17.0 for Windows, SPSS Inc. 2008). The best regression model (linear or nonlinear) was chosen according to the coefficient of determination. Pearson correlation analysis was used to explore correlations between the environmental variables and the abundances of dominant soil bacterial and fungal phyla. Stepwise regression analysis was used to determine the relationship between the environmental variables and the α-diversity measurements. Due to the sufficient sample size for grasslands and forests, we examine the relationships of plant richness (i.e., number of grass, or tress), soil bacterial and fungal richness (i.e., number of observed OTUs) with stand age of grasslands or forests, and the relationships among plant richness and soil microbial richness were also estimated.

Results

Soil bacterial community composition

A total of 1,255,819 effective 16SrRNA sequences with 35,054–51,265 sequences per stand (mean 43,303) were obtained, and the Good's coverage estimates for 16S sequencing ranged from 0.946 to 0.977 across sites. The number of bacterial in soils ranged from 3126 to 5048 with a mean of 4328 (Table 1).

Table 1 The number of quality sequences, taxon sequences, average length of sequences, Good's coverage index, and the number of OTUs (at the 97% similarity level) of soil samples

Site	Bacteria					Fungi				
	Sequences	Taxon Sequence	Average Length	Good's coverage	OTU	Sequences	Taxon Sequence	Average Length	Good's coverage	OTU
C1	50,017	45,963	256	0.972	4132	49,277	48,665	243	0.998	780
C2	48,000	44,478	256	0.974	3655	38,842	38,056	253	0.997	860
C3	47,287	42,517	256	0.976	3758	44,348	43,294	254	0.998	811
C4	47,548	44,783	256	0.977	3126	41,601	37,788	258	0.999	502
C5	49,410	45,320	256	0.977	3270	34,819	34,086	250	0.999	614
C6	55,651	50,185	256	0.966	4702	49,435	48,357	250	0.998	931
C7	55,239	51,029	256	0.963	4694	46,167	45,445	249	0.998	858
G1	44,038	40,381	256	0.973	4165	44,533	43,311	257	0.998	796
G2	46,704	43,938	256	0.971	4115	55,168	54,462	254	0.998	889
G3	55,327	51,265	256	0.959	5048	42,676	41,355	254	0.997	1276
G4	46,038	42,034	256	0.963	4856	53,295	51,915	252	0.994	1365
G5	51,748	47,673	256	0.961	5012	50,579	49,459	251	0.996	1294
G6	48,832	45,440	256	0.963	4654	52,243	51,337	268	0.993	1285
G7	44,129	40,510	256	0.970	4388	43,036	41,859	253	0.996	1106
G8	38,825	35,929	256	0.973	3879	45,355	44,636	244	0.998	708
S1	39,115	35,599	256	0.974	4067	36,030	34,811	265	0.997	1136
S2	41,971	38,235	256	0.968	4591	39,796	38,320	260	0.995	1507
S3	47,687	44,613	256	0.968	4308	47,683	46,828	260	0.995	1198
S4	43,833	40,290	256	0.968	4349	54,464	52,246	264	0.993	1604
S5	43,316	40,119	256	0.967	4512	45,660	44,630	264	0.994	1186
F1	49,384	45,699	256	0.963	4709	38,821	38,184	275	0.997	552
F2	53,962	49,846	256	0.967	4487	39,980	39,198	281	0.996	772
F3	42,821	39,255	256	0.969	4255	43,147	41,805	247	0.995	1518
F4	45,432	41,881	256	0.969	4263	45,243	43,910	274	0.996	704
F5	46,780	43,845	256	0.968	4335	46,181	45,288	262	0.996	978
F6	48,645	45,249	256	0.966	4631	47,417	46,357	270	0.994	926
F7	44,212	41,135	256	0.969	4264	45,221	44,156	266	0.995	805
F8	37,865	35,054	256	0.946	4945	55,315	54,687	263	0.994	646
F9	46,911	43,554	256	0.968	4364	46,618	45,226	264	0.995	1149

To ensure equal sampling across all stands, bacterial and fungal sequences were respectively rarefied to 34,086 and 35,054 sequences per stand to estimated the number of OTUs

The results of partial RDA (constraining the space with time) showed that the soil bacterial community displayed successional trends along with vegetation succession (Table 2, Fig. S1, Fig. S2), and the stand age explained 11.0 and 8.7% variations of bacterial community at phylum and order level, respectively (Table 2). The Proteobacteria was the most abundant bacterial phylum cross all stands (Fig. 1). The vegetation successional stage did not affect the dominance of Proteobacteria (ANOVA, $P > 0.05$, Table 3), and its relative abundances were 38.66%, 38.88%, 40.31%, and 40.77% in croplands, grasslands, shrublands, and forests, respectively (Table 3, Fig.1). Moreover, relative abundances of Proteobacteria did not change with stand age (Fig. S3). The relative abundances of Acidobacteria, Firmicutes, Actinobacteria, Gemmatimonadetes, Planctomycetes, Verrucomicrobia, Nitrospirae, Cyanobacteria Armatimonadetes, and Spirochaetes were significantly affected by vegetation successional stage (ANOVA, $P < 0.05$, Table 3). The relative

Table 2 Results of partial RDA constraining the space with time (i.e., stand age) for soil bacterial and fungal communities (Phylum and Order levels)

	Variable	%Var	Pseudo-F	*P*
Phylum level	Bacteria			
	Stand age	11.0	3.1	0.024
	Fungi			
	Stand age	28.0	9.7	0.002
Order level	Bacteria			
	Stand age	8.7	2.4	0.058
	Fungi			
	Stand age	31.6	11.5	0.002

%Var: the percentage of variance in community composition data explained by explanatory variable

abundances of Acidobacteria, Planctomycetes, Verrucomicrobia, Nitrospirae, and Spirochaetes increased significantly with stand age, while the Firmicutes, Actinobacteria, Gemmatimonadetes, Cyanobacteria, and Armatimonadetes showed an opposite trend (Fig. S3, Fig. S4). The relative abundances of Acidobacteria was positively correlated with soil total C, total N, available N, and soil moisture, whereas negatively correlated with soil bulk density, total K, and available P ($P < 0.05$, Table S3). The relative abundances of Bacteroidetes, Chloroflexi, Nitrospirae, and Spirochaetes were positively correlated with soil pH, while the relative abundances of Firmicutes and Armatimonadetes were negatively correlated with soil pH (Table S3). There were no significant relationships between the relative abundances of Proteobacteria and the studied environmental variables (Table S3).

Table 3 Relative abundances (%) of the dominant soil bacterial phylum (Mean ± SE) under different successional stages (*P*-values indicate statistically significant differences among successional stages (*$P < 0.05$;**$P < 0.01$;***$P < 0.001$)

	Successional stage				*P*-value
	Cropland	Grassland	Shrubland	Forest	
Proteobacteria	38.66 ± 1.32	38.88 ± 1.49	40.31 ± 1.9	40.77 ± 1.29	0.66
Acidobacteria	11.62 ± 0.77^{a}	15.03 ± 1.04^{b}	15.32 ± 0.92^{b}	17.25 ± 1.09^{b}	**
Firmicutes	10.74 ± 2.01^{a}	4.22 ± 0.51^{b}	4.08 ± 0.51^{b}	3.79 ± 0.41^{b}	***
Actinobacteria	7.82 ± 0.81^{a}	5.76 ± 0.42^{b}	6.17 ± 0.62^{ab}	5.53 ± 0.46^{b}	*
Bacteroidetes	5.92 ± 0.54	5.86 ± 0.22	5.78 ± 0.49	6.15 ± 0.30	0.91
Gemmatimonadetes	5.24 ± 0.44^{a}	3.17 ± 0.21^{bc}	3.84 ± 0.24^{b}	2.89 ± 0.16^{c}	***
Chloroflexi	3.78 ± 0.37	4.39 ± 0.33	4.08 ± 0.21	4.09 ± 0.30	0.62
Planctomycetes	3.75 ± 0.44^{b}	6.13 ± 0.48^{a}	5.62 ± 0.44^{a}	5.85 ± 0.44^{a}	**
Verrucomicrobia	3.33 ± 0.49^{b}	6.09 ± 0.38^{a}	5.01 ± 0.54^{a}	5.87 ± 0.49^{a}	**
TM7	1.62 ± 0.19^{a}	0.63 ± 0.08^{b}	0.31 ± 0.05^{b}	0.42 ± 0.04^{b}	***
Nitrospirae	0.88 ± 0.15^{c}	2.18 ± 0.29^{b}	3.27 ± 0.17^{a}	2.22 ± 0.30^{b}	***
Cyanobacteria	0.85 ± 0.2^{a}	0.44 ± 0.12^{b}	0.13 ± 0.01^{b}	0.13 ± 0.02^{b}	**
Armatimonadetes	0.62 ± 0.05^{a}	0.42 ± 0.06^{b}	0.22 ± 0.05^{c}	0.27 ± 0.03^{c}	***
Elusimicrobia	0.57 ± 0.13	0.80 ± 0.11	0.47 ± 0.05	0.66 ± 0.06	0.14
WS3	0.37 ± 0.08^{b}	1.60 ± 0.3^{a}	1.55 ± 0.24^{a}	1.25 ± 0.23^{a}	**
OD1	0.34 ± 0.07	0.37 ± 0.08	0.17 ± 0.03	0.22 ± 0.03	0.07
Fibrobacteres	0.23 ± 0.06	0.28 ± 0.02	0.22 ± 0.03	0.20 ± 0.02	0.35
AD3	0.21 ± 0.06	0.18 ± 0.04	0.09 ± 0.04	0.17 ± 0.10	0.80
Chlorobi	0.16 ± 0.02	0.24 ± 0.02	0.22 ± 0.03	0.18 ± 0.02	0.06
TM6	0.12 ± 0.04	0.11 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	0.58
Spirochaetes	0.09 ± 0.03^{b}	0.18 ± 0.02^{a}	0.17 ± 0.01^{a}	0.21 ± 0.02^{a}	**
OP3	0.08 ± 0.03^{b}	0.20 ± 0.03^{a}	0.22 ± 0.05^{a}	0.18 ± 0.02^{a}	*

Letters on superscript indicate differences at $P < 0.05$ among successional stages)

Soil fungal community composition

A total of 1,289,671 effective ITS sequences with 34,086–54,687 per stand (mean 44,471) were obtained, and the Good's coverage for ITS sequencing ranged from 0.993 to 0.999 across plots. The number of fungal OTUs in soils ranged from 502 to 1604 with a mean of 991 (Table 1).

The results of partial RDA (constraining the space with time) showed that the soil fungal community displayed obvious successional trends along with vegetation succession (Table 2, Fig. S1, Fig. S2). The stand age affected the fungal composition and explained 28.0 and 31.6% variations of fungal community at phylum and order level, respectively (Table 2). The most abundant fungal phylum in early successional stages was Ascomycota, and its dominant status was replaced by the Basidiomycota in the later successional stages (Fig. 1). The mean relative abundance of Ascomycota was the highest in croplands (52.55%), followed by grasslands (35.85%), shrublands (34.11%), and forests (22.9%). The highest abundance of Basidiomycota was observed in forests with a mean of 63.75%, followed by shrublands (32.73%), grasslands (22.59%), and croplands (10.87%) (Table 4).

The relative abundances of Ascomycota decreased significantly with stand age, while the Basidiomycota, Zygomycota, Chytridiomycota, and Cercozoa showed an opposite pattern (Fig. S3, Fig. S5). The relative abundances of Ascomycota, Basidiomycota, Zygomycota, Chytridiomycota, and Glomeromycota varied significantly among the vegetation successional stages (ANOVA, $P > 0.05$, Table 4). The relative abundance of Basidiomycota increased significantly with increase of vegetation coverage, soil moisture, total C, total N, available N, CEC, C:N, and C:P, while the abundances of Ascomycota was correlated negatively with these environmental variables (Fig. 2, Table S4). The highest abundance of Glomeromycota was observed in grasslands with a mean of 2.67%, followed by shrublands (0.83%), croplands (0.17%), and forests (0.15%) (Table 4). The relative abundance of Glomeromycota was positively correlated with soil bulk density and negatively correlated with soil moisture, total C, total N, and available N (Table S4).

α-Diversity of soil bacteria and fungi

Vegetation successional stage significantly affected the α-diversity indexes of soil bacteria and fungi ($P < 0.05$, Fig.3). Soil bacteria of croplands showed the lowest richness and diversity, as indicated by the number of OTUs, Chao1, Shannon, and Faith's Phylogenetic diversity index (Fig. 3). The observed bacteria OTUs averaged 3905, 4515, 4365, and 4473 in croplands, grasslands, shrublands, and forests, respectively. The all α-diversity measurements of soil bacteria increased logarithmically with increasing stand age ($P < 0.01$, Fig. 4a–d). There were no significant relationships between soil fungal α-diversity measurements of soil fungi and stand age (Fig. 4e–h). The mean number of observed fungal OTUs in croplands, grasslands,

Table 4 Relative abundances (%) of the dominant soil fungal phylum (Mean ± SE) under different successional stages (P-values indicate statistically significant differences among successional stages (*$P < 0.05$;**$P < 0.01$;***$P < 0.001$)

	Successional stage				*P*-value
	Cropland	Grassland	Shrubland	Forest	
Ascomycota	52.55 ± 2.94^{a}	35.85 ± 2.71^{b}	34.11 ± 3.27^{b}	22.90 ± 4.23^{c}	***
Basidiomycota	10.87 ± 1.82^{c}	22.59 ± 3.62^{bc}	32.73 ± 4.45^{b}	63.75 ± 6.90^{a}	***
Zygomycota	9.74 ± 1.23^{a}	11.76 ± 1.81^{a}	10.98 ± 1.49^{a}	4.00 ± 1.18^{b}	**
Chytridiomycota	2.01 ± 0.43^{a}	0.64 ± 0.14^{b}	0.69 ± 0.23^{b}	0.16 ± 0.06^{b}	***
Fungi_unidentified	0.85 ± 0.08^{abc}	0.98 ± 0.15^{ab}	1.07 ± 0.26^{a}	0.43 ± 0.17^{c}	*
Glomeromycota	0.17 ± 0.09^{b}	2.67 ± 0.68^{a}	0.83 ± 0.13^{b}	0.15 ± 0.04^{b}	***
Cercozoa	0.16 ± 0.05	0.12 ± 0.02	0.20 ± 0.06	0.06 ± 0.03	0.09
Blastocladiomycota	0.06 ± 0.05	0.02 ± 0.02	0.01 ± 0.00	0.00 ± 0.00	0.44
Rozellomycota	0.02 ± 0.01	0.37 ± 0.14	0.42 ± 0.15	0.22 ± 0.09	0.08

Letters on superscript indicate differences at $P < 0.05$ among successional stages)

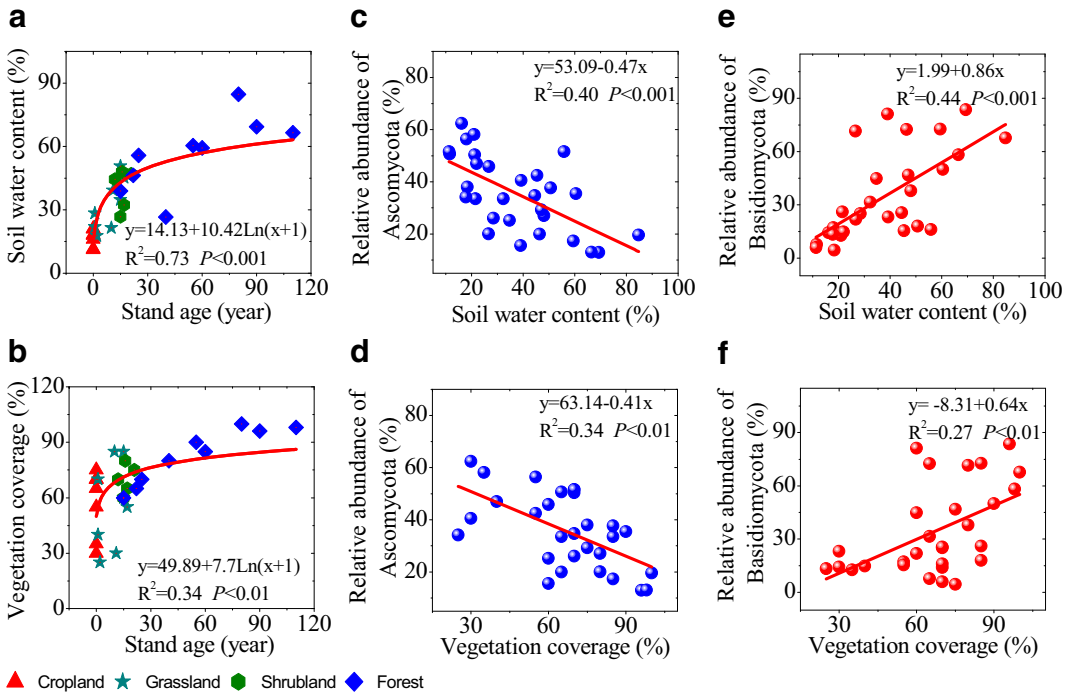

Fig. 2 Changes in soil moisture (**a**) and vegetation coverage (**b**) during secondary succession in Qinling Mountains, Central China. The relationships of the relative abundance of Ascomycota and Basidiomycota with soil moisture (**c**, **e**) and vegetation coverage (**d**, **f**)

shrublands, and forests were 765, 1090, 1326, and 894. The number of OTUs, Chao1, and Faith's Phylogenetic diversity index of soil fungi in the shrubland were significantly higher than those in croplands and forests (Fig. 3). The Shannon index of soil fungi in forests was significantly lower than that in shrublands, grasslands,

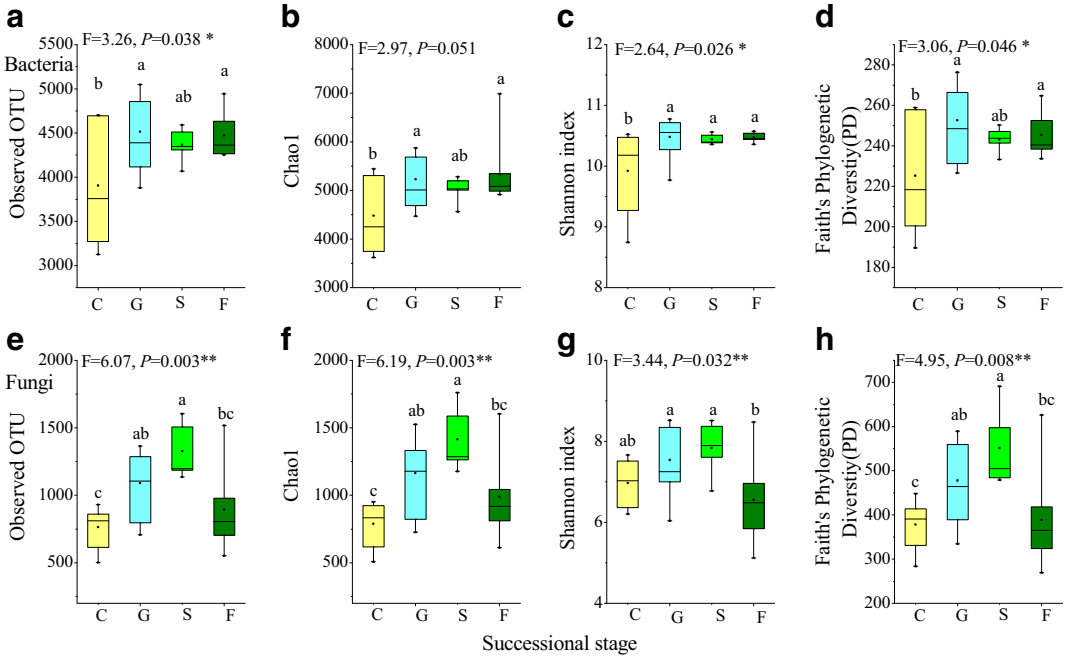

Fig. 3 Bacterial (**a**-**d**) and fungal (**e**-**h**) α-diversity measurements of soils under different successional stages. (C, Cropland; G, Grassland; S, Shrubland; F, Forest)

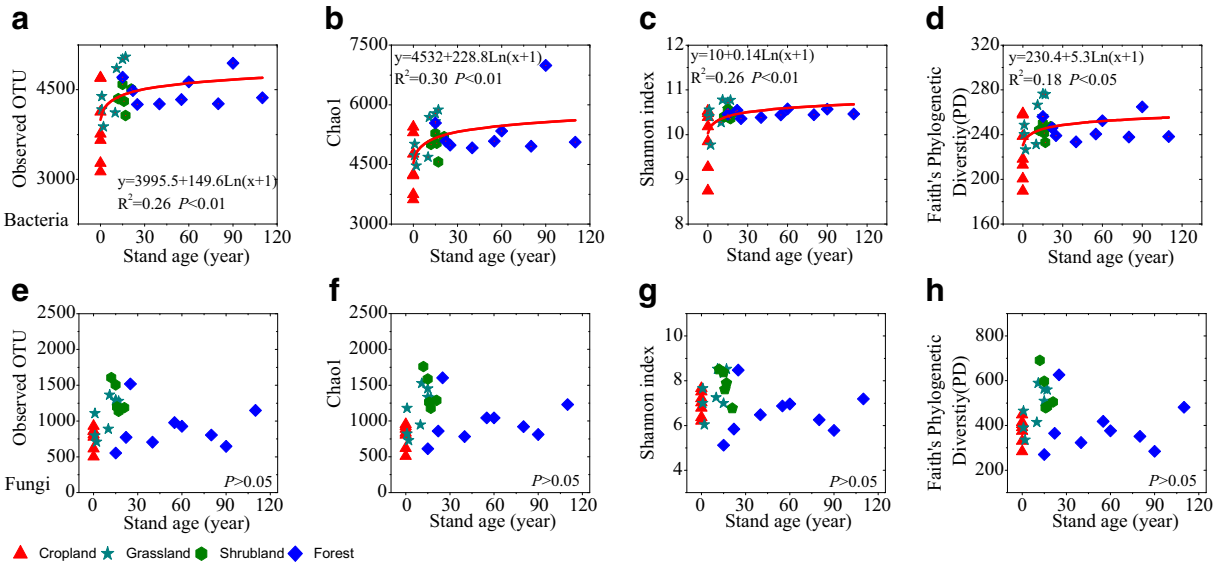

Fig. 4 Relationships between stand age and α-diversity measurements of soil bacteria (**a**-**d**) and fungi (**e**-**h**)

and croplands (Fig. 3). Stepwise-regression models showed that soil pH was the main environmental factor affecting the soil fungal α-diversity measurements (Table S5). The Shannon index of soil bacteria was affected by soil pH and N/P ratio, while the Chao1 was affected by soil total N, available N, and available P. The soil pH and available P were the main environmental factors explaining the variations in the bacterial Faith's Phylogenetic diversity index and number of OTUs (Table S5). For the grasslands, the bacterial and fungal richness (no. of observed OTUs) increased significantly with stand age (Fig. S6). The fungal richness was correlated positively with the plant richness of grassland, while there was no significant relationship between bacterial richness and plant richness (Fig. S6). For forest stands, there were no significant relationships between the richness of bacteria and fungi and stand age, and the bacterial and fungal richness did not correlated with the tree richness (Fig. S6).

Environmental factors and β-diversity of soil bacteria and fungi

Vegetation successional stage significantly affect soil pH, moisture, bulk density, total C, total N, CEC, available N, available P, C:N ratio, C:P ratio and N:P ratio, as well as the vegetation coverage (ANOVA, $P < 0.05$) (Table S6). The soil pH showed an increase trend from croplands to shrublands stage, and then followed by a decreasing trend (Fig. S7). The vegetation coverage, soil moisture, soil total C, N, and available N increased significantly with stand age (Fig. 2, Fig. S7).

The results of ANOSIM test showed that soil bacteria ($R = 0.47$, $P = 0.001$) and fungi ($R = 0.52$, $P = 0.001$) community composition (based on OTUs) differed significantly among the successional stages, except that dissimilarity between the grasslands and shrublands was not significant (Table 5). The heatmap of Phylogenetic β-

Table 5 Dissimilarities in total bacterial and fungal OTU community composition between successional stages, as determined by analysis of similarities (ANOSIM) R value

Successional stage	Cropland	Grassland	Shrubland
Bacterial OTU			
Grassland	**0.69**		
Shrubland	**0.84**	0.16	
Forest	**0.93**	**0.26**	**0.28**
Successional stage	Cropland	Grassland	Shrubland
Fungal OTU			
Grassland	**0.72**		
Shrubland	**0.95**	0.17	
Forest	**0.86**	**0.69**	**0.38**

An R value near +1 means that there is dissimilarity between the groups, while a R value near 0 indicates no significant dissimilarity between the groups. Values in bold indicate significant dissimilarity ($P < 0.05$)

diversity of soil fungi and bacteria based on weighted UniFrac distances also showed the differences among the different successional stage (Fig. S8, Fig. S9). Principal coordinate analysis (PCoA) using UniFrac distances showed clear clustering of the fungal communities in different stages except for few stands (e.g., F3) (Fig. 5). The weighted UniFrac PCoA explained 38% (PC1 and PC2) of total variation of soil fungal communities, while the unweighted UniFrac PCoA explained 24% of total variation (Fig. 5a, b). The UPGMA cluster analysis based on weighted UniFrac distances of fungal communities showed that, all the forest stands except the F3 could cluster into a group, and all the croplands except the C5 could cluster into a group (Fig. 5c). For bacterial communities, most of the stands in different successional stages could cluster into clear groups based on the PCoA of the UniFrac distances. However, the PCoA and UPGMA cluster analysis showed that the bacterial communities in different successional stages did not cluster as clearly as the fungal communities (Fig. 5, Fig. S10). The weighted UniFrac PCoA explained 53% (PC1 and PC2) of total variation of soil bacterial communities (Fig. S10).

The soil pH, total C, and available P were closely correlated with the total bacterial community composition at order level, and together explained 60.2% of variations (Table 6, Fig. 6). The soil pH explained 33.5% of the total variation of bacterial community composition, which provided the greatest explanatory power. For the fungal communities, total C, available P, bulk density, total K, and vegetation coverage were related significantly to the community composition at order level, and together explained 46.1% of variations (Table 6, Fig. 6). The soil total C explained 31.2% of the total variation of fungal community composition, which provided the greatest explanatory power.

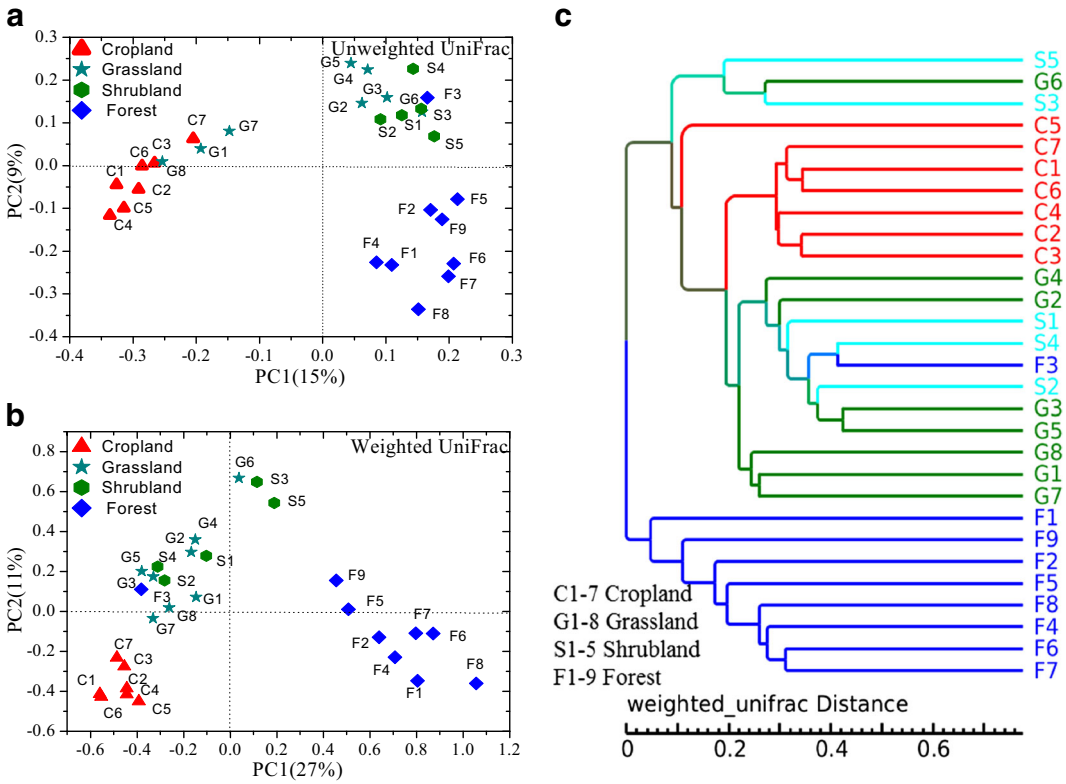

Fig. 5 Phylogenetic β-diversity of soil fungi. (**a**, **b**) Principal coordinate analysis of unweighted and weighted UniFrac distances. (**c**) UPGMA (Unweighted Pair-group Method with Arithmetic Mean) cluster analysis based on weighted UniFrac distances

Table 6 Results of redundancy analysis (RDA) for soil bacterial and fungal communities (Order level) showing the percentage of variation explained by environmental variable

Variable	%Var	Pseudo-F	*P*
Bacteria			
Simple Term Effects:			
Soil pH	33.5	13.6	**0.002**
Total C	29.3	11.2	**0.002**
Available P	17.1	5.6	**0.004**
All selected Variables			
Soil pH, total C, and available P	60.2	12.6	**0.002**
Fungi			
Simple Term Effects:			
Total C	31.2	12.2	**0.001**
Bulk density	18.1	6.0	**0.002**
Total K	15.1	4.8	**0.006**
Vegetation cover	15.1	4.8	**0.004**
Available P	12.8	4.0	**0.014**
All selected Variables			
Total C, bulk density, total K, available P, and vegetation cover	46.1	3.9	**0.002**

%Var: the percentage of variance in community composition data explained by the variable. Values in bold indicate significant correlation ($P < 0.05$)

Discussion

Patterns of soil bacterial and fungal community composition change during secondary vegetation succession

Our results indicated that both the soil bacterial and fungal communities displayed successional trends along with vegetation succession (Tables 2 and 5). The bacterial succession seems less obvious than fungal succession due to only a small part of variations in bacterial communities could be explained by the successional time (i.e., stand age) (Table 2).

The results of partial RDA indicated that the stand age was not a significant variable ($P = 0.058$) for soil bacterial community at order level, which is in contrast to soil fungal communities, where stand age showed a significant effect ($P = 0.002$). Moreover, the most abundant soil bacterial and fungal phyla displayed different dynamic patterns during secondary succession (Fig. 1). The Proteobacteria, the most abundant bacterial phylum cross all stands, kept its absolute dominance constantly during succession even though the vegetation changed obviously from crop to grass, shrub, and forest (Fig. 1). For the fungi, a distinct replacement was observed along with the secondary succession gradient and the most abundant soil fungal phylum shifted from Ascomycota in early successional stages to Basidiomycota in older stands (Fig.1). All these results indicated that the soil bacteria and fungi exhibited differences in successional pattern during secondary succession. The differences between soil bacteria and fungi succession might be attributed to their differences in metabolic types and adaptations to the shifts in environmental conditions and nutrient availability (Duc et al. 2009; Schmidt et al. 2014; Alfaro et al. 2017).

The extremely abundant Proteobacteria and their relatively stable abundance in all successional stages possibly linked with the high resource availability of our studied ecosystems (Table S6). The three most dominant orders within the Proteobacteria phylum of our soils, i.e., Xanthomonadales, Myxococcales, and Rhizobiale, belong to chemoorganotrophic groups and together accounted for 14.5% 13.9%, 16.2%, and 17.1% OTUs in croplands, grasslands, shrublands, and forests, respectively (Table S7, Fig. S11). Contrasting to the primary succession which occurs on poor habitat with low carbon and mineral nutrient availability (Schmidt et al. 2014), the initial abandoned croplands inherited soil organic matters and mineral nutrients from the croplands. Thus, the chemoorganotrophic bacteria would dominate even in the early stages of secondary succession.

The decreasing abundance of Cyanobacteria along with successional gradient was as expected because the Cyanobacteria belong to photoautotrophs and sunlight availability was decreasing during succession due the increasing vegetation coverage (Fig. 2, Fig. S4). This inference was supported by the negative correlations between the relative abundances of the Cyanobacteria and vegetation coverage (Table S3). In addition, the vegetation coverage may also affect the abundances of Armatimonadetes and Elusimicrobia due to the significantly correlations were observed (Table S3). The increasing abundances of Nitrospirae during secondary succession could be attributed to the rising total N in soils because the Nitrospirae was positively corrected with soil total N (Figs. S4, S7).

The increase of Basidiomycota abundance and the decrease of Ascomycota abundance could be attributed to the changes in habitat because their abundances

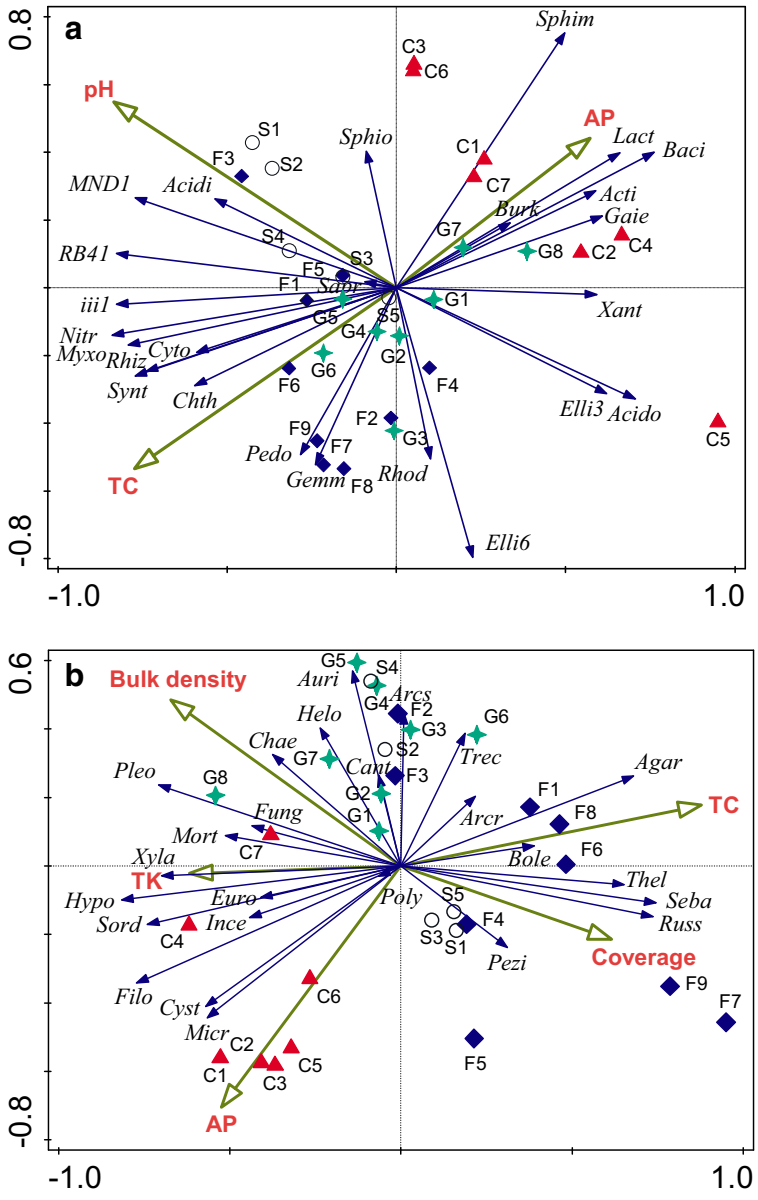

Fig. 6 The RDA (Redundancy Analysis) ordination maps based on the relative abundances of the 25 most abundant bacterial (**a**) and fungal (**b**) orders (See Table S7 and Table S8 for the full name). Orders (solid arrows), environmental variables (hollow arrows), and sampling sites (triangle: C1 to C7, star: G1 to G8, circle: S1 to S5, diamond: F1 to F9) were showed in the plots of RDA. TC: total C; TK: total K; AP: available P; Coverage: Vegetation coverage

strongly correlated with vegetation coverage and soil moisture which both increased significantly with stand age (Fig. 2). Most surprisingly, the shift in dominant fungal phylum in our soils was consistent with the fungal dynamic patterns in a primary succession in Damma glacier forefield in central Switzerland, where the fungi shifted from an Ascomycota-dominated community in young soils to a more Basidiomycota-dominated community in old soils (Zumsteg et al. 2012). Currently, we are not sure whether the similar shifts from Ascomycota-dominant to Basidiomycota-dominant during secondary succession and primary succession present a universal pattern, because the fungal community in Damma glacier forefield was studied by the Terminal Restriction Fragment Length Polymorphism (T-RFLP) which differed from our method used in the present study (Zumsteg et al. 2012). However, it is worth noting that the sampling sites in the Damma glacier forefield ranged from fine granite sand without vegetation (close to the glacier terminus) to well-developed soils covered with vegetation (up to 140 years old) (Zumsteg et al. 2012). Also, our sampling sites

involved a century of secondary succession with stand age ranging from 0 to 110 years (Fig.1). Within the Basidiomycota phylum of our soils, Agaricales was the dominant order, and its abundance increased significantly with increasing stand age and averaged 3.7%, 13.5%, 24.0%, and 20.5% respectively in the croplands, grasslands, shrublands, and forests (Fig. S12, Table S8). Thus, the dominance of Basidiomycota in the older stands can be explained by the mycorrhizal associations of the Agaricales and the plant litter decomposing activities of other Basidiomycota fungi (Zumsteg et al. 2012). With the Ascomycota phylum of our soils, the Hypocreales and Sordariales were the most dominant orders, and their abundance both decreased significantly with increasing stand age (Fig. S12). The relative abundance of Hypocreales averaged 14.3%, 5.6%, 6.0%, and 2.4% respectively in the croplands, grasslands, shrublands, and forests, and the relative abundance of Sordariales averaged 9.7%, 5.2%, 3.0%, and 2.2% respectively in the croplands, grasslands, shrublands, and forests (Fig. S12, Table S8). Most Sordariales are saprobic, and they are commonly found on dung or decaying plant matter (Kendrick 2000). Thus, the dominance of Ascomycota in the early successional stage soils can be explained by the dung associations of the Sordariales, due to the fact that the animal and human manure are the common fertilizer in croplands in our study region.

We found that the abundance of arbuscular mycorrhizal (AM) fungi (i.e., Glomeromycota fungi, Schappe et al. 2017) differed significantly among successional stages, and the abundance of AM fungi peaked in grasslands and then decreased (Table 4, Fig. S5). The exact reasons for the AM fungi dynamic patterns during secondary succession were unclear because there was no environmental factor correlated with the abundance of AM fungi in our study. One possible explanation is that the increasing competition from Agaricales fungi may reduce the abundance of AM fungi in later successional stages.

Different responses of soil bacteria and fungi diversity to vegetation succession

Our results evidenced that the soil bacteria and fungi diversity responded differently to vegetation succession (Figs 3 and 4). The soil bacterial richness and diversity measurements increased logarithmically with increasing stand age, indicating that vegetation succession had a strong effect on bacteria diversity and the community diversity gradually reached equilibrium. The increasing soil bacterial richness and diversity could be attributed to the increasing resource availability and changing soil chemistry along vegetation succession, because we found the soil variables, e.g., soil pH, total N, N/P ratio, available P, etc., significantly affected the α-diversity measurements of soil bacteria (Table S5). The patterns of soil bacterial richness change during succession were consistent with the findings of Schuette et al. (2010) who reported a linearly increasing richness pattern during primary succession in an Arctic glacier forefield, but inconsistent with the results of Dini-Andreote et al. (2014, 2015) who observed a linearly decreasing richness and diversity pattern in a salt marsh successional chronosequence, and inconsistent with the findings of Jangid et al. (2013) who found that all of the α-diversity indices of soil bacterial community declined along the along the Franz Josef soil and vegetative succession chronosequence ranging in age from 60 to 120,000 years since glacial retreat. In addition, in a secondary succession chronosequence of abandoned farmland in China's Loess Plateau, Zhang et al. (2016) found that the soil bacterial community richness and diversity in the non-abandoned croplands were higher than that in the abandoned cropland being at early successional stage, which was inconsistent with our result that the croplands displayed the lowest bacterial α-diversity indices (Fig. 3). The inconsistencies indicated that more efforts should be made to test whether there are universal patterns of soil bacterial community diversity change during secondary vegetation succession.

Unlike bacteria, fungi community diversity tended to increase in the early successional stages and then followed by a decreasing trend (Fig. 3). The changes in fungi community diversity during succession could be attributed to the changes in soil chemistry (Fig.3, Fig. S7). For example, we found that the soil pH was the most important environmental factor affecting soil fungal α-diversity measurements, and the soil pH increased first and then decreased along the succession gradient (Fig. S7, Table S5). Moreover, the shrublands displayed the highest soil pH and highest fungal α-diversity measurements (Table S6, Fig. 3). Thus, the changes in soil pH during vegetation succession could partly explain the patterns of fungi community diversity changes. The fungal community diversity dynamics in our study sites did not follow the primary successional patterns characterized by some previous studies. For example, Brown

and Jumpponen (2014) found that fungal richness and diversity indices were static across the Lyman glacier chronosequence representing ~70 years of deglaciation. Cutler et al. (2014) observed that the fungal richness and Shannon diversity increased with terrain age during primary succession on an 850-year chronosequence of lava flows in Iceland. Thus, these findings together with our present study indicated that the fungal community diversity dynamics in secondary succession seem to differ from the patterns in primary succession.

Linkages among vegetation succession, soil microbial succession, and dynamics of soil physico-chemical properties

We found that vegetation succession played a strong role in shaping soil bacterial and fungal communities and soil physico-chemical properties (Tables 2, and 5, Fig. 5, Fig. S10). The results of ANOSIM test, PCoA, and UPGMA clustering indicated that the soil microbial communities showed clear dissimilarities across the successional gradient (Table 5). However, the soil microbial communities in grasslands and shrublands did show significant discrepancy, which could be attributed to the unsignificant differences in the vegetation coverage and soil nutrients between grasslands and shrublands (Table S6). Following agricultural abandonment in our study area, the natural vegetation restored spontaneously and the soil physicochemical attributes of the abandoned croplands changed markedly (Fig.1). The increasing vegetation coverage, soil moisture, and soil nutrients (e.g., soil C and N) represented the changes in soil habitat, which consequently structured the soil microbial communities (Fig. S7).

The RDA results showed that the soil total C, total K, and available P were the main factors associated with the fungal order composition (Table 6), suggesting the importance of soil nutrients in shaping fungal community. This result is in accordance with expectation, because previous researches have suggested some fungal groups, e.g., AM fungi, are closely related to the availability of P (Read 1994). We found that the soil pH was the main factor explaining the variation of bacterial order composition, and the soil pH increased first and then decreased during the vegetation succession (Table 6, Fig. S7). The increases of soil pH in early successional stage possibly caused by the land-use history and the new vegetation growing on abandoned croplands. Chemical fertilizer could result in the acidification in croplands (Guo et al. 2010), thus, we observed the lowest soil pH in croplands (Table S6). Since the croplands were abandoned and the chemical fertilizer input was ceased, the aftereffect of chemical fertilizer faded away with vegetation succession. Thus, the soil pH increased in the early years after abandonment. The decrease of soil pH in later successional stages could be attributed to the natural progression of soil acidification during forest restoration which could be explained by the uptake of nutrient cations and deposition of acidic litter by trees (Bautista-Cruz and del Castillo 2005). Therefore, overall, our results evidenced that the changes in soil pH and nutrients driven by vegetation succession shaping soil microbial communities.

The grass richness, soil bacterial and fungal richness in grasslands increased with stand age (Fig. S6), indicating that the plant and soil microbial communities develop towards species abundant status in early successional stage following agricultural abandonment. Compared to bacteria, the fungal richness was more closely associated with grass richness (Fig. S6). As fungi readily disperse by spores, increasing fungal richness is most likely due to new habitat niches created by the newly colonized plant species, thus, the increased fungal richness is likely to be associated with increased plant richness (Read 1994; Cutler et al. 2014). In the forest stage, the soil bacterial and fungal richness seems to reach equilibrium, and the tree species displayed a large variation and tended to show a unimodal pattern (but not significant in statistics) with increasing stand age. The relationships among the tree richness, soil microbial richness, and stand stage were not significant (Fig. S6), suggesting the stability of species richness in later successional stage. However, the reasons for this species richness patterns in forest stage are unclear. Further studies could focus on the linkages among tree richness and soil microbial richness and their responses to succession.

Conclusions

The novel contribution of our study comes from simultaneous measurements of soil bacterial and fungal community succession during a century of secondary vegetation development following agricultural abandonment. Our data clearly evidenced that both soil bacterial and fungal communities displayed successional trends

along with vegetation succession, and the soil bacteria and fungi exhibited marked differences in successional pattern during secondary succession. The Proteobacteria, dominated by chemoorganotrophic bacteria, kept its absolute dominance status constantly during succession even though the vegetation changed obviously from crop to grass, shrub, and forest. A distinct shift in fungal communities from Ascomycota -dominant in young stands to Basidiomycota -dominant in older stands was observed, which could be attributed to the increase of vegetation coverage and soil moisture during succession. The soil bacterial richness and diversity increased logarithmically with increasing stand age and gradually reached equilibrium in late-successional stage. Soil fungi community diversity tended to increase in the early successional stages and then followed by a decreasing trend. The soil pH was the most important environmental factor predicating the soil fungal α-diversity measurements. The increasing soil bacterial richness and diversity could be attributed to the increasing resource availability and changes in soil chemistry along vegetation succession. The vegetation coverage, soil moisture, pH, and soil nutrients (e.g., total C, total N, available N, available P etc.) changed significantly with stand age, suggesting that vegetation succession played a strong role in shaping the habitats structuring soil bacterial and fungal communities. Our findings highlight the importance of vegetation coverage and soil moisture in shaping soil microbial communities during secondary succession following agricultural abandonment.

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