Science of the Total Environment 820 (2022) 153137

Contents lists available at ScienceDirect

Science of the Total Environment

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

Vegetation restoration facilitates belowground microbial network complexity and recalcitrant soil organic carbon storage in southwest China karst region

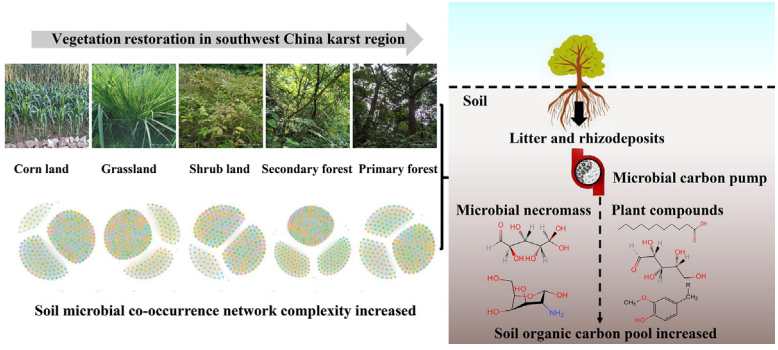
Linan Hu [a,b,c], Qiang Li [b,c,*], Jiahui Yan [b,c], Chun Liu [a,d,**], Juxin Zhong [b,c]

[a] Institute of Groundwater and Earth Sciences, Jinan University, Guangzhou 510632, China
[b] Key Laboratory of Karst Dynamics, MNR & GZAR, Institute of Karst Geology, Chinese Academy of Geological Sciences, Guilin 541004, China
[c] International Research Center on Karst under the Auspices of UNESCO, Guilin 541004, China
[d] Department of Ecology, Jinan University, Guangzhou 510632, China

HIGHLIGHTS

- Vegetation restoration in karst rocky desertification area enhanced soil organic carbon storage.
- Vegetation restoration facilitated the complexity of soil bacterial and fungal networks.
- Soil *Bryobacter*, *Haliangium* and MND1 play key roles in vegetation restoration processes.
- Dominant functional groups involved in C and N cycling shift with vegetation restoration process.
- Recalcitrant soil organic carbon storage linked to dominant microbial functional groups.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 7 November 2021
Received in revised form 11 January 2022
Accepted 11 January 2022
Available online 15 January 2022

Editor: Elena Paoletti

Keywords:
Karst rocky desertification
Vegetation restoration
Microbial community
Co-occurrence network
Recalcitrant soil organic carbon storage

ABSTRACT

Soil organic carbon (SOC) is an important component of soil ecosystems, and soils are a hotbed of microorganisms playing critical roles in soil functions and ecosystem services. Understanding the interaction between SOC and soil microbial community is of paramount significance in predicting the C fate in soils following vegetation restoration. In this study, high-throughput sequencing of 16S rRNA and ITS genes combined with ^{13}C NMR spectroscopy analysis were applied to characterize SOC chemical compounds and elucidate associated soil microbial community. Our results indicated that the contents of SOC, total nitrogen, total phosphorus, microbial biomass carbon and biomass nitrogen, dissolved organic carbon, available potassium, exchangeable calcium and soil moisture increased significantly ($P < 0.05$) along with the vegetation restoration processes from corn land, grassland, shrub land, to secondary and primary forests. Moreover, the Alkyl C and *O*-alkyl C abundance increased with vegetation recovery, but no significant differences of Alkyl C were observed in different successional stages. In contrast, the relative abundance of Methoxyl C showed an opposite trend. The dominate phyla Proteobacteria, Acidobacteria, Actinobacteria, Ascomycota and Basidiomycota were strongly related to SOC. And, SOC was found to be the determining factor shaping soil bacterial and fungal communities in vegetation restoration processes. The complexity of soil bacteria and fungi interactions along the vegetation restoration chronosequence increased. Determinism was the major assembly mechanism of bacterial community while stochasticity dominated the assembly of fungal community. *Bryobacter*, *Haliangium*, and MND1 were identified as keystone genera in co-occurrence network. Besides, the dominant functional groups across all

* Correspondence to: Q. Li, Key Laboratory of Karst Dynamics, MNR & GZAR, Institute of Karst Geology, Chinese Academy of Geological Sciences, Guilin 541004, China.
** Correspondence to: C. Liu, Institute of Groundwater and Earth Sciences, Jinan University, Guangzhou 510632, China.
E-mail addresses: glqiangli@hotmail.com (Q. Li), lspring@hnu.edu.cn (C. Liu).

http://dx.doi.org/10.1016/j.scitotenv.2022.153137

vegetation restoration processes were mainly involved in soil C and N cycles and linked to the enhanced recalcitrant SOC storage. Our results provide invaluable reference to advance the understanding of microbe response to vegetation restoration processes and highlight the impact of microbes on recalcitrant SOC storage.

1. Introduction

The 540,000 km^2 karst region including Yunnan, Guizhou and Guangxi provinces of southwest China is the core of east Asia karst areas, which is one of three major karst areas in the world (Fan et al., 2011). Karst landscape typically has its own particularities, such as soluble rock, calcium-rich, double-layer structure and so forth (Yuan, 1997, 2001). These distinct geological and hydrological characters make it extremely vulnerable to anthropogenic interference and lead to ecological environmental degradation (Jiang et al., 2014). From 1949 to 1970s, under the pressure of 1.7 million people living there, deforestation to reclaim land for food production, consequently, led to severe rocky desertification in these areas (Song et al., 2019). To remediate land degradation and restore degraded soils, several nationwide ecological restoration projects, such as 'Grain to Green Project' and 'Karst Rocky Desertification Restoration Project' were implemented by Chinese government (Bai et al., 2019). With the enforcement of these projects, large amount of farmlands were returned to forests. Thus, the manual intervention or reforestation provides us a unique model to observe changes in the content and quality of soil organic matter and microbial components (Wang et al., 2011; Lu et al., 2018).

Soil microorganisms, possessing tremendous physiological and functional versatility, are ubiquitous and essential to the biogeochemical cycling processes in terrestrial ecosystems, including plant nutrient assimilation, litter decomposition, material transformation, carbon cycling, and nutrient availability (Delgado-Baquerizo et al., 2018, 2019). Several studies provided evidence that the growth of vegetation will influence soil physical and chemical conditions through organic inputs from litters and roots, and soil microbial communities were largely determined by the diversity, composition and production of vegetation (Zhu et al., 2012; Qiu et al., 2020; Li et al., 2021). Thus, the response of the soil microbial community to vegetation changes can increase our understanding of the above- and below-ground interaction (Qu et al., 2020). To better understanding their interactions, the new generation of high-throughput sequencing technology was emerged. Compared with traditional micro-organism culture method and PCR-DGGE, clone library and other methods, high-throughput sequencing provides an extraordinary scientific and practical tool to understand microbial community structures, diversities, interactions and co-occurrence patterns (Liang et al., 2017; Schmidt et al., 2019; Li et al., 2021).

The restoration or rehabilitation of vegetation in degraded environments has been studied extensively in recent years. Previous studies have focused largely on soil bacterial community during vegetation restoration process. For example, Qu et al. (2020) found that bacterial community and function shift drastically in the early stages of forest succession caused by forest disease, and the soil pH, total nitrogen (TN) and soil organic carbon (SOC) were the dominant factors affecting the bacterial community. Cui et al. (2018) found that soil nutrients (SOC, TN, and NH_4^+) increased while the soil bacterial community structures did not show any changes during vegetation succession process in Loess Plateau, China. However, there is a paucity of studies discussing the changes in bacterial and fungal community simultaneously, especially in fragile karst ecosystem. It is essential to synchronously study the bacterial and fungal communities in soil to fully understand microbial response to ecosystem processes, because these communities mediate different ecological functions in soil (Romanowicz et al., 2016). In addition, current research has gradually moved from exploring the distribution patterns of microbial communities in different habitats to the intrinsic community assembly mechanisms (Dini-Andreote et al., 2015). Then, clarifying the relationship between community ecological construction processes and microbial community structure has been a hot topic in microbial ecology in recent years (Knelman and Nemergut, 2014). The assembly processes of the microbial community are generally influenced by two types of ecological process, including deterministic and stochastic processes (Stegen et al., 2012; Zhao et al., 2019). Extensive data demonstrate that a range of factors, such as nutrition, resource availability, successional stage, and perturbations, can influence the relative importance of different community assembly processes (Zhao et al., 2019). Gao et al. (2021) found that long-term fertilization of agricultural fields led to a targeted enrichment of microbial taxa and enhanced the deterministic process of community assembly. How karst soil microbial community assembly process responding to vegetation restoration currently remains elusive, which limits our understanding of microbial community ecology in karst soils.

SOC is an important component of soil ecosystems, providing essential nutrients for plant growth and improving soil fertility. The chemical structure and stability of organic carbon is the key to study soil fertility and carbon cycling (Paul, 2016). Nevertheless, current knowledge of the specific association between SOC molecular properties and microbiota is limited, since most previous studies focused on analysis of quantitative changes in organic carbon pools. How SOC quality changes with time series is still lacking a general consensus. Recent evidence suggests that microbes can progressively exploiting leaf litter and alter its chemical compounds which in turn affects microbial community composition during leaf litter decomposition process (Bonanomi et al., 2018). In this regard, we speculated that SOC chemical dynamics during vegetation restoration were associated with the changes of soil microbial community composition. Microbial processing of organic matter involves not only consumption and degradation, but also the accumulation of new molecules (Hu et al., 2021). The relationship between soil microbe and SOC chemical compounds could be more sophisticated than expected. Hence, understanding the relationship between SOC chemical compounds and soil microbial community is of paramount significance in predicting the C fate in soil following vegetation restoration.

In order to fill the knowledge gap, the soil samples from five typical vegetation types, including corn land, grassland, shrub land, secondary forest and primary forest, representing a spontaneous successional sequence in subtropical karst rocky desertification control ecosystems at national observation and research station in Baise, China were selected as the research objects by using 'space for time' substitution ('Chronosequence') method. Despite its drawbacks, the 'space for time' substitution approach is widely acknowledged as the sole means to determine long-term ecological processes (Li et al., 2021). High-throughput sequencing of the 16S rRNA and ITS gene combined with ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR hereafter) analysis were used to investigate the structural characteristic and interactions between microbial communities, characterize the SOC chemical compounds, and determine the specific relationship between SOC chemical compounds and microbial community across the restoration chronosequence. Given vegetation variations contributing significantly to shaping the microbial community (Zhao et al., 2019; Li et al., 2021; Khalid et al., 2022), we hypothesized that both bacterial and fungal communities and their assembly mechanisms would change with vegetation restoration process in karst area. Due to soil microorganisms determining the fate of soil organic matter (Hu et al., 2021), we then hypothesized that the dominant microbial functional groups are interlinked with SOC chemical compounds in revegetation processes. Briefly, our study focuses on investigating microbial community change pattern during vegetation restoration and the driving of soil microorganisms on C fate in karst rocky desertification area by addressing the following questions: 1. Does vegetation restoration alter the soil microbial community? 2. What are the dominant community assembly mechanisms at different successional stages? 3. How do soil physicochemical and organic carbon chemical compounds respond to vegetation restoration, and do these changes associate with the microbial community? This research will advance our understanding of

the relationships among microbes and soil environment under the influence of vegetation restoration in karst area.

2. Materials and methods

2.1. Study sites

The national observation and research station in Baise (104°26′-107°54′ E, 22°51′-25°07′N) is located in the western part of Guangxi Zhuang Autonomous Region, which is situated in the transition zone from the Yunnan-Guizhou Plateau to the Guangxi Hilly Basin. Its rocks are mainly composed of pure limestone and siliceous limestone. Moreover, this area is dominated by subtropical monsoon climate with abundant light and heat. The average annual temperature is 19.0–22.3 °C, and the average annual precipitation is 1113–1713 mm. In the past, the rocky desertification was severe, the vegetation coverage was limited, the composition of tree species was monotonous, and there were invasive exotic species such as *Eupatorium odoratum* L. After almost 30 years' rocky desertification control, the forest coverage rate has risen from 35.5% to 66.62%, which not only successfully curbed the further aggravation of rocky desertification but also protected part of the forest ecosystem (Yin and Yin, 2010).

2.2. Field measurement and bulk soil samples collection

Field measurements and sample collections were carried out in August 2020. In the transition zone from corn land to primary forest, we selected five different habitat types: corn land (YM) covered with *Zea mays*, grassland (CD) mainly covered with *Achnatherum splendens* (Trin.) Nevski, shrub land (XG) mainly covered with *Vitex negundo* L. and *Pennisetum alopecuroides* (L.) Spreng., secondary forest (CSL) mainly covered with *Schizophragma integrifolium* (Franch.) Oliv., *Derris fordii*, *Acacia concinna* (Willd.) DC., *Mallotus tenuifolius*, *Cipadessa baccifera* and *Ficus erecta*, and primary forest (YS) mainly covered with *Photinia komarovii*, *Xylosma conqesta* and *Sloanea hemsleyana* to represent the different stages of the typical vegetation successional process in this area. The distance between different successional stage sample plots was approx. 1000 m. Three 10 m^2 quadrats were established along the "S" type for each sample plot, and each quadrat was approx. 10 m apart. Due to the high spatial heterogeneity features of karst soil (Li et al., 2021), five sampling cores with 5 cm diameter and 20 cm depth in each quadrat were hybridized to obtain a composite soil sample, and a total of 15 soil samples were collected. Soil samples were packed in sterile plastic bags and immediately transferred to the laboratory to remove plant roots, gravel and other external intruder. After that, each sample was homogenized and divided into two sub-samples. One fresh subset was passed through 2.00 mm sieve and then stored at −80 °C for 16S rRNA and ITS gene sequencing. The remaining subset was further separated into two portions. One portion was screened through 2.00 mm sieve and stored at 4 °C to determine soil moisture (SM), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) contents, while the other portion was air-dried and passed through 0.15 mm or 2.00 mm sieve for the measurement of pH, SOC, TN, dissolved organic carbon (DOC), total phosphorous (TP), exchangeable calcium (E-Ca), exchangeable magnesium (E-Mg) and available potassium (AK).

2.3. Soil physicochemical analysis

SOC content was determined by potassium dichromate-sulfuric acid oxidation method (Bremner and Jenkinson, 2010). TN content was determined by Kjeldahl method (Baethgen and Alley, 1989). TP content was determined by acid solution-molybdenum antimony colorimetric method (Parkinson and Allen, 2009). SM content was determined by gravimetric analysis. Potentiometric method was used for measuring soil pH value (soil/water ratio = 1:5, M/V) (Li et al., 2015). E-Ca, E-Mg and AK were extracted from 6 g of soil using 40 mL 0.1 mol/L $BaCl_2$ solution after 2 h of oscillation, and the extracted solution was determined by ICP-AES (Hendershot and Duquette, 1986). DOC content was extracted by high purity deionized water, and the supernatant was filtered through 0.45 μm membrane, then measured by total organic carbon analyzer (TOC-VCPH, SHIMADZU, Japan) (Lu, 1999). MBN and MBC contents were determined by chloroform fumigation-K_2SO_4 extraction method (Lu, 1999).

SOC chemical data were obtained from ^{13}C NMR analysis. The NMR instrument used for analysis is Bruker Avance 400 at Nanjing University of Science and Technology. The test conditions were spectral frequency - 100 MHz, contact time - 1 ms, delay time - 0.5 s, sampling time - 0.05 s, and number of scan - 30,720 times. The chemical displacement and integration range of various organic carbon functional groups selected in this study were alkyl C (0–45 ppm), methoxyl C (46–60 ppm), *O*-alkyl C (61–90 ppm), di-*O*-alkyl C (91–110 ppm), H- and C- substituted aromatic C (111–140 ppm), *O*-substituted aromatic C (141–160 ppm) and carboxylic C (161–190 ppm).

2.4. DNA extraction and bioinformatic analysis

DNA was extracted from 0.25 g soil samples with the Power Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) following the manufacturer's instructions. The concentration and purity of the extracted DNA were determined by using Quawell Q5000 (Quawell Technology, Inc., San Jose, USA). The primers 515F (GTGCCAGCMGCCGCGGTAA) and 907R (CCGTCAATTCMTTTRAGTTT) were used for PCR amplification of the 16S rRNA V4-V5 variable region (Caporaso et al., 2011), and ITS1F (CTTGGTCATTTAGAGGAAGTAA) combined with ITS2R (GCTGCGTTCTT CATCGATGC) were used for PCR amplification of the ITS rRNA region (Limon et al., 2017). Specific barcode sequences were added to the above primers 907R and ITS2R to differentiate the different samples. The amplification products were sequenced on the next-generation Illumina Novaseq platform at Guangdong Meige Gene Technology Co., Ltd. (Guangzhou, China). The obtained sequencing downstream data were divided into different sample data according to barcode sequence, and the barcode sequence was truncated. Flash version 1.2.11 software was used to splice each sample sequence (Mago and Salzberg, 2011). Primer sequences and sequences less than 200 bp were removed using Cutadapt version 1.9.1 software (Martin, 2011). Usearch version 8.1.1861 software was used to remove chimeras and perform cluster analysis on the processed sequences, and sequences >97% similarity levels were grouped into an OTU (operational taxonomic unit). Bacterial and fungal OTU taxonomic information were obtained by comparing the representative sequences of OTUs with Silva version 132 16S rRNA and UNITE version 8.0 databases, respectively, using Uclust version 1.2.22 software (Edgar, 2010). To avoid biases caused by the different sequencing depths, OTU tables were rarefied to the lowest number of sequences per sample (47,704 and 48,732 for bacteria and fungi, respectively) using single_rarefaction.py script. Based on the rarefied OTU tables, QIIME version 1.9.1 software was used to calculate alpha diversity index (Chao1, Simpson, Shannon, and Observed OTUs) and beta diversity distance (Weighted UniFrac) matrices (Caporaso et al., 2010). The 16S rRNA and ITS genes sequences were deposited into the NCBI Sequence Read Archive (SRA) database with the number of PRJNA778339 and PRJNA778453.

2.5. Statistical analysis

SPSS version 25.0 software (IBM Corp., Armonk, NY, USA) were used for statistical analyses of soil physicochemical property parameters, ^{13}C-CPMAS NMR-derived parameters and microbial diversity indices in different successional stages. One-way-analysis of variance (one-way ANOVA) followed by least-significant difference (LSD) multiple comparison test at 0.05 level was used for significance analysis. The NMR functional group area integration was carried out with the MestReNova software version 14.0 to obtain the relative abundance of different C-types. The Origin version 8.5 software was used to map the abundance of bacterial and fungal communities at the phylum level. R environment (http://www.r-project.com) with version 3.6.1 was also used to perform statistical computation. Venn diagrams were generated to explore the distribution patterns of bacteria and fungi at OTU level by using Venn package in R. Principal co-

ordinates analysis (PCoA) (based on Bray-Curtis distance algorithm) and analysis of similarity (ANOSIM) plots were drawn to assess the similarity of soil bacterial and fungal community structures by applying the Vegan package in R. Heat maps of dominant OTUs (>0.5%) and the Pearson correlations between edaphic variables and dominant OTUs with P values <0.05 were created by Pheatmap package in R to determine the influence of environmental factors on the change in relative abundance of microbial taxa. The β-mean nearest taxon distance (βMNTD) and β-nearest taxon index (βNTI) were calculated by Picante package in R following the protocol outlined by Stegen et al. (2012) to shed light on whether deterministic or stochastic processes shaped the microbial community along vegetation restoration processes. If $|\beta NTI| \geq 2$, then a deterministic process accounts for differences between microbial communities in two samples, conversely, if $|\beta NTI| < 2$, then a stochastic process explains observed differences in microbial community composition between two samples. Subsequently, deterministic and stochastic processes can be further categorized into five ecological processes according to βNTI and Bray-Curtis-based Raup-Crick Index (RC_{Bray}) values, including homogeneous selection (βNTI>2), heterogeneous selection (βNTI<−2), dispersal limitation ($|\beta NTI| < 2$ and $RC_{Bray} > 0.95$), homogenizing dispersal ($|\beta NTI| < 2$ and $RC_{Bray} < -0.95$), and undominated ($|\beta NTI| < 2$ and $|RC_{Bray}| < 0.95$). Redundancy analysis (RDA) of dominant OTUs and soil physicochemical parameters was made by CANOCO for Windows 5.0 (Ithaca, NY, United States) to detect the strength of edaphic drivers upon bacterial and fungal communities' structure. Gephi version 0.9.2 software was used to plot the Pearson correlation networks to characterize the interactions with P values < 0.05 among the dominant OTUs and between soil physicochemical factors and dominant OTUs (Li et al., 2021). Based on the reference of Barberan et al. (2012), the OTUs with more than 5 sequences in each group were selected for further analysis, and the co-occurrence networks of soil bacteria and fungi across five successional stages were mapped via Gephi version 0.9.2 software. To determine the potential microbial ecological functions in soils in different successional stages, the dominant OTUs obtained were compared with the FAPROTAX (script version 1.1) and FUNGuild v1.1 databases to predict the potential metabolic functions of soil bacterial and fungal communities. Finally, Pearson correlation matrix was calculated to determine the specific relationships between the dominant microbial functional groups and resulted C-types.

3. Results

3.1. Bulk soil physicochemical and SOC chemical compound characteristics

Significant differences of soil physicochemical properties in vegetation restoration processes were observed (Table 1). SOC, TN, MBC, MBN, DOC, AK, E-Ca and SM contents generally increased along with the restoration chronosequence. High spatial heterogeneity in soil TP and E-Mg contents emerged among the five successional stages. Interestingly, the variation of pH ranged from 6.41 to 7.25, with YS having a considerable lower pH than that in YM, CD and CSL, but no significant difference appeared between YS and XG. ^{13}C NMR spectra revealed the dynamics in the relative abundance of different C chemical compounds during vegetation restoration (Table 1). It is notable that the relative abundance of Alkyl C and *O*-alkyl C showed an increasing trend with vegetation recovery, although the change of Alkyl C was not significant. Conversely, the relative abundance of Methoxyl C followed an opposite trend, with significant differences between YM and other successional stages. The relative abundance of Carboxylic C did not change substantially in vegetation restoration processes.

3.2. Soil microbial community composition

The distribution of microorganisms differed at the phylum level across all of the soil samples (Fig. 1A and C). The dominant bacterial phyla in the studied soil samples were Proteobacteria, Acidobacteria and Actinobacteria. The total proportions of the three dominant bacteria were YM (53.32%), CD (69.98%), XG (62.06%), CSL (65.62%) and YS (68.11%) (Table S1). Ascomycota, Basidiomycota and Zygomycota were the dominant fungal phyla, and the total proportions of the three dominant fungi were YM (92.81%), CD (78.99%), XG (76.36%), CSL (90.97%) and YS (89.52%) (Table S1). It is remarkable that the relative abundance of Proteobacteria was significant lower in YM and XG than that in CD, CSL and YS, the relative abundance of Basidiomycota was highest in YS, and the relative abundance of Ascomycota was lowest in XG. In addition, the relative abundance of soil bacterial and fungal communities varied in each soil sample, which in turn reflected the highly heterogeneous characteristics in karst soils (Fig. 1B and D).

Venn diagrams demonstrated that OTUs of soil bacteria and fungi varied in vegetation restoration processes (Fig. S1A and S1B). The number of

Table 1
Soil physicochemical properties and SOC chemical traits in vegetation restoration processes.

Factors	Vegetation restoration process				
	YM	CD	XG	CSL	YS
pH	7.25 ± 0.17a	7.21 ± 0.14a	6.68 ± 0.08b	6.82 ± 0.14a	6.41 ± 0.24b
SM(%)	21.34 ± 0.51c	24.10 ± 0.68bc	24.05 ± 0.92bc	25.35 ± 0.89b	31.34 ± 1.45a
SOC(g/kg)	19.68 ± 0.79d	35.73 ± 1.65c	40.56 ± 1.54c	65.06 ± 1.69b	115.25 ± 4.86a
TN(g/kg)	2.03 ± 0.39bc	1.53 ± 0.20c	2.76 ± 0.66b	2.80 ± 0.05b	4.56 ± 0.20a
TP(g/kg)	0.78 ± 0.06d	1.35 ± 0.03b	1.09 ± 0.03c	0.63 ± 0.09d	2.05 ± 0.10a
MBC(mg/kg)	84.55 ± 20.19b	63.10 ± 4.86b	164.1 ± 29.13b	371.3 ± 57.96a	473.5 ± 89.18a
MBN(mg/kg)	7.14 ± 1.81b	15.51 ± 0.65b	16.70 ± 2.95b	15.43 ± 2.98b	46.40 ± 7.21a
DOC(g/kg)	0.45 ± 0.02b	0.49 ± 0.02b	0.39 ± 0.06b	0.66 ± 0.07ab	0.89 ± 0.23a
AK(mg/kg)	30.29 ± 2.13b	37.82 ± 5.07b	28.45 ± 4.03b	35.29 ± 4.01ab	42.40 ± 3.73a
E-Ca(cmol/kg)	11.56 ± 1.17c	21.47 ± 2.67b	18.87 ± 1.52b	24.72 ± 2.74b	37.58 ± 1.38a
E-Mg(cmol/kg)	0.37 ± 0.02c	0.48 ± 0.05bc	0.41 ± 0.03c	1.29 ± 0.19a	0.75 ± 0.05b
Alkyl C - 0-45 ppm	0.25 ± 0.03a	0.23 ± 0.01a	0.23 ± 0.02a	0.25 ± 0.01a	0.26 ± 0.01a
Methoxyl C - 46-60 ppm	0.15 ± 0.02a	0.10 ± 0.01b	0.10 ± 0.01b	0.11 ± 0.01b	0.08 ± 0.02b
O-alkyl C - 61-90 ppm	0.20 ± 0.02b	0.23 ± 0.01ab	0.23 ± 0.02ab	0.28 ± 0.02a	0.26 ± 0.01ab
di-*O*-alkyl C - 91–110 ppm	0.04 ± 0.00b	0.04 ± 0.00b	0.05 ± 0.00b	0.06 ± 0.00a	0.06 ± 0.00a
H-C-substituted aromatic C - 111–140 ppm	0.14 ± 0.00b	0.19 ± 0.01a	0.18 ± 0.02ab	0.11 ± 0.01b	0.15 ± 0.02ab
O-substituted aromatic C - 141-160 ppm	0.05 ± 0.00a	0.04 ± 0.01a	0.05 ± 0.01a	0.03 ± 0.00a	0.04 ± 0.01a
Carboxylic C - 161-190 ppm	0.17 ± 0.01a	0.16 ± 0.01a	0.16 ± 0.00a	0.16 ± 0.01a	0.16 ± 0.01a

Note: Values are mean ± standard error (mean ± SE) (n = 3). Statistical significance was assessed by one-way ANOVA followed by LSD multiple comparison test. Different lowercase letters (a, b, c and d) per line indicate the significant difference ($P < 0.05$). YM, corn land; CD, grassland; XG, shrub land; CSL, secondary forest; YS, primary forest; SM, soil moisture; SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; AK, available potassium; E-Ca, exchangeable calcium; E-Mg, exchangeable magnesium. For each soil sample, ^{13}C CPMAS NMR data refer to abundance of C-types relative to the whole spectrum.

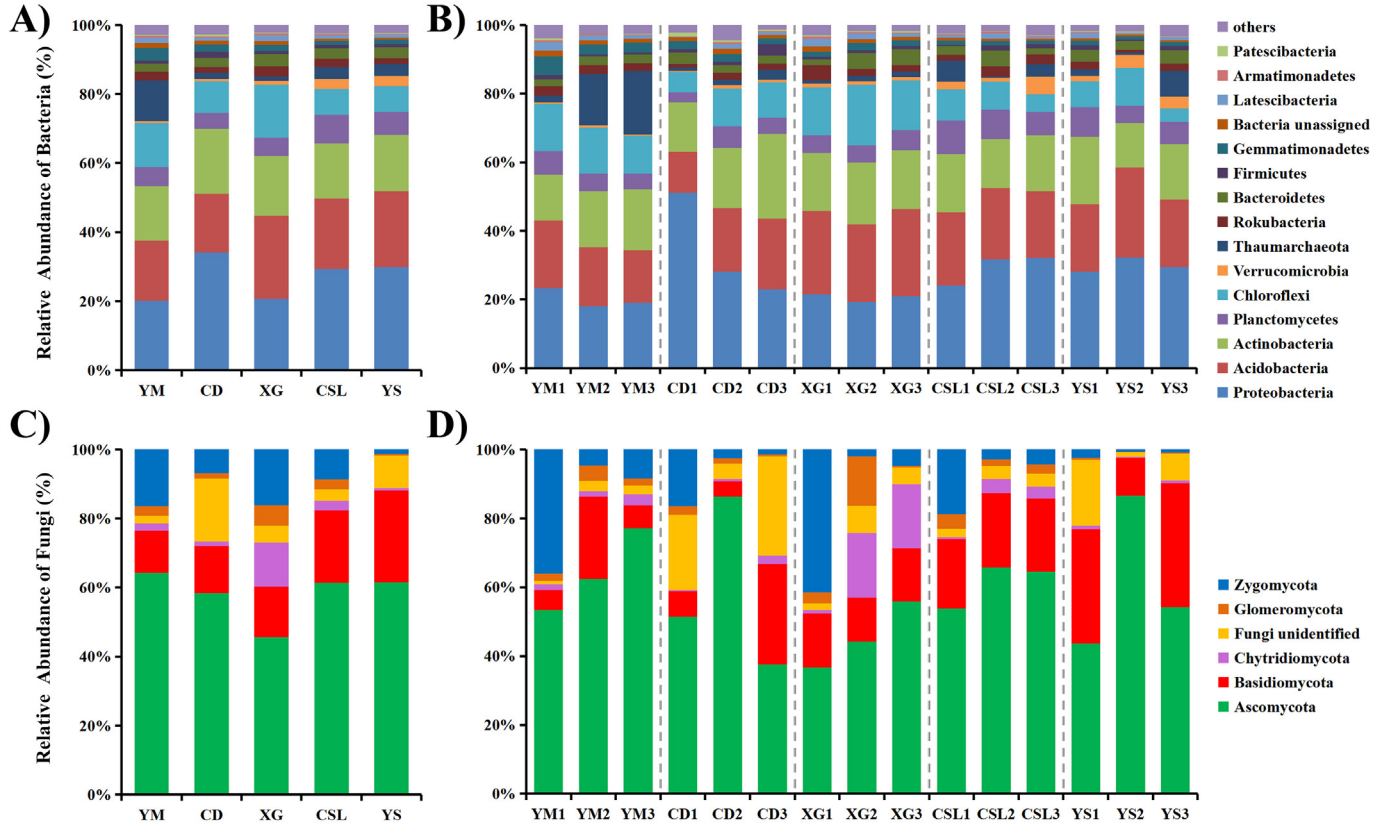


Fig. 1. Soil bacterial (A) and fungal (C) dominant phyla in vegetation restoration processes and in every single soil samples (B and D). Sequences not classified to any known phylum are included as unassigned bacteria. In each sample, bacterial phyla with relative frequency of less than 0.1% are included as others. YM, corn land; CD, grassland; XG, shrub land; CSL, secondary forest; YS, primary forest.

site-shared bacterial OTUs was 1979, accounting for 19.48% of the total bacterial OTUs, and the OTUs specific to each of the different sites accounted for 9.80%, 9.78%, 7.73% and 9.50% of the total OTUs in the different sites respectively. The number of site-shared fungal OTUs was 290, accounting for 2.51% of the total fungal OTUs, and the OTUs specific to each of the different sites accounted for 7.78%, 8.74%, 10.45% and 6.79% of the total OTUs, respectively. The ratios of bacterial to fungal OTUs in different soils were 1.74, 1.47, 1.44, 1.45 and 1.56, respectively. Generally, the site-shared bacterial and fungal OTUs are defined as generalists, which were assigned into Proteobacteria, Acidobacteria, Ascomycota and Basidiomycota. Moreover, the heat map showed the distribution of the 29 dominant bacterial and 41 dominant fungal OTUs (Fig. 2A and B) in different successional stages, indicating that vegetation restoration exerted a greater effect on most frequent OTUs occurrences. The dominant OTUs differed across the five successional stages, e.g., YM was dominated by OTUs 21 (*Gaiellales*), 18 (*Rokubacteriales*), 1 (*Mortierella*), and 8 (*Nectriaceae*), whereas CD was dominated by OTUs 6 (*Methylobacterium*), 4 (*Sphingomonas*), 3 (*Dongia*), 177 (*Luedemannella*), 9 (*Derxomyces*), 20 (*Candida*), 49 (Fungi unidentified), 38 (*Hortaea*), 3 (*Mycoarthris*), 7 (Fungi unidentified), 47 (Fungi unidentified), 26 (*Paecilomyces*), and 27 (Fungi unidentified).

3.3. Soil microbial diversity

The microbial alpha diversity varied among the studied samples (Table 2). According to OTU diversity estimated by Shannon index, the greatest bacteria diversity was in XG, followed by CSL, YM and YS, whereas CD showed the lowest bacteria diversity. The greatest fungi diversity was in CSL, followed by YM, XG and YS, likewise, the lowest fungi diversity was in CD. The richness of bacteria (estimated by Chao1 index) fluctuated and the maximum result was in YS, whereas fungal richness tended to increase from CD to YS. The overall change in diversity and richness of bacteria was not significantly different with the revegetation duration, and fungi did not also, except for CD.

The beta diversity of soil microbial communities in different successional stages was analyzed by PCoA based on Bray-Curtis dissimilarity to visualize the effects of vegetation restoration on soil microbial communities. There was a certain distance between different soil samples, indicating that the bacterial and fungal community varied in different successional stages (Fig. 3A and B). Notably, the magnitude of the shift in fungal community was greater than that in bacterial community. Moreover, the ANOSIM analysis tested significant differences of soil bacterial ($R = 0.346$, $P = 0.001$) and fungal ($R = 0.228$, $P = 0.031$) community structure among the five successional stages (Fig. 3C and D). Further ANOSIM analysis between every two successional stages showed the more different of CSL/YM and less different of CSL/YS in bacterial community, whereas CD/YM showed less different and CD/YS showed more different in fungal community (Fig. S2A and S2B). Additionally, a higher relative contribution of deterministic processes mainly belonging to heterogeneous selection was observed for bacterial community assembly, while stochastic processes belonging to homogenizing dispersal dominated the assembly of soil fungal communities in different successional stages according to the resulting βNTI and RC_{Bray} values (Fig. S3A and S3B).

3.4. The relationship between edaphic characters and microbes

RDA plots on the most abundant OTUs (>0.5%) (29 and 41 for bacteria and fungi, respectively) were performed to explore the key drivers shaping soil microbial communities (Fig. 4C and D). According to the RDA plots, the first two axes explained 59.64% and 44.92% of the variability of the bacterial and fungal community structure respectively. The major edaphic variables driving soil bacterial community composition were SOC ($F = 3.9$,

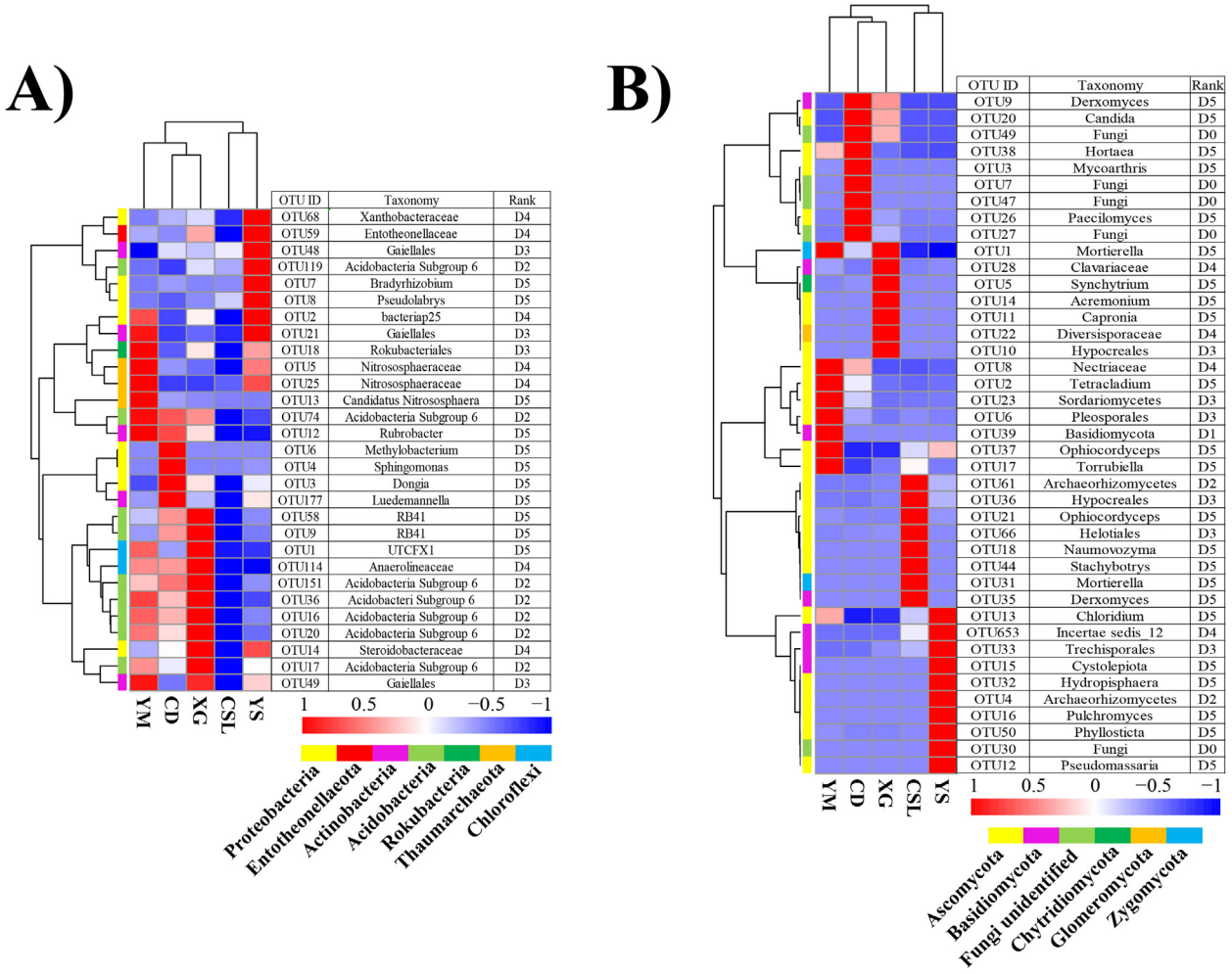


Fig. 2. Heat map illustrating the mean relative frequency of the 29 most abundant bacterial OTUs (A) and 41 most abundant fungal OTUs (B) with abundances >0.5% in vegetation restoration processes. Taxonomic assignment of the OTUs is provided at the lowest level of classification possibly. (D0: kingdom, D1: phylum, D2: class, D3: order, D4: family, and D5: genus). YM, corn land; CD, grassland; XG, shrub land; CSL, secondary forest; YS, primary forest.

$P = 0.014$), E-Ca ($F = 3.2$, $P = 0.014$) and SM ($F = 3.0$, $P = 0.001$), and the 41 top fungal OTUs across all samples were significantly correlating with SOC ($F = 3.1$, $P = 0.008$), TN ($F = 2.9$, $P = 0.006$), pH ($F = 2.3$, $P = 0.030$) and SM ($F = 2.2$, $P = 0.028$). Pearson correlation-based heat maps graphically demonstrated that the distribution of 29 most abundant bacterial OTUs and 41 most abundant fungal OTUs was strongly affected by edaphic variables (Fig. 4A and B). Network analysis showed associations between highly connected genera and edaphic parameters (Fig. 4E and F). The nodes in the network were assigned to six bacterial phyla and five fungal phyla, of which Acidobacteria, Actinobacteria, Proteobacteria, Ascomycota and Basidiomycota were widely distributed, accounting for more than 50% of total nodes. Noteworthy, the relative abundances of most frequent OTUs from Proteobacteria, Acidobacteria, Actinobacteria, Ascomycota and Basidiomycota had a significant negative or positive relationship with SOC content.

Table 2
Alpha diversity of the soil bacterial and fungal community in vegetation restoration processes.

	Sample ID	Chao1	Shannon	Simpson	Observerd OTUs
Bacteria	YM	3941 ± 607.45a	9.01 ± 0.22a	0.99 ± 0.00a	3045 ± 352.19a
	CD	2835 ± 1055.21a	8.43 ± 0.83a	0.97 ± 0.01a	2326 ± 822.47a
	XG	4100 ± 855.80a	9.13 ± 0.02a	0.99 ± 0.00a	3098 ± 539.22a
	CSL	3687 ± 434.05a	9.04 ± 0.28a	0.99 ± 0.00a	2883 ± 270.05a
	YS	4201 ± 259.36a	8.90 ± 0.33a	0.99 ± 0.00a	3177 ± 240.82a
Fungi	YM	1255 ± 291.29a	5.45 ± 0.61a	0.90 ± 0.03a	1055 ± 297.56a
	CD	1106 ± 241.41a	4.17 ± 0.71b	0.77 ± 0.13a	781 ± 158.09a
	XG	1486 ± 105.86a	5.08 ± 0.03a	0.89 ± 0.02a	1015 ± 55.69a
	CSL	1428 ± 96.06a	6.14 ± 0.15a	0.94 ± 0.00a	1105 ± 133.04a
	YS	1503 ± 51.86a	4.87 ± 0.59a	0.86 ± 0.05a	1030 ± 119.35a

Note: Values are mean ± standard error (mean ± SE) (n = 3). Statistical significance was assessed by one-way ANOVA followed by LSD multiple comparison test. Different lowercase letters (a and b) in the same column indicate significant difference ($P < 0.05$). YM, corn land; CD, grassland; XG, shrub land; CSL, secondary forest; YS, primary forest.

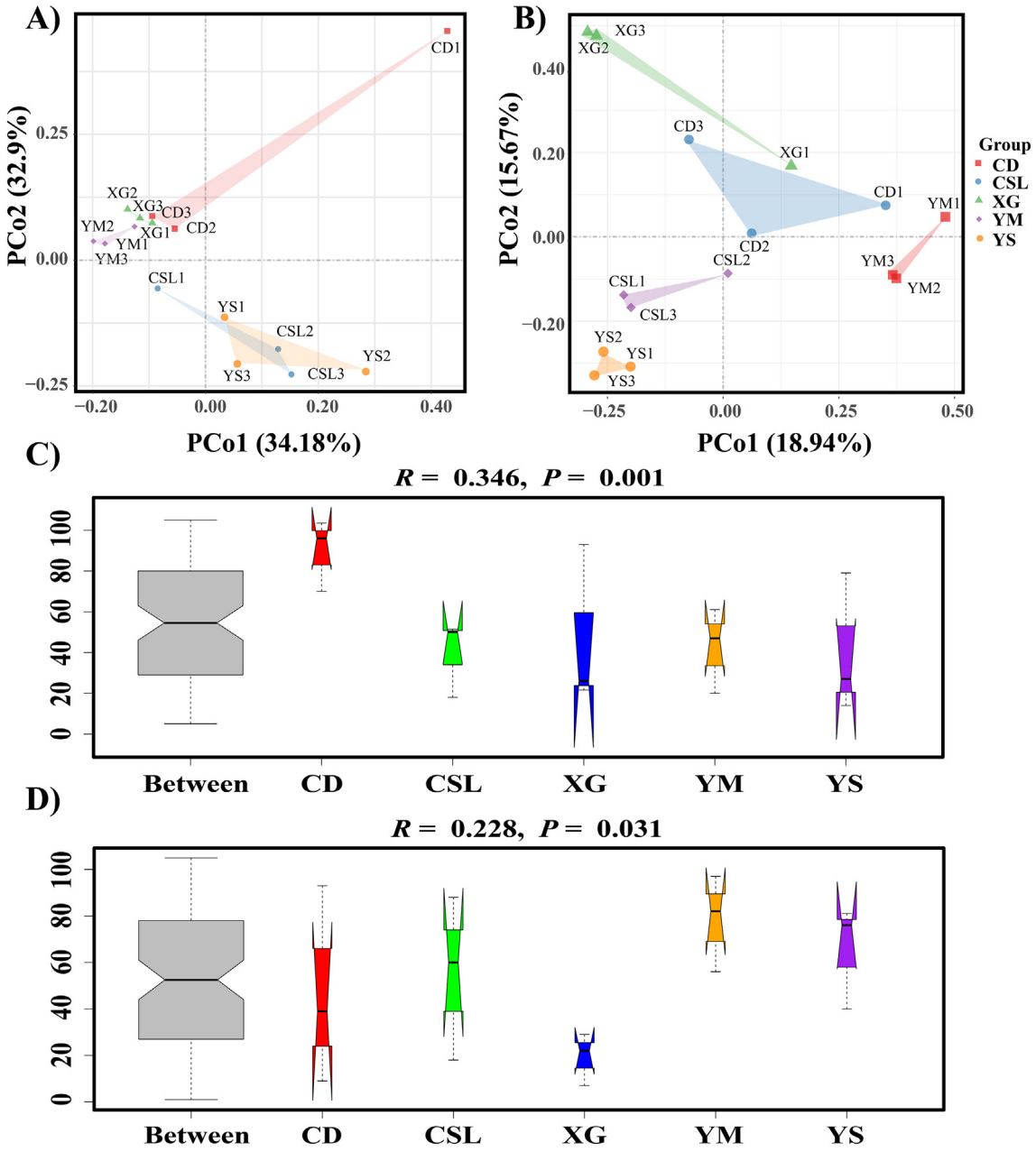


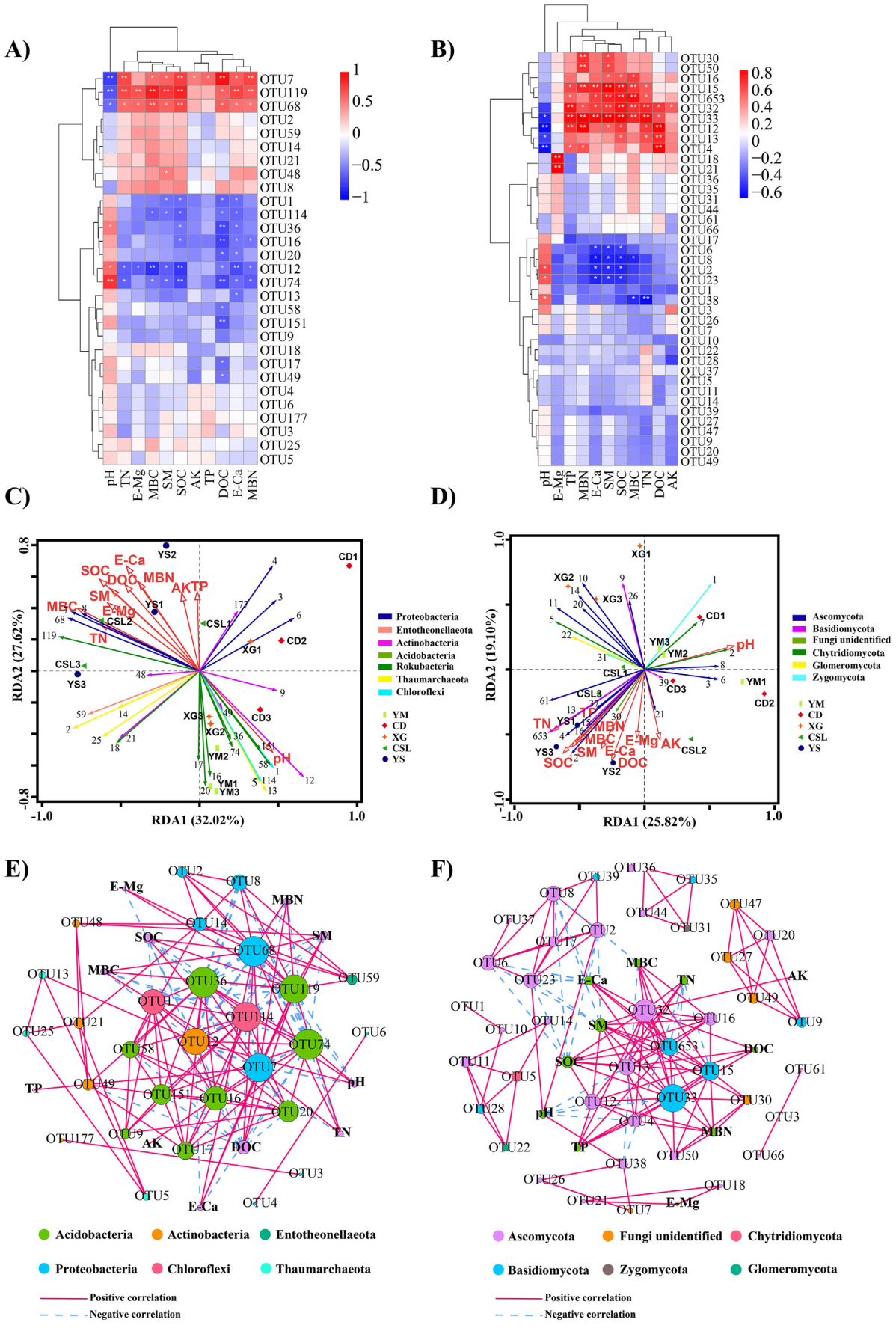
Fig. 3. PCoA plots and ANOSIM analysis based on Bray-Curtis distances representing the soil bacterial (A/C) and fungal (B/D) community similarity/dissimilarity. Ordinate–the rank of the distance between samples; Abscissa–Between is the result between the five different successional stages, and the other five are the results within their groups, respectively. YM, corn land; CD, grassland; XG, shrub land; CSL, secondary forest; YS, primary forest.

3.5. Microbial co-occurrence network analysis

Co-occurrence network and its topological properties were generated to better decipher the potential interactions between soil bacterial and fungal communities (Fig. 5). The formed soil microbial interaction networks in YM, CD, XG, CSL and YS included 240, 247, 234, 326 and 230 nodes (OTUs) as well as 24,246, 26,947, 20,342, 40,166 and 18,990 edges, respectively. The average degree or node connectivity in YM, CD, XG, CSL and YS was 101.025, 109.097, 86.932, 123.209 and 82.565, respectively. The clustering coefficient in YM, CD, XG, CSL and YS was 0.998, 0.998, 0.996, 0.997 and 0.996, respectively, and the modularity index in YM, CD, XG, CSL and YS was 0.438, 0.503, 0.565, 0.441 and 0.547, respectively. Interestingly, the ratio of bacterial nodes was the highest across all soil samples, whereas the proportion of fungal nodes did not differ significantly in YS and XG and were both higher than that in CD and CSL, indicating that the vegetation restoration process promotes the growth of fungi to some extent. However, the highest percentage of fungal nodes in YM maybe relate to anthropogenic disturbances such as fertilizer/tillage. It should be noted that all the top four bacteria and two fungi in the network were assigned into Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Ascomycota and Basidiomycota. Meanwhile, *Bryobacter*, *Haliangium*, and MND1 were identified as the top three genera in the co-occurrence network.

3.6. The potential microbial ecological functions

Soil microbial communities in vegetation restoration processes have various ecological functions. Five main bacterial ecological functions were identified in the studied samples (Fig. 6A-6E), which were aerobic ammonia oxidation, nitrification and nitrate reduction involved in N cycle, and aerobic chemoheterotrophy and chemoheterotrophy involved in C cycle. Among them, the abundance of aerobic ammonia oxidation


A)
B)
C)
D)
E)
F)
RDA1 (32.02%)
RDA2 (27.62%)
RDA1 (25.82%)
RDA2 (19.10%)
Proteobacteria
Entotheonellaeota
Actinobacteria
Acidobacteria
Rokubacteria
Thaumarchaeota
Chloroflexi
Ascomycota
Basidiomycota
Fungi unidentified
Chytridiomycota
Glomeromycota
Zygomycota
YM
CD
XG
CSL
YS
Positive correlation
Negative correlation

and nitrification functional groups were highest in YM, and tended to increase from CD to YS, while aerobic chemoheterotrophy, nitrate reduction and chemoheterotrophy groups showed a decreasing trend with the restoration process. With respect to fungal communities, they can be distinguished into six trophic types (Fig. 6F-K), which were Pathotroph, Saprotroph, Symbiotroph, Saprotroph-Symbiotroph, Pathotroph-Symbiotroph, and Pathotroph-Saprotroph-Symbiotroph flora. Among them, Pathotroph flora had the highest abundance in XG, and tended to decrease with the restoration process. Saprotroph flora was more abundant in YS, followed by YM and CD, while XG and CSL showed the lowest abundance. The groups of Symbiotroph flora were mostly distributed in XG, with extremely lower abundance in other successional stages. The groups of Saprotroph-Symobiotroph flora showed the lowest abundance in YS, and fluctuated in abundance from YM to CSL. As to Pathotroph-Symbiotroph flora, higher abundance was found in CSL and YS, with the lowest abundance in CD and XG. Additionally, there were higher abundances of Pathotroph-Saprotroph-Symbiotroph flora in YM, XG and YS soils.

The above mentioned different microbial functional groups had significant correlations with the measured SOC chemical compounds (Table S2). Specifically, the aerobic ammonia oxidation, nitrification and Pathotroph-Saprotroph-Symbiotroph flora were positively correlated with Carboxylic C. Nitrate reduction flora was negatively correlated with di-*O*-alkyl C and *O*-alkyl C. The groups of Saprotroph-Symbiotroph flora were negatively correlated with *O*-alkyl C, and groups of Pathotroph-Saprotroph-Symbiotroph flora were positively correlated with Carboxylic C.

4. Discussion

4.1. The influence of vegetation restoration on soil physicochemical properties and SOC chemical compounds

Previous studies have demonstrated significant effects of vegetation restoration on soil physicochemical properties (Li et al., 2014; Jiang et al., 2014; Chang et al., 2018; Bai et al., 2019). Our study showed that SOC, TN, TP, MBC, MBN, DOC, AK, E-Ca and SM generally increased with time series, suggesting that vegetation restoration not only accelerates organic matter accumulation but also increases the soil water-holding capacity in karst ecosystems. The increased SOC as well as N accumulation driven by the translation of plant residues and root exudation to belowground C and N storage suggested the co-evolution process of the C and N cycle in karst calcareous soil (Shao et al., 2019). Soil TP content exhibited a high spatial heterogeneity, which might be explained by derived-P mainly from the weathering release of soil minerals, which was a long-term biological cycle process (Wen et al., 2018). Besides that, DOC and MBC are part of soil active organic carbon (Hu et al., 2021). Although it only accounts for a small part of SOC, it is fast-moving, unstable, easily oxidized and mineralized, as well as the driving force of soil microbial activity energy and soil nutrients. In the present study, the variation trend of soil active organic carbon in vegetation restoration processes was basically similar to that of organic carbon with a good positive correlation (Table S3). This result indicates that the content of soil active organic carbon was largely determined by the content of SOC, and the content of SOC was affected by the different organic matter content input to soil in vegetation restoration processes. Soil calcium also plays a significant role in maintaining SOC pool through a chemical bonding mechanism and chemical structure stability (Rowley et al., 2018).

Except for the change in the amount of SOC, the SOC chemical compounds also changed in vegetation restoration processes. The relative abundance of Alkyl C fractions, which correspond to long chain aliphatic and waxes, slightly increased along the successional trajectory. Alkyl C is positively associated with soil organic carbon stability (Bonanomi et al., 2018). Thus, the increased abundance of Alkyl C may indicate the enhanced SOC stability companied with vegetation restoration. The preservation of recalcitrant C compounds also may have been due to some labile C compounds quickly consumed by microorganisms (Hu et al., 2021). Besides, the amount and type of litter and plant root exudates can lead to the differentiation of SOC molecules. It has been reported that there is a good correlation between plant residues and *O*-alkyl C in soil with the extension of vegetation restoration years (Erhagen et al., 2013). In the present study, the relative abundance of *O*-alkyl C increased in concert with the relative abundance of Alkyl C. Yet, no significant difference of Alkyl C abundance was observed as vegetation restoration progressed, which may have been due to land restoration generally accompanied by labile C pools increasing more quickly than recalcitrant C pools (Zhang et al., 2019c).

Calcareous soils with high Ca^{2+} concentrations developed from carbonate bedrock are normally characterized by high pH (Bárcenas-Moreno et al., 2011). In the present study, the soil exhibited pH values >6.41 which reflects the characteristics of higher pH in karst soils. A decreasing trend in pH was also observed as vegetation restoration progressed. A plausible explanation for the significant lower pH value in YS could be attributed to a large amount of deciduous aggregates on soil surface in primary forests that can release various organic acids and CO_2 in the process of organic matter being decomposed by soil microorganisms. The high soil humus content exist in primary forests may also contribute to the decrease of soil pH (Brand et al., 1986), in accordance with previous studies (Schipper et al., 2001; Holtkamp et al., 2008). Moreover, the alkali environment restricts the improvement of fertility in lime soil and appropriately reduces the soil pH values, which effectively improve soil fertility (Deng et al., 2010). In the present study, vegetation restoration has significant impacts on edaphic properties and SOC chemical compounds dynamics. Nonetheless, these changes can not be fully understood without soil microorganism since it plays a pivotal role in biogeochemical cycle in terrestrial ecosystem (Delgado-Baquerizo et al., 2018, 2019; Wagg et al., 2019).

4.2. The influence of vegetation restoration on microbial distribution and assembly

Soil properties play a vital role in determining microbial composition and changes in soil microbial community are important indicators of soil quality (Liang et al., 2017; Shan et al., 2017). Thus, understanding the dynamics of microbial communities is of great significance in studying the entire terrestrial ecosystem. Consistent with our hypothesis, bacterial and fungal beta diversity varied significantly along vegetation restoration chronosequence indicating that returning farmland to forests disrupted the microbial niches created by karst ecosystem in each phase of succession, and soil microbial communities differing dramatically were associated with shifts in specific soil characteristics (Li et al., 2021). Besides, the transition in bacterial communities was more gradual and overlapped, whereas fugal communities were more distinct in different restoration stages (Fig. 3A and B). This is congruent to prior observations that plant-fungi symbioses are more prevalent than that with bacteria (Shan et al., 2017) and most bacteria inhabiting tiny-scale niches in bulk soil have less direct contact with plants than fungi (Bonfante and Anca, 2009). This phenomenon was also validated by the Venn diagrams (Fig. S1A and S1B). Interestingly, both bacterial and fungal communities from corn land were significantly different from those in primary forest (Fig. S2A and S2B), implying that the rate of species replacement was relatively fast, possibly due to the lack of soil nutrients in corn land and increased competition during succession process, which intensifies the species replacement between communities (Sun

Fig. 4. Heat map representing the relationship between the soil physicochemical parameters and the most abundant bacterial (A) and fungal (B) OTUs with abundances >0.5% in vegetation restoration processes, * $P < 0.05$ (Pearson correlation, two-tailed), ** $P < 0.01$ (Pearson correlation, two-tailed). RDA plots revealing the relationship between the most abundant bacterial (C) and fungal (D) OTUs (color corresponds to taxonomic classification) with abundances >0.5% and soil physicochemical parameters. Correlation network of significant correlations (Pearson correlation, $P < 0.05$, two-tailed) among bacterial (E) and fungal (F) OTUs and between OTUs and edaphic variables, nodes are colored at the phylum level, and the size of each node is proportional to the number of connections.

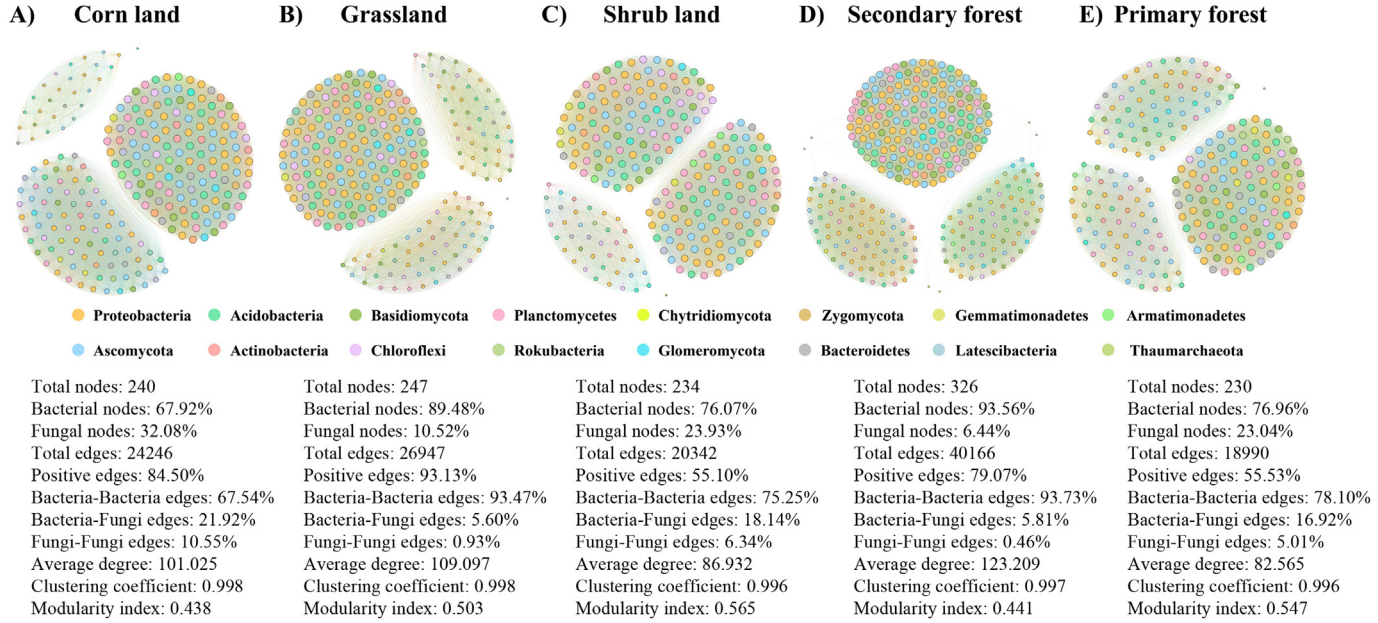


Fig. 5. Co-occurring networks and topological properties of soil bacterial and fungal communities across five different successional stages (A-E). A connection stands for a Spearman correlation with a coefficient of >0.6 and statistically significant ($P < 0.01$). The size of each node is proportional to the number of connections. The colors of nodes represent their classification at the phylum level.

et al., 2017; Qiu et al., 2020). The abundance of fungal communities tended to increase after the farmland was converted to forest, however, no conspicuous change patterns were observed in diversity, suggesting that reforestation maybe have more obvious influence on their abundances than their diversities. Moreover, the peak richness of bacteria and fungi was found in YS, which may have been due to the coexistence of both *r*- and *K*-selected species at the end of succession (Mouquet et al., 2002).

Uncovering the microbial assembly processes is essential to advance fundamentally understanding of ecosystem diversity and functioning (Sessitsch et al., 2019; Singh et al., 2020; Trivedi et al., 2020). Previous studies have discovered that in severe or extremely variable circumstances, a certain environmental factor will become the limiting factor impacting microbial community succession and deterministic processes (Graham et al., 2017). However, stochastic processes play a dominant role in relatively mild and less disturbed environments, where microbial community changes are more likely to come from probabilistic dispersal and random birth-death events, etc. (Dini-Andreote et al., 2015). In the present study, soil bacterial communities were mainly controlled by deterministic processes, more specifically, heterogeneous selection, while stochastic processes (homogenizing dispersal) dominated the assembly of soil fungal communities across the five vegetation successional processes, contradicting our hypothesis. Generally, heterogeneous selection is determined by dynamic selection under biotic or abiotic conditions and can lead to large changes in microbial community (Zhang et al., 2019a). Heterogeneous selection dominating bacterial community assembly process maybe relate to the significant changes in edaphic properties during vegetation restoration. By contrast, fungi are more capable of adapting to complex environmental changes, and some of them will adopt multiple nutritional approaches simultaneously to enhance their autotrophic survival (Xiong et al., 2020). Therefore, stochastic processes (homogenizing dispersal) of soil fungal communities were found in our study.

4.3. Impact of edaphic factors on soil microorganisms

The varied structure of microbial communities is closely related to edaphic properties (Shan et al., 2017; Li et al., 2021). Previous studies have illustrated that SOC was the key factor influencing the bacterial community structure (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011). In the present study, SOC was closely related to soil bacterial distribution in vegetation restoration processes. The strong correlation between SOC and bacterial distribution could be explained by the copiotrophic-oligotrophic life strategy of soil bacteria. For example, bacteria belonging to Acidobacteria phylum were considered as *K*-strategists and were most abundant in nutrient-poor environments (Gao et al., 2021). By contrast, Proteobacteria exhibited copiotrophic attributes and were enriched in soils with high C availability (Qu et al., 2020). Calcium, as a nutrient element necessary for microbial growth and development, has the function of participating in cellular energy metabolism, regulating cell proliferation and differentiation, stimulating microbial growth and improving microbial activity, which can be used to maintain microbial metabolism under adverse soil conditions (Xue et al., 2017; Wang et al., 2019). Therefore, it was significantly correlated with most bacteria, and this finding was concur with the conclusions of Xue et al. (2017) and Li et al. (2021) that Ca^{2+} is an essential nutrient that affects bacterial communities in karst soils. In addition, maybe SM was another important factor driving microbial diversity in the present study. Changes of soil moisture will affect oxygen levels and substrate availability, consequently, affecting microbial community (Delgado-Baquerizo et al., 2018). Moreover, pH, SOC and TN exerted the most significant effects on fungal community, in accordance with previous studies (Gao et al., 2021). The negative correlation between TN and Basidiomycetes may have been due to nitrogen inhibiting the production of extracellular catabolic enzymes and hydroxyl groups of some Basidiomycetes, thus reducing its ability to break down lignin (Fog, 1988). Each microorganism has an optimum pH for their thriving, and slight changes in pH may favor different fungal taxa (Fierer and Jackson, 2006; Xue et al., 2017). In the present study, the strong correlation between pH and fungal community was resonating with previous works in Qinghai-Tibetan Plateau (Gao et al., 2021) and marsh land in Sanjiang Plain (Xu et al., 2016). Though multiple edaphic variables were jointly involved in regulating soil bacterial and fungal community structures, the determining factor was SOC, which parallel with the previous studies (Xue et al., 2017; Zhao et al., 2019), indicating that soil microbial community structure was closely related to soil nutrients especially SOC content (Liang et al., 2017). Besides, the diversities of fungi and bacteria in YS were lower than those in YM and XG, implying that early successional stages containing low amounts of SOC lead to more unique niches and relatively high-resource habitats are less inclined to microbial niche differentiation (Shao et al., 2019).

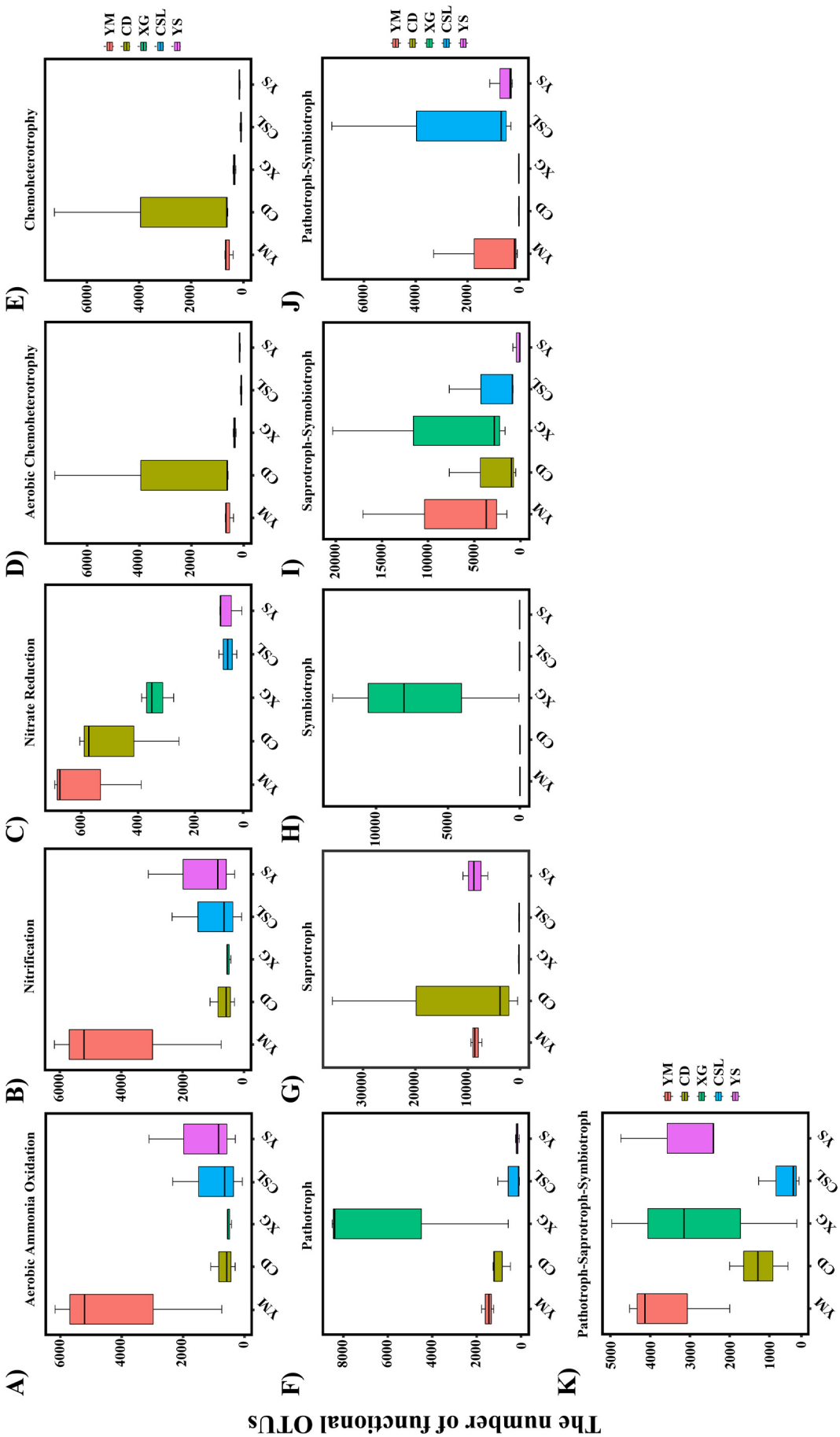


Fig. 6. Changes of soil bacterial (A-E) and fungal (F-K) functional groups in vegetation restoration processes. Box bounds show 25-75th percentiles, vertical lines show 10-90th percentiles, and horizontal thick black lines within boxes are median values (n = 3). YM, corn land; CD, grassland; XG, shrub land; CSL, secondary forest; YS, primary forest.

4.4. The influence of vegetation restoration on microbial co-occurrence patterns

Microorganisms develop interactions within certain ecological niches, such as commensalism, competition, and predation, etc. (Barberan et al., 2012; Faust and Raes, 2012), which can be revealed by microbial co-occurrence analysis (Li et al., 2021). In our case, the modularity index > 0.4 suggests that the network has modular characteristics (Xue et al., 2017). The most abundant phyla in co-occurrence network were Proteobacteria and Ascomycota, which was in accordance with the result of existing studies (Gao et al., 2021), indicating that these microorganisms were adapted to a variety of environments (Jiao et al., 2016). Proteobacteria are the main soil bacterial groups in various habitats (Cui et al., 2018; Qiu et al., 2020; Chen et al., 2020), and it is assumed that they are able to assimilate easily available organic compounds and grow rapidly (Fierer and Jackson, 2006). Ascomycota are known as degraders of a variety of plant cellulose and hemicellulose, suggesting their important roles in decomposition of macromolecular organic matter in karst soils (Shan et al., 2017). Moreover, *Bryobacter*, *Haliangium* and MND1 were identified as the top three genera in the co-occurrence network. *Bryobacter*, an mesophilic and psychrotolerant chemoheterotroph bacterium that utilizes various sugars, polysaccharides, and fatty acids, have the ability to grow under anoxic conditions and capability to reduce nitrate (Dedysh et al., 2016). *Haliangium* was confirmed as a crucial species in soil biogeochemical cycle, yet further research into their roles in soil microbial co-occurrence network is still required in the future (Kundim et al., 2003). MND1 belongs to β-Proteobacteria which has variable degradation variable capacity, and is widely distributed in nutrient-poor environments (Zhao et al., 2020). Thus, all three keystone taxa may play crucial roles in karst soils. Nevertheless, Li et al. (2021) found that the key taxon bacteria from karst soils in Yunnan were *Candidatus Udaeobacter*, *Chthoniobacterales* and *Pedosphaeraceae*. This is unsurprising that the high heterogeneity of karst soils and the seasonal variation will change the structure of microbial communities (Banerjee et al., 2019). Therefore, the key species may only occur in specific seasons or time periods (Banerjee et al., 2019).

Our results also disclosed that the network properties varied throughout the vegetation restoration process. For instance, the proportion of bacterial nodes in the co-occurrence network declined, while the proportion of fungal nodes showed an opposite trend with the extension of succession time series. This may indicate that bacteria dominated a pioneer taxon in the early succession stage, and as the soil nutrients increased, the competition of fungi, which had acquired enough organic matter, also increased. Previous studies have shown that forest secondary succession contributes to the formation of dense root systems and continuously increases nutrient release, which increases the activity of soil fungi, making the fungal community structure more complex and enhancing its resilience to environmental changes (Bai et al., 2019; Liang et al., 2017). In general, the more complex and diverse the microbial community structure in soil, the more stable the soil ecosystem, the higher the ecological function of the ecosystem, and thus the more obvious the buffering effect on external environmental changes. It is worth noting that the lower the bacteria/fungi ratio in soil the more stable the ecosystem (Wal et al., 2006). The present study showed that the abundance of soil bacteria and fungi increased and the bacteria/fungi ratio decreased in vegetation restoration processes, indicating that vegetation restoration enhanced the stability of the karst ecosystem. Altered bacteria/fungi ratios induced the different contributions from microbial catabolism and anabolism driving soil C and N dynamics (Wagg et al., 2019), which may lead to asynchrony of soil carbon and nitrogen. Fungi-dominated soil communities may sequester more C than that with lower fungal abundance (Kallenbach et al., 2016). Moreover, strong positive correlations were observed in the co-occurrence network, while negative correlations were rare, revealing that microorganisms might cooperate in order to adapt to similar ecological niches (Liang et al., 2017).

4.5. The influence of vegetation restoration on potential ecological function of microbial community

Versatile microbial metabolic functions were observed across the successional choronosequence. Among the identified microbial functions, the relative abundance of aerobic ammonia oxidation and nitrification groups exhibited an increasing trend in the order of CD, XG, CSL, YS and YM. On the contrary, bacteria with aerobic chemoheterotrophy and chemoheterotrophy potential followed an opposite trend. Previous studies have found that some soil microorganisms participate in the nitrogen cycle including nitrogen fixation, nitrification, denitrification and ammonia oxidation to promote nitrogen transformation (Parkinson and Allen, 2009). Among them, ammonia oxidation, as the first link of nitrification, is the key rate-limiting reaction of nitrification process (Cui et al., 2018). Considering that microorganisms can boost plant growth via increasing the bioavailability of soil-borne nutrients, in which the organic N forms can be transferred to more inorganic forms through microbial depolymerization and mineralization. Thus, the increased abundance of nitrate respiration and nitrogen flora during the restoration process could be the consequence of nutrient demand for plant growth (Xiong et al., 2020). In this study, the observed discrepancy of TN content in different successional stages may result from the enhancement of ammonia oxidation and nitrification processes in soil during vegetation restoration. Correspondingly, the soil N contents have a significant impact on microbial nitrogen reduction functions (Table S4). It is notable that the abundance of bacteria with chemoheterotrophy potential decreased during the succession process, considering that this type of bacteria requires organic matter for growth and that the SOC content increased continuously in vegetation restoration choronosequence (Chen et al., 2020). A potential explanation is that the increased water content during the succession process may reduce the oxygen level in soil, consequently, vegetation restoration posed a negative effect on chenmoheterotrophy bacteria.

In contrast to bacteria, fungi are indispensable to breakdown refractory organic matter and mediators of slower carbon cycling (Tolkkinen et al., 2015). Increasing evidences have revealed that fungi have more complex niches and formed different functional guilds that implement different roles in nutrient cycling and carbon storage (Wal et al., 2006; Liu et al., 2015). In this study, Saprotroph and Saprotroph-Symbiotroph flora were the major functional taxa in each sample. Indeed, previous studies demonstrated that saprophytic fungi produce a range of hydrolytic and oxidative enzymes that decompose dead or senescent plant residues and increase soil organic matter, which is important for accelerating decomposition of organic matter and nutrient cycling (Liu et al., 2015; Shan et al., 2017). Generally, saprophytes were mainly from OTUs 12 (*Pseudomassaria*), 3 (*Mycoarthris*), 2 (*Tetracladium*), 15 (*Cystolepiota*) and 32 (*Hydropisphaera*) and they were positively correlated with SOC and TN contents, indicating that these groups may play a key role in leaf-decomposition and nutrient cycling. Notably, the abundance of Pathotroph-Saprotroph-Symbiotroph trophic mode fungi tends to increase with revegetation process. This may be due to the fact that nutrient input changes during vegetation restoration affecting the survival environment of fungi, consequently making the parthenogenic fungi dominated and the diversity of fungal metabolic functions increased (Yang et al., 2021). Pathotrophic fungi obtain nutrients mainly from host cells and can be harmful to plant growth (Shan et al., 2017). Thus, the higher abundance of pathotrophic fungi in XG may imply the higher risk of fungal diseases than other ecosystem types. The declining trend of pathotrophic fungal abundance in the late successional stages indicates that natural vegetation restoration process has reduced the risk of fungal diseases to some extent, which is beneficial to the growth of plants. Meanwhile, we found that substantial saprophytic fungi existed in YS, probably due to the fact that primary forest has a large amount of vegetation apoplast on the soil surface, which enhanced soil water-holding capacity. As a consequence, the higher soil moisture will stimulate the growth of saprophytic fungi to produce a series of hydrolytic and oxidative enzymes to complete the decomposition of organic matter (Zhang et al., 2019b). Multiple nutrient types of fungi were detected in soil samples at each successional stage, suggesting the relative complexity of karst habitats to accommodate the survival of different trophic mode fungi. Collectively, these results suggested that bacteria taxa mainly contributed to soil nitrogen fixation while fungi taxa played key roles in regulating soil C cycle in karst vegetation restoration processes.

4.6. Linking microbial modulators to soil recalcitrant carbon storage

Vegetation restoration is widely recognized as an effective approach to substantially increase vegetation coverage and terrestrial carbon sink (Shi and Han, 2015), yet the stable soil C pool and its dynamics are an enigma that has puzzled scientists for decades. The formation and accumulation of SOC is the long-term evolution of complex organic compounds under specific climate and biological environment conditions (Paul, 2016). It includes part of the decomposition of plant macromolecular residues and microbial metabolic residues, excrement, secretions and soil humite substances (Dungait et al., 2011). It should be of note that microbial-derived substances, such as chitin and polysaccharide, accumulate continuously in soil along with the microbial growth, metabolism and death, which contribute more than 50% to soil organic matter (Liang et al., 2017). Due to its own biochemical properties, microbial necromass C contributed to the stabilized soil organic matter considerably. In the present study, both Alkyl and *O*-alkyl C derived from soil microbial metabolites or plant residues showing an increasing trend in vegetation restoration processes revealed the dual carbon sink effect with vegetation restoration in karst areas, which further reflect the huge carbon sequestration potential of southwest China karst region that has been underestimated (Wang et al., 2020). In according with our initial hypothesis, the dominant microbial functional groups had been the complex interaction between different C-types (Table S2). The interrelationship may stem from the changes in microbial enzyme activities that can give rise to selective decomposition of specific compounds, modifying the structures of plant materials differently, and leading to distinct inter-relationships, depending on plant & tissue types and which microbial populations are active (Liang et al., 2017). Interestingly, SOC chemical compounds that had a significant correlation with soil microbial functional groups mainly belonged to amino acids/lignin-like structures (e.g., Carboxylic C and *O*-alkyl C), which were characterized as readily available to microorganisms, i.e., easily degraded carbon (Paul, 2016). This finding support the recent proposed concept of soil microbial carbon pump, which portrayed the pathways of plant- and microbial- derived carbon flow, that are soil microbial dual regulation pathways- "*ex vivo* modification" and "*in vivo* turnover" (Liang et al., 2017). Soil microorganisms transform easily degradable substrates into microbial biomass and metabolites through "in vivo turnover" process, and when soil microorganisms die their necromass contributes to the stable SOM (Bonanomi et al., 2018). Considering the formation mechanism of complex SOC chemical compounds metabolized by microorganisms is extraordinary sophisticated, the contribution of microbial- and plant-derived compounds to stable SOM is far from being elucidated. Therefore, further research focused on the formation and composition of stable SOM is still warranted.

5. Conclusions

In the present study, we revealed the effects of vegetation restoration on microbial interactions and recalcitrant SOC storage in southwest China karst region. Revegetation in karst area could alter soil physiochemical properties which in turn affected soil microbial community structure. The determining factor shaping soil bacterial and fungal communities in vegetation restoration processes was SOC. The relative abundance of different substances in SOC exhibited different trends in response to vegetation restoration, e.g., the relative abundance of Alkyl C showing an increasing trend, while the relative abundance of Methoxyl C decreased. Functionally, bacteria played a vital role in maintaining N cycling and nutrient requirements while fungi played an increasing role in regulating C cycling. Besides that, the complexity of soil bacteria and fungi interactions along the restoration chronosequence increased. Moreover, a dominant effect of determinism on bacterial communities and stochastic on fungal communities under vegetation restoration scenario were found. The dominant functional groups shift with vegetation restoration process, which could be linked to the enhanced recalcitrant SOC storage. Taken together, our results illustrated that ecological restoration facilitated belowground microbial network complexity and recalcitrant SOC storage in southwest China karst region, which support the theory that recovery is the appropriate way for restoration and rehabilitation of degraded karst ecosystems and simultaneously pave the way for further understanding about the microbial responses to vegetation restoration in degraded karst regions.

Declaration of competing interest

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

Acknowledgements

This work was supported by the Special Funds for Local Science and Technology Development Guided by the Central Government, China (GuikeZY20198009), National Natural Science Foundation of China (42172341 and 42007049), and the Key Research and Development Program of Guangxi (GuikeAD20297091).

CRediT authorship contribution statement

Q.L. conceived the study, completed the soil sample collection and designed the experiment. L.H., J.Y. and J.Z. completed the data analysis and DNA extraction. L.H. drafted the manuscript. Q.L. and C.L. polished the text and improved the structure and logic of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153137.

References

Baethgen, W.E., Alley, M.M., 1989. A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant Kjeldahl digests. Commun. Soil Sci. Plant Anal. 20 (9–10), 961–969.

Bai, Y., Zha, X., Chen, S., 2019. Effects of the vegetation restoration years on soil microbial community composition and biomass in degraded lands in Changting County, China. J. For. Res. 31, 1295–1308.

Banerjee, S., Walder, F., Büchi, L., Meyer, M., Held, A.Y., Gattinger, A., et al., 2019. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. ISME J. 13 (7), 1722–1736.

Barberan, A., Bates, S.T., Casamayor, E.O., Fierer, N., 2012. Using network analysis to explore co-occurrence patterns in soil microbial communities. ISME J. 6, 343–351.

Bárcenas-Moreno, G., Rousk, J., Bååth, E., 2011. Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments. Soil Biol. Biochem. 43 (5), 1023–1033.

Bonanomi, G., Filippis, F.D., Cesarano, G., Storia, A.L., Incerti, G., 2018. Linking bacterial and eukaryotic microbiota to litter chemistry: combining next generation sequencing with 13C CPMAS NMR spectroscopy. Soil Biol. Biochem. 129, 110–121.

Bonfante, P., Anca, I.A., 2009. Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu. Rev. Microbiol. 63 (1), 363–383.

Brand, D.G., Kehoe, P., Connors, M., 1986. Coniferous afforestation leads to soil acidification in Central Ontario. Can. J. For. Res. 16, 1389–1391.

Bremner, J.M., Jenkinson, D.S., 2010. Determination of organic carbon in soil: i. Oxidation by dichromate of organic matter in soil and plant materials. Eur. J. Soil Sci. 11 (2), 394–402.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al., 2010. Qiime allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., et al., 2011. Global patterns of 16s rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108 (1), 4516–4522.

Chang, J., Zhu, J., Xu, L., Su, H., Gao, Y., Cai, X., et al., 2018. Rational land-use types in the karst regions of China: insights from soil organic matter composition and stability. Catena 160, 345–353.

Chen, J., Li, Q., He, Q., Schröder, H.C., Lu, Z., Yuan, D., 2020. Influence of CO_2/HCO_3on microbial communities in two karst caves with high CO_2. J. Earth Sci. https://doi.org/10.1007/s12583-020-1368-9.

Cui, Y., Fang, L., Guo, X., Wang, X., Wang, Y., Zhang, Y., et al., 2018. Responses of soil bacterial communities, enzyme activities, and nutrients to agricultural-to-natural ecosystem conversion in the loess plateau, China. J. Soils Sediments 19 (3), 1–14.

Dedysh, S.N., Kulichevskaya, I.S., Huber, K.J., Overmann, J., 2016. Defining the taxonomic status of described subdivision 3 acidobacteria: the proposal of bryobacteraceae fam. nov. Int. J. Syst. Evol. Microbiol. 67, 498–501.

Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D.J., Bardgett, R.D., et al., 2018. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325.

Delgado-Baquerizo, M., Bardgett, R.D., Vitousek, P.M., Maestre, F.T., Williams, M.A., Eldridge, D.J., et al., 2019. Changes in belowground biodiversity during ecosystem development. Proc. Natl. Acad. Sci. 116 (14), 6891–6896.
Deng, Y., Jiang, Z., Luo, W., Qi, X., Qin, X., 2010. Effects of vegetation restoration on soil nutrient in typical karst area. Earth Environ. 19 (1), 96 (In Chinese with English abstract).
Dini-Andreote, F., Stegen, J.C., Elsas, J.V., Salles, J.F., 2015. Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proc. Natl. Acad. Sci. 112 (11), 1326–1332.
Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, O.C., Brookes, P.C., Evershed, R.P., 2011. Variable response of the soil microbial biomass to trace concentrations of 13C-labled glucose, using 13C-PLFA analysis. Eur. J. Soil Sci. 62 (1), 117–126.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than blast. Bioinformatics 26 (19), 2460.
Erhagen, B., Öquist, M., Sparrman, T., Haei, M., Ilstedt, U., Hedenström, M., Schleucher, J., Nilsson, M.B., 2013. Temperature response of litter and soil organic matter decomposition is determined by chemical composition of organic material. Glob. Chang. Biol. 19 (12) (3858-387).
Fan, F., Wang, K., Xiong, Y., Xuan, Y., Zhang, W., Yue, Y., 2011. Assessment and spatial distribution of water and soil loss in karst regions, Southwest China. Acta Ecol. Sin. 31, 6353–6362 (in Chinese with English abstract).
Faust, K., Raes, J., 2012. Microbial interactions: from networks to models. Nat. Rev. Microbiol. 10, 538–550.
Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. 103, 626–631.
Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63 (3), 433–462.
Gao, X., Dong, S., Xu, Y., Li, Y., Li, S., Wu, S., et al., 2021. Revegetation significantly increased the bacterial-fungal interactions in different successional stages of alpine grasslands on the Qinghai-tibetan plateau. Catena 205, 105385.
Graham, E.B., Crump, A.R., Resch, C.T., Fansler, S., Arntzen, E., Kennedy, D.W., et al., 2017. Deterministic influences exceed dispersal effects on hydrologically-connected microbiomes. Environ. Microbiol. 19 (4), 1552.
Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of british soils. Environ. Microbiol. 13, 1642–1654.
Hendershot, W.H., Duquette, M., 1986. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. Soil Sci. Soc. Am. J. 50 (3), 605–608.
Holtkamp, R., Kardol, P., Wal, A., Dekker, S.C., Putten, W.H., Ruiter, P.C., 2008. Soil food web structure during ecosystem development after land abandonment. Appl. Soil Ecol. 39, 23–34.
Hu, H., Umbreen, S., Zhang, Y., Bao, M., Huang, C., Zhou, C., 2021. Significant association between soil dissolved organic matter and soil microbial communities following vegetation restoration in the loess plateau. Ecol. Eng. 169, 106305.
Jiang, Z., Lian, Y., Qin, X., 2014. Rocky desertification in Southwest China: impacts, causes, and restoration. Earth-Sci. Rev. 132 (3), 1–12.
Jiao, S., Liu, Z., Lin, Y., Yang, J., Chen, W., Wei, G., 2016. Bacterial communities in oil contaminated soils: biogeography and co-occurrence patterns. Soil Biol. Biochem. 98, 64–73.
Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nat. Commun. 7, 13630.
Khalid, M., Tan, H.X., Ali, M., Rehman, A., Liu, X.X., Su, L.T., et al., 2022. Karst rocky desertification diverged the soil residing and the active ectomycorrhizal fungal communities thereby fostering distinctive extramatrical mycelia. Sci. Total Environ. 807, 151016.
Knelman, J.E., Nemergut, D.R., 2014. Changes in community assembly may shift the relationship between biodiversity and ecosystem function. Front. Microbiol. 5, 424.
Kundim, B.A., Itou, Y., Sakagami, Y., Fudou, R., Iizuka, T., Yamanaka, S., Ojika, M., 2003. New haliangicin isomers, potent antifungal metabolites produced by a marine myxobacterium. J. Antibiot. 56 (7), 630–638.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.
Li, Q., Liang, J.H., He, Y.Y., Hu, Q.J., Yu, S., 2014. Effect of land use on soil enzyme activities at karst area in nanchuan, Chongqing, Southwest China. Plant Soil Environ. 60 (1), 15–20.
Li, Q., Hu, Q., Zhang, C., Müller, W.E., Schröder, H.C., Li, Z., Jin, Z., 2015. The effect of toxicity of heavy metals contained in tailing sands on the organic carbon metabolic activity of soil microorganisms from different land use types in the karst region. Environ. Earth Sci. 74 (9), 6747–6756.
Li, Q., Song, A., Yang, H., Müller, W.E.G., 2021. Impact of rocky desertification control on soil bacterial community in karst graben basin, southwestern China. Front. Microbiol. 12, 636405.
Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nat. Microbiol. 2 (8), 17105.
Limon, J.J., Skalski, J.H., Underhill, D.M., 2017. Commensal fungi in health and disease. Cell Host Microbe 22 (2), 156–165.
Liu, J., Chi, F., Xu, X., Kuang, E., Zhou, B., 2015. Effect of long-term fertilization on microbial community functional diversity in black soil. J. Appl. Ecol. 26 (10), 3066–3072.
Lu, R.K., 1999. Soil Chemical Analysis. China Agricultural Science and Technology Press, Beijing (In Chinese).
Lu, F., Hu, H., Sun, W., Zhu, J., Liu, G., Zhou, W., et al., 2018. Effects of national ecological restoration projects on carbon sequestration in China from 2001 to 2010. Proc. Natl. Acad. Sci. 115 (16), 4039–4044.
Mago, T., Salzberg, S.L., 2011. Flash: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27 (21), 2957–2963.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Embnet J. 17 (1), 10–12.
Mouquet, N., Moore, J.L., Loreau, M., 2002. Plant species richness and community productivity: why the mechanism that promotes coexistence matters. Ecol. Lett. 5, 56–65.
Parkinson, J.A., Allen, S.E., 2009. A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. Commun. Soil Sci. Plant Anal. 6 (1), 1–11.
Paul, E.A., 2016. The nature and dynamics of soil organic matter: plant inputs, microbial transformations, and organic matter stabilization. Soil Biol. Biochem. 98, 109–126.
Qiu, J., Cao, J., Lan, G., Liang, Y., Li, Q., 2020. The influence of land use patterns on soil bacterial community structure in the karst graben basin of Yunnan province, China. Forests 11 (1), 51.
Qu, Z.L., Liu, B., Ma, Y., Xu, J., Sun, H., 2020. The response of the soil bacterial community and function to forest succession caused by forest disease. Funct. Ecol. 34 (12), 2548–2559.
Romanowicz, K.J., Freedman, Z.B., Upchurch, R.A., Argiroff, W.A., Zak, D.R., 2016. Active microorganisms in forest soils differ from the total community yet are shaped by the same environmental factors: the influence of ph and soil moisture. FEMS Microbiol. Ecol. 92 (10), fiw149.
Rowley, M.C., Grand, S., Verrecchia, É.P., 2018. Calcium-mediated stabilisation of soil organic carbon. Biogeochemistry 137 (1–2), 27–49.
Schipper, L.A., Degens, B.P., Sparling, G.P., Duncan, L.C., 2001. Changes in microbial heterotrophic diversity along five plant successional sequences. Soil Biol. Biochem. 33 (15), 2093–2103.
Schmidt, R., Ulanova, D., Wick, L.Y., Bode, H.B., Garbeva, P., 2019. Microbe-driven chemical ecology: past, present and future. ISME J. 13 (11), 2656–2663.
Sessitsch, A., Pfafenbichler, N., Mitter, B., 2019. Microbiome applications from lab to field: facing complexity. Trends Plant Sci. 24, 194–198.
Shan, S., Song, L., Avera, B.N., Strahm, B.D., Badgley, B.D., 2017. Soil bacterial and fungal communities show distinct recovery patterns during forest ecosystem restoration. Appl. Environ. Microbiol. 83 (14) e00966-17.
Shao, P., Liang, C., Lynch, L., Xie, H., Bao, X., 2019. Reforestation accelerates soil organic carbon accumulation: evidence from microbial biomarkers. Soil Biol. Biohem. 131, 182–190.
Shi, S., Han, P., 2015. Estimating the soil carbon sequestration potential of china's grain for green project. Glob. Biogeochem. Cycles 28 (11), 1279–1294.
Singh, B.K., Trivedi, P., Egidi, E., Macdonald, C.A., Delgado-Baquerizo, M., 2020. Crop microbiome and sustainable agriculture. Nat. Rev. Microbiol. 18, 601–602.
Song, M., Peng, W., Du, H., Xu, Q., 2019. Responses of soil and microbial C:N: P stoichiometry to vegetation succession in a karst region of Southwest China. Forests 10 (9), 755.
Stegen, J., Lin, X., Konopka, A.E., Fredrickson, J., 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. ISME J. 6 (9), 1653–1664.
Sun, C., Chai, Z., Liu, G., Sha, X., 2017. Changes in species diversity patterns and spatial heterogeneity during the secondary succession of grassland vegetation on the Loess Plateau, China. Front. Plant Sci. 8, 1465.
Tolkkinen, M., Mykrä, H., Annala, M., Markkola, A.M., Vuori, K.M., Muotka, T., 2015. Multistressor impacts on fungal diversity and ecosystem functions in streams: natural vs. anthropogenic stress. Ecology 96 (3), 672–683.
Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Plant–microbiome interactions: from community assembly to plant health. Nat. Rev. Microbiol. 18 (11), 607–621.
Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E.E., van der Heijden, M.G., 2019. Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. Nat. Commun. 10 (1), 1–10.
Wal, A., Veen, J., Smant, W., Boschker, H., Bloem, J., Kardol, P., et al., 2006. Fungal biomass development in a chronosequence of land abandonment. Soil Biol. Biochem. 38 (1), 51–60.
Wang, B., Guo, B., Xue, S., Zhu, B., 2011. Changes in soil physico-chemical and microbiological properties during natural succession on abandoned farmland in the loess plateau. Environ. Earth Sci. 62 (5), 915–925.
Wang, T., Flint, S., Palmer, J., 2019. Magnesium and calcium ions: roles in bacterial cell attachment and biofilm structure maturation. Biofouling 35 (9), 959–974.
Wang, J., Feng, L., Palmer, P.I., Liu, Y., Fang, S., Bösch, H., et al., 2020. Large chinese land carbon sink estimated from atmospheric carbon dioxide data. Nature 586, 720–723.
Wen, J., Ji, H., Sun, N., Tao, H., Du, B., Hui, D., et al., 2018. Imbalanced plant stoichiometry at contrasting geologic-derived phosphorus sites in subtropics: the role of microelements and plant functional group. Plant Soil 430, 113–125.
Xiong, D., Ou, J., Li, L., Yang, S., He, Y., Li, C., 2020. Community composition and ecological function analysis of endophytic fungi in the roots of Rhododendron simsii in Pinus massoniana forest in Central Guizhou. Acta Ecol. Sin. 40 (4), 1228–1239 (in Chinese with English abstract).
Xu, F., Cai, T., Yang, X., Ju, C., Tang, Q., 2016. Effect of cultivation and natural restoration on soil bacterial community diversity in marshland in the sanjiang plain. Acta Ecol. Sin. 36 (22), 7412–7427 (in Chinese with English abstract).
Xue, L., Ren, H., Li, S., Leng, X., Yao, X., 2017. Soil bacterial community structure and co-occurrence pattern during vegetation restoration in karst rocky desertification area. Front. Microbiol. 8, 2377.
Yang, Y., Zhang, X., Hartley, I.P., Dungait, J., Wen, X., Li, D., et al., 2021. Contrasting rhizosphere soil nutrient economy of plants associated with arbuscular mycorrhizal and ectomycorrhizal fungi in karst forests. Plant Soil https://doi.org/10.1007/s11104-021-04950-9.
Yin, R., Yin, G., 2010. China's primary programs of terrestrial ecosystem restoration: initiation, implementation, and challenges. Environ. Manag. 45 (3), 429–441.
Yuan, D.X., 1997. Rock desertification in the subtropical karst of South China. Z. Geomorphol. 108, 81–90.
Yuan, D.X., 2001. On the karst ecosystem. Acta Geol. Sin. 75 (3), 336–338.
Zhang, Q., Feng, J., Wu, J., Zhang, D., Chen, Q., Li, Q., Long, C., Feyissa, A., Cheng, X., 2019c. Variations in carbon-decomposition enzyme activities respond differently to land use change in Central China. Land Degrad. Dev. 30, 459–469.
Zhang, J., Chen, M., Huang, J., Guo, X., Zhang, Y., Liu, D., Wang, J., 2019a. Diversity of the microbial community and cultivable protease-producing bacteria in the sediments of the Bohai Sea, Yellow Sea and South China Sea. PLoS One 14 (4), e0215328.

Zhang, W.H., Sun, R.B., Xu, L., Liang, J.N., Wu, T.Y., Zhou, J., 2019b. Effects of micro-/nano-hydroxyapatite and phytoremediation on fungal community structure in copper contaminated soil. Ecotoxicol. Environ. Saf. 174, 100–109.

Zhao, P., Bao, J., Wang, X., Liu, Y., Chai, B., 2019. Deterministic processes dominate soil microbial community assembly in subalpine coniferous forests on the loess plateau. PeerJ 7, e6746.

Zhao, Y., Mao, X., Zhang, M., Yang, W., Di, H.J., Ma, L., et al., 2020. Response of soil microbial communities to continuously mono-cropped cucumber under greenhouse conditions in a calcareous soil of North China. J. Soils Sediments 20 (5), 2446–2459.

Zhu, H., He, X., Wang, K., Su, Y., Wu, J., 2012. Interactions of vegetation succession, soil biochemical properties and microbial communities in a karst ecosystem. Eur. J. Soil Biol. 51, 1–7.