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Rare prokaryotic sub-communities dominate the complexity of ecological networks and soil multinutrient cycling during long-term secondary succession in China's Loess Plateau

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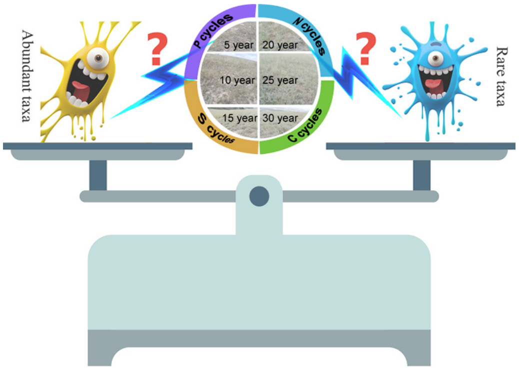
HIGHLIGHTS

- Natural restoration altered the diversity and function of abundant and rare taxa.
- Abundant and rare sub-communities present contrasting assemblage patterns.
- Rare taxa dominated the stability of entire microbial ecological networks.
- Soil multi-nutrients cycling were mainly driven by rare sub-communities.

GRAPHICAL ABSTRACT



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ABSTRACT

Unraveling the succession of microbial communities is a core ecological research topic. Yet few studies have focused on how long-term secondary succession affects the functional profiles and ecological processes of abundant and rare microbial subcommunities. Here, we used amplicon sequencing and GeoChip analysis to explore the ecological functions of abundant and rare biospheres and their correlation with soil multinutrient cycling. Samples for this study were collected from a well-established secondary succession chronosequence that spans >30 years of dryland ecosystem development on the Loess Plateau of China. Although both abundant and rare subcommunities shifted with succession, the changing of beta-diversity of the microbial communities was primarily driven by species replacement of the rare biosphere. Phylogenetic changes of abundant and rare taxa were associated with their functional traits, which dominated the diversity-related selection along all succession ages. Neutral theory analysis indicated that the assemblage of abundant taxa over all successional ages was regulated by dispersal homogenizing and ecological drift. The null model revealed that homogeneous and variable selection were the dominant assembly processes for rare subcommunities compared with abundant species. pH and nitrogen content were the paramount drivers determining the assembly of microbial communities and functional genes, consistent with the importance of environmental filtering. Furthermore, the rare biosphere

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had a paramount role in the entire ecological network and was the major driver for most soil processes such as C, N, and S cycling. Nonetheless, a significant portion of soil P cycling was regulated by abundant taxa. Collectively, our study provides insight into the mechanisms underlying microbial community assembly and soil microbe-driven functional changes in biogeochemical processes during secondary succession.

1. Introduction

Human activities, such as shifting cultivation, nomadic herding, rudimentary sedentary tillage, and commercial plantations, have profoundly influenced every ecosystem on our planet (Cardinale et al., 2012). These anthropogenic pressures have led to a decline in species richness, genetic diversity, and ecosystem functions (Miraldo et al., 2016; Newbold et al., 2015). Consequently, local terrestrial diversity faces extraordinarily pressures, such as habitat conversion, fragmentation, and habitat degradation (Haddad et al., 2015; Moreno-Mateos et al., 2020). In the last few decades, governments and international agencies have implemented multiple restoration large-scale strategies to counteract biodiversity and ecosystem loss (Moreno-Mateos et al., 2020; Yu et al., 2020). Secondary succession has attracted growing attention as a reestablishment of original community members to offset biodiversity loss (Hannula et al., 2017; Lozano et al., 2014; Zhou et al., 2017). The ecology of secondary succession has been well-studied in plant communities and has provided comprehensive insight into the mechanisms linking complexity, the stability of natural ecosystems, and the successional dynamics of aboveground natural communities (Bonet, 2004). However, recent evidence has demonstrated that belowground soil communities are also key for natural restoration progress (Liu et al., 2020; Zhou et al., 2017). The dynamics of belowground soil microbial communities can serve as useful indicators of soil physiochemical properties related to soil quality (Hermans et al., 2020; Zhang et al., 2016) and element turnover (Morriën et al., 2017; Zhong et al., 2018), and also had positive direct and indirect effects on the aboveground plant communities (Guo et al., 2019). Although these studies provide considerable information on the aboveground-belowground associations in restored habitats, little is known about how long-term secondary succession affects soil microbial structure-function relationships and the contribution of soil microorganisms to soil biogeochemical processes. Therefore, understanding the nature and intensity of the response of belowground soil communities to secondary succession is of utmost importance to accelerate the restoration of abandoned fields.

Soil organisms are the engineers of earth's biogeochemical cycles (Falkowski et al., 2008) that play pivotal roles in soil functions, such as nutrient cycling (for example, carbon, nitrogen, phosphorus, and sulfur), soil fertility, and soil carbon sequestration (Fierer, 2017). The complexity of these communities is immense, with a relatively few abundant taxa coexisting with a considerable proportion of rare species (termed “rare biosphere”) (Lynch and Neufeld, 2015). Given the abundance determines the functional role of soil microbial communities (Rivett and Bell, 2018), previous literatures of soil communities primarily focused on the contribution of relatively abundant taxa to ecological functions (Delgado-Baquerizo et al., 2018). Nonetheless, rapid advances in molecular analysis technologies have substantially boosted our understanding of the rare biosphere. In particular, multiple omics sequencing approaches have been successfully applied to unravel ecological drivers of distinctive microbial communities assembly in various environments such as coastal waters (Wu et al., 2017), inland waters (Liu et al., 2015), reservoirs (Xue et al., 2018), and oil polluted soil (Jiao et al., 2017). For instance, the abundant taxa in subtropical bays (Mo et al., 2018) and agricultural ecosystems (Jiao and Lu, 2020) are mainly regulated by deterministic processes, but stochastic processes are the principal drivers for the assemblage of rare taxa. Contrasting results were observed in the epipelagic waters of oceanic (Wu et al., 2017) and inland waters (Xue et al., 2018). These studies indicate that abundant and rare species present contrasting diversity patterns and are regulated by distinct ecological processes due to the heterogeneity of their habitats.

Understanding the linkages between microbial communities and soil element cycles is emerging as a major challenge in microbial ecology (Fierer, 2017). A previous study in terrestrial ecosystems provides empirical evidence that soil microbial diversity drives ecosystem multifunctionality (Delgado-Baquerizo et al., 2016b). The ecological importance of rare species has become increasingly recognized since the rare subcommunity contains a higher proportion of metabolically active microorganisms than the abundant subcommunity (Campbell et al., 2011). Rare species act as keystone species in mediating the functions of different environments (Lynch and Neufeld, 2015). In view of carbon cycling, some rare species may be pivotal for methane consumption, organic substrate use, and straw decomposition (Jousset et al., 2017). Nitrogen cycling processes, including ammonia oxidation, nitrogen fixation, denitrification, and dissimilatory nitrate reduction were also detected in the rare members (Liang et al., 2020). Soil phosphorus turnover was also regulated by rare taxa in a paddy field (Wei et al., 2019). Additionally, rare species in peatland play an important role in sulfate reduction and sulfur oxidation. Although these studies effectively linked abundant and rare subcommunities to nutrient cycles in independent systems, they neither account for the quantitative contribution of these microbial subcommunities to key soil processes on the level of functional genes nor determine the fluctuation of metabolic traits of these microorganisms over a soil chronosequence. Especially, linking abundant and rare subcommunities to key soil processes in response to long-term secondary succession has not been investigated.

Loess Plateau, a temperate semiarid area, is well known for its low aboveground vegetation coverage and serious soil erosion due to its adverse land-use management. The Chinese government has therefore launched the “Grain to Green” program (GCP: 1999–present) to mitigate the degradation of land quality and declines of above- and belowground biodiversity by returning cropland and to grassland (Yu et al., 2020). Long-term secondary succession in this area has formed distinct landscapes at different succession ages by implementing several decades of active and passive restoration strategies (Zhang et al., 2016). Nevertheless, studies assessing the mechanisms of ecological processes controlling the abundant and rare subcommunities and their associations with key soil processes, particularly in long-term secondary succession, remain scarce. To fill the knowledge gap of structure-function relationships, the community assembly processes of the abundant and rare biospheres in long-term secondary succession soil has been investigated in the present study.

Herein, we hypothesized that positive associations between multifunctionality (especially linked to C, N, P, and S cycles) and belowground microbial diversity are maintained in the argo-ecosystems, as they have been well validated in the natural ecosystem (Delgado-Baquerizo et al., 2017). More specifically, the following key questions will be addressed: (1) Are abundant and rare taxa present in similar or contrasting diversity and co-occurrence patterns? (2) What are the relative contributions of ecological processes modulating the community assemblage of abundant and rare species? (3) Which microbial subcommunities are the key regulators for soil C, N, P, and S cycles?

2. Materials and methods

2.1. Research sites selection, soil sampling, and physicochemical analysis

The field experiment was performed in a natural grassland ecosystem located in a national nature reserve of Yunwu mountain, Ningxia Province, China (spanning approximately 36.10–36.17° N and 106.21–106.27° E). This area is in a temperate semiarid climate with a mean annual temperature of 5 °C, and a mean annual precipitation of 445 mm. The main type of soil in this area is gray cinnamonic soils (FAO classification) that are distributed at a range of 1999 to 2148 m of mean altitude. The predominant plants in the selected areas are *Artemisia sacrorum* and *Stipa bungeana*.

Six restoration habitats left for 5, 10, 15, 20, 25, and 30 years in the core area of the national natural reserve were selected as experimental locations based on their similar landforms, altitudes, slope gradients, and well-dated successional chronosequence. Within each habitat, three landscape positions including an upper slope, middle slope, and foot slope were selected. Four plots were established at each position. Then, a composite soil sample including five discrete soil samples with 0–20 cm depth were collected from each plot. After plant roots were removed by sifting through 2 mm mesh UV-sterilized sieves, the homogenized fresh soil samples were immediately transported to the laboratory by bubble chamber with ice packages. Aliquots of the soil samples were stored at -80 °C for later genomic DNA extraction. A subset of samples was air-dried for the determination of edaphic properties including soil moisture, pH, total organic carbon (TOC), total nitrogen (TN), available nitrogen (AN), available potassium (AK), and available phosphorus (AP). All edaphic properties and samples locations are described in supplementary data (Table S1, Fig. S1).

2.2. Soil DNA extraction, Illumina sequencing, and GeoChip analysis

Approximately 0.5 g of homogenized fresh soil was used to extract genomic DNA from each sample using FastDNA® SPIN Kit for Soil (Mp Biomedicals, Illkirch, France) following the manufacturer's procedure. Prior to sequencing, the primer set 515F (5′-GTGCCAGCMGCCGCG GTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with adaptors and barcodes was applied to amplify the hypervariable V4 region of bacterial 16S rRNA. Three individual PCR amplifications were conducted for each sample in order to minimize amplification bias. The protocols for PCR amplification and PCR product purification were performed following to the manufacturer's instructions. Finally, the paired-end (2 × 250) sequencing for the constructed amplicon libraries were conducted on an Illumina HiSeq 2500 platform (Personal Biotechnology Co., Ltd., Shanghai, China). For processed sequences, all raw reads were analyzed using QIIME 2 (2019.4, https://qime2.org)-Cenots 7.6 environment. The DADA2 plugin (Callahan et al., 2016) in QIIME2 then produced an amplicon sequence variants (ASVs) count table containing 551,488 high quality reads and 15,278 ASVs for downstream analyses. The QIIME2 plus DADA2 pipeline for present study is found at https://github.com/Duntao/Project_of_Yunwu_mountain.

GeoChip 5.0 is a comprehensive functional gene array containing 167,044 distinct probes, covering 1500 functional gene families from 12 major functional categories included in microbial nutrient cycling, energy metabolism, stress response, etc. Genomic DNA from each sample was initially labeled with the fluorescent dye Cy-3 using a random priming method. Then, the DNA was purified with a QIA quick PCR purification kit, dried at 45 °C for 45 min (SpeedVac, ThermoSavant), and hybridized at 67 °C with 10% formamide for approximately 16 h. Subsequently, the washed and dried arrays were imaged with a MS 200 Microarray Scanner (NimbleGen, Roche) and data were extracted using Agilent's Feature Extraction program (v.12.1.1.1). The raw signals were further cleaned and normalized using the Microarray Data Manager pipeline (http://ieg.ou.edu/microarray). In brief, spots were removed when the signal-to-noise ratio was <2 or the signal intensity was <200. The LnMR method was then used to transform and normalize signals. Together, a total of 61,643 functional gene probes, including 9917 carbon cycle genes, 2658 nitrogen cycle genes, 1328 phosphorus cycle genes, and 1786 sulfur cycle genes, were assigned across all time-gradient soil samples.

2.3. Null-based and neutral community model

To assess the phylogenetic community assembly within a sample, the standardized effect size measure of mean nearest taxon distance (ses. MNTD) was calculated in the "picante" R package (Kembel et al., 2010). In this study, the Nearest Taxon Index (NTI) could represent ses. MNTD. Lower values of NTI (<-2) or mean NTI < 0 indicates that co-occurring ASVs are more influenced by phylogenetic clustering, whereas higher values of NTI ($>+2$) or mean NTI > 0 indicates dispersal limitation over phylogenetic clustering. To infer the community assembly processes, the faith's phylogenetic diversity (PD), nearest relative index (NRI), mean nearest taxon distance between communities (βMNTD), and standard deviation of βMNTD (β-nearest taxon index, βNTI) were further calculated in the "picante" R package. $|\beta\text{NTI}| > +2$ indicates that deterministic processes play pivotal roles in shaping the community composition. Briefly, βNTI values < -2 suggest the predominance of homogeneous selection, whereas βNTI $> +2$ indicates variable selection making a greater contribution to the deterministic process. $|\beta\text{NTI}| < +2$ indicates the dominance of stochastic processes in the bacterial assemblage patterns.

Due to the contributions of homogenizing dispersal, ecological drift, dispersal limitation, diversification, and undominated processes in the stochastic process, Bray-Curtis-based Raup-Crick (RC_{bray}) was further calculated. To be specific, the relative effects of dispersal limitation were quantified as the proportion of pairwise comparisons with $|\beta\text{NTI}| < +2$ and $\text{RC}_{\text{bay}} > +0.95$. Homogenizing dispersal was quantified as the percentage of pairwise comparisons with $|\beta\text{NTI}| < +2$ and $\text{RC}_{\text{bay}} < -0.95$. The relative contributions of ecological drift, diversification, and undominated fraction were evaluated as the proportion of pairwise comparisons with $|\beta\text{NTI}| < +2$ and $|\text{RC}_{\text{bay}}| < +0.95$. The logic equations and simulation models for these approaches were based on previous studies.

To further determine the potential contribution of stochastic processes on the assemblage of the soil bacterial community, a neutral community model (NCM) was applied to predict the association between ASV assignment frequency in regional communities and their relative abundance across wider metacommunities. The model used here predicts that abundant taxa would be widely distributed across the metacommunity due to their dispersion by chance among distinct sample sites, whereas rare taxa would be lost with succession time because of ecological drift. In this model, *Nm* represents the relationship between ASV detection frequency and local relative abundance. The parameters R^2, and m indicate overall fit to the neutral model and immigration rate, respectively. Boot strapping (10^3 bootstrap replicates) was performed to fit the 95% confidence for this model. The related calculating processes were conducted in R (Version 3.6.1, https://r-project.org).

To further confirm whether the niche-based mechanisms determined the soil bacterial communities, the local relative abundance distributions of ASVs were fitted to preemption, null, log-normal, Zipf, and Zipf-Mandlebrot models. These models representing the niche theory were conducted using the "*radfit*" function in "vegan" R package (Oksanen et al., 2016). To assess the fit qualities of statistical models, the Akaike Information Criterion (AIC) was employed to compare these models. The lower values in different models indicate the best fit.

2.4. Statistical analyses

In this study, ASVs were defined as regionally abundant taxa when their average relative abundance was ≥0.1% in all samples and ASVs were defined as regionally rare taxa when their had an average relative abundance was ≤0.05% across the entire dataset. The ASVs with a relative abundance between 0.05% and 0.1% belong to a transient subcommunity. In this study, significant differences between succession groups were calculated by Wilcoxon rank sum tests in R. p-Values were adjusted by using the "*fdrtool*" package (Strimmer, 2008) and were considered significant with $p\text{-value}_{\text{adjust}} < 0.05$ unless indicated otherwise.

α-Diversity estimators, including Species richness, Pielou evenness, Shannon index, and Simpson index, were calculated in the "vegan" R package for each sample. β-diversity was further measured on the basis of Bray-Curtis dissimilarity matrix. Briefly, non-metric multidimensional scaling (NMDS) was applied to visualize different community compositions in the "vegan" package. The principal coordinate analysis (PCoA) was then conducted to demonstrate community dissimilarity between groups in the "vegan" package. Moreover, a permutational ANOVA (PERMANOVA), an analysis of similarity (ANOSIM), and a multiple response permutation procedure (MRPP) were implemented in the "vegan" package to elucidate that different succession time gradients had involved remarkable discrepancies in microbial subcommunity structures. Additionally, a time-lag model was applied to determine the Bray-Curtis dissimilarity between each group of succession years. In order to further explore the relative contribution of nestedness and turnover to the β-diversity, the β-diversity values of different subcommunities were divided into components, including balanced variation and abundance gradient, by implementing in the "betapart" package (Baselga and Orme, 2012). Finally, Levin's niche breadth index was calculated individually for abundant and rare microbial subcommunities to explain the patterns of β-diversity by using the "EcolUtils" package (Salazar, 2018).

To get a deeper understanding of the metabolic profiles of abundant and rare subcommunities, each ASV across the dataset was analyzed by using the PICRUSt2 pipeline (Douglas et al., 2020). The relative abundance of functional traits and functional genes were then performed to elucidate discrepancies between groups based on the KO level 1 and level 2, respectively.

In order to control the compositional effects of sequencing on the microbial interactome patterns (Gloor et al., 2017), the filtered ASV sequence counts were normalized by using the "trimmed means of M" (TMM) method with the "edgeR" package and expressed the normalized counts as relative abundance counts per million (Hartman et al., 2018). Co-occurrence network analysis was then constructed by using the "WGCNA" package with pairwise Spearman's rank correlation matrix (Fan et al., 2020; Langfelder and Horvath, 2008) to infer the relative importance and complex co-occurrence patterns of abundant and rare subcommunities in the whole succession period. Only robust ($|\text{soft threshold }(\rho)| > 0.85$) and statistically significant ($p_{\text{adjust}} < 0.05$) correlations were integrated into the network analysis. Moreover, six succession stages were further incorporated into three subnetworks based on the whole network to distinguish the complexity of soil subnetworks. Node-level topological characteristics involved average degree, betweenness, closeness, and eigenvector were measured in the "igraph" package (Csárdi, 2019). In order to compare with the real network, 1000 Erdös–Réyni random networks with real number of nodes and edges were also constructed in "igraph" package. In addition, topology properties, including modularity, average path length, and clustering coefficient were also calculated for random and empirical networks. In the empirical network, nodes with low betweenness centrality (<5000) and high average degree (>100) are identified as keystone taxa. Finally, the co-occurrence network and hubs with different modules were visualized using the Gephi platform (Version 0.9.2, https://gephi.org) and the "ggtern" package (Hamilton and Ferry, 2018).

Based on the longest gradient length values from the detrended correspondence analysis (DCA) and Hellinger-transformed ASVs tables, coordinates principal analysis (CAP) was performed by using the "vegan" package to assess the influence of environmental variables on different microbial subcommunities and functional profiles. Moreover, the related edaphic factors were $\log(x + 1)$ transformed without pH and then performed using distance-based linear modeling (distLM) analysis with an adjusted-R^2 selection procedure in PRIMER 7.0.12 (http://www.primer-e.com) to confirm the importance of environmental variables. Mantel test (permutations = 9999) was used to quantify the relative contribution of edaphic variables on the taxonomic patterns, module profiles, and functional traits by using the "vegan" package. Furthermore, the paramount drivers of the relative abundance of keystone taxa and dominant functional categories were measured by random forest analysis using the "rfPermute" and "rfUtilities" packages (Archer, 2016; Evans and Murphy, 2015).

Finally, ecological associations between community contributors and soil multinutrient cycling (i.e., carbon, nitrogen, phosphorus, and sulfur cycling) were determined by random forest analysis combined with the ecosystem function results from GeoChip analysis and high-throughput sequencing. In the random forest model, β-diversity indices (NMDS1), α-diversity estimators (ASV richness and Shannon index), and module eigenvalue (first axis of principal component analysis for module matrix) served as predictors to estimate the contribution of different microbial subcommunities to multiple nutrient-cycling-related functions. The observations of random forest analysis were further validated by the multiple regression model using the "relaimpo" package (Grömping, 2006).

3. Results

3.1. Diversity patterns and functional traits of abundant and rare subcommunities

Among all assigned ASVs, *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, and *Chloroflexi* were the dominant phyla in abundant and rare subcommunities based on the phylum-level (Fig. 1A and B). However, their average relatives differed substantially in each succession stage. For instance, *Proteobacteria* was the predominant phyla in the 5 year (averaging at 36.3%), 15 year (averaging at 32.1%), and 30 year (averaging at 27.2%) samples, while *Actinobacteria* was the paramount phyla in the 10 year (averaging at 29.9%), 20 year (averaging at 32.2%), and 25 year (averaging at 25.1%). In addition, *Actinobacteria* was always the principal phyla across the whole successional chronosequence ages in the rare subcommunity. These similar results could be observed in the transient, core and whole taxa (Fig. S2). Based on the taxonomy level, rare taxa involved more taxonomic group members than the abundant taxa.

In terms of α-diversity indices, species richness ($p < 0.001$), Pielou's evenness ($p = 0.037$), Shannon diversity ($p < 0.001$), and Simpson estimator ($p = 0.007$) were significantly influenced by the secondary succession time in both the abundant and rare subcommunities (Table S2). Although these four indices showed an analogous increasing tendency during the initial stage of succession in the range of 5 to 15 years, they displayed distinct patterns during the later stage of succession from 20 to 30 years (Fig. S3). Considering β-diversity, rare taxa displayed a remarkable separation compared with the entire and abundant taxa on the base of Bray-Curtis dissimilarity. Simultaneously, it could be observed that dissimilarity in different bacterial subcommunities across all periods declined with increasing soil chronosequence. This result was further confirmed by comparing succession groups among different periods and the results of ANOSIM and MRPP analyses (Fig. 1C and Table S3). Moreover, NMDS and PERMANOVA analyses revealed that abundant (stress: 0.153, $p < 0.001$) and whole taxa (stress: 0.122, $p < 0.001$) clustered more strongly by time than rare taxa (stress: 0.235, $p < 0.001$) (Fig. 1D and Table S3).

Regarding the abundance of metabolic profiles derived from PICRUSt2, abundant and rare subcommunities shared the top 20 functional traits such utilization of sugar, TCA cycle, fatty acid oxidation, EMP, CAZy, etc. Among these metabolic pathways, transporters,

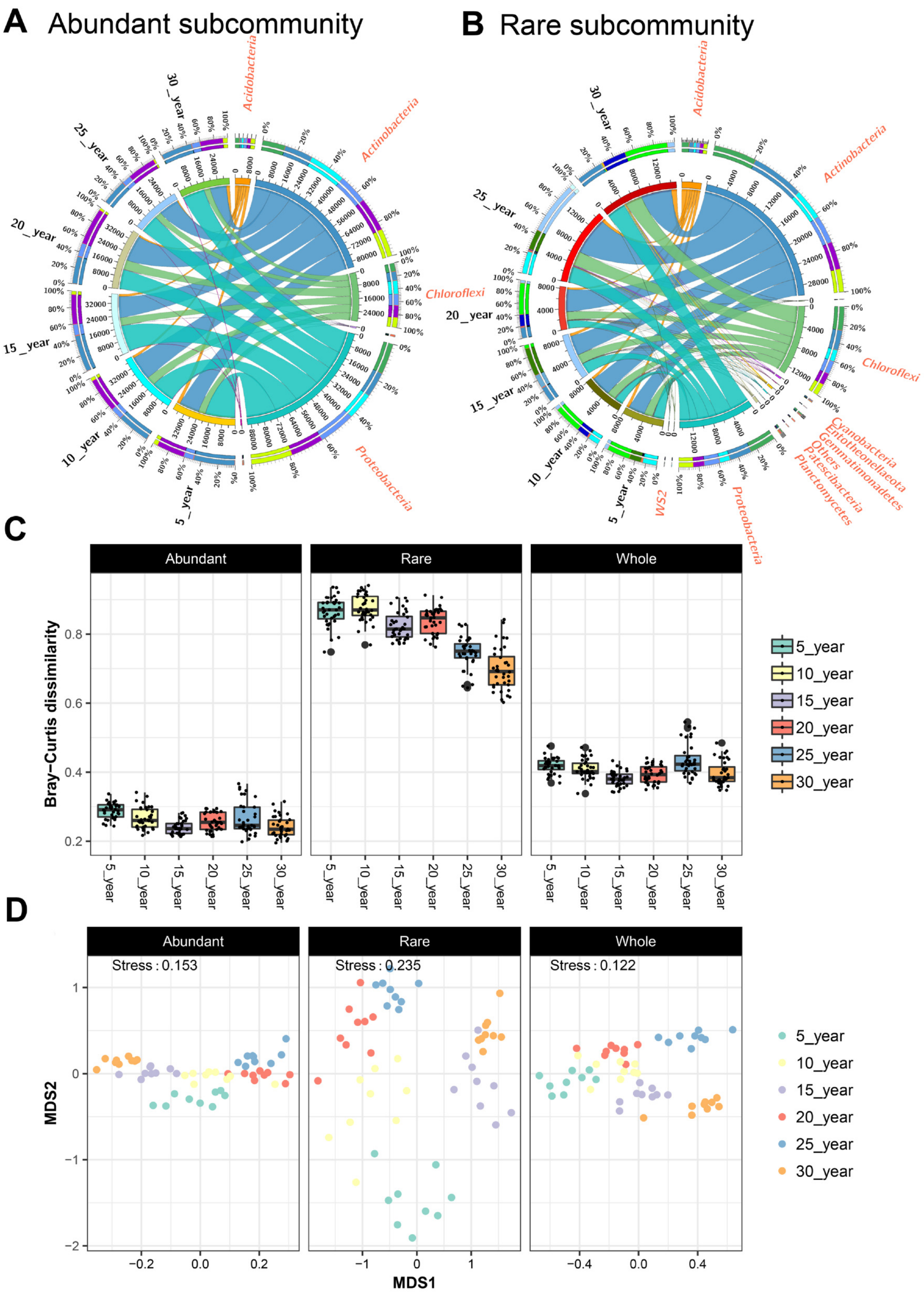

Fig. 1. Community structure of soil microbial communities. (A) and (B) Distribution of dominant phyla in the abundant and rare subcommunities along the soil successional chronosequences. The thickness of each ribbon represents the abundance of each ASV. The absolute tick above the inner segment and relative tick above the outer segment stand for the reads abundances and relative abundance of each ASV, respectively. The data were visualized using Circos (Version 0.69, http://circos.ca/). (C) The Bray-Curtis dissimilarity of soil bacterial communities in different successional ages. (D) Nonmetric multidimensional scaling (NMDS) ordination of abundant, rare and whole communities based on Bray-Curtis distances.

saccharide and derivative synthesis, nitrogen cycle, and homoacetogenesis were the dominant functional traits in the abundant subcommunity. In contrast, the rare subcommunity had a larger proportion of the remaining functional traits involving EMP, CAZy, TCA cycle, etc. (Fig. 2A). Other functional traits (Fig. 2B), including fatty acid oxidation, nucleic acid metabolism, hydrocarbon degradation,

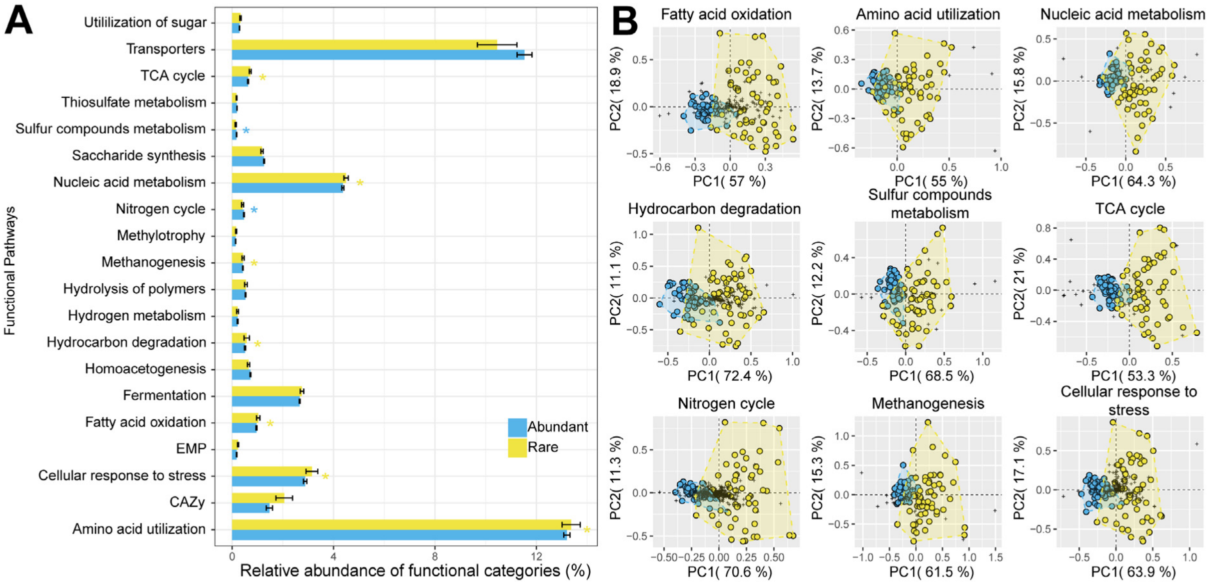

Fig. 2. Functional traits of soil microbial communities. (A) The relative abundance of community groups of functional traits in abundant and rare subcommunities based on the FOAM Database level 1. The error bars represent standard errors of sample replicates and blue asterisks (*) indicate metabolic categories that are significantly more predominant in abundant subcommunity and orange asterisks (*) indicate categories that are significantly more predominant in rare subcommunity (p value < 0.05, Wilcoxon test). (B) Distinct metabolic pathways between abundant and rare subcommunities were shown by principal coordinate analysis ordinations (PCoA) based on the FOAM Database level 2. The circles represent the clustering of the abundant and rare species, respectively.

nitrogen cycle, and cellular response to stress, etc., showed significant differences between abundant and rare subcommunities (Wilcoxon rank sum tests, $p < 0.05$). Moreover, the principal coordinates analysis showed that hydrocarbon degradation (72.4% of explained variation) and nitrogen cycle (70.6% of explained variation) were the two most important processes during the microbial succession (Fig. 2).

PCoA results revealed that abundant and rare subcommunities presenting distinct diversity patterns could be attributed to succession periods, confirmed by the Mantel statistic tests ($R = 0.7245$, $p = 0.001$). The first PCoA axis explained the larger fraction of variances (25.7% on the taxonomy level and 63.2% on the functional level) in the abundant subcommunity than in rare subcommunity (11.8% on the taxonomy level and 50.3% on the functional level) (Fig. S4A and S4B). Although the functional traits matrix between the bacterial subcommunities did not show significant differences, the functional traits of the bacterial subcommunities overlapped more by comparing with the community species composition. Moreover, abundant taxa (63.2% of PC1) showed more functional capability than rare taxa (50.3% PC1). Rare subcommunities, however, displayed more functional redundancy (22.2% of PC2) than abundant taxa (14.9% of PC2) (Fig. S4B).

Based on the β-diversity, time-lag regression analysis showed that there were a significant positive slopes for both abundant ($R = 0.220$, $p < 0.001$) and rare taxa ($R = 0.163$, $p < 0.001$) (Fig. S5A), suggesting that both bacterial subcommunities and edaphic variables were undergoing a directional change. To further reveal the mechanisms underlying the discrepancies observed between the abundant and rare subcommunities, the Sørensen index (β_{SOR}), which involves both incidence-based (β_{SIM}) and abundance-based (β_{NES}) dissimilarities, was used to decompose the β-diversity. The estimated β_{SOR} value of the abundant taxa among different periods was 0.847 ± 0.03, with a strong contribution of species turnover ($\beta_{SIM} = 0.802 \pm 0.02$) and a small fraction of nestedness ($\beta_{SIM} = 0.044 \pm 0.03$). Likewise, the β_{SOR} value of the rare subcommunity was 0.964 ± 0.05, with a large proportion of species turnover ($\beta_{SIM} = 0.948 \pm 0.05$) and a small contribution of nestedness ($\beta_{SIM} = 0.015 \pm 0.02$). Although the slope for abundant taxa in the partitioning β-diversity was significantly steeper than that for rare taxa, balanced variation and abundance gradients in the abundant taxa were lower than that in the rare taxa (Fig. S5B).

3.2. Quantitative importance of deterministic and stochastic processes

Considering the assembly processes of abundant and rare taxa, results of the phylogenetic distance based on the null model revealed that rare taxa had a larger phylogenetic diversity than abundant taxa (Fig. S6A). Moreover, the mean NTI (Fig. 3A) and NRI (Fig. S6B) in the rare taxa were found positive, while NTI had both negative and positive values in the abundant taxa. These results indicated that phylogenetic clustering was dominant in rare taxa assembly, while both clustering and dispersal limitation were responsible for controlling the abundant subcommunity. Furthermore, the mean βNTI values for abundant taxa in all successional ages ranged between −2 and +2 but were less than −2 for the rare taxa in the 15 and 30 year samples (Fig. 3B). Combining the values of βNTI and RC_{bray} (Fig. 3B and C), we found that stochastic processes provided over 72.2% of the contribution over all succession periods (Fig. S7A). In the rare subcommunity, the contribution of deterministic processes increased from 5.55 to 55.5% from 5 to 15 years. It declined to 13.8% at 20 years, and the contribution of deterministic process then increased from 13.8 to 63.8% in the later stage of succession (Fig. S7A). These results suggested that stochastic processes regulate the assemblage of abundant subcommunity. When it comes to rare taxa, community construction was governed by both deterministic and stochastic processes.

Subsequently, the relative contributions of each assembly process for the different bacterial subcommunities over successional ages were further calculated (Fig. S7B). Species sorting (homogeneous and variable selection) (0–27.7% for abundant taxa and 5.55–63.8% for rare taxa), dispersal limitation (0–61.1% for abundant taxa and 2.77–16.6% for rare taxa), and ecological drift (30.5–69.4% for abundant taxa and 19.4–88.8% for rare taxa) showed significant influences in all successional soils. To be specific, dispersal limitation (61.1% at the 5 year) contributed the largest fraction to the assembly of abundant taxa in the early successional ages. Although ecological drift slightly declined from 69.4% to 52.7% in the mid-successional soils (15–20 years) and declined from 69.4% to 50.0% at the late successional ages (25–30 years), it was also the dominant process compared with other processes. Consistent with abundant taxa, ecological drift was also the predominant process (88.3–88.8%) in the early successional ages for rare taxa. Species

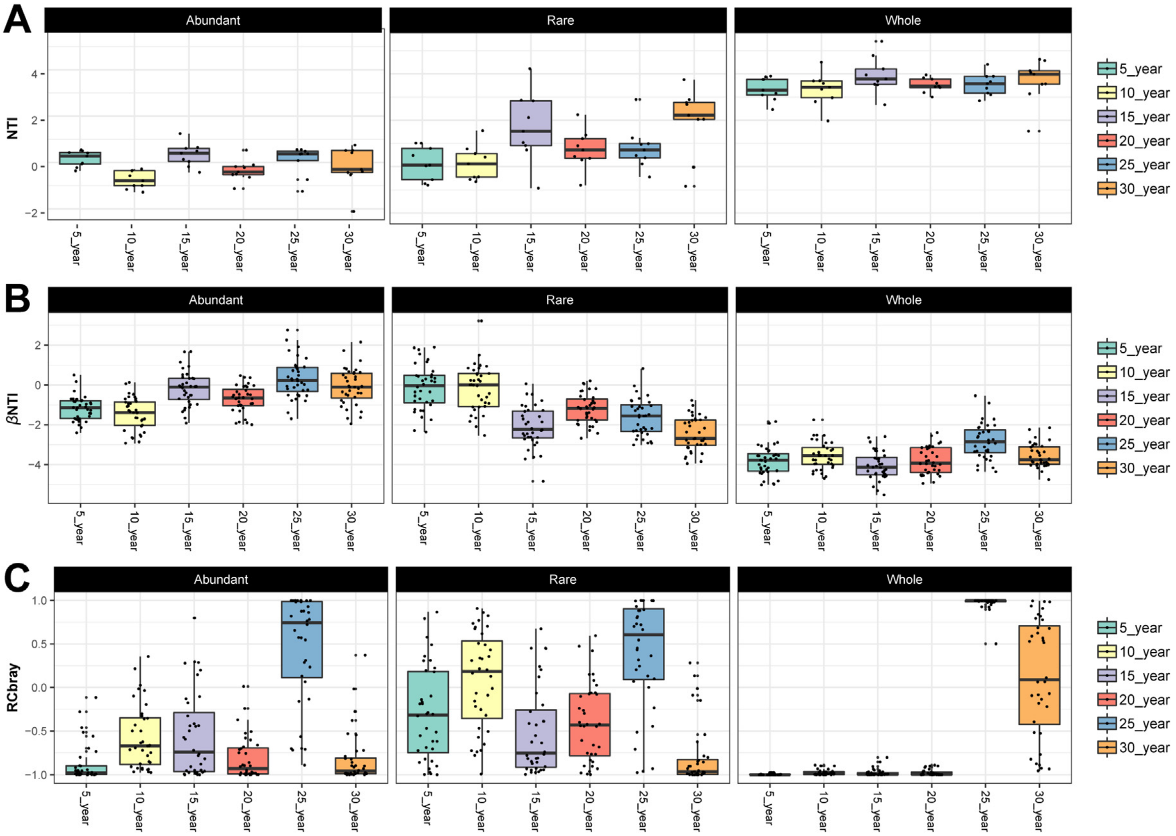

Fig. 3. Ecological process of soil microbial community assembly. (A) and (B) Null model analysis results for NTI and βNTI distributions across the different successional ages in abundant, rare and whole communities based on phylogenetic turnover. (C) Taxonomic turnover (RC_{bray}) of abundant, rare and whole communities along the soil successional chronosequences based on the Null model.

sorting (55.5% at the 15 year) and ecological drift (75.0% at the 20 year), however, contributed the largest fraction to the mid-successional soils. In the late successional soils, the fraction of species sorting assembly increased from 13.8% to 63.8%, whereas ecological drift sharply dropped from 75.0% to 19.4%. These results indicated that the assemblage of abundant taxa in all successional ages was regulated by dispersal homogenizing and ecological drift. These two processes also dominated the rare taxa assembly in the early successional soil, while homogeneous and variable selection were the principal processes in the mid- and late-successional ages.

The niche-based null model and neutral community model (NCM) were further successfully applied to confirm the assemblage processes of the meta-community. Based on the null model, rare taxa had lower AIC values than that in abundant taxa across all successional ages (Fig. S8), suggesting that the dominant ecological process for rare taxa was consistent with the niche-based theory. The higher coefficients between occurrence frequency of ASVs and their relative abundance in all successional soils ranged from 76.3% ($m = 0.1102$) to 80.2% of explained community variance ($m = 0.0526$) (Fig. S9A), indicating the abundant subcommunity assembly was in accordance with neutral theory. Moreover, the value of m in the NCM varied differently indicating that the migration rates of abundant taxa changed with species dispersal and ecological drift. Additionally, 54.0% of ASVs affiliated with abundant taxa were better fitted with neutral prediction, thus indicating the significance of the neutral theory for the abundant taxa. Analogous results could be observed in the rare taxa (Fig. S9B) indicating that some rare taxa were also affected by the neutral processes. This was consistent with the $|\beta NTI| > 2$ at the 5, 10, and 20 year successional soils. Finally, 40.9% of the ASVs belonging to abundant taxa that occurred more frequently than predicted by NCM, revealing that these species have a higher migration ability to disperse to other locations. This result was further confirmed by the wider niche breadth and overlap of abundant species (Fig. S10), confirming that dispersal acting with drift together controlled the assembly of abundant subcommunities.

3.3. Microbial interactome and characteristics of constructed networks

Based on the correlation relationships, a metacommunity co-occurrence network was constructed with 1613 nodes and 9356 associations. The degree distribution of the global network fitted well with the power-law model ($R^2 = 0.947$), suggesting that the global network was non-random and scale-free. Compared with Erdös-Réyni random networks, global empirical networks had greater values of modularity, average clustering, and average path length indicating that the empirical network had "small-world" properties and modular structures. In addition, 88.8% and 6.14% of nodes were affiliated with rare and abundant taxa, respectively (Fig. 4A). Among these edges, rare taxa had a large proportion of positive associations and minor negative links. Abundant taxa, however, exhibited the opposite trend. Moreover, abundant taxa had lower co-occurrence instances (edge/node ration = 2.58) than rare taxa (edge/node ration = 5.79), indicating that rare taxa tend to co-occur more frequently and tightly.

Subsequently, subnetworks for abundant and rare taxa were further constructed and node-level topological features were calculated. Mean betweenness, closeness centrality, and degree in rare subnetworks were higher than in abundant networks. However, eigenvector centrality showed higher values in the abundant taxa (Fig. 4C). These results suggested that rare taxa have taken a core location in the global empirical network. Moreover, rare sub-networks in the three individual sub-networks showed stronger intra- and inter-taxon co-occurrence

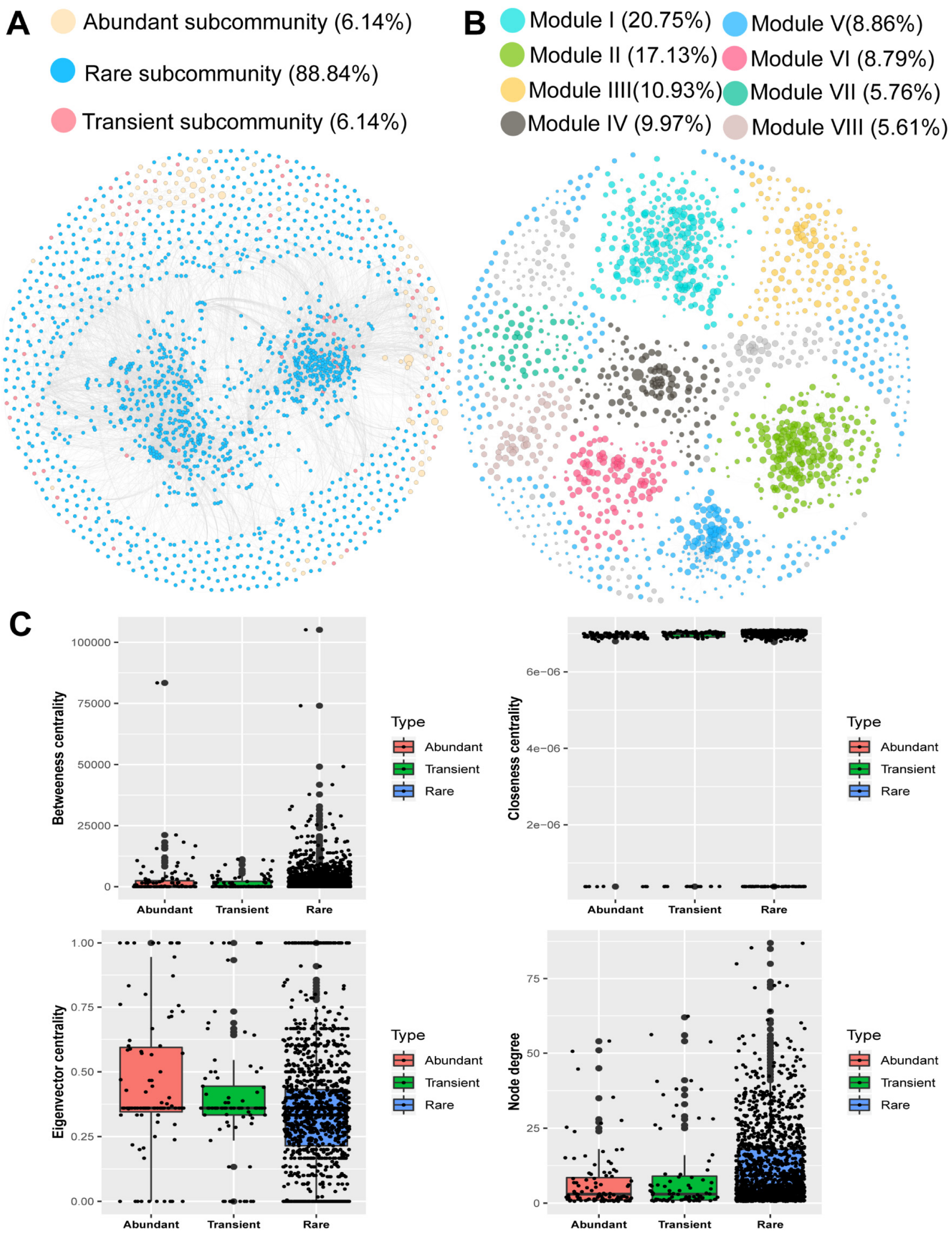

Fig. 4. Network of co-occurring bacterial ASVs in long-term successional soil. The nodes were colored according to different sub-communities (A) and modularity class (B), respectively. The connection represents a robust (Spearman's correlation coefficient $|\rho| > 0.85$) and significant ($p < 0.05$) correlation. The size of each node is proportional to the relative abundance of each ASVs with normalizing. (C) The node-level topological features of different sub-communities, including betweenness, closeness, transitivity, and degree.

patterns than abundant taxa (Fig. S11), which was confirmed by the high value of network-level topological characteristics in early, middle, and last successional ages in rare taxa (Table S4).

Modularity analysis further revealed that the global network could be separated into 8 major modules, of which modules I, II, and III accounted for 20.75%, 17.13%, and 10.93% of the entire network, respectively (Fig. 4B). Results provided by the separate subnetworks (Fig. S10) showed that modules of both abundant and rare subnetworks increased with increasing successional age. Compared with abundant taxa, three separate rare sub-networks had higher values of modularity (Table S4), indicating that the complexity and resistance increased with increasing successional age. Ternary plot analysis demonstrated that the majority of modules presented yearly patterns (Fig. S12). For instance, ASVs from modules I and IV had higher relative abundances in late successional stages than in the other stages, while modules III and V tend to occur in the early stages. Most importantly, taxonomic relatedness played a pivotal role in determining the modular structure of the global subnetworks. The 8 major modules, which were composed of 90.8% rare taxa and 4.86% abundant taxa, were dominantly occupied by *Actinobacteria* (46.1%), *Chloroflexi* (22.3%), and *Proteobacteria* (21.49%). Additionally, class *Acidimicrobiia* dominated modules I, II, IV, VI, and VIII, while modules III, V, and VII primarily belonged to *Alphaproteobacteria* in the rare taxa. In terms of abundant taxa, modules II, III, and VI were affiliated with relatively low proportion of *Alphaproteobacteria*; and module III was composed of *Acidimicrobiia*. These results indicate that *Acidimicrobiia* and *Alphaproteobacteria* could be considered keystone species in the microbial subnetworks.

3.4. Abiotic predictors for community assemblage and function profiles

Since divergent assembly processes in abundant and rare subcommunities were observed in distinct successional ages, the intrinsic variables driving the global taxonomic patterns, modularity profiles of the networks, and functional traits were further explored. Mantel tests and DistLM analysis revealed that moisture and AP were significantly associated with global taxonomic patterns in the abundant subcommunity. TOC and TN were the strongest correlates of global modularity profiles and functional traits, respectively (Fig. S12). In terms of the rare subcommunity, the global taxonomic patterns were remarkably correlated with all edaphic factors. Compared with abundant taxa, TOC and AK had no significant association with modularity profiles in the rare taxa. Similar results were observed in the driving force of functional traits, indicating that pH, TOC, TN, AN, and AK rather than moisture and AP had significant influences on the function of rare taxa (Fig. S13). These results suggest that the ecological patterns of abundant and rare subcommunities were regulated by distinct driving forces. Moreover, deterministic processes (i.e., environmental filtering) had a higher influence on the assembly process of rare taxa than abundant taxa.

Finally, multiple regression modeling and random forest analysis were applied to verify the relative contribution of edaphic variables on the distribution of the microbial community and gene functions. Bacterial taxonomic diversity and the relative abundance of major bacterial classes can be explained by soil moisture, pH, and soil available nutrient level. The abundance of bacterial classes, including *Verrucomicrobiae*, *Rubrobacteria*, *α-Proteobacteria*, and Actinobacteria were important factors for predicting soil nutrient levels in the abundant taxa (Fig. 5A). In contrast, the classes such as *Gemmatimonadetes*, *Chloroflexia*, and *Acidimicrobiia* were pivotal variables for determining soil nutrient concentrations in the rare taxa (Fig. 5B). In terms of metabolic pathways, most of the soil nutrient levels, including AK, AN, AP, TN, and TOC, have strong and positive effects on saccharide synthesis, hydrogen metabolism, and homoacteogenesis for abundant taxa (Fig. 5C). As for metabolic pathways in the rare taxa, methanogenesis, hydrogen metabolism, and fermentation were vital factors for predicting the five soil nutrient levels (Fig. 5D). Based on these results, pH (71.08% of explained variation) (Fig. 5A) and TN (66.58% of explained variation) (Fig. 5B) were the strongest predictors for the dissimilarities of abundant and rare subcommunities. TN (60.56% of explained variation) (Fig. 5C) and AN (45.85% of explained variation) (Fig. 5D) played predominant roles for the gene functions of these bacterial subcommunities. These results indicate that the concentration of nitrogen in the successional soils was a key factor in determining the assembly of the microbial communities and gene functions.

3.5. Importance of microbial subcommunities in multinutrient cycling

To disentangle the contribution of multifunctionality (especially-linked to C, N, P, and S cycling), GeoChip technique was applied in the present study. Results showed that cycling genes differed substantially among the six successional ages (Fig. S14). Carbon fixation genes, including starch, hemicellulose, cellulose, chitin, pectin, aromatics, and lignin increased in the early successional ages. Then they decreased in the mid-successional ages and increased again in the later successional ages (Fig. S14A). Similar patterns could be observed in the carbon fixation genes for methaneogenesis and methane oxidation (Fig. S14B). Variability was also apparent for nitrogen cycling (Fig. S14C). In total, functional genes affiliated with ammonification (*gdh* and *ureC*), nitrogen fixation (*nifH*), denitrification (*nosZ*), and dissimilatory N reduction (DNRA, *narG*) were more abundant than other genes in related nitrogen processes. The increasing or decreasing trend for nitrogen cycling was in accordance with carbon cycling. Strikingly, the detected abundance of AOA *amoA* genes was higher than that of AOB *amoA* genes along the successional soils. Additionally, the *hzsA* gene affiliated with Anammox and the *nrfA* genes belonging to DNRA increased over the 5–15 year samples, but decreased in the 20–25 year soils. For sulfite reduction (Fig. S14D), *dsrA*, *dsrB*, and sir genes involved in the sulfite reduction showed an increasing trend from 5 to 25 years. After a minor decline in the 25 year soils, their abundance increased once again. In contrast to sulfite reduction, genes affiliated with sulfite oxidation varied with those for carbon cycling as did those for phosphate cycling (Fig. S14E). However, *pstS*, *ppn*, *ptxD*, and *phlD*, which are affiliated with polyphosphate assimilation, polyphosphate degradation, phosphorus oxidation, and polyphosphate synthesis, respectively, slightly declined in the 10 year soils. These results revealed that alterations in functional gene abundance shape the soil multinutrient cycling.

To further disentangle the association between microbial subcommunities and soil multinutrient cycling in the successional soils, a multivariate regression analysis was applied to quantify the contributions of α-diversity, β-diversity, and network modularity (Table S5). For abundant taxa, the β-diversity and module profiles contributed most to carbon, nitrogen, phosphate, and sulfur cycling. The explained contributions of module profiles were higher than β-diversity, especially to most of nitrogen cycling processes without assimilatory nitrogen reduction. In terms of rare taxa, the α-diversity such as Shannon-Wiener index had a higher contribution to the soil multinutrients cycling. By contrast, some processes, including carbon fixation, methanogenesis, assimilatory nitrogen reduction (ANR), denitrification, dissimilatory nitrate reduction to ammonium (DNRA), Anammox, polyphosphate degradation, and sulfite oxidation/reduction were also affected by the richness and module profiles. These results indicated that soil multinutrient cycling was primarily regulated by microbial interaction due to the higher habitat breath of abundant taxa. Nevertheless, species diversity and relative abundance in the soil ecosystem determine the soil multinutrient cycling of rare subcommunities.

All detected functional genes based on the GeoChip analysis were further divided into four soil multinutrient cycling groups such as carbon, nitrogen, phosphate, and sulfur cycles. The linear least-squares regression revealed that the dissimilarities for both abundant and rare subcommunities have positive correlations with carbon, nitrogen, and sulfur cycling, while they were negatively associated with phosphate cycling (Fig. 6). Moreover, the soil multinutrient dissimilarities, including carbon ($R^2 = 0.465$, $p = 0.008$), nitrogen ($R^2 = 0.346$, $p = 0.015$), and sulfur cycling ($R^2 = 0.316$, $p = 0.009$), had more remarkable associations with rare subcommunity dissimilarity than that in the abundant taxa. Nevertheless, this association for phosphate cycling ($R^2 = 0.124$, $p = 0.036$) in the abundant subcommunity presented the opposite trend. These results indicate that, soil carbon, nitrogen, and sulfur cycling are primarily regulated by rare taxa in secondary successional soils, whereas abundant taxa dominant phosphate cycling.

4. Discussion

4.1. Abundant and rare bacterial communities present contrasting temporal patterns and functional traits

Recent methodological advances like next generation sequencing have massively improved our understanding of the role of the rare

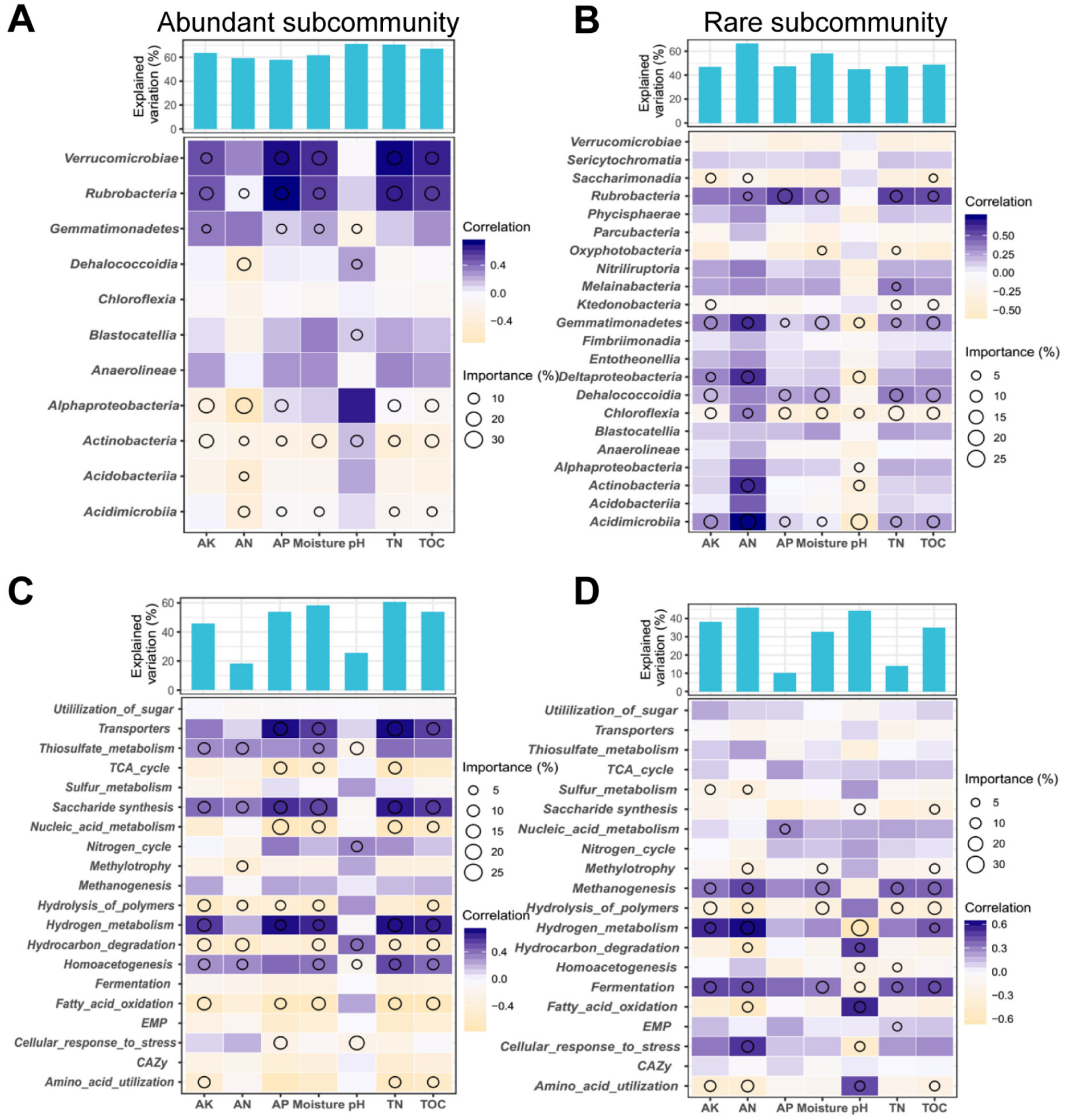

Fig. 5. Potential biological contributions of the taxonomy and functional traits to soil nutrient properties. (A) and (B) Correlation and best random forest model for dominant taxonomic at the class level of abundant and rare subcommunities. (C) and (D) Correlation and best random forest model for metabolic pathways of abundant and rare subcommunities. Circle size represents the variable's importance (i.e., percentage of explained variation calculated via multiple regression modeling). Colors represent Spearman correlations. AK, available potassium; AN, available nitrogen; AP, available phosphorus; TN, total nitrogen; TOC, total organic carbon.

biosphere and its shifts in response to anthropogenic perturbation (Jousset et al., 2017; Lynch and Neufeld, 2015). The results that we present here suggest that the diversity patterns and functional traits differ substantially between abundant and rare microbial taxa. Several reasons could account for this phenomenon. First, this result may be due to the changing edaphic profiles, including soil pH, nitrogen availability, soil organic carbon content, and soil moisture, have far-reaching influences on the communities of abundant and rare taxa (Jiao and Lu, 2020; Liu et al., 2015). With the variation of edaphic profiles during secondary succession, the population density of abundant and rare taxa would have been changed in order to adapt to the local environment. During the adaptation process, the abundant taxa were more widespread or had a more ubiquitous distribution than rare taxa. This conclusion is confirmed by the notion that abundant species occupy wider niche breadths and overlaps, agreeing with previous studies (Wu et al., 2017) but was not consistent with another study in marine sandy sediments (Zhang et al., 2018), and reasons may be correlation with the local soil type, the differences in the abundant bacteria phyla, and the scale of research regions.

Another reason for the differentiation may be a discrepancy in competitive ability for limited resources between abundant and rare species. In other words, abundant and rare taxa applied distinct life history strategies to confront varying conditions. Given the trait-based theories of life history in microbial ecology, soil communities could be divided into growth yield (Y), resource acquisition (A) and stress tolerance strategists (S) (Malik et al., 2020). In the present study, functional traits of microbial communities suggest that abundant species belonged to resource acquisition strategists with more transporters in their cells. In this view, abundant species would outcompete other microbial subcommunities for space and resources especially under less intensively

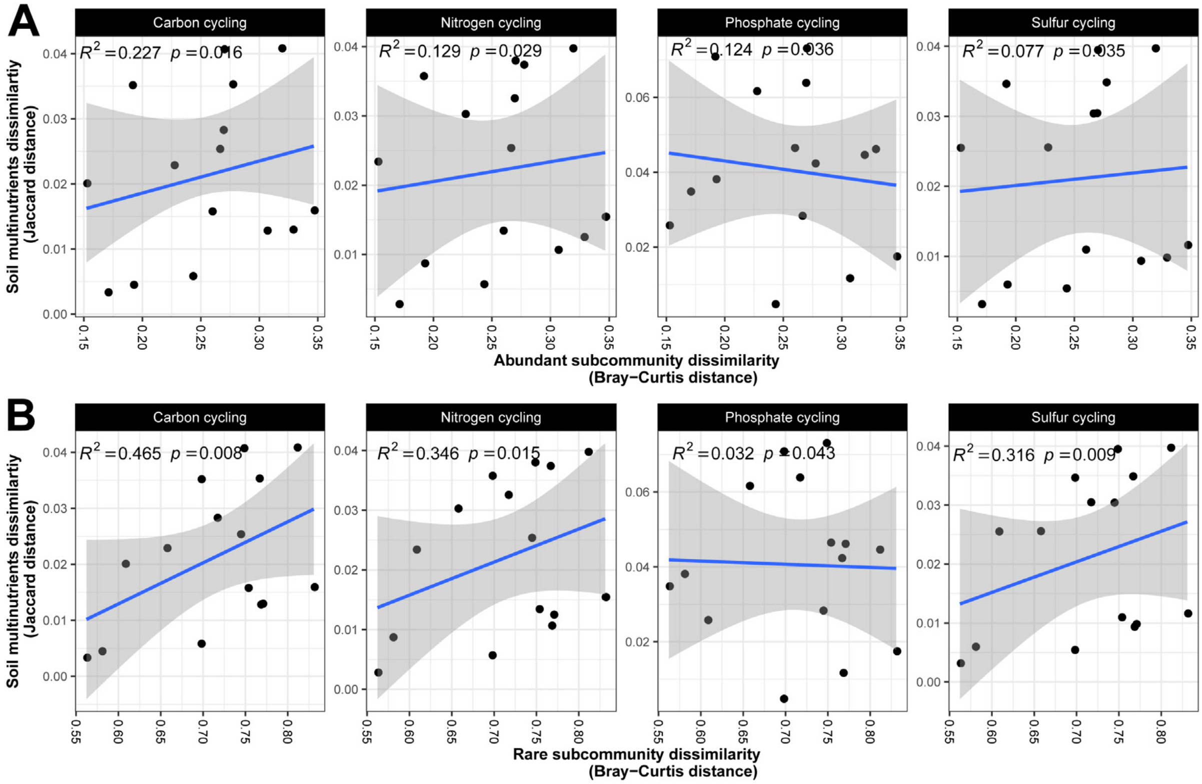

Fig. 6. Associations between compositional β-diversity of distinctive microbial sub-communities and dissimilarities of soil multinutrients index based on Jaccard distance, estimated by Mantel test. Solid blue line represents a significant relationship that fitted the linear regression model, and gray shaded area shows 95% confidence interval of the fit. Carbon cycling, carbon cycling related genes. N cycling, N cycling related genes phosphate cycling, phosphate cycling related genes. Sulfur cycling, sulfur cycling related genes.

managed soils. Rare taxa, however, became stress tolerant strategists owing to their function traits. They can thus persist under limited resources and suboptimal abiotic conditions (Fierer, 2017). The life history strategies of microbial communities and another previous study on rhizosphere soil (Yan et al., 2017) corroborated our findings that functional traits dominated the diversity-related selection of abundant and rare taxa during secondary succession.

4.2. Controlling mechanisms and relative contribution of assembly processes in shaping the bacterial subcommunities

A vital issue in microbial ecology is uncovering the underlying mechanisms driving community assembly, particularly in the continuum of deterministic and stochastic processes (Tripathi et al., 2018). Revealing this detailed information would be beneficial for the development of reasonable management strategies to remediate soil microbial communities exhibiting adverse functionality after human perturbation (Fierer, 2017). The compelling result of this study suggested that abundant taxa are predominantly governed by stronger stochastic but weaker deterministic assembly processes. A potential explanation is that less phylogenetically clustered bacterial communities are highly diverse and are mediated by abundant taxa capable of using many alternative nutrient resources, resulting in weak selection from environment variation. This is partially in line with a recent study (Jiao and Lu, 2020) of maize fields and paddy soils, which demonstrated that stochastic processes may outcompete deterministic processes under less environmental perturbation. It is worth noting that stronger stochastic assembly processes for rare taxa were also observed in the early and mid-successive stages. This phenomenon was supported by the results that most of rare taxa have a good fit to the neutral model. Nevertheless, rare taxa in the late stages were more regulated by deterministic processes. In other words, rare taxa were more constrained by the local environment in the later stages than in the other stages, indicating that rare taxa with a narrow niche breadth tend toward a more restricted distribution and are more governed by environmental filtering. Additionally, our findings are supported by a previous study (Dini-Andreote et al., 2015), suggesting that rare taxa are primarily mediated by stochastic processes during the initial stages of microbial succession due to the resource rich environment and smaller population sizes. The selective power from environmental filtering increased with the progressively increasing population size owing to the emergence of stable environments resulting in the rare taxa thriving and recovering their previous growth rates and activities (Campbell et al., 2011; Jousset et al., 2017).

A central challenge in microbial ecology is quantifying the relative contributions of deterministic and stochastic processes to abundant and rare bacterial assembly. Linking microbial subcommunities to the relative importance of these ecological processes is vital for a better understanding of how environmental variables act on biotic communities in the context of human perturbation (Soininen, 2014). In the present study, dispersal limitation played a major role in the assembly of abundant subcommunities but not of rare subcommunities. This finding is in line with a previous study, which showed that abundant taxa were more influenced by dispersal limitation than rare taxa in the surface layer of the Pacific Ocean and the upland soil of agricultural ecosystems (Jiao and Lu, 2020; Wu et al., 2017). Nevertheless, other studies demonstrated that rare taxa were more shaped by dispersal limitation than abundant taxa in subtropical bays (Mo et al., 2018). These discrepancies may result from habitat heterogeneity (Wang et al., 2017b) and spatial scale (Tripathi et al., 2018), as well as the different definitions of microbial subcommunities (Logares et al., 2014).

In contrast to the abundant subcommunity, the rare subcommunity in our study was significantly affected by species sorting. In a relative sense, species sorting, including homogeneous selection and variable selection, is usually defined as the selective power of an environment that alters microbial communities to survive under constant environmental conditions. As such it is a deterministic process (Zhou and Ning, 2017). Our findings are partially supported by other studies in a subtropical reservoir (Xue et al., 2018; Zhang et al., 2018), which demonstrated that rare taxa present stronger response to environmental disturbance. An alternative explanation for this pattern may be due to habitat specialists with narrower niche breadths (Lynch and Neufeld, 2015) or relatively lower abundance microorganisms on the base of the size-dispersal hypothesis (Farjalla et al., 2012) that are affected more by environmental filtering. From an eco-evolutionary perspective, rare taxa have often adopted dormancy strategies to cope with environmental stresses (Lennon and Jones, 2011), indicating that their growth rates, activities, and metabolic profiles often change with unfavorable environment conditions (Shade and Gilbert, 2015). Clearly, our findings therefore suggest that environmental selection mainly mediated the assembly of rare subcommunities, while assembly of the abundant subcommunities is dominated by dispersal limitation, a processes that can be explained by neutral theory.

4.3. Rare subcommunity dominated the complexity of microbial ecological networks and multinutrient cycling

Not only can network analysis potentially reveal the ecological rules guiding community assembly across the temporal gradients, it can also provide unique perspectives on the species interactions that reflect habitat heterogeneity and distinct selection regimes (Barberán et al., 2012). Consistent with previous observations (Jiao et al., 2017; Shu et al., 2018), our results showed that abundant and rare species tend to co-occur more than expected by chance. Moreover, the rare subcommunity was nonrandomly regulated by taxonomic relatedness. This may be due to the synergistic relationships or strong niche differentiation in closely related species (Ju et al., 2014). This is in line with the phenomenon of phylogenetic clustering of soil microorganisms reported elsewhere, suggesting the pivotal roles of environmental filtering and niche differentiation. This pattern was evident in the relative contribution of ecological processes governing the microbial assembly.

Regarding the topological properties of the microbial network, our results demonstrated that, similar to microbial guilds in activated sludges (Ju et al., 2014), bioreactor system (Shu et al., 2018) and soils (Jiao et al., 2017; Ma et al., 2016) the co-occurrence network of the abundant and rare subcommunities exhibited a power-law distribution and modular structures. Topological features such as node degree, betweenness centrality, and closeness centrality indicate that the rare subcommunity formed remarkably more complex networks and play irreplaceable roles in maintaining the stability of the entire network, which is consistent with a later study (Xue et al., 2018). In a pairwised co-occurrence network, positive correlations are usually regarded as commensalism and mutualism. The more positive links in the rare taxa subnetworks indicate that cooperation may contribute to the resilience of the rare taxa over long-term succession. A potential explanation is that coexisting and clustering species can encode the same energy-yielding metabolic functions, resulting in functional redundancy of species, which may provide a better buffer to cope with changing environment (Louca et al., 2018).

The modularity of the taxa profoundly influences the acting role of species in a microbial network (Faust and Raes, 2012). In the present study, the dominant modules in the entire network were comprised largely of rare taxa. At the same time, the complexity and the modules of the rare sub-network increased with succession age, indicating that the disappearance of these rare taxa or modules may cause networks and modules to disassemble. Previous studies have reported that even rare taxa can act as keystone taxa in soil networks (Shi et al., 2016). Thus, rare taxa can be as more important than abundant taxa in maintaining the complexity of microbial networks.

Understanding the linkages between the soil microbiome and the capacity of soil ecosystems to conduct multiple nutrient cycling simultaneously has been a core ecological research topic in recent years (Delgado-Baquerizo et al., 2013; Gross et al., 2017). Soil biogeochemical processes, including carbon, nitrogen, phosphorus and sulfur cycling can be directly and indirectly modulated by microbial taxa (Fierer, 2017; Wang et al., 2017a). Recent studies using metagenomics and meta-transcriptomics in acid mine drainage demonstrated that dominant and rare taxa have distinct metabolic potentials and ecological roles and that nitrogen and sulfur cycling were performed by the rare taxa, especially nitrogen fixation and sulfur oxidation. Similarly, denitrification activity in the soil was regulated by the rare species (Philippot et al., 2013). In long-term fertilized farmland, recent research indicated that the rare taxa rather than the abundant taxa were the major drivers of multi-functionality (Chen et al., 2020). Rare microbial taxa also have central roles in many other soil processes, such as sulfate reduction (Hausmann et al., 2016), methane consumption (Kleindienst et al., 2016), carbon utilization (Shu et al., 2018) and degradation of organic matter (Delgado-Baquerizo et al., 2016a). Our results corroborated those previous findings, and provided further evidence from long-term secondary succession to support the concept that rare bacteria have an over-proportional role in soil carbon, nitrogen, and sulfur cycling.

Previous studies have reported that rare taxa contributed substantially to the protein synthesis potential associated with phosphorus removal and phosphorus mineralization in enhanced biological phosphorus removal (Lawson et al., 2015) and phosphorus fertilized soils (Wei et al., 2019), respectively. These findings were not in line with our results, where phosphorus cycling was predominantly regulated by abundant taxa, suggesting the disproportional role of microbial subcommunities in soil biogeochemical processes. One potential explanation is attributed to the large proportion of microbial taxa that are conditionally rare taxa, may be not permanent, changing among transient, rare, and persistent taxa (Shade and Gilbert, 2015). This transition is usually exerted by external abiotic and biotic factors, including edaphic variables and features of microbial diversity such as β-diversity, species richness and Shannon index. Prior research has demonstrated that microbial diversity drives ecosystem multifunctionality in terrestrial ecosystems (Delgado-Baquerizo et al., 2016b). Additionally, the modularity of a microbial co-occurrence network not only reflects the stability of soil microbial communities but determines the complementarity and redundancy effects of the microbial taxa (Allison and Martiny, 2008; Xue et al., 2018). Thus, it would not be surprising to find that microbial diversity and microbial interactions were somehow related to soil multinutrient cycles. Through these results, our study reveals the distinct roles of abundant and rare taxa in microbial community assembly and soil nutrient cycles. To the best of our knowledge, the present work, based on >30 years of successional chronosequences, is the first demonstration of the roles of microbial subcommunities with the distinct community and functional traits in driving soil biogeographical processes over long-term secondary succession in a dryland area. Further studies should combine meta-transcriptomics and stable isotope trace technologies to quantify the contribution of microbial subcommunities to key soil processes.

5. Conclusions

In this study, natural restoration affects the assembly processes of abundant and rare subcommunities by changing their functional traits, microbial interactions and soil properties, thus altering multinutrient cycling in the soil. By comparing the abundant and rare taxa in community and functional level, our findings suggest that both of their successional orientations were determined by metabolic traits due to the substantial discrepancies in the life history strategies and niche breadth of these communities. Neutral theory and null model indicate that both deterministic and stochastic processes affected the community assemblage of rare subcommunities, while abundant taxa assembly was mediated by stochastic processes. Furthermore, soil carbon, nitrogen, and sulfur cycling were mainly driven by the rare subcommunity, while the abundant taxa regulated soil phosphorus cycling. To conclude, these findings could provide a deeper perspective on the ecological significance of abundant and rare taxa, and reinforcing the prediction of key soil processes that are mediated by the response of microbial communities to global change in secondary succession of dryland.

Data availability statement

The R code that supports the findings of this study is available upon reasonable request. GeoChip data are available online (http://ieg4.rccc.ou.edu/) with the accession number raw1482582747, raw1482575044, raw1482567079, raw1482559166, raw1482551352, and raw 1482543-437. HiSeq 2500 data are available in the NCBI SRA database with the accession number PRJNA644329.

CRediT authorship contribution statement

Duntao Shu: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Yanqing Guo:** Investigation, Writing – review & editing. **Baogang Zhang:** Investigation, Writing – review & editing. **Chunfang Zhang:** Writing – review & editing. **Joy D. Van Nostrand:** Investigation, Writing – review & editing. **Yanbing Lin:** Conceptualization, Methodology, Writing – review & editing. **Jizhong Zhou:** Investigation, Writing – review & editing. **Gehong Wei:** Conceptualization, Methodology, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145737.

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