Nutrients Changed the Assembly Processes of Profuse and Rare Microbial Communities in Coals

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Abstract
Nutrient stimulation is considered effective for improving biogenic coalbed methane production potential. However, our knowledge of the microbial assembly process for profuse and rare microbial communities in coals under nutrient stimulation is still limited. This study collected 16S rRNA gene data from 59 microbial communities in coals for a meta-analysis. Among these communities, 116 genera were identified as profuse taxa, and the remaining 1,637 genera were identified as rare taxa. Nutrient stimulation increased the Chao1 richness of profuse and rare genera and changed the compositions of profuse and rare genera based on nonmetric multidimensional scaling with Bray-Curtis dissimilarities. In addition, many profuse and rare genera belonging to Proteobacteria and Acidobacteria were reduced, whereas those belonging to Euryarchaeota and Firmicutes were increased under nutrient stimulation. Concomitantly, the microbial co-occurrence relationship network was also altered by nutrient addition, and many rare genera mainly belonging to Firmicutes, Bacteroides, and Euryarchaeota also comprised the key microorganisms. In addition, the compositions of most of the profuse and rare genera in communities were driven by stochastic processes, and nutrient stimulation increased the relative contribution of dispersal limitation for both profuse and rare microbial community assemblages and that of variable selection for rare microbial community assemblages. In summary, this study strengthened our knowledge regarding the mechanistic responses of coal microbial diversity and community composition to nutrient stimulation, which are of great importance for understanding the microbial ecology of coals and the sustainability of methane production stimulated by nutrients.

Key words: dispersal limitation, variable selection, methanogenic archaea, co-occurrence network, nutrient stimulation

Introduction
Coal is the most vital fossil fuel on earth (Sekhohola et al. 2013; Iram et al. 2017), the value of which is far greater than that of petroleum and natural gas. The formation of coal is driven by geological events (Emery et al. 2020), geologic settings (Li et al. 2018), and microorganisms (Liu et al. 2019). Microbes are the dominant form of life in subsurface ecosystems, including coals, and play vital roles in biogeochemical cycles such as the carbon cycle (Iram et al. 2017). In addition, the synergistic interaction of microbial complexes in
coal seams drives the production of a large proportion (20–40%) of global methane reserves (Thielemann et al. 2004; Faiz and Hendry 2006; Rathi et al. 2019). Therefore, the development of biogenic coalbed methane has gradually attracted more attention, and scholars expect to stimulate the production potential of biogenic methane in coalbeds through various methods, particularly nutrient addition, to improve coalbed methane (CBM) production (Jones et al. 2010; in ’t Zandt et al. 2018).

Researchers have mainly focused on the abundance and activity of methanogenic archaea in addition to microbial diversity under nutrient stimulation (in ’t Zandt et al. 2018; Wang et al. 2019b; Bucha et al. 2020; Pytlak et al. 2020). These methanogenic groups are the drivers of the final step in degrading organic matter into methane in coal seams (Vick et al. 2019). However, little attention has been given to the process of microbial assembly (including profuse and rare taxa) under nutrient stimulation. This knowledge is of great importance for understanding the microbial ecology of coal seams and judging the sustainability of methane production stimulated by nutrients.

Generally, the dominant taxa in microbial community changes have received more attention (Wu et al. 2017). However, microbial taxa with low abundance are often identified as the “rare biosphere”; these taxa represent most of the biodiversity on Earth (Ji et al. 2020), undertake essential ecological functions (Ji et al. 2020), and play vital roles in community function and stability in ecosystems (Jousset et al. 2017). For example, the majority of turnover in community composition was observed in rare taxa in sandy soils (Gobet et al. 2012). These rare microbes also drive anaerobic respiration, such as sulfate reduction (Pester et al. 2010) and respiratory denitrification (Philippot et al. 2013) in anaerobic environments. Thus, understanding the assembly process for profuse and rare microbial taxa is vital for knowledge on microbe-driven biogenic methane production processes in coals.

It is generally believed that deterministic and stochastic processes co-occur and control the aggregation of microbial communities (Chase 2010; Chase and Myers 2011). Traditional niche theory assumes the dominant role of deterministic processes and holds that deterministic factors, including species characteristics, interspecific interactions, and environmental conditions, determine community structure (Chesson 2000; Fargione et al. 2003). In contrast, neutral theory considers that stochastic processes control the aggregation of microbial communities, including birth, death, colonization, extinction, and speciation, which are independent of species characteristics (Chesson 2000; Fargione et al. 2003). The importance of stochastic processes in controlling microbial diversity has received little attention until recently (Zhou et al. 2014; Stegen et al. 2015). Many studies have found that changes in microbial communities can be driven by stochastic processes such as historical contingency, ecological drift, and dispersal limitations (Chase 2010; Oﬁírîju et al. 2010; Zhou et al. 2014; Evans et al. 2017). However, our knowledge of microbial assembly processes in underground environments, particularly in coals and profuse and rare microbial communities in coals under nutrient stimulation. It limits our understanding of the mechanistic responses of coal microbial diversity and community composition to nutrient stimulation.

This study extracted 16S rRNA data on coal sample microbial composition under different treatments from the NCBI database and reanalyzed the assembly process of profuse and rare taxa under nutrient stimulation. This knowledge is of great importance for understanding the microbial ecology of coals and the sustainability of methane production stimulated by nutrients.

Experimental

Materials and Methods

Datasets. Up to and including September 2021, published papers on “coal” and “microbial communities” were sourced through the Web of Science database, the accession numbers of the 16S rRNA gene obtained, and the associated FASTQ files downloaded. 16S rRNA gene data from 59 microbial communities in coals were collected for a meta-analysis, and the effect of nutrient addition on the assembly processes of profuse and rare microbial communities was analyzed. Two types of experiments were considered: 1) 19 coals cultured with nutrients (nutrient group) and 2) 40 in situ coals without treatments (ck group). The detailed sample information is shown in Table I. The base properties of coals have been reported elsewhere (Su et al. 2018; Liu et al. 2019; Wang et al. 2019a), including total carbon (TC, 72.82–87.65% in the ck group and 37.67–91.67% in the nutrient group), total nitrogen (TN, 0.93–1.79% in the ck group and 0.36–1.80% in the nutrient group), total oxygen (TO, 4.53–20.70% in the ck group and 3.47–17.39% in the nutrient group), total hydrogen (TH, 4.09–6.16% in the ck group and 2.58–5.58% in the nutrient group), dry ash-free volatiles (Vdaf, 5.34–32.05% in the ck group and 32.58–91.67% in the nutrient group), air dry ash (Ad, 6.77–25.46% in the ck group and 3.73–20.50% in the nutrient group), air dry moisture (Mad, 1.44–7.50% in the ck group and 3.73–20.50% in the nutrient group), air dry moisture (Mad, 1.44–7.50% in the nutrient group) and fixed carbon (FC, 44.54–82.63% in the ck group and 31.03–80.99% in the nutrient group).
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contents of ammonium, sodium, and phosphate (Davis et al. 2018; Detman et al. 2018; in ’t Zandt et al. 2018; Su et al. 2018; Wang et al. 2019b; Bucha et al. 2020; Pytlak et al. 2020). Thus, nutrient concentration ranges used for the present study were: organic carbon (OC), 0–12 g/l; ammonium (NH$_4^+$), 0–0.34 g/l; sodium (Na$^+$), 0–4.33 g/l; chloride (Cl$^-$), 0–7.05 g/l; potassium (K$^+$), 0–0.67 g/l; phosphate as HPO$_4^{2-}$/H$_2$PO$_4^-$, 0–6.46 g/l; sulfophosphate (SO$_4^{2-}$), 0–2.75 g/l; magnesium (Mg$^{2+}$), 0–0.70 g/l; and calcium (Ca$^{2+}$), 0–0.05 g/l.

Bioinformatics analysis. For the microbial community (bacteria and archaea) analysis, the reads from 16S genes were merged, and the raw sequences were filtered for quality using the QIIME pipeline. The chimeric sequences were identified by the “identify_chimeric_seqs.py” command and removed with the “filter_fasta.py” command.

Table I
Detailed sample information on 59 microbial communities in coals for meta-analysis.

| ID/NCBI accession number | Sites | Primer sequences | Coal rank | Treatment | References |
|--------------------------|-------|------------------|-----------|-----------|------------|
| SRR9312778-SRR9312784    | Erlian Basin | 515F: GTGCCAGCMGCCGCGGG 907R: CCGTCAATTCMTTTRAGTTT | Lignite | (Wang et al. 2019a) |
| SRR6998887                | Moghla | 515F: GTGCCAGCMGCCGCGGTAA 806R: GGACTACHVGGGTWTCTAAT | Bituminous | (Sharma et al. 2019) |
| SRR1695964                | Queensland | 926F: AAACYAAGAATTTGACGG 1392R: ACGGGCGGGTGTGTCR | Bituminous | (Raudsepp et al. 2016) |
| SRR1695967                |          |                  |           |           |            |
| SRR1695971                |          |                  |           |           |            |
| SRR5342611                | Powder River Basin | 341F: CCTACGGGNBGCASCAG 805R: GACTACNVGGGTATCTAATCC | Bituminous | (Davis et al. 2018) |
| SRR8373697-SRR8373705     | Anhui |                  |           |           |            |
| SRR8373722-SRR8373724     |          |                  |           |           |            |
| SRR8373719-SRR8373721     | Guizhou | 338F: ACTTCTACGGAGGGGACGAG 806R: GGACTACHVGGGTWTCTAAT | Bituminous | (Liu et al. 2019) |
| SRR8373696                | Shangsi |                  |           |           |            |
| SRR8373718                |          |                  |           |           |            |
| SRR8373734-SRR8373745     | Shanxi |                  |           |           |            |
| SRR8373746                |          |                  |           |           |            |
| SRR8373747                |          |                  |           |           |            |
| SRR7271165                | Huaibei Coalfield | 515F: GTGCCAGCMGCCGCGGG 907R: CCGTCAATTCMTTTRAGTTT | Bituminous | (Wang et al. 2019b) |
| SRR11128868               | Konin Basin |                  | Lignite | (Bucha et al. 2020) |
| SRR5826886                | New South Wales | 341F: CCTACGGGNBGCCGCAG 785R: GACTACNVGGGTATCTAATCC | Bituminous | (in ’t Zandt et al. 2018) |
| SRR5826889                |          |                  |           |           |            |
| SRR11241403               | Upper Silesian Coal Basin | 515F: GTGCCAGCMGCCGCGGG 907R: CCGTCAATTCMTTTRAGTTT | Bituminous | (Pytlak et al. 2020) |
| SRR5397976                | Konin Basin |                  | Bituminous | (Detman et al. 2018) |
| SRR7422168                | Jiaozuo |                  | Anthracite | (Su et al. 2018) |
| SRR7422169                | Neimeng |                  | Bituminous | (Su et al. 2018) |
| SRR7422170                | Suzhou |                  | Bituminous | (Su et al. 2018) |
| SRR7422171                | Jingcheng | 314F: CCTACGGGNBGCCGCAG 805R: GACTACNVGGGTATCTAATCC | Anthracite | (Su et al. 2018) |
| SRR7422172                | Hebi |                  | Bituminous | (Su et al. 2018) |
| SRR7422173                | Shaq |                  | Bituminous | (Su et al. 2018) |
| SRR7422174                | Liyazhuang |                  | Bituminous | (Su et al. 2018) |
| SRR7422175                | Yima |                  | Bituminous | (Su et al. 2018) |
| SRR7422176                | Pingdingshan |                  | Bituminous | (Su et al. 2018) |
| SRR7422177                | Shoushan |                  | Bituminous | (Su et al. 2018) |
| SRR5342597                | Powder River Basin | 341F: CCTACGGGNBGCCGCAG 805R: GACTACNVGGGTATCTAATCC | Bituminous | (Davis et al. 2018) |
| SRR5342605                |          |                  |           |           |            |
command according to the UCHIME algorithm. The selection and taxonomic assignment of operational taxonomic units (OTUs) were performed based on the SILVA reference data (version 128) at 97% similarity. Reads that did not align to the anticipated region of the reference alignment were removed as chimeras by the UCHIME algorithm. Reads that were classified as "chloroplast," "mitochondria," or "unassigned" were removed.

**Data analysis.** To avoid differences in amplified fragments among different samples, the microbiological analysis was performed at the genus level based on the classification. Bacterial and archaeal genera with relative abundances above 0.1% were considered profuse microbial taxa. Similarly, the bacterial and archaeal genera with a relative abundance of less than 0.1% were considered rare microbial taxa.

Manhattan plots were used to analyze the enrichment of genera based on their taxonomy using the Tutools platform (https://www.cloudtutu.com). The Shannon diversity and Chao1 richness indices were determined based on the relative abundance of profuse and rare genera. In addition, Bray-Curtis dissimilarity was calculated based on a matrix of the relative abundance of profuse and rare genera in the vegan package of Rs 4.1.2. Nonmetric multidimensional scaling (NMDS) was applied based on Bray-Curtis dissimilarity using the vegan metaMDS function. The Wilcoxon test was used to compare differences in microbial diversity and Bray-Curtis dissimilarity. Permutational multivariate analysis of variance (adonis) was used to analyze the difference in profuse and rare microbial compositions based on Bray-Curtis dissimilarity using the adonis functions in vegan. A redundancy analysis (RDA) in Rs was used to identify the factors that were most important to the profuse and rare microbial community compositions using the “rda” and “envfit” functions of the vegan package, allowing for full permutation of the raw data and Monte Carlo tests with 999 permutations using the “permutest” function of the vegan package.

The Raup-Crick index (RCI) and the β nearest taxon index (βNTI) were calculated to determine the assembly processes for profuse and rare microbial taxa. A value of −2 < βNTI < 2 was interpreted as indicating that the turnover of a group of communities was primarily due to stochastic processes, and the turnover of communities was interpreted as governed primarily by probabilistic dispersal when |RCI| > 0.95 (homogenizing dispersal when RCI < −0.95 and dispersal limitation when RCI > 0.95). In addition, turnover between a pair of communities was mainly due to deterministic processes when |βNTI| > 2 (homogeneous selection when βNTI < −2 and variable selection when βNTI > 2). In addition, the communities were driven by undominated processes when |RCI| < 0.95, which mostly involved weak selection, weak dispersal, diversification, and/or drift (Stegen et al. 2015).

Network analysis was used to explore the co-occurrence patterns of profuse and rare microbial taxa. Spearman’s correlations between the relative abundance of genera for profuse and rare microbial groups were considered for a Spearman’s correlation coefficient (p) > 0.55 and a p-value < 0.05. Gephi (v0.9.1) was used to visualize the co-occurrence networks.

**Results**

**The effect of nutrients on the diversity of profuse and rare microbial communities in coals.** The Chao1 richness of profuse and rare genera in the ck group was 1,744 ± 458 and 1,052 ± 261, respectively. Adding nutrients increased the Chao1 richness of the profuse and rare genera by 17,389 ± 2,485 and 6,881 ± 1,565, respectively (Fig. 1a). Nutrients mainly increased the Chao1 richness of the profuse and rare genera in anthracite and bituminous coals (Fig. 1b and 1c). Simultaneously, nutrients can significantly change the microbial community structure of profuse and rare genera. The ordering of the coal samples by NMDS based on profuse and rare genera composition and using Bray-Curtis distances (Fig. 2a and 2b) showed a separation of the coal samples between nutrient and ck groups along the first axis. In addition, we found that nutrient addition changed the composition of profuse and rare microbial communities in coals; i.e., the average Bray-Curtis dissimilarity of community composition in the nutrient group was significantly greater than that in the ck group (Fig. 2c). The effects of nutrients on the compositions of profuse and rare microbial communities also differed in various coal ranks. Nutrients mainly caused shifts in the compositions of profuse and rare microbial communities in bituminous coal (Table SI).

**Nutrient addition changed the profuse and rare microbial communities in coals.** In the coal microbial communities, 116 genera were identified as profuse taxa, which accounted for 86.55% of the total sequence reads. Among them, unclassified_Oxalobacteraceae_ was the most profuse taxon (35.17%); profuse archaea accounted for 13.33% of the total microbial community, and _Methanoseta_ was the most profuse archaeal genus (5.74%). A total of 1637 rare genera accounted for 13.45% of the total sequence reads, of which 0.66% were rare archaea. The addition of nutrients considerably changed the microbial composition in coals (Fig. 3, Tables SII and SIII); many profuse and rare genera belonging to Proteobacteria and Acidobacteria were reduced, whereas those belonging to Euryarchaeota and Firmicutes were increased. The profuse and rare known genera (detected in more than 30% of samples) were analyzed (Fig. 4). Nutrients increased a small number of known profuse
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Fig. 1. a) Chao1 index and b) Shannon index of the abundant and rare genera in coals. The Wilcoxon test was used to compare the differences in microbial diversities.

Fig. 2. a) Ordering of the abundant community compositions at the genus level by nonmetric multidimensional scaling (NMDS) based on the Bray-Curtis dissimilarity index; b) ordering of the rare community compositions at the genus level by NMDS based on the Bray-Curtis distance index; c) difference for Bray-Curtis dissimilarities among nutrient and ck groups. The Wilcoxon test was used to compare the differences in Bray-Curtis dissimilarities.
and rare genera such as the methanogenic profuse archaea genera *Methanoseta* and *Methanobacterium* and the profuse bacteria genera *Tissierella, Soehnengenia, Bacillus*, and *Clostridium*. Nutrients also increased the rare bacteria genera *Planctomycetes, Gemmata, Lysinibacillus*, and *Proteiniclasticum* in bituminous coals.

The redundancy analysis (RDA) showed that coal characteristics, including TC, TN, TO, TH, Vdaf, Ad, and Mad, strongly affect the compositions of profuse and rare microbes in coals without nutrients (Fig. 5a and 5b). In the coal samples with added nutrients, the factors affecting the compositions of profuse and rare microbes were reduced (Fig. 5c and 5d). TN and TO affected the composition of profuse microbes, and TC and TO affected the composition of rare microbes. In addition, the organic carbon (OC) and ammonium ion (NH$_4^+$) contents in the nutrients were important factors affecting the compositions of profuse and rare microbes at the phylum level (Fig. 5e and 5f). Herein, an increase in NH$_4^+$ may have an effect on the Euryarchaeota phylum in profuse and rare taxa, and an increase in OC could affect the Firmicutes phylum in profuse and rare taxa.
Fig. 4. Heatmap for a) the profuse and b) rare known genera (detected in more than 30% of samples).
Assembly processes for profuse and rare microbial communities under nutrient stimulation. RCI and βNTI were used to quantify the deterministic and stochastic assembly of microbial communities in profuse and rare communities, most of which were driven by stochastic processes. The relative contribution of stochastic processes (particularly dispersal limitation) to the nutrient group was higher than that of the ck group. The relative contribution of stochastic processes (mainly undominated) to the nutrient group in rare communities was lower than that of the ck group, whereas the dispersal limitation and variable selection...
in the nutrient group were significantly increased (Fig. 6, Tables SIV–SVII).

**Interaction network between profuse and rare microbial communities under nutrient stimulation.** Based on Spearman’s correlation coefficient, the community co-occurrence relationship network of profuse and rare genera was constructed, and the co-occurrence patterns of different treatments were evaluated (Fig. 7, Tables SVIII–SXII). The results showed that the number of connections was 1,350 and 1,443 in the nutrient and ck groups, respectively. Notably, there were more positive connections (profuse-profuse, rare-rare, and profuse-rare) than negative connections, whereas the number of negative connections in the treatment group was lower than in the ck group. In addition, changes were observed in the network relationship between

![Fig. 6. Deterministic and stochastic processes in community assembly.](image)

(a) βNTI index in abundant and rare groups; b) the relative influences of deterministic and stochastic assembly processes in shaping abundant and rare groups. Detailed information is shown in Tables SIV–SVII.

![Fig. 7. Co-occurrence networks of abundant and rare groups in coals based on the correlation analysis.](image)

(a) profuse (b) ck

Pro – Proteobacteria, Act – Actinobacteria, Bact – Bacteroidetes, Fir – Firmicutes, Ver – Verrucomicrobia, Pla – Planctomycetes, Spi – Spirochaetes, Acid – Acidobacteria, Eury – Euryarchaeota, Cren – Crenarchaeota, Gem – Gemmatimonadetes, Chl – Chloroflexi, and Syn – Synergistetes.
the nutrient and ck groups. The hub microorganisms in the microbial co-occurrence relationship network of the ck group were mainly profuse groups, including Proteobacteria and Actinobacteria. In addition, hub microorganisms can be intuitively divided into two groups in the symbiotic network of nutrients: one was mainly composed of bacteria, and the other was mainly composed of bacteria and archaea. However, rare genera mainly belonging to Firmicutes, Bacteroides, and Euryarchaeota also formed a significant constituent of the hub microorganisms.

**Discussion**

This study assessed the assembly processes of profuse and rare microbial communities in coals under nutrient (such as organic carbon, and nitrogen) stimulation. The analyzed taxonomic unit was used at the genus level to avoid OTU sequence differences caused by various amplified primers. In microbial research on coal seams, including these referenced studies, the most significant attention has been given to groups related to the formation of biogenic coalbed methane (Szanfranek-Nakonieczna et al. 2018; Plyatsuk et al. 2020). These studies are critical hubs for applying microbial knowledge to practical production.

Coal seams are important habitats for the co-existence of underground microbial communities, and improving the activity of functional microorganisms also requires consideration of the relationships between multiple microbial groups. Coal seams possess many bacterial taxa, including Firmicutes, Spirochetes, Bacteroidetes, and Proteobacteria (Dawson et al. 2012; Chen et al. 2018). This study found that nutrients have a selective stimulating effect on profuse and rare groups. In addition, these investigated studies highlighted that nutrient addition can effectively accelerate CBM production and that biomethane production is closely related to coal decomposition (in ’t Zandt et al. 2018; Plyatsuk et al. 2020). Therefore, the focus of this study was to identify the core profuse and rare genera stimulated by nutrients, which have potential value in the study of biological CBM.

The main components of nutrients added in the surveyed studies were similar (Davis et al. 2018; Detman et al. 2018; in ’t Zandt et al. 2018; Su et al. 2018; Wang et al. 2019b; Bucha et al. 2020; Plytlak et al. 2020), mainly including organic carbon such as tryptone and yeast, ammonia salts, and potassium and sodium salts. The changes in profuse and rare taxa caused by adding nutrients are different for different coal ranks. Most studies also support the finding that the microbial community structure of coals differs among various ranks (Su et al. 2018; Liu et al. 2019). In this study, the shifts in the composition of profuse and rare microbial communities caused by nutrients mainly occurred in bituminous and anthracite coals. This result may have been overlooked in previous studies (Davis et al. 2018; Detman et al. 2018; in ’t Zandt et al. 2018; Su et al. 2018; Wang et al. 2019b; Bucha et al. 2020; Pytlak et al. 2020). In addition, the Chao1 richness for lignites was higher when nutrients were added to both profuse and rare genera. This may be related to the selective stimulation of nutrients (Bucha et al. 2020). For example, this study found that Euryarchaeota phylum in profuse and rare taxa may prefer increased NH$_4^+$, and Firmicutes phylum in profuse and rare taxa may prefer increased organic carbon. Increased organic carbon stimulated the development of the profuse and rare genera associated with Firmicutes in bituminous coals, including the profuse bacterial genera Tissierella_Soehngenia, Bacillus, Clostridium, and the rare bacterial genera Lysinibacillus and Proteiniclasticum.

Firmicutes were often detected in coal seams with high microbial abundance (Midgley et al. 2010; Wang et al. 2019b; Bucha et al. 2020), which played a vital role in coal decomposition. These groups were the main active heterotrophic and syntrophic bacterial consortia and dominated kerogen degradation, and the abundance of these fermentation bacteria can even restrict the generation of coal biomethane (Meslé et al. 2013). In addition, it was key that nutrients increased the methanogenic archaea, particularly the profuse archaea genera Methanoseta and Methanobacterium, in a study covering multiple research areas. The activation of these methanogens directly affects the yield increase of biogenic coalbed methane (Lupton et al. 2020; Pytlak et al. 2020). Coal quality properties can directly restrict microbial community structure (Meyer et al. 2018; Plyatsuk et al. 2020), which was also evident in this study (Fig. 5a and 5b). However, not all coal quality properties can influence profuse and rare microbial communities under nutrient stimulation (Fig. 5c and 5d), and the main components of nutrients added in the surveyed studies restricted the assembly process of profuse and rare microbial communities (Fig. 5e and 5f). Little difference in microbial coal structures of the ck group was found among multiple regions, indicating that the microbial coal structures in different areas have high similarity at the genus level. Nutrients stimulated the profuse and rare microbial Chao1 richness and affected community structure, and the average Bray-Curtis dissimilarity of community composition in the nutrient group was significantly greater than that in the ck group. This result indicated that nutrient deficiency, particularly in available organic carbon, NH$_4^+$, and Na$, may be an important factor limiting the development of microorganisms in coal seams. Once the nutrients in coal seams are supplemented, the change in the
microbial community may have undergone spatial niche partitioning (Vick et al. 2019).

The positive interaction in a co-occurrence network was mainly regarded as cooperation (Ju et al. 2014). In this study, there were more positive connections (profuse-profuse, rare-rare, and profuse-rare) than negative connections, and the number of negative connections in the treatment group was lower than that in the control group. In coals, the interactions between microorganisms might be an important factor in maintaining the stability of underground communities (Abreu and Taga 2016). Frequent cooperation within profuse and/or rare taxa may contribute to community resilience in changing environments because of the buffering function of the interaction network among microbes against environmental disturbances (Konopka et al. 2015). In addition, nutrients can enhance the interaction between rare taxa, including archaea (particularly methanogenic archaea) and bacteria with profuse taxa, which may be another potential factor influencing yield enhancement of biogenic coalbed methane. The process of biological methane production in coals requires the collective action of microorganisms involving at least three major metabolic groups, including hydrolyzing and fermenting bacteria, hydrogen- and acetogen-producing bacteria, and methanogenic archaea (Wang et al. 2018; Vick et al. 2019). To our knowledge, bacteria attach to the surface of the coal seams (Vick et al. 2016; McLeish et al. 2021) and drive the anaerobic fermentation of these organic materials in coal seams (Strapoć et al. 2008; Penner et al. 2010). Methanogens also require bacterial partners to depolymerize and oxidize complex organic molecules into simple fermentation products (CO$_2$, H$_2$, acetate, formate, or other compounds). For methanogenic archaea in coal seams, symbiosis and aggregation with bacteria may be the main factor impacting their survival and sustainable methane production in coal seams (He et al. 2020).

Stochastic processes drive the most rich and rare communities in coals. Similarly, in many cases, microbial community changes may occur due to stochastic processes in communities via historical contingency (such as priority effects), ecological drift, and/or dispersal limitation (Chase 2010; Ofiţeru et al. 2010; Zhou et al. 2014; Evans et al. 2017). In previous experiments adding nutrients directly affected the carbon and nitrogen in the coal environments and caused changes in the microbial community. Thus, intuitively, the microbial community structure governed by environmental conditions such as the nutrients in this study should be referred to as deterministic processes (Fargione et al. 2003). It is despite the nutrient group increasing dispersal limitation (a stochastic process) for profuse and rare microbial community assembly and only increasing the variable selection (a deterministic process) for rare microbial community assembly. A previous study considered that stochastic processes could play more important roles than the functional differences of species in community pattern generation (Zhou and Ning 2017). The samples selected for this study came from coal seams in different regions, and dispersal limitation is the most important factor shaping large-scale biogeographic patterns (Hanson et al. 2012; Meyer et al. 2018). In addition, the increased contribution of variable selection by nutrient stimulation in the rare community suggested that heterogeneous abiotic and biotic factors, particularly chemical properties, can impose selective solid pressure by filtering rare species (Li et al. 2021) and drive changes in rare community compositions (Bottos et al. 2018). Nutrients have been demonstrated to drive a highly deterministic process for rare groups in various ecosystems and influence the diversity of rare microbial communities (He et al. 2018; Guo et al. 2020; Cao et al. 2021; San Roman and Wagner 2021).

In conclusion, this study is the first to focus on the assembly processes of profuse and rare microbial communities in coals under nutrient stimulation and showed that dispersal limitation played an important role in changing the profuse and rare microbial communities in coals. Nutrient stimulation intensified the relative contribution of dispersal limitation for both profuse and rare microbial community assemblies. It is the most crucial reason for shifts in microbial community diversity. In addition, nutrients increased the variable selection for rare microbial community assembly and enhanced the role of rare groups in the microbial co-occurrence network. Overall, this study strengthened our knowledge of the mechanistic response of coal microbial diversity and community composition to nutrient stimulation.

### Availability of data and material

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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### Author contributions

YZ, XC and XY conducted the bulk of the data analysis for the study and coauthored the manuscript. YL provided the funding for the study and was involved in the conceptualization of the study as well as assisting in writing of the manuscript. All authors read and approved the final manuscript.
Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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