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## Distinct succession patterns of abundant and rare bacteria in temporal microcosms with pollutants<sup>☆</sup>

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## ABSTRACT

Elucidating the driving forces behind the temporal dynamics of abundant and rare microbes is essential for understanding the assembly and succession of microbial communities. Here, we explored the successional trajectories and mechanisms of abundant and rare bacteria via soil-enrichment subcultures in response to various pollutants (phenanthrene, *n*-octadecane, and CdCl<sub>2</sub>) using time-series Illumina sequencing datasets. The results reveal different successional patterns of abundant and rare sub-communities in eighty pollutant-degrading consortia and two original soil samples. A temporal decrease in  $\alpha$ -diversity and high turnover rate for  $\beta$ -diversity indicate that deterministic processes are the main drivers of the succession of the abundant sub-community; however, the high cumulative species richness indicates that stochastic processes drive the succession of the rare sub-community. A functional prediction showed that abundant bacteria contribute primary functions to the pollutant-degrading consortia, such as amino acid metabolism, cellular responses to stress, and hydrocarbon degradation. Meanwhile, rare bacteria contribute a substantial fraction of auxiliary functions, such as carbohydrate-active enzymes, fermentation, and homoacetogenesis, which indicates their roles as a source of functional diversity. Our study suggests that the temporal succession of microbes in polluted microcosms is mainly associated with abundant bacteria rather than the high proportion of rare taxa. The major forces (i.e., stochastic or deterministic processes) driving microbial succession could be dependent on the low- or high-abundance community members in temporal microcosms with pollutants.

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### 1. Introduction

The investigation of community successional dynamics has been a central theme of ecological research; however, it has mainly been focused on animal and plant communities. Microbial communities have a high taxonomic and metabolic diversity (Schloss and Handelsman, 2007; Prosser et al., 2007), and perform important ecological functions (Falkowski et al., 2008). Studies focused on microbial succession have become more common with the development of high-throughput sequencing (Gonzalez et al., 2012), and to this point have mainly explored the dynamics of microbial communities in response to environmental changes in diverse systems (Kazemi et al., 2016; Brown and Jumpponen, 2014;

Mikkonen et al., 2011; Jiao et al., 2016a,b). The temporal variability in microbial communities is directional according to environmental conditions and is consistent across similar environments (Shade et al., 2013). However, elucidating the successional trajectories and mechanisms of microbial communities remains a major challenge.

There is an ongoing debate regarding how stochastic and deterministic processes influence microbial community assembly over time (Dini-Andreote et al., 2015). Deterministic processes are associated with ecological selection, where abiotic and biotic factors determine the community assembly (Vellend, 2010). The succession of communities driven by deterministic processes is directional and converges towards a stable state, with decreasing dissimilarities amongst patches and successional rates over time (Anderson, 2007). In contrast, stochastic processes reflect random changes in the relative abundance of species (i.e., ecological drift) and are not associated with environmentally derived fitness (Chase and Myers, 2011; Hubbell, 2001). Communities structured by stochastic processes should remain divergent, with  $\beta$ -diversity being

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maintained or even increasing over time. The lack of convergence can be related to stochastic events, such as stochastic dispersal, colonization, and local extinction (Chase, 2003). Using time-series datasets, it is possible to estimate the directionality, convergence, and rate of successional change to provide insights into community assembly.

In nature, most microbial communities are comprised of a large number of species, the distribution of which is often unbalanced. A few of these species are abundant; meanwhile, a large number of species, often called the “rare biosphere”, are represented by only a few individuals (Sogin et al., 2006; Pedrós-Alió, 2012). The rare biosphere is typically defined as the long tail in the rank-abundance curves used to depict diversity (Pedrós-Alió, 2012). Explorations of the rare microbial biosphere have occurred in the Arctic Ocean (Galand et al., 2009) and coastal Antarctic lakes (Logares et al., 2014), as well as coastal marine (Gobet et al., 2012) and salt marsh sediments (Bowen et al., 2012). These studies have shown that abundant taxa have a log-normal species-abundance distribution and rare taxa have a log-series species-abundance distribution (Galand et al., 2009). Additionally, the dispersal probability of rare bacteria is limited compared with that of abundant taxa (Nemergut et al., 2011). Typically, the abundant species occupy core niche positions and are strongly impacted by deterministic filtering (Umaña et al., 2015); meanwhile, rare species are typically impacted more by demographic stochasticity due to their small population sizes (Orrock and Watling, 2010). However, previous studies have typically focused on the biogeography of communities and have not examined the role of abundant and rare species in the context of successional trajectories; this lack of knowledge hinders our ability to fully understand the temporal mechanisms of microbial community assembly.

Rare taxa provide a nearly limitless reservoir of genetic and functional diversity, and serve to ensure the maintenance of microbial diversity (Jones and Lennon, 2010). They may also hold significant roles as keystone species in microbial consortia (Sauret et al., 2014) or in maintaining biogeochemical functions (Leininger et al., 2006; Sjöstedt et al., 2012). When “passing through” favorable conditions, some rare microorganisms can become abundant community members (Shade et al., 2014). A study in the Western English Channel showed that rare microorganisms were persistent over time and some occasionally bloomed (Caporaso et al., 2012). Some rare community members are hypothesized to be less important for the community; for example most members of the rare biosphere in coastal sands did not become abundant at any point over the course of a year (Gobet et al., 2012). The rare archaeal biosphere shows seasonal patterns similar to those of abundant microbes and cannot be considered a seed bank (Hugoni et al., 2013). Bio-prospecting for members of the rare biosphere may provide novel products and processes (Reid and Buckley, 2011). It is clear that we still lack a complete understanding of the dynamics of abundant and rare microorganisms, as well as the forces driving their general incidence.

Environmental contamination is a global problem, and most of the contaminated sites are characterized by the presence of complex pollutants (González et al., 2011). Microbial remediation is an effective option for environment cleanup, and learning the key organisms in the procedures of bioremediation is relevant to the development of optimal in situ bioremediation strategies (Liebeg and Cutright, 1999; Abed et al., 2002). Most relevant studies have focused on the microbial assembly and dynamics during hydrocarbon or oil degradation via next generation sequencing (Liu et al., 2016; Bacosa et al., 2015, 2016; Severin et al., 2016; Kimes et al., 2013). These works have concentrated on abundant bacterial groups in hydrocarbon degradation; however, the significance of the rare biosphere remains obscure. On the other hand, the

temporal dynamics of microbial communities in natural environments are often influenced by multiple environmental factors simultaneously, which makes it difficult to determine the effect of individual factors on microbial succession (Jiao et al., 2016a,b). Microcosms are a closed system with tightly controlled conditions, and are suitable for estimating the influence of single environmental factors on microbial succession (Jiao et al., 2016a,b; Viñas et al., 2005). Our previous studies indicated that bacterial succession in microcosms is influenced by the directionality of pollutants, and exhibits temporal decays in community similarity (Jiao et al., 2016a,b). Based on the existing time-series datasets from temporal microcosms, we aimed to reveal the successional trajectories and mechanisms of abundant and rare community members. The datasets examined the response of the microbial community composition to various pollutants via high-throughput sequencing of the 16S rRNA gene. Specifically, we addressed the following questions: (Schloss and Handelsman, 2007): do abundant and rare taxa show distinct succession patterns and converge towards a similar community state over time in microcosms (Prosser et al., 2007); what are the main forces (i.e., stochastic or deterministic) driving the microbial succession of abundant and rare sub-communities, and (Falkowski et al., 2008); what are the potential functions of abundant and rare sub-communities?

## 2. Materials and methods

### 2.1. Datasets

The microbial time series used in this study have been previously published (Jiao et al., 2016a,b). The original soils were collected from two oil refineries (108°46′09″ E and 34°21′35″ N; 108°50′10″ E and 37°35′35″ N) in the northwestern area of China. The sampling sites have been continuously contaminated with crude oil for the last 20 years. The total petroleum hydrocarbons were as high as  $5.69 \pm 0.01$  g kg<sup>-1</sup> of dry soil, and the physico-chemical properties of the two soil samples were markedly different (see Table S1 in supplementary material).

Illumina V4-V5 16S rRNA datasets were obtained from eighty pollutant-degrading consortia (Jiao et al., 2016a,b); these were generated by ten batches of soil-enrichment microcosms from two original soils corresponding to four treatments: (Schloss and Handelsman, 2007): 500 mg/L phenanthrene (PHE) (Prosser et al., 2007); 500 mg/L *n*-octadecane (C18) (Falkowski et al., 2008); 250 mg/L phenanthrene and 250 mg/L *n*-octadecane (PC), and (Gonzalez et al., 2012); 250 mg/L phenanthrene, 250 mg/L *n*-octadecane, and 50 mg/L CdCl<sub>2</sub> (PCC). Successive subcultures were performed at 10-day intervals over a 100-day incubation period. The soil-enrichment microcosms were performed in triplicate for all of the treatments. Cells from the consortium of each subculture were collected for DNA extraction.

Both phenanthrene and *n*-octadecane are components of petroleum and can be used by bacteria as carbon source in these microcosms. Cadmium (Cd) was used because it is a heavy metal that is highly toxic to animals, plants, and microorganisms (Thavamani et al., 2012). The pollutant concentrations used in this experiment are common in oil-contaminated soils, as reported in previous studies (Thavamani et al., 2012; Hassanshahian et al., 2012; Yang et al., 2015). The tightly regulated microcosms were used as a model to understand the complex interactions between environmental factors and microbial communities, and ensured that succession was caused by the pollutants (Jiao et al., 2016a,b).

### 2.2. Sequence analyses

The bacterial community in each of eighty pollutant-degrading

consortia (two original soils  $\times$  ten batches  $\times$  four treatments) and two original soils samples was characterized by sequencing a portion of V4–V5 hypervariable region of the 16S rRNA gene using the primers 515F (5'-GTG CCA GCM GCC GCG GTA A-3') and 926R (5'-CCG YCA ATT YMT TTR AGT TT-3') (Yu et al., 2015) on an Illumina MiSeq (250-bp paired-end reads). The data were derived from PCR amplification of environmental DNA using primer pairs designed to amplify the gene region from all, or nearly all, known bacterial taxa. The acquired sequences were quality filtered (Caporaso et al., 2011) and chimeras were removed in USEARCH using the UCHIME algorithm (Edgar et al., 2011). The high quality sequences from all samples were clustered into OTUs at 97% sequence similarity using the "uclust" model, which searches and clusters orders of magnitude faster than BLAST. The OTUs that contained  $<2$  reads were not used to avoid possible biases. The representative sequences for each OTU were assigned to taxonomic groups using the RDP classifier at an 80% confidence threshold.

### 2.3. Data analyses

The same number of sequences from each sample (Chase, 2003) was used to avoid potential side effects based on sample size. Rarefaction curves for each individual samples were performed to estimate the sequence coverage. In this study, we defined OTUs as "abundant" when they had relative abundances above 0.1% of total sequences or "rare" when their abundances were below 0.1% (Alonso-Saez et al., 2015; Campbell et al., 2011). OTU richness and Shannon index were calculated using three iterations (step size = 100 sequences per sample) for analysis of  $\alpha$ -diversity. The Bray–Curtis similarity matrix is considered to be one of the most robust similarity coefficients, widely used and appropriate for microbial ecology (Kent, 2011), and was used to analyze the relative abundance of bacterial OTUs. A NMDS ordination was used to investigate differences between the whole, abundant, and rare bacterial communities. Mantel's test was used to calculate the correlations between the similarity matrices of the whole, abundant, and rare bacterial communities; this test also determines significance.

For each treatment, species-time relationships (STRs) were constructed based on the degree of richness using the moving window (P White et al., 2006). STRs mainly focus on changes in alpha-diversity and species richness over time (Rosenzweig, 1995; Preston, 1960). Briefly, each time series was partitioned into as many subsets (windows) as possible based on the number of observations and the STRs model (i.e., the power law relationship between time and richness) was fitted for each window. We estimated the STRs for the whole, abundant, and rare bacterial communities. Changes in  $\beta$ -diversity between adjacent batches along the succession for the whole, abundant and rare bacterial communities were calculated using the Bray–Curtis distance matrices.

To investigate the potential functions of abundant and rare taxa, 16S rRNA gene amplicon data were analyzed using Tax4Fun to predict functional capabilities of bacterial communities (Aßhauer et al., 2015). Identified microbial gene families (specified by KEGG Orthology groups) were screened to the FOAM database and grouped into different FOAM levels (Prestat et al., 2014). To show the functional differences between abundant and rare taxa, we combined the FOAM level 1 matrices of the abundant and rare sub-communities and performed redundancy analysis by constraining the category of abundant and rare samples. The functional differences in FOAM level 1 and level 2 between the abundant and rare sub-communities were estimated by Wilcoxon rank-sum tests (Wilcoxon et al., 1970). The functional predictions made in this work are therefore considered only as an indication of the functional potential held in each sub-community and not the ground-

truth. We are aware that prediction approaches cannot replace whole metagenome or metatranscriptome profiling.

All statistical analyses were performed in the R environment (<http://www.r-project.org>) using the vegan (Oksanen and Blanchet, 2015), gplots (Warnes et al., 2009), agricolae (De Mendiburu, 2014), car (Fox and Weisberg, 2010), or Tax4Fun (Aßhauer et al., 2015) packages.

## 3. Results

### 3.1. Distributions of abundant and rare bacterial taxa in the pollutant-degrading consortia

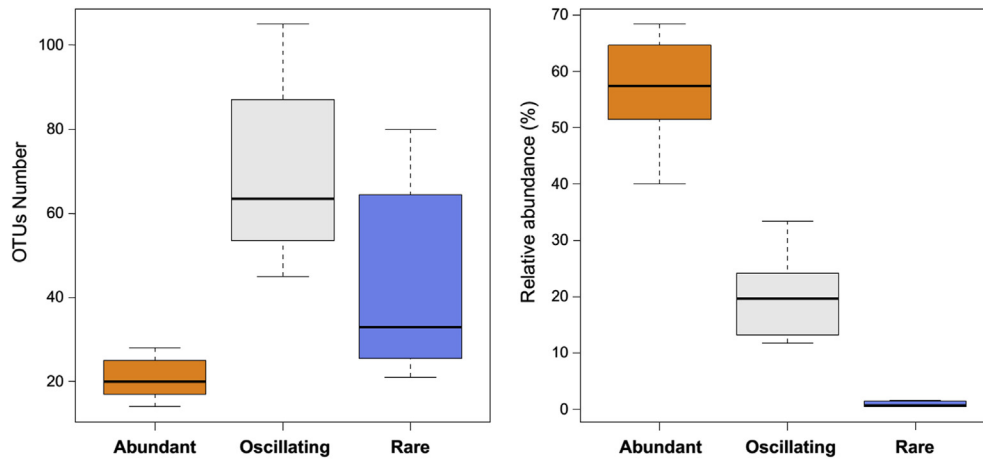
After the quality filtering and the removal of chimeric sequences, 3,281,843 high quality sequences from the entire sequencing dataset of all the eighty microcosm consortia and the two original soil samples were clustered into 41,354 operational taxonomic units (OTUs, 97% similarity) (see Table S2 in supplementary material). The number of sequences per sample ( $n = 82$ ) ranged from 70,838 to 15,006. Rarefaction curves suggest that the majority of the bacterial taxa were recovered (see Fig. S1 in supplementary material). The abundant taxa generally accounted for a significantly lower proportion of OTUs (mean = 2.8%) and a larger proportion of average relative abundance (mean = 80.5%) in each sample compared with the rare taxa (mean = 97.2% and 19.5%, respectively).

To explore persistent bacterial taxa, any OTUs present in ten consortia samples from each treatment were denoted as generalists. There were between 86 and 109 (average proportion: 5.6%) generalist OTUs in each treatment, with average relative abundances between 70.2 and 85.5%; the abundance of the generalist OTUs significantly increased with time (see Fig. S2 in supplementary material). Among the generalists, there were between 14 and 28 abundant taxa and 21–80 rare taxa (Fig. 1); this demonstrates their persistence during the enrichments. These generalist and abundant taxa accounted for 56.9% of sequences; meanwhile, the generalist and rare taxa accounted for only 0.9% of sequences. We also estimated the local occupancy of abundant and rare taxa across ten batches of each microcosm consortia (see Fig. S3 in supplementary material). The number of rare taxa OTUs decreased with increasing local occupancies; meanwhile, the number of abundant taxa OTUs did not regularly change with local occupancies. Specifically, we observed that the number of abundant taxa in ten batches consortia were more than in nine batches consortia for all treatments. This indicates that the distribution patterns of abundant and rare taxa were distinct.

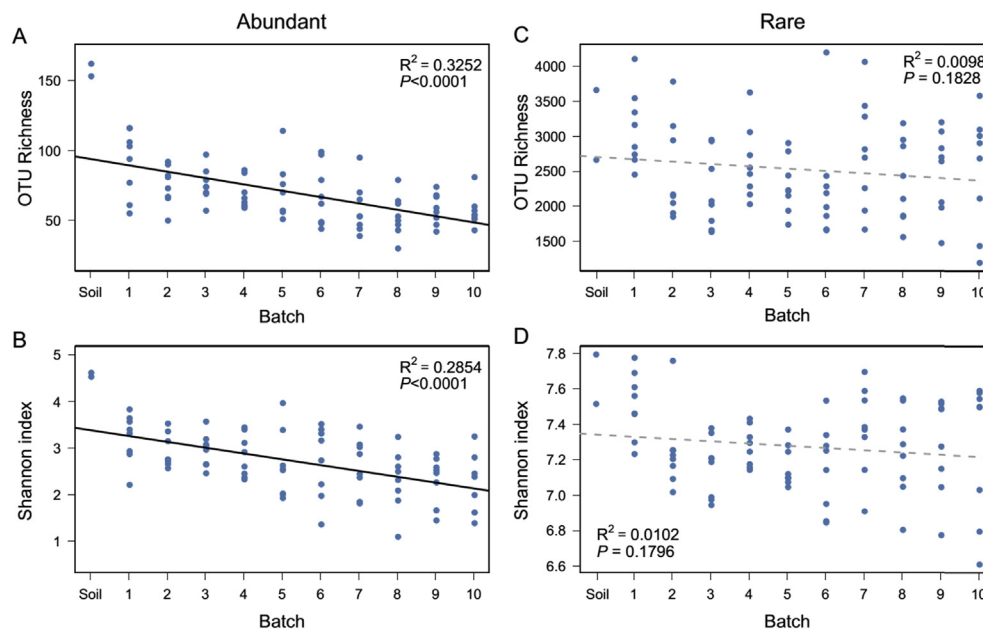
The composition of the abundant and rare sub-communities differed: *Gammaproteobacteria*, *Actinobacteria*, *Spartobacteria*, and *Planctomycetia* were most prevalent abundant taxa, and; *Sphingobacteriia*, *Alphaproteobacteria*, *Anaerolineae*, and *Bacilli* were most prevalent rare taxa (see Fig. S4 in supplementary material). The rare taxa contained more taxonomic groups than the abundant taxa.

### 3.2. Succession of abundant vs. rare sub-communities

The  $\alpha$ -diversity of the abundant taxa significantly decreased with time (Fig. 2A and B); meanwhile, the  $\alpha$ -diversity of the rare taxa was not correlated with time (Fig. 2C and D). The rare sub-community had higher OTU richness and Shannon–Weiner values than the abundant sub-community ( $P < 0.01$ ; Wilcoxon rank-sum). This suggests that rare biosphere contained a more diverse taxonomy. During enrichment, the proportion of OTUs and the relative abundance of the rare taxa significantly increased and decreased, respectively (see Fig. S5 in supplementary material); the opposite trends were observed for the abundant taxa (see Fig. S6 in



**Fig. 1.** The number and relative abundance of the generalist operational taxonomic units (OTUs) categorized into: always abundant (i.e., >0.1% abundance in all batches), always rare (i.e., <0.1% in all batches), or oscillating (i.e., not falling in the other categories) for eighty pollutant-degrading consortia and two original soils samples.



**Fig. 2.** Changes in  $\alpha$ -diversity of the abundant (A, B) and rare taxa (C, D) between the original soils and the successive batches. Linear regressions of operational taxonomic unit (OTUs) richness (A, C) and Shannon index (B, D).

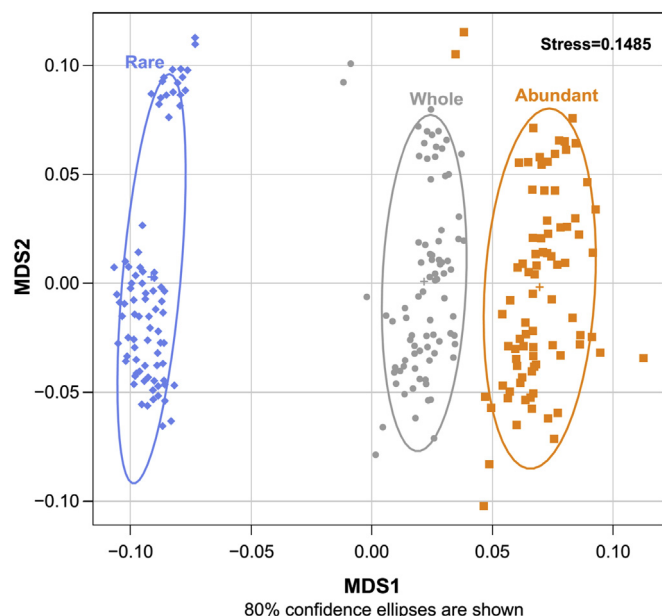
supplementary material). This indicates that a decreasing number of abundant taxa were enriched and dominated the consortia during enrichment.

Nonmetric dimensional scaling (NMDS) revealed that the structure of the abundant bacterial sub-community was more similar to the overall (i.e., whole) community than that of the rare sub-community (Fig. 3); this was confirmed by mantel tests (abundant vs. whole:  $r = 0.9934$ ,  $P < 0.01$ ; rare vs. whole:  $r = 0.7629$ ,  $P < 0.01$ ). The  $\beta$ -diversity was calculated based on Bray-Curtis distance for different sub-communities. The results showed that the  $\beta$ -diversity of the rare sub-community was significantly higher than that of the abundant sub-community. The whole community had the lowest  $\beta$ -diversity (see Fig. S7 in supplementary material).

The accumulation of Species richness was estimated by species-time relationships (STRs). The STRs were significant for different sub-communities of all microcosm consortia ( $P < 0.05$ ), indicating

an increase in the cumulative species richness over time (Table 1). A higher STRs exponent means that a higher number of newly introduced taxa are present. For each treatment, the STR exponents of the rare sub-community were higher than those of the abundant sub-community; they were also marginally higher than those of the whole community. This might indicate that rare taxa contributed most of species accumulation to the whole community.

Moreover, plotting the changes in community structure from one batch to the next revealed that the turnover of different sub-communities significantly decreased ( $P < 0.001$ ) with time (Fig. 4). The slope of temporal turnover ( $w$ ) of the abundant sub-community was steeper than that of the rare sub-community, which indicates a more stable community structure over time for the abundant sub-community. Additionally, the slope of temporal turnover ( $w$ ) of the whole community was closer to that of the abundant sub-community than the rare sub-community, suggesting a similar succession rate between the whole and abundant sub-



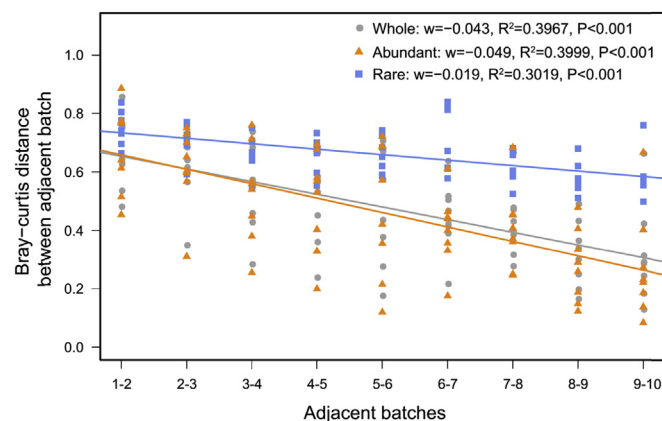
**Fig. 3.** Nonmetric multidimensional scaling ordination for bacterial communities from eighty pollutant-degrading consortia and two original soils samples. Whole: all bacterial taxa; Abundant: abundant taxa, and; Rare: rare taxa.

communities.

To further explore the succession of abundant and rare sub-communities, the temporal dynamics of the top five most abundant phyla were estimated for different sub-communities (see Fig. S8 in supplementary material). Based on our previous study (Jiao et al., 2016a,b), the ten-stage enrichment process can be divided into three phases: I (subcultures 1–3), II (subcultures 4–7), and III (subcultures 8–10). We found similar dynamics of the phyla in the abundant and whole communities, which differed from the dynamics of the phyla in the rare sub-community. This might suggest that the abundant sub-community was the largest driver of succession and temporary turnover for the whole community.

### 3.3. Potential functions of abundant vs. rare taxa

A functional ontology provided by FOAM (Functional Ontology Assignments for Metagenomes) was used to classify gene functions relevant to environmental microorganisms. Based on FOAM level 1, the abundant and rare taxa formed distinct clusters in a redundancy analysis (RDA), suggesting that they possessed different functional profiles (Fig. 5A). The functions related to amino acid metabolism, cellular response to stress, hydrocarbon degradation, and fatty acid oxidation were more prevalent in the abundant taxa; meanwhile, carbohydrate-active enzymes, fermentation, homoacetogenesis, methylotrophy, Embden Meyerhof–Parnos, and gluconeogenesis were more prevalent in the rare taxa. These differences were significant between the abundant and rare taxa



**Fig. 4.** Changes in  $\beta$ -diversity, as well as the succession of the whole, abundant, and rare bacterial communities. Variations in community dissimilarity were calculated with Bray-Curtis distance between two adjacent batches. Whole: all bacterial taxa; Abundant: abundant taxa, and; Rare: rare taxa, and; w: the slope of regression line.

( $P < 0.05$ , Wilcoxon rank-sum test) (Fig. 5B).

To further investigate the functions associated with environmental pollution, we selected a functional subset (FOAM level 2) of cellular response to stress and hydrocarbon degradation, and examined the differences between the abundant and rare sub-communities (see Fig. S9 in supplementary material). These traits, including cellular response to stress, response to oxidative stress, polycyclic aromatic hydrocarbon degradation, and aromatic carboxylic acid degradation, were significantly more prevalent in the abundant sub-community. The results indicate that primary features associated with environmental pollution and nutrition metabolism were more active in the abundant taxa compared with the rare taxa.

To explore the functional succession of the abundant and rare sub-communities, we estimated the dynamics of functional traits based on FOAM level 2 for different sub-communities. The Spearman correlations of every functional trait were calculated between the abundant and rare sub-communities, as well as with the whole community (see Fig. S10 in supplementary material). The proportions of functional traits with significant correlations ( $P < 0.01$ ), as well as the correlation coefficients, were larger in the abundant sub-community compared with the rare sub-community; this suggests that the abundant sub-community was more similar to the whole community in regards to functional succession.

## 4. Discussion

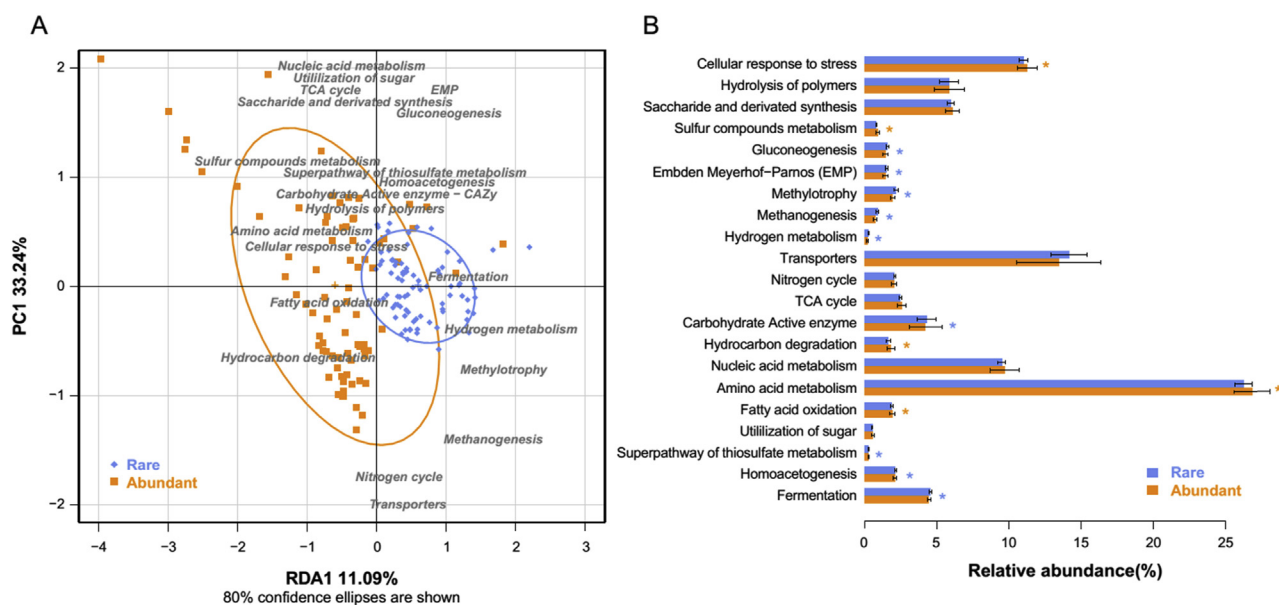
Revealing the mechanisms of microbial succession is critical for understanding the assembly of microbial communities, which is a central issue in microbial ecology (Shade et al., 2013). Furthermore, elucidating the temporal dynamics of abundant and rare microbes is helpful in understanding successional trajectories, convergence,

**Table 1**

The exponents of species-time relationships for the whole, abundant, and rare bacterial communities in different consortia.

Community	J-C18	J-PHE	J-PC	J-PCC	X-C18	X-PHE	X-PC	X-PCC
Whole	0.7903	0.7607	0.7983	0.7984	0.7612	0.7157	0.7701	0.7454
Abundant	0.246	0.4103	0.3687	0.4344	0.4009	0.3096	0.3794	0.2596
Rare	0.799	0.7683	0.8053	0.8042	0.7672	0.7216	0.7857	0.7514

"J" means consortia enriched from the soils of 108°50'10" E and 37°35'35" N; "X" means consortia enriched from the soils of 108°46'09" E and 34°21'35" N; C18 = 500 mg l<sup>-1</sup> n-octadecane; PHE = 500 mg l<sup>-1</sup> phenanthrene; PC = 250 mg l<sup>-1</sup> phenanthrene +250 mg l<sup>-1</sup> n-octadecane; and PCC = 250 mg l<sup>-1</sup> phenanthrene +250 mg l<sup>-1</sup> n-octadecane + 50 mg l<sup>-1</sup> CdCl<sub>2</sub>.



**Fig. 5.** The differences in potential functions of abundant and rare taxa in temporal microcosms. Redundancy analysis performed on FOAM level 1 matrix of abundant and rare sub-communities by constraining the category of abundant and rare samples (A). The functional differences in FOAM level 1 between abundant and rare sub-communities were estimated by Wilcoxon rank-sum test (B). \*:  $P < 0.05$ ; Abundant: abundant taxa. Rare: rare taxa.

and temporal turnover in community assembly. In an effort to determine the successional trajectories and mechanisms of the abundant and rare microbial biospheres, we conducted a meta-analysis of bacterial community structure in temporal microcosms with pollutants. The results indicate that the succession of abundant sub-community is mainly driven by deterministic processes, while stochastic processes drive the succession of the rare sub-community. The temporal succession of bacterial community in microcosms is mainly associated with abundant bacteria rather than rare taxa. This finding suggests that the forces driving microbial succession (i.e., stochastic or deterministic processes) might be dependent on the low or high abundance community members in temporal microcosms with pollutants.

New molecular tools and increased sampling efforts have confirmed the existence of the “rare biosphere” (Pedrós-Alió, 2012; Logares et al., 2014; Hugoni et al., 2013; Liu et al., 2015). The abundance of a given bacterial species is the result of a balance between its growth rate and loss factors (Pedrós-Alió, 2012). In the present study, the high proportion and  $\alpha$ -diversity of rare taxa suggest that the rare biosphere is an important contributor to  $\alpha$ -diversity (Lynch and Neufeld, 2015). R. Logares et al (Logares et al., 2014). found a substantial amount of metabolically active lineages in the rare biosphere; this suggests that the rare sub-community constitutes a diversity reservoir that can respond rapidly to environmental change. Within the microcosms, the organic and inorganic pollutants appear to generate stronger selection pressure. The amount of persistent rare taxa (21–80; Fig. 1) in each consortium indicates that they can survive at low abundances under selection pressure; this may be because rare species can benefit from active losses via both viral lysis and predation (Pedrós-Alió, 2006). Rare taxa consist of dormant microorganisms that can be resuscitated under different environmental conditions (Pedrós-Alió, 2006).

In the current study, we found that between 14 and 28 abundant OTUs were persistent in the pollutant-degrading consortium and accounted for a substantial fraction of sequences (40.0%–68.4%; Fig. 1). The persistent abundant taxa may play important roles in the degradation of organic pollutants that serve as the sole energy and carbon source in the microcosms. In particular, several other

persistent taxa were abundant in one batch but rare in other batches, indicating large oscillations in abundance during culture enrichment. This is similar to the fluctuation patterns seen in a study examining the temporal dynamics of microbial communities (Anderson, 2007). The rare bacteria with lower abundance (<0.1%) could already attain a more stable community, while the abundant bacteria can fluctuate significantly depending on environmental conditions and toxicity of the pollutants. The local distributions of the abundant and rare taxa were different (see Fig. S4 in supplementary material), which may be a result of their distinct life strategies; various species have different life habits and occupy different ecological niches (Potapov et al., 2016). For instance, rare taxon *Desulfosporosinus* could contribute most of the sulfate reduction in the peat (Pester et al., 2010). In addition, some bacteria may be adapted to use only a few specific substrates or high nutrient. Teira et al. (2007). found that the abundance of *Cycloclasticus* (a hydrocarbon-degrading gammaproteobacterium) depended on the presence of polycyclic aromatic hydrocarbons (PAHs). *Cycloclasticus* became abundant when PAHs added, and reduced and returned to the rare biosphere once the PAHs disappeared.

The decrease in  $\alpha$ -diversity of the abundant sub-community during the succession indicated that selection drove the predictable assembly of the abundant taxa, which was determined by deterministic processes (Dini-Andreote et al., 2015). Meanwhile, the  $\alpha$ -diversity of rare taxa did not change temporally, suggesting that random succession (stochastic process) drove this sub-community. Abundant species can occupy a wide variety of niches, competitively utilize an array of resources, and effectively adapt to the environment; this supports the notion that they may be strongly influenced by deterministic filtering (Umaña et al., 2015). On the other hand, rare species may be more strongly influenced by demographic stochasticity due to their small population sizes (Orrock and Watling, 2010). Our previous study showed that  $\alpha$ -diversity of the whole bacterial community decreased during enrichment subculture (Jiao et al., 2016a,b), suggesting that deterministic processes are the primary drivers of whole community succession. Furthermore, the similar trends in  $\alpha$ -diversity

variation between the abundant sub-community and the whole community indicate that the microbial succession of the whole community can be primarily attributed to the abundant bacteria.

In the microcosms, the rare sub-community possessed higher  $\alpha$ - and  $\beta$ -diversity than the abundant sub-community. The high  $\beta$ -diversity of this sub-community can be attributed to new microbial inputs (Dini-Andreote et al., 2014). The higher rate of stochastic microbial inputs in the rare sub-community may be due to the limited capacity of each community. Limited community sizes could effectively make local competition more neutral and less predictable, leading to more divergent community structures (Orrock and Watling, 2010). In the tightly controlled microcosms, the newly detected taxa may be derived from taxa that are present at low levels in the original inoculum. A previous study demonstrated that the variations in community composition are due to changes in the relative abundance of taxa, rather than extinction and recolonization (Caporaso et al., 2012). Therefore, high dynamics in relative abundance of microbial taxa among batches and treatments could cause “new microbial inputs” for the rare and abundant community. This could explain the higher  $\beta$ -diversity of these sub-communities than the whole community.

Exponents of STRs can estimate the rate at which new taxa are observed in a community over time; a higher exponent means that a higher number of newly introduced taxa are present. The higher rate of cumulative species richness in the rare sub-community could also indicate intrinsically high and stochastic microbial inputs (Table 1). Likewise, these results support the effect of stochastic processes on the succession of rare bacteria. Moreover, the higher rate of temporary turnover in the abundant sub-community than in the rare sub-community indicates a more stable community structure in the abundant bacteria as succession proceeds (Fig. 4). This also suggests that deterministic processes contribute more to the succession of the abundant sub-community than they do to the rare sub-community.

The succession of the microbial community can be further elucidated by examining the partitioned temporal dynamics of the abundant and rare sub-communities. We found that the exponents of STRs for rare sub-communities were similar to those of whole communities, indicating that rare taxa accounted for most of the species accumulation across each whole community. This could be because rare bacteria are an important contributor to  $\alpha$ -diversity (Lynch and Neufeld, 2015) and act as a seed bank for community stability that can respond rapidly to environmental change (Logares et al., 2014). The structure of the abundant sub-community was also similar to that of the whole community (Fig. 3). Additionally, the abundant sub-community accounted for most of the succession and temporal turnover in the whole community. Studies have suggested that abundant species can occupy wide niches and competitively utilize an array of resources (Umaña et al., 2015), and that community succession is dependent on species abundance (Sp et al., 2016). The variations in dominant taxa are responsible for a large fraction of overall community  $\beta$ -diversity (Shade et al., 2014); thus, our results confirm that abundant and rare bacteria play distinct roles in the succession and species accumulation of microbial communities within microcosms.

Microbial communities are the core contributors to ecosystem function (Miki et al., 2010), especially in the pollutant-degrading consortia in this study. The functions related to cellular response to stress and hydrocarbon degradation were prevalent in the abundant sub-community. These functions can be enriched and become abundant within the consortia, as organic pollutants (i.e., *n*-octadecane and phenanthrene) serve as sole energy and carbon source in the microcosms and inorganic pollutants (e.g., cadmium) exert selection pressures on bacteria. While, the rare bacteria could have been reduced or outcompeted by the dominant because the

pollutants might be toxic to them. Additionally, vital functions associated with energy synthesis and metabolism, such as amino acid metabolism and fatty acid oxidation, were prevalent in the abundant sub-community. These results suggest that the abundant bacteria are likely responsible for primary ecosystem functions in the consortia. This is also supported by the fact that the abundant sub-community was more similar to the whole community in functional succession compared with the rare sub-community (see Fig. S10 in supplementary material). In contrast, the rare sub-community contributed a substantial fraction of auxiliary functions, such as carbohydrate-active enzymes, fermentation, and homoacetogenesis. This could contribute to the functional redundancy of the community (i.e., the insurance hypothesis) and enable the ecosystem to respond to and counteract disturbances (Yachi and Loreau, 1999). Studies have revealed that the rare microorganisms may mediate ecosystem function and stability. In the Arctic Ocean, the rare microorganisms exhibit biogeography, which indicates that some rare taxa, similar to more abundant taxa, are distributed based on their ecological requirements (Galand et al., 2009). Additionally, nitrification-mediated pathways are facilitated by ammonia-oxidizing archaea through aerobic ammonia oxidation (Kool et al., 2010), and methane production occurs through an anaerobic process (i.e., methanogenesis) by methanogenic archaea (Aydin et al., 2015); these archaea are often present in low abundance. In this case, the rare microbial biosphere may act as a diverse pool and play a role in maintaining ecosystem functions.

## 5. Conclusion

Our results show the distinct succession patterns of abundant and rare sub-communities in microcosms in response to various pollutants. The succession of the abundant sub-community is mainly driven by deterministic process; meanwhile, the intrinsically high and stochastic microbial inputs of the rare sub-community indicate the major roles of stochastic processes in succession. In particular, the abundant bacteria are the largest contributors to temporary microbial turnover, while the rare taxa are primarily responsible for species accumulation during microbial succession in microcosms. Abundant bacteria likely contribute primary functions in the consortia, while the rare microbial biosphere may act as a source of functional diversity; therefore, both the abundant and rare sub-communities are essential to microbial community succession, and should be distinguished in future studies.

## Conflict of interest

The authors declare no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2017.03.015>.

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