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Longfei Jiang and Mengke Song contributed equally to this manuscript.

Key Points:
- Taxonomic and degrading genes of active PAH degraders were identified by stable isotope probing
- Decoupled distribution and diversity of active PAH degraders was determined by 16S and PAH-degrading genes
- Taxonomic instead of the functional genes explain the influence of distance on functional microbial community

Supporting Information:
- Supporting Information S1

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Decoupled Spatial Distribution of PAHs Degraders Determined by Taxonomic 16S rRNA and Degrading Genes Across Chinese Forest Soils

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Abstract Knowing the structure and distribution of microbial communities and the underlying mechanisms shaping microbial geographic patterns is crucial for soil ecology and biogeochemical cycles of elements. Studies have explored the diversity, composition, and distribution of whole microbial communities based on taxonomic (16S rRNA) genes in many habitats. However, it is unclear whether the active microbes characterized by other genetic elements are driven by the same environmental variables and follow similar distribution patterns owing to technical limits on identifying the active functional genes in complex communities. Here, we employed ubiquitous phenanthrene as a model compound and applied DNA-stable isotope probing to investigate the active phenanthrene degraders by 16S rRNA genes and phenanthrene-degrading genes in forest soils. The effects of environmental variables and geographic distance on the diversity and composition of both genetic elements were examined. The diversity and similarity of whole microbial communities was closely linked with the total phenanthrene-degrading genes. However, the abundance and diversity of the active phenanthrene-degrading genes mismatched those of the active 16S rRNA genes, suggesting their distinct responses to environment variables and geographic distance. Geographic distance had a strong effect on the active phenanthrene-degrading community identified by taxonomic genes but not the active phenanthrene-degrading genes. Dispersal and mutation might explain the decoupled biogeographic patterns between the active taxonomic 16S rRNA and phenanthrene-degrading genes. This study provides new insights into the different driving forces for the active functional microbes characterized by various genetic elements, implying the diverse evolutionary mechanisms between functional genetic elements and 16S rRNA genes.

Plain Language Summary As the most abundant organisms on Earth, microbes play important roles in ecosystem and biogeochemical cycles of elements. However, the information about the active functional microbes is limited due to deficient approaches to identify the active ones. Here we give a multiangle view about the active functional microbes by analyzing the two different genetic elements with the aid of stable isotope probing for the first time. A huge diversity of functional microbes characterized by taxonomic 16S rRNA and PAH-RHDα genes were identified from eight Chinese forest soils. We observed the uncoupling distribution between 16S rRNA and PAH-RHDα genes, and the inconsistent responses of whole microbial communities, functional microbes, and the functional genes to environmental changes, suggesting both active taxonomic and active functional genes of microbial communities should be considered in future studies. This work expands our knowledge on the diversity and environmental behavior of specific functional microbes in the environment and provides some clues on the mechanisms underlying the geographic distribution patterns of soil microbes.

1. Introduction

Knowledge about biodiversity, the spatial distribution pattern of organisms, and the driving forces shaping biotic communities is key to predict the response of ecosystems to future environmental changes (Martiny

et al., 2011). Microorganisms are the most abundant and diverse group of living organisms on our planet, playing integral and often unique roles in the biogeochemical cycling of various elements and minerals that are crucial to ecosystem functioning (Zhou et al., 2008). These functions depend particularly on the microbes actively participating in specific ecological processes and the corresponding functional genes. Herein, diversity of both microbes and functional genes has received increasing attention (Herrick et al., 1997; Song et al., 2015).

The underlying causes of microbial diversity and distribution are proposed as the interplay of mutation, selection, drift, and dispersal (Angermeyer et al., 2016; Hanson et al., 2012), and the contribution of each process to the microbial diversity depends on environmental variables (Hanson et al., 2012). In addition, geographic distance plays an important role in the diversity of the whole microbial community (Angermeyer et al., 2016; Ma et al., 2016; Zhou et al., 2008), and interplays with other environmental variables making the spatial distribution of microbial communities follow a distance-decay pattern in various environments (Angermeyer et al., 2016; Finkel et al., 2012; Martiny et al., 2011). Nevertheless, previous studies are confined to the whole community or total functional genes with the aid of metagenomics (a genomic analysis of microbial assemblages in the environment) (Handelsman, 2004; Jiang et al., 2016; Mueller et al., 1997; Zhou et al., 2008) or microarray (He et al., 2007; Liang et al., 2011; Tu et al., 2014; Zhou et al., 2008). Alternatively, researches based on stable isotope probing (SIP) targeted the active functional microbes or genes in complex environmental matrices by sequencing the isotope-labeled 16S rRNA or functional genes, which uncovered the identities, abilities, and functional genes of the active microbes responsible for organic compound metabolism (Jones et al., 2011; Lemmel et al., 2019; Schmidt et al., 2015; Singleton et al., 2007), methane oxidation (Lu & Conrad, 2005; Shiau et al., 2018), ammonium oxidation (Avrahami et al., 2011; Daebeler et al., 2014), and sulfur cycling (Coskun et al., 2019). SIP has a unique feature of utilizing an environmentally relevant concentration of target compounds to identify the active microbes from in situ habitats, possessing minimal disturbance on microenvironment and indigenous microbial community (Jiang et al., 2018). It is still unclear for the diversity and distribution of the active functional microbes characterized by both taxonomic genes and functional genes under the forces of environmental variables. Besides, considering that operational genes and informational genes were affected differently by the forces of mutation, selection, drift, and dispersal (Angermeyer et al., 2016; Brown, 2003; Rivera et al., 1998), it is necessary to study the differences between the diversity and distribution pattern of the functional microbes determined by 16S rRNA and functional genes.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous substances in nature, and their fate is an important biogeochemical process drawing wide concerns. Microbes play an important role in the occurrence and fate of PAHs by biotransformation or metabolism (Liu et al., 2014; Zheng et al., 2014). Additionally, PAH ring-hydroxylating dioxygenases (PAH-RHD) are responsible for the initial step of PAH degradation, and their encoding genes have remarkable diversity across PAH degraders and are often used as an indicator of these microbes (Hilyard et al., 2008; Iwai et al., 2010; Liang et al., 2011). Hence, PAH degradation offers good opportunities to study the active functional microbes determined by either taxonomic genes or functional genes. Until now, total microbes and alpha subunit of PAH-RHD (PAH-RHDα) genes instead of the active ones have been identified at PAH contaminated sites by traditional isolation or metagenomics (Hilyard et al., 2008; Iwai et al., 2010; Jones et al., 2011; Song et al., 2016). Few SIP studies give sufficient information to link the active PAH degraders with the active PAH-RHDα genes (Lemmel et al., 2019; Singleton et al., 2012; Song et al., 2015), challenging our understanding of their diversity and driving forces in nature.

In this work, phenanthrene (PHE) was used as the model compound of PAHs to analyze the diversity and composition of the active PHE degraders defined by both16S rRNA and PAH-RHDα genes across eight Chinese mountain forest soils with the aid of DNA-SIP and high-throughput sequencing. Our aims are to answer the following questions: (1) What are the differences between the diversity of whole microbial community and active PHE-degrading community? (2) What is the link between diversities of the total and active PAH-RHDα genes? (3) Is the relationship between whole microbial community and total PAH-RHDα genes different from the active ones? (4) Do the forces of mutation, selection, drift, and dispersal act differently on the diversity of taxonomic and functional genes? Our hypothesis is that the diversity of different microbial communities (whole *vs*. active) and different genetic elements (taxonomic *vs*. functional) are shaped by distinct environmental variables, exhibiting inconsistent distribution patterns.

Figure 1. Map of the sampling sites in eight mountain forests located in different geographical regions across China. Xiaoxinganling (XXAL) and Changbai (CB) in North China; Shennongbei (SNB) in Central China; Fanjing (FJ), Leigong (LG), and Nanling (NL) in South China; Ailao (AL) in Southwest China; and Longquan (LQ) in East China.

2. Materials and Methods

2.1. Soil Samples

Soil samples were collected from eight different mountains (Mt.) located in China, (Figure 1, and detailed information is listed in supporting information Table S1). At each sampling site, five small pits with 5 m apart from each other were hand dug. After removing vegetation litters, the topsoils (0–10 cm) were collected separately from each pit using a soil corer (3 cm in diameter, stainless steel) and pooled as one sample. Mts. Changbai, Longquan, Fanjing, Nanling, Xiaoxinganling, Leigong, Ailao, and Shennongjia were designated as CB, LQ, FJ, NL, XX, LG, AL, and SNB, respectively. After being transferred to the laboratory with ice packs, the soils were homogenized, sieved through a 2-mm mesh, and immediately used to set up SIP microcosms. The remaining soils were kept at 4°C and −20°C for physiochemical analysis and DNA extraction, respectively.

2.2. Soil Physicochemical Analysis

Contents of soil total organic carbon (TOC) and total nitrogen (TN) were determined with an elemental analyzer (Jiang et al., 2016). Other soil properties including pH, electric conductivity (EC), ammonium nitrogen

(NH_4-N), total phosphorus (TP), available phosphorus (AP), total potassium (TK), available potassium (AK), aluminum (Al), calcium (Ca), and magnesium (Mg) were measured following the standard methods in Bao Shidan (2000). PAHs were analyzed using the methods described previously (Li et al., 2019; Song et al., 2016) with some modifications. Briefly, 20 g of soil sample was subjected to freeze drying, pulverizing, and extraction with dichloromethane (DCM). After purifying the contracted extracts based on a multilayer silica gel/alumina column, PAHs were eluted using DCM:HEX (v/v = 1:1, HEX: *n*-hexane). The eluted fraction was concentrated to ~50 μl, added with internal standard, and performed PAHs detection on an Agilent 7890 gas chromatograph equipped with a capillary column and a mass spectrometric detector with the methods reported by Song et al. (2016). Detailed information about the soil physicochemical properties and PAHs contents is listed in Table S1.

2.3. PHE-Degrading Microcosms

SIP microcosms were prepared as previously described with some modifications (Li et al., 2019; Song et al., 2015, 2016). Briefly, 3.0 g of soil (dry weight) was placed in a 150-ml serum bottle containing 10 ml of phosphate-buffered mineral medium. After bottles were sealed with rubber stoppers and compressed with an aluminum seal, ^{12}C-labeled PHE (99%, Cambridge Isotope Laboratories, Inc., USA) or ^{13}C-labeled PHE ($^{13}C_6$-PHE, 99%, Cambridge Isotope Laboratories, Inc.) was added to the bottles using a gastight syringe to obtain a final PHE concentration of 1 mg/kg. The PHE content in these microcosms was slightly higher than that in the original soils (Table S1), thus allowing the incorporation of ^{13}C into DNA to identify the active PHE degraders without significantly altering bacterial community structure. For each soil, two control treatments were carried out as follows: non–PHE addition control (CK) and nonbioactive control (radicidation, ^{12}C-NB). To prepare nonbioactive controls, soils were γ-irradiated (50 kGy) for 2 hr as previously described (Song et al., 2015). Two positive treatments were added with ^{12}C- and ^{13}C-labeled PHE as carbon sources, named as ^{12}C-PT and ^{13}C-PT, respectively. Each treatment was prepared in duplicates for PHE degradation and SIP investigation. All the microcosms were incubated at room temperature (~20°C) with reciprocal shaking at a speed of 120 rpm. On Day 7, the duplicated samples from each treatment were sacrificed for PHE analysis and DNA extraction, respectively. All the PHE stock solutions were filtered through 0.2-μm pore size filters and stored in dark brown bottles.

2.4. DNA Extraction and Gradient Fraction

Microcosms were sacrificed for genomic DNA extraction using a PowerSoil DNA extraction kit (MO BIO Laboratories Inc., USA) following the manufacturer's instructions. The DNA content was quantified with an ND-2,000 UV-Vis spectrophotometer (NanoDrop Technologies, USA). Thereafter, equilibrium density gradient centrifugation was performed to separate the ^{13}C-labeled DNA from the unlabeled DNA according to a protocol described by Li and Song (Li et al., 2019; Song et al., 2015). Following centrifugation, the centrifuge tubes were placed on a fraction recovery system (Beckman Coulter, USA), and fractions were collected. The buoyant density (BD) of each fraction was then measured by refractometry using an AR200 automatic digital refractometer (Reichert Technologies, USA), and CsCl was removed (Song et al., 2015).

2.5. Quantification of PAH-RHDα Genes

The copy numbers of PAH-RHD_α genes in fractions from the ^{12}C-PT and ^{13}C-PT microcosms were determined by quantitative polymerase chain reaction (qPCR) using a primer set of 396F (5′-ATTGCGCTT AYCAYGGBTGG-3′) and 696R (5′-ATAGGTGTCTCCAACRAARTT-3′) as described previously (Ding et al., 2010). The qPCR and the construction of the standard curve were carried out according to the protocol described previously (Li et al., 2019). The "light" and "heavy" DNA fractions were selected based on the copy numbers of PAH-RHD_α genes in the ^{12}C-PT and ^{13}C-PT microcosms and then subjected to the following Illumina sequencing. Detailed information about the amplification procedures and qPCR mixture is provided in supporting information Text S1.

2.6. Amplification and Sequencing of 16S rRNA and PAH-RHDα Gene Amplicons

The V4 hypervariable region (~300 bp) of 16S rRNA genes from original soils, "heavy" and "light" DNA fractions in SIP microcosms were amplified to provide sufficient resolution for accurately classifying the taxonomy of bacterial and archaeal sequences with a primer set of 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011; Thompson et al., 2017). Meanwhile,

PAH-RHDα genes were amplified with another primer set (396F and 696R) (Ding et al., 2010), and a sample-specific 12-bp barcode was added to the forward primer as previously reported (Caporaso et al., 2011). PCR conditions and sample preparation for Illumina sequencing were previously described (Ding et al., 2010; L. F. Jiang et al., 2016).

The 16S rRNA gene sequencing data were processed and analyzed using the Mothur (v.1.34.4) (Schloss et al., 2009) and Quantitative Insights Into Microbial Ecology (QIIME 1) (Caporaso et al., 2010) following the published procedure (Edgar, 2010; Jiang et al., 2016; Li et al., 2019; McDonald et al., 2012). Mothur was employed to assemble the raw sequences and search the chimera in the aligned sequences. QIIME was used to process the rest steps. Briefly, reads with quality score of <25 or length of <250 bp were discarded. Qualified sequences were then assembled and assigned to samples. The singletons were removed to reduce the error rate. Then the sequences were assigned using an operational taxonomic unit (OTU)-based method with the cutoff of 97% to generate microbiome profiles (Edgar, 2010; McDonald et al., 2012; Werner et al., 2012). The taxonomic identification of the sequences was determined against SILVA database using QIIME (Glockner et al., 2017; Quast et al., 2013). The relative abundance of each taxon within each community was determined by comparing the number of representative sequences versus that of the total sequences. A subset of 14,613 sequences per sample was randomly selected to normalize the sequences and used for the calculation of alpha diversity including the indices of Shannon, Inverse Simpson (InSimpson) and Pielou, and beta diversity (Bray-Curtis distance) metrics.

For the PAH-RHDα genes from original soils and heavy DNA fractions in SIP microcosms, raw sequencing data were processed and analyzed according to Mothur (v.1.34.4) and QIIME 1. Briefly, sequences were assembled based on overlap and assigned to samples according to their unique barcodes. Low-quality reads with a quality score <25 or a length >360 bp or <150 bp were removed. Then, the singletons were discarded to reduce the error rate with a small reduction in sensitivity. OTUs with 95% similarity were picked out using Uclust (Xiong et al., 2018). Next, the representative sequence set was picked and aligned. The OTUs were annotated using Basic Local Alignment Search Tool (BLAST) based on the NCBI nonredundancy database (Altschul et al., 1990). The sequences were considered to be PAH-RHDα-like sequences with the similarity ≥90% to the reference sequences over 25 amino acids (Kristiansson et al., 2011). The relative abundance of each OTU was determined by comparing the number of representative sequences with the total sequences obtained from each sample. A subset of 14,926 sequences per sample was randomly selected to normalize the sequencing depth.

2.7. Statistical Analysis

Statistical analyses were performed using SPSS 18.0, QIIME (http://qiime.org/) and functions in the VEGAN package in R v.3.3.2 (http://www.r-project.org) (Oksanen et al., 2019). The diversity of taxonomic 16S rRNA genes and PAH-RHDα genes and the correlations between microbes and environmental variables were constructed based on the original soils without amendments. The alpha diversity indices were calculated to estimate within-sample diversity using functions "diversity" and "specnumber" in VEGAN package. The relationship between alpha diversity indices and environmental variables was analyzed using Pearson correlations (two-tailed) because these data fitted the normal distribution tested by SPSS, and p values were adjusted using the function "p.adjust" in the R. For 16S rRNA and PAH-RHD$_\alpha$ gene taxa, Bray-Curtis community similarities between samples were determined following the scripts in QIIME. Principle coordinate analysis (PCoA) with Bray-Curtis distance was used to estimate the composition and heterogeneity of bacterial community and PAH-RHDα genes by using the relative abundances of OTUs. The samples were then grouped based on their distribution pattern, and the significant difference between groups were tested using analysis of similarities (ANOSIM). Mantel tests were used to evaluate the linkages between microbial community composition, PAH-RHDα gene structure, and environmental variables, and p values were adjusted using the function "p.adjust" in the R.

3. Results

3.1. Overview of Soil Bacterial Communities Across Eight Chinese Mountains

To examine the structure of the whole microbial community, 16S rRNA genes from all the soil samples were amplified and sequenced. A total of 248,896 quality-filtered, chimera-free sequences were obtained, ranging

Table 1
Alpha Diversity Indices of Microbial Community in Soils From Eight Mountain Forests

Diversity indices	CB	LQ	FJ	NL	XXAL	LG	AL	SNB
Active PHE degrader community								
Shannon	2.02	3.21	3.00	3.34	2.10	3.29	2.89	2.78
InSimpson	3.41	14.21	10.07	16.70	3.45	16.73	7.88	9.10
Pielou	0.51	0.76	0.73	0.81	0.51	0.79	0.69	0.70
Whole microbial community								
Shannon	6.27	5.98	5.44	5.46	5.67	5.96	5.98	6.71
InSimpson	67.46	109.14	59.77	23.57	44.28	127.83	90.58	281.63
Pielou	0.82	0.80	0.75	0.73	0.77	0.81	0.80	0.87

Note. Higher values of Shannon and InSimpson indices represent higher diversity. Higher values of Pielou index represent higher evenness.

from 14,613 to 54,016 sequences per sample. The taxonomic analysis revealed 31 bacterial phyla and the dominate lineages were in the following order: Proteobacteria (36.6%) > Acidobacteria (25.3%) > Antinobacteria (11.3%) > Verrucomicrobia (10.6%) > Planctomycetes (3.79%) > Chlorolexi (3.20%) > Bacteroidetes (2.98%) > WPS-2 (1.88%) > Gemmatimonadetes (1.03%) (Figure S1a). The rare phyla (abundance <1%) held 0.59% of the total sequences, and 0.38% were classified as unknown phyla affiliated with bacteria.

All the sequences were assigned to 9,512 distinct OTUs at the 97% identity with a range from 1,352 to 3,937 OTUs per sample. The α-diversity indices (Shannon, InSimpson and Pielou) were calculated and listed in Table 1. They ranged from 5.44 to 6.71 (Shannon), 8.79 to 10.71 (InSimpson), and 0.73 to 0.87 (Pielou). The distribution pattern of the whole microbial community in each sample based on the Bray-Curtis community similarities showed that Mts. LQ, AL, LG, FJ and NL were clustered together and separated from Mts. CB, Xiaoxinganling (XXAL), and SNB ($p < 0.01$, Figure 2a).

3.2. Microbial PHE Metabolism and Total PAH-RHDα Genes in Soils

The PHE removal efficiency varied across the eight microcosms after 7-day degradation. Since significantly more PHE declined in the ^{12}C-PT microcosms than that in ^{12}C-NB (nonbioactive control), we confirmed the major contribution of microbial metabolism to PHE degradation (Table S2). The absolute abundance of the total PAH-RHDα genes quantified by qPCR, ranging from 0.02×10^3 to 5.36×10^3 copies/g soil, was not significantly correlated with the removal efficiency of PHE ($p > 0.05$) in this work (Table S2).

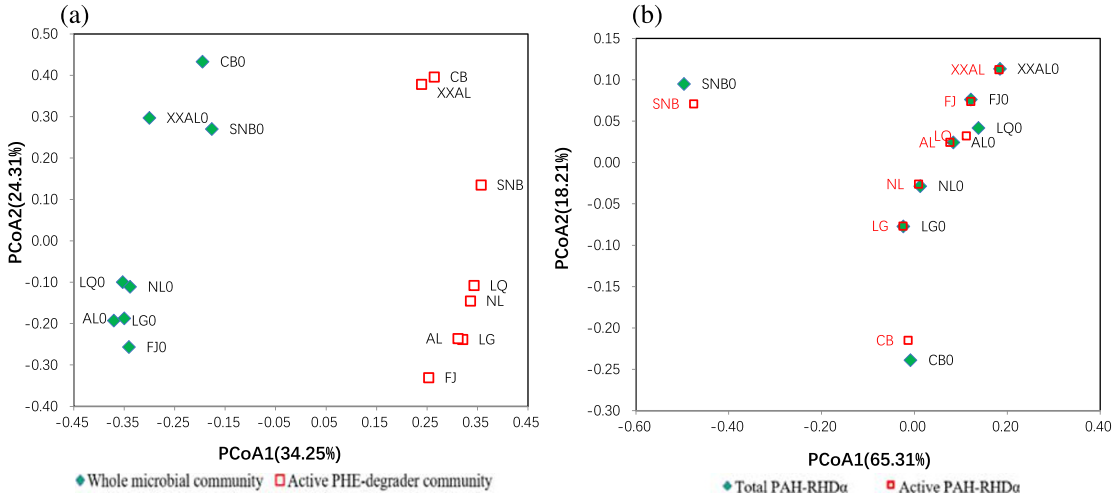

Figure 2. Score plots of principle coordinate analysis (PCoA) for the structure differences in microbial communities (a) and PAH-RHDα genes (b) of the eight mountain forest soils. XXAL = Xiaoxinganling, CB = Changbai, SNB = Shennongbei, FJ = Fanjing, LG = Leigong, NL = Nanling, AL = Ailao, LQ = Longquan.

Table 2
Alpha Diversity Indices of PAH-RHDα Genes in Soils From Eight Mountain Forests

Diversity indices	CB	LQ	FJ	NL	XXAL	LG	AL	SNB
Active PAH-RHDα genes								
Shannon	1.21	0.73	0.20	0.28	0.58	0.45	0.29	0.39
InSimpson	1.95	1.57	1.07	1.11	1.35	1.23	1.10	1.17
Pielou	0.32	0.19	0.05	0.07	0.16	0.12	0.08	0.11
Total PAH-RHDα genes								
Shannon	1.66	1.23	0.27	0.44	0.65	0.48	0.52	1.28
InSimpson	2.29	2.04	1.09	1.15	1.37	1.23	1.19	1.99
Pielou	0.32	0.24	0.06	0.09	0.14	0.11	0.11	0.28

Note. Higher values of Shannon and InSimpson indices represent higher diversity. Higher values of Pielou index represent higher evenness.

A total of 311,060 quality-filtered, chimera-free sequences of PAH-RHDα genes were obtained from eight mountain soils, ranging from 14,926 to 52,286 sequences per sample. Most of sequences (>95%) were assigned to the PAH-RHDα genes from uncultured bacteria and were affiliated to 330 distinct functional OTUs at 95% identity with a range from 87 to 175 functional OTUs per sample. The α-diversity indices were calculated and listed in Table 2, ranging from 0.27 to 1.66 (Shannon), 1.09 to 2.29 (InSimpson), and 0.06 to 0.32 (Pielou). Compared to 16S rRNA genes, the total PAH-RHDα genes had much lower diversities. The Bray-Curtis community similarity, representing the similarity of the total PAH-RHDα genes among samples, showed that all samples except for SNB and CB clustered together ($p < 0.05$), indicative of the more similar structure of PAH-RHDα genes (Figure 2b).

3.3. Active Microbes Involved in PHE Metabolism

Figure S2 shows the relative abundance of PAH-RHDα genes *versus* BD values. Fractions with the highest relative abundance of PAH-RHDα genes in the ^{12}C-PT microcosms were selected as the "light" DNA fractions (BD value ~1.7122 g/ml) and marked with red stars in Figure S2. The "heavy" DNA fractions (BD value ~1.7300 g/ml) were the ones in which PAH-RHDα genes were enriched in the ^{13}C-PT but not ^{12}C-PT microcosms, marked with blue stars in Figure S2. Microorganisms possessing the ability of ^{13}C-PHE assimilation were identified by a comparison of the relative abundance of specific taxonomic 16S rRNA genes (OTUs) between the ^{13}C-PT and ^{12}C-PT microcosms from both light and heavy DNA fractions.

After paired-end joining and quality trimming, 799,033 16S rRNA gene sequences were obtained from the light and heavy DNA fractions, ranging from 10,491 to 48,297 sequences per sample, classified into 8,518 OTUs at the 97% identity. Overall, the relative abundance of 63 OTUs from eight soils increased in the heavy DNA fractions and decreased in the light DNA fractions of the ^{13}C-PT microcosms compared with the corresponding heavy and light DNA fractions of the ^{12}C-PT microcosms (Figure S3), indicating microbes represented by these OTUs were the active PHE degraders assimilating ^{13}C-PHE and producing ^{13}C-DNA. All of them accounted for 17.89% of the total populations in the original soils, covering eight bacterial phyla and one archaeal phylum. Proteobacteria (10.19%), Verrucomicrobia (4.92%) and Acidobacteria (1.29%) were the dominant taxa (Figure S1b). The alpha diversity indices of the active PHE degrader community and their Bray-Curtis community similarities were also calculated (Table 1 and Figure 2a). The Shannon, InSimpson, and Pielou indices ranged from 2.02 to 3.34, 3.45 to 16.73, and 0.51 to 0.81, respectively. The active PHE degrader community of each sampling location differentiated from the whole microbial community of original soils based on Bray-Curtis community similarities ($p < 0.01$), as shown in Figure 2a.

Bacteria affiliated to 19 OTUs were involved in the PHE degradation in soils from Mt. LQ (Figure 3a), showing a high diversity of the active PHE-degrading microbes comparing to other mountains. Among these active PHE degraders, bacteria represented by OTU 1110303 affiliated with the class Gammaproteobacteria possessed the highest relative abundance (3.17% in the whole community, Figure 3b). In the soils from Mt. LG, 13 OTUs were responsible for in situ PHE degradation and classified as the members of the classes Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, Thermoleophilia, Planctomycetia, Acidobacteriia, and Group 1.1c Thaumarchaeota (archaea). Among these 13 OTUs, OTU 580625 (Alphaproteobacteria) was the major PHE degrader exhibiting the highest relative abundance (2.39% in the whole community, Figure 3b). The same archaeon in the class Group 1.1c Thaumarchaeota (phylum Thaumarchaeota, OTU 3106281) was also associated with the PHE degradation in soils from Mt. FJ. In addition, eight OTUs affiliated to bacteria including the classes Alphaproteobacteria, Acidobacteriia and Pedosphaerae (phylum Verrucomicrobia) were directly linked to in situ PHE metabolism in Mt. FJ. For Mt. SNB, seven OTUs were involved in ^{13}C-PHE biodegradation with OTU 580625 (class Alphaproteobacteria) as the dominant one. The soils from Mts. XX, AL, and CB had six different OTUs representing PHE degraders. The class Verrucomicrobiae (phylum Verrucomicrobia) behaved as dominant PHE degrader in soils from Mt. XXAL and CB. Gammaproteobacteria dominated the active PHE

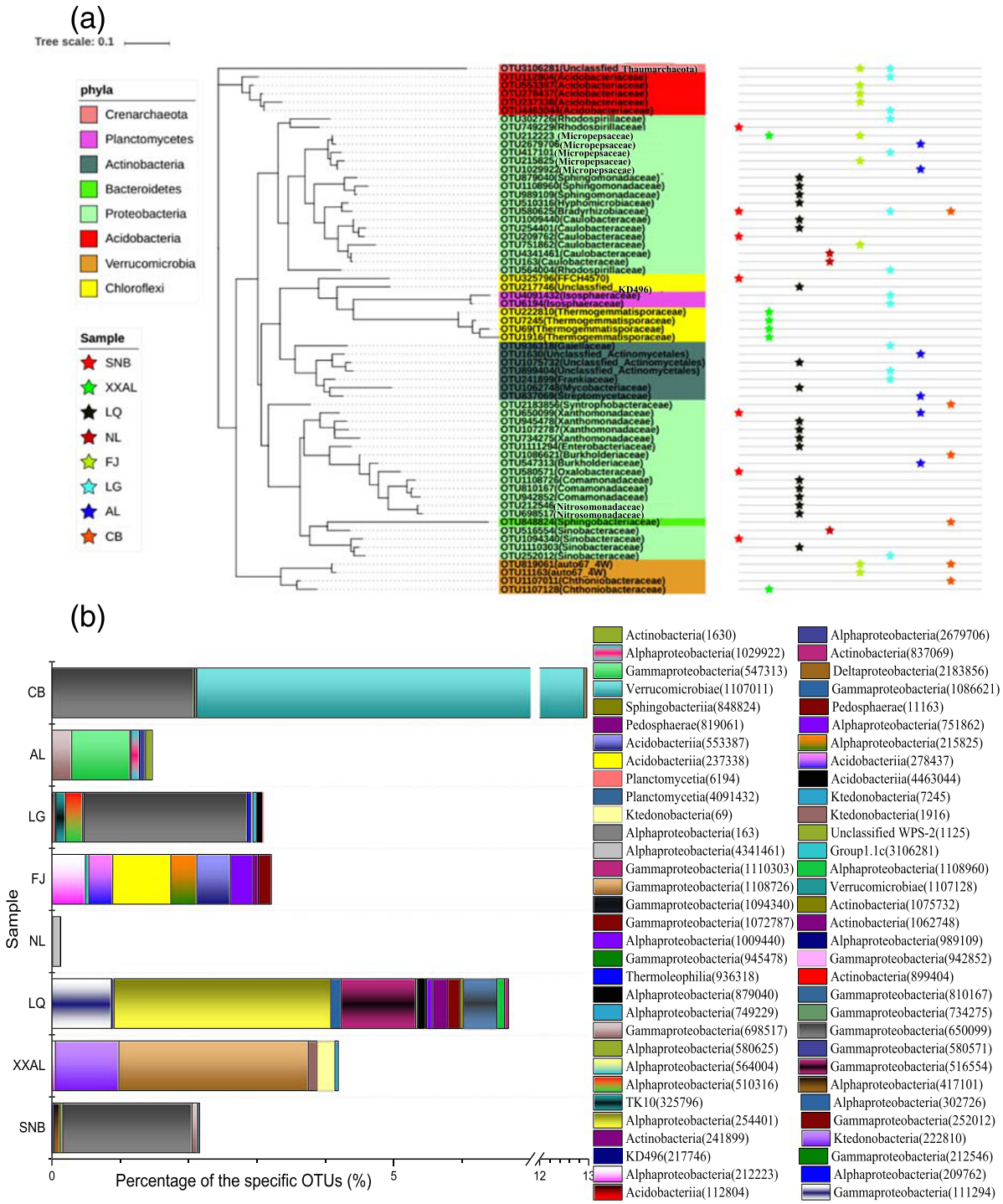

Figure 3. Taxonomic information and abundance of OTUs representing the active PHE degraders. (a) Phylogenetic tree based on OTU representative sequences of the active PHE degraders detected in all soils. Stars represent the presence of the active PHE degraders in each sample. (b) Percentage of the OTUs representing the active PHE degraders in all soils. The numbers in the bracket are the OTU ID. XXAL = Xiaoxinganling, CB = Changbai, SNB = Shennongbei, FJ = Fanjing, LG = Leigong, NL = Nanling, AL = Ailao, LQ = Longquan.

degrader community in the soil from Mt. AL. For Mt. NL, only three OTUs classified as the classes Gammaproteobacteria and Alphaproteobacteria were capable of metabolizing ^{13}C-PHE.

Among the 63 OTUs associated with in situ PHE degradation, 5 OTUs were shared by soils from more than one mountain. Although OTU 580625 was found in the whole microbial communities from all the eight mountains, it only contributed to ^{13}C-PHE biodegradation in soils from Mts. SNB, LG, and CB. OTUs 212223, 650099, and 819061, which were shared by the whole soil microbial communities from seven mountains, showed the ability of metabolizing ^{13}C-PHE only in soils from Mts. XXAL, and FJ (OTU 212223), SNB and AL (OTU 650099), and FJ and CB (OTU 819061), respectively. Although OTU 3106281 was detected in the whole microbial communities from six mountains, it was only involved in ^{13}C-PHE degradation of soils from Mts. FJ and LG.

3.4. Active PAH-RHDα Genes Involved in Initial PHE Metabolism

After quality control, 1,177,671 sequences were obtained from the light and heavy DNA fractions of soils from eight mountains, and 324 functional OTUs were identified at the 95% sequence identity to known PAH-RHDα genes (Xiong et al., 2018). They were classified as PAH-RHDα gene from environmental samples and accounted for 93.28% of the total sequences in eight soils. The active PAH-RHDα genes involved in the ^{13}C-PHE metabolism and labeled by ^{13}C were identified by a comparison of the relative abundance of specific PAH-RHDα genes (functional OTUs) between the ^{13}C-PT and ^{12}C-PT microcosms in the light and heavy DNA fractions, respectively (Figure S4). In total, 50 functional OTUs were linked with the in situ ^{13}C-PHE metabolism (Figure 4a). The Shannon, InSimpson, and Pielou indices of these active PAH-RHDα genes ranged from 0.20 to 1.21, 1.07 to 1.95, and 0.05 to 0.32, respectively, indicating a low alpha diversity (Table 2). The composition of the active PAH-RHDα genes was similar to the total PAH-RHDα genes based on Bray-Curtis community similarities ($p > 0.05$) (Figure 2b and Table S7).

In total, 27 functional OTUs were linked with the PHE degradation in soils from more than one mountain (Figure 4b). Functional OTU 827 was shared by soils from Mts. SNB, FJ, LG, LQ, and AL, and functional OTU 1026 was involved in the PHE metabolism in Mts. SNB, XXAL, AL, CB, and NL. Functional OTUs 837, 1139, 192, 990, and 767 were responsible for PHE metabolism in soils from three different mountains. Functional OTU 521 dominated the active PAH-RHDα genes in soils from Mts. XXAL, LQ, CB, and NL. Mt. SNB had the highest numbers of functional OTUs representing the active PAH-RHDα genes (18 OTUs); however, the relative abundance of all these functional OTU was lower than 1%. In soils from Mts. FJ, AL, and CB, functional 14 OTUs were associated with in situ PHE degradation, and functional OTU 990 was dominant in Mt. AL with the relative abundance of 2.25%. Although 11 functional OTUs were identified as the active PAH-RHDα genes in Mt. NL, their relative abundance was lower than 0.1% except for functional OTU 521. In soils from Mts. LQ, LG, and XXAL, 10, 7 and 6 functional OTUs were correlated with the PHE degradation, respectively, and functional OTU 521 was the major active PAH-RHDα genes for Mt. XXAL with the relative abundance of 84.61% (Figure 4).

3.5. Environmental Variables Affecting the Diversity of the Whole Microbial Community and Active PHE Degrader Community by 16S rRNA Genes

Table 1 shows the microbial diversity indices in soils from eight mountains. For the whole microbial communities, there were significantly negative correlations between NH_4-N and Shannon ($r = -0.921$, $p = 0.017$) and Pielou indices ($r = -0.939$, $p = 0.017$) (Table S3). Shannon index also had significantly positive correlations with pH ($r = 0.773$, $p = 0.048$). The diversity indices of the active PHE degrader community had positive correlations with temperature (T, $r = 0.776 \sim 0.911$, $p = 0.002 \sim 0.024$) (Table S3). In addition, Shannon and InSimpson indices were positively related to precipitation (PR, $r = 0.856$ and 0.854, $p = 0.042$). There was no significant relationship between the diversity indices of the whole microbial community and active PHE degrader community (Table S4).

Mantel test showed that the whole microbial community and active PHE degrader community were affected by different environmental variables. The similarity of the whole microbial community among eight mountains correlated with Mg ($r = 0.465$, $p = 0.044$), EC ($r = 0.715$, $p = 0.024$), TP ($r = 0.441$, $p = 0.038$) and AP ($r = 0.558$, $p = 0.024$), whereas the similarity of the active PHE degrader community was significantly related to T ($r = 0.679$, $p = 0.024$) and distance ($r = 0.643$, $p = 0.024$) (Table S5). The PCoA score plot of 16S rRNA genes (Figure 2a) suggested that all the eight active PHE degrader communities in the original soils were

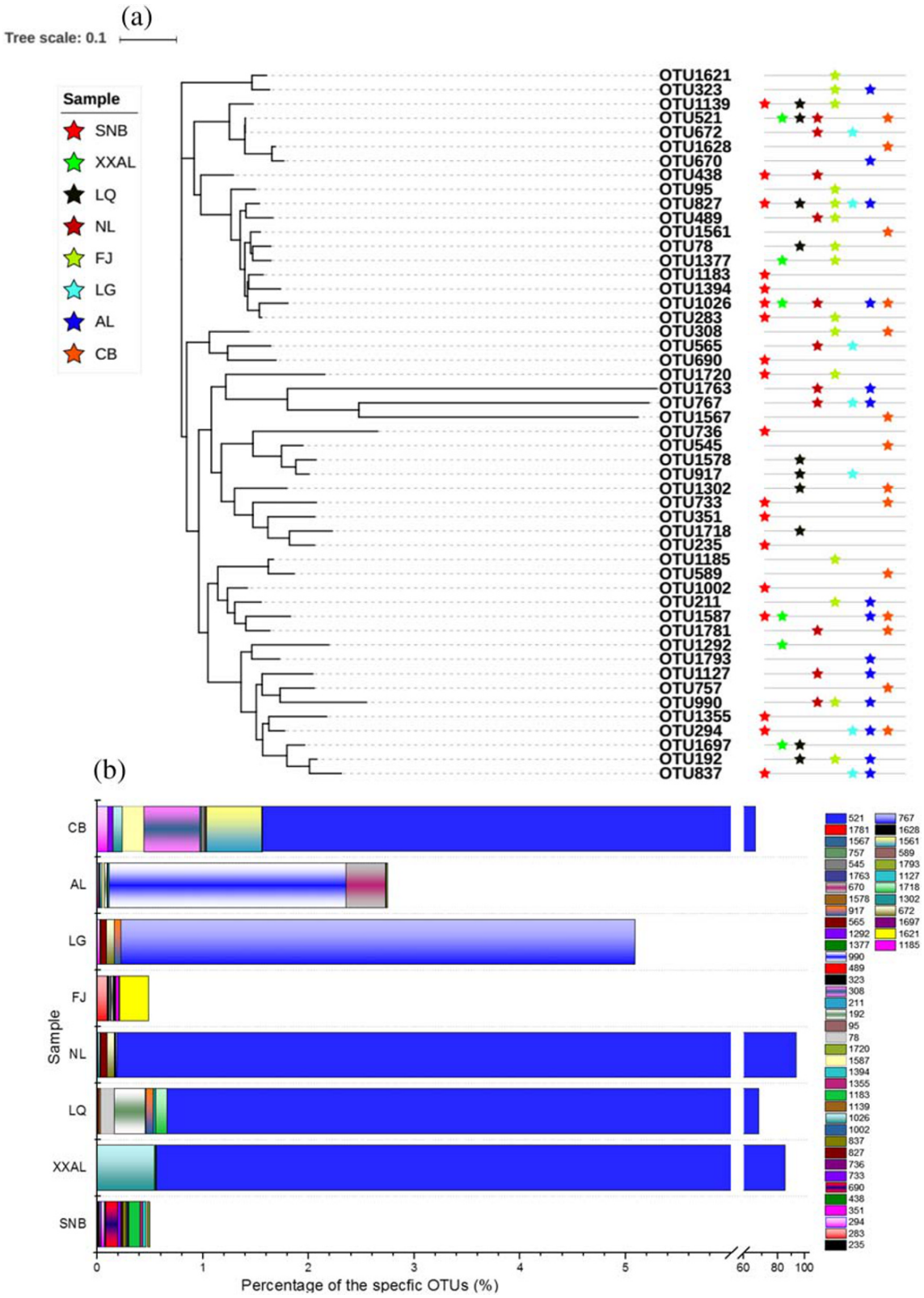

Figure 4. Taxonomic information and abundance of functional OTUs representing the active PAH-RHDα genes. (a) Phylogenetic tree based on functional OTU representative sequences of the active PAH-RHDα genes detected in all soils. Stars represent the presence of the active PAH-RHDα genes in each sample. (b) Percentage of functional OTUs representing the active PAH-RHDα genes in all soils. The numbers in the bracket are the functional OTU ID. XXAL = Xiaoxinganling, CB = Changbai, SNB = Shennongbei, FJ = Fanjing, LG = Leigong, NL = Nanling, AL = Ailao, LQ = Longquan.

separated from their corresponding whole microbial communities along PCoA1. It was in accordance with the different responses of the active PHE degrader communities and whole microbial communities to environmental variables, suggesting the unique ecological characteristics of PHE degraders that constituted averagely 4.24% of the whole community in comparison with predominant non–PHE degraders. However, the active PHE degrader communities were derived from the whole community resulting in the similarity of positions along PCoA2.

3.6. Environmental Variables Affecting the Total and Active PAH-RHDα Genes Responsible for Initial PHE Metabolism

Table 2 shows the diversity indices of the PAH-RHDα genes associated with ^{13}C-PHE in soils from eight mountains. Pearson correlation analysis illustrated positive correlations between the diversity indices of the total PAH-RHDα genes and pH, whereas there was a negative correlation between the diversity indices of the active PAH-RHDα genes and NH_4-N (Table S6, $p < 0.05$). Additionally, the diversity indices of the total PAH-RHDα genes positively and significantly connected with those of the whole microbial community (Table S4, $p < 0.05$).

Bray-Curtis community similarities of the total and active PAH-RHDα genes among soils were positively related with EC ($r = 0.735$, $p = 0.024$; $r = 0.725$, $p = 0.024$), AP ($r = 0.883$, $p = 0.044$; $r = 0.881$, $p = 0.041$), and the whole microbial community ($r = 0.528$, $p = 0.039$; $r = 0.534$, $p = 0.39$) from Mantel tests (Tables S5 and S7). A positive correlation was also observed between the total and active PAH-RHDα genes ($r = 0.993$, $p = 0.011$). Surprisingly, there was no significant relationship between the active PHE degrader community and the active PAH-RHDα genes (Table S7). According to the PCoA score plot of PAH-RHDα gene, eight groups of the active PAH-RHDα genes from each mountain did not cluster together. Instead, they were located closer to the total PAH-RHDα genes from the same location, indicating the same environmental variables affecting the active and total PAH-RHDα genes (Figure 2b).

4. Discussion

SIP revealed a wealth of microbes, including bacteria and archaea, as the putative PHE metabolizers. Of them, 17 OTUs affiliated to 10 bacterial orders and 1 archaeal order were firstly linked with PHE degradation, including Gaiellales and Frankiales (phylum Actinobacteria), Isosphaerales (phylum Planctomycetes), Syntrophobacterales, Rhizobiales and Micropepsales (phylum Proteobacteria), Chthoniobacterales and Pedosphaerales (phylum Verrucomicrobia), and Group 1.1c of the phylum Thaumarchaeota. Members of the phylum Verrucomicrobia were known to degrade various polysaccharides (Herlemann et al., 2013). Thaumarchaeota are important contributors to ammonia oxidation in terrestrial habitats, but Group 1.1c Thaumarchaeota is abundant in acidic forest soils and is not associated with ammonia oxidation for the absence of *amoA* gene, and our study firstly linked this order to PAHs degradation (Weber et al., 2015). Rhizobiales possesses the ability of degrading chlorobiphenyl and biphenyl in previous studies (Abbasian et al., 2016; Koubek et al., 2013). Micropepsales is involved in polysaccharide degradation and plant litter decomposition (Harbison et al., 2017). Members of Syntrophobacterales (class Deltaproteobacteria, phylum Proteobacteria) were found in PHE-contaminated soils contaminated by PHE (Liu & Conrad, 2017; H. Wang et al., 2016). Planctomycetes has the ability to degrade various polysaccharides and the family Isosphaeraceae plays an important role in biphenyl degradation (Leigh et al., 2007). Several members of Actinobacteria were reported to have PHE degradation capabilities (Chaudhary et al., 2011; Sharma et al., 2016), whereas it is still unknown whether the order Frankiales is capable of degrading PAHs. Gaiellales is the keystone species in the co-occurrence networks of soil microbiotas at continental scale in eastern China (Ma et al., 2016).

Comparing with PHE-degrading bacteria, there is even less information about the active PHE-degrading genes, for example, PAH-RHD, responsible for in situ PHE degradation. Accordingly, all the active PAH-RHDα genes identified in the present study were affiliated to those from uncultured bacteria and shared high similarity with the putative PAH-degrading genes from environmental samples without clear taxonomic information. Our results indicated an unexpected extensive diversity of PAH-RHDα genes in the environment and the gap of their affiliation, as evidenced by the abundant novel aromatic dioxygenase genes revealed using gene-targeted metagenomics and pyrosequencing (Iwai et al., 2010; Wang et al., 2014).

Understanding the environmental variables influencing the diversity and composition of the functional microbial community is an important goal in microbial ecology. Temperature has a significant impact on soil hydrocarbon utilizer community, and different temperatures lead to unique dominant microbes in soils (Coulon et al., 2007; Leahy & Colwell, 1990; Zhou et al., 2012). In the present study, members of phylum *Verrucomicrobia* dominated the soil microbial communities from Mts. CB and XXAL in the temperate zone with relatively low temperature, as they have powerful adaptive capacity for extreme conditions (Wagner & Horn, 2006). Another explanation might be the lower bioavailability of organic matters under low temperature conditions (Coulon et al., 2007), which facilitates the growth of microbes as "oligotrophic" bacteria such as *Verrucomicrobia* (Wagner & Horn, 2006). In subtropical soils with high temperatures, the elevated available carbon sources benefit the growth of "copiotrophic" bacteria and encourage the activities of soil *beta*- and *alpha-Proteobacteria*, which rapidly colonize and flourish under labile C-rich conditions (Leewis et al., 2016). It is worth mentioning that geographic distance had impacts on the distribution of the active PHE degrader community instead of the whole microbial community, indicating PAH degraders may be more sensitive to dispersal comparing with the whole populations in mountain forest soils. Since the whole microbiota is consisted of many guilds with distinct functions, the driving forces of selection, drift, dispersal and mutation probably act differently on the evolution of each guild (Yergeau et al., 2007; Zhou et al., 2008). The dispersal limitation of the active PAH degraders in this work might be hidden by other driving forces affecting other guilds (Rivera et al., 1998), resulting in the different impacts of geographic distance on the whole microbial community and active PHE degrader community. Our results are different from previous studies in which the contamination level of organic compounds had significant impacts on soil functional degrader communities (Aislabie et al., 2004; Liang et al., 2011). It might be explained by the extremely low levels of PAHs in mountain forest soils (10.37–56.22 pg/g) in our study, similar to another study in which low PHE content in grassland soils showed limited influence on the functional microbial diversity (Niepceron et al., 2014).

Another finding worth highlighting is the uncoupling pattern of PAH degraders identified by taxonomic 16S rRNA and PAH-RHDα genes across eight mountain soils. It is possible explained by the differences in the horizontal gene transfer ability between the functional degrading genes and taxonomic 16S rRNA genes among species (Rivera et al., 1998). The 16S rRNA genes crucial for microbes and imposed by demanding functional constraints are reported to form a tightly integrated set and exhibit little transfer ability (Rivera et al., 1998); hence, microbial taxonomic identities by 16S rRNA genes are influenced mainly by dispersal limitation and exhibit distance-decay patterns across different habitats (Martiny et al., 2011; Schauer et al., 2010; Song et al., 2016). This geographic pattern consists with our results of the active PHE-degrading community. In contrast, the functional genes, such as PAH-RHDα genes in the present study, are easily transferred horizontally among species (Brown, 2003; Rivera et al., 1998). Such features might cover up the effects of dispersal limitation and result in the limited impacts of distance (Herrick et al., 1997; Song et al., 2015; Wilson et al., 2003), leading to the different geographic patterns of functional genes from 16S RNA genes. For instance, Angermeyer et al. (2016) found the decoupled distance effect between *dsrA* and 16S rRNA genes among salt marsh sulfate-reducing bacteria (Angermeyer et al., 2016), and our work suggested a similar inconsistent influence of distance on PAH-RHDαand 16S rRNA genes related to PHE metabolism (carbon cycle). Additionally, different from the decoupled response of active 16S rRNA genes and active PAH-RHDα genes to environmental variables, the diversity and similarity of the whole microbial community and total PAH-RHDα genes had positive relationship (Tables S4 and S7) and shared the influence of some environmental variables (Tables S3, S5, and S6). The results emphasize the necessity to explore the active functional microbes by studying both taxonomic and functional genes. Additionally, the uncoupling pattern of PAH degraders defined by taxonomic 16S rRNA and PAH-RHDα genes might be attributed to the limitation of SIP method to some extent due to cross feeding. It is possible that DNA of some predatory bacteria and those feeding off necromass are labeled by ^{13}C, bringing about the deviation of the PHE degraders and PAH-RHDα genes.

5. Conclusions

In this work, we coupled SIP and high-throughput sequencing to investigate the diversities and compositions of the whole microbial community, active PHE degrader community, total alpha subunit of PAHs ring-hydroxylating dioxygenases (PAH-RHDα) genes, and active PAH-RHDα genes. The whole microbial

community was positively related to the total PAH-RHDα genes, both influenced by EC and AP. The active PHE degrader community was significantly affected by temperature and distance, whereas the active PAH-RHDα genes had significant correlations with EC and AP. Such uncoupling influence highlights the different evolutionary mechanisms and processes between 16S rRNA and PAH-RHDα genes and the significance of determining the active degraders, suggesting both taxonomic and functional elements of the active microbial communities should be considered in future studies of microbial ecology and biogeochemical cycles. Besides the knowledge about in situ active microbes defined by 16S rRNA and functional genes related to PHE degradation, mechanisms shaping the diversity and spatial distribution patterns of different genetic elements will help us predict ecosystem responses to future environmental changes and then design appropriate strategies to mediate biogeochemical processes.

Data Availability Statement

The authors comply with the AGU's data policy; the sequences of 16S rRNA genes and PAH-RHDα genes obtained in this study for Tables 1, 2 and Figures 2–4, and were deposited in https://www.ncbi.nlm.nih.gov/sra under the accession numbers SRR7738373 and SRR7738413. The data for Figures 1–4 and Tables 1, 2, and were provided online (in https://doi.org/10.6084/m9.figshare.8326676.v1).

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References

Abbasian, F., Lockington, R., Megharaj, M., & Naidu, R. (2016). The biodiversity changes in the microbial population of soils contaminated with crude oil. *Current Microbiology*, *72*(6), 663–670. https://doi.org/10.1007/s00284-016-1001-4

Aislabie, J. M., Balks, M. R., Foght, J. M., & Waterhouse, E. J. (2004). Hydrocarbon spills on Antarctic soils: Effects and management. *Environmental Science & Technology*, *38*(5), 1265–1274. https://doi.org/10.1021/es0305149

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Angermeyer, A., Crosby, S. C., & Huber, J. A. (2016). Decoupled distance-decay patterns between dsrA and 16S rRNA genes among salt marsh sulfate-reducing bacteria. *Environmental Microbiology*, *18*(1), 75–86. https://doi.org/10.1111/1462-2920.12821

Avrahami, S., Jia, Z. J., Neufeld, J. D., Murrell, J. C., Conrad, R., & Kusel, K. (2011). Active autotrophic ammonia-oxidizing bacteria in biofilm enrichments from simulated creek ecosystems at two ammonium concentrations respond to temperature manipulation. *Applied and Environmental Microbiology*, *77*(20), 7329–7338. https://doi.org/10.1128/AEM.05864-11

Bao, S. (2000). *Soil and Agricultural Chemistry Analysis* (Vol. 3, pp. 42–241). Beijing: China Agriculture Press.

Brown, J. R. (2003). Ancient horizontal gene transfer. *Nature Reviews. Genetics*, *4*(2), 121–132. https://doi.org/10.1038/nrg1000

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 4516–4522. https://doi.org/10.1073/pnas.1000080107

Chaudhary, P., Sharma, R., Singh, S. B., & Nain, L. (2011). Bioremediation of PAH by *Streptomyces* sp. *Bulletin of Environmental Contamination and Toxicology*, *86*(3), 268–271. https://doi.org/10.1007/s00128-011-0211-5

Coskun, O. K., Ozen, V., Wankel, S. D., & Orsi, W. D. (2019). Quantifying population-specific growth in benthic bacterial communities under low oxygen using (H_2O)-O-18. *ISME Journal*, *13*(6), 1546–1559. https://doi.org/10.1038/s41396-019-0373-4

Coulon, F., McKew, B. A., Osborn, A. M., McGenity, T. J., & Timmis, K. N. (2007). Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. *Environmental Microbiology*, *9*(1), 177–186. https://doi.org/10.1111/j.1462-2920.2006.01126.x

Daebeler, A., Bodelier, P. L. E., Yan, Z., Hefting, M. M., Jia, Z. J., & Laanbroek, H. J. (2014). Interactions between Thaumarchaea, *Nitrospira* and methanotrophs modulate autotrophic nitrification in volcanic grassland soil. *ISME Journal*, *8*(12), 2397–2410. https://doi.org/10.1038/ismej.2014.81

Ding, G. C., Heuer, H., Zuhlke, S., Spiteller, M., Pronk, G. J., Heister, K., et al. (2010). Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes by using a novel PCR detection system. *Applied and Environmental Microbiology*, *76*(14), 4765–4771. https://doi.org/10.1128/AEM.00047-10

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Finkel, O. M., Burch, A. Y., Elad, T., Huse, S. M., Lindow, S. E., Post, A. F., & Belkin, S. (2012). Distance-decay relationships partially determine diversity patterns of phyllosphere bacteria on *Tamarix* trees across the Sonoran Desert. *Applied and Environmental Microbiology*, *78*(17), 6187–6193. https://doi.org/10.1128/AEM.00888-12

Glockner, F. O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., et al. (2017). 25 years of serving the community with ribosomal RNA gene reference databases and tools. *Journal of Biotechnology*, *261*, 169–176. https://doi.org/10.1016/j.jbiotec.2017.06.1198

Handelsman, J. (2004). Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews*, *68*(4), 669–685. https://doi.org/10.1128/MMBR.68.4.669-685.2004

Hanson, C. A., Fuhrman, J. A., Horner-Devine, M. C., & Martiny, J. B. H. (2012). Beyond biogeographic patterns: Processes shaping the microbial landscape. *Nature Reviews Microbiology*, *10*(7), 497–506. https://doi.org/10.1038/nrmicro2795

Harbison, A. B., Price, L. E., Flythe, M. D., & Brauer, S. L. (2017). *Micropepsis pineolensis* gen. nov., sp. nov., a mildly acidophilic alphaproteobacterium isolated from a poor fen, and proposal of *Micropepsaceae* fam. nov. within *Micropepsales* ord. Nov. *International Journal of Systematic and Evolutionary Microbiology*, *67*(4), 839–844. https://doi.org/10.1099/ijsem.0.001681

He, Z. L., Gentry, T. J., Schadt, C. W., Wu, L. Y., Liebich, J., Chong, S. C., et al. (2007). GeoChip: A comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *ISME Journal*, *1*(1), 67–77. https://doi.org/10.1038/ismej.2007.2

Herlemann, D. P. R., Lundin, D., Labrenz, M., Jurgens, K., Zheng, Z. L., Aspeborg, H., & Andersson, A. F. (2013). Metagenomic de novo assembly of an aquatic representative of the verrucomicrobial class Spartobacteria. *MBio*, *4*(3), e00569–e00512. https://doi.org/10.1128/mBio.00569-12

Herrick, J. B., StuartKeil, K. G., Ghiorse, W. C., & Madsen, E. L. (1997). Natural horizontal transfer of a naphthalene dioxygenase gene between bacteria native to a coal tar-contaminated field site. *Applied and Environmental Microbiology*, *63*(6), 2330–2337. https://doi.org/10.1128/AEM.63.6.2330-2337.1997

Hilyard, E. J., Jones-Meehan, J. M., Spargo, B. J., & Hill, R. T. (2008). Enrichment, isolation, and phylogenetic identification of polycyclic aromatic hydrocarbon-degrading bacteria from Elizabeth River sediments. *Applied and Environmental Microbiology*, *74*(4), 1176–1182. https://doi.org/10.1128/AEM.01518-07

Iwai, S., Chai, B. L., Sul, W. J., Cole, J. R., Hashsham, S. A., & Tiedje, J. M. (2010). Gene-targeted-metagenomics reveals extensive diversity of aromatic dioxygenase genes in the environment. *ISME Journal*, *4*(2), 279–285. https://doi.org/10.1038/ismej.2009.104

Jiang, B., Jin, N. F., Xing, Y., Su, Y. P., & Zhang, D. Y. (2018). Unraveling uncultivable pesticide degraders via stable isotope probing (SIP). *Critical Reviews in Biotechnology*, *38*(7), 1025–1048. https://doi.org/10.1080/07388551.2018.1427697

Jiang, L. F., Song, M. K., Yang, L., Zhang, D. Y., Sun, Y. T., Shen, Z. G., et al. (2016). Exploring the influence of environmental factors on bacterial communities within the rhizosphere of the Cu-tolerant plant, *Elsholtzia splendens*. *Scientific Reports*, *6*(1), 36302. https://doi.org/10.1038/Srep36302

Jones, M. D., Crandell, D. W., Singleton, D. R., & Aitken, M. D. (2011). Stable-isotope probing of the polycyclic aromatic hydrocarbon-degrading bacterial guild in a contaminated soil. *Environmental Microbiology*, *13*(10), 2623–2632. https://doi.org/10.1111/j.1462-2920.2011.02501.x

Koubek, J., Mackova, M., Macek, T., & Uhlik, O. (2013). Diversity of chlorobiphenyl-metabolizing bacteria and their biphenyl dioxygenases in contaminated sediment. *Chemosphere*, *93*(8), 1548–1555. https://doi.org/10.1016/j.chemosphere.2013.07.073

Kristiansson, E., Fick, J., Janzon, A., Grabic, R., Rutgersson, C., Weijdegard, B., et al. (2011). Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. *PLoS ONE*, *6*(2), e17038. https://doi.org/10.1371/journal.pone.0017038

Leahy, J. G., & Colwell, R. R. (1990). Microbial-degradation of hydrocarbons in the environment. *Microbiological Reviews*, *54*(3), 305–315. https://doi.org/10.1128/MMBR.54.3.305-315.1990

Leewis, M. C., Uhlik, O., & Leigh, M. B. (2016). Synergistic processing of biphenyl and benzoate: Carbon flow through the bacterial community in polychlorinated-biphenyl-contaminated soil. *Scientific Reports*, *6*(1), 6. https://doi.org/10.1038/Srep22145

Leigh, M. B., Pellizari, V. H., Uhlik, O., Sutka, R., Rodrigues, J., Ostrom, N. E., et al. (2007). Biphenyl-utilizing bacteria and their functional genes in a pine root zone contaminated with polychlorinated biphenyls (PCBs). *ISME Journal*, *1*(2), 134–148. https://doi.org/10.1038/ismej.2007.26

Lemmel, F., Maunoury-Danger, F., Leyval, C., & Cebron, A. (2019). DNA stable isotope probing reveals contrasted activity and phenanthrene-degrading bacteria identity in a gradient of anthropized soils. *FEMS Microbiology Ecology*, *95*(12). https://doi.org/10.1093/femsec/fiz181

Li, J. B., Luo, C. L., Zhang, D. Y., Cai, X. X., Jiang, L. F., & Zhang, G. (2019). Stable-isotope probing-enabled cultivation of the indigenous bacterium *Ralstonia* sp. strain M1, capable of degrading phenanthrene and biphenyl in industrial wastewater. *Applied and Environmental Microbiology*, *85*(14). https://doi.org/10.1128/AEM.00511-19

Liang, Y. T., Van Nostrand, J. D., Deng, Y., He, Z. L., Wu, L. Y., Zhang, X., et al. (2011). Functional gene diversity of soil microbial communities from five oil-contaminated fields in China. *ISME Journal*, *5*(3), 403–413. https://doi.org/10.1038/ismej.2010.142

Liu, P. F., & Conrad, R. (2017). Syntrophobacteraceae-affiliated species are major propionate-degrading sulfate reducers in paddy soil. *Environmental Microbiology*, *19*(4), 1669–1686. https://doi.org/10.1111/1462-2920.13698

Liu, X., Li, J., Zheng, Q., Bing, H. J., Zhang, R. J., Wang, Y., et al. (2014). Forest filter effect versus cold trapping effect on the altitudinal distribution of PCBs: A case study of Mt. Gongga, eastern Tibetan Plateau. *Environmental Science & Technology*, *48*(24), 14,377–14,385. https://doi.org/10.1021/es5041688

Lu, Y. H., & Conrad, R. (2005). In situ stable isotope probing of methanogenic archaea in the rice rhizosphere. *Science*, *309*(5737), 1088–1090. https://doi.org/10.1126/science.1113435

Ma, B., Wang, H. Z., Dsouza, M., Lou, J., He, Y., Dai, Z. M., et al. (2016). Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME Journal*, *10*(8), 1891–1901. https://doi.org/10.1038/ismej.2015.261

Martiny, J. B. H., Eisen, J. A., Penn, K., Allison, S. D., & Horner-Devine, M. C. (2011). Drivers of bacterial beta-diversity depend on spatial scale. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(19), 7850–7854. https://doi.org/10.1073/pnas.1016308108

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., et al. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal*, *6*(3), 610–618. https://doi.org/10.1038/ismej.2011.139

Mueller, J. G., Devereux, R., Santavy, D. L., Lantz, S. E., Willis, S. G., & Pritchard, P. H. (1997). Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology*, *71*(4), 329–343. https://doi.org/10.1023/A:1000277008064

Niepceron, M., Beguet, J., Portet-Koltalo, F., Martin-Laurent, F., Quillet, L., & Bodilis, J. (2014). Low impact of phenanthrene dissipation on the bacterial community in grassland soil. *Environmental Science and Pollution Research*, *21*(4), 2977–2987. https://doi.org/10.1007/s11356-013-2258-9

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). vegan: Community ecology package. R package, The Comprehensive R Archive Network: The R foundation. version 2.5–6. https://CRAN.R-project.org/package=vegan

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

Rivera, M. C., Jain, R., Moore, J. E., & Lake, J. A. (1998). Genomic evidence for two functionally distinct gene classes. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(11), 6239–6244. https://doi.org/10.1073/pnas.95.11.6239

Schauer, R., Bienhold, C., Ramette, A., & Harder, J. (2010). Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. *ISME Journal*, *4*(2), 159–170. https://doi.org/10.1038/ismej.2009.106

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Schmidt, O., Horn, M. A., Kolb, S., & Drake, H. L. (2015). Temperature impacts differentially on the methanogenic food web of cellulose-supplemented peatland soil. *Environmental Microbiology*, *17*(3), 720–734. https://doi.org/10.1111/1462-2920.12507

Sharma, A., Singh, S. B., Sharma, R., Chaudhary, P., Pandey, A. K., Ansari, R., et al. (2016). Enhanced biodegradation of PAHs by microbial consortium with different amendment and their fate in in-situ condition. *Journal of Environmental Management*, *181*, 728–736. https://doi.org/10.1016/j.jenvman.2016.08.024

Shiau, Y. J., Cai, Y. F., Jia, Z. J., Chen, C. L., & Chiu, C. Y. (2018). Phylogenetically distinct methanotrophs modulate methane oxidation in rice paddies across Taiwan. *Soil Biology & Biochemistry*, *124*, 59–69. https://doi.org/10.1016/j.soilbio.2018.05.025

Singleton, D. R., Hu, J., & Aitken, M. D. (2012). Heterologous expression of polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes from a novel pyrene-degrading Betaproteobacterium. *Applied and Environmental Microbiology*, *78*(10), 3552–3559. https://doi.org/10.1128/AEM.00173-12

Singleton, D. R., Hunt, M., Powell, S. N., Frontera-Suau, R., & Aitken, M. D. (2007). Stable-isotope probing with multiple growth substrates to determine substrate specificity of uncultivated bacteria. *Journal of Microbiological Methods*, *69*(1), 180–187. https://doi.org/10.1016/j.mimet.2006.12.019

Song, M. K., Jiang, L. F., Zhang, D. Y., Luo, C. L., Wang, Y., Yu, Z. Q., et al. (2016). Bacteria capable of degrading anthracene, phenanthrene, and fluoranthene as revealed by DNA based stable-isotope probing in a forest soil. *Journal of Hazardous Materials*, *308*, 50–57. https://doi.org/10.1016/j.jhazmat.2016.01.009

Song, M. K., Luo, C. L., Jiang, L. F., Zhang, D. Y., Wang, Y. J., & Zhang, G. (2015). Identification of benzo[a]pyrene-metabolizing bacteria in forest soils by using DNA-based stable-isotope probing. *Applied and Environmental Microbiology*, *81*(21), 7368–7376. https://doi.org/10.1128/AEM.01983-15

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., et al. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*(7681), 457–463. https://doi.org/10.1038/nature24621

Tu, Q. C., Yu, H., He, Z. L., Deng, Y., Wu, L. Y., Van Nostrand, J. D., et al. (2014). GeoChip 4: A functional gene-array-based high-throughput environmental technology for microbial community analysis. *Molecular Ecology Resources*, *14*(5), 914–928. https://doi.org/10.1111/1755-0998.12239

Wagner, M., & Horn, M. (2006). The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. *Current Opinion in Biotechnology*, *17*(3), 241–249. https://doi.org/10.1016/j.copbio.2006.05.005

Wang, H., Wang, B., Dong, W. W., & Hu, X. K. (2016). Co-acclimation of bacterial communities under stresses of hydrocarbons with different structures. *Scientific Reports*, *6*(1), 34588. https://doi.org/10.1038/Srep34588

Wang, W. P., Zhong, R. Q., Shan, D. P., & Shao, Z. Z. (2014). Indigenous oil-degrading bacteria in crude oil-contaminated seawater of the Yellow Sea, China. *Applied Microbiology and Biotechnology*, *98*(16), 7253–7269. https://doi.org/10.1007/s00253-014-5817-1

Weber, E. B., Lehtovirta-Morley, L. E., Prosser, J. I., & Gubry-Rangin, C. (2015). Ammonia oxidation is not required for growth of Group 1.1c soil Thaumarchaeota. *FEMS Microbiology Ecology*, *91*(3). https://doi.org/10.1093/femsec/fiv001

Werner, J. J., Koren, O., Hugenholtz, P., DeSantis, T. Z., Walters, W. A., Caporaso, J. G., et al. (2012). Impact of training sets on classification of high-throughput bacterial 16s rRNA gene surveys. *ISME Journal*, *6*(1), 94–103. https://doi.org/10.1038/ismej.2011.82

Wilson, M. S., Herrick, J. B., Jeon, C. O., Hinman, D. E., & Madsen, E. L. (2003). Horizontal transfer of phnAc dioxygenase genes within one of two phenotypically and genotypically distinctive naphthalene-degrading guilds from adjacent soil environments. *Applied and Environmental Microbiology*, *69*(4), 2172–2181. https://doi.org/10.1128/AEM.69.4.2172-2181.2003

Xiong, W. G., Wang, Y. L., Sun, Y. X., Ma, L. P., Zeng, Q. L., Jiang, X. T., et al. (2018). Antibiotic-mediated changes in the fecal microbiome of broiler chickens define the incidence of antibiotic resistance genes. *Microbiome*, *6*(1), 6. https://doi.org/10.1186/s40168-018-0419-2

Yergeau, E., Kang, S., He, Z., Zhou, J., & Kowalchuk, G. A. (2007). Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. *ISME Journal*, *1*(2), 163–179. https://doi.org/10.1038/ismej.2007.24

Zheng, Q., Nizzetto, L., Mulder, M. D., Sanka, O., Lammel, G., Li, J., et al. (2014). Does an analysis of polychlorinated biphenyl (PCB) distribution in mountain soils across China reveal a latitudinal fractionation paradox? *Environmental Pollution*, *195*, 115–122. https://doi.org/10.1016/j.envpol.2014.08.021

Zhou, J. Z., Kang, S., Schadt, C. W., & Garten, C. T. (2008). Spatial scaling of functional gene diversity across various microbial taxa. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(22), 7768–7773. https://doi.org/10.1073/pnas.0709016105

Zhou, J. Z., Xue, K., Xie, J. P., Deng, Y., Wu, L. Y., Cheng, X. H., et al. (2012). Microbial mediation of carbon-cycle feedbacks to climate warming. *Nature Climate Change*, *2*(2), 106–110. https://doi.org/10.1038/Nclimate1331