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Contrasting microbial functional genes in two distinct saline-alkali and slightly acidic oil-contaminated sites ☆

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HIGHLIGHTS

- Microbial diversity was distinctly different in two oil contaminated sites.
- Lower abundance of functional genes was detected in saline-alkali soils.
- Soil pH was a major factor influencing the microbial distribution pattern.

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ABSTRACT

To compare the functional gene structure and diversity of microbial communities in saline-alkali and slightly acidic oil-contaminated sites, 40 soil samples were collected from two typical oil exploration sites in North and South China and analyzed with a comprehensive functional gene array (GeoChip 3.0). The overall microbial pattern was significantly different between the two sites, and a more divergent pattern was observed in slightly acidic soils. Response ratio was calculated to compare the microbial functional genes involved in organic contaminant degradation and carbon, nitrogen, phosphorus, and sulfur cycling. The results indicated a significantly low abundance of most genes involved in organic contaminant degradation and in the cycling of nitrogen and phosphorus in saline-alkali soils. By contrast, most carbon degradation genes and all carbon fixation genes had similar abundance at both sites. Based on the relationship between the environmental variables and microbial functional structure, pH was the major factor influencing the microbial distribution pattern in the two sites. This study demonstrated that microbial functional diversity and heterogeneity in oil-contaminated environments can vary significantly in relation to local environmental conditions. The limitation of nitrogen and phosphorus and the low degradation capacity of organic contaminant should be carefully considered, particularly in most oil-exploration sites with saline-alkali soils.

1. Introduction

The ongoing global increase in oil consumption has resulted in the frequent occurrence of oil contamination as a result of exploration, production, maintenance, transportation, storage, and accidental release. Hundreds of micrograms of oil per gram of soil were detected in some heavily oil-contaminated sites (Liang et al., 2009). Oil contamination decreases soil permeability and inhibits oxygen transportation to plant roots, resulting in plant injury or death. Mutagenic components, such as polycyclic aromatic hydrocarbons (PAHs), also present a high risk to the environment.

Understanding the functional structure of the microbial community and the factors influencing microbial functions is important for the bioremediation of oil-contaminated sites. Previous studies demonstrated that changes in soil microbial communities in contaminated sites are strongly correlated with the differences in contaminant components and concentrations, as well as with soil geochemical variables, such as nitrogen and phosphorus nutrients, water content, and soil types (Atlas, 1981; Van Hamme et al., 2003; Hamamura et al., 2006; Allen et al., 2007). However, most studies have focused on microbial communities in neutral soils. Soil pH and alkalinity strongly influenced the composition and diversity of soil microbial communities, as well as ecological functions in some cases (Fierer and Jackson, 2006; Kleinsteuber

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et al., 2006; Lauber et al., 2009; Rousk et al., 2010). These factors are rarely studied in oil-contaminated sites. Metabolically active microorganisms in acidic or saline-alkali environments are of interest because of their unique ecology and physiology, as well as for their potential applications in bioremediation. For example, hydrocarbon-degrading acidophiles are potential microorganisms for the bioremediation of contaminated acidic environments (Hamamura et al., 2005). A better understanding of microbial suitability and function in natural environments is necessary for successful bioremediation.

Microbial functional genes provide direct information about the functionality of microbial communities in the environment. The presence or absence of genes involved in the degradation of specific compounds in hydrocarbon-contaminated environments can be used as indicators of the biodegradation potential of the respective compounds, as well as of bioavailability and transport in the environment (Stapleton and Sayler, 2000; Marlowe et al., 2002; Mesarch et al., 2004; Tuomi et al., 2004). Change in the abundances of contaminant degrading genes is correlated with a decrease in contaminant concentrations, as well as to the efficiency at which the hydrocarbons are mineralized (Fleming et al., 1993; Park and Crowley, 2006; Salminen et al., 2008). In some PAH-contaminated soils and sediments, the abundance or expression of naphthalene degrading genes has also been correlated with naphthalene concentrations (Dionisi et al., 2004; Cébron et al., 2008). Functional genes are useful in monitoring the dynamics of contaminant-degrading bacteria in microcosms (Ringelberg et al., 2001; Sei et al., 2003). Recent studies showed that these genes are also useful in evaluating the effect of different bioremediation treatments on hydrocarbon biodegradability (Cavalca et al., 2004; Baldwin et al., 2008). All these previous studies indicated that environmental conditions significantly affect microbial functional genes.

The extreme diversity and complexity of microbial communities and their yet uncultured characteristics inhibit comprehensive detection, characterization, and quantification of microbial functional genes in oil-contaminated sites. The recently developed metagenomic analysis involves the direct isolation of nucleic acids from environmental samples followed by high-throughput DNA sequencing or microarray and data processing. This analysis provides a robust method by which to detect all organisms of interest as a whole and understanding their communities more comprehensively. Metagenomic analysis can be used to assess the effects of oil on microbial communities under different environmental conditions by monitoring the changes in DNA sequences and sequence diversities.

In this study, we examined the changes in microbial functional genes using a robust metagenomic tool, GeoChip 3.0 (He et al., 2010) in saline-alkali and slightly acidic oil-contaminated soils to discuss the following: (1) the distribution pattern of microbial functional genes in saline-alkali and slightly acidic oil-contaminated soils; (2) the differences among the microbial functional groups involved in organic contaminant degradation and cycling of carbon, nitrogen, phosphorus, and sulfur in the two sites; and (3) the relationship between the microbial functional diversity and environmental variables.

2. Material and methods

2.1. Site description and sampling

Forty soil samples were collected from two oil-contaminated sites: Shengli oilfield (SL, 20 samples) in the Yellow River area in North China and Baise oilfield (BS, 20 samples) in South China. The geoclimate and soil geochemicals of the two sites significantly vary. SL (37°28′N, 118°29′E) has a warm temperate continental semi-humid monsoon climate with a mean annual rainfall of 550 mm. BS (23°43′N, 107°04′E) has a subtropical humid monsoon climate with a mean annual rainfall of 1159.8 mm. The oil concentration, along with the following soil physical and chemical parameters, was measured: pH, water content, total nitrogen (TN, nitrogen in all organic and inorganic forms), available nitrogen (N, NO_3^--N, NO_2^--N and NH_4^+-N), total phosphorus (TP, phosphorus in all organic and inorganic forms), available phosphorus (P, PO_4^{3-}-P), total organic carbon (TOC), soluble salts, and soil texture. The detailed sampling procedure and soil geochemical variables could be found in a previous study (Liang et al., 2012).

2.2. Soil microbial DNA extraction and GeoChip hybridization

Microbial genomic DNA was extracted from 5 g of well-mixed soil for each sample using a combination of freeze-grinding and sodium dodecyl sulfate for cell lysis, as previously described (Zhou et al., 1996). Crude DNA was purified by agarose gel electrophoresis, followed by phenol–chloroform–butanol extraction (Moore and Dowhan, 2002). The purified DNA was quantified using agarose gel electrophoresis, ND-1000 spectrophotometer (Nanodrop Inc., Wilmington, DE, USA), and Quant-It PicoGreen kit (Invitrogen, Carlsbad, CA, USA). An aliquot of 2 μg of DNA from each sample was directly labeled, purified, and resuspended in 50 μL of hybridization solution, which contained 45% formamide, 5× SSC, 0.1% SDS, 0.1 mg/mL of Salmon sperm DNA, and 2 μL of common oligonucleotide reference standard (0.1 pmol/μL) (Wu et al., 2006; Liang et al., 2010). The fluorescence-labeled DNA was hybridized with GeoChip 3.0 on a MAUI® Hybridization System (BioMicro Systems, Salt Lake City, UT, USA) at 42 °C for 12 h. Microarrays were scanned using a ScanArray 5000 microarray analysis system (PerkinElmer, Wellesley, MA, USA) at 95% laser power and 68% photomultiplier tube gain. Scanned images were saved in 16-bit TIFF format, quantified using an ImaGene® version 6.0 (BioDiscovery, Inc., Los Angeles, CA, USA), and processed in the Microarray Data Manager system in the Institute for Environmental Genomics website (http://ieg.ou.edu/microarray). Spots with signal-to-noise ratios lower than 2.0 were removed prior to statistical analysis, as previously described (He et al., 2010).

2.3. Data analysis

All SL and BS samples (40 samples) were analyzed to compare microbial functional diversity. Detrended correspondence analysis (DCA) was used to represent all samples in ordination space. Dissimilarity test of the microbial functional genes between the two sites was performed using three non-parametric multivariate statistical tests, analysis of similarities (ANOSIM), non-parametric multivariate analysis of variance (MANOVA) with the adonis function, and multiple response permutation procedure (MRPP), a nonparametric procedure independent of assumptions, such as normally distributed data and homogeneous variances, but is dependent on the internal variability of data. The community dispersion among the two fields was subjected to the permutational analysis of dispersion (Anderson et al., 2006). Response ratio of the genes functioning in organic contaminant degradation and cycling of carbon, nitrogen, phosphorus, and sulfur was calculated between the alkaline and acidic samples (Luo et al., 2006). Subsets of soil geochemical variables and oil concentration were selected for canonical correspondence analysis (CCA) to reduce noise. Variances in inflation factors and Bio-Env procedure were used to select environmental variables for CCA modeling. All analyses were performed in R version 2.15.2 (http://www.r-project.org/) with vegan and ecodist packages.

3. Results

3.1. Soil geochemical and overall microbial functional patterns

Soil geochemical characteristics considerably varied between the two oil-contaminated sites (Liang et al., 2012). Soils were slightly acidic in BS with pH of 5.5 to 6.5, but were saline-alkali in SL with pH of 8.0 to 8.9 and salinity of 0.1 to 4.5% NaCl (1% an average). In addition, soils in BS had higher TOC, nitrogen and phosphorus than the soils in SL. Oil contamination in BS and SL ranged from 12 mg/g to 168 mg/g with

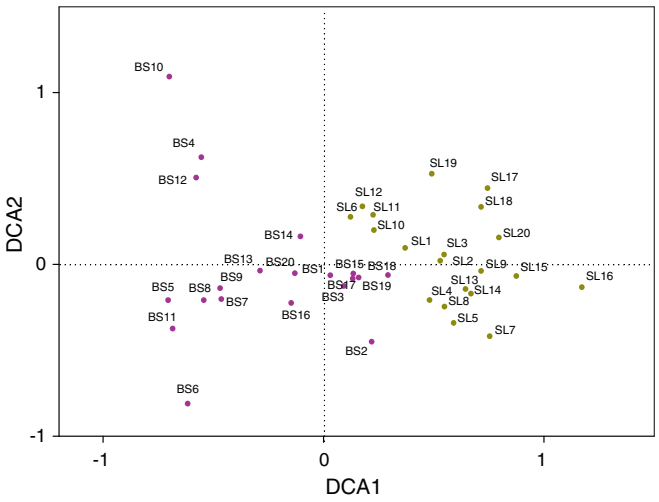

Fig. 1. Detrended correspondence analysis (DCA) of all functional genes in alkaline (SL) and acid (BS) oil contaminated sites.

an average of 74 mg/g, and 13 mg/g to 183 mg/g with an average of 80 mg/g, respectively.

The number of detected bacterial genes was significantly higher in BS than in SL ($t = 2.07$, $P < 0.05$). However, the number of detected fungal genes exhibited no significant difference between the two sites. DCA of all functional genes was performed to determine the overall pattern of soil microbial functional genes in alkaline and acidic soils in oil-contaminated sites (Fig. 1). The samples in the two sites were clearly separated from one another. Three non-parametric multivariate statistical tests, ANOSIM, adonis and MRPP, confirmed that the difference in community structures was significant (Table 1). The samples in SL were relatively tightly clustered, whereas a more divergent pattern was observed in BS, indicating dissimilar microbial community pattern in the two sites. Divergence in microbial composition and structure was also evident in the results of permutational analysis of dispersion, in which the microbial dispersion was significantly higher in BS than in SL ($P = 0.007$).

3.2. Comparison of key functional genes in alkaline and slightly acidic soils

The changes in the gene groups observed in organic contaminant degradation and cycling of carbon, nitrogen, phosphorus and sulfur were examined in detail. Response ratios were calculated to reveal the gene changes in the two oil-contaminated sites (Fig. 2). A positive value indicated an increase in gene group abundance in alkaline to slightly acidic soils, whereas a negative value indicated the opposite. Three CIs (90%, 95% and 99%) were used to examine the significance of changes. In oil-contaminated sites, organic compounds, such as alkanes, aromatics, alicyclics and branched hydrocarbons, were major contaminants. Thus, we first compared the related genes in terms of organic contaminant degradation in the two sites. In total, the response ratios ranged from −0.86 to 1.27 (−0.29 in average), indicating a low abundance of most organic degradation genes in saline-alkali soils. For example, the gene groups of *alkB*, *alkH*, and *alkK* involved in alphatic hydrocarbon degradation, *xylC*, *catB*, *bphA*, *bphB*, *bphD*, and *phd* involved in aromatic degradation; and *phaB*, *pobA*, *nagG*, *nagI*, *nagK*, *mhpA*, and *mhpC* involved in aromatic carboxylic acid degradation showed a significantly lower abundance in saline-alkali soils in the SL site than in the slightly acidic soils of the BS site. Only *tomA* and *xylG* genes were high in abundance in the SL site.

For carbon, nitrogen, sulfur, and phosphorus cycling genes, the response ratios ranged from −1.19 to 0.42 (−0.32 in average). Most genes (79.5%) in carbon cycling exhibited no significant difference in the two sites. Exoglucanase for cellulose degradation, *pulA* for starch degradation, and pectinase for pectin degradation, as well as limonene epoxide hydrolase (*limEH*), alkylsuccinate synthase (*assA*), and vanillin dehydrogenase (*vdh*) for the metabolism of other carbon compounds, were significantly different in the two sites. Interestingly, all four gene-coding carbon-fixation enzymes (ribulose-l,5-bisphosphate carboxylase/oxygenase for the Calvin cycle, carbon monoxide dehydrogenase for the reductive acetyl-CoA pathway, propionyl-CoA/acetyl-CoA carboxylase for the 3-hydroxypropionate/malyl-CoA cycle, and ATP citrate lyase for the reductive acetyl-CoA pathway) had similar abundances in both sites. Nitrogen-cycling genes: *ureC* and *gdh* for ammonification; *narG*, *nirS/K*, and *nos* for denitrification; *nifH* for nitrogen fixation; and *nrfA* and *nirA* for nitrogen reduction, had significantly lower signal intensities in the SL samples (Figs. 2b and 3). For phosphorus utilization, *ppk* and *ppx* encoding polyphosphate kinase and exopolyphosphatase, respectively, also exhibited lower abundance in SL, indicating a potentially lower capacity of indigenous microbes in modulating the formation and metabolism of cellular poly(P) reserves to environmental phosphorus limitation. In addition, *dsrA/B* for sulfite reductase was in lower abundance in SL.

Table 1
Significance tests of dissimilarity of the overall microbial functional structure in the two sites with three different statistical approaches.

Microbial functional genes		
Statistical approaches	Parameters	*P*
ANOSIM*	$R = 0.122$	0.014
Adonis**	$F = 9.348$	<0.001
Mrpp***	$\delta = 0.705$	0.012

All three tests are based on 999 permutations.
* Analysis of similarities ANOSIM.
** Non-parametric multivariate analysis of variance (MANOVA) with the adonis function.
*** A nonparametric procedure that does not depend on assumptions such as normally distributed data and homogeneous variances, but rather depends on the internal variability of the data.

3.3. Relationship between community structure and environmental variables

CCA was performed to identify possible linkages between the microbial functional structure and soil geochemical parameters (Fig. 4). Of all the environmental variables, only the significant geochemical parameters were included (oil concentration, pH, TN, available phosphorus, salt, water, clay, Cu and Zn based on the variances in inflation factors and Bio-Env procedure). For all functional genes, 31.6% of the total variance can be explained by the first two constrained axes, with the first axis explaining 25.0%. The specified CCA model was significant ($P = 0.020$). The first axis was positively correlated with pH and salt but negatively correlated with water, Zn, Cu, and available phosphorus, whereas the second axis was positively correlated with oil and TN and negatively correlated with clay. As expected, the samples in each site were grouped separately. Based on the relationship between the environmental variables and microbial functional structure, pH was a major factor that influenced the microbial distribution pattern. The SL samples were closely clustered with higher pH, whereas BS samples were clustered with lower pH. In addition, TN, available phosphorus and Zn were key factors that manipulated the microbial functional structure in BS, where soils were richer in nutrient contents.

4. Discussion

Although the well-known hydrocarbon-degrading microorganisms, such as *Pseudomonas*, *Rhodococcus*, *Caulobacter* and *Sphingomonads*, have been found to be ubiquitous in hydrocarbon-contaminated soil and water environments, several studies indicated that overall microbial populations differ with environmental conditions (Van Hamme et al., 2003; Head et al., 2006; Hazen et al., 2010; Sutton et al., 2013). A spiked experiment showed that indigenous microbial communities respond

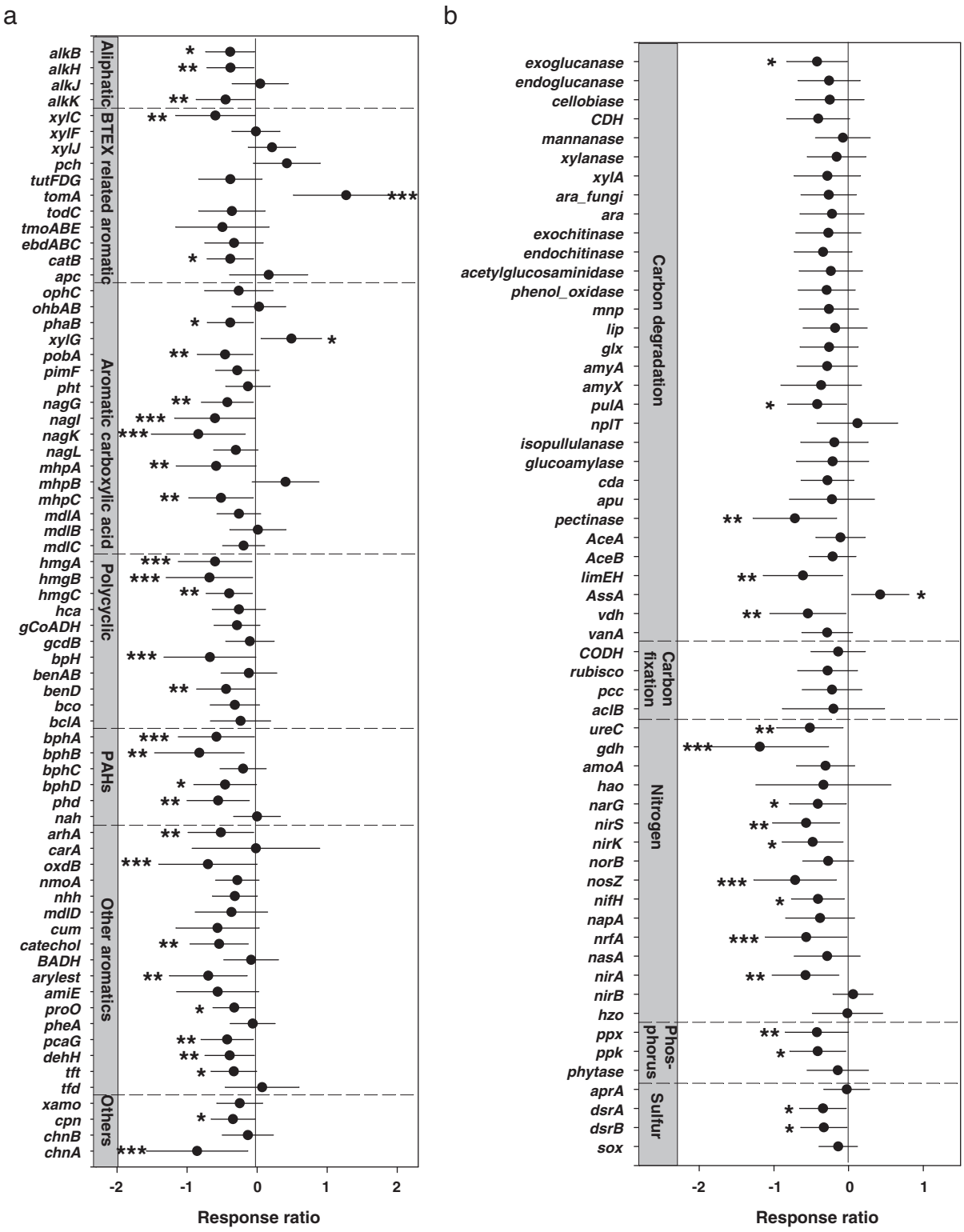

Fig. 2. Response ratios (mean ± SE for each gene) of genes in alkaline soils to acid soils in oil contaminated sites, (a) organic contaminant degradation-related genes and (b) carbon, nitrogen, phosphorus, and sulfur cycling-related genes. *Significant at 90% CI; **significant at 95% CI; ***significant at 99% CI.

differently to crude oil contamination across different soil types, even with other parameters (e.g., water potential, nutrients, temperature, and aeration) held constant (Hamamura et al., 2006). Our previous study showed that in-situ microbial community structures distinctly differ in five oil-contaminated sites (Liang et al., 2011). The endemism of microbial communities indicated that a variety of optimized strategies might be required to stimulate and maintain the desired populations based on the local conditions for in-situ bioremediation.

Many oilfields are located in semiarid regions characterized by soils with high natural salinity and alkalinity, particularly in the area with dry climate and high groundwater level. Microbial communities have been highly influenced by soil salinity, alteration of microbial structure, and

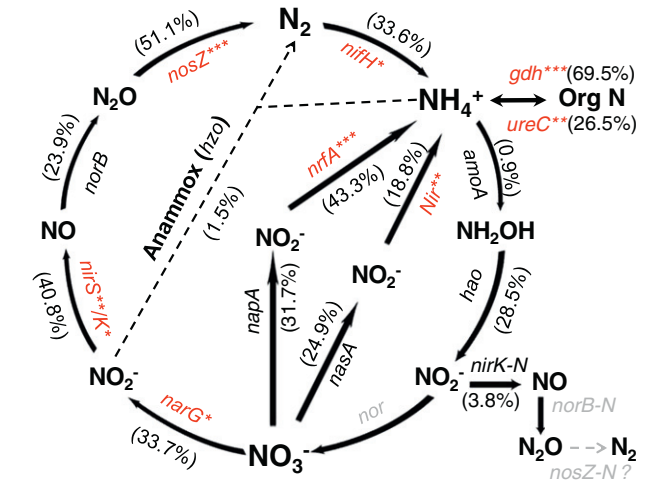

Fig. 3. Comparison of gene changes involved in the nitrogen cycling in the two sites. The signal intensity for each gene detected was normalized by all detected gene sequences using the mean. The percentage of a functional gene in a bracket was the sum of signal intensity of all detected sequences of this gene divided by the grand sum of signal intensity of the detected nitrogen cycle genes, and weighted by the fold change of the signal intensity of this gene in BS site to that in SL site. For each functional gene, red indicates that this gene had significantly higher signal intensity in BS than in SL. Gray-colored genes were not targeted by this GeoChip, or not detected in those samples. The presence of *nosZ* homologs in nitrifiers remains unknown.

reduction in functional diversity with increasing salinity (Zahran, 1997; Pankhurst et al., 2001). In this study, microbial organic contaminant degradation genes were significantly low in saline-alkali soils, indicating their relatively low organic contaminant degradation potential. Bacterial communities in saline environments may include extreme (halophilic) and facultative (halotolerant) bacteria. Accumulated evidence has shown that these bacteria are essential elements in the saline environment because of their activity such as the degradation of contaminants. Al-Mailem et al. (2010) reported that two halophilic *Marinobacter* strains successfully mineralized crude oil in hypersaline soils. Moreover, the activity of catechol 1,2 dioxygenase of the ortho-cleavage pathway and the genes of halophilic bacteria were detected in a previous study (Erdogmus et al., 2013). In the current research, a total of 20 functional genes were detected in the saline-alkali oil-contaminated

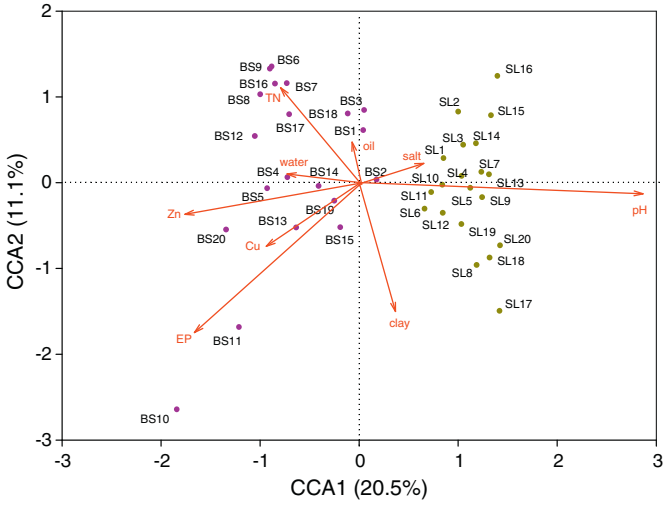

Fig. 4. Canonical correspondence analysis (CCA) of total functional genes and soil geochemical variables: oil concentration, pH, total nitrogen (TN), available phosphorus (AP), salt, water, clay, Cu, and Zn in alkaline (SL) and slightly acidic (BS) oil-contaminated sites. The percentage of variation explained by each axis is shown, and the relationship is significant ($P = 0.016$).

site. These genes were derived from halophilic bacteria as *Marinobacter* sp. ELB17, *Marinobacter aquaeolei* VT8, and *Marinobacter algicola* DG893. Halotolerant bacteria, particularly hydrocarbon-degrading microorganisms that have adapted to grow and thrive in saline-alkali environments, serve an important function in the biological treatment of hydrocarbon-contaminated sites. Kleinsteuber et al. (2006) studied the diversity and dynamics of a bacterial community extracted from an exploited oilfield with high natural soil salinity, and observed a clear community shift responsible for the microbial adaptation of saline environments. In this study, several functional genes involved in organic contaminant degradation, such as *alkB*, *alkK*, catechol, *mdlA*, *mdlC*, *nmoA*, *pcaG*, and *pimF*, were detected in saline-alkali soils of the SL oil exploration site. These functional genes were mainly derived from *Mycobacterium*, *Bacillus*, *Rhodococcus*, *Sphingomonas*, *Pseudomonas*, *Flavobacterium*, *Ralstonia*, and so on, indicating the potential of these microbes in treating oil pollution in saline-alkali soils.

By contrast, most soils in South China are acidic and acid rain erosion accelerates the acidification process. Compared with neutral soils, microbial diversity is lower in acidic soils at the continental scale (Fierer and Jackson, 2006). The overall bacterial community compositions are significantly affected by changes in the relative abundances of *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* (Lauber et al., 2009). In this study, the abundant functional genes in the oil-contaminated soils in BS were mainly derived from hydrocarbon-degrading microbes, such as *Mycobacterium*, *Pseudomonas*, *Ralstonia*, *Rhodococcus*, *Burkholderia*, *Bradyrhizobium*, *Aspergillus*, *Sphingomonas*, *Azotobacter*, *Bacillus*, *Polaromonas*, and so on, indicating the oil-degrading potential in slightly acidic soils. The biodegradation of hydrocarbons in acidic soils has been reported previously. Petroleum hydrocarbon utilizers were found in a tropical, acidic forest soil (pH varied from 4 to 6) 17 years after an extensive oil spillage (Amadi et al., 1996). The presence of an *alkB* gene homologue indicates the alkane-degrading capability of the microbial populations that are indigenous to acidic hydrocarbon seep soils (Hamamura et al., 2005). In addition to the dominant hydrocarbon-degrading microbes, we also observed that several functional genes related to contaminant degradation were derived from *Acidiphilium*, *Acidobacteria*, *Acidothermus* and so on, indicating the potential function of acidophilic microorganisms in oil-contaminated soils. Stapleton et al. (1998) reported the biodegradation of aromatic hydrocarbons and PAHs in extremely acidic environments, and 16S rRNA gene sequence analyses suggested the presence of acidophilic bacteria in the soil samples. However, a microbial consortium, including eukaryotes, rather than individual acidophiles was suggested to be involved in the biodegradation in the acidic environment. Hamamura et al. (2005) described the bacterial communities associated with natural hydrocarbon seeps in acidic soils at Rainbow Springs, and the majority of the sequences recovered (>75%) are related to the sequences of heterotrophic acidophilic bacteria, including *Acidisphaera* spp. and *Acidiphilium* spp. of the α-Proteobacteria. Compared with saline-alkali oil-contaminated soils, most functional genes in organic contaminant degradation were significantly higher in abundance in slightly acidic oil-contaminated soils. One possible reason for the higher organic contaminant degradation genes detected in the BS site is the lower soil salinity. High salinities limit the microbial access to hydrocarbon and the availability of oxygen (Al-Mailem et al., 2010). In addition, soils in the BS site were richer in nitrogen and phosphorus, which are important for microbial metabolic activities and hydrocarbon biodegradation (Van Hamme et al., 2003).

Oil exposure has been proven to alter essential microbial biogeochemical cycling processes (Haines et al., 1981). Our previous study also indicated a reduction in several functional genes in the families *pgl*, *rbcL*, *nifH*, and *nor*, as well as those encoding cellulase, laccase, chitinase, urease, and key enzymes in organic metabolization, particularly under high contamination stress (Liang et al., 2009). In this study, we observed that microbial functional genes involved in the cycling of carbon, nitrogen, phosphorus and sulfur varied in different oil-contaminated sites, depending on local environmental conditions. The nitrogen fixation potential of the indigenous microbial communities in oil-contaminated sites is

interesting and important for bioremediation. Nitrogen limitation is always considered as one of the key factors that influences bioremediation of organic compounds (Van Hamme et al., 2003; Head et al., 2006). Thus, the potential for nitrogen fixation could be important for biodegradation. In this work, the *nifH* gene functioning in nitrogen fixation exhibited significantly lower abundance in the SL site. In addition, several other genes involved in nitrogen cycling were also lower in abundance in SL. Previous studies reported that the mineralization and immobilization of nitrogen, nitrification, and ammonification are decreased in saline soils (Bandyopadhyay and Bandyopadhyay, 1983; Wollenweber and Zechmeister–Boltenstern, 1989). Another study also indicated that oil contamination can reduce microbial nitrogen fixation and denitrification rate (Haines et al., 1981). In this study, the inhibition on nitrogen cycling could be more serious in oil-contaminated sites with saline-alkali soils in SL, suggesting that nitrogen-addition strategies may vary in different geographic oil-contaminated sites during the bioremediation process.

Interestingly, most genes in carbon degradation and all genes in carbon fixation were similar in abundance in saline-alkali and slightly acidic sites. However, the soil geochemical variables and regional climate varied considerably in the two sites. This finding might be attributed to the high resistance of soil fungi, which was evident in the non-significant difference in fungal gene number between the two sites. Soil fungi serve important functions in carbon cycling under extreme environments because of their capability to colonize, penetrate, and spread in various soils (Novotny et al., 1999; Husaini et al., 2008). Rousk et al. (2010) reported that the relative abundance of soil fungi is unaffected by pH, and that fungal diversity is weakly related to pH. Our previous study also indicated that fungi are more resistant to severe oil contamination than bacteria and archaea in an oil-contaminated site (Liang et al., 2009). In this study, the genes involved in carbon cycling in both sites were mainly derived from *Aspergillus*, *Botryotinia*, *Chaetomium*, *Coprinopsis*, *Hypocrea*, *Magnaporthe*, *Penicillium*, *Trichoderma*, *Pleurotus*, *Rhizopus*, *Piloderma*, *Polyporus* and so on, some of which are able to degrade hydrocarbons (Bezalel et al., 1996; Lotfinasabasl et al., 2012).

This study compared the microbial functional gene diversity and gene changes in organic contaminant degradation and in the cycling of carbon, nitrogen, phosphorus, and sulfur in saline-alkali and slightly acidic soils from the two oil-contaminated sites. This research elucidated microbial functional diversity in contaminated environments, which is important for understanding the microbial community for the bioremediation of oil-contaminated sites. Further studies may be focused on comparisons of active indigenous microorganisms in different oil-contaminated sites using DNA stable isotope probing integrated with metagenomics and detection of responsive genes by analyzing microbial messenger RNA on a meta-transcriptomic level.

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