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Long-term oil contamination increases deterministic assembly processes in soil microbes

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Abstract. The mechanisms that drive microbial turnover in time and space have received considerable attention but remain unclear, especially for situations with anthropogenic perturbation. To understand the impact of long-term oil contamination on microbial spatial turnover, 100 soil samples were taken from five oil exploration fields located in different geographic regions across China. The microbial functional diversity was analyzed with a high-throughput functional gene array, GeoChip. Our results indicated that soil microbial α-diversity (richness and Shannon diversity index) decreased significantly with contamination. All contaminated and uncontaminated samples exhibited significant spatial autocorrelation between microbial community similarity and spatial distance, as described by a distance–decay relationship (DDR). However, long-term oil exposure flattened the slopes of the DDRs of all of the functional genes and each functional group involved in C/N/P/S cycling, particularly of those involved in contaminant degradation. The relative importance of deterministic and stochastic processes in microbial assembly was determined. The decrease in microbial spatial turnover with long-term oil contamination was coupled with an increase in the proportion of deterministic processes that structured microbial assembly based on null model analysis. The results indicated long-term oil contamination significantly affects soil microbial community spatial structure by acting as an environmental filter to decrease the regional differences distinguishing soil microbial communities.

Key words: *deterministic processes; distance–decay relationship; microbial turnover; oil contamination; perturbation; stochastic processes.*

Introduction

Over the last few decades, biogeographic patterns have been recognized among microbes in various habitats (Green and Bohannan 2006, Martiny et al. 2006, Ramette and Tiedje 2007, Zinger et al. 2013). The classical ecological theories that measure the "variation or rate of species turnover," such as the distance–decay relationship (how community composition changes with geographic distance; Green et al. 2004, Martiny et al. 2011) and the taxa–area relationship (the tendency that species richness increases with area; Green et al. 2004, Horner-Devine et al. 2004, Zhou et al. 2008) have been applied to a wide range of microbial communities. Beyond the biogeographic patterns, the mechanisms driving the turnover of microbes in space and time have not been easy to uncover, even in field work dedicated to the question (Hanson et al. 2012, Nemergut et al. 2013). Deterministic processes driven by contemporary environmental heterogeneity (Fierer and Jackson 2006, Lozupone and Knight 2007, Ranjard et al. 2013) and stochastic processes driven by dispersal limitation (Woodcock et al. 2007, Peay et al. 2010, Caruso et al. 2011, Martiny et al. 2011) are both considered important in structuring microbial communities. The combined effects of determinism and stochasticity on the structure of microbial communities have been the topic of recent studies (Dumbrell et al. 2010, Ofiteru et al. 2010, Langenheder and Szekely 2011, Wang et al. 2013, Zhou et al. 2014), but their relative importance remains difficult to resolve (Chase et al. 2011).

Intense anthropogenic activities result in strong perturbations of the diversity, composition, and ecological functions of both macrobes and microbes (Nogales et al. 2011, Vačkář et al. 2012). Recent research also implied that anthropogenic disturbances may affect organisms' fundamental distribution pattern. For example, fishing significantly depressed the slope of the species–area relationship in reef fish assemblages (Tittensor et al. 2007). Long-term mechanical perturbation attenuated the spatial structure of soft-bottom meiofaunal communities (Boldina et al. 2014). The impact on

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microbial spatial pattern has also been reported in a few studies, such as one on the effect of grass land management on soil bacterial and fungal communities (Sayer et al. 2013) and another on the effect of farming practices on arbuscular mycorrhizal fungi (van der Gast et al. 2011). However, the mechanisms by which disturbance drives microbial β-diversity (variation in community composition) are still unknown. How do stochastic vs. deterministic processes change microbial assemblages under anthropogenic perturbations? This question is important for maintaining microbial diversity and spatial structure, which are essential for protecting the biological patrimony and ecosystem services of the soil, and also key to predict ecosystem responses to further environmental changes.

Contamination is another type of undesirable anthropogenic perturbation. Various types of organic and/or inorganic contamination have significantly impacted ecological environments (Giller et al. 1998, Kingston 2002, Peterson et al. 2003). Oil contamination has occurred often around the world in a variety of regions, including the sea, offshore areas, coastal beaches, and land. Spills can occur during exploration, transportation, storage, usage, or accidents and pose a great threat to ecological safety and human health. Once the site has been contaminated, recovery may require years to decades, with continuing impacts and risk (Kingston 2002, Peterson et al. 2003). One of the central goals of investigating microorganisms in contaminated sites is for environment cleanup. Understanding the drivers of microbial community structure and assembly has important implications for ecosystem restoration and environmental management. However, few studies have examined the impact of contamination on microbial biogeographic patterns and potential alterations in ecosystem services and biogeochemical cycling, as well as the driving forces of microbial community assembly in contaminated soils. Our previous study showed that oil contamination significantly decreased microbial functional diversity and that microbial communities in oil exploration sites showed spatial differences (Liang et al. 2011). However, only limited samples (a pair of contaminated and uncontaminated soils from each field) were obtained, and theses samples were not sufficient to verify the importance of determinism/stochasticity in shaping microbial composition. Thus, in this study, we conducted intensive sampling based on the previous study. A total of 100 soil samples were taken, of which one-half were historically contaminated and the other one-half were pristine, from five crude-oil exploration fields across China with long histories of exploration (three sites were also used in the previous study [Liang et al. 2011], and the other two were different, covering a broader range of climate regions and soil types). Soil microbial communities were analyzed with a powerful, high-throughput functional microarray, GeoChip 3.0, which contains tens of thousands of functional genes involved in the biogeochemical cycling of carbon, nitrogen, phosphorus, sulfur, and metals and in the degradation of various organic contaminants (He et al. 2010). As the contamination events occurred more than ten years ago, we believe that the soil microbial community has reached a new stable state during long-term succession. Thus, studying the microbial diversity and their assembly processes is feasible and worthwhile to understand how human perturbations affect microbial diversity and succession. We made the following predictions: (1) long-term oil contamination poses significant impact on soil microbial α and β-diversity, (2) both determinism and stochasticity play important roles in controlling the assembly of soil microbial community, and (3) deterministic process increases with long-term oil contamination.

Methods

Study sites and sample collection

A total of 100 soil samples were collected from five oil fields (fields in areas where oil is extracted) across China (23°–46° N, 84°–125° E): Daqing (DQ, 20 samples) in northeast China; Talimu (TLM, 20 samples), in northwest China; Shengli (SL, 20 samples) in the Yellow River area in north China; Jianghan (JH, 20 samples) in the Yangtze River area in central China; and Baise (BS, 20 samples) in south China. Sampling was conducted from May to July of 2008, beginning in the south and ending in the north. DQ (46°35′ N, 125°18′ E) has a temperate continental monsoon climate with a mean annual rainfall of 427.5 mm. TLM (41°28′ N, 84°13′ E) has a temperate continental arid climate with a mean annual rainfall of 58.6 mm. SL (37°28′ N, 118°29′ E) has a warm temperate continental semi-humid monsoon climate with a mean annual rainfall of 550 mm. JH (30°21′ N, 114°20′ E) and BS (23°43′ N, 107°04′ E) have a subtropical humid monsoon climate with a mean annual rainfall of 1159.8 mm and 1115.0 mm, respectively. One-half of all soil samples were samples of contaminated soils where contamination had occurred more than 10 years ago. Sampling sites in the same regions were located in an area of 2 km^2. A randomized block design was performed and the longitude and latitude of each sample was recorded. The same sampling design was conducted in the local region where soils were not contaminated and we also measured oil content in uncontaminated soil to ensure they were not contaminated. All samples were collected from surface soil (0–10 cm) and transported to the lab on ice. Detailed sampling information and information on variations in oil contamination and soil geochemical variables can be found in a previous study (Liang et al. 2012).

DNA extraction and GeoChip hybridization of all 100 samples

Microbial genomic DNA was extracted from 5 g of well-mixed soil for all samples by combining freeze-grinding and sodium dodecyl sulfate (SDS) 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 with agarose gel electrophoresis, an ND-1000 spectrophotometer (Nanodrop, Wilmington, Delaware, USA) and Quant-It PicoGreen kit (Invitrogen, Carlsbad, California, USA). An aliquot of 2 μg of DNA from each sample was directly labeled, purified, and resuspended in 50 μL of hybridization solution, which contains 45% formamide, 5× saline sodium citrate (SSC), 0.1% SDS, and 0.1 mg/mL salmon sperm DNA (0.1 pmol/μL) (Wu et al. 2006). The fluorescently labeled DNA was hybridized with GeoChip 3.0 on a MAUI Hybridization System (BioMicro Systems, Salt Lake City, Utah, USA) at 42°C for 12 hours. Microarrays were scanned by a ScanArray 5000 Microarray Analysis System (PerkinElmer, Wellesley, Massachusetts, USA) at 95% laser power and 68% photomultiplier tube gain. Scanned images were saved in 16-bit TIFF format and were quantified using ImaGene version 6.0 (BioDiscovery, Los Angeles, California, USA) and processed in the Microarray Data Manager system in the Institute for Environmental Genomics (IEG) website (*available online*).[5] Spots with signal-to-noise ratios lower than 2.0 were removed before statistical analysis, as described previously (He et al. 2010).

Data analysis

We compared the microbial functional diversity among all the samples. Microbial α-diversity (Shannon index) was calculated and compared between contaminated and uncontaminated soils for all samples, both across sites and within each site. Based on all the GeoChip data, soil microbial distribution pattern were further analyzed. The rate of distance-decay of the microbial communities was calculated as the slope of a linear least-squares regression on the relationship between the (ln-transformed) geographic distance and the (ln-transformed) microbial similarity, where similarity = 1 − β-diversity (dissimilarity based on Bray-Curtis dissimilarity metric). To test if the slope of the similarity–distance relationship was different from zero, bootstrapping was used for regressing variables that violate the assumption of independence (Efron and Tibshirani 1993). The slope was tested by an one-sample *t* test between original sloe and a mean of bootstrapped slopes by random pairing of the original set (9999 times with replacement; Horner-Devine et al. 2004, Zhou et al. 2008). We tested for significant differences using a nonparametric analysis method, NPMANOVA (nonparametric MANOVA, also known as PERMANOVA). Microbial functional gene distribution pattern was determined by nonmetric multidimensional scaling (NMDS). The Mantel test was used to calculate the correlations between environmental factors and the soil microbial community of contaminated and uncontaminated soils. All analyses were performed in R using the packages vegan and ecodist (version 3.0.2; R Development Core Team 2013).

The null model analysis is based on the method proposed by Chase et al. (2011). This technique uses a null model to create stochastically assembled communities from the regional species pool to determine the degree to which observed β-diversity patterns deviate from stochastic assembly. A quantitative estimate of the role of deterministic selection processes in shaping community composition and structure was calculated as the proportion of the difference between the observed similarity and the similarity expected under the null hypothesis divided by the observed similarity (Kraft et al. 2011), which is defined as selection strength (SS). The complement of the selection strength (1 − SS) should provide a quantitative assessment of the importance of stochastic processes in regulating community composition and structure. The null analysis was performed on the website of the Institute for Environmental Genomics (see footnote 5).

Results

Influence of contamination on microbial functional gene diversity

After long-term oil contamination, the total number of genes detected in the "gene pool" in contaminated soils was reduced by 0.24% for all sites and 16.44%, 18.72%, 18.65%, 28.84%, and 21.85% for DQ, SL, TLM, JH, and BS, respectively (Appendix: Table A1). Oil contamination also significantly reduced microbial α-diversity based on both gene richness (Fig. A1) and the Shannon index (Fig. A2; $P < 0.01$). In addition, we observed a significant decay relationship between microbial Shannon diversity and oil concentration across the five sites for all functional genes ($P < 0.001$; Fig. A2) and for functional groups such as the genes/proteins involved in nitrogen cycling (*nifH*, *nirS*, *nirK*, and *nosZ*; $P < 0.001$; Fig. A3), carbon fixation (*pcc*, rubisco, and CODH; $P < 0.001$; Fig. A4), carbon degradation (starch, cellulose, chitin, and lignin; $P < 0.001$; Fig. A5) and phosphorus utilization (*ppk* and *ppx*; $P < 0.001$; Fig. A6).

Changes in microbial spatial structure

Microbial β-diversity was estimated as community similarity vs. geographic distance for each pair-wise set of samples, defined as distance–decay relationships (DDR; Fig. 1). The results revealed a significant, negative distance–decay curve for both uncontaminated samples (slope = −0.171, $P < 0.001$) and contaminated samples (slope = −0.095, $P < 0.001$). Both slopes were significantly different from zero according to a permutation test ($P < 0.0001$). However, the contaminated samples had a shallower slope ($P < 0.001$), indicating that there was less β-diversity under oil contamination. The slopes of the DDR for each functional group (C/N/

[5] http://ieg.ou.edu/microarray

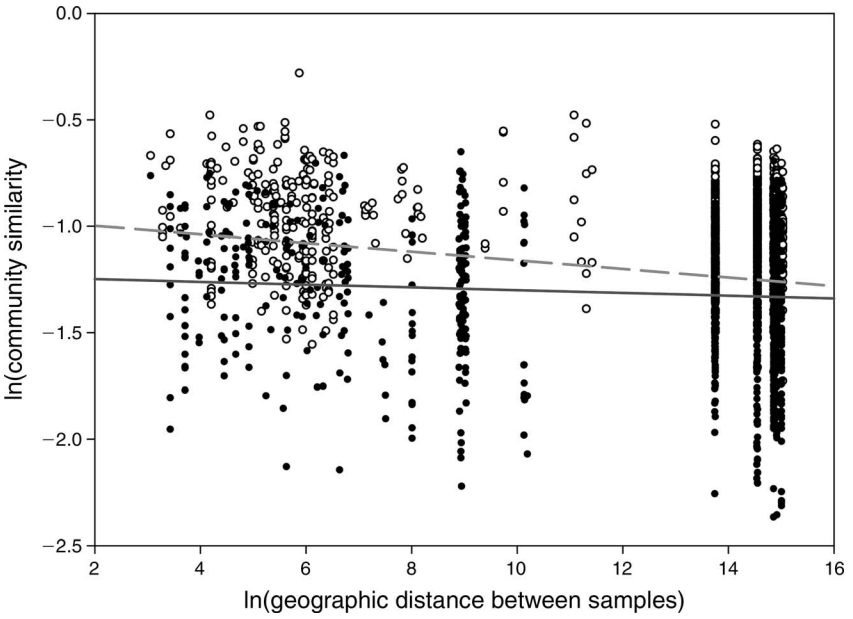

FIG. 1. Distance–decay curves for microbial communities in uncontaminated soils (open circles, dashed line, slope = −0.171, $P < 0.001$) and contaminated soils (solid circles, solid line, slope = −0.095, $P < 0.001$). The line denotes the least-squares linear regression across the five sites. The slope of the contaminated soils is significantly different from the slope of the uncontaminated soils ($P < 0.001$). Geographic distance was measured in meters.

S/P cycling, organic remediation, metal resistance and energy processing) were also calculated (Table 1). All functional groups showed significant DDRs in the uncontaminated soils ($P < 0.001$). However, some groups did not have significant DDRs in the contaminated soils, including nitrogen fixation, nitrate reduction and aromatic and aliphatic hydrocarbons degradation. Shallower slopes were also observed with oil contamination for each functional group. The test for dissimilarity with NPMANOVA indicated significant differences of microbial community composition among the five sites in both contaminated and uncontaminated soils. However, the contaminated soils were more homogeneous and lower r/F values (Table A1). Similar results could be found in the pair-wise site analyses (Table A2). Oil contamination appeared to eliminate the significant differences between BS and DQ, BS and SL, DQ and SL, and TLM and SL (Fig. 2). The dissimilarity

TABLE 1. Distance–decay relationships of microbial functional gene groups in contaminated and uncontaminated soils.

	Uncontaminated soils				Contaminated soils			
	Correlation		Permutation test		Correlation		Permutation test	
Functional category	*r*	*P*	*t*	*P*	*r*	*P*	*t*	*P*
All functional genes	**−0.171**	<0.001	−123.22	<0.0001	**−0.095**	0.001	−71.13	<0.0001
Carbon cycling	**−0.125**	<0.001	−91.32	<0.0001	**−0.121**	<0.001	−87.85	<0.0001
C degradation	**−0.126**	<0.001	−85.50	<0.0001	**−0.113**	<0.001	−82.57	<0.0001
C fixation	**−0.111**	<0.001	−78.29	<0.0001	**−0.104**	<0.001	−82.60	<0.0001
Nitrogen cycling	**−0.184**	<0.001	−135.01	<0.0001	**−0.090**	0.002	−69.03	<0.0001
Nitrate reduction	**−0.135**	<0.001	−93.73	<0.0001	−0.020	0.484		
Denitrification	**−0.231**	<0.001	−167.12	<0.0001	**−0.137**	<0.001	−109.53	<0.0001
Nitrogen fixation	**−0.158**	<0.001	−108.62	<0.0001	−0.057	0.052		
Ammonification	**−0.164**	<0.001	−115.28	<0.0001	**−0.045**	0.032	−40.57	<0.0001
Phosphorus utilization	**−0.184**	<0.001	−130.13	<0.0001	**−0.091**	0.002	−69.67	<0.0001
Sulfate cycling	**−0.238**	<0.001	−157.68	<0.0001	**−0.056**	0.046	−41.60	<0.0001
Hydrocarbon degradation	**−0.176**	<0.001	−128.06	<0.0001	−0.051	0.089		
Aromatics	**−0.180**	<0.001	−126.29	<0.0001	−0.050	0.111		
Aliphatic hydrocarbons	**−0.133**	<0.001	−92.24	<0.0001	−0.044	0.416		
Metal Resistance	**−0.142**	<0.001	−93.87	<0.0001	**−0.115**	<0.001	−83.93	<0.0001
Energy process	**−0.140**	<0.001	−101.99	<0.0001	**−0.079**	0.007	−57.81	<0.0001

Notes: *P* and *t* values are from one-sample *t* tests on bootstrapping (9999 times). Significant ($P < 0.05$) correlations are shown in boldface type. If correlations were not significant, no *t* test was performed.

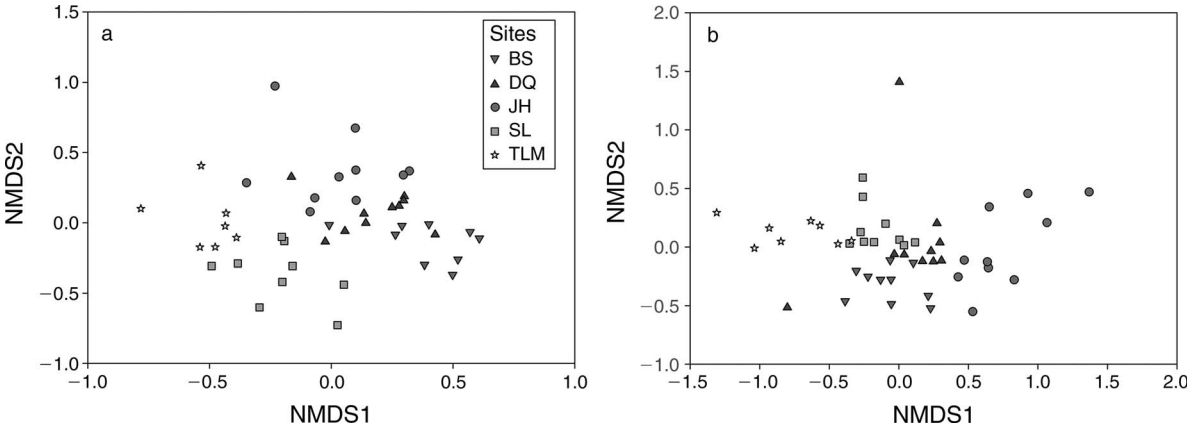

FIG. 2. Nonmetric multidimensional scaling (NMDS) of (a) uncontaminated soil samples, and (b) contaminated soils. See *Methods: Study sites and sample collection* for site descriptions.

test further confirmed the DDR analysis that microbial β-diversity decreased with oil contamination.

Relationship between microbial communities and environmental variables

The relationship between microbial communities and environmental variables was different in the contaminated and uncontaminated soils among the five sites, according to the Mantel test (Table 2). Significant correlations were found between soil microbial functional genes and all of the environmental variables (spatial distance, oil concentration and soil geochemical parameters) in the contaminated soils ($P < 0.05$). Spatial distance was found to be significantly correlated with the microbial communities in both uncontaminated ($P < 0.001$) and contaminated soils ($P < 0.01$) but showed higher correlation coefficients in the former, which was in accord with steeper slopes of DDR in uncontaminated soils. Of all soil geochemical variables, soil texture and salt content were significantly correlated with the microbial communities in uncontaminated soils, where soil texture, total organic carbon (excluding oil concentration), and nitrogen content were significantly correlated with the microbial communities in contaminated soils. In addition, the oil concentration was highly correlated with the microbial communities and had the highest correlation coefficient ($r = 0.403$) of all environmental variables.

Stochasticity vs. determinacy under oil contamination

A quantitative estimation process based on a null model analysis (Chase et al. 2011) was conducted to verify our hypothesis that oil contamination changes the stochastic and deterministic processes in the microbial assembly (Fig. 3). The proportion of the difference between the observed similarity for each pairwise comparison and the null expected similarity divided by the observed similarity was calculated. The results indicated that microbial assembly processes were determined by both stochastic and deterministic processes, of which determinacy accounted for ~30.0–55.8% and stochasticity accounted for ~70.0–44.2%. There was an increase of deterministic selection processes that shape the community composition and structure in contaminated soils compared to uncontaminated soils, increasing 56.9% for all the sites, 6.7% in DQ, 32.3% in SL, 1.5% in TLM, 42.9% in JH, and 68.0% in BS (Fig. 2).

DISCUSSION

After years of exposure to oil, the soil microbial diversity changed substantially. Dramatic decreases in microbial diversity were found across all sites and within each site, indicating that some of the regional species pool was eliminated or inhibited. The overall microbial population diversity in oil-contaminated sites declined, as has been previously observed using both culture-

TABLE 2. Mantel test of GeoChip data and environmental attributes.

	Uncontaminated samples	Contaminated samples
All environmental variables	0.018	**0.129***
Spatial distance	**0.222** ***	**0.187****
Oil content		**0.403***
Texture (sand, silt)	**0.2441*****	**0.142***
Total organic carbon	0.077	**0.086***
Nitrogen content (TN, EN)	−0.017	**0.132***
Phosphorus content (TP, EP)	0.001	−0.061
pH	0.081	−0.051
Moisture	0.034	0.047
Salt content	**0.108***	0.079
Metal element	0.003	−0.001

Notes: Values are the correlation r between GeoChip data and environmental attributes. Significant differences are highlighted in boldface type. Abbreviations are TN, total nitrogen (nitrogen in all organic and inorganic forms); AN, available nitrogen (NO_3^--N, NO_2^--N and NH_4^+-N); TP, total phosphorus (phosphorus in all organic and inorganic forms); and AP, available phosphorus (PO_4^{3-}-P).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

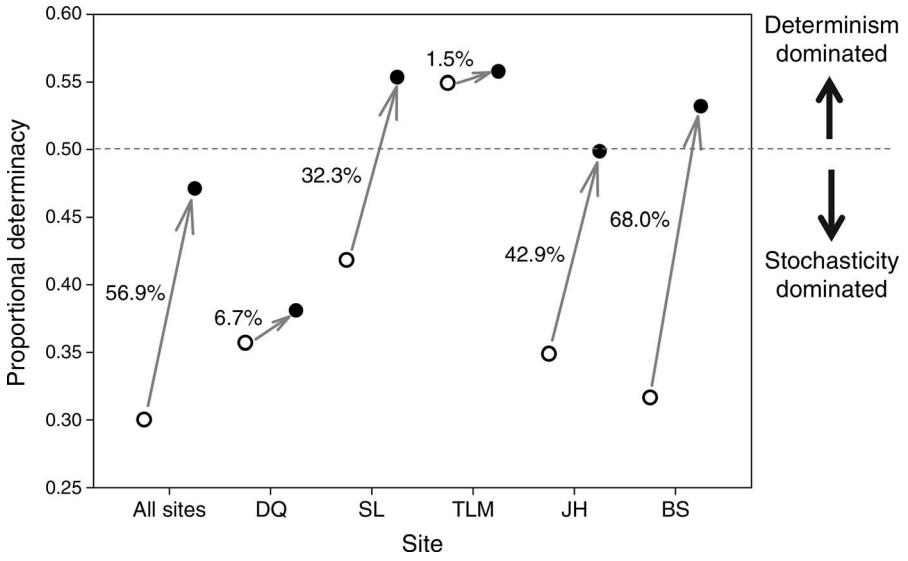

FIG. 3. Changes in the proportion of determinacy in uncontaminated (open circles) and contaminated (solid circles) soils across all sites and in each site from north to south China (DQ, SL, TLM, JH, and BS). The dashed line denotes equal roles for both. Below the line, stochasticity is dominant, and above it, determinacy is dominant. The numbers associated with each line indicate a percentage increase in determinacy.

dependent and culture-independent approaches (Van Hamme et al. 2003). The diversity loss was more significant for microbes that function in carbon and nitrogen cycling (Liang et al. 2009). Consistent with these observations, in our study, the α-diversity of all functional genes and several functional groups was very negatively correlated with oil concentration at a regional spatial scale in historically contaminated sites (Appendix: Figs. A2–A6). In contrast to long-term oil contamination, new oil spills generally increase certain microbial communities, especially those functioning in hydrocarbon degradation, resulting in an increase of overall functional diversity (Hazen et al. 2010, Lu et al. 2012). However, when the biodegradable hydrocarbons are consumed, the microbial diversity began to decrease as the available resources declined (Beazley et al. 2012), especially in the older contaminated sites where high molecular weight aliphatic hydrocarbons and polycyclic aromatic hydrocarbons as well as their alkylated derivatives and non-degradable asphaltenes persisted in the soils (Liang et al. 2012). Species richness is considered to be highly related with ecosystem function (Chapin et al. 2000). The loss of microbial diversity and changes in community composition changed their functional processes (Salonius 1981, Allison and Martiny 2008, Strickland et al. 2009, Nogales et al. 2011).

The similarity among soil microbial communities declined significantly with increasing spatial distance in both contaminated and uncontaminated soils across a regional spatial scale. This distance–decay relationship revealed that different locations had unique microbial compositions and this variation was spatially autocorrelated between microbial community similarity and spatial distance. The slopes ranged from −0.238 to −0.111 in uncontaminated soils and from −0.137 to −0.045 in contaminated soils, which is in the same range as found in previous studies, such as the slopes reported for ascomycete fungi in soil (−0.147; Green et al. 2004), bacteria in marsh sediments (−0.080; Horner-Devine et al. 2004), Nitrosomonadales in marshes (−0.27 to approximately −0.04, based on spatial scale; Martiny et al. 2011), and bacterial communities in lake sediments (−0.443; Xiong et al. 2012). The results all indicate that the microbial community is spatially structured. Thus, how does oil contamination influence microbial spatial turnover? In this study, long-term oil-contamination causes a negative effect on microbial temporal or spatial turnover. A previous study indicated that the turnover of the bacterial taxa–time relationship decreased as organic contaminant (industrial wastewater) concentrations increased (van der Gast et al. 2008). Here, we observed a decrease in microbial spatial turnover in historically oil-contaminated sites (Fig. 1, Table 1). In addition, the dissimilarities among the five locations and between pair-wise locations all decreased (Tables A1 and A2). The different responses of soil microbial communities to anthropogenic perturbations imply that the mechanisms underlying the community succession could vary with the type of disturbance (Houseman et al. 2008, Chase 2010). Although new oil spills could be considered sources of carbon substrate enrichment, after long-term contamination, the microbial community could be more deterministic selective. The role of contamination as an environmental filter decreases the dissimilarity of microbial communities in different locations, resulting in lower β-diversity. Due to the filtering effects from oil contamination, the local communities also showed lower α-diversity compared

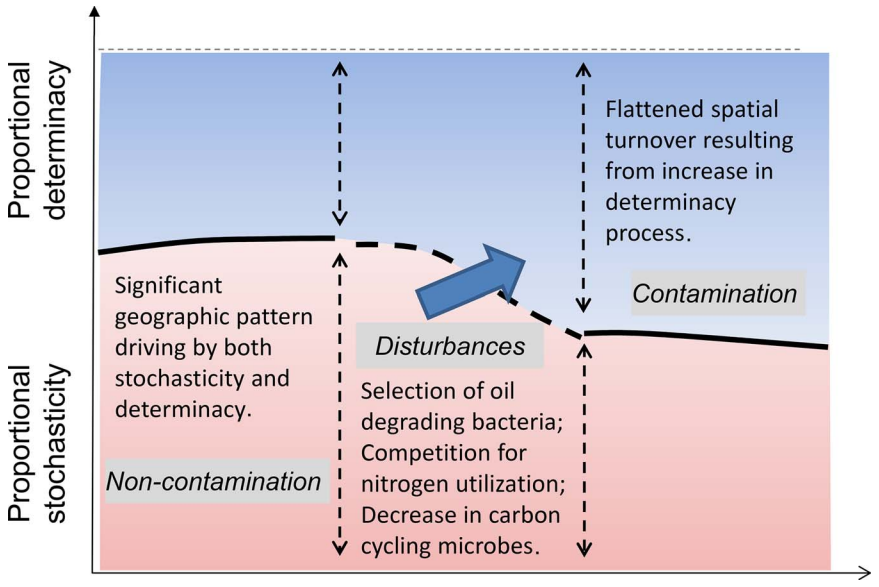

FIG. 4. A conceptual model for the effects of long-term oil contamination on microbial assembly and a lot showing the proportional changes of stochastic and deterministic factors in structuring microbial assemblages based on null model analysis. The solid line denotes the observed values in uncontaminated and contaminated soils across the five sites. The dashed line denotes hypothetical dynamics during the contamination event, which were unmeasured.

with that before disturbance (Liang et al. 2011). These decreases in diversity imply that soil microbial communities are not resistant or resilient to long-term oil exposure, indicating that they do not converge to their previous composition (Allison and Martiny 2008).

The relative roles of deterministic and stochastic processes were quantified to determine the influence of oil contamination on microbial succession. Deterministic processes and stochastic processes were found to be jointly responsible for structuring the microbial communities across all sites and within each site, regardless of the contamination state, which is consistent with the widely held idea that community assembly is simultaneously influenced by stochastic and deterministic factors (Dumbrell et al. 2010, Ofiteru et al. 2010, Langenheder and Szekely 2011, Ferrenberg et al. 2013). In several cases, stochastic processes have been considered dominant (Sloan et al. 2006, Woodcock et al. 2007, Ofiteru et al. 2010, Caruso et al. 2011). Herein, we observed a higher proportion of stochastic drivers in uncontaminated soils: 70% for the overall structure at a regional scale and ~58.2–68.3% in every site except TLM (45.1%), which might be because the study area in TLM is desert. It has been considered that the drylands constitute unique microbial assemble (Pointing and Belnap 2012). The results indicated that dispersal limitation might have an important role in structuring microbial communities, especially at large spatial scales with decreasing migration rates.

Recently, a few studies have indicated that disturbance alters the relative influence of stochastic and deterministic processes, such as drought (Chase 2007) and wildfire disturbance (Ferrenberg et al. 2013). In this study, after long-term contamination, when the microbial community's succession neared a new stable state, the proportion of stochastic vs. deterministic processes also changed. A potential increase in the deterministic processes was found in oil-contaminated soils. As the spatial distance among contaminated samples was the same as that among uncontaminated samples, dispersal limitation was predicted to be similar, especially at the regional scale. Thus, the decrease in compositional dissimilarity among sites was largely due to the filtration of oil contamination stress, which increased the relative importance of deterministic effect. In addition, the Mantel test revealed that the influence of contemporary environmental heterogeneity on microbial communities might increase with oil contamination. A conceptual model was proposed to illustrate the effects of long-term oil contamination on microbial assembly (Fig. 4). The significant spatial pattern of microbial communities found different geographic locations is driven by both stochasticity and determinacy, with stochasticity dominant in non-contaminated soils. Oil contamination has a significant effect on soil microbes, e.g., the selection of oil-degrading bacteria (Liang et al. 2011, Lu et al. 2012), a potential increase in microbial competition for nitrogen and phosphorus utilization due to an imbalance of carbon and nitrogen in soils, a decrease in carbon fixation and degradation, and a decrease in sulfur cycling (Liang et al. 2009). Under continuous long-term disturbance, microbial spatial differences are weakened due to the increase in environmental selection (determinacy process). Furthermore, recent studies

indicated that the changes in assembly processes in microbial communities following disturbance changed over time (Ferrenberg et al. 2013, Zhou et al. 2014). Because we did not measure the microbial community structure soon after oil contamination, we could only obtain a stable state after long-term contamination, and then compare it with the current stable state of uncontaminated soils. Further work is required to elucidate the microbial stochastic and deterministic assembly processes following recent and older disturbances.

Long-term oil contamination not only significantly influenced the microbial biodiversity and spatial pattern but also altered the mechanisms underlying the biological assembly. Oil contamination reduces potential microbial functional processes such as nitrogen fixation, denitrification and phosphorus utilization. Such impact may last for decades. Furthermore, oil contaminations decrease site-to-site variation in species composition and deterministic process plays more roles in microbial assembly, indicating the potential usage of bioaugmentation in contaminant removal in a more predictable way. Of all geochemical variables, oil content, total organic carbon, soil texture, and nitrogen content may be the most important deterministic factors that influencing the microbial communities in contaminated soils. Furthermore, when bioremediation is chosen, site-specific remediation strategy should be considered since significant spatial autocorrelation between microbial community similarity and spatial distance was observed among sites. Our study provided insight into understanding the variation in the relative importance of deterministic and stochastic processes of soil microbial assembly under long-term anthropogenic contamination, which is important for predicting and protecting ecosystem functions and biodiversity (Hanson et al. 2012, Nemergut et al. 2013).

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Supplemental Material

Ecological Archives

The Appendix is available online: http://dx.doi.org/10.1890/14-1672.1.sm

Data Availability

The microarray data presented in this article are available online: http://ieg.ou.edu/4download/