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Carbon dioxide (CO_2) flooding of oil reservoirs has some merits such as extensive adaptability, low cost, and high recoverability. However, the risk of CO_2 leakage during the CO_2-EOR process is not a concern to take lightly. There are many sources of CO_2 emission during this process, such as the CO_2 transportation process, oil production wells and the CO_2 injection well. Studies have shown that elevated concentrations of CO_2 might modify soil properties through altering the soil mineralogy, the pH of underground water and surface vegetation. These changes could impact microbial communities and consequently ecosystems processes. High-throughput sequencing was used to investigate the effects of CO_2 emission on the composition and structure of soil bacterial communities. The diversity of bacterial community notably decreased along the CO_2 flux gradient and the composition of the community also varied along the gradient. These results could be useful for evaluating the impact of potential CO_2 leakages on ecosystems associated with CO_2-EOR processes.

Effects of CO_2 leakage on soil bacterial community from simulated CO_2-EOR areas

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Abstract: CO_2-EOR (enhance oil recovery) has been proposed as a viable option for flooding oil and reducing anthropogenic CO_2 contribution to the atmospheric pool. However, a potential risk of CO_2 leakage from the process is the threat to the ecological system. High-throughput sequencing was used to investigate the effects of CO_2 emission on the composition and structure of soil bacterial communities. The diversity of bacterial community notably decreased with increasing CO_2 flux. The composition of bacterial community varied along the CO_2 flux, with increasing CO_2 flux accompanied by increases in the relative abundance of *Bacteroidetes* and *Firmicutes* phyla, but decreases in the relative abundance of *Acidobacteria* and *Chloroflexi* phyla. Within the *Firmicutes* phylum, the genus *Lactobacillus* increased sharply when the CO_2 flux was at its highest point. Alpha and beta diversity analysis revealed that differences in bacterial communities were best explained by CO_2 flux. The redundancy analysis (RDA) revealed that differences in bacterial communities were best explained by soil pH value which related with CO_2 flux. These results could be useful for evaluating the risk of potential CO_2 leakages on the ecosystems associated to CO_2-EOR process.

Keywords: High-throughput sequencing; CO_2 enhance oil recovery; potential CO_2 leakages; soil bacterial communities; *Lactobacillus*; Redundancy analysis

Introduction

Recent global warming and climate change have been attributed to anthropogenic CO_2 emissions from the burning and consumption of fossil fuels. CO_2 capture, utilization and sequestration (CCUS) is a useful technology toward reducing such emissions from stationary sources [1,2]. It has been proven that CO_2 can be used to enhance oil recovery (named CO_2-EOR). As a promising technology, CO_2-EOR has been widely adopted by many oil companies around the world since it could improve the recovery ratio of oil reservoir characterized by extensive adaptability, low cost and high recoverability [3,4]. However, the risk of CO_2 leakage during the CO_2-EOR process is a continuing concern due to CO_2 emission originating from CO_2 transportation process, as well as oil production and CO_2 injection well [5,6].

Numerous studies have shown that elevated concentrations of CO_2 can modify soil physical and chemical properties, microbial respiration and activity. At a natural geological CO_2 emission point in Italy, Beaubien et al. found that soil pH significantly decreased near the CO_2 exhaust port, also the soil water content and O_2 concentration decreased. They implied that decreasing soil pH value and O_2 concentration created an acidic soil environment, which inhibited the soil microbial activity and enzyme activity, and had a great impact on the activity of the rhizosphere microorganisms [7]. Pierce and Sjögersten have shown that elevated CO_2 concentration influenced microbial biomass and activity in different degree in a short term. The microorganism had a higher tolerance to higher CO_2, but the research did not involve soil microbial community structure and diversity of change [8]. Moreover, negative effects of high CO_2 concentrations on soil microorganisms were observed by detection of decreased microbial respiration rates [9]. The study published in 2015 by Cunha involved the effect of CO_2 seepage on microorganisms of soil surface in the Atlantic forest region. Results showed that the soil microbial activity decreased with long term exposure to CO_2, and the increase of CO_2 concentration affected the microbial biomass, species richness and metabolic activity [10]. These changes can ultimately impact microbial communities and, consequently, the ecosystem function [11]. Elevated CO_2 has also been shown to enhance plant biomass production thereby increasing the size of root-associated microbial populations. Together this can result in a modification in plant-microbial interactions [12]. As a consequence, these changes may influence biological cycles and the overall function of the ecosystem [13-15].

As the most abundant and diverse group of all soil organisms, bacteria play an indispensable role in soil ecology, particularly due to their central role in decomposition. Due to their enormous metabolic versatility, they also serve as keystones in biogeochemical cycling [16,17]. Soil bacterial community composition associated with CO_2 emission sites has been investigated using DNA fingerprinting, quantitative polymerase chain reaction analyses (qPCR), polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and assessment on specific genes [7,8,18,19]. Shen et al. have given a comprehensive analysis of the soil bacterial community composition and diversity using the Roche 454 pyrosequencing technique [20]. Also, microbial community diversity in a real industrial PAH-polluted soil was analyzed using this technique [21]. However, few studies have investigated the effects of increasing CO_2 concentrations on soil bacterial communities through Illumina Miseq sequencing, a more sensitive method to detect overall microbial community changes and minor populations [22,23]. The use of Illumina MiSeq has provided a sufficient number of sequences with adequate length to enable extrapolations that estimate bacterial alpha diversity based on richness and evenness [16]. This approach has been applied to decipher the microbial communities in distinct samples, such as samples taken from polychlorinated biphenyl contaminated environments [24], river water [25] and iron mining soil [26].

Changes in microbial community structure may affect both the below- and above-ground processes, thus influencing ecosystem function [27]. Microorganisms can be extremely sensitive to changes in soil characteristics, thereby acting as good indicators of soil quality. An increase in CO_2 concentration could cause changes in soil biochemical conditions, which could lead to a shift in the functionality or diversity of inhabiting microorganisms [20,28-30]. The goal of the present study was to better understand how elevated CO_2 emissions affect the composition and structure of soil bacterial community in a simulated CO_2 gas vent, in order to assess the potential impact of CO_2 leaking from the CO_2-EOR process on the surface ecosystem.

Materials and Methods

Study site and experimental design

The study was carried out in an open field, at the CO_2 experimental site (34°12′31.735″N, 117°08′08.328″E) within the campus of China University of Mining and Technology, in Xuzhou City, Jiangsu Province, China (Fig. 1). The soil is haplic brown soil with CEC value 14.91±3.27 cmol/kg, and granulometric composition is 15.12: 22.34: 9.15: 53.39 (<0.001mm: 0.001-0.005: 0.005-0.01: >0.01). This CO_2 experimental site consists of a factorial experiment of 16 plots (2.5m × 2.5m each) in which fluxes of CO_2 were conducted from 15th July 2014 to 15th September 2014 and 4 replicates for each treatment were assayed. Plots were filled from the bottom to the top with a 10 cm coarse sand layer covered by 10 cm of fine sand, followed by a nano porous plate and 80-cm top soil layer, respectively. The CO_2 was injected from centre of the plot at 1.0 m below the surface. Four sampling sites were selected according to the soil CO_2 emission flux intensity. Flux intensity categories consisted of the most extreme category (E, pure CO_2 injection 1200 $g \cdot m^{-2} \cdot d^{-1}$), a high intensity category (H, pure CO_2 injection 800 $g \cdot m^{-2} \cdot d^{-1}$), a medium intensity category (M, pure CO_2 injection 400 $g \cdot m^{-2} \cdot d^{-1}$), a low intensity category (L, pure CO_2 injection 200 $g \cdot m^{-2} \cdot d^{-1}$), a control (C, stands for background level, without CO_2 injection) and a resilience category (R, after CO_2 injection terminated). Soil samples, one composite sample on three occasions from each plot, (10-30 cm depth) were collected (15th September 2014) for L, M, H and E categories. The C and R soil samples were collected at 15th July and 15th October 2014, respectively. The soil samples were packed in sterile Ziploc bags, and the bags were sealed and transported to the laboratory for further analysis. A fraction of the mixed sample was immediately treatment for biodiversity analysis. Another fraction was dried at room temperature for about a week and sieved through a 2-mm grid to remove stones and visible plant fragments. A portion of this was ground and sieved through a 0.25-mm sieve for physicochemical analysis. All samples were stored at 4 ℃. Organic matter was measured using the Walkley-Black wet oxidation method [31]. For pH and electrical conductivity determination, 1 g of soil sample was combined with 5 mL of deionized water and shaken for 5 min, and then allowed to stand for 1 hour prior to measurement. Nitrate content (NO_3^-) was measured with a dual wavelength spectrometry [32].

DNA extraction, PCR amplification and Illumina MiSeq sequencing

DNA was extracted from 0.5 g of sieved soil sample using FastDNA^{TM} SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instruction. The V4 regions of the bacterial 16S rRNA gene were amplified using an Eppendorf thermal cycler (Model 5332). Amplification conditions were as follows: An initial denaturation step at 98 ℃ for 5 min, followed by 25 cycles of 98 ℃ for 30 s, 50 ℃ for 30 s, and 72 ℃ for 30 s and a final extension step at 72 ℃ for 5 min. The primers used in the reaction were 520F (5'-barcode+ GCACCTAAYTGGGYDTAAAGNG-3'), 802R (5'- TACNVGGGTATCTAATCC-3'). The barcode is a seven-base sequence unique to each sample.

The PCR reactions were performed in triplicate in a 25-μL mixture containing 5 μL of 5×Q5 Reaction Buffer, 5 μL of 5×Q5 GC high Enhancer, 2 μL of 2.5 mM dNTPs, 1 μL of each primer (10 μM), 0.25 μL of Q5 Polymerase, 8.75μL sterilizing ultrapure water and 2 μL of template DNA (20 ng/μL). Three PCR products per sample were pooled and purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions. Quantification of purified PCR product was performed with a Quant-iTPicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Personalbio, Shanghai) according to a standard protocol.

Processing and analyzing of sequencing data

Raw FASTQ files were de-multiplexed and quality-filtered using QIIME (version 1.7.0) with the following criteria: (1) 300-bp reads were truncated at any site that acquired an average quality score of ＞20 over a 10-bp sliding window, and truncated reads shorter than 150 bp were discarded; (2) Two nucleotide mismatch in primer matching and reads containing ambiguous characters were removed; (3) Only overlapping sequences longer than 10 bp were assembled according to their overlapped sequences. Reads that could not be assembled were discarded. Operational taxonomic units (OTUs) with 97 % similarity cutoff were clustered using UPARSE (version 7.1), and chimeric sequences were identified and removed using UCHIME method.

Rarefaction analysis based on Mothur v.1.21.1 [33] was conducted to reveal the diversity indices, Chao richness estimate, Shannon diversity index were also estimated from OTUs using software Mothur. Beta diversity analysis was performed using weighted UniFrac and Nonmetric multidimensional scaling (NMDS) index [34]. The results of the redundancy analysis (RDA) were compared using the Canoco windows for 4.5.

Results

Soil chemical characteristics and CO_2 emission fluxes

No significant differences in soil properties were observed among low, medium, high and extreme CO_2 sites (Table 1). Soil pH ranged from mild alkaline in the control samples (7.90 ± 0.16) and slightly acidic in the extreme sites (6.70 ± 0.27). Organic matter ranged from 0.70 ± 0.05 % in control site to 0.84 ± 0.05 % in the extreme sampling site.

Strong negative correlations were observed between the natural logarithm of CO_2 flux and NO_3^- nitrogen ($r = -0.991$; $p < 0.005$) and EC value ($r = -0.900$; $p < 0.005$). Also, strong negative linear correlations were observed between the CO_2 flux and pH value ($r = -0.984$; $p < 0.005$). By contrast, a positive correlation was detected between organic matter and ln of CO_2 flux ($r = 0.929$; $p < 0.005$).

Sequencing results and alpha diversity measurements

A total of 1121,986 reads were obtained from the 24 samples through Miseq sequencing analysis. The libraries ranged in sizes, from 34,555 reads at site R3 to 69,433 reads at site C2. All rarefaction curves tended to approach the saturation at any sequencing depth. The rarefaction curve indicated that a large variation in the total number of retrieved OTUS was observed among samples (data not shown). Compared to the high CO_2 concentration soil (e.g., H1, H2, and E1, E2), all samples from control and resilience sites had higher OTU density. The OTU densities of samples from the L and M sites were also lower than the average OTU densities of the C and R sites. In addition, the average OTUs of group R attained a higher value, but its average reads were significantly lower than those of group M.

Moreover, the calculation of the alpha diversity species richness (Chao) and Shannon index confirmed the decreasing diversity in the high CO_2 flux sites, especially in group E, compared to

group C (Fig. 2). The bacterial diversity of the 24 soil samples was analyzed by calculating the alpha diversity indices. Chao 1 richness estimator values ranged from 4041 (E2) to 5851 (C3), whereas Shannon diversity index varied between 6.39 (E2) and 7.06 (R4). Both index values decreased (Fig. 2), indicating that community richness and uniformity declined increasing CO_2 fluxes.

Bacterial taxonomy composition

Sequences that could not be classified into any known group were assigned as unclassified. Greengene (Release 13.8， http://greengenes.secondgenome.com/) was used as the annotation database. The bacterial OTUs were assigned to 30 different phyla, 164 families, or 456 genera. Nine of the 30 phyla comprised more than 90 % of the total read in every sample: *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Planctomycetes*, *Proteobacteria*, *Gemmatimonadetes* and *Verrucomicrobia*. *Actinobacteria* was the most abundant group (Fig. 3A), comprising approximately 35.51%. *Proteobacteria* consisted of 20.41 % in all libraries. However, the proportion of *Bacteroidetes* in the different samples showed high variation, in the range of 2.9 to 21.10 %. The members from *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Firmicutes*, *Gemmatimonadetes* and *Verrucomicrobia* comprised 12.18 %, 8.86 %, 5.48%, 3.01%, 5.44%, 2.28 % and 2.83%, respectively, in all libraries. The average reads of the unclassified group accounted for 2.26 %, but this value fluctuated for different sites. Phylogenetic analysis revealed clear differences in community composition related to soil CO_2 emission values (Fig. 3A).

The relative abundance of *Bacteroidetes* phylum increased along the CO_2 gradient from 9.40-10.60% in the control sampling site to 18.70-19.20% in the extreme sampling site, with *Cytophagales* being the more abundant order within this phylum. However, the *Bacteroidales* order showed the most obvious increasing trend with respect to the CO_2 flux (Fig. 3B). On the other hand, relative abundance of *Firmicutes* phylum increased from control (3.50-4.20%) to extreme sampling sites (8.70-9.70%). Moreover, the relative abundance of the genus *Lactobacillus* increased sharply from 1.10% to 6.95% (Fig. 3B). By contrast, the relative abundances of *Acidobacteria* and *Chloroflexi* phyla decreased along the CO_2 flux, from 17.70-20.80 % in the control to 6.20-10.50% in the extreme sample sites. *Anaerolineae*, *Chloroflexi* and *Thermomicrobia* were the dominant genera in the *Chloroflexi* phylum and their relative abundances decreased along the CO_2 gradient (Fig. 3B). Changes in the relative abundance of *Actinobacteria* and *Proteobacteria* phyla were not so obvious. Overall, the *Actinobacteria* class, which is mainly composed of the *Actinomycetales* order, was the most abundant *Actinobacteria*.

Soil bacterial community comparison

Beta diversity analysis based on the quantitative weighted unifrac metric (Fig. 4A) and nonmetric multidimensional scaling was performed (Fig. 4B). The unifrac metric provides a robust index of community phylogenetic distances, whereas the nonmetric multidimensional scaling retains the original ecological abundance-based similarity. Low, medium, high and extreme CO_2 bacterial communities were considered as a single group, and separated from the control communities by the first axis. In contrast, the high and extreme CO_2 bacterial communities were assigned into one group, and separated from low and medium communities by the second axis. The calculated plot obtained using the NMDS grouped bacterial communities following a similar pattern, with the first axis explaining most of the variations. However, the low CO_2 bacterial communities were divided into C group. Fig. 4B also showed that bacterial communities sampled in the high and extreme CO_2 sites were phylogenetically closer than the group formed by bacterial communities sampled in the low and medium CO_2 sites. The bacterial communities were different from each other with respect to the CO_2 flux categories ($p < 0.001$).

The redundancy analysis (RDA) ordination diagram is shown in Fig. 5. The goodness of fit statistic for environmental variables showed that ordination was highly correlated with CO_2 and pH, NO_3^- nitrogen and EC value ($p < 0.01$) with long arrow (Fig. 5A). Among the environmental factors, pH, NO_3^- nitrogen and EC value were highly correlated with Axis 1, which explains most of the variation in the bacterial communities. This is not surprising that pH is correlated with CO_2 as it was previously commented. The pH arrow was the longest one, which might indicate that it was the most important variable regarding bacterial community ordination. Also, bacterial community distribution follows a gradient from low (control, low and medium samples) to high CO_2 flux (high and extreme samples). Moreover, organic matter distinguished category of low and high bacterial community samples along Axis 2 (Fig. 5A).

In terms of bacterial community composition, the RDA also showed several OTUs particularly characteristic of some communities since these OTUs were plotted around particular samples (Fig. 5B). Considering the frequent OTUs, high and extreme CO_2 bacterial communities were associated with OTUs related to *Bacteroidetes* and *Firmicutes* phyla: *Bacteroidales* (OTU0003 and OTU0004), and *Lactobacillus* (OTU0009, OTU0010, OTU0011 and OTU0013). By contrast, control samples were characterized by OTUs related to *Acidobacteria-6* (OTU0006, OTU0017 and OTU0018) and *Chloroflexi* phylum: *Gitt-GS-136*(OTU0005), *Thermomicrobia* (OTU0008), *Anaerolineae* (OTU0016) and *Ellin6529* (OTU0001 and OTU0014).

Discussion

Possible impacts of CO_2 leaks from the CCUS-EOR process on the ecosystem needs to be assessed in order to understand how CO_2 fluxes might affect soil bacterial communities before introducing this technology to oil displacement. For a better understanding of the effects of CO_2 emissions on soil bacterial communities, high-throughput 16S RNA gene sequencing was used to provide detailed information on soil bacterial communities. Our results showed that simulated soil CO_2 emissions significantly altered the diversity and structure of bacterial communities, especially for certain genera.

Within the area investigated, four sampling sites were designed according to CO_2 fluxes. The sites were defined as low, medium, high and extreme sites, with CO_2 flux varying from the rate of 200 g m^{-2} d^{-1} at the low site to 1200 g m^{-2} d^{-1} at the extreme site. Along this gradient, soil pH decreased slightly, showing a negative correlation with CO_2 flux. Beaubien et al. [7] and Oppermann et al. [29] have reported decreasing pH values in CO_2 leaking sites. H_2CO_3 is assumed to be formed by CO_2 gas which reacted with soil pore water or filled the pore space, which in turn lowered the pH of soil solution [35,36]. Thus in this study, it was speculated that decreases in pH might be attributed to the acidification of soil water as a result of the dissolution of leaking CO_2 gas. On the other hand, McFarland et al. [21] did not find significant variation in soil pH in response to CO_2 flux. Moreover, several other studies have revealed that different soil pHs often give rise to changes in soil microbial communities across different locations [37,38]. Organic matter is positively correlated to CO_2 flux. Miera et al. [39] also reported similar result, and inferred that it might be related to the low turnover of organic matter under reducing conditions. The analyzed soil parameters were not statistically significant among sampling sites, so we could assume that CO_2 flux could be the main factor that led to changing soil bacterial structure and composition.

Plant species have been reported to affect the diversity and composition of bacterial communities [16,40], so we designed the experimental sampling sites without plant cultivation. Diversity and richness of the bacterial communities decreased with increasing CO_2 fluxes (Fig. 2). Decreases in diversity have also been reported in other studies [38]. However, several influential factors such as the use of different

regions of the 16S rRNA genes, the sequence length and definition of OTU have made comparison of diversity and richness estimates between different reports unrealistic [41].

The bacterial phyla in the soil represented in this study were consistent with the results from other soils [39,42-44] in that most sequences belonged to nine major phyla, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Gemmatimonadates* and *Verrucomicrobia*, although the last three phyla accounted for a lesser portion of the sequences. Nevertheless, the distributions of sequences among and within phyla were different for different sampling sites, indicating a possible effect of soil CO_2 flux. Further examination of those phyla revealed differences among some special bacterial species, either at the class or lower levels. The *Acidobacteria* phylum did not show an obvious changing trend in the CO_2 emission sites, which was different from its increases in CO_2 gas vents as suggested by Oppermann et al. [29]. In *Chloroflexi*, the relative abundance of *Ellin6529* class, which accounts for the majority of the phyla, decreased noticeably along the CO_2 gradient. Two other dominant classes, *Anaerolineae* and *Thermomicrobia*, also showed a declining trend. *Thermomicrobium* is known to exist as an aerobic Chemoheterotroph [45]. As the CO_2 gradient increases, the proportion of O_2 gas in the soil decreases. Decreases in the number of bacteria from the *Thermomicrobia* phylum (Fig. 3) conformed to the CO_2 gradient. On the contrary, Miera et al. [39] have also reported that the relative abundance of *Chloroflexi* noticeably increases along the CO_2 gradient. Furthermore, the *Bacteroidales* order, which belongs to the *Bacteroidetes* phylum also increased significantly. In a simulated underground storage site, Morales et al. [46] have also investigated the effects of elevated CO_2 on microbial community richness and composition using pyrosequencing analysis. They demonstrated the similar results with ours that bacterial richness decreased, and bacterial community composition shifted in response to CO_2. Moreover, their study also expressed that *Bacteroidetes* phylum was the most affected, with 11 OTUs either decreasing or increasing in response to CO_2 enrichment. One the other side, the relative abundance of the genus *Lactobacillus*, which was included in the *Firmicutes* phylum, increased significantly. These results were the first report to demonstrate the presence of increasing *Lactobacillus* along the CO_2 gradient. It is difficult to explain why some bacteria increased or decreased in the high and extreme communities. Since many reports of CO_2 gas vent were aimed at specific bacterial activities, such as methane production and sulphate oxidation [18,29]. In CO_2 rich hypoxic soils Šibanc et al. [47] reported that methanogenic taxa dominated with significant increase in abundance of *Methanomicrobia* and anaerobic *Chloroflexi* and *Firmicutes* were also predominantly. However, information about the presence of bacteria related to *Chloroflexi* and *Firmicutes* phyla are still not sufficient available. The high-throughput 16S RNA gene sequencing used in this study has provided extensive information about the taxa present in bacterial communities along the CO_2 gradient, but gave little insight into the functional role of the *Lactobacillus* and *Lactococcus* genera. The ecological function of these taxa is not completely known and a more extensive research of its metabolism and diversity might provide new information to complete this study.

The beta analysis results demonstrated that the microbial community composition significantly changed under different CO_2 flux pressures. This finding was consistent with the alpha analysis results. The effect of soil properties on the community difference has been described. RDA result demonstrated that the larger gap between C group and other groups under CO_2 flux pressures. It is in accordance with the high EC and NO_3^- nitrogen value in C group. This indicated that there might be some correlation between high EC, NO_3^- nitrogen value and different community composition. In Fig. 5B, the arrows of OTU0003, OTU0005, OTU0008, OTU0009 and OTU0010 were longer, which indicated that these four OTUs were the most important variable OTUs regarding sample groups.

The relationship between OTU and sample showed that the abundance order of OTU0005 and OTU0008 in each sample was C>L>M>H>E. On contrast, the abundance order of OTU0003, OTU0009 and OTU0010 was E>H>M>L>C. This result also confirmed that *Bacteroidales*, *Thermomicrobia* and *Lactobacillus* were significantly impacted under different CO_2 flux pressure.

Conclusion

Shifts in diversity and composition of bacterial communities in a simulated CO_2 gradient area were defined by soil CO_2 flux. Parameters (NO_3^- nitrogen, EC and pH) correlated with CO_2 flux have provided some explanations for the differences among the low, medium, high and extreme CO_2 bacterial communities. Little research has focused on understanding the impact of high soil CO_2 fluxes on soil bacterial communities. New findings are considered important in the development of remedial measures for handling CO_2 leakage from CCUS-EOR. The data obtained here for the characterization of soil communities, in particular the obvious changing trend related to *Chloroflexi* and *Firmicutes* phyla warrant further research on this environmental pattern and the CO_2 effect on bacterial communities. In the future, such knowledge will be useful to understand the potential effects of CO_2 leakages from EOR sites on soil ecosystems.

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TABLE 1. Soil physicochemistry and soil CO_2 flux in sampling point. C = control; L = Low CO_2 sites; M = Medium CO_2 sites; H = High CO_2 sites; E = Extreme CO_2 sites; R= Resilience. 1, 2, 3, 4 stand for 4 parallel sample plots of each CO_2 flux level.

Sampling point		pH	EC	Organic matter (%)	NO_3^- (mg/kg)
Control	C1	7.83	255	0.65	5.99
	C2	8.06	226	0.75	6.10
	C3	7.73	239	0.64	5.62
	C4	7.97	242	0.74	6.59
Low	L1	7.66	188	0.89	2.83
	L2	7.60	154	0.77	2.97
	L3	7.70	175	0.84	2.79
	L4	7.74	179	0.79	2.91
Medium	M1	7.47	192	0.76	1.87
	M2	7.58	173	0.81	1.91
	M3	7.50	187	0.84	1.85
	M4	7.42	185	0.78	1.88
High	H1	7.31	183	0.83	1.55
	H2	7.16	179	0.87	1.27
	H3	7.25	172	0.80	1.37
	H4	7.18	175	0.78	1.42
Extreme	E1	6.59	188	0.83	1.19
	E2	6.98	179	0.75	1.02
	E3	6.78	165	0.89	1.13
	E4	6.43	181	0.87	1.08
Resilience	R1	7.51	140	0.87	0.11
	R2	7.3	156	0.75	0.09
	R3	7.2	167	0.65	0.10
	R4	7.37	160	0.62	0.45

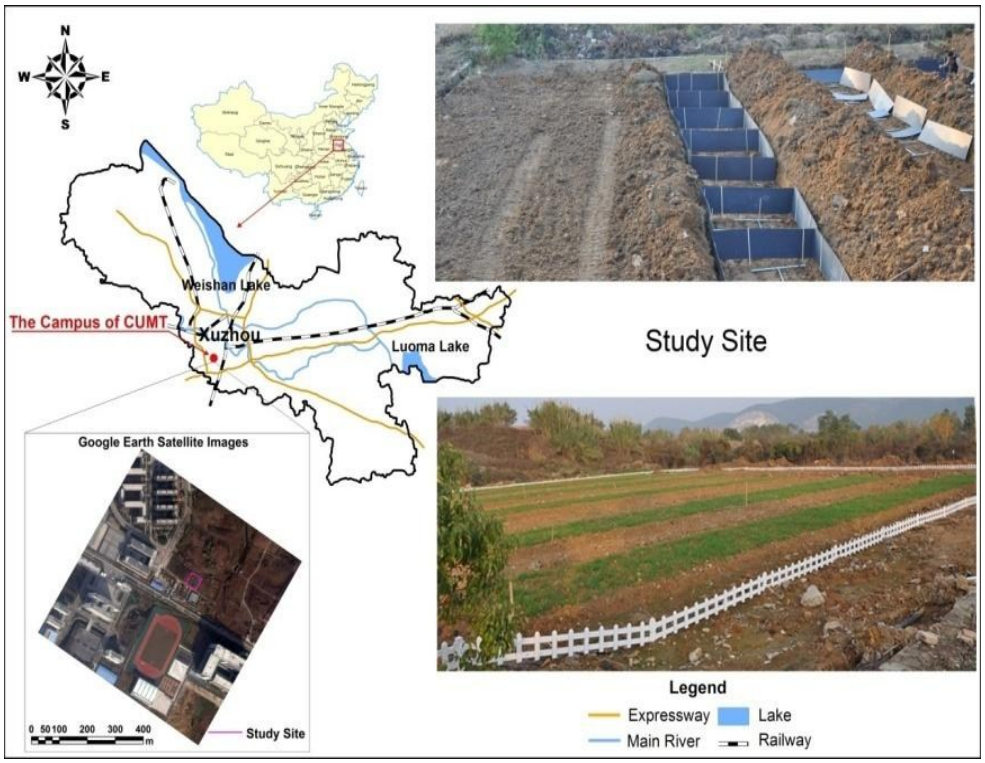

FIG. 1. Location map and studied area photos

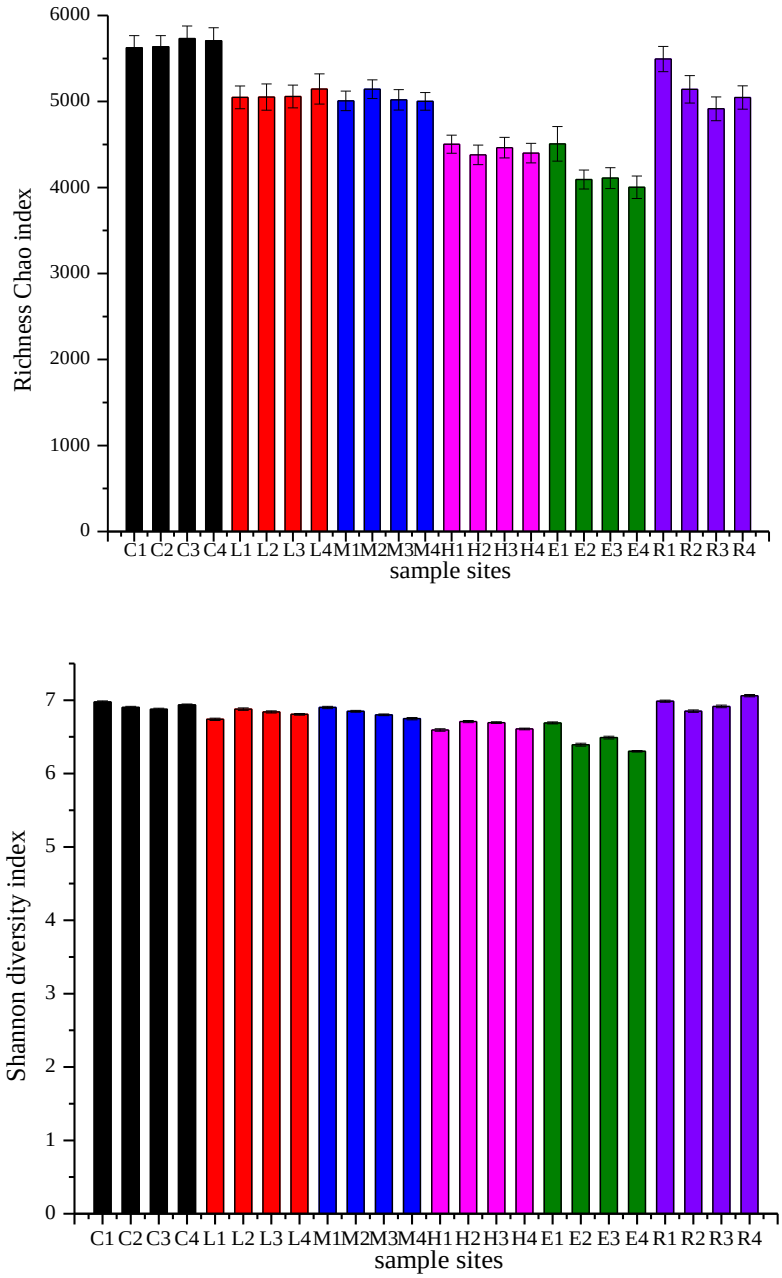

FIG. 2. Richness (Chao index) and diversity (Shannon diversity index) plotted against CO_2 flux (C = control; L = Low CO_2 sites; M = Medium CO_2 sites; H = High CO_2 sites; E = Extreme CO_2 sites; R= Resilience. 1, 2, 3, 4 stand for 4 parallel sample plots of each CO_2 flux level.)

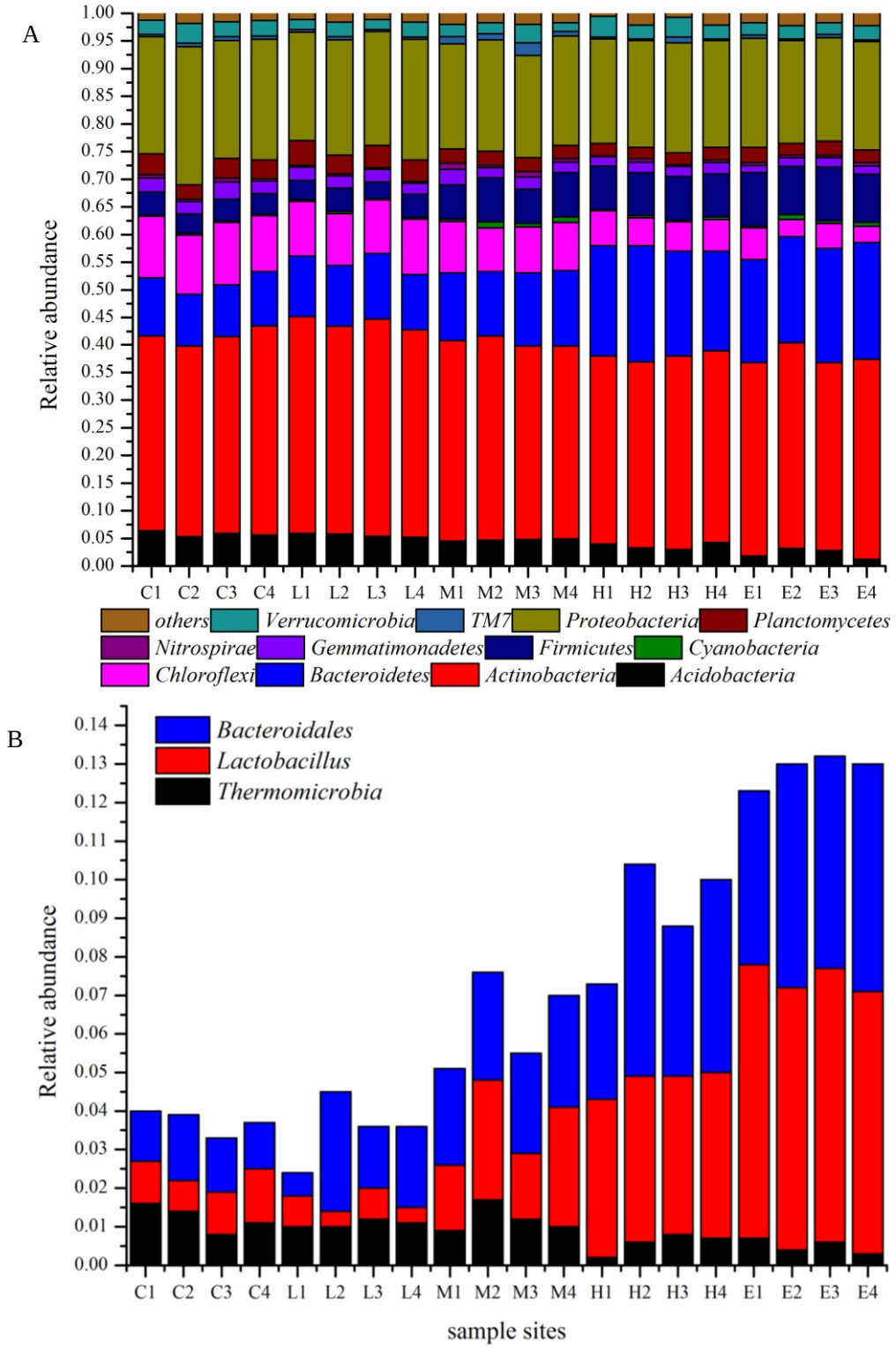

FIG. 3. Taxonomic distributions of soil samples. A, Phylum distribution level; B, dominant genus in *Chloroflexi*, *Firmicutes* and *Bacteroidetes*(C = control; L = Low CO_2 sites; M = Medium CO_2 sites; H = High CO_2 sites; E = Extreme CO_2 sites. 1, 2, 3, 4 stand for 4 parallel sample plots of each CO_2 flux level.)

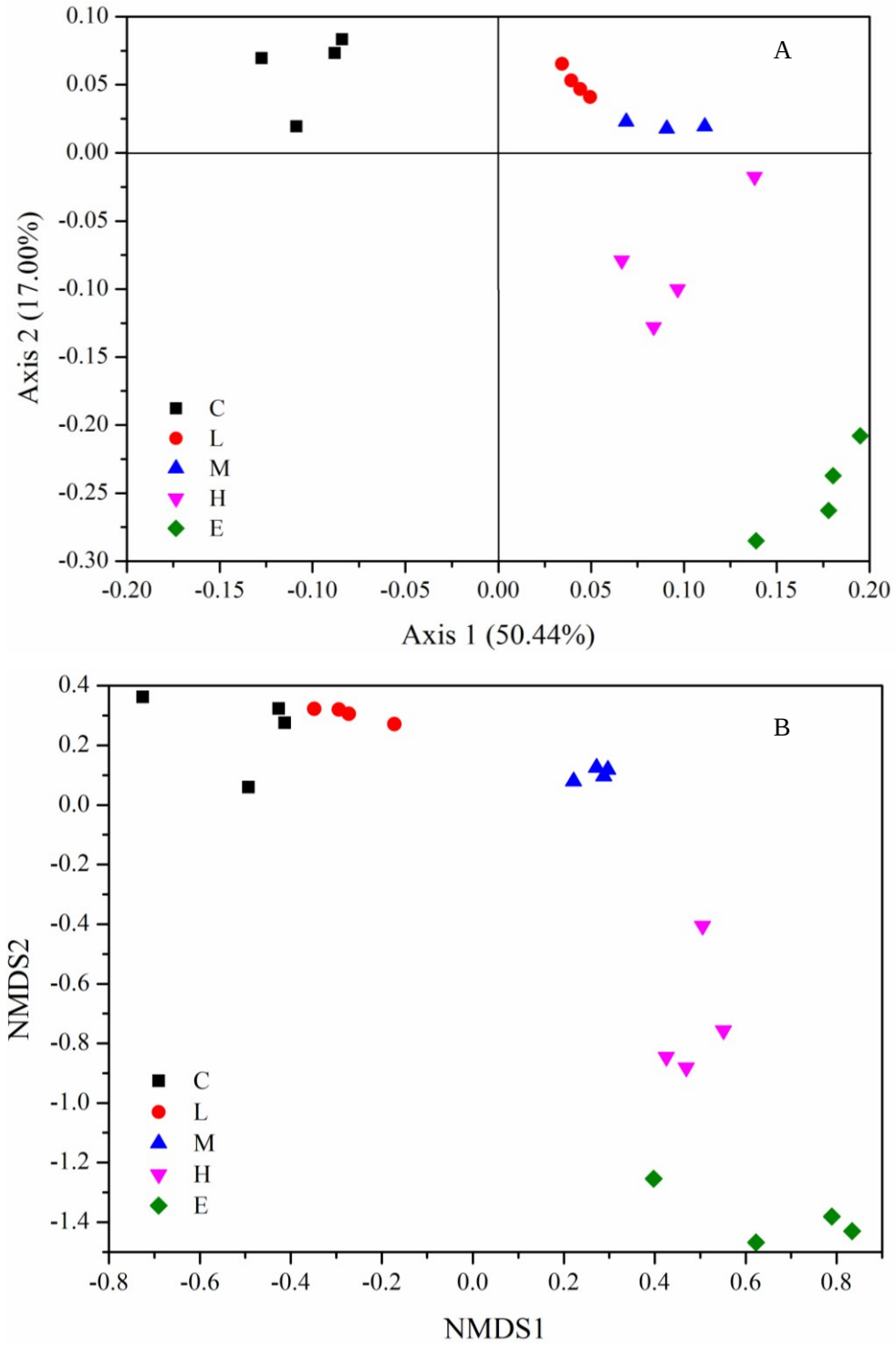

FIG. 4. Beta diversity analysis plots derived from Unifrac distances (A) and NMDS (B) (C = control; L = Low CO_2 sites; M = Medium CO_2 sites; H = High CO_2 sites; E = Extreme CO_2 sites. 1, 2, 3, 4 stand for 4 parallel sample plots of each CO_2 flux level.)

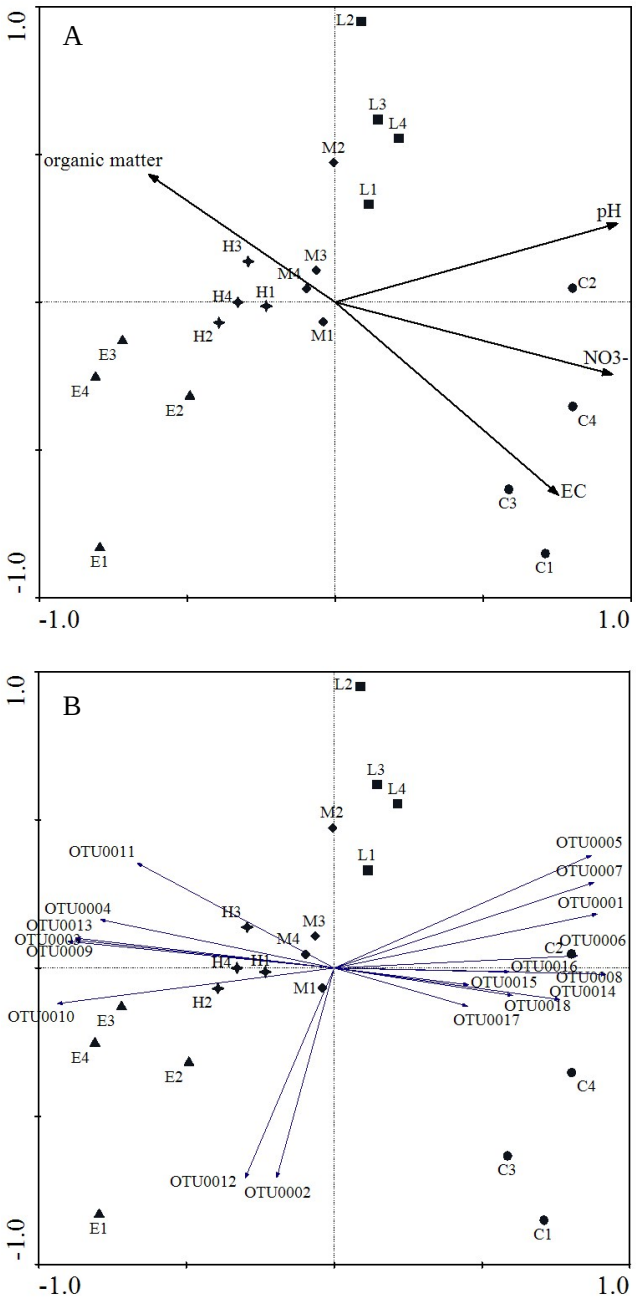

FIG. 5. Redundancy analysis ordination plot showed the relationship between the bacteria community and soil properties (A) or some frequent OTUs (B) (C = control; L = Low CO_2 sites; M = Medium CO_2 sites; H = High CO_2 sites; E = Extreme CO_2 sites. 1, 2, 3, 4 stand for 4 parallel sample plots of each CO_2 flux level.)