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Original Research

Impact of CO_2 Leakage from Geological Storage on the Eco-environment: Rhizosphere Microenvironment of Soybean Case

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Abstract

As a crucial technology for global CO_2 reduction, CO_2 capture and storage (CCS) faces leakage risks that endanger surrounding organisms and the environment. This study aimed to clarify how CO_2 leakage impacts the rhizosphere soil's physicochemical properties and microbial communities, supporting CCS environmental risk assessments. A simulation platform was built, and the 16S rDNA high-throughput sequencing, correlation, and redundancy analyses were used to examine soybean cultures. Results showed that as soil CO_2 concentration rose, pH and O_2 significantly declined, while Soil Organic Carbon and Ammonium Nitrogen increased. Total Nitrogen and Nitrate Nitrogen decreased, with the latter dropping notably. Microbial Biomass Carbon and Nitrogen in soybean rhizosphere soil rose at 10% and 30% CO_2 but fell at 50%. CO_2 leakage reduced the Chao1 index of rhizosphere soil bacteria yet increased the Pielou's evenness index. Although dominant bacterial phyla remained consistent, Bacteroidetes became more abundant, while Proteobacteria, Acidobacteria, and Nitrospirae decreased. O_2 and pH were key factors shaping bacterial diversity and phylum-level community abundance.

Keywords: bacterial diversity, bacterial community structure, NovaSeq sequencing, CO_2 leakage, carbon dioxide capture and storage

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Introduction

China's "dual carbon" goal is a crucial national strategy in actively addressing global climate change [1, 2]. Carbon dioxide capture and storage (CCS) involves utilizing the captured CO_2 from high-emission sources, such as the energy industry, and injecting it into geological formations, which is a crucial technology for reducing CO_2 emissions [3]. Nevertheless, the potential for CO_2 leakage during the storage phase remains a critical concern. Leakage pathways, including injection wells, geological faults, and other subsurface discontinuities, pose significant risks [4, 5]. Such leakage events can have far-reaching consequences, potentially endangering the surrounding biotic communities and disrupting ecological functions [6, 7].

Since the release of the Intergovernmental Panel on Climate Change (IPCC) special report on carbon dioxide capture and storage in 2005, considerable progress has been made in studying the potential impacts of CO_2 leakage from carbon sequestration projects on near-surface terrestrial ecosystems [8]. This research has been particularly focused on North America, Europe, South America, and Australia. The primary methods of study have involved observing natural CO_2 leakage systems and conducting field injection tests intentionally designed to generate shallow CO_2 leakage through the soil and into the atmosphere. Research conducted at both natural CO_2 leakage points and shallow CO_2 leakage field experiments has centered on directly or indirectly evaluating the impact on surrounding plants and soil microecology [9]. These leakage observations and experiments have yielded a wealth of information about the effects of increased soil CO_2 gas concentration and soil CO_2 flux on surface ecosystems.

The scientific community widely agrees that CO_2 leakage results in a significant increase in soil CO_2 concentration. The soil CO_2 flux, caused by CO_2 leakage in carbon sequestration projects, can be substantial enough to increase the CO_2 concentration in the atmosphere, while also significantly elevating the soil CO_2 concentration, typically achieving a volume ratio of 10-95% of soil CO_2 [10]. The soil's physical and chemical properties, as well as the response of soil microorganisms to CO_2 leakage, are more immediate and direct. Both natural CO_2 leakage points and CO_2 injection field experiments have observed significant decreases in soil pH and oxygen content [11, 12]. Concurrently, an increase in soil total organic carbon (TOC) and cation exchange capacity (CEC) was observed at the epicenter of natural leakage points such as Latera and Laacher See [13]. Leakage simulation experiments revealed that CO_2 leakage increased soil TOC, decreased total nitrogen (TN), and reduced nitrate nitrogen (NO_3^--N) [14-16]. Moreover, CO_2 leakage significantly impacts microbial activity in the rhizosphere soil of plants, primarily manifested in the weakening of microbial respiration and the reduction of total microbial quantity [17, 18]. Extensive monitoring data indicate that CO_2 leakage leads to a significant increase in the concentration of surface CO_2 in the soil environment, resulting in changes in soil physical and chemical properties and microbial activity processes. These soil physical and chemical properties and microbial activities further interact with each other, leading to changes in the soil microecological environment. As the most active component in the soil environment, changes in bacterial diversity and community structure significantly impact the functioning of soil ecosystems, as well as the decomposition of soil organic matter, nitrogen fixation and transformation, and the supply of rhizosphere nutrients [19, 20]. Therefore, studying the effects and interactions of CO_2 leakage on soil physicochemical properties, bacterial diversity, and community structure is of paramount importance.

The Ordos Basin, a large-scale cratonic sedimentary system in northern China, has been identified as the prime potential area for geological carbon dioxide storage, primarily by virtue of its exceptional tectonic stability and unique stratigraphic configuration [21]. This study concentrates on the rhizosphere soil of soybeans, a plant species known for its sensitivity to CO_2 leakage of the Ordos Basin [22]. A CO_2 leakage simulation platform was established, and a high-throughput sequencing technology was employed to investigate changes in physicochemical properties, bacterial diversity, and community structure in soybean cultures under CO_2 leakage conditions. Furthermore, it analyzed the interrelationships between these factors based on correlation analysis and redundancy analysis. This research not only deepens our understanding of the impact of CO_2 leakage on soil microorganisms but also provides a scientific basis for assessing the environmental impact and risks associated with CCS projects.

Materials and Methods

Experimental Design

To simulate the impact of CO_2 leakage in CCS on physicochemical properties, bacterial diversity, and community structure in soybean cultures, this experiment employed a CO_2 leakage simulation platform. The CO_2 leakage simulation platform primarily comprises four components: a CO_2 sensor, a CO_2 controller, a solenoid valve, and an online monitoring system (Fig. 1). The soil CO_2 concentrations were set at four levels: normal concentration (CK), low (10%), medium (30%), and high (50%) (Fig.1). For the detailed design of the CO_2 leakage simulation platform, the planting box and gas distribution mode, please refer to the article Xue et al. [23].

This experiment utilized the CO_2 leakage-sensitive soybean crop (Shanning 17), previously selected by the research group, as the subject of study [22]. The soil used in the experiment is a typical silty clay loam,

Fig. 1. The CO_2 leakage simulation platform and experimental site

composed of 11.80% sand, 68.47% silt, and 19.73% clay. The simulation experiment commences at the five-leaf stage of the soybean and continues until maturity, spanning a period of 80 days of CO_2 aeration planting.

Indicator Measurement and Methods

Upon completion of the 80-day CO_2 aeration planting, soybean rhizosphere soil samples were collected from a depth of 0-30 cm using the multi-point mixing method. For each leakage level, three replicate samples were collected, resulting in a total of 12 soil samples. A portion of the soil samples was pretreated to determine various soil physicochemical properties, including soil pH, O_2, total organic carbon (TOC), total nitrogen (TN), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN). Another portion of the fresh soil samples was stored at -80°C for subsequent determination of bacterial diversity and community structure in the rhizosphere soil.

Among the measurements taken, soil pH was determined using an in-situ soil pH meter (TZS-pH-IG, China), soil O_2 was measured using a handheld oxygen content meter (Apogee MO-200, USA), TOC and TN were quantified using a fully automated elemental analyzer (EA3000, Italy), NH_4^+-N and NO_3-N were measured using a fully automated intermittent chemical analyzer (Cleverche 200+, Germany), and MBC and MBN were determined using a total organic carbon analyzer (Isotope TOC cube, Germany).

Shanghai Personal Biotechnology Co., Ltd (Shanghai, China) was commissioned to conduct high-throughput sequencing of 16S rRNA V3-V4 using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit for rhizosphere soil bacteria. The process included:

(1) Extraction and Amplification of Total Bacterial DNA: DNA was extracted from 12 soil samples using a soil DNA extraction kit, and the quality of the extracted DNA was assessed via 1.2% agarose gel electrophoresis. Primers 338F (5'-ACTCCTACGGGGCAGCA-3') and 806R (5'-GACTACHVGGGTWTCTAAT-3'), designed based on conserved regions in the sequence, were used to perform PCR amplification on the V3-V4 region of 16S rRNA. The specific amplification program included an initial denaturation at 98°C for 2 minutes, followed by 25-30 cycles of denaturation at 98°C for 15 seconds, annealing at 55°C for 30 sec, and extension at 72°C for 30 sec, with a final extension at 72°C for 5 min.

(2) Purification and Quantification of Amplification Products: Following the acquisition of PCR amplification products of soil bacteria via DNA polymerase, the amplification products were purified and recovered using magnetic beads with a sample volume of 0.8 times. Fluorescent reagents (Quant-iT PicoGreen dsDNA Assay Kit) and an enzyme-linked immunosorbent assay (BioTek, FLx800) were then used to perform fluorescence-quantitative analysis of the recovered PCR amplification products.

(3) Sequencing Library Preparation and Sequencing: Sequence end repair was performed on the purified and quantified amplification products to ensure the target sequence was connected to the sequencing sequence connector. PCR amplification was performed on the DNA fragments connected to the adapter to enrich the sequencing library template. The enriched library products were then selected and purified. The Promega QuantiFluor system was reused for quantitative analysis of the library, with the qualified library concentration being above 2 nM. The qualified sequencing library was diluted, mixed in proportion, and denatured into a single strand using NaOH for sequencing. Paired-end sequencing of soil bacterial DNA fragments was conducted using the Illumina NovaSeq-PE250 platform.

(4) Sequence Denoising and Taxonomic Annotation: The DADA2 method was used for quality control, denoising, splicing, and chimerism removal on the original sequence. Each deduplicated sequence generated after quality control is referred to as ASVs (amplicon sequence variants), and the rationality of sequencing depth was assessed through rarefaction curves. Based on the SILVA database, a pre-trained Naive Bayes classifier was used to annotate the species

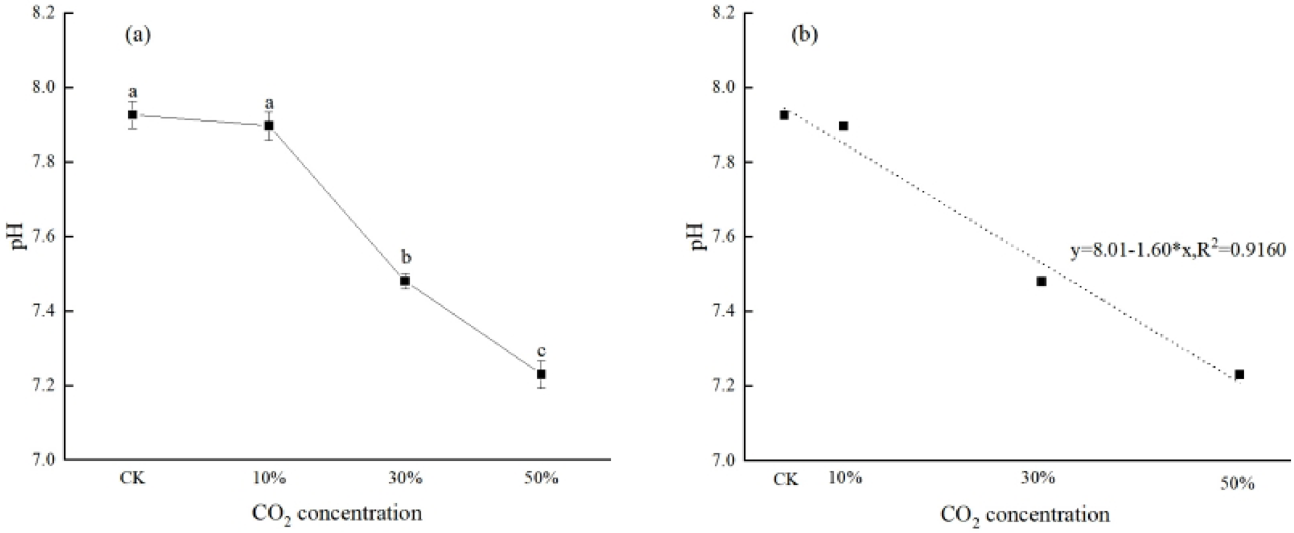

Fig. 2. Change in pH in soybean rhizosphere soil under CO_2 leakage conditions.
Note: Different lowercase letters indicate a significant difference (*P<0.05*) among treatments; The same applies to subsequent figures.

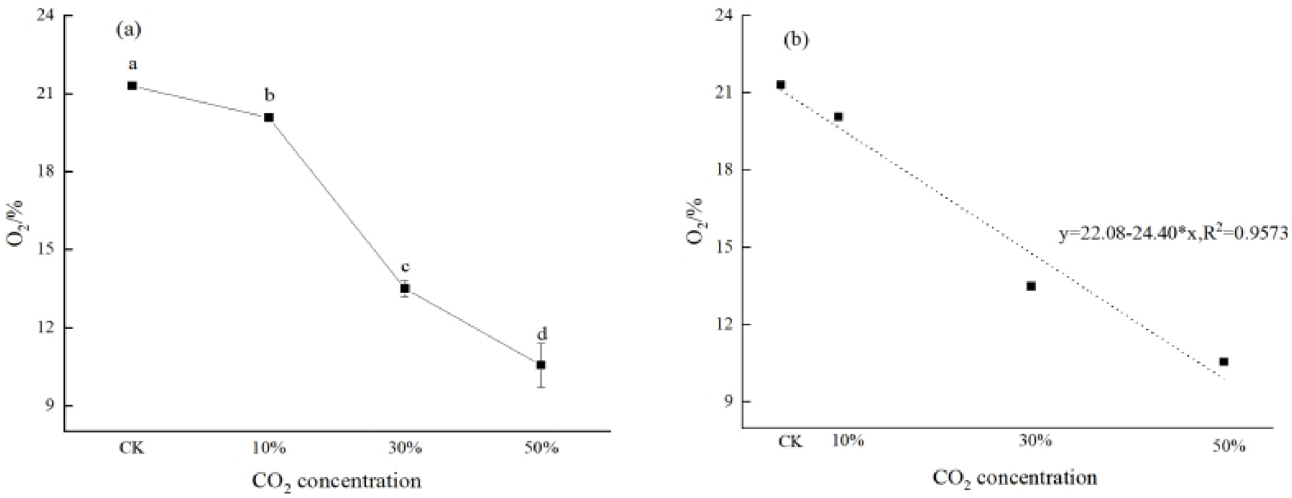

Fig. 3. Change in O_2 in soybean rhizosphere soil under CO_2 leakage conditions.

of each ASV feature sequence using QIIME2's classify-sklearn algorithm.

Data Statistics and Analysis

The alpha diversity index (Chao1, Simpson, Pielou's evenness, and Good's coverage) of bacterial communities was calculated using QIIME2 2019.4 software (Lab of Rob Knight and Greg Caporaso, USA). Redundancy analysis (RDA) was employed to analyze the relationship between environmental factors, microbial diversity, and community structure, and the permutation test was utilized to ascertain whether these factors significantly impact microbial structure and function. SPSS 22.0 (SPSS Inc., USA) was used to conduct the significance test and correlation analysis for soil physicochemical properties, the alpha diversity index, and the relative abundance of bacteria at the phylum level in different groups, among which the least significant difference (LSD) method was applied for multiple group comparisons. All statistical analyses and mapping of bacterial diversity and community structure were performed using various R software packages (3.5.1).

Results

Impact of CO_2 Leakage on Soil Physical and Chemical Properties

Changes in pH and O_2

CO_2 leakage significantly influences the pH and O_2 levels of soybean rhizosphere soil, both of which exhibit a marked decreasing trend with the increase

Table 1. Changes of soil organic carbon (SOC), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) in soybean rhizosphere soil under CO_2 leakage conditions.

Soil CO_2	SOC g·kg-1	TN g·kg-1	NH_4^+-N mg·kg-1	NO_3^--N mg·kg-1	MBC mg·kg-1	MBN mg·kg-1
CK	6.44±0.59a	1.31±0.04a	2.87±0.04b	0.21±0.02a	10.53±0.37c	9.56±0.31c
10%	6.69±0.60a	1.22±0.08a	2.9±0.10b	0.18±0.01b	11.11±0.06b	10.55±0.14b
30%	7.11±0.03a	1.21±0.15a	3.02±0.01a	0.15±0.01c	11.61±0.08a	12.44±0.26a
50%	7.18±0.02a	1.09±0.06b	3.09±0.01a	0.11±0.01d	7.37±0.34d	7.77±0.05d

Note: Different lowercase letters in the same column indicate a significant difference ($p < 0.05$) among treatments.

Table 2. Changes of alpha diversity in soybean rhizosphere soil under CO_2 leakage conditions.

Soil CO_2	Chao1	Simpson	Pielou's evenness	Good's coverage
CK	7255.55±151.96a	11.36±0.02a	0.8994±0.0012a	0.9831±0.0013a
10%	7215.17±56.99b	11.37±0.08a	0.8992±0.0013a	0.9818±0.0009a
30%	7115.26±56.79c	11.35±0.04a	0.9005±0.0013a	0.9827±0.0005a
50%	6945.38±122.12d	11.36±0.05a	0.9023±0.0009b	0.9836±0.0008a

in soil CO_2 concentration (Fig. 2 and Fig. 3). Notably, a significant difference ($P<0.05$) was observed in pH and the CK of soybean rhizosphere soil when the soil CO_2 concentration reached 30% and 50% (Fig. 2a)). Similarly, a significant difference ($P<0.05$) was found in O_2 and CK of soybean rhizosphere soil when the soil CO_2 concentration was at 10%, 30%, and 50% (Fig. 3a)). A robust linear relationship exists between soil pH, O_2, and soil CO_2 concentration, with R_2 values of 0.9106 and 0.9573, respectively (Fig. 2b) and Fig. 3b)).

Changes in Soil Carbon and Nitrogen Components

Under conditions of CO_2 leakage, the SOC of the soybean rhizosphere exhibited an increasing trend with the rise in soil CO_2 concentration, although no significant difference was observed compared to the CK (Table 1). The TN in the soybean rhizosphere soil demonstrated a decreasing trend with the increase in soil CO_2 concentration. Notably, a significant difference ($P<0.05$) was observed between TN and CK in the soybean rhizosphere soil when the soil CO_2 concentration reached 50% (Table 1). Under CO_2 leakage conditions, the NH_4^+-N in the soybean rhizosphere soil showed a slight increase with the rise in soil CO_2 concentration, while NO_3^--N exhibited a significant decrease. Compared to CK, increases of 1.28%, 5.40%, and 7.72% were observed in NH_4^+-N in the soybean rhizosphere soil when the soil CO_2 concentration was at 10%, 30%, and 50%, respectively. Moreover, significant differences ($P<0.05$) were observed between NH_4^+-N and CK when the soil CO_2 concentration was at 30% and 50%. In contrast, reductions of 14.73%, 30.23%, and 44.96% were observed in NO_3^--N in the soybean rhizosphere soil when the soil CO_2 concentration was at 10%, 30%, and 50%, respectively, all of which showed significant differences ($P<0.05$) (Table 1).

CO_2 leakage significantly impacts the MBC and MBN of the soybean rhizosphere soil. Compared to CK, the MBC and MBN of the soybean rhizosphere soil significantly increased ($P<0.05$) when the soil CO_2 concentration was at 10% and 30%, and significantly decreased ($P<0.05$) when the soil CO_2 concentration reached 50% (Table 1).

Impact of CO_2 Leakage on Bacterial Communities in Soybean Rhizosphere Soil

Impact of CO_2 Leakage on Bacterial Diversity

The Chao1, Simpson, Pielou's evenness, and Good's coverage diversity indices represent the richness, diversity, evenness, and species coverage of soil microbial communities, respectively. CO_2 leakage resulted in a significant decrease in the Chao1 index of soybean soil bacteria ($P<0.05$). Specifically, at soil CO_2 concentrations of 10%, 30%, and 50%, the Chao1 index decreased by 0.56%, 1.93%, and 4.27% compared to CK, respectively (Table 2). Under conditions of CO_2 leakage, Pielou's evenness index of soybean soil bacteria demonstrated an overall upward trend ($P<0.05$) (Table 2). However, no significant differences were observed in the Simpson and Good's coverage indices of soybean soil bacteria under varying CO_2 leakage conditions ($P>0.05$) (Table 2).

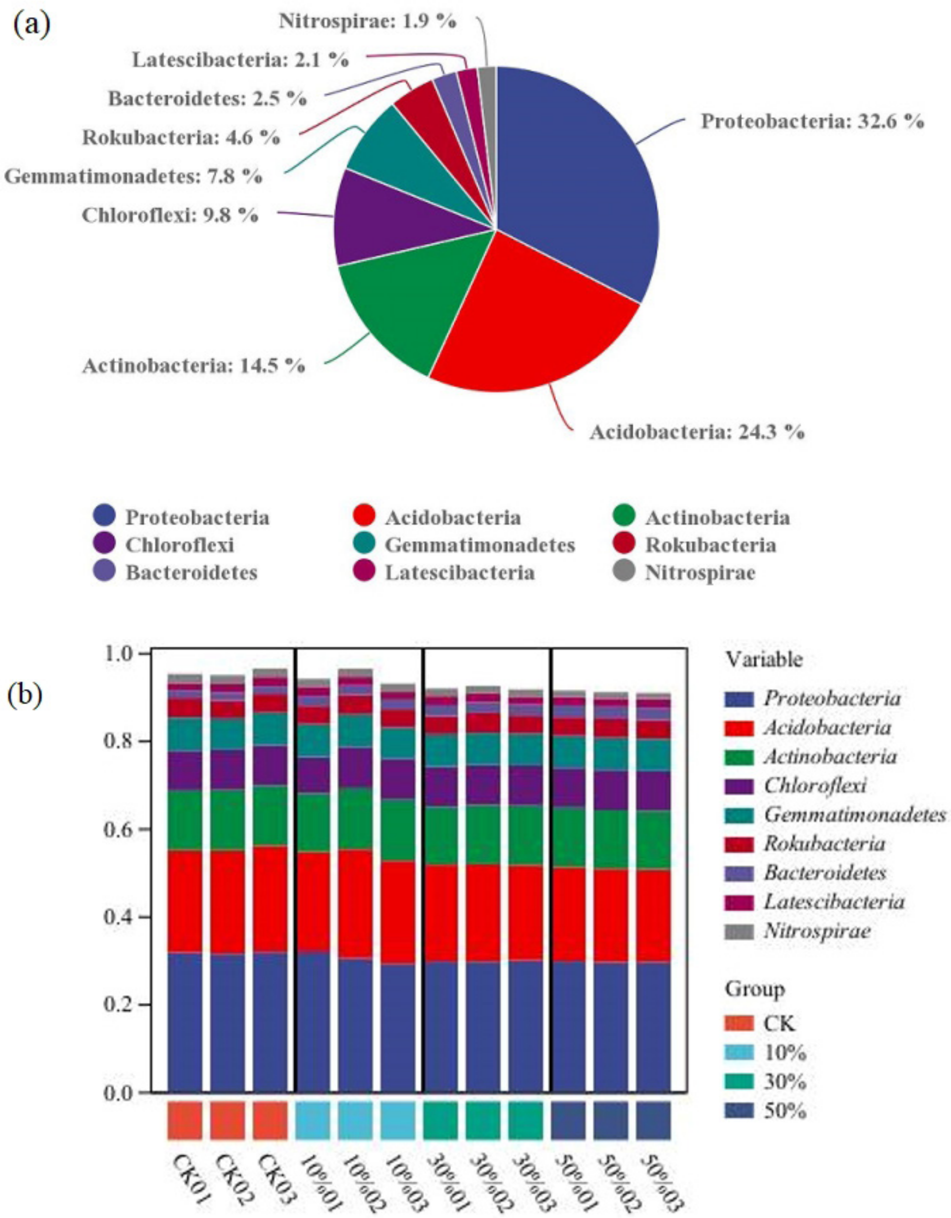

Fig. 4. a) Changes in composition and b) relative abundance of dominant bacteria at the phylum level in soybean rhizosphere soil under CO_2 leakage conditions.

Impact of CO_2 Leakage on Bacterial Community Structure

High-throughput sequencing of 12 soil samples from the soybean rhizosphere yielded a total of 1,121,907 high-quality sequences, with an average of 93,492 sequences per sample, ranging from 77,495 to 111,204. The sequencing identified a total of 42 phyla, 149 classes, 452 orders, 804 families, and 1,380 genera. The dominant bacterial phyla in the soybean rhizosphere soil samples included Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Rokubacteria, Bacteroidetes, Latescibacteria, and Nitrospirae, which collectively accounted for 93.25% of the relative abundance. Among these, Proteobacteria, Acidobacteria, and Actinobacteria were the most abundant, accounting for 32.60%, 24.30%, and 14.50% of the relative abundance, respectively (Fig. 4a)).

Under varying CO_2 leakage conditions, the composition of the dominant bacterial phyla in the soybean rhizosphere soil remained similar, but the relative abundance of some phyla varied. As the soil CO_2 concentration increased, the relative abundance of Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, and Nitrospirae decreased, while that of Bacteroidetes increased. The relative abundance of Rokubacteria and Latescibacteria did not change significantly (Fig. 4b)). Significant differences ($P<0.05$) were observed in the relative abundance of Proteobacteria, Acidobacteria,

Table 3. Changes in the abundance of dominant bacteria in soybean rhizosphere soil under different CO_2 leakage conditions.

Soil CO_2	Proteobacteria	Acidobacteria	Bacteroidetes	Nitrospirae
CK	31.67%±0.0023a	23.68%±0.0046a	1.78%±0.0005d	2.00%±0.0008a
10%	30.51%±0.0138b	23.67%±0.0108a	2.23%±0.0004c	1.85%±0.0005b
30%	29.76%±0.0013c	21.99%±0.0023b	2.44%±0.0003b	1.71%±0.0005c
50%	29.62%±0.0009d	21.27%±0.0021c	2.77%±0.0006a	1.41%±0.0001d

Bacteroidetes, and Nitrospirae under different CO_2 leakage conditions. When the soil CO_2 concentration was at 10%, 30%, and 50%, the relative abundance of Proteobacteria decreased by 1.16%, 1.91%, and 2.05% compared to CK, respectively, while the relative abundance of Acidobacteria decreased by 0.01%, 1.69%, and 2.40% compared to CK, respectively. The relative abundance of Bacteroidetes increased by 0.46%, 0.67%, and 0.99% compared to CK, respectively, and the relative abundance of Nitrospirae decreased by 0.16%, 0.29%, and 0.59% compared to CK, respectively (Table 3).

Analysis of the Relationship between Soil Physicochemical Properties and Bacterial Communities under CO_2 Leakage Conditions

Correlation Analysis of Soil Physical and Chemical Properties

The correlation analysis revealed that under CO_2 leakage conditions, soil pH exhibits a significant positive correlation with soil O_2. Both NH_4^+-N and nitrate nitrogen (NO_3^--N) show significant correlations with soil pH and soil O_2. Specifically, NO_3^--N is positively correlated with soil pH and soil O_2, while NH_4^+-N is negatively correlated with both. The SOC, TN, and MBC are significantly correlated with soil pH and soil O_2. In particular, SOC is negatively correlated with soil pH and soil O_2, while TN and MBC show positive correlations with both. However, MBN does not exhibit a correlation with either soil pH or soil O_2 (Table 4).

Redundancy Analysis of Soil Physicochemical Properties and Bacterial Diversity

Eight physicochemical properties of soybean rhizosphere soil, namely pH, O_2, SOC, TN, NH_4^+-N, NO_3^--N, MBC, and MBN, were used as explanatory variables, while alpha diversity data (Chao1, Simpson, Pielou_e, Observed_specifics) were used as response variables. Through redundancy analysis, the relationship between bacterial diversity and soil physicochemical properties was explored.

The redundancy analysis revealed that the characteristic value of axis 1 (RDA1) is 0.5155, explaining 51.55% of bacterial diversity differences, while the characteristic value of axis 2 (RDA2) is 0.0916, accounting for 9.16% of bacterial diversity differences. Cumulatively, RDA1 and RDA2 explain 60.71% of bacterial diversity differences (Fig. 5), indicating that these axes effectively reflect the correlation between bacterial diversity and soil physicochemical factors, primarily determined by RDA1. Among the eight physicochemical properties, all except SOC, which correlates more with RDA2, have a higher correlation with RDA1. NH_4^+-N correlates with both RDA1 and RDA2. In the RDA ranking chart (Fig. 5), Chao1 and Observed_specifics play significant roles. Chao1 is highly correlated with eight physicochemical indicators and is positively correlated with pH, O_2, NO_3^--N, TN, MBC, and MBN, but negatively correlated with SOC and NH_4^+-N. Observed_specifics, except for having little correlation with NH_4^+-N, have a certain positive correlation with pH, O_2, MBC, MBN, and SOC. Among the eight soil physicochemical properties, O_2, pH,

Table 4. Correlation analysis of physicochemical properties of soybean rhizosphere soil under CO_2 leakage conditions.

Index	pH	O_2	SOC	TN	NH_4^+-N	NO_3^--N	MBC	MBN
pH	1							
O_2	0.955**	1						
SOC	-0.695*	-0.647*	1					
TN	0.584*	0.655*	-0.192	1				
NH_4^+-N	-0.906**	-0.886**	0.699*	-0.486	1			
NO_3^--N	0.841**	0.905**	-0.423	0.737*	-0.819*	1		
MBC	0.630*	0.595*	-0.314	0.499	-0.519	0.539	1	
MBN	0.250	0.203	0.026	0.301	-0.198	0.240	0.889**	1

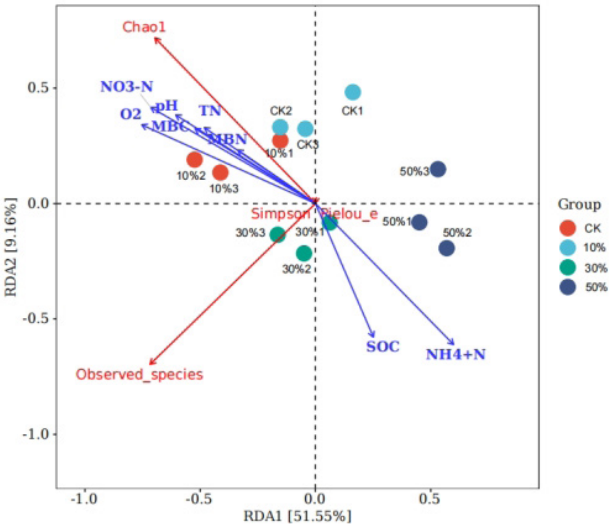

Fig. 5. RDA sequence of physicochemical properties and bacterial diversity of soybean rhizosphere soil under CO_2 leakage conditions.

Table 5. Redundancy analysis of physicochemical properties and bacterial diversity of soybean rhizosphere soil under CO_2 leakage conditions.

Soil environment factors	Explains %	Contribution %	pseudo-F	*P*
O_2	30.4	50.0	4.4	0.05
pH	7.8	12.9	1.1	0.284
NH_4^+-N	3.8	6.3	0.5	0.55
MBN	3.9	6.5	0.5	0.434
MBC	6.2	10.2	0.8	0.406
NO_3^--N	5.2	8.6	0.6	0.498
TN	2.1	3.5	0.2	0.702
SOC	1.3	2.1	0.1	0.796

NH_4^+-N, and MBN are the main properties affecting bacterial diversity under leakage conditions, with explanatory powers of 30.4%, 7.8%, 3.8%, and 3.9%, respectively. The Monte Carlo permutation test showed that O_2 is the key physicochemical factor affecting the differences in bacterial diversity in soybean rhizosphere soil (P<0.05) (Table 5).

Redundancy Analysis of Soil Physicochemical Properties and Bacterial Community Structure

Eight indicators of soil physicochemical properties, including pH, O_2, SOC, TN, NH_4^+-N, NO_3^--N, MBC, and MBN, were used as explanatory variables in the soybean rhizosphere soil. The relative abundance data of dominant bacterial communities (Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Rokubacteria, Bacteroidetes, Latescibacteria, Nitrospirae) at the phylum level in the soybean rhizosphere soil were used as response variables. Redundancy analysis was conducted to further explore the relationship between bacterial community structure at the phylum level and soil physicochemical properties.

The redundancy analysis of the physicochemical properties of the soybean rhizosphere soil and the relative abundance of dominant bacterial communities at the phylum level revealed that the characteristic value of axis 1 (RDA1) was 0.7171, explaining 71.71% of the differences in dominant bacterial communities at the phylum level. The characteristic value of axis 2 (RDA2) was 0.0763, accounting for 7.63% of the differences. Cumulatively, RDA1 and RDA2 explained 79.35% of the differences (Fig. 6), indicating that these axes effectively reflect the correlation between dominant microbial communities and soil physicochemical factors at the phylum level, primarily determined by RDA1 (Fig. 6). Among the eight physicochemical properties, pH, O_2, SOC, and NH_4^+-N correlated more with RDA1, while MBN correlated more with RDA2. In the RDA ranking chart (Fig. 6), Proteobacteria, Acidobacteria, Bacteroidetes, and Nitrospirae showed high correlations

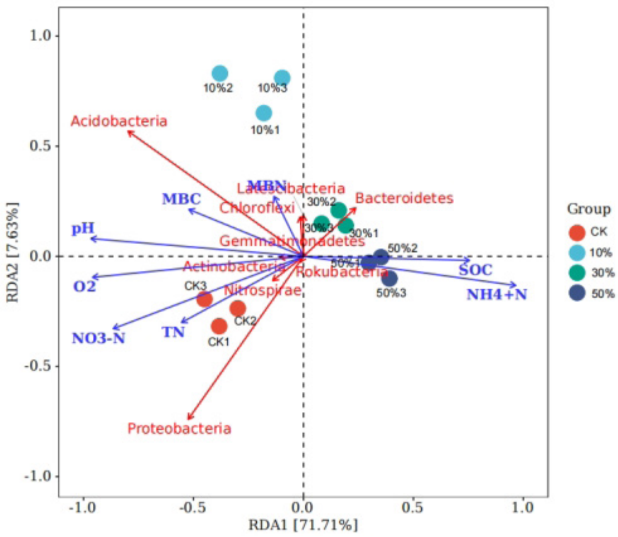

Fig. 6. RDA sequence of physicochemical properties and bacterial community structure of soybean rhizosphere soil under CO_2 leakage conditions.

Table 6. Redundancy analysis of physicochemical properties and bacterial community structure of soybean rhizosphere soil under CO_2 leakage conditions.

Soil environment factors	Explains %	Contribution %	pseudo-F	P
pH	67.1	82.1	20.4	0.002
NO_3^--N	5.0	6.1	1.6	0.198
SOC	4.0	4.9	1.3	0.278
NH_4^+-N	3.7	4.5	1.3	0.276
MBN	1.0	1.3	0.3	0.722
O_2	0.3	0.4	<0.1	0.936
MBC	0.3	0.4	<0.1	0.948
TN	0.3	0.4	<0.1	0.966

with various physicochemical indicators, with varying positive and negative correlations. Among the eight soil physicochemical properties, pH, NO_3^--N, SOC, and NH_4^+-N were the main properties affecting the dominant bacterial community at the phylum level under leakage conditions, with explanatory powers of 67.1%, 5.0%, 4.0%, and 3.7%, respectively. The Monte Carlo permutation test showed that pH is the key physicochemical factor affecting the differences in the dominant bacterial community at the phylum level in the soybean rhizosphere soil ($P<0.05$) (Table 6).

Discussion

Examination of Alterations in the Physicochemical Properties of Soybean Rhizosphere Soil Subjected to CO_2 Leakage Conditions

CO_2 leakage significantly impacts the pH and O_2 levels of soybean rhizosphere soil, both of which show a marked decreasing trend with increasing soil CO_2 concentration (Figs 2a) and 3a)). This finding aligns with data from natural CO_2 leakage points and simulated CO_2 leakage experiments [12, 24], demonstrating a strong correlation between soil pH, O_2, and soil CO_2 concentration (Figs 2b) and 3b)). The correlation coefficient between soil O_2 and soil CO_2 concentration (0.9573) is higher than that between soil pH and soil CO_2 concentration (0.9106). This result corroborates the findings of Zhang et al. [12] but contradicts Liu et al.'s observation of the pH of rice field water decreasing exponentially over time [25]. The discrepancy may be attributed to the differing properties of dryland soil and semi-aquatic soil in rice fields.

CO_2 leakage concurrently affects soil carbon and nitrogen components. Under CO_2 leakage conditions, the SOC of soybean rhizosphere soil increases with the rise in soil CO_2 concentration, a finding consistent with the research of Deng et al. [26]. This increase may be due to CO_2 leakage augmenting the soil carbon source. However, this study found no significant difference in SOC between soybean rhizosphere soil and the CK under CO_2 leakage conditions. Compared to changes in SOC, CO_2 leakage has a more pronounced impact on nitrogen elements in soybean rhizosphere soil. Under CO2 leakage conditions, both TN and NO_3^--N in soybean rhizosphere soil exhibit a decreasing trend with the increase in soil CO_2 concentration. The content of NO_3^--N shows a significant decreasing trend with the increase in soil CO_2 concentration, differing significantly from CK (P<0.05). The NH_4^+-N content in soybean rhizosphere soil shows a slight increasing trend with the increase in soil CO_2 concentration, and there is a significant difference (P<0.05) between NH_4^+-N and CK when the soil CO_2 concentration is 30% and 50% (Table 1). The significant decrease in NO_3^--N and the slight increase in NH_4^+-N may be due to the anaerobic environment caused by CO_2 leakage, which inhibits the soil nitrification process and promotes the denitrification process. This affects the aerobic process of NH_4^+-N to NO_3^--N in the soil, resulting in a significant decrease in NO_3^--N content [27].

Soil microbial biomass carbon and nitrogen can reflect microbial activity to some extent. This experiment observed that soil MBC and MBN increased with the increase in soil CO_2 concentration to a certain extent, while decreasing when soil CO_2 concentration exceeded a certain concentration (Table 1). A plausible explanation for the observed phenomena is that the rise in soil CO_2 concentration supplies a carbon source for soil microorganisms, thereby augmenting their quantity and activity [28]. However, the hypoxic acidic environment engendered by sustained high-concentration CO_2 stress is unfavorable for the survival of most microorganisms, leading to a reduction in their quantity and activity. In this study, when the soil CO_2 concentration exceeded 50%, the decline in MBC and MBN did not align with the turning point of soil MBC and MBN reduction (soil CO_2 concentration of 15%) as determined by Zhang et al. [29]. This discrepancy may be attributed to the inconsistency of climate factors such as weather, temperature, and water during the simulated CO_2 leakage process, as well as the variation in experimental soil types.

Alterations in Bacterial Diversity and Community Structure in Soybean Rhizosphere Soil under CO_2 Leakage Conditions

The bacterial diversity in soybean rhizosphere soil under CO_2 leakage conditions revealed that CO_2 leakage significantly decreases the Chao1 index of soil bacteria. Conversely, Pielou's evenness index exhibits an overall increasing trend, while the Simpson and Good's coverage indices remain unchanged (Table 2). Although CO_2 leakage alters the relative abundance of certain bacteria, the dominant populations maintain a similar composition at the phylum level (Fig. 4a)), aligning with the findings of Shelton et al. [30].

CO_2 leakage results in a significant increase in the relative abundance of the dominant bacterial phylum Bacteroidetes, while the relative abundance of Proteobacteria, Acidobacteria, and Nitrospirae significantly decreases (Fig. 4b) and Table 3). This increase in the relative abundance of Bacteroidetes is consistent with the research results of Chen et al. [31]. Generally, the anaerobic environment induced by CO_2 leakage increases the abundance of Bacteroidetes, a type of anaerobic bacteria. Moreover, Kim et al. observed an increase in N_2O emissions through CO_2 leakage simulation experiments and suggested that CO_2 leakage might enhance soil denitrification processes [32], while the nitrogen transformation functional bacterial community, which includes ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, and denitrifying bacteria, belongs to Bacteroidetes. Meanwhile, some groups of Bacteroidetes also possess denitrification functions [33]. These explain the increase in the relative abundance of Bacteroidetes and the decrease in Nitrospirae, a typical functional bacterium with nitrification function, observed in this experiment. These observations suggest that CO_2 leakage may increase soil denitrification while inhibiting soil nitrification, which aligns with the increase in the relative abundance of Bacteroidetes. The decrease in the relative abundance of Acidobacteria in this experiment contradicts the research results of Tian et al. [34]. The CO_2 leakage rates (500, 1000, 1500, and 2000 $g(m^2d)^{-1}$) used by Tian et al. are not comparable with the soil CO_2 concentration in this experiment; the two experimental soils also differ in acidity and alkalinity: Tian et al. used acidic common cinnamon soil, while we used alkaline silty clay loam. Moreover, Acidobacteria belong to aerobic bacteria, and the anaerobic environment caused by CO_2 leakage can lead to a decrease in some aerobic bacteria, which may be the primary reason for the decrease in the relative abundance of Acidobacteria.

CO_2 leakage induces changes in soil physical and chemical properties, leading to alterations in soil bacterial diversity and community structure. Among these changes, soil pH and O_2 are the primary physicochemical factors influenced by CO_2 leakage (Table 4). Redundancy analysis (RDA) can more directly reflect the relationship between soil physicochemical properties, bacterial diversity, and community structure. The length of the arrow in the RDA plot represents the magnitude of the impact; a longer arrow indicates a greater influence of the factor on bacterial composition/function, while a shorter arrow suggests a smaller impact. The angle between the environmental factors in the graph reflects the degree of their correlation; an acute angle indicates a positive correlation between two factors, a right angle suggests no correlation, and an obtuse angle signifies a negative correlation. The angle between the arrow ray and the coordinate axis represents the correlation between a specific environmental factor and the coordinate axis, with a smaller angle indicating a higher correlation [35, 36]. Redundancy analysis reveals that under CO_2 leakage conditions, O_2 is the key physicochemical factor affecting bacterial diversity in soybean rhizosphere soil among eight soil physicochemical properties (P<0.05) (Fig. 5, Table 5). pH is the key physicochemical factor influencing the differences in dominant bacterial communities at the bacterial phylum level in soybean rhizosphere soil (P<0.05) (Fig. 6, Table 6). Compared to other carbon and nitrogen physicochemical indicators, CO_2 leakage directly affects soil O_2 and pH, thereby influencing soil microbial diversity and community structure.

This study provides an initial exploration of the relationship between soil physicochemical properties and bacterial community structure under CO_2 leakage conditions. A substantial amount of data suggests that CO_2 leakage in carbon sequestration engineering will significantly impact soil physicochemical properties, microbial activities, and soil microbial community functions. Future research should focus more on the effects of soil microbial community growth, metabolic rate, and metabolic function to provide more fundamental data for the environmental impact and risk assessment of CCS projects.

Screening of Soil Monitoring Indexes of CO_2 under Leakage Conditions

The CO_2 injected into geological storage migrates underground, forming a CO_2 plume and a pressure transmission range. As the injection time extends and the amount of CO_2 injected increases, CO_2 may escape through cracks or ascend through injection wells due to various factors during underground migration. This process can affect groundwater, surface water, soil, plants, and the atmosphere [37, 38]. To ensure the safety of CO_2 geological storage, it is necessary to develop a series of technical methods for the measurement, monitoring, and verification (MMV) of CO_2. These methods should be incorporated into international and Chinese standards for CO_2 geological storage, such as "Carbon dioxide capture, transportation and geological storage-Geological storage" and "Technical Guideline on Environmental Risk Assessment for Carbon Dioxide Capture, Utilization and Storage (on Trial)" [39, 40]. Environmental monitoring of carbon dioxide storage is a core component of the CO_2 geological storage monitoring system and plays a pivotal role in the success of CO_2 geological storage projects [41]. The study of the impact characteristics of CO_2 leakage on vegetation, soil, and microorganisms aims to clarify the impact of CO_2 leakage on the surface environment. More importantly, it aims to identify environmental indicators that can be used for CO_2 monitoring. The goal of this study is to identify soil properties and microbial indicators that can be used for CO_2 leakage monitoring in storage areas. This is based on a deepened understanding of the effects of CO_2 leakage on soil physicochemical properties, soil bacterial diversity, and community structure.

The key to identifying CO_2 leakage is the difference between various soil indices and the CK under soil CO_2 stress. Soil monitoring indicators of CO_2 leakage should exhibit statistically significant changes under soil CO_2 stress compared with CK conditions. Particularly, a significant increase or decrease in a single direction is more meaningful for monitoring. Based on the data results of various soil physicochemical property indices, bacterial diversity index, and relative abundance of each dominant microphyla in this study, soil O_2, NO_3^--N, Chao1 index, and the relative abundance of Proteobacteria and Nitrospirae are significantly different from CK under CO_2 leakage conditions. They show a significant increase or decrease with the increase in soil CO_2 concentration. Therefore, soil O_2, NO_3^--N, Chao1 index, and the relative abundance of Proteobacteria and Nitrospirae can be used as soil monitoring indices for CO_2 leakage. Considering the technical cost, monitoring cost, and periodicity of data acquisition, soil O_2 and NO_3^--N are recommended as conventional monitoring indices for CO_2 leakage in soybean rhizosphere soil. The Chao1 index and the relative abundance of Proteobacteria and Nitrospirae can be used as supplementary monitoring indices for CO_2 leakage in soybean rhizosphere soil.

CO_2 soil monitoring index established in this study primarily targets the study of the rhizosphere soil of soybean, a widely existing dry farming plant in the Ordos Basin. Future studies should investigate the impact of CO_2 leakage on a broader range of plant rhizosphere soil types and geographical environments. This will place the ecological impact of CO_2 leakage in a wider environmental scope and select more timely and accurate soil monitoring indices for CO_2 leakage.

Conclusions

This study primarily establishes a CO_2 leakage simulation platform and employs 16S rDNA Illumina NovaSeq high-throughput sequencing technology, correlation analysis, and redundancy analysis to investigate the response of soybean rhizosphere soil physicochemical properties and bacterial community changes to CO_2 leakage, as well as their interrelationships. The main conclusions were as follows:

(1) CO_2 leakage results in a significant decrease in soil pH and O_2. Soil organic carbon and NH_4^+-N increase with the rise in soil CO_2 concentration, while TN and NO_3^--N decrease with the increase in soil CO_2 concentration, with NO_3^--N showing a significant decrease.

(2) CO_2 leakage causes a significant decrease in the Chao1 index of soybean soil bacteria, while Pielou's evenness index exhibits an overall upward trend. Under different CO_2 leakage conditions, the composition of dominant bacterial phyla in soybean rhizosphere soil remains similar, but the relative abundance of some phyla varies. The relative abundance of Bacteroidetes significantly increases, while the relative abundance of Proteobacteria, Acidobacteria, and Nitrospirae significantly decreases.

(3) Among the eight soil physicochemical properties, O_2 is the key physicochemical factor affecting the diversity of bacteria in soybean rhizosphere soil. Meanwhile, pH is the key physicochemical factor influencing the relative abundance differences of some bacterial communities at the bacterial phylum level in soybean rhizosphere soil.

(4) Soil O_2 and NO_3^--N are recommended as conventional monitoring indicators for CO_2 leakage, and the Chao1 index, relative abundance of Proteobacteria and Nitrospirae can be used as supplementary monitoring indicators for CO_2 leakage.

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Conflict of Interest

The authors declare no conflict of interest.

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