



## OPEN ACCESS

## EDITED BY

Marika Pellegrini,  
University of L'Aquila, Italy

## REVIEWED BY

Carl-Eric Wegner,  
Friedrich Schiller University Jena, Germany  
Rihab Djebaili,  
Consorzio Interuniversitario Nazionale per le  
Scienze Ambientali, Italy  
Beatrice Farda,  
University of L'Aquila, Italy, in collaboration  
with reviewer RD

## \*CORRESPONDENCE

Sascha M. B. Krause  
✉ ssa@des.ecnu.edu.cn

RECEIVED 21 September 2023

ACCEPTED 03 January 2024

PUBLISHED 23 January 2024

## CITATION

Liu R, Wei Z, Dong W, Wang R, Adams JM,  
Yang L and Krause SMB (2024) Unraveling the  
impact of lanthanum on methane consuming  
microbial communities in rice field soils.  
*Front. Microbiol.* 15:1298154.  
doi: 10.3389/fmicb.2024.1298154

## COPYRIGHT

© 2024 Liu, Wei, Dong, Wang, Adams, Yang  
and Krause. This is an open-access article  
distributed under the terms of the [Creative  
Commons Attribution License \(CC BY\)](#). The  
use, distribution or reproduction in other  
forums is permitted, provided the original  
author(s) and the copyright owner(s) are  
credited and that the original publication in  
this journal is cited, in accordance with  
accepted academic practice. No use,  
distribution or reproduction is permitted  
which does not comply with these terms.

# Unraveling the impact of lanthanum on methane consuming microbial communities in rice field soils

Ruyan Liu<sup>1</sup>, Ziting Wei<sup>1</sup>, Wanying Dong<sup>1</sup>, Rui Wang<sup>1</sup>,  
Jonathan M. Adams<sup>2</sup>, Lin Yang<sup>2</sup> and Sascha M. B. Krause<sup>1\*</sup>

<sup>1</sup>School of Ecology and Environmental Sciences, East China Normal University, Shanghai, China,

<sup>2</sup>School of Geographic and Oceanographic Sciences, Nanjing University, Nanjing, China

The discovery of the lanthanide requiring enzymes in microbes was a significant scientific discovery that opened a whole new avenue of biotechnological research of this important group of metals. However, the ecological impact of lanthanides on microbial communities utilizing methane (CH<sub>4</sub>) remains largely unexplored. In this study, a laboratory microcosm model experiment was performed using rice field soils with different pH origins (5.76, 7.2, and 8.36) and different concentrations of La<sup>3+</sup> in the form of lanthanum chloride (LaCl<sub>3</sub>). Results clearly showed that CH<sub>4</sub> consumption was inhibited by the addition of La<sup>3+</sup> but that the response depended on the soil origin and pH. 16S rRNA gene sequencing revealed the genus *Methylobacter*, *Methylosarcina*, and *Methylocystis* as key players in CH<sub>4</sub> consumption under La<sup>3+</sup> addition. We suggest that the soil microbiome involved in CH<sub>4</sub> consumption can generally tolerate addition of high concentrations of La<sup>3+</sup>, and adjustments in community composition ensured ecosystem functionality over time. As La<sup>3+</sup> concentrations increase, the way that the soil microbiome reacts may not only differ within the same environment but also vary when comparing different environments, underscoring the need for further research into this subject.

## KEYWORDS

methane, soil health, rare earth elements, ecotoxicology, microbial communities, lanthanum, global change

## 1 Introduction

Methane (CH<sub>4</sub>) is an important greenhouse gas after carbon dioxide (CO<sub>2</sub>) with a global warming potential of 85 or 32 for 20- or 100-y time horizons, respectively (IPCC, 2021). Rice fields stand out as one of the major anthropogenic contributors to atmospheric CH<sub>4</sub>, accounting for 14.1 to 48% of total agricultural CH<sub>4</sub> emissions (Carlson et al., 2017; Saunio et al., 2020; Gupta et al., 2021; Li et al., 2022; Duan et al., 2023; Qian et al., 2023).

Methylophs constitute a microbial group that can utilize single-carbon or multi-carbon compounds without carbon-carbon bonds, including methanol and methylamine, for growth and as energy source (Vu et al., 2021). CH<sub>4</sub>-oxidizing bacteria (methanotrophs) are a special case in this group because they are additionally capable of utilizing CH<sub>4</sub> as their primary carbon and energy source (Hanson and Hanson, 1996). In rice fields, methanotrophs can oxidize up to 90% of the CH<sub>4</sub> before it reaches the atmosphere (Conrad and Rothfuss, 1991).

Consequently, methanotrophs play a vital role as a biofilter, significantly contributing to the regulation of the global climate in agro-ecosystems like rice fields.

Lanthanides, a group of 15 elements from lanthanum ( $\text{La}^{3+}$ ,  $Z=57$ ) to lutetium ( $Z=71$ ) on the periodic table, along with scandium and yttrium, collectively known as Rare Earth Elements (REEs), have garnered increased interest over the past four decades. Their pivotal role in modern technology, serving as essential components in portable electronic devices and clean energy technologies, has fueled this growing interest (Leal Filho et al., 2023). Notably, REEs are also of interest in agriculture, being used as additives in animal feed to enhance livestock yield and as components in fertilizers to boost crop productivity (Balaram, 2019; Tommasi et al., 2021). REEs can be introduced to the soil through phosphate fertilizers, contributing to their accumulation in the soil (Silva et al., 2019; Dushyantha et al., 2020). For instance, the concentration of  $\text{La}^{3+}$  in the soil can vary widely from 1.1 to 413 mg/kg, but also can reach exceptionally high levels in areas close to mining areas, ranging from 1800.44 to 6905.24 mg/kg (Tyler, 2004; Li et al., 2010; Liang et al., 2014; Ramos et al., 2016).

Lanthanides were long considered biologically inactive due to their low bioavailability (Bulman, 2003) but a biological significance has recently been discovered. In the first step of  $\text{CH}_4$ -oxidation,  $\text{CH}_4$  is converted to methanol which is then further oxidized to formaldehyde by an enzyme called methanol dehydrogenase (MDH). Calcium (II) ions have been thought of as the cofactor of MDHs (Anthony, 2000), but a novel form of MDHs is requiring lanthanides (Hibi et al., 2011; Pol et al., 2014; Skovran and Martinez-Gomez, 2015; Picone and op den Camp, 2019; Kato et al., 2020). It was demonstrated that the newly discovered functional MDH is primarily expressed even in the presence of 95  $\mu\text{M}$   $\text{Ca}^{2+}$  in the cultivation medium and with as little as 1  $\mu\text{M}$  supplemental  $\text{La}^{3+}$  (Chu and Lidstrom, 2016). Despite these discoveries in physiology, there is still a knowledge gap for the role and function of  $\text{La}^{3+}$  in natural and agroecosystems. It is largely unknown whether methanotrophic communities in soils are inhibited or stimulated by the addition of  $\text{La}^{3+}$ .

Because the diversity of methanotrophs is limited, elevated levels of  $\text{La}^{3+}$  in the soil may lead to a loss of function that could not be compensated by other microbes with dramatic consequences for atmospheric  $\text{CH}_4$  concentrations and global warming. However,  $\text{La}^{3+}$  is an important element in the metabolism of methanotrophs, and increasing concentrations in the environment could be beneficial with a positive effect on ecosystem processes, bioremediation, and biotechnological applications (Skovran and Martinez-Gomez, 2015; Dance, 2023). To the best of our knowledge, only Kravchenko and colleagues (Kravchenko et al., 2022) have examined the implications of  $\text{La}^{3+}$  addition on the composition and activity of methanotrophs in sod-podzolic soil ( $\text{pH}=5.4$ ). The addition of 5  $\mu\text{g}$   $\text{La}^{3+}$  stimulated the growth of the methanotrophic genus *Methylobacter* and the non-methanotrophic genus *Methylothera*. In addition, a stimulating effect on  $\text{CH}_4$  oxidation was observed at 200 parts per million (ppm) in the headspace of their experimental setup (Kravchenko et al., 2023).

In this study, we focused on the impact of increasing amounts of  $\text{La}^{3+}$  in rice field soil. Therefore, we performed a comprehensive lab incubation study combining gas chromatography, mass spectrometry, sequencing, and quantitative real-time polymerase chain reaction (qPCR) to get a better understanding of the ecological implications of the increasing amounts of REEs on the soil microbiome, which plays a crucial role in mitigating atmospheric  $\text{CH}_4$  emissions. We selected

three rice field soils with different pH conditions to explore the relationship between the soil microbiome and the bioavailability and mobility of  $\text{La}^{3+}$  within this soil ecosystem. Previous studies clearly demonstrated the direct impact of pH on the solubility, mobility, and bioavailability of La in the soil (Iftekhhar et al., 2018; Kotelnikova et al., 2021; Zhao et al., 2021). We hypothesized that  $\text{La}^{3+}$  in the form of lanthanum chloride ( $\text{LaCl}_3$ ) will stimulate methanotrophic activity and growth due to their availability to utilize lanthanides in their enzyme machinery. We further hypothesized that the soil origin and their specific soil properties (e.g., pH) will influence the  $\text{La}^{3+}$  retention capacity and consequently the effect on the soil microbiome.

## 2 Materials and methods

### 2.1 Study sites

In October 2020, three rice field soil samples with different pH (0–20 cm) were collected from Jiangsu Province, China, each originating from varying pH levels of 5.76, 7.2, and 8.36, representing acidic, neutral, and alkaline soils, respectively. Acidic soil samples were collected from Yangzhou City (32°24' N, 119°2' E). Neutral soil samples were collected from Xuzhou City (34°15' N, 118°8' E). Alkaline soil samples were collected from Yancheng City (33°17' N, 120°34' E). For incubation experiments, soil samples were stored at 4°C. However, soil physicochemical properties were determined from fresh or frozen soil (–20°C) except for the determination of La content in the original soil samples. All measured properties are summarized in Table 1. Detailed description of methods was published in a previous study (Guo et al., 2022) and carried out by Nanjing innovation biotechnology Co., LTD. The determination of La was carried out by TÜV Rheinland (Shanghai) Co., Ltd., China using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

### 2.2 Experimental setup and sampling

In August 2022, 2 grams (g) of rice field soils and 25 milliliters (mL) of diluted nitrate mineral salt (NMS)  $\text{Ca}^{2+}$  medium (0.1 × strength, Dedysh and Dunfield, 2017) were mixed in 100 mL transparent serum bottles, these bottles were then placed in the shaker (MQT-60R, Shanghai Minquan Instrument Co., Ltd., China) and incubated in the dark at 20°C and 120 revolutions per minute (rpm) for 4 weeks. The diluted NMS medium consisted of 22.5 mL different concentrations of  $\text{La}^{3+}$  in the form of  $\text{LaCl}_3$  (Sangon Biotech (Shanghai) Co., Ltd., China) and 2.5 mL NMS ( $\text{Ca}^{2+}$ ) medium. To find suitable concentrations of  $\text{La}^{3+}$  for our experiment, we first searched the literature for ecotoxicological studies that applied  $\text{La}^{3+}$  (e.g., Chu et al., 2003; Li et al., 2018; Kotelnikova et al., 2019). We then chose  $\text{LaCl}_3$  concentrations ranging from 2 mg/L to 1,200 mg/L to determine the levels that depicted the strongest response in  $\text{CH}_4$  oxidation. Finally, the chosen concentrations included no added  $\text{La}^{3+}$  (control), 300 mg/L (low), 600 mg/L (intermediate), and 1,200 mg/L (high), denoted as control CK, L, M, and H levels, respectively.

All chemical reagents were purchased through Sangon Biotech (Shanghai) Co., Ltd., China. Each soil sample has four  $\text{La}^{3+}$  treatments in triplicate, 36 bottles in total. All bottles for the experiment were thoroughly washed with 1 mol/L hydrochloric acid (HCl) overnight

TABLE 1 Main soil characteristics of the initial rice field soil samples.

Sample name soil properties	Acidic sample	Neutral sample	Alkaline sample
pH	5.67	7.2	8.36
La (mg/kg dry soil)	46.93	47.37	32.78
Conductivity (ms/cm)	0.09	0.1	0.35
Water content (fresh)	0.23	0.23	0.25
Organic matter (g/kg)	19.48	26.98	11.16
Organic carbon (g/kg)	11.3	15.65	6.47
Total phosphorus (P) (g/kg)	0.33	0.86	1.07
Total potassium (K) (g/kg)	10.71	13.77	18.65
Ca (mg/kg)	359.50	791.50	672.87
Cu (mg/kg)	2.33	3.10	1.45
Magnesium (Mg) (mg/kg)	2152.20	1167.82	273.91
N (g/kg)	1.34	1.76	0.79
Cl <sup>-</sup> (g/kg)	0.05	0.08	0.32
NO <sub>3</sub> <sup>-</sup> (mg/kg)	5.35	3.90	0.70
NH <sub>4</sub> <sup>+</sup> (mg/kg)	2.17	2.25	3.86
Available P (mg/kg)	4.40	33.13	37.47

and autoclaved before use, sealed with butyl rubber stoppers, and capped with plastic aluminum shells.

All 36 bottles were pre-incubated on a shaker at 20°C and 120 rpm in the dark for 24 h. During the experiment, on the first day of each week, the headspace of the bottles was flushed three times with air using a 60 mL syringe. Subsequently, 1.5 mL of 99.999% high-purity CH<sub>4</sub> (Shanghai Jiaya Chemical Co., Ltd., China) was added resulting in a starting CH<sub>4</sub> concentration of approximately 10,000 parts per million volume (ppmV) (~1%) in the headspace reflecting on typical *in-situ* methane concentrations in rice fields.

On the seventh day of each week, 2.5 mL of a well-mixed cultivation solution was extracted from the bottles. The extracted solution was then transferred to centrifuge at 7,800 rpm for 12 min using an Eppendorf Microcentrifuge 5.430 R (Eppendorf, Germany). Subsequently, the resulting supernatant underwent analysis for pH (S220-K, METTLER TOLEDO, Switzerland), and the remaining samples were stored in a -80°C refrigerator for subsequent molecular analyses and La<sup>3+</sup>, NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> determination. The CH<sub>4</sub> concentration in the headspace was determined using GC (Nexis GC-2030, Shimadzu, Japan) equipped with a Flame Ionization Detector (FID).

### 2.3 Determination of La<sup>3+</sup> in the soil

The analysis of La<sup>3+</sup> levels in the soil during the fourth week was carried out by Shanghai WEIPU Testing Technology Group Co., Ltd. (Shanghai, China). In brief, approximately 0.1 g of each soil sample was placed into a microwave digestion vessel and 4 mL of nitric acid was added for microwave digestion. Subsequently, each sample was tested using the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (PerkinElmer Avio 500, United States).

### 2.4 Determination of La<sup>3+</sup>, NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> ions in the supernatant

All analyses were performed by the Shanghai WEIPU Testing Technology Group Co., Ltd. (Shanghai, China). In brief, for the La<sup>3+</sup> determination 0.1 mL of the supernatant was transferred into digestion tubes, followed by the addition of 4 mL of nitric acid. The tubes were then heated at 120°C for 4 h until the solution became clear and transparent. Subsequently, the digestive solution was adjusted to a constant volume of 10 mL. La<sup>3+</sup> was determined using either ICP-MS (PerkinElmer NexION 2000, United States) or ICP-OES (PerkinElmer AvioVIO 500, United States), depending on the concentration range. NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> concentrations were determined by diluting each sample 10–20 times with water, and then the aqueous solution was filtered through a 0.22 μM membrane into a 2 mL Agilent vial and an injection tube for ion chromatography analysis using the Dionex ICS-5000<sup>+</sup> ion chromatography system (Thermo Fisher Scientific, USA).

### 2.5 Soil DNA extraction and 16S rRNA gene analysis

The DNeasy PowerSoil<sup>®</sup> Pro Kit (Qiagen, Germany) was used to extract total soil community genomic DNA from 0.2–0.25 g of each sample, following the manufacturer's steps. The concentration of the DNA solution was checked through NanoDrop<sup>™</sup> One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, United States). The variable regions V1-V3 of bacterial 16S rRNA gene were amplified using the primers of 27F (AGRGT<sup>TT</sup>GATCMTGGCTCAG) (Lane, 1991) and 519R (GWATTACCGCGGCKGCTG) (Lane et al., 1985). The process of PCR amplification of the 16S rRNA gene, followed by library preparation, quantification, and sequencing was executed using the Illumina MiSeq platform (Illumina MiSeq, United States) at Sangon Biotech (Shanghai) Co., Ltd., China. The reaction system of PCR was used as follows: microbial DNA (10–20 ng/μL) 2 μL; amplicon PCR forward primer (10 μM) 1 μL; amplicon PCR reverse primer (10 μM) 1 μL; 2 × Hieff<sup>®</sup> Robust PCR Master Mix (Yeasen, 10105ES03, China) 15 μL; 30 μL in total. The following PCR program was used: 1 cycle of denaturing at 94°C for 3 min, first 5 cycles of denaturing at 94°C for 30 s, annealing at 45°C for 20 s, elongation at 65°C for 30 s, then 20 cycles of denaturing at 94°C for 20 s, annealing at 55°C for 20 s, elongation at 72°C for 30 s and a final extension at 72°C for 5 min.

16S rRNA gene high-throughput raw sequencing data was analyzed with the package of DADA2 version 1.26.0 (Callahan et al., 2016) in R 4.2.2 (Team, R. C., 2022). In brief, 16S rRNA gene forward and reverse reads were trimmed at positions 290 and 250 from the end or any position. Error models were constructed with random sample picking. The resulting amplicon sequence variances (ASVs) were taxonomically classified by using the SILVA ribosomal RNA database SSU version 138 (Pruesse et al., 2012). ASVs that were identified as mitochondrial or chloroplast sequences, or not classified into prokaryotic phyla were removed from the dataset. Removing ASVs less than 0.001% left 5,794 ASVs in the final dataset, 95.12% of total reads were retained.

## 2.6 qPCR of the *pmoA* gene

This experiment was conducted by Sangon Biotech (Shanghai) Co., Ltd., China. In brief, the *pmoA* gene was quantified using a specific qPCR assay to enumerate the total abundance of methanotrophs (MTOT assay; Kolb et al., 2003). The *pmoA* gene encodes the  $\beta$ -subunit of the particulate methane monooxygenase (pMMO) which catalyzes the first step in  $\text{CH}_4$ -oxidation. It has been widely used as a functional marker to identify methanotrophs from the environment (Dedysh and Knief, 2018). The primer sequences were A189 F (GGN GAC TGG GAC TTC TGG) and mb661R (CCG GMG CAA CGT CYT TAC C) (Holmes et al., 1995; Costello and Lidstrom, 1999). In addition, this primer set was shown to have a broad coverage of methanotroph diversity (Bourne et al., 2001). The qPCR protocols for the *pmoA* gene were executed as follows: initial pre-denaturation at 95°C for 4 min; followed by denaturation at 95°C for 15 s; annealing at 67°C for 20 s; and extension at 72°C for 30 s. The previous steps comprised 10 cycles, with the annealing temperature decreasing by 1°C after each cycle. Subsequently, denaturation was carried out at 95°C for 15 s, followed by annealing at 57°C for 20 s, and extension at 72°C for 30 s, repeated for a total of 35 cycles. Copy numbers of target genes were derived from plasmid DNA. The PCR efficiency was on average 92.8% with an  $R^2$  value of 0.9972. Amplicon specificity was determined from melt curve analysis and confirmed by 1.5% agarose gel electrophoresis.

## 2.7 Statistical analysis

The  $\text{CH}_4$  concentration in the headspace was calculated by using a  $\text{CH}_4$  standard gas (1,002 ppmV) measured on the GC before sample measurements. *pmoA* gene copy numbers were expressed as per gram dry weight.

The Shannon index was calculated using the R package *vegan* 2.6.4 (Oksanen et al., 2022), with prior sequence flattening. The variation in microbial community compositions with different  $\text{La}^{3+}$  treatments and time points were assessed through non-metric multidimensional scaling (NMDS). First, ASVs with relative abundance below 0.05% were removed, reducing sparsity from 94 to 62.73%. Next, Bayesian-Multiplicative replacement of count zeros (*cmultRepl*) in the R package *zCompositions* package version 1.4.0.1 (Palarea-Albaladejo and Martín-Fernández, 2015) was employed to impute zeros in compositional count data. Then, a centered log-ratio (CLR) transformation was applied using R package *compositions* version 2.0.4 (van den Boogaart et al., 2022). For assessing the dissimilarity among various treatments and time points, Permutational multivariate analysis of variance (PERMANOVA) was performed using the function of *adonis2* in the R package *vegan* version 2.6.4. NMDS plots at a 95% confidence level were generated using the “*stat\_ellipse*” function in the R package *ggplot2* version 3.4.1 (Wickham, 2016). R package *reshape2* version 1.4.4 (Wickham, 2007), *dplyr* version 1.1.0 (Wickham et al., 2023), and *tidyverse* version 2.0.0 (Wickham et al., 2019) were utilized to process the data to calculate relative abundance plots. The *ddply* function in the R package *plyr* version 1.8.8 (Wickham, 2011) was employed to calculate the mean value  $\pm$  standard deviation of pH in the supernatant. The *leveneTest* function in the R package *car* version 3.1.2 (Fox and Weisberg, 2019) was used to conduct the homogeneity of variance test of data. Analysis

of variance (ANOVA) was performed using the *stat\_compare\_means* function in the R package *ggpubr* version 0.6.0 (Kassambara, 2023). Following that, *post hoc* tests were performed using the Least Square Difference (LSD) test in R package *agricolae* version 1.3.5 (de Mendiburu, 2021).

## 3 Results

### 3.1 $\text{CH}_4$ Consumption is affected by the addition of $\text{La}^{3+}$

We observed a consistent trend of inhibited  $\text{CH}_4$  consumption across all treatments, with the degree of inhibition corresponding to the concentrations of  $\text{La}^{3+}$  added to the soil slurries. It is important to note that the extent of inhibition correlated with the pH of the soil slurries, each derived from rice fields with distinct acidity levels: acidic, neutral, and alkaline (Figure 1).

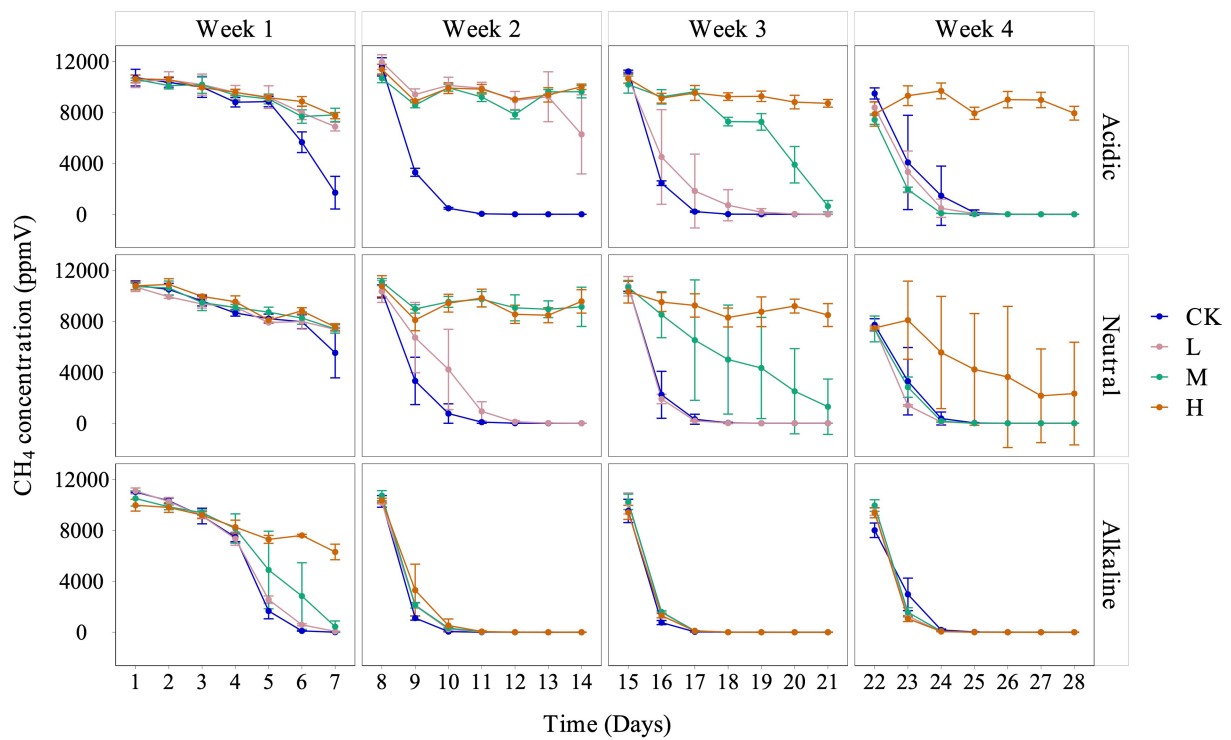
Although there was a clear inhibition of  $\text{CH}_4$  consumption, the data revealed an interesting trend: during the fourth week, in soil slurries from acidic and neutral rice fields, the low and intermediate treatments exhibited a faster rate of  $\text{CH}_4$  consumption between 22 and 24 days. This trend was also observed across all  $\text{La}$  treatments throughout the entire fourth week cultivation period, in soil slurries from alkaline rice fields (Figure 1).

### 3.2 The effect of $\text{La}^{3+}$ on the methanotrophic community composition, microbial diversity, and methanotroph abundance

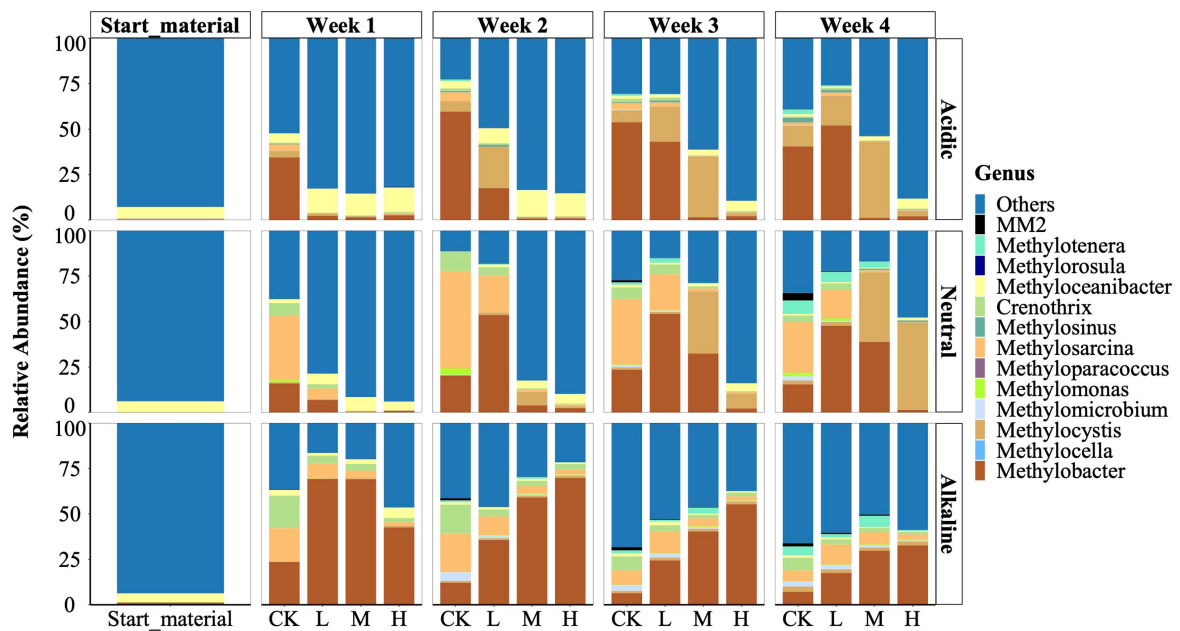
We identified a total of 13 genera of methylotrophs using Illumina sequencing of the 16S rRNA gene (Figure 2). These types were distributed among five families: *Beijerinckiaceae* (comprising *Methylocella*, *Methylorosula*); *Crenotrichaceae* (*Crenothrix*); *Hyphomicrobiaceae* (*Methyloceanibacter*); *Methylococcaceae* (including *Methyloparacoccus*, *Methylobacter*, *Methylomicrobium*, *Methylomonas*, and *Methylosarcina*); *Methylocystaceae* (comprising *Methylocystis* and *Methylosinus*); and *Methylophilaceae* (*Methylotenera* and *Methylovorus* sp. strain MM2). Among these, *Methyloceanibacter*, *Methylorosula*, *Methylotenera*, and *Methylovorus* sp. strain MM2 were identified as facultative methylotrophs, while the remaining 9 genera were classified as obligate methanotrophs. Notably, *Methylocella*, *Methyloparacoccus*, and *Methylorosula* were absent from the neutral and alkaline soil samples.

We observed that the genus *Methyloceanibacter* was a prevalent microbe across the three initial rice field soil samples with a relative abundance of 6.3, 6.0, and 4.6% in the acidic, neutral, and alkaline samples, respectively (Figure 2). Representatives of *Methyloceanibacter* have been described to also oxidize  $\text{CH}_4$ , but possess solely a SMMO, and were mainly found in marine environments (Vekeman et al., 2016).

We further observed that during the incubation period, the genera *Methylobacter*, *Methylocystis*, and *Methylosarcina* emerged as the dominant methanotrophs in the control treatment. However, their response to  $\text{La}^{3+}$  addition displayed variations among soil slurries with different initial pH levels: acidic, neutral, and alkaline (Figure 2). In



**FIGURE 1**  
Variation of CH<sub>4</sub> concentration in the headspace with different pH conditions for control (CK), 300 (L), 600 (M), and 1200 (H) mg/L La<sup>3+</sup> treatments, from top to bottom are acidic, neutral, and alkaline samples, respectively. Error bars indicate the standard deviation (*n* = 3).



**FIGURE 2**  
Relative abundance of the methylotrophs of starting material, control (CK), 300 (L), 600 (M), and 1200 (H) mg/L La<sup>3+</sup> treatments in varying pH rice field soil samples, from top to bottom, respectively, are acidic, neutral, alkaline samples. Data is based on 16s rRNA gene sequencing data (see Material and Methods for details).

both soil slurries from acidic and neutral rice fields, the application of higher doses of  $\text{La}^{3+}$  consistently resulted in lower relative abundances of the genus *Methylobacter*. For instance, in the treatments with high amount of added  $\text{La}^{3+}$  (referred to as the “H treatments”), *Methylobacter*'s relative abundance ranged from 0.9 to 2.6%. In contrast, in the corresponding control treatments (referred to as the “CK treatments”) for acidic samples, *Methylobacter*'s relative abundance remained notably higher, ranging from 34.4 to 59.6% (Figure 2). Once  $\text{CH}_4$  consumption was resumed (Figure 1) we also observed higher relative abundance of the genus *Methylobacter* (Figure 2). Intriguingly, when examining soil slurries from alkaline soil, we observed a distinct relationship between the relative abundance of the genus *Methylobacter* and the amount of added  $\text{La}^{3+}$ . Specifically, in the CK and treatments with low, intermediate, and high levels of added  $\text{La}^{3+}$ , the relative abundance of *Methylobacter* increased progressively between treatments, such as its abundance was 12.1% (CK), 35.7% (L), 59.1% (M), and 69.8% (H) in week 2 (Figure 2).

Next, we noticed a significant shift in the predominant methanotroph population from the genus *Methylobacter* (Type I) to the genus *Methylocystis* (Type II) in soil slurries from acidic and neutral rice field soils at higher  $\text{La}^{3+}$  concentrations (Figure 2). In soil slurries from the acidic rice field, *Methylobacter* dominated the CK treatments consistently throughout the cultivation process, while *Methylocystis* gradually took over in the M treatment after the second week. For instance, in the M treatments, *Methylocystis* showed a notably higher relative abundance during week 3 (33.4%) and week 4 (41.9%) compared to *Methylobacter*, which only accounted for 1.5% during week 3 and 1.2% during week 4. In soil slurries from the neutral rice field, *Methylocystis* progressively established as the dominant species within the M and H treatments. For instance, in week 4, *Methylocystis* showed a significantly higher relative abundance (48%) in the H treatment compared to *Methylobacter*, which had only 1.3% relative abundance.

In general, the relative abundance of non-methanotrophic taxa such as *Afipia*, *Pseudolabrys*, and *Pseudarthrobacter* increased with  $\text{La}^{3+}$  addition in acidic soil slurry (Supplementary Figure S1A). Noteworthy, in alkaline soil slurries, the *ASV6-Bdellovibrionaceae-Bdellovibrio* emerged as the most predominant non-methanotrophic organism. Notably, the *Bdellovibrio* genus is recognized as a bacterial predator (Feng et al., 2017). The relative abundance of the *ASV6-Bdellovibrionaceae-Bdellovibrio* in L treatment was higher than that in the CK treatment. Specifically, in the L treatments, its relative abundance was 12.9, 8.3, and 11.7% from week 2 to week 4, while in the CK treatments it was 7.2, 5.2, and 4.3%, respectively (Supplementary Figure S1C). Finally, in soil slurries that displayed inhibition of  $\text{CH}_4$  consumption (Figure 1) we observed that the community composition resembled its original inoculum suggesting that the community did not exhibit any growth (Supplementary Figure S1).

We used NMDS and PERMANOVA to provide further evidence for the observed shifts in community composition. NMDS plots clearly illustrated a significant effect of added  $\text{La}^{3+}$  on the composition of microbial communities in soil slurries from the acidic ( $p < 0.05$ ), neutral ( $p < 0.001$ ), and alkaline ( $p < 0.001$ ) rice fields (Supplementary Figure S2).

The Shannon diversity index (SDI) was used to describe the response of the total microbial diversity to the addition of different amounts of  $\text{La}^{3+}$ . The results showed that, in all experimental

treatments including the control, the complexity of microbial communities simplified when compared to the starting material over the course of the experiment (Supplementary Figure S3). However, we observed that the diversity values for the H treatments of soil slurries from the acidic rice field in week 3 (SDI = 5.70) and week 4 (SDI = 5.52) nearly matched that of the initial material (SDI = 5.59). A similar trend was observed for the diversity values for the M (SDI = 5.37) and H (SDI = 5.48) in week 2 and the H (SDI = 5.38) treatment in week 3 of soil slurries from the neutral rice field. This similarity to the starting material indicated an artifact and suggested that the addition of  $\text{La}^{3+}$  likely inhibited the total microbial community (see Supplementary Figure S3).

Overall, *pmoA* gene copy numbers (per g) did not vary significantly by the end of the experiment (Figure 3). However, in soil slurries from the acidic rice field, a significant inhibitory effect of added  $\text{La}^{3+}$  was observed but this diminished in the last week of the experiment. For instance, during the second week, the *pmoA* gene copy numbers (per g) in the CK, L, M, and H groups were  $7.12 \times 10^7$ ,  $2.35 \times 10^7$ ,  $2.38 \times 10^6$ , and  $2.20 \times 10^6$ , respectively. In soil slurries from the neutral rice field, we observed no significant differences but observed a similar trend in the second week of the experiment. In the second week, *pmoA* gene copy numbers (per g) for the CK, L, M, and H groups were  $1.27 \times 10^8$ ,  $7.33 \times 10^7$ ,  $8.49 \times 10^6$ , and  $5.15 \times 10^5$ , respectively. In soil slurries from the alkaline rice field, we only observed a significant inhibition of added  $\text{La}^{3+}$  in the second week, the *pmoA* gene copy numbers (per g) in the CK, L, M, and H groups were  $1.01 \times 10^8$ ,  $2.63 \times 10^7$ ,  $1.69 \times 10^7$ , and  $3.32 \times 10^7$ , respectively (Figure 3).

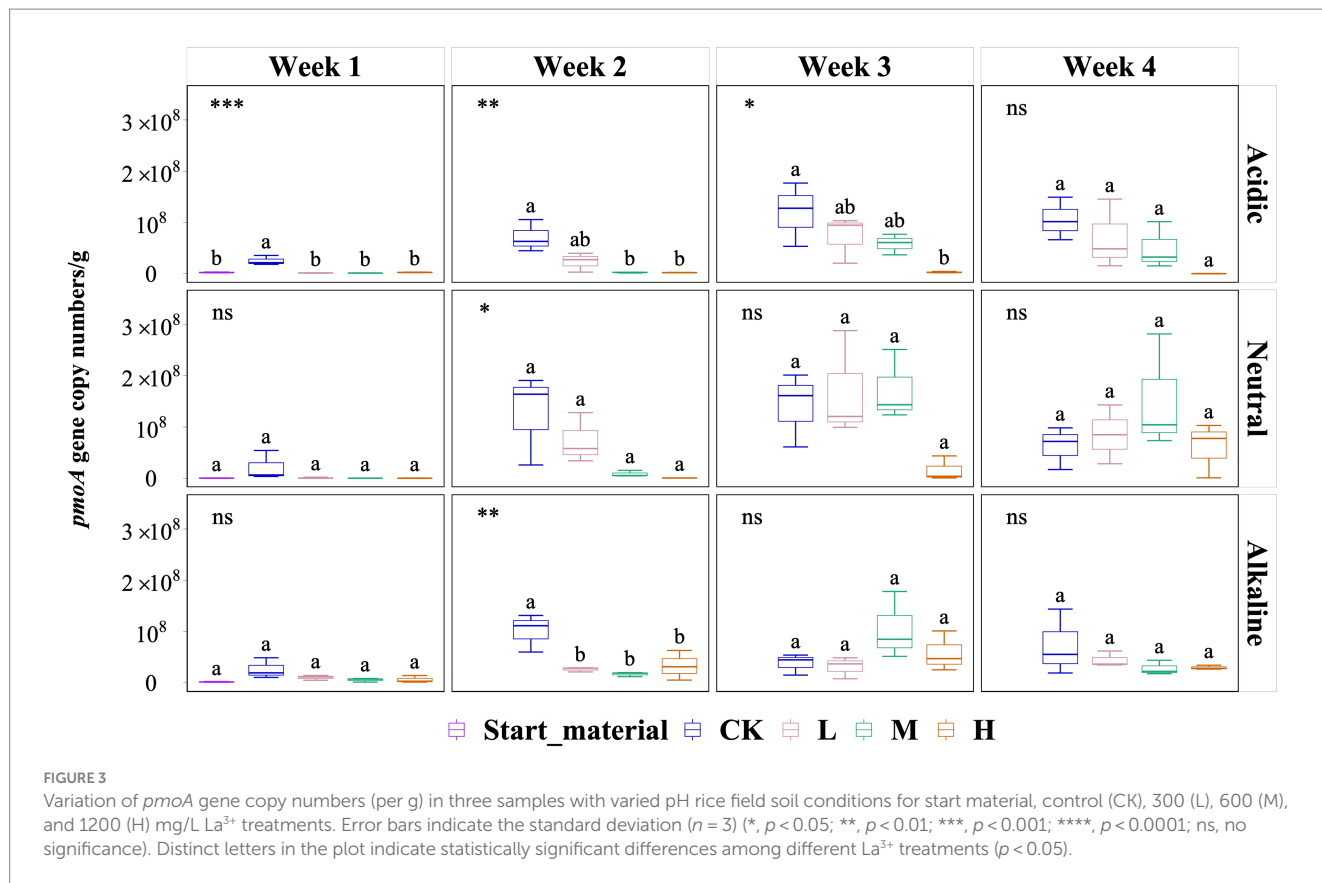
### 3.3 Added $\text{La}^{3+}$ affected pH in soil slurries and the retention of $\text{La}^{3+}$ in soil particles

The pH in the supernatant indicated a clear relationship between added  $\text{La}^{3+}$  and acidity of the soil slurries from acidic and neutral rice fields (Table 2). In contrast, soil slurries from the alkaline rice field did not show any effect of added  $\text{La}^{3+}$  on the pH indicating a higher buffer capacity of that soil (Table 2).

The concentration of  $\text{La}^{3+}$  in soil particles at the end of the experiment was similar across soil slurries from rice fields with different pH values (Figure 4). There were no significant differences in  $\text{La}^{3+}$  absorption capacity between CK, L, and M treatments from all soil types, except for soil slurries from the acidic rice field in the H treatment ( $p < 0.05$ ). In this case the  $\text{La}^{3+}$  content reached values of 4,560 mg/kg (acidic soil condition), 7,830 mg/kg (neutral soil condition), and 6,982 mg/kg (alkaline soil condition).

We also measured the  $\text{La}^{3+}$  content in the supernatant at the end of each week of the experiment to identify patterns in the bioavailable  $\text{La}^{3+}$  (Supplementary Figure S4). No consistent patterns were observed. However, a notable discrepancy in  $\text{La}^{3+}$  concentration was observed between the H and the L treatments in soil slurries from acidic rice fields during weeks 2, 3, and 4. Specifically, the  $\text{La}^{3+}$  concentration in the H treatments measured 140.73 mg/L, 133.53 mg/L, 143.27 mg/L, respectively, surpassing that of the L treatments in week 2 (64.93  $\mu\text{g/L}$ ), week 3 (171.83  $\mu\text{g/L}$ ), and week 4 (191.33  $\mu\text{g/L}$ ).

In soil slurries from the neutral rice field, the  $\text{La}^{3+}$  concentrations in the CK and L treatments were below the detection limit of the machine (5  $\mu\text{g/L}$ ) but we observed a similar trend as seen before that the contents of  $\text{La}^{3+}$  in the H treatments far greater than the M



**TABLE 2** pH of the supernatant for control (CK), 300 (L), 600 (M), and 1200 (H) mg/L La<sup>3+</sup> treatments in samples with different pH levels.

pH	Treatment	Week 1	Week 2	Week 3	Week 4
Acidic	CK	6.49 ± 0.27 a	7.06 ± 0.05 a	7.12 ± 0.05 a	6.97 ± 0.15 a
	L	5.16 ± 0.09 b	5.24 ± 0.39 b	4.57 ± 0.25 b	5.86 ± 0.23 b
	M	4.14 ± 0.17 c	4.4 ± 0.28 c	4.1 ± 0.14 c	4.76 ± 0.03 c
	H	4.1 ± 0.2 c	4.04 ± 0.24 c	3.95 ± 0.16 c	4.24 ± 0.18 d
Neutral	CK	7.25 ± 0.12 a	7.35 ± 0.07 a	7.63 ± 0.18 a	7.4 ± 0.17 a
	L	5.99 ± 0.99 ab	7.02 ± 0.03 a	7.03 ± 0.22 ab	7.3 ± 0.03 a
	M	5.59 ± 0.44 ab	5.31 ± 0.54 b	6.5 ± 0.5 b	6.8 ± 0.14 a
	H	4.54 ± 0.54 b	4.01 ± 0.05 c	4.2 ± 0.13 c	5.42 ± 0.52 b
Alkaline	CK	7.53 ± 0.14 b	7.63 ± 0.03 b	7.73 ± 0.23 a	7.74 ± 0.03 a
	L	7.85 ± 0.08 a	7.92 ± 0.02 a	7.78 ± 0.47 a	7.85 ± 0.03 a
	M	7.67 ± 0.13 ab	7.82 ± 0.06 a	7.8 ± 0.25 a	7.75 ± 0.02 a
	H	7.45 ± 0.07 b	7.7 ± 0.04 b	7.82 ± 0.12 a	7.54 ± 0.07 b

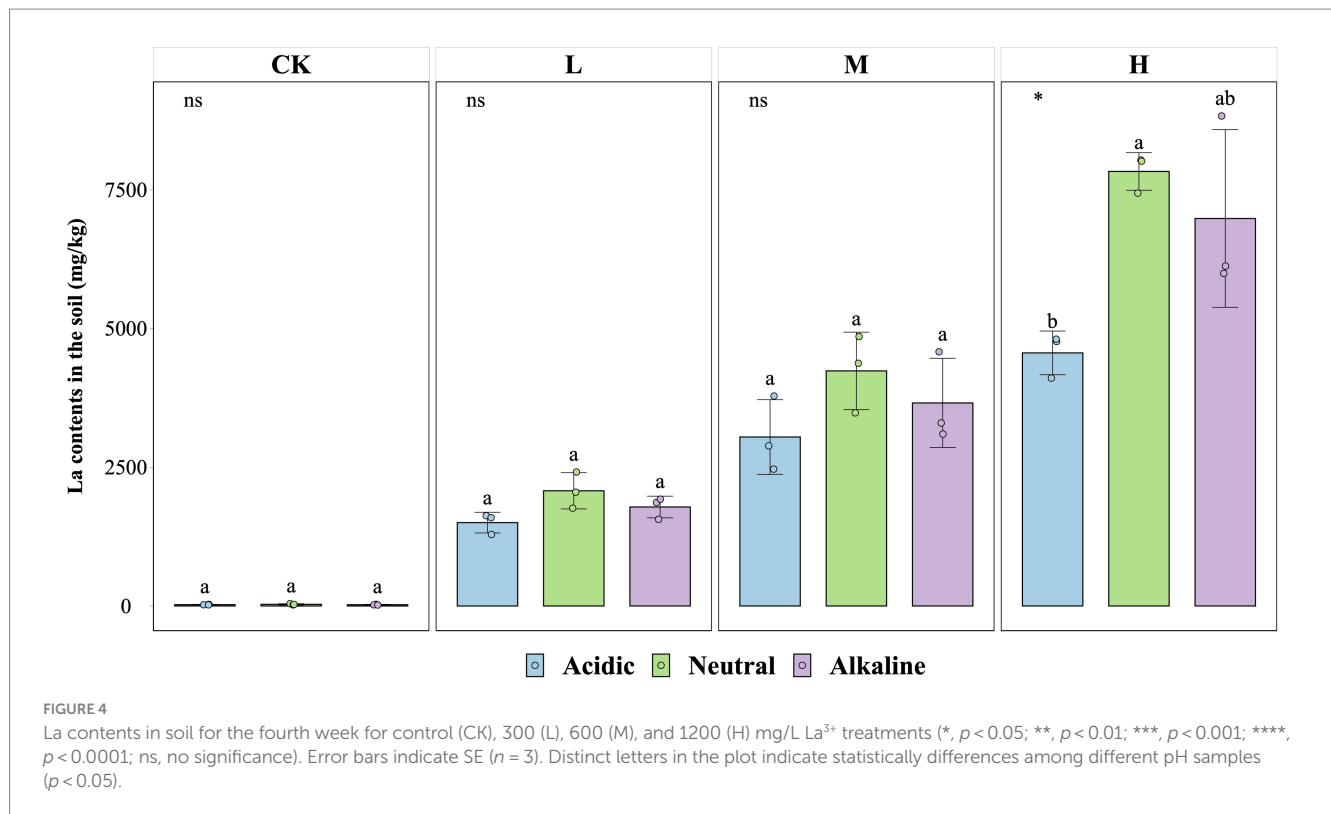
Mean ± SD is shown (*n* = 3), and distinct letters in the table indicate statistically differences among different La<sup>3+</sup> treatments (*p* < 0.05).

treatments (Supplementary Figure S4). The La<sup>3+</sup> concentrations in the H treatments were 16.77 mg/L (week 1), 14.98 mg/L (week 2), 13.67 mg/L (week 3), and 16.04 mg/L (week 4), whereas in the M treatments, the corresponding concentrations were 346 μg/L (week 1), 200.23 μg/L (week 2), 100.97 μg/L (week 3), and 326.87 μg/L (week 4). Moreover, when analyzing soil slurries from the alkaline rice field, it was evident that the La<sup>3+</sup> content was lower compared to acidic and neutral samples. The concentrations of La<sup>3+</sup> in week 1, in the L treatments in week 2 and 3, and in the M treatments in week 3, and CK treatments in week 4 were all below the detectable limit of the machine (5 μg/L). Interestingly, the La<sup>3+</sup> concentration in solution

displayed a positive correlation with the amount of added La<sup>3+</sup> in week 4 (76.60, 170.83, and 228.93 μg/L for L, M, and H treatments, respectively).

### 3.4 NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, and PO<sub>4</sub><sup>3-</sup> concentration changes in supernatant of soil slurries of different soil types

We also measured important soil chemical data in the first and last week of the experiment (Supplementary Figure S5). Firstly,



concentration levels of  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$  in the supernatant were found to be below the detection limit of the machine (0.1 mg/kg and 0.5 mg/kg, respectively, [Supplementary Figure S5](#)). Secondly,  $\text{NO}_3^-$  concentration levels did not show any notable differences in the first week. By week 4 an overall decrease was observed in all setups. In the supernatant of soil slurries from acidic rice fields the  $\text{NO}_3^-$  concentration in week 4 decreased by 49.89% (CK), 44.58% (L), 31.85% (M), and 3.28% (H) compared to their respective concentrations in week 1. In the supernatant of soil slurries from neutral rice fields the  $\text{NO}_3^-$  concentration decreased by 31.30, 50.96, 36.73, and 14.46% for CK, L, M, and H treatments, respectively. In the supernatant of soil slurries from alkaline rice fields the  $\text{NO}_3^-$  concentration decreased by roughly 22.55, 45.63, 44.07, and 43.79% for CK, L, M, and H treatments, respectively. Finally, we observed a positive correlation between the  $\text{Ca}^{2+}$  concentration in the supernatant of soil slurries and  $\text{La}^{3+}$  dosage in week 1 and 4 of the experiment ([Supplementary Figure S5](#)). In week 4,  $\text{Ca}^{2+}$  concentration in the supernatant of soil slurries from the acidic rice field reached the following levels: 1.80 mg/kg (CK), 33.83 mg/kg (L), 72.29 mg/kg (M), and 103.77 mg/kg (H). In neutral rice field soil slurries, its contents were 28.60 mg/kg (CK), 49.74 mg/kg (L), 93.77 mg/kg (M), and 175.18 mg/kg (H).  $\text{Ca}^{2+}$  concentration in the supernatant of soil slurries from the alkaline samples reached the following levels in week 4: 40.48 mg/kg (CK), 56.40 mg/kg (L), 98.56 mg/kg (M) and 193.65 mg/kg (H) ([Supplementary Figure S5](#)).

## 4 Discussion

In this laboratory microcosm study, we aimed to gain a better understanding of the ecological implications of increasing amounts of REEs, in terms of the soil microbiome which plays a crucial role in

mitigating  $\text{CH}_4$  emissions in agricultural soils. We specifically chose three rice field soils with different pH conditions because the pH is an important regulating factor for the bioavailability of  $\text{La}^{3+}$  in aquatic and soil ecosystems ([Ran and Liu, 1993](#); [Cao et al., 2001](#)).

The addition of external  $\text{La}^{3+}$  changed the activity of  $\text{CH}_4$ -consuming microbial communities in the soil slurries. We observed an inhibition of  $\text{CH}_4$  consumption based on the amount of added  $\text{La}^{3+}$ , time, and soil origin. In line with the  $\text{CH}_4$  consumption data ([Figure 1](#)), the response of the soil microbiome followed a similar pattern with no significant changes in community composition from the starting material at higher concentrations of added  $\text{La}^{3+}$ , especially in slurries with soil from acidic rice fields ([Supplementary Figure S1](#)). As such, our experimental data did not support our hypothesis that the soil microbiome, involved in  $\text{CH}_4$  consumption, will be stimulated by the addition of  $\text{La}^{3+}$  ([Figure 2](#)). Our data rather suggests that there are complex interactions between the soil origin, environmental conditions (e.g., pH), and the soil microbiome, involved in  $\text{CH}_4$  consumption. Hence, the soil microbiome's response to increasing concentrations of  $\text{La}^{3+}$  may not only vary within a single ecosystem but also between ecosystems, calling for additional research on this topic.

One possible explanation for the inhibitory effect of  $\text{La}^{3+}$  on the soil microbiome in our experiment may be attributed to chemical interactions between this element and other significant elements important in microbial cellular functions. For instance,  $\text{Ca}^{2+}$  are versatile signaling molecules that impact almost every aspect of cellular life and play crucial roles in physiological functions such as cell migration and growth ([Clapham, 2007](#); [Giorgi et al., 2018](#)). Numerous studies have reported analogous chemical properties between  $\text{La}^{3+}$  and  $\text{Ca}^{2+}$  owing to their closely matched ionic radius, charge, ligand specificities, and biological roles (e.g., [Reeves and Condrescu, 2003](#)). These similarities suggest that  $\text{La}^{3+}$  can effectively



substitute  $\text{Ca}^{2+}$ , with its trivalent ion displaying a higher affinity for given binding sites compared to  $\text{Ca}^{2+}$  (Peng et al., 2004; Dudev et al., 2005; Kotelnikova et al., 2021).

First, the  $\text{La}^{3+}$  initially present in the solution could have facilitated an exchange with the  $\text{Ca}^{2+}$  present in the soil, resulting in the release of  $\text{Ca}^{2+}$  into the solution while  $\text{La}^{3+}$  integrated into the soil. Most of the available  $\text{Ca}^{2+}$  from the soil might have been replaced this way. Our data supports this idea, because we observed an increasing concentration of available  $\text{Ca}^{2+}$  in the supernatant of soil slurries when higher amounts of  $\text{La}^{3+}$  were added, particular in alkaline soil slurry in week 1 (Supplementary Figure S5); and most of the  $\text{La}^{3+}$  was found in the soil particles of the soil slurries (Figure 4) and less  $\text{La}^{3+}$  in the supernatant (Supplementary Figure S4).

Second,  $\text{Ca}^{2+}$  ions in biological cells could have been replaced by  $\text{La}^{3+}$  ions which has been shown to inhibit cellular functions of microorganisms (Aussel et al., 1996; Mu et al., 2006; Jiang et al., 2008). In addition, the excessive amount of  $\text{La}^{3+}$ , may have blocked  $\text{Ca}^{2+}$  channels on the cell membrane and subsequently interacting with membrane related enzymes and impairing the functionality of  $\text{Ca}^{2+}$ -dependent processes (Aussel et al., 1996; Wang et al., 2007). These findings suggest that the described processes involving  $\text{La}^{3+}$  and  $\text{Ca}^{2+}$  exchange may similarly apply to methanotrophs, potentially influencing their cellular functions and disrupting  $\text{Ca}^{2+}$ -dependent processes.

The low pH in soil slurries from the acidic and neutral rice fields is another possible driving factor of the observed activity and community composition of  $\text{CH}_4$  consuming communities in our laboratory microcosm system. The soil microbiome mainly consuming  $\text{CH}_4$  faced challenges in adapting to acidic conditions (pH = 5.76), limiting the activity of their main MMO through changes in available metal ions such as copper and iron (Yao et al., 2023). However, low pH conditions did not correlate with the observed  $\text{CH}_4$  consumption patterns and are likely not the main explanation for the observed inhibition patterns. For instance, we observed a pH of 4.57 in week 3, but in week 4, the pH in the L treatments in the supernatant of soil slurries from acidic rice fields was 5.86 (see Table 2). However, the  $\text{CH}_4$  consumption activity and community composition remained similar. Therefore, we suggest that the effect of  $\text{La}^{3+}$  perhaps had a greater impact on  $\text{CH}_4$  consumption at lower pH levels, possibly due to alterations in the solubility and bioavailability of  $\text{La}^{3+}$ .

Next, to pH, other soil properties are important contributing factors that can influence the effect of  $\text{La}^{3+}$  on the soil microbiome. The mineral composition of the parent material can affect soil cation exchange capacity, which determines the soil's ability to retain and release nutrients, including  $\text{La}^{3+}$ . In addition, organic matter content plays a crucial role in buffering pH and retaining nutrients. More importantly, organic matter can also bind to  $\text{La}^{3+}$  ions, affecting their availability to soil microbes (Luo et al., 2021). In our three rice field soils the organic matter content differed substantially, with neutral samples (26.98 g/kg) having the highest concentration, followed by acidic samples (19.48 g/kg), and the lowest in alkaline samples (11.16 g/kg) (Table 1). Soil origin can also influence the availability of essential nutrients, such as nitrogen, potassium, and phosphorus. It is well known that the addition of small quantities of  $\text{LaCl}_3$  forms small precipitates of  $\text{LaPO}_4$  in the presence of phosphates (Ding et al., 2007; Phuruangrat et al., 2012; Silva et al., 2019). As such, higher available phosphorus content in alkaline (37.47 mg/kg) and neutral (33.13 mg/kg) rice field samples may have contributed to the precipitation of  $\text{La}^{3+}$  thereby changing the bioavailability of  $\text{La}^{3+}$ .

It should be noted that  $\text{CH}_4$  was the sole carbon source added to the soil slurries over the course of the experiment. Our data demonstrated that the soil microbiome simplified in their community composition, with the *Methylobacter* genus as the dominant methanotroph but also non-methanotrophic methylotrophs such as the genus *Methylotenera* and *Methylovorus*. As such, our findings align with previous metagenomic studies that have examined the distribution of carbon derived from  $\text{CH}_4$  across various bacterial populations (Kalyuzhnaya et al., 2008; Hernandez et al., 2015; Oshkin et al., 2015). As shown by Krause et al. (2017) the *Methylobacter* genus can provide carbon in the form methanol to non-methanotrophic methylotrophs such as the *Methylotenera* genus in the presence of  $\text{La}^{3+}$ . Hence, our results further provide evidence for the concept that these communities collectively contribute to  $\text{CH}_4$  cycling, rather than a single type of microorganism. In addition, both methylotrophs are known to possess lanthanide (Ln)-dependent alcohol dehydrogenases (ADH) which may confer an advantage in the presence of high  $\text{La}^{3+}$  concentrations (Krause et al., 2017; Huang et al., 2018). In this context, we observed that the *Methylocystis* genus, a type II methanotroph, depicted higher relative abundance than the *Methylobacter* genus (Type I) in soil slurries with acidic and neutral rice field soil at higher  $\text{La}^{3+}$  concentrations (Figure 2). Previous studies already indicated that *Methylocystis* is more flexible than *Methylobacter*, mainly exhibited by their substrate utilization, oxygen requirements, and environmental adaptability (Krause et al., 2015; Hakobyan and Liesack, 2020; Nguyen et al., 2021). Interestingly, the *Methylocystis* genus has been demonstrated to harbor Ln-dependent ADHs (Jung et al., 2020). Our results indicate that *Methylocystis* genus also exhibits increased flexibility under high  $\text{La}^{3+}$  conditions as well. Hence, we suggest that the soil microbiome involved in  $\text{CH}_4$  consumption can generally cope with the addition of excessive amount of  $\text{La}^{3+}$  and changes in community composition ensured ecosystem functionality.

While our primary hypothesis lacked support from the data, we did make several noteworthy observations. Firstly, in the fourth week of the experiment, the  $\text{CH}_4$  consumption data revealed a faster decrease in  $\text{CH}_4$  concentration in the L and M treatments when compared to the control treatment for all soil slurries with different initial pH levels (Figure 1). We believe that the experimental design, utilizing only a 1%  $\text{CH}_4$  concentration in the headspace, may have constrained the  $\text{CH}_4$  oxidation potential of these communities and hindered the identification of clear stimulatory effects.

Secondly, we observed an increase in the relative abundance of the genus *Methylobacter* in response to varying levels of added  $\text{La}^{3+}$  in soil slurries from alkaline rice fields (Figure 2). We conducted qPCR analysis of the *pmoA* gene using the A189F and mb661R primer set (Holmes et al., 1995; Costello and Lidstrom, 1999); however, we were unable to confirm this trend with this primer set. A possible explanation lies in the coverage of the used primer set that fails to detect phylogenetic clusters that fall between *pmoA* and *amoA* (subunit of ammonium monooxygenase) sequences like *pxmA* cluster and high-affinity  $\text{CH}_4$  oxidizers cluster. Previously, the genera *Methylomonas*, *Methylobacter* and *Methylomicrobium* have been shown to encode a sequence-divergent particulate monooxygenase (pXMO) (Tavormina et al., 2011). In addition, members of the family  *Beijerinckiaceae*  are not well amplified (Bourne et al., 2001; Knief, 2015). Hence, considering our *pmoA* gene copy number data using A189F/mb661R, it appears that a single primer might not be enough to fully investigate the abundance of methanotrophs in these rice field samples during our experiments, which might not cover all the active methanotrophs.

More importantly, we also did not pick methanotrophs that only have soluble form of the methane monooxygenase (SMMO) next to the pMMO such as *Methyloceanibacter*, which was an essential member of the total microbial community in all our initial soil slurries. Although it is not categorized as a methanotroph, this microbe has been identified to possess a SMMO and gene copies of Ln-dependent PQQ ADHs in marine environments, making it a putative methanotroph that can benefit from the addition of  $\text{La}^{3+}$  (Vekeman et al., 2016).

It is worth noting another contributing factor to our findings. As mentioned before, the presence of  $\text{La}^{3+}$  can enhance the activity of methanotrophs by increasing the production of MDH, particularly XoxF, and higher conversion of  $\text{CH}_3\text{OH}$  into  $\text{HCHO}$  (Chu and Lidstrom, 2016). However, the metabolites produced in this process may have an impact on the activity of formaldehyde dehydrogenase (FDH) and other enzymes, inhibiting the conversion of  $\text{CH}_4$  into biomass. Consequently, certain intermediates in the  $\text{CH}_4$  oxidation pathway could potentially increase the cell toxicity of the methanotrophs and inhibit overall cell growth.

While our study contributes valuable insights to the ecological effect of added  $\text{La}^{3+}$  on methanotrophic communities in a microcosm model system, it is essential to acknowledge certain limitations that may impact the interpretation of our findings. One notable constraint is the detection of only approximately 37% of  $\text{La}^{3+}$  retained in the soil (Supplementary Figure S6). Together with the presence of negligible amounts in the supernatant (approximately 1%), it raises the question about potential precipitation and sequestration mechanisms for the remaining unaccounted fraction (Supplementary Figure S6). As mentioned before  $\text{La}^{3+}$  has a strong tendency to bind phosphates. Interestingly, we did not detect any  $\text{PO}_4^{3-}$  in the supernatant of samples during the experiment (Supplementary Figure S5) supporting this as a mechanism for  $\text{La}^{3+}$  precipitation. In addition, the portion that remains unaccounted for could also be ascribed to potential interactions within microbial cells, possibly through bioaccumulation. For instance, at an exposure concentration of  $20\ \mu\text{M}$ , the  $\text{La}^{3+}$  concentration within bacteria was measured at a level of  $10832\ \mu\text{g g}^{-1}$  dry weight (Zhang et al., 2010). These limitations highlight a need for further studies into the fate and behavior of  $\text{La}^{3+}$  in soil ecosystems. Rather than relying solely on  $\text{La}^{3+}$  in the supernatant as a proxy for its bioavailability, employing sequential extractions followed by ICP-MS could enhance our understanding of the unaccounted portion of  $\text{La}^{3+}$ .

## 5 Conclusion

This study offers valuable insights into the potential impacts of increasing amounts of REEs in the form  $\text{La}^{3+}$  on the soil microbiome originating from rice fields. Our findings imply that the consumption of  $\text{CH}_4$  in soil is influenced by intricate relationships between the soil origin, environmental factors like pH, and the community of microorganisms inhabiting the soil. As such, these findings contribute to a better understanding of the crucial role that microbial communities play in mitigating greenhouse gas emissions and combating climate change. In future research, it is crucial to better account for potential precipitation and bioaccumulation effects and concentrate on the cellular responses of the  $\text{CH}_4$ -consuming community. This involves obtaining metagenome-assembled genomes coupled with transcriptomics to elucidate the biological and chemical components during the observed interactions with  $\text{La}^{3+}$ .

## Data availability statement

The data presented in the study are deposited in the National Center for Biotechnology Information (NCBI) repository, BioProject accession number PRJNA1014707.

## Author contributions

RL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. ZW: Writing – original draft, Writing – review & editing. WD: Methodology, Formal analysis, Writing – review & editing. RW: Writing – original draft, Writing – review & editing. JA: Resources, Writing – review & editing. LY: Resources, Writing – review & editing. SK: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. SK was funded by National Natural Science Foundation of China (NSFC) International (Regional) Cooperation and Exchange Program (grant number 32050410288). JA and LY were supported by the Fundamental Research Funds for the Central Universities (grant number 020914380079). ChatGPT version 3.5 was used to improve English grammar and language.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2024.1298154/full#supplementary-material>

## References

- Anthony, C. (2000). Methanol dehydrogenase, a PQQ-containing quinoprotein dehydrogenase. *Subcell. Biochem.* 35, 73–117. doi: 10.1007/0-306-46828-x\_3
- Aussel, C., Marhaba, R., Pelassy, C., and Breittmayer, J. P. (1996). Submicromolar La<sup>3+</sup> concentrations block the calcium release-activated channel, and impair CD69 and CD25 expression in CD3- or thapsigargin-activated Jurkat cells. *Biochem. J.* 313, 909–913. doi: 10.1042/bj3130909
- Balaran, V. (2019). Rare earth elements: a review of applications, occurrence, exploration, analysis, recycling, and environmental impact. *Geosci. Front.* 10, 1285–1303. doi: 10.1016/j.gsf.2018.12.005
- Bourne, D., McDonald, I., and Murrell, J. (2001). Comparison of pmoA PCR primer sets as tools for investigating Methanotroph diversity in three Danish soils. *Appl. Environ. Microbiol.* 67, 3802–3809. doi: 10.1128/AEM.67.9.3802-3809.2001
- Bulman, R. (2003). Mobilization of lanthanides through the terrestrial biosphere. *Met. Ions Biol. Syst. The lanthanides and their interrelations with biosystems* eds A. Sigel A and H. Sigel (Marcel Dekker, Inc, New York, NY) 4, 39–67.
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869
- Cao, X., Chen, Y., Wang, X., and Deng, X. (2001). Effects of redox potential and pH value on the release of rare earth elements from soil. *Chemosphere* 44, 655–661. doi: 10.1016/S0045-6535(00)00492-6
- Carlson, K. M., Gerber, J. S., Mueller, N. D., Herrero, M., MacDonald, G. K., Brauman, K. A., et al. (2017). Greenhouse gas emissions intensity of global croplands. *Nat. Clim. Chang.* 7, 63–68. doi: 10.1038/nclimate3158
- Chu, F., and Lidstrom, M. E. (2016). XoxF acts as the predominant methanol dehydrogenase in the type I Methanotroph *Methylobacterium buryatense*. *J. Bacteriol.* 198, 1317–1325. doi: 10.1128/JB.00959-15
- Chu, H. Y., Zhu, J. G., Xie, Z. B., Zeng, Q., Li, Z. G., and Cao, Z. H. (2003). Availability and toxicity of exogenous lanthanum in a haplic Acrisols. *Geoderma* 115, 121–128. doi: 10.1016/S0016-7061(03)00081-8
- Clapham, D. E. (2007). Calcium Signaling. *Cells* 131, 1047–1058. doi: 10.1016/j.cell.2007.11.028
- Conrad, R., and Rothfuss, F. (1991). Methane oxidation in the soil surface layer of a flooded rice field and the effect of ammonium. *Biol. Fertil. Soils* 12, 28–32. doi: 10.1007/BF00369384
- Costello, A. M., and Lidstrom, M. E. (1999). Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments. *Appl. Environ. Microbiol.* 65, 5066–5074. doi: 10.1128/aem.65.11.5066-5074.1999
- Dance, A. (2023). Microbial miners take on rare-earth metals. *Nature* 623, 876–878. doi: 10.1038/d41586-023-03611-4
- de Mendiburu, F. (2021). *Agricolae: Statistical procedures for agricultural research*. Available at: <https://CRAN.R-project.org/package=agricolae> (accessed June 05, 2021).
- Dedysh, S. N., and Dunfield, P. F. (2017). “Cultivation of Methanotrophs” in *Hydrocarbon and Lipid Microbiology Protocols: Isolation and Cultivation*. eds T. J. McGenity K.-N. Timmis and B. Nogales (Cham: Springer Berlin Heidelberg)
- Dedysh, S. N., and Knief, C. (2018). “Diversity and phylogeny of described aerobic Methanotrophs” in *Methane biocatalysis: Paving the way to sustainability*. eds M. G. Kalyuzhnaya and X.-H. Xing (Cham: Springer International Publishing)
- Ding, S., Liang, T., Yan, J., Zhang, Z., Huang, Z., and Xie, Y. (2007). Fractionations of rare earth elements in plants and their conceptive model. *Sci. China Life Sci.* 50, 47–55. doi: 10.1007/s11427-007-2040-7
- Duan, Y., Gao, Y., Zhao, J., Xue, Y., Zhang, W., Wu, W., et al. (2023). Agricultural methane emissions in China: inventories, driving forces and mitigation strategies. *Environ. Sci. Technol.* 57, 13292–13303. doi: 10.1021/acs.est.3c04209
- Dudev, T., Chang, L.-Y., and Lim, C. (2005). Factors governing the substitution of La<sup>3+</sup> for Ca<sup>2+</sup> and Mg<sup>2+</sup> in Metalloproteins: a DFT/CDM study. *J. Am. Chem. Soc.* 127, 4091–4103. doi: 10.1021/ja044404t
- Dushyantha, N., Batapola, N., Ilankoon, I. M. S. K., Rohitha, S., Premasiri, R., Abeyasinghe, B., et al. (2020). The story of rare earth elements (REEs): occurrences, global distribution, genesis, geology, mineralogy and global production. *Ore Geol. Rev.* 122:103521. doi: 10.1016/j.oregeorev.2020.103521
- Feng, S., Tan, C. H., Constancias, F., Kohli, G. S., Cohen, Y., and Rice, S. A. (2017). Predation by *Bdellovibrio bacteriovorus* significantly reduces viability and alters the microbial community composition of activated sludge flocs and granules. *FEMS Microbiol. Ecol.* 93. doi: 10.1093/femsec/fix020
- Fox, J., and Weisberg, S. (2019). *An R companion to applied regression*. Thousand Oaks, CA: Sage Publications.
- Giorgi, C., Marchi, S., and Pinton, P. (2018). The machineries, regulation and cellular functions of mitochondrial calcium. *Nat. Rev. Mol. Cell Biol.* 19, 713–730. doi: 10.1038/s41580-018-0052-8
- Guo, Y., Song, B., Li, A., Wu, Q., Huang, H., Li, N., et al. (2022). Higher pH is associated with enhanced co-occurrence network complexity, stability and nutrient cycling functions in the rice rhizosphere microbiome. *Environ. Microbiol.* 24, 6200–6219. doi: 10.1111/1462-2920.16185
- Gupta, K., Kumar, R., Baruah, K. K., Hazarika, S., Karmakar, S., and Bordoloi, N. (2021). Greenhouse gas emission from rice fields: a review from Indian context. *Environ. Sci. Pollut. Res.* 28, 30551–30572. doi: 10.1007/s11356-021-13935-1
- Hakobyan, A., and Liesack, W. (2020). Unexpected metabolic versatility among type II methanotrophs in the Alphaproteobacteria. *J. Biol. Chem.* 401, 1469–1477. doi: 10.1515/hsz-2020-0200
- Hanson, R. S., and Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiol. Rev.* 60, 439–471. doi: 10.1128/mr.60.2.439-471.1996
- Hernandez, M. E., Beck, D., Lidstrom, M., and Chistoserdova, L. (2015). Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation. *PeerJ.* 3:e801. doi: 10.7717/peerj.801
- Hibi, Y., Asai, K., Arafuka, H., Hamajima, M., Iwama, T., and Kawai, K. (2011). Molecular structure of La<sup>3+</sup>-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. *J. Biosci. Bioeng.* 111, 547–549. doi: 10.1016/j.jbiosc.2010.12.017
- Holmes, A. J., Costello, A., Lidstrom, M. E., and Murrell, J. C. (1995). Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiol. Lett.* 132, 203–208. doi: 10.1111/j.1574-6968.1995.tb07834.x
- Huang, J., Yu, Z., and Chistoserdova, L. (2018). Lanthanide-dependent methanol dehydrogenases of XoxF4 and XoxF5 clades are differentially distributed among methylophilic Bacteria and they reveal different biochemical properties. *Front. Microbiol.* 9:1366. doi: 10.3389/fmicb.2018.01366
- Iftekhhar, S., Ramasamy, D. L., Srivastava, V., Asif, M. B., and Sillanpää, M. (2018). Understanding the factors affecting the adsorption of lanthanum using different adsorbents: a critical review. *Chemosphere* 204, 413–430. doi: 10.1016/j.chemosphere.2018.04.053
- IPCC (2021). *The physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press.
- Jiang, Z., Weng, B., Huang, Y., Jiang, Z., and Wang, Y. (2008). Effects of lanthanum on soil microorganism. *J. Chin. Rare Earth Soc.* 26, 498–502. doi: 10.3321/j.issn:1000-4343.2008.04.021
- Jung, G. Y., Rhee, S. K., Han, Y. S., and Kim, S. J. (2020). Genomic and physiological properties of a facultative methane-oxidizing bacterial strain of *Methylocystis* sp. from a Wetland. *Microorganisms* 8:1719. doi: 10.3390/microorganisms8111719
- Kalyuzhnaya, M. G., Lapidus, A., Ivanova, N., Copeland, A. C., McHardy, A. C., Szeto, E., et al. (2008). High-resolution metagenomics targets specific functional types in complex microbial communities. *Nat. Biotechnol.* 26, 1029–1034. doi: 10.1038/nbt.1488
- Kassambara, A. (2023). Ggpubr: 'ggplot2' based publication ready plots. Available at: <https://CRAN.R-project.org/package=ggpubr> (accessed June 05, 2021).
- Kato, S., Takashino, M., Igarashi, K., and Kitagawa, W. (2020). Isolation and genomic characterization of a Proteobacterial Methanotroph requiring lanthanides. *Microbes Environ.* 35:ME19128. doi: 10.1264/jsme2.ME19128
- Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic Methanotrophic Bacteria evaluated based on pmoA as molecular marker. *Front. Microbiol.* 6:1346. doi: 10.3389/fmicb.2015.01346
- Kolb, S., Knief, C., Stubner, S., and Conrad, R. (2003). Quantitative detection of methanotrophs in soil by novel pmoA-targeted real-time PCR assays. *Appl. Environ. Microbiol.* 69, 2423–2429. doi: 10.1128/aem.69.5.2423-2429.2003
- Kotelnikova, A., Fastovets, I., Rogova, O., Volkov, D. S., and Stolbova, V. (2019). Toxicity assay of lanthanum and cerium in solutions and soil. *Ecotoxicol. Environ. Saf.* 167, 20–28. doi: 10.1016/j.ecoenv.2018.09.117
- Kotelnikova, A. D., Rogova, O. B., and Stolbova, V. V. (2021). Lanthanides in the soil: routes of entry, content, effect on plants, and genotoxicity (a review). *Eurasian Soil Sci.* 54, 117–134. doi: 10.1134/S1064229321010051
- Krause, S. M. B., Johnson, T., Samadhi Karunarathne, Y., Fu, Y., Beck, D. A. C., Chistoserdova, L., et al. (2017). Lanthanide-dependent cross-feeding of methane-derived carbon is linked by microbial community interactions. *Proc. Nat. Acad. Sci. U. S. A.* 114, 358–363. doi: 10.1073/pnas.1619871114
- Krause, S., Niklaus, P. A., Badwan Morcillo, S., Meima Franke, M., Lüke, C., Reim, A., et al. (2015). Compositional and functional stability of aerobic methane consuming communities in drained and rewetted peat meadows. *FEMS Microbiol. Ecol.* 91:fiv119. doi: 10.1093/femsec/fiv119
- Kravchenko, I. K., Sizov, L. R., and Lysak, L. V. (2023). Laboratory study of the effect of ammonium and lanthanum salts on methane oxidation and composition of microbial communities in Soddy-Podzolic soil. *Eurasian Soil Sci.* 56, 573–583. doi: 10.1134/S1064229322602773
- Kravchenko, I. K., Sizov, L. R., Tikhonova, E. N., and Lysak, L. V. (2022). The effect of lanthanum on the composition of Methanotrophic Community of sod-Podzolic Soil. *Microbiology* 91, 599–603. doi: 10.1134/S002621672260135X

- Lane, D. J. (1991). "16S/23S rRNA sequencing" in *Nucleic acid techniques in bacterial systematics*. ed. E. G. S. Michael (New York, USA: John Wiley & Sons), 115–175.
- Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., and Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *PNAS* 82, 6955–6959. doi: 10.1073/pnas.82.20.6955
- Leal Filho, W., Kotter, R., Özyayar, P. G., Abubakar, I. R., Eustachio, J. H. P. P., and Matandirotya, N. R. (2023). Understanding rare earth elements as critical raw materials. *Sustainability* 15:1919. doi: 10.3390/su15031919
- Li, S., Chen, L., Han, X., Yang, K., Liu, K., Wang, J., et al. (2022). Rice cultivar renewal reduces methane emissions by improving root traits and optimizing photosynthetic carbon allocation. *Agriculture* 12:2134. doi: 10.3390/agriculture12122134
- Li, J., Hong, M., Yin, X., and Liu, J. (2010). Effects of the accumulation of the rare earth elements on soil macrofauna community. *J. Rare Earths* 28, 957–964. doi: 10.1016/S1002-0721(09)60233-7
- Li, J., Verweij, R. A., and van Gestel, C. A. M. (2018). Lanthanum toxicity to five different species of soil invertebrates in relation to availability in soil. *Chemosphere* 193, 412–420. doi: 10.1016/j.chemosphere.2017.11.040
- Liang, T., Li, K., and Wang, L. (2014). State of rare earth elements in different environmental components in mining areas of China. *Environ. Monit. Assess.* 186, 1499–1513. doi: 10.1007/s10661-013-3469-8
- Luo, Y., Yuan, H., Zhao, J., Qi, Y., Cao, W.-W., Liu, J.-M., et al. (2021). Multiple factors influence bacterial community diversity and composition in soils with rare earth element and heavy metal co-contamination. *Ecotoxicol. Environ. Saf.* 225:112749. doi: 10.1016/j.ecoenv.2021.112749
- Mu, K., Zhao, X., Hu, L., Zhang, F., Zhang, W., and Cui, J. (2006). Toxicity of lanthanum to pathogenic Fungi and its morphological characteristics. *J. Rare Earths* 24, 607–612. doi: 10.1016/S1002-0721(06)60173-7
- Nguyen, D. T. N., Lee, O. K., Nguyen, T. T., and Lee, E. Y. (2021). Type II methanotrophs: a promising microbial cell-factory platform for bioconversion of methane to chemicals. *Biotechnol. Adv.* 47:107700. doi: 10.1016/j.biotechadv.2021.107700
- Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., et al. (2022). *Vegan: Community ecology package*. Available at: <https://CRAN.R-project.org/package=vegan>
- Oshkin, I. Y., Beck, D. A. C., Lamb, A. E., Tchesnokova, V., Benuska, G., McTaggart, T. L., et al. (2015). Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. *ISME J.* 9, 1119–1129. doi: 10.1038/ismej.2014.203 (accessed June 05, 2021).
- Palarea-Albaladejo, J., and Martín-Fernández, J. (2015). ZCompositions - R package for multivariate imputation of left-censored data under a compositional approach. *Chemometr. Intell. Lab. Syst.* 143, 85–96. doi: 10.1016/j.chemolab.2015.02.019
- Peng, L., Yi, L., Zhaxue, L., Juncheng, Z., Jiabin, D., Daiwen, P., et al. (2004). Study on biological effect of La<sup>3+</sup> on *Escherichia coli* by atomic force microscopy. *J. Inorg. Biochem.* 98, 68–72. doi: 10.1016/j.jinorgbio.2003.08.012
- Phuruangrat, A., Ekthammathat, N., Thongtem, S., and Thongtem, T. (2012). Preparation of LaPO<sub>4</sub> nanowires with high aspect ratio by a facile hydrothermal method and their photoluminescence. *Res. Chem. Intermed.* 39, 1363–1371. doi: 10.1007/s11164-012-0692-9
- Picone, N., and op den Camp, H. J. M. (2019). Role of rare earth elements in methanol oxidation. *Curr. Opin. Chem. Biol.* 49, 39–44. doi: 10.1016/j.cbpa.2018.09.019
- Pol, A., Barends, T. R. M., Dietl, A., Khadem, A. F., Eygensteyn, J., Jetten, M. S. M., et al. (2014). Rare earth metals are essential for methanotrophic life in volcanic mudpots. *Environ. Microbiol.* 16, 255–264. doi: 10.1111/1462-2920.12249
- Pruesse, E., Peplies, J., and Glöckner, F. O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 28, 1823–1829. doi: 10.1093/bioinformatics/bts252
- Qian, H., Zhu, X., Huang, S., Linquist, B., Kuzakov, Y., Wassmann, R., et al. (2023). Greenhouse gas emissions and mitigation in rice agriculture. *Nat. Rev. Earth Environ.* 4, 716–732. doi: 10.1038/s43017-023-00482-1
- Ramos, S. J., Dinali, G. S., Oliveira, C., Martins, G. C., Moreira, C. G., Siqueira, J. O., et al. (2016). Rare earth elements in the soil environment. *Curr. Pollut. Rep.* 2, 28–50. doi: 10.1007/s40726-016-0026-4
- Ran, Y., and Liu, Z. (1993). The characteristics of adsorption and desorption of rare earth elements by the Main types of soils of China. *J. Rare Earths* 1, 46–51.
- Reeves, J. P., and Condrescu, M. (2003). Lanthanum is transported by the sodium/calcium exchanger and regulates its activity. *Am. J. Physiol. Cell Physiol.* 285, C763–C770. doi: 10.1152/ajpcell.00168.2003
- Saunio, M., Stavert, A., Poulter, B., Bousquet, P., Canadell, J., Jackson, R., et al. (2020). The global methane budget 2000–2017. *Earth Syst. Sci. Data* 12, 1561–1623. doi: 10.5194/essd-12-1561-2020
- Silva, F. B. V., Nascimento, C. W. A., Alvarez, A. M., and Araújo, P. R. M. (2019). Inputs of rare earth elements in Brazilian agricultural soils via P-containing fertilizers and soil correctives. *J. Environ. Manag.* 232, 90–96. doi: 10.1016/j.jenvman.2018.11.031
- Skovran, E., and Martinez-Gomez, N. C. (2015). Just add lanthanides. *Science* 348, 862–863. doi: 10.1126/science.aaa9091
- Tavormina, P. L., Orphan, V. J., Kalyuzhnaya, M. G., Jetten, M. S., and Klotz, M. G. (2011). A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. *Environ. Microbiol. Rep.* 3, 91–100. doi: 10.1111/j.1758-2229.2010.00192.x
- Team, R. C. (2022). R: a language and environment for statistical computing. R Foundation for Statistical Computing patent application. Available at: <https://www.R-project.org/> (accessed June 05, 2022).
- Tommasi, F., Thomas, P. J., Pagano, G., Perono, G. A., Oral, R., Lyons, D. M., et al. (2021). Review of rare earth elements as fertilizers and feed additives: a knowledge gap analysis. *Arch. Environ. Contam. Toxicol.* 81, 531–540. doi: 10.1007/s00244-020-00773-4
- Tyler, G. (2004). Rare earth elements in soil and plant systems - a review. *Plant Soil* 267, 191–206. doi: 10.1007/s11104-005-4888-2
- van den Boogaart, K. G., Tolosana-Delgado, R., and Bren, M. (2022). Compositions: compositional data analysis. Available at: <https://CRAN.R-project.org/package=compositions> (accessed June 05, 2021).
- Vekeman, B., Kerckhof, F.-M., Cremers, G., de Vos, P., Vandamme, P., Boon, N., et al. (2016). New Methyloceanibacter diversity from North Sea sediments includes methanotroph containing solely the soluble methane monooxygenase. *Environ. Microbiol.* 18, 4523–4536. doi: 10.1111/1462-2920.13485
- Vu, H. N., Subuyuj, G. A., Crisostomo, R. V., and Skovran, E. (2021). Chapter seven - transposon mutagenesis for methylotrophic bacteria using *Methylorubrum extorquens* AM1 as a model system. *Methods Enzymol.* J. 650, 159–184. doi: 10.1016/bs.mie.2021.01.015
- Wang, X., Shi, G. X., Xu, Q. S., Xu, B. J., and Zhao, J. (2007). Lanthanum- and cerium-induced oxidative stress in submerged *Hydrilla verticillata* plants. *Russ. J. Plant Physiol.* 54, 693–697. doi: 10.1134/S1021443707050184
- Wickham, H. (2007). Reshaping data with the reshape package. *J. Stat. Softw.* 21, 1–20. doi: 10.18637/jss.v021.i12
- Wickham, H. (2011). The Split-apply-combine strategy for data analysis. *J. Stat. Softw.* 40, 1–29. doi: 10.18637/jss.v040.i01
- Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., et al. (2019). Welcome to the Tidyverse. *J. Open Source Softw.* 4:1686. doi: 10.21105/joss.01686 (accessed January 29, 2023).
- Wickham, H., François, R., Henry, L., Müller, K., and Vaughan, D. (2023). Dplyr: a grammar of data manipulation. Available at: <https://CRAN.R-project.org/package=dplyr>
- Yao, X., Wang, J., and Hu, B. (2023). How methanotrophs respond to pH: a review of ecophysiology. *Front. Microbiol.* 13:1034164. doi: 10.3389/fmicb.2022.1034164
- Zhang, H., He, X., Bai, W., Guo, X., Chai, Z., and Zhao, Y. (2010). Ecotoxicological assessment of lanthanum with *Caenorhabditis elegans* in liquid medium. *Metallomics* 2, 806–810. doi: 10.1039/C0MT00059K
- Zhao, D., Luo, Y., Feng, Y. Y., He, Q.-P., Zhang, L.-S., Zhang, K.-Q., et al. (2021). Enhanced adsorption of phosphorus in soil by lanthanum-modified biochar: improving phosphorus retention and storage capacity. *Environ. Sci. Pollut. Res.* 28, 68982–68995. doi: 10.1007/s11536-021-15364-6