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pH dependence of arsenic speciation in paddy soils: The role of distinct methanotrophs☆

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ABSTRACT

Arsenic (As) is a priority environmental pollutant in paddy field. The coupling of arsenate (As(V)) reduction with anaerobic methane (CH_4) oxidation was recently demonstrated in paddy soils and has been suggested to serve as a critical driver for As transformation and mobilization. However, whether As(V)-dependent CH_4 oxidation is driven by distinct methanotrophs under different pH conditions remains unclear. Here, we investigated the response of As(V)-dependent CH_4 oxidation to pH shifts (pH 5.5–8.0) by employing isotopically labelled CH_4. Furthermore, the underlying mechanisms were also investigated in well-controlled anoxic soil suspension incubations. Our results showed that As(V)-dependent CH_4 oxidation is highly sensitive to pH changes (1.6–6.8 times variation of arsenite formation). A short-term (0–10 d) pH shift from near-neutral pH to acidic conditions (i.e., pH 5.5, −85% arsenite formation) had an inhibitory effect on As(V)-dependent CH_4 oxidation. However, prolonged acidic conditions (i.e., >15 d) had no significant influence on As(V)-dependent CH_4 oxidation. The microbial analyses indicated that As reduction in paddies can be driven by anaerobic CH_4 oxidation archaea (ANME) and methanotrophs. And, methanotrophs may serve as a critical driver for As(V)-dependent CH_4 oxidation. Moreover, type I methanotrophs *Methylobacter* were more active in oxidizing CH_4 than type II methanotrophs *Methylocystis* when the pH $\geq$ 6.5. However, *Methylocystis* had a higher tolerance to soil acidification than *Methylobacter*. This study illustrates that As(V)-dependent CH_4 oxidation could be dominated by distinct methanotrophs along with pH shifts, which eventually enhances As release in paddy soils.

1. Introduction

Rice paddies are substantial sources of anthropogenic methane (CH_4) emission and provide rich substrates for CH_4-oxidizing microbes. Anaerobic CH_4 oxidation is a crucial biochemical process that mitigates 200 Tg or 50% CH_4 emission into the atmosphere from wetlands (Wassmann and Aulakh, 2000; Segarra et al., 2015). Many electron acceptors (e.g., nitrate (Raghoebarsing et al., 2006), sulfate (Timmers et al., 2016), ferric iron (Fe^{3+}) (Fan et al., 2020), tetravalent manganese (Mn^{4+}) (Ettwig et al., 2016; Leu et al., 2020), and arsenate [As(V)] (Shi et al., 2020) etc.) are feasible to anaerobic CH_4 oxidation. Most recently, coupling of As(V) reduction with anaerobic CH_4 oxidation was reported (Shi et al., 2020). The As(V)-dependent CH_4 oxidation poses a limited influence on CH_4 emissions, since As is only a minor element of the Earth's crust (Sarkar and Paul, 2016). However, the As(V)-dependent CH_4 oxidation can significantly influence the behavior of As in paddy fields, which is a priority environmental pollutant that poses an imminent health threat to more than half of the world's population through rice consumption (Zhu et al., 2008; Sohn, 2014; Yang et al., 2020). Shi et al. (2020) estimated that coupling of As(V) reduction with anaerobic CH_4 oxidation contributes between 26 and 49% of total As release in rice paddies, but its impact on As behavior in rice paddies under different environmental conditions is largely unknown.

Soil pH is one of the most important factors affecting As behavior in rice paddies (Antoniadis et al., 2017). During rice cultivation, rapid pH shifts occur under various scenarios. The soil pH tends to converge

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toward near-neutral conditions along with flooding (Gambrell, 1994; Kögel-Knabner et al., 2010; Chen et al., 2020). In the meanwhile, ferrous Fe (Fe^{2+}) oxidation by radial oxygen loss and the exudation of organic acids by the rice roots result in rhizospheric soil acidification (Maisch et al., 2019; Yuan et al., 2022). Furthermore, the application of sulfur or nitrogen fertilizers can lead to soil pH decrease (Kissel et al., 2020; Yuan et al., 2021). The dynamic changes in pH greatly influence the adsorption/desorption of As on/from soil minerals. For example, a pH increase will enhance As desorption from minerals due to the lower stability of otherwise stable metal oxide-As complexes (Al-Abed et al., 2007). In addition, Cudennec and Lecerf (2006) reported that a dramatic soil pH increase (i.e., from pH 6 to pH 10–14) or decrease (i.e., from pH 6 to pH 2–5) leads to higher crystallinity of Fe minerals, which would decrease As adsorption by Fe minerals, a major sink for soil As.

In addition to influencing As abiotically, pH can also influence the microbial transformation of As (Tripathi et al., 2018). Low pH (i.e., pH < 6.0) enhances microbial-mediated As methylation and volatilization (Huysmans and Frankenberger Jr, 1991; Chen et al., 2019); in contrast, alkaline pH conditions (i.e., pH > 6.5) favor As thiolation (Wang et al., 2020). Furthermore, pH also reshapes methanotrophs responsible for CH_4 oxidation (Wang et al., 1993; Rosentreter et al., 2021). Within circumneutral paddy soils, CH_4-oxidizing bacteria (like type I methanotrophs *Methylosarcina* and *Methylobacter*) were found to actively participate in CH_4 oxidation; in contrast, type II methanotrophs (like *Methylosinus*) tend to dominate CH_4 oxidation under acidic soil conditions (e.g., pH = 5.17) (Shiau et al., 2018). Similarly, to type I methanotrophs, anaerobic methane oxidation archaea (ANME) also favor a relatively high pH condition (Hilger et al., 2000). Shi et al. (2020) found that ANME actively participate in coupling of As(V) reduction with anaerobic CH_4 oxidation under neutral incubations. However, whether distinct methanotrophs can be involved in the As(V)-dependent CH_4 oxidation remains unclear. Considering that methanotrophs are sensitive to pH (Hilger et al., 2000; Shiau et al., 2018), it is therefore reasonable to hypothesize that distinct methanotrophs may sustain As(V)-dependent CH_4 oxidation under different pH conditions.

Hence the objectives of this study were to: 1) validate As(V)-dependent CH_4 oxidation in typical paddy soils; 2) illustrate the response of As(V)-dependent CH_4 oxidation to pH shifts; and 3) identify the microorganisms involved in As(V)-dependent CH_4 oxidation under different pH conditions. By employing isotopically labelled CH_4 in well-controlled anoxic soil suspension incubations, we demonstrated that As(V)-dependent CH_4 oxidation was highly sensitive to pH shifts. Through further microbial analysis, distinct methanotrophs were found to drive As(V)-dependent CH_4 oxidation under different pH conditions. These results provide novel insights for our full understanding of As(V)-dependent CH_4 oxidation in a varying environment.

2. Materials and methods

Experimental preparation. Three soil samples were collected from different paddy fields located in Chengdu (30°29′N, 103°45′E), Mianyang (31°16′N, 105°22′E) and Maguan (22°55′N, 104°31′E), China. Before the soil was sampled, flat land in the paddy field was selected; obvious stones and plant debris were mechanically removed by shovels. In total, ~10 kg plow layer (0–20 cm) soils were collected and immediately transported to the lab. The soil samples were preserved at −20 °C before further use. Chengdu, Mianyang, and Maguan soils belong to silt loam, loam, and clay loam, respectively. And, they have a total organic carbon content ranging from 0.96 to 13.23 g/kg, and total As content from 9.6 to 4334.8 mg/kg. The detailed soil properties are depicted in Table S1.

Anoxic soil suspension incubations were used in this study. The mineral medium was prepared following the method previously reported by Shi et al. (2020). The incubation was designed to study the response of As(V)-dependent CH_4 oxidation to pH shifts in paddies. Briefly, the incubation was prepared as follows: 1.0 g of thawed fresh soil was weighed into 120 mL serum bottles containing 50 mL sterile mineral medium. The resulting soil suspensions were then degassed using pure dinitrogen for 30 min. After degassing, the serum bottles were tightly covered with a rubber cap and sealed using a crimper. The serum bottles were incubated under constant temperature (25 ± 0.8 °C) and continuous dark conditions. The pH of the medium was adjusted by using 1 M HCl or NaOH. Each treatment has three replicates.

Experiment 1. validation of As(V)-dependent CH_4 oxidation in paddies. Three paddy soils with distinct physico-chemical characteristics were used to validate As(V) reduction driven by CH_4 oxidation. Before the experiment, external As(V) was added to the 50 mL soil suspension medium, with a concentration of 50 μM (3.75 mg L^{-1}) As(V). Then, 7.0 mL of ^{13}C-labelled CH_4 was injected into the bottles to maintain a $^{13}CH_4$ content of ~10% (v/v) in the headspace. The anoxic soil suspensions were incubated for seven days under near-neutral conditions, and manually shaken for 2 min every day to avoid diffusion constraints. The sampling was undertaken before (Day-0) and after the incubation (Day-7). The $^{13}CH_4$ and $^{13}CO_2$ concentrations as well as As speciation were also measured after each sampling event.

Experiment 2. identification of the response of As(V)-dependent CH_4 oxidation to pH shifts. Maguan soil has higher As concentration than the other two paddy soils selected (Table S1), we assumed Maguan soil should be more representative. Therefore, the influence of pH shifts on As(V)-dependent CH_4 oxidation was further investigated in Maguan soil. Before the experiment, external As(V) was added to the 50 mL soil suspension medium, with a concentration of 50 μM (3.75 mg L^{-1}) As(V). The anoxic soil suspensions with different pH values (pH 5.5, 6.5, and 8.0) were incubated with or without the addition of $^{13}CH_4$ into the headspace of the serum bottles. The ^{13}C-labelled CH_4 and CO_2 concentrations as well as As speciation were monitored every 2–5 d in a short- (0–15 d) or mid-term (15–30 d) incubation, mimicking the intermittent pH shift in the real paddy field (Takahashi et al., 2004; Wang et al., 2019). During the transition from short-to mid-term incubation, fresh medium and $^{13}CH_4$ gas were added to re-supply the nutrients, electron acceptors and donors. The detailed process was: 30 mL of deoxygenated fresh mineral medium containing 50 μM (3.75 mg L^{-1}) As(V) was injected into each serum bottle; afterward, 3 mL of $^{13}CH_4$ was injected into the headspace to maintain a sufficient $^{13}CH_4$ content during the mid-term incubation.

Experiment 3. identifying the microorganisms involved in As(V)-dependent CH_4 oxidation under different pH conditions. During Experiment 2, the soil samples were taken at Day-0, Day-15 and Day-30 to extract the microbial community shift. For the Day-0 samples, 0.50 g well-mixed wet soils were taken; for the Day-15 and Day-30 samples, 20 mL soil suspension containing ~0.50 g wet soils were taken from the serum bottles with a 20 mL syringe, and the solid soils were obtained by centrifuging the suspension at 5000 rpm for 30 min.

Chemical analysis. Headspace $^{13}CH_4$ and $^{13}CO_2$ were quantified by using Agilent gas chromatography-mass spectrometry (GC-MS, GC 6890 N (G1530N), MS 5975 B, injection volume: 10 μL) equipped with an HP-PLOT U column (19091 P-UO4, ID: 30 m × 0.32 mm × 10 μm). Dissolved $^{13}CH_4$ and $^{13}CO_2$ were measured after transferring a 0.25 mL aliquot to a vial (2 mL) containing 50 μL 6 M HCl. Vials were stored upside-down for 24 h, and the headspace $^{13}CH_4$ and $^{13}CO_2$ were determined by GC-MS. The limits of detection for $^{13}CH_4$ and $^{13}CO_2$ were 7.46 and 24.1 ppm (v/v), respectively. Standard samples were tested every 20 samples to assure the data quality.

The As(III) and As(V) in the liquid phase were analyzed by high-performance liquid chromatography (HPLC, PerkinElmer, Inc., USA)-inductively coupled plasma mass spectrometry (ICP-MS NEXION300X, PerkinElmer, Inc., USA) (Zhai et al., 2020). The measurement conditions were as follows: dynamic reaction cell (DRC) mode (O_2, gas flow, 0.65 mL/min); $^{91}AsO^+$ was detected; RF power 1650 W; plasma gas flow rate 16 L/min; auxiliary gas flow 1.3 L/min; nebulized gas flow 0.98 L/min;

nickel sampling and skimmer cones. An anion-exchange column (PRP-X100, 250 × 4.1 mm) was used to separate the different As species by a mobile phase of 20 mM $NH_4H_2PO_4$ (pH = 6.5). The limit of detection for As was 0.490 μg L^{-1}. A spiked standard was tested every 20 samples to assure the data quality.

Microbial analysis. The genomic DNA of the soil was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc. Carlsbad, USA) following the manufacturer's protocols.

Extracted DNA was subjected to barcode amplification of the V3–V4 hypervariable region of the 16 S rRNA gene at Hangzhou KaiTai Biotechnology Co., Ltd. (Hangzhou, China). The effective sequences were selected using the same method used in our previous study (Yuan et al., 2022). The detailed DNA sequencing and raw data processing information can be found in the supplementary information. The average length of the remaining sequences was 450 bp. The effective sequences were grouped into operational taxonomic units (OTUs) at a similarity of ≥97%. The alpha and beta diversity analyses were performed in QIIME 1.8.0. Indices of Chao 1, Shannon, and Good's coverage were selected for the alpha diversity analysis. The principal coordinate analysis (PCoA) was applied to calculate the differences between samples. And, the averaged top 10 genera were analyzed and displayed. The 16 S rRNA gene sequence data have been deposited at NCBI-GenBank under the accession KFUI00000000.

In addition, As and CH_4 metabolic genes were quantified by real-time qPCR. The oligonucleotide primers used in this study are detailed in Table S2. Seven genes, including *Bacteria*, *Archaea*, aerobic CH_4 oxidation (*pmoA*), anaerobic CH_4 oxidation (*mcrA*), As(V) reduction (*arrA*, *arsC*), and As(III) oxidation (*aioA*) were quantified; and, different *pmoA* types (i.e., *pmoA1* and *pmoA2*) were also quantified to reveal the involvement of type I and II methanotrophs in As(V)-dependent CH_4 oxidation.

Statistical analysis. All the data were plotted by R3.5.0 software. One-way analysis of variance (ANOVA) was employed to determine statistical significance ($p < 0.05$) between different treatments, using SPSS 22 software (IBM SPSS, Armonk, NY, USA).

3. Results

As(V)-dependent CH_4 oxidation in paddies. As(V)-dependent CH_4 oxidation was validated in three paddy soils with distinct physicochemical properties (Table S1). We measured the oxidation of $^{13}CH_4$ to $^{13}CO_2$ and the simultaneous reduction of As(V) to As(III), at the beginning (Day-0) and end (Day-7) of the anoxic soil suspension incubation. The suspension remained at a constant pH around 7.4 (Fig. S1) and reducing conditions around −101 mV (*vs.* Ag/AgCl reference electrode) during the incubation. As(V) reduction driven by CH_4 oxidation was observed (Fig. 1, Fig. S2).

After 7 d incubation, $^{13}CH_4$ decreased from ~350 μM to 218, 201, and 240 μM in Chengdu, Maguan, and Mianyang paddy soils, respectively (Fig. S2). At the same time, 143, 165, and 158 μM $^{13}CO_2$, accounting for an average of 121 ± 13.4% of $^{13}CH_4$ decrease, were generated in the three soils, respectively (Fig. 1). Similarly, considerable As(V) was reduced to As(III) (average 1.36 μM; equal to ~50% of the added As(V)) during the incubation. The concomitant formation of $^{13}CO_2$ and As(III) indicates that As(V)-dependent CH_4 oxidation was active in the three paddy soils.

Effect of pH shifts on As(V)-dependent CH_4 oxidation. As(V)-dependent CH_4 oxidation was significantly influenced by changes in soil suspension pH. We therefore tested the response of As(V)-dependent CH_4 oxidation to pH from 5.5 to 8.0 under short- (0–15 d) and mid-term (15–30 d) anoxic incubations, mimicking the intermittent pH shift in paddy soils. The suspension pH was maintained during the incubation (Fig. S3), and reducing conditions (i.e., 9.50, −144 and −183 mV for pH 5.5, pH 6.5 and pH 8.0, respectively) were also maintained.

When $^{13}CH_4$ was not added, we did not observe an obvious increase of $^{13}CO_2$ and As(III) under the different pH and incubation periods

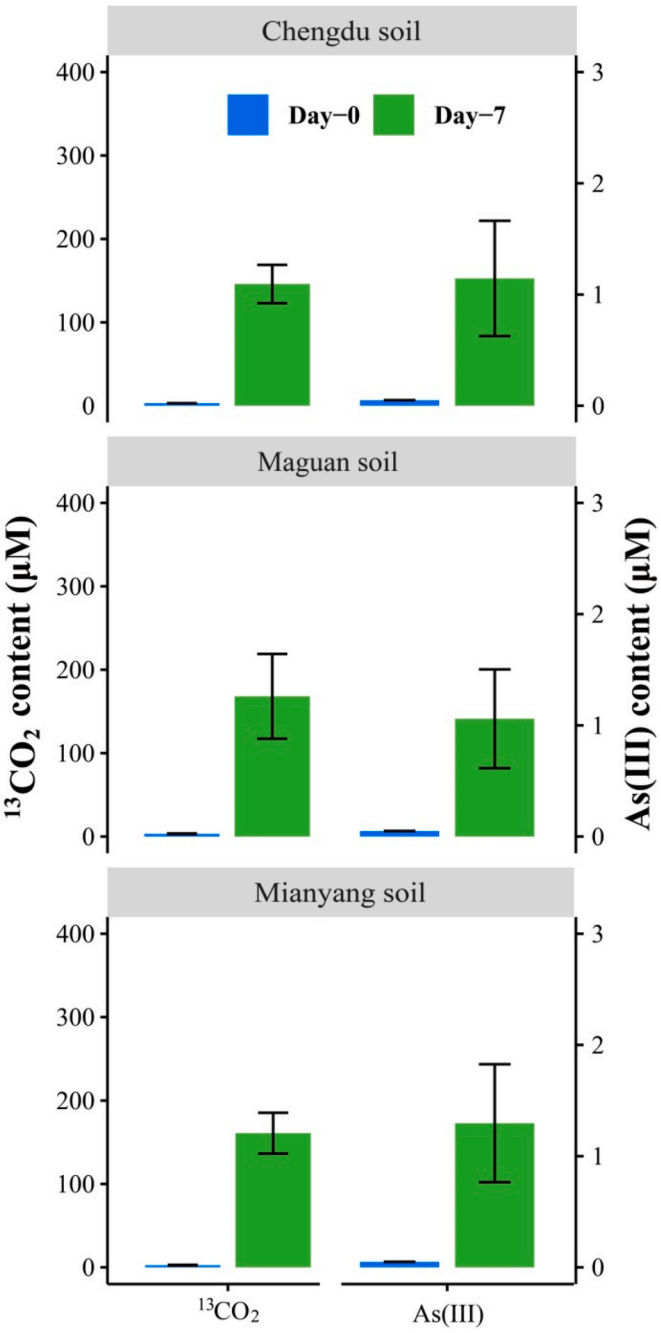

Fig. 1. Formation of ^{13}C-labelled carbon dioxide ($^{13}CO_2$) and arsenite (As(III)) in the anoxic serum bottles. $^{13}CO_2$ content was calculated by pooling gaseous and aqueous $^{13}CO_2$ in each treatment. Data are presented as the absolute value. Error bars are standard deviations ($n = 3$).

(Fig. 2). In contrast, a concomitant increase of $^{13}CO_2$ (105 ± 63.6 μM) and As(III) (0.553 ± 0.596 μM) was observed when $^{13}CH_4$ and As(V) were supplied. The As(V) was rapidly reduced by electrons stemming from microbial oxidation of $^{13}CH_4$ (0.128 μM As d^{-1}, Fig. 2) at near-neutral pH conditions (pH 6.5) from Day-0 to Day-10. However, As(V) reduction was slightly inhibited (0.0805 μM As d^{-1}) under moderately alkaline (pH 8.0) conditions, but significantly inhibited (0.0189 μM As d^{-1}, $p = 0.003$) under moderately acidic (pH 5.5) conditions. The variation of As(III) formation rate varied up to 1.6–6.8 times between the different pH treatments. $^{13}CH_4$ oxidation also changed greatly in different pH conditions, especially during the first 5 days of incubation. For example, $^{13}CH_4$ oxidation rate under pH 6.5 (4.52 ± 0.375 μM d^{-1})

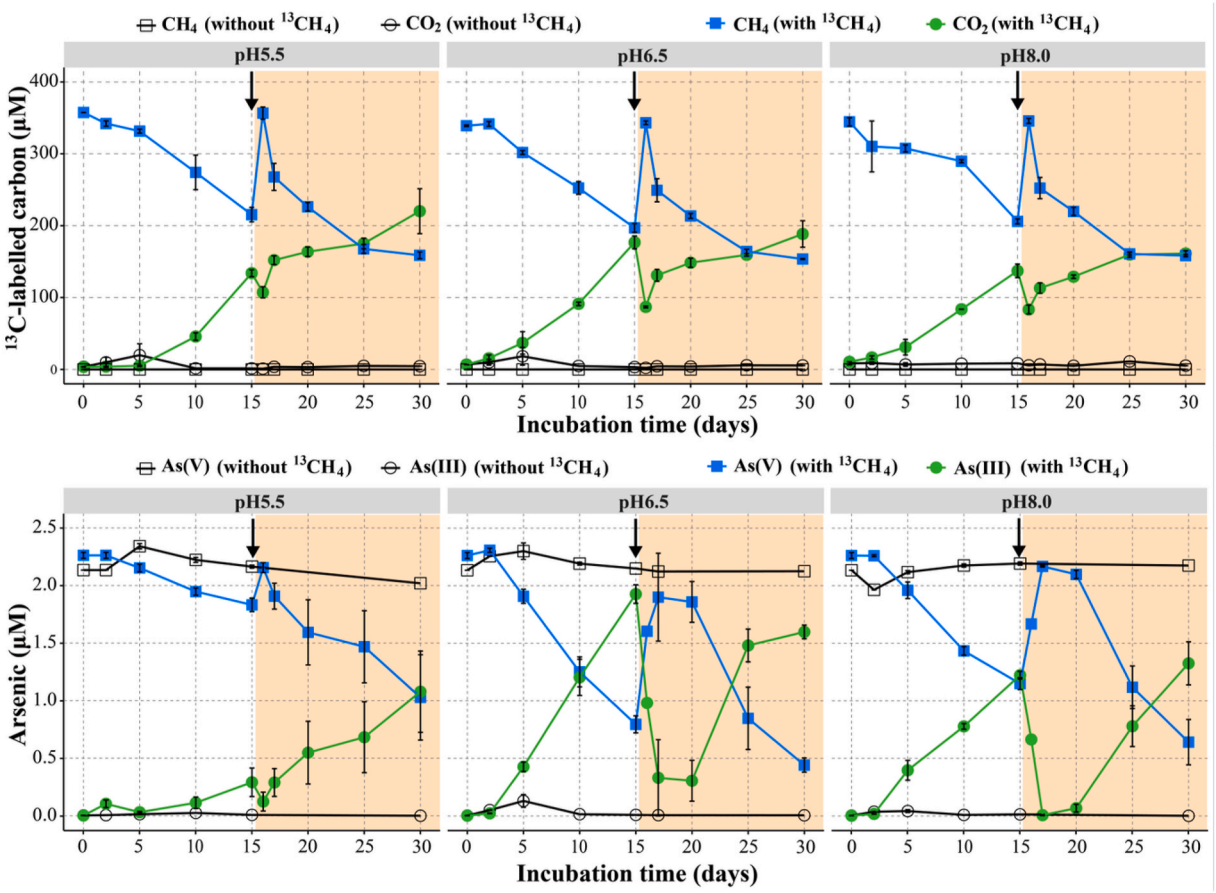

Fig. 2. Changes of ^{13}C-labelled carbon and arsenic speciation in the anoxic serum bottles at different pH conditions. $^{13}CO_2$ and $^{13}CH_4$ were calculated by pooling gaseous and aqueous $^{13}CO_2$ and $^{13}CH_4$ in each treatment, respectively. Data are presented as the absolute value. Error bars are standard deviations ($n = 3$). Black arrows indicate the timepoint when As(V) and $^{13}CH_4$ were amended for the mid-term incubation.

was much higher than that under pH 8.0 (3.95 ± 0.676 μM d^{-1}) and 5.5 (1.54 ± 0.380 μM d^{-1}) during the noted period. These results indicate that a rapid pH change, which always occurs in paddies with intensive water or fertilizer management, would greatly influence As(V)-dependent CH_4 oxidation. After the 15 d incubation, the As(V) reduction by $^{13}CH_4$ oxidation at all pH conditions (average 0.101 μM As d^{-1}) was relatively the same. This indicates that the influence of pH shifts on As(V)-dependent CH_4 oxidation was insignificant in a mid-term incubation.

In addition, a gradual increase of As(V) was observed around Day 15 (Fig. 2), this may be due to two causes: i) As(V) was re-supplemented at Day 15; ii) As(III) in the medium was oxidized to As(V).

Molecular evidence for distinct methanotrophs mediating As (V)-dependent CH_4 oxidation under different pH **conditions.** To reveal potentially important microorganisms involved in As(V)-dependent CH_4 oxidation under different pH conditions, we analyzed the soil microbial community and seven associated genes (i.e., *Bacteria*, *Archaea*, *pmoA*, *mcrA*, *arrA*, *arsC*, and *aioA* genes). The distinct preference of different types of methanotrophs for As(V)-dependent CH_4 oxidation was observed under different pH conditions (Figs. 3 and 4).

Analysis of 16 S rRNA gene, at Day-0, -15 and −30, yielded a high Good's coverage value (0.950 ± 0.0114, Table S3), indicating the sequencing depth was sufficient to cover the bacterial communities. Up to 9204 OTUs were obtained, however, only 772 OTUs were shared by the different treatments (Fig. S4A). Alpha analysis indicated pH shifts (pH 5.5 and 8.0) alone only slightly decreased Chao 1 (4289 ± 168) and Shannon (6.34 ± 0.123) compared to the original soil (4854 ± 311 and 6.60 ± 0.0316, respectively) (Table S3). By contrast, adding $^{13}CH_4$ and shifting pH together significantly decreased Chao 1 (2627 ± 116) and Shannon (4.42 ± 0.562), except Chao 1 (4946 ± 362) in the pH 5.5 treatment, compared to the control ($p < 0.05$; 4584 ± 439 and 6.42 ± 0.154, respectively). This indicates that pH shifts coupled with the supply of $^{13}CH_4$ significantly altered the bacterial community in paddy soils. Similarly, principal coordinate analysis PCoA1 *vs.* PCoA2 (explaining 59.2% variance) showed that the bacterial communities were significantly altered between the different treatments (Fig. S4B). Adding $^{13}CH_4$ promoted type I methanotrophs (i.e., *Methylobacter*) under relatively high pH conditions (pH ≥ 6.5, average 39.7%; 10.7% under pH 5.5), while type II methanotrophs (i.e., *Methylocystis*) were mainly enhanced under acidic conditions (pH 5.5, 8.49%; average 3.00% when pH ≥ 6.5) (Fig. 3). This indicates that pH shifts can significantly influence As(V)-dependent CH_4 oxidation in paddy soils via reshaping the methanotrophic groups.

Furthermore, qPCR analysis revealed that supplying $^{13}CH_4$ significantly promoted *Bacteria* and *Archaea* genes under all pH conditions, compared to the control setups (Fig. 4). This suggests that both archaea and bacteria actively participate in As(V)-dependent CH_4 oxidation in paddies. However, the active microbial groups may differ under different pH conditions. *pmoA* gene harbored by methanotrophic bacteria preferred the pH range from 5.5 to 8.0, while *mcrA* harbored by ANME favored a relatively high pH (pH ≥ 6.5). It should be noted that the *pmoA* gene was 19.1–187 times higher than the *mcrA* gene under the different pH conditions (Table S4). The acidic pH conditions were dominated by *pmoA2* (relative abundance, 82.0%), and relatively high pH conditions (pH ≥ 6.5) were dominated by *pmoA1* (relative abundance, ~60%, Fig. 4).

In addition, the *arrA* gene preferred a relatively high pH, while the opposite was true for the *arsC* gene. Significant promotion of the As-

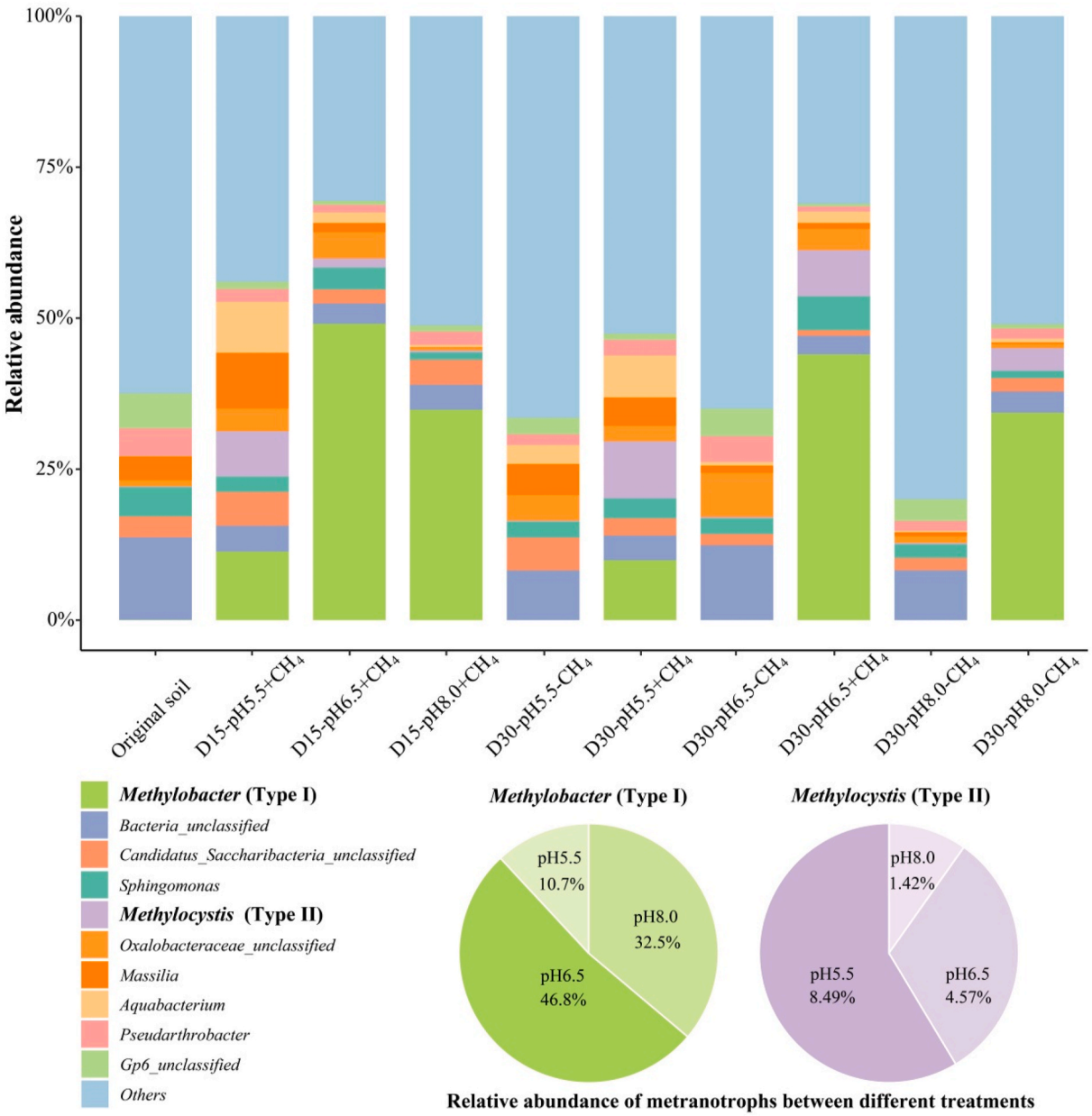

Fig. 3. Microbial community composition in microcosms during the incubation. The relative abundance of the top 10 most genera in different treatments was depicted. Type I&II methanotrophs of the treatments with $^{13}CH_4$ addition were remapped in the pie plot.

oxidizing gene (i.e., *aioA*) was detected, particularly when the pH was ≥6.5. The correlation matrix analysis vividly showed a significantly negative correlation between pH and *arsC* gene abundance ($r = -0.888$; $p < 0.05$). While a significantly positive correlation between pH with *Archaea*, *mcrA* and *arrA* gene abundance was observed ($r = 0.865$, 0.845 and 0.864, respectively; $p < 0.05$).

4. Discussion

Coupling of As(V) reduction with anaerobic CH_4 oxidation was recently shown to significantly influence As redox chemistry and its behavior in paddy soils. Although soil pH is known to be one of the most important factors affecting As behavior, the influence of pH on As(V)-dependent CH_4 oxidation was unclear so far. This study demonstrated that pH shifts can greatly influence As(V)-dependent CH_4 oxidation in paddies through reshaping the methanotrophic microorganisms.

First, As(V)-dependent CH_4 oxidation was validated in three different paddy soils in this study (Fig. 1). However, As(V) is not the main electron acceptor in the soil suspension. Other electron acceptors (e.g., oxygen (Shi et al., 2020), sulfate (Timmers et al., 2016), Fe^{3+} (Fan et al., 2020) or humic fraction of natural organic matters (Valenzuela et al., 2017) etc.) may have actively participated in CH_4 oxidation. Shi et al. (2020) estimated oxygen, due to incomplete deaeration and oxygen diffusion through rubber stoppers, could oxidize ~20% of $^{13}CH_4$ in the serum bottles. We used similar procedures to prepare serum bottles as that of Shi et al. (2020), hence aerobic $^{13}CH_4$ oxidation could take place. In addition, we detected Fe^{2+} formation during our incubation (0.208 μM Fe^{2+}). Hence, Fe^{3+}-dependent $^{13}CH_4$ oxidation could take place. However, we did not detect obvious sulfide formation (< the detection limit 0.15 mg L^{-1}). This may be due to the fact that sulfate reducers mainly compete with methanogens for organic substrates in freshwater systems, rather than participate in CH_4 oxidation (Scholz et al., 2020). The potential involvement of other electron acceptors such as Fe^{3+} and Mn^{4+} (released by the added soils) in CH_4 oxidation was also

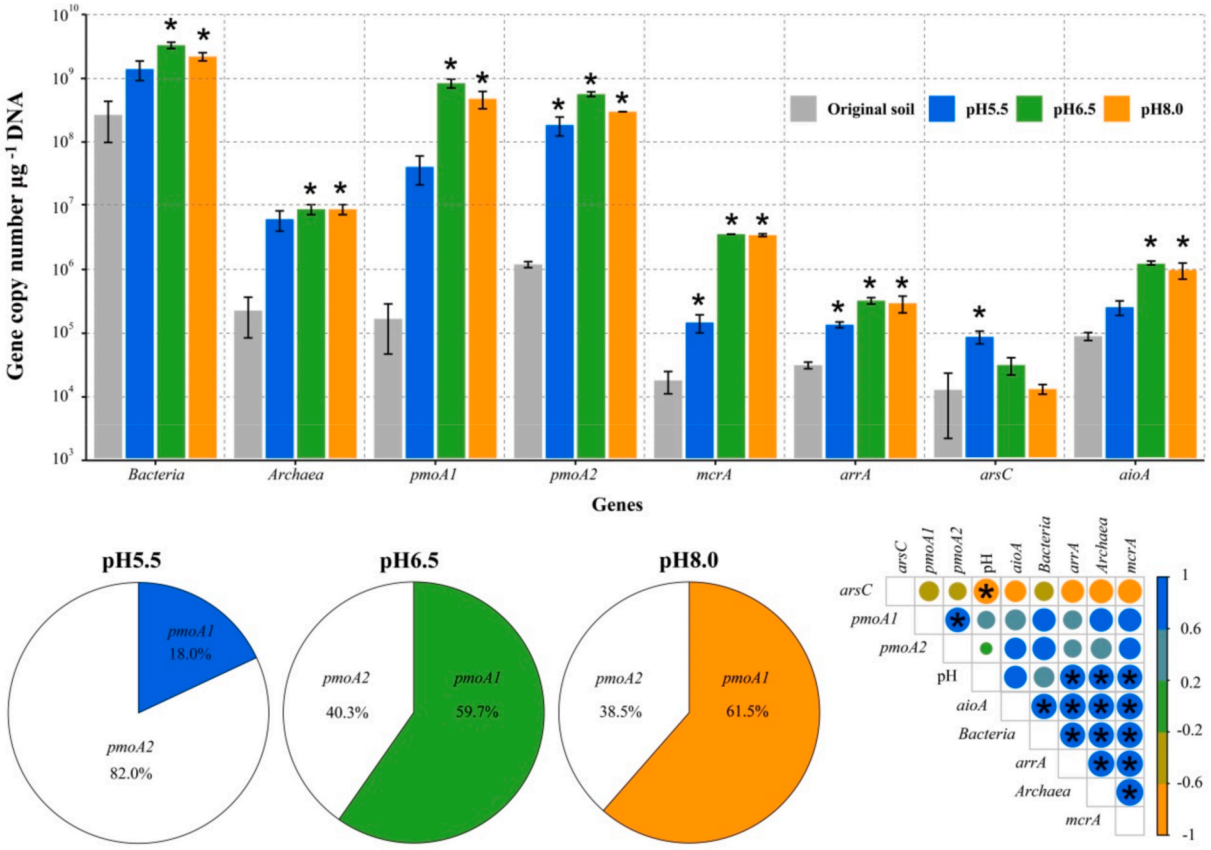

Fig. 4. Gene abundance in microcosms and the correlation matrix between pH with gene abundance. *pmoA1* and *pmoA2* refer to *pmoA* genes in type I&II methanotrophs, respectively. The star means a significant difference at $p < 0.05$. *pmoA1* and *pmoA2* of different treatments were remapped in the pie plot.

described by Pienkowska et al. (2021) when verifying the coupling of Fe^{3+} reduction with CH_4 oxidation in anoxic soil suspension incubations. Moreover, CH_4 oxidation could also be enhanced by cryptic cycling of redox-sensitive elements (e.g., As, Fe etc.) (Peng et al., 2019; Saunders et al., 2019), since those cycles are able to provide adequate substrates for CH_4 oxidation.

We found that As(V)-dependent CH_4 oxidation is sensitive to pH changes. Upon soil flooding, soil pH will converge toward near-neutral pH conditions, where the pH of acidic soils raises owing to proton consumption by metal-oxide reduction and the pH of alkaline soils drops due to CO_2 and its bicarbonate formation (Kögel-Knabner et al., 2010; Pan et al., 2015). Both methanogens and methanotrophs favor neutral conditions (Wang et al., 1993; Rosentreter et al., 2021). This may be due to two causes: i) both kinds of microorganisms grow optimally around neutral conditions and are very sensitive to pH changes in soils (Le Mer and Roger, 2001); ii) their key enzyme is similar, for example, methyl-coenzyme M reductase (*Mcr*) catalyzes the last step of CH_4 production in methanogenesis and the first step of CH_4 activation in anaerobic CH_4 oxidation (Timmers et al., 2017). Nevertheless, soil acidification alleviates CH_4 production and CH_4 oxidation (Le Mer and Roger, 2001). Hence, a future soil acidification scenario may not significantly stimulate As(V)-dependent CH_4 oxidation (Larssen and Carmichael, 2000; Singh and Agrawal, 2007; Zeng et al., 2017), but attempts to increase the soil pH via liming or applying biochar could cause high As mobilization through stimulating the CH_4 production and As(V)-dependent CH_4 oxidation (Lombi et al., 2004; Dai et al., 2017; Chen et al., 2020). Hence, attention should be paid to soil As release when amending soils with lime or biochar to repair the soil acidification in As contaminated soils. Acidification of rice rhizosphere, caused by protons generated during Fe^{2+} oxidation and plant exudation of protons and organic acids (Kuzyakov and Razavi, 2019; Maisch et al., 2019; Yuan et al., 2022), widely occurs in paddy fields. The acidification may be good for controlling As release in rhizosphere via inhibiting As (V)-dependent CH_4 oxidation (Chen et al., 2005; Yuan et al., 2022), however, it will significantly decrease the rhizosphere CH_4 consumption (Nauhaus et al., 2005; Shiau et al., 2018). Hence, the growing acidification zone along root development may open a gate for CH_4 diffusion from hypoxic paddy soils to the atmosphere through aerenchymatous tissues of rice (Hosono and Nouchi, 1997; Walter et al., 1996; Jeffrey et al., 2019).

Microbial As(V) reduction may rely on As(V) detoxification process mediated by *arsC* gene or As(V) respiratory process mediated by *arrA* gene. Compared with *arsC* gene, *arrA* gene showed a parallel change with that of *mcrA* and *pmoA* genes (Fig. 4), indicating *arrA* gene was actively involved in As(V)-dependent CH_4 oxidation. A similar indication was also proposed by Shi et al. (2020) when demonstrating the coupling of As(V) reduction with anaerobic CH_4 oxidation. During the process, ANME were reported to mediate the As(V)-dependent CH_4 oxidation independently or in syntrophy with As(V) reducing bacteria harboring *arrA* gene. In addition, methanotrophs were also suggested to perform the coupling of CH_4 oxidation with certain electron acceptors reduction (e.g., selenate, nitrous oxide) independently or synergistically (Campbell et al., 2011; Shi et al., 2021). Hence, methanotrophs and ANME in this study may sustain the As(V)-dependent CH_4 oxidation either independently or synergistically with arsenate reducing bacteria harboring *arrA* gene. ANME in this study were suggested to actively participate in As(V)-dependent CH_4 oxidation under relatively high pH conditions (pH $\geq$ 6.5), which is in line with the report of As (V)-dependent CH_4 oxidation under neutral conditions by Shi et al. (2020). In addition, this study further provided evidence showing that methanotrophs harboring *pmoA* gene could also play a critical role in driving As(V)-dependent CH_4 oxidation in paddies. The *pmoA* gene

detected in this study was much higher than the *mcrA* gene under different pH conditions. A similar phenomenon was also described by previous studies in paddies and sediments (Wang et al., 2018; Pienkowska et al., 2021). Nonetheless, much higher *mcrA* than *pmoA* gene abundance was also found in diverse aquatics (Inagaki et al., 2004; Kong et al., 2019). These results indicate that methanotrophs and ANME may dominate CH_4 oxidation under different conditions. Yet, it should be noted that direct DNA extraction and sequencing have their limitation, since relic DNA from dead cells (Gustave et al., 2019) and inactivate DNA that is not transcription-active (Yoshida et al., 2012) cannot be excluded. Our results showed soil pH is an important factor for influencing their activities. pH shifts may influence As(V)-dependent CH_4 oxidation via influencing methanotrophic cell physiology, nutrients or substrates availability of functional microorganisms (Schuchmann and Müller, 2014; Xiao et al., 2016), but the detailed mechanism warrants further research.

Methanotrophs determined in this study showed an obvious difference in pH optima, i.e., type I and II methanotrophs (i.e., *Methylobacter*; *Methylocystis*) favor near-neutral to moderate alkaline and acidic pH conditions, respectively. This is in line with the finding by Shiau et al. (2018). Shiau et al. (2018) detected the same type II methanotrophs *Methylocystis* in a paddy field suffering from soil acidification (pH 5.17). Different methanotrophs were revealed to mediate CH_4 oxidation and the further reduction of available electron acceptors (e.g., oxygen (Shi et al., 2020), nitrate (Raghoebarsing et al., 2006), sulfate (Timmers et al., 2016), Fe^{3+} (Fan et al., 2020), Mn^{4+} (Ettwig et al., 2016; Leu et al., 2020)). However, whether methanotrophs containing *pmoA* genes can drive hypoxic CH_4 oxidation has long been debated (Wrede et al., 2012; Chistoserdova, 2015; Martinez-Cruz et al., 2017; Hao et al., 2020). Our study strongly supported that they are active under hypoxic conditions. Additionally, *Methylobacter* were also frequently observed in hypoxic aquatic delta (Hao et al., 2020), paddy soil (Shiau et al., 2018), lake sediment (Martinez-Cruz et al., 2017), and groundwater (Kuloyo et al., 2020). Therefore, they may play a critical role in driving CH_4 oxidation in hypoxic aquatics. In contrast, although type II methanotrophs *Methylocystis* can be better adapted to acidic soil conditions than type I methanotrophs *Methylobacter* (Fig. 3), an extended period may be required (e.g., 10 d, Fig. 2) to induce the essential genes to perform CH_4 oxidation. A similar phenomenon was also observed by Shiau et al. (2018) when testing anaerobic CH_4 oxidation in acidic paddies. This reflects the diversity of microbes and microbial associations in CH_4 oxidation.

5. Conclusion

Through this study, we demonstrated that As(V)-dependent CH_4 oxidation is highly sensitive to pH shifts. The pH optima of As(V)-dependent CH_4 oxidation in paddies is around near-neutral conditions (i.e., pH 6.5), and pH shifts (e.g., pH 5.5 and 8.5) from the optima tend to inhibit As(V)-dependent CH_4 oxidation, especially when the pH decreased to acidic conditions (i.e., pH 5.5, −85% As(III) formation). Moreover, the microbial analysis in this study showed that diverse methanotrophs can drive As(V) reduction in paddies. Type I methanotrophs *Methylobacter* harboring the *pmoA1* gene were more active in oxidizing CH_4 than type II methanotrophs *Methylocystis* harboring the *pmoA2* gene under near-neutral conditions. However, *Methylocystis* had a higher tolerance to soil acidification than *Methylobacter*. Nonetheless, further studies are required to strengthen our understanding of As(V)-dependent CH_4 oxidation, which is the prerequisite for making efficient strategies to mitigate As risk caused by As(V)-dependent CH_4 oxidation in wetlands.

Credit statements

Zhao-Feng Yuan, Conceptualization; Funding acquisition; Investigation; Formal analysis; Visualization; Writing – original draft; Yu-Jie Zhou, Writing – review & editing, Lina Zou, Writing – review & editing, Zheng Chen, Visualization; Writing – review & editing, Williamson Gustave, Writing – review & editing, Dechao Duan, Writing – review & editing, Andreas Kappler, Writing – review & editing, Xianjin Tang, Supervision; Conceptualization; Project administration; Funding acquisition; Writing – review & editing, Jianming Xu, Writing – review & editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120880.

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