



The effect of nitrogen input on methane uptake in a wet and a dry year from a temperate desert steppe

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ABSTRACT

Methane is the second most important greenhouse gas, and soils in arid region can oxidise large amounts of atmospheric methane, thereby contributing to mitigating climate warming. Elevating input of atmospheric nitrogen (N) and precipitation change significantly affect the strength of methane sink (uptake from the atmosphere), but this is still unclear in the desert steppe. Therefore, a field simulation N input (N_{ip}) control experiment with a wet year (2019) and a dry year (2021) was done to elucidate the impact of N_{ip} on methane sink in a typical desert steppe of Eurasia. The result showed that this desert steppe was a net sink of atmospheric methane with annual uptake rate of $3.88 \text{ kg CH}_4 \text{ ha}^{-1}$. And found that methane uptake was much lower in a wet year ($33.9 \pm 1.6 \mu\text{g C m}^{-2} \text{ h}^{-1}$, 2019) than that in a dry year ($46.9 \pm 3.1 \mu\text{g C m}^{-2} \text{ h}^{-1}$, 2021), which was mainly mediated by soil water-filled pore space. The effect of N_{ip} on methane uptake was varied, both promoting (0.4 % – 1317%) and inhibiting (0.5% – 270.5%). And the inconsistent response of methane uptake was observed to N_{ip} in a wet and a dry year: the methane uptake was decreased significantly with the increase of N_{ip} rate in a wet year ($p < 0.05$); however, N_{ip} did not significantly affect methane uptake overall in a dry year ($p > 0.05$). This may attribute to the inhibitory effect of N_{ip} on methane uptake depended on soil moisture ($p < 0.01$). The abundance ratio of *pmoA* to *mcrA* gene was identified as the most significant influencing factors of methane uptake rather than soil inorganic N ($\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$) content. Furthermore, soil moisture had an important indirect effect on methane uptake, mainly through influencing the abundance ratio of *pmoA* to *mcrA* gene. Overall, we suggest that the role of soil water-filled pore space and the abundance ratio of *pmoA* to *mcrA* gene should be considered when developing biochemical models of methane uptake in arid areas.

1. Introduction

Methane, the second largest greenhouse gas in the atmosphere, significantly affects climate warming. Fortunately, numerous studies have shown that aerobic soil, such as desert ecosystems, can greatly consume atmospheric methane 28 Tg yr^{-1} and mitigate climate warming (Striegl et al., 1992; IPCC, 2013). The uptake ability of these drylands on atmospheric methane was strongly affected by soil environmental and biological factors (Gomez-casanovas et al., 2016; Yue et al., 2019; 2022). As the large increase in atmospheric N input (N_{ip}) and precipitation change have had a significant impact on soil

moisture, soil inorganic nitrogen (N , $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) contents, plant net primary productivity, biodiversity, carbon and N turnover, soil acidification, the soil microbial diversity, activity and abundance (Hao et al., 2018; 2020; Galloway et al., 2008; LeBauer and Treseder, 2008; Zhang et al., 2018), all of which could greatly impact the strength of methane sinks in dry-land ecosystems (Kou et al., 2017; Chen et al., 2019; Zhuang et al., 2013). However, it is unclear that the interactive effect of N_{ip} and precipitation change on methane uptake in temperate desert steppe.

Nitrogen input significantly affects methane uptake in arid areas (Yue et al., 2019; 2022), and the responses in different systems are

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inconsistent (Chen et al., 2013; Fang et al., 2014). As methane uptake was significantly enhanced from a desert soil by a low N_{ip} ($3 \text{ g N m}^{-2} \text{ yr}^{-1}$), with no further effect of higher N_{ip} ($6 \text{ g N m}^{-2} \text{ yr}^{-1}$) rate on its uptake, with uptake being mainly driven by soil water content and plant underground productivity (Yue et al., 2019). However, the rate of methane uptake in temperate grassland was not significantly changed by increasing N_{ip} (Chen et al., 2013). Meta-analyses have therefore suggested that a small and medium amount of N_{ip} promoted methane uptake in aerobic soil, in contrast, a high amount of N_{ip} inhibited its uptake (Aronson and Helliker, 2010; Peng et al., 2019). The effects of N_{ip} on methane uptake are also influenced by temperature and precipitation change (Yue et al., 2019; 2022), which may explain the inconsistency response of methane uptake to N_{ip} across different ecosystems (Fang et al., 2014; Peng et al., 2019). However, how methane uptake in desert steppes corresponds to N_{ip} remains unclear.

As we know that N_{ip} increases the content of soil inorganic N, which can influence methane uptake because methane oxidizing bacteria can oxidize $\text{NH}_4^+\text{-N}$ and CH_4 due to their very similar molecular structure (King and Schnell, 1994). In arid areas, plant growth and microbial activity were significantly limited by soil moisture and N availability (Kou et al., 2017), therefore N_{ip} may affect the activity of methane oxidizing bacteria and methanogens, affecting ecosystem methane net sink (Peng et al., 2019). And N_{ip} also increases plant biomass, which can also influence methane uptake (Chen et al., 2019), primarily through the effect of soil environment and nutrient conditions (Wilschut et al., 2019). Overall, soil environment changes and plant growth caused by N_{ip} will affect methane uptake in arid areas (Yue et al., 2019; 2022).

In addition, the structure and activity of soil microorganism were significantly changed by N_{ip} (Liu et al., 2018). Especially functional microorganisms, methanogens (*mcrA* gene) and methane oxidizing bacteria (*pmoA* gene), the key microorganisms affecting methane production and consumption, were very sensitive to environmental change (Yue et al., 2019). This effect of N_{ip} on methane flux has been reported for desert soil, where N_{ip} also significant changed the copy number of functional genes of methane oxidizing bacteria and methanogens (Yue et al., 2019). Variation in soil moisture content caused by changes in precipitation was reported as an important influencing factor for the activity and copy number of functional genes of methane oxidizing bacteria (Yue et al., 2022), and showing a unimodal methane uptake trend, moderate soil moisture promoting methane uptake and low or high soil moisture reducing its uptake (Yue et al., 2022). Soil temperature was also widely acknowledged to affect soil microbial activity (Frey et al., 2013). Overall, N_{ip} and precipitation changes will significantly affect soil properties and functional microorganisms, which will inevitably significantly affect the sink of methane in desert soil.

In summary, N_{ip} and annual precipitation change significantly affect plant growth, nutrient cycling, activity of soil functional microorganisms and increase soil inorganic N content, and these changes are bound to significantly affect the soil methane sink capacity in arid areas (Hao et al., 2018, 2020; Bodelier and Laanbroek, 2004). The desert steppe is a transitional region from a steppe to desert ecosystem, with arid and highly aerobic soils, which are good conditions for methane uptake. Therefore, these natural conditions determine that the desert steppe has great potential to uptake atmospheric methane (Yue et al., 2022). And methane sink in this area was very sensitive to global changes (including atmospheric N_{ip} and precipitation change; Yue et al., 2022). To date, the effects and influencing factors of increasing N_{ip} on methane uptake specifically for a desert steppe ecosystem are still unclear. Therefore, a field N_{ip} experiment was done in a typical Eurasian temperate desert steppe. The purpose was to clarify the following issues: (1) assessing the change in the methane uptake capacity of a desert steppe to N_{ip} in wet and dry years; and (2) identifying the key driving factors regulating methane uptake with the increase in N_{ip} .

2. Materials and methods

2.1. Study area

Our research was conducted at the Chinese Academy of Sciences' Desert Steppe Research Station in the heart of the Eurasian temperate zone ($106^\circ 58'E$, $41^\circ 25'N$, 1615 m a.s.l.). Average precipitation at this location in the last 30 years was about 150 mm, the 70 % precipitation mainly concentrated in the growing season (Zuo et al., 2020). Annual evapo-transpiration exceeds 2000 mm. During the study period, the precipitation in the 2019 was much greater than that in 2021: the 2019 was a wet year with 207 mm of precipitation, over 40 % more than the average annual precipitation (148 mm, in the last 37 years; Figure S1). In contrast, the 2021 was a relative dry year, with 107 mm of precipitation, 28 % below the average annual precipitation in the last 37 years (Figure S1). In addition, based on precipitation and air temperature data from 1985 to 2022, we calculated the palmer drought severity index (PDSI), and indicated a wet state in 2019 and a dry state from May to December 2021 (Figure S2). The dominant plants in the study area were *Stipa caucasica* subsp. and *glareosa* (P. A. Smirnov) Tzvelev with the plant coverage was about 30–50 %. The soil was gray brown desert soil, and mainly composed of coarse sand and fine sand content, and the clay powder content was only 10 %, with very low content of total carbon (5.72 g kg^{-1}), total N (0.32 g kg^{-1}) and available phosphorus (5.78 mg kg^{-1}).

2.2. Experimental design

There were six N treatments in this experiment, including no N application (N_0 , atmospheric N deposition) and annual N input based on atmospheric N deposition (1 g N m^{-2} , N_1 , simulated the most beneficial N deposition for plant growth (Bragazza et al., 2004); 3 g N m^{-2} , N_3 , simulated average N deposition in china (Zhang et al., 2008); 6 g N m^{-2} , N_6 , simulated N deposition level in 2050 in China (Galloway et al., 2008); 12 and 24 g N m^{-2} , N_{12} , N_{24} , simulated N contaminated, including manure from cattle and sheep and so on (Novák and Slamka, 2003). In addition, there are six plots per treatment, and the area of each plot was $6 \times 6 \text{ m}^2$ with a corridor of 1 m in each plot. Urea was used to simulate N_{ip} . The N input experiment began in 2018. Nitrogen was input twice per year, mainly in June and mid-July each year, the amount of N input each time was half of the annual N input. Firstly, the quality of urea was measured according to the amount of N_{ip} to each sample site, and then gradually dissolved in the same amount of water from low N_{ip} to high N_{ip} , and then evenly sprayed to the plot with the corresponding amount of N_{ip} . The amount of dissolved N we chose to simulate 2 mm of precipitation. And to remove the effect of adding water, we added the same amount of water to the control (i.e. without N_{ip} treatment).

2.3. Determination of methane uptake

Methane flux (a net balance of methane uptake and emission) was determined *in situ* with the plant from June 2019 to January 2020 and March to September 2021 using the static chamber (diameter of 25 cm and height of 17 cm) and gas chromatography (Yue et al., 2019). Measurements from February 2020 to February 2021 were unable to be taken because of the COVID-19 pandemic. The gas sample of methane were taken once or twice a week between 10 a.m. and 12 a.m. On a clear day, the gas sample was collected in the static tank lid tightly after 0, 10, 20, 30 min (Yue et al., 2019). The gas concentration of methane was analyzed by Agilent gas chromatography (7890A) over a week. The flux of methane was calculated from the slope of methane concentration derived from four measurements at 10 min intervals from 0 to 30 min and normalized to standard temperature and pressure according to Eq. (1).

Table 1

Effects of nitrogen (N) input on soil total carbon (TC), total nitrogen (TN), NO_3^- -N and NH_4^+ -N contents and plant aboveground net primary productivity (ANPP) in a wet and a dry year. Different letters indicated significant differences ($p < 0.05$). Lowercase letters indicated the difference between treatments, while uppercase letters indicated the difference between a dry and a wet year.

Treatments	TC g kg ⁻¹	TN g kg ⁻¹	NO_3^- -N mg kg ⁻¹	NH_4^+ -N mg kg ⁻¹	ANPP g m ⁻²
In a wet year					
N0	4.2 ± 0.2Aa	0.32 ± 0.02Aa	1.2 ± 0.5Bc	1.4 ± 0.3Bb	37.3 ± 8.4Ab
N1	4.6 ± 0.5Aa	0.31 ± 0.02Aa	1.7 ± 0.3Bc	1.3 ± 0.1Ab	44.6 ± 7.6Aa
N3	4.1 ± 0.3Ba	0.30 ± 0.05Aa	2.1 ± 0.7Bc	1.3 ± 0.1Ab	40.4 ± 2.0Aab
N6	3.9 ± 0.2Aa	0.31 ± 0.07Aa	2.0 ± 0.7Bc	1.4 ± 0.3Ab	44.0 ± 7.1Aab
N12	4.5 ± 0.3Aa	0.33 ± 0.03Aa	5.2 ± 4.1Bb	3.4 ± 1.4Aa	57.2 ± 3.8Aa
N24	4.3 ± 0.2Aa	0.37 ± 0.06Aa	16.1 ± 0.2Aa	19.6 ± 3.4Aa	50.02 ± 8.0Aab
In a dry year					
N0	4.5 ± 0.4Aa	0.27 ± 0.03Ab	4.4 ± 1.64Ab	2.8 ± 0.6Aa	26.3 ± 5.7Ab
N1	5.2 ± 0.6Aa	0.32 ± 0.02Aab	8.0 ± 2.2Ab	1.6 ± 0.2Ab	50.3 ± 9.7Aa
N3	5.2 ± 0.3Aa	0.33 ± 0.03Aab	17.2 ± 4.6Ab	2.2 ± 0.8Aab	40.6 ± 4.8Aab
N6	4.9 ± 0.4Aa	0.31 ± 0.04Aab	11.9 ± 1.8Aab	2.0 ± 0.1Ab	32.1 ± 3.5Ab
N12	4.9 ± 0.6Aa	0.33 ± 0.05Aab	49.3 ± 19.4Aa	4.2 ± 0.8Aa	39.0 ± 4.3Bab
N24	4.9 ± 0.4Aa	0.38 ± 0.03Aa	28.3 ± 21.2Aab	6.2 ± 0.1Ba	55.5 ± 5.7Aa

$$F = \rho \cdot h \cdot \frac{dC}{dt} \cdot \frac{273}{273 + T} \quad (1)$$

Where, F was the CH_4 flux ($\mu\text{g} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$), ρ was the CH_4 gas density ($\mu\text{g} \cdot \text{m}^{-3}$) of the standard conditions in the chamber, h was the actual height (m) from the top of the sampling box to the water surface, $\frac{dC}{dt}$ was the slope of the change with the concentration of CH_4 in the box at different times, T was the average atmospheric temperature ($^{\circ}\text{C}$) at the sampling time, 273 was a constant for converting degrees Celsius to degrees Fahrenheit.

The average hourly fluxes in each month were scaled to monthly periods and summed to calculate the annual flux according to Eq. (2).

$$\text{Annual } \text{CH}_4 \text{ flux} = \sum_{m=1}^{m=12} F(m) \times d \times 24 \quad (2)$$

Where, F(m) was the average monthly flux ($\text{kg } \text{CH}_4 \text{ ha}^{-1} \cdot \text{month}^{-1}$) and d was the corresponding number of days per month, m was the month of the year and 24 was hours per day.

Soil moisture and temperature were determined by TDR-350 at each methane uptake measurement occasion. Topsoil samples (0–10 cm) were taken in growing season in 2019 and 2021. A flow analyzer (AA3, Seal Analytical, Norderstedt, Germany) was used for the determination of the soil inorganic N content (NH_4^+ -N and NO_3^- -N) following extraction with calcium chloride (Yue et al., 2022). The contents of soil total N and total carbon were measured by the element analyzer (Costech ECS 4010 CHNSO, Italy). Aboveground biomass of plants was determined by cutting each plot in mid-August each year. The soil water-filled pore space (WFPS) was calculated based on soil moisture and bulk density according to formula (3) (Peng et al., 2019). In addition, the effect of N input on methane flux was calculated based on the formula (4).

$$\text{WFPS} = \frac{\text{SM} \times \text{BD}}{1 - \frac{\text{BD}}{\text{PD}}} \times 100\% \quad (3)$$

Where, SM was soil moisture (%), BD was soil bulk density (g cm^{-3}), and PD was the soil particle density (2.65 g cm^{-3}).

$$\text{N effect on methane flux} = \frac{\text{FN} - \text{FC}}{\text{FC}} \times 100\% \quad (4)$$

Where, FN was the methane flux in the N input treatment, FC was the methane flux in control plots.

2.4. Determination of the abundance of *pmoA* and *mcrA* genes

The net flux of methane was a balance between uptake and emission. Therefore, the abundance of methanotrophic bacteria (regulate the oxidation of methane, *pmoA* gene) and methanogens (regulate the production of methane, *mcrA* gene) in each plot was determined by absolute real-time quantitative PCR (Yue et al., 2022). Genes abundance of *pmoA* and *mcrA* were measured (three replicates per samples) through the method of Kolb et al. (2003). The primers for the *pmoA* gene were 5'-GGNGACTGGGACTTCTGG-3' and 5'-CCGGMGCAACGTCYTTACC-3', and for the *mcrA* gene were 5'-GGTGGTGTGGMATTACACARTAYG CWACAGC-3' and 5'-TCATTGCRTAGTTWGGRTAGTT-3'. DNA from soil samples was obtained using an extraction kit (Tiangen Biochemical Technology (Beijing) Co. LTD). Amplification was done in 18 μL reaction system (10 μL 2 × Master Mix, 0.5 μL of premier F, 0.5 μL of premier R, 1 μL of

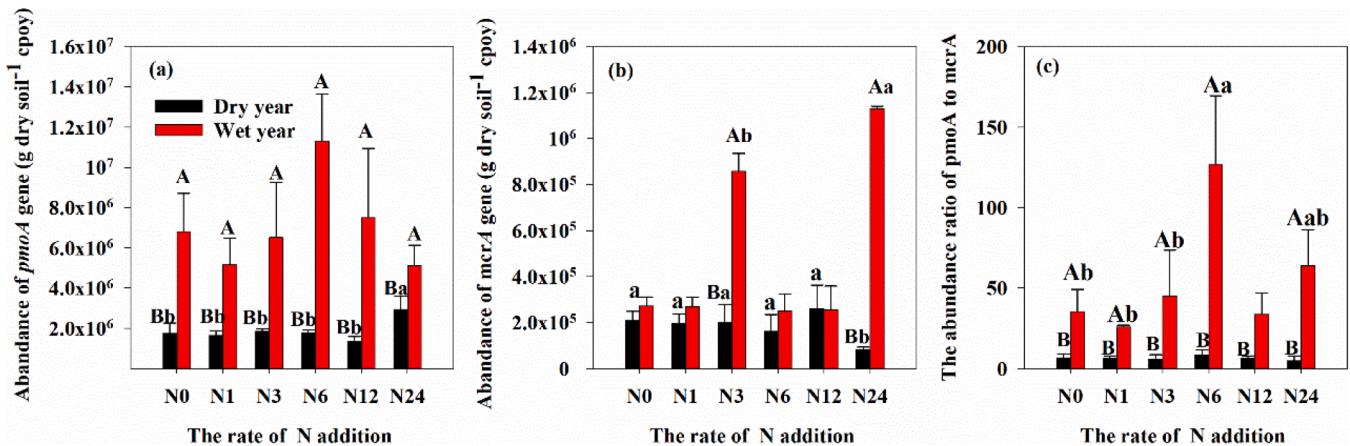


Fig. 1. The impact of nitrogen (N) input on the abundance of the functional gene *pmoA* of methane oxidizing bacteria (a), the functional gene *mcrA* of methanogens (b) and the abundance ratio of *pmoA* to *mcrA* (c). Lowercase letters indicated the effect of N input on the abundance of *pmoA* and *mcrA* genes and the abundance ratio of *pmoA* to *mcrA*, uppercase letters indicated the difference between a dry and a wet year. Different letters indicated significant differences ($p < 0.05$).

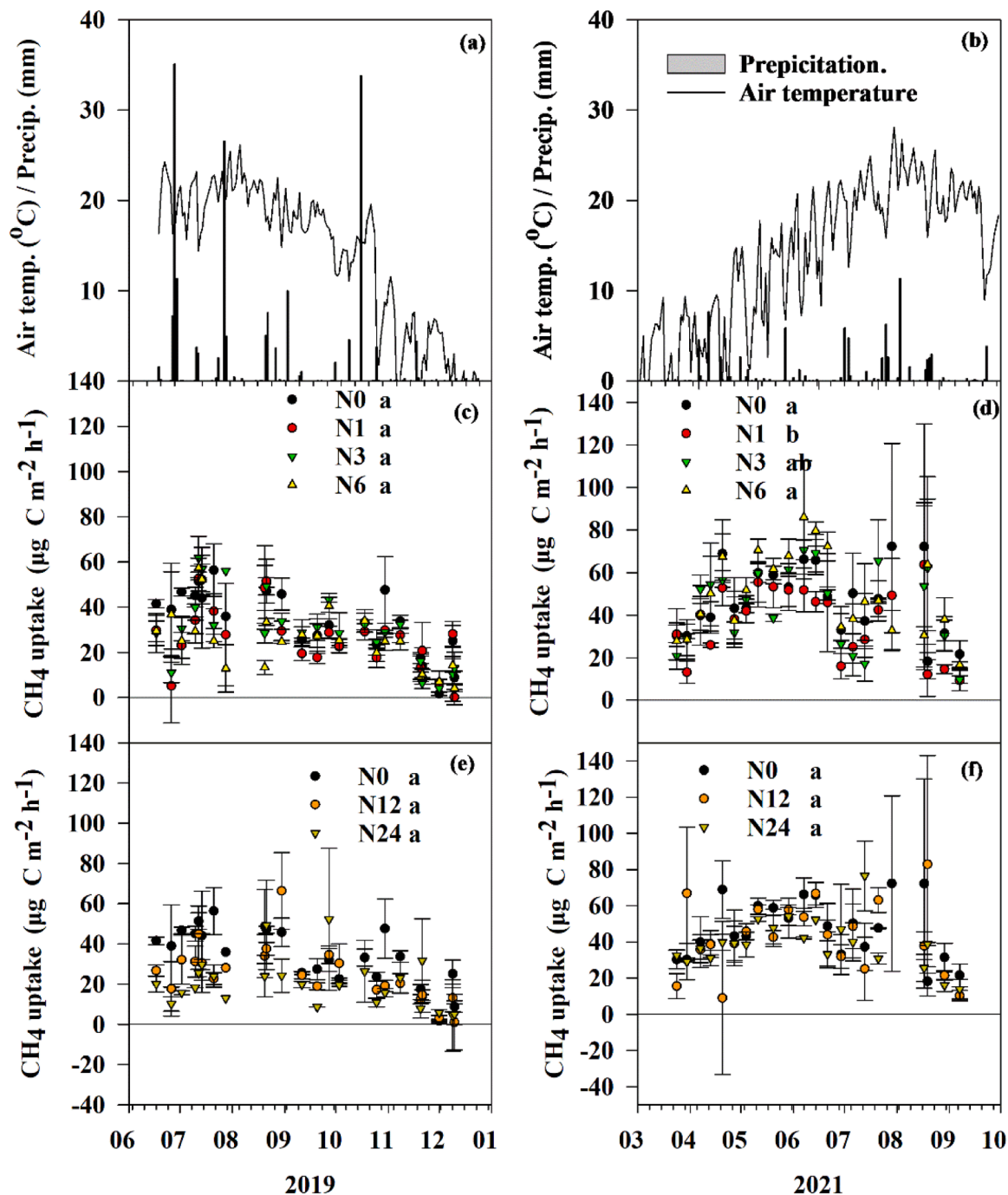


Fig. 2. Air temperature and precipitation in the period of 2019 (a) and 2021 (b), and methane (CH₄) uptake in response to nitrogen (N) input for the lower N input rates (c, d) and higher N input rates (e, f). A positive value represented CH₄ uptake from the atmosphere and a negative value represented CH₄ emission to the atmosphere. Lowercase letters a and b indicated the effect of N treatment on methane uptake, with different letters indicating significant differences between treatments ($p < 0.05$).

DNA template, 6 μL of ultrapure water) according to the following procedure: 95°C, 30 s; 40 PCR cycles (95°C, 5 s; 60°C, 40 s (collect fluorescence)). In order to establish the melting curve of PCR products, after the amplification reaction, press (95°C, 10 s; 60°C, 60 s; 95 °C, 15 s); And slowly heating from 60 °C to 99 °C. And then according to the standard curve of the *pmoA* and *mcrA* genes, the their copy number in the sample was determined.

2.5. Statistical analyses

The significant differences the *pmoA*, *mcrA* genes, soil TC, TN, soil

inorganic N contents, plant aboveground biomass and methane uptake were tested in different N_{ip} by one-way ANOVA. The influencing of soil moisture, inorganic N, plants aboveground biomass, and the functional gene abundance on methane uptake was analyzed by regression analyses. The repeated measures ANOVA was used to analyze the effect of sampling date (Time) and N_{ip} in a wet and dry a year. The direct and indirect effects of soil moisture, soil inorganic N content, ANPP and functional microorganisms on methane uptake were analyzed by structural equation model (SEM, Amos (22.0) plug-in in SPSS). All data analysis was completed by SPSS Statistics 22.0 (IBM, SPSS, Chicago, Illinois, U.S.A.). All figures were completed using sigmplot 12.5 software

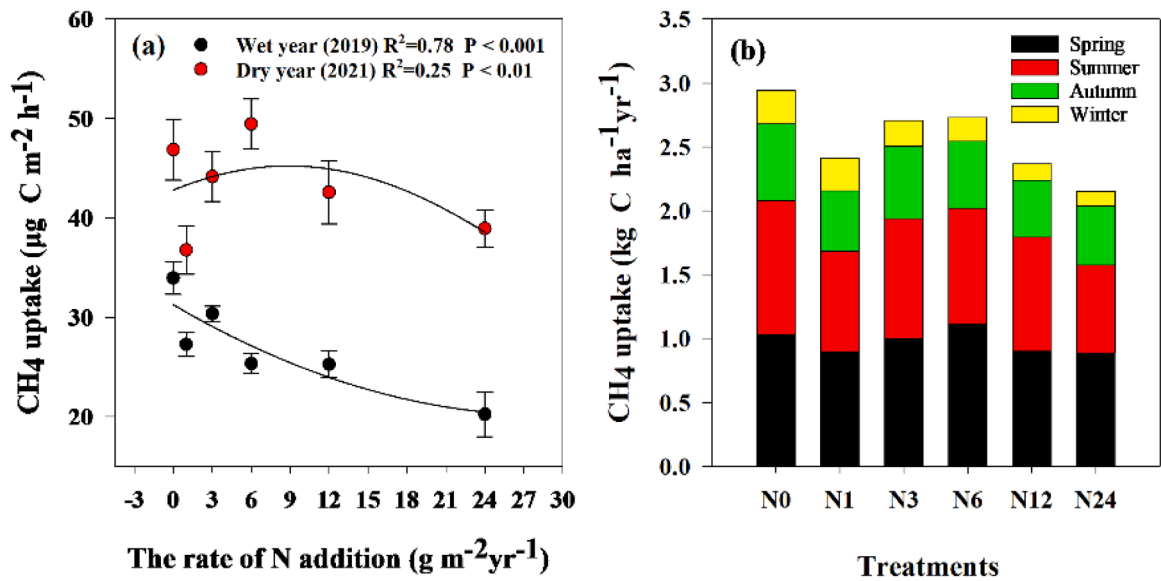


Fig. 3. The effect of N input on methane uptake in wet year and dry year and seasonal of methane uptake.

Table 2
Effect of sampling date (Time) and nitrogen (N) input on methane uptake was analyzed by repeated measures ANOVA. * and *** represent levels of significance at $p < 0.05$ and $p < 0.001$, respectively.

Repeated measures ANOVA	wet year			dry year		
	df	F	P	df	F	P
Time	3.617	9.293	0.000***	3.377	3.139	0.032*
N	5	5.045	0.010*	5	2.447	0.100
N × Time	18.087	1.230	0.281	16.887	1.025	0.455

package (SyStat Software Inc., San Jose, CA).

3. Results

3.1. Impact of N input on soil properties and functional genes

No significant influence on soil total carbon or N content was observed in a wet and a dry year by N_{ip} (Table 1). Low and medium N_{ip} (< 6 g N m⁻² yr⁻¹) had no significant impact on soil available N content in a wet year (NH₄⁺-N and NO₃⁻-N), while this was significantly increased by high N_{ip} (12 and 24 g N m⁻²; Table 1). And soil NO₃⁻-N content in a dry year was higher than that in a wet year (Table 1). A tendency, although non-significant, plant aboveground net productivity (ANPP) was enhanced by N_{ip}, except N₁₂ plots in a wet year and N₃ and N₂₄ plots in a dry year (Table 1). The abundance of *pmoA* gene (methane oxidizing bacteria) was significantly higher (by more than 6-fold) than that of the *mcrA* gene of methanogens in all treatments (Fig. 1). There was not significantly influence on the abundances of either *pmoA*, *mcrA* genes or the ratio of *pmoA* to *mcrA* genes by N_{ip}, except for a significant increase was observed at the highest N_{ip} (Fig. 1). And the abundances of *pmoA* gene and the ratio of *pmoA* to *mcrA* genes was much higher in a wet year (2019) than that in a dry year (2021, Fig. 1).

3.2. Impact of N input on methane uptake

The methane flux showed a net uptake with the obvious seasonal changes in the wet or dry year (Fig. 2). And the methane uptake was much lower in a wet year than that in a dry year (Fig. 3). Nitrogen input tended to reduce methane uptake, although this was not significant, except for in the N₂₄ plots (Fig. 2). In contrast, there was occasional increased in methane uptake by N_{ip}, and being more pronounced in a dry

year (2021) than that in a wet year (2019, Fig. 2). And the response of methane uptake was inconsistent to N_{ip} in a wet and a dry year (Fig. 3). The methane uptake rate in a wet year was decreased significantly with the increase of N_{ip} rate (Fig. 3). Compared with a dry year, methane uptake was increased firstly and then decreased with the increase rate of N_{ip} (Fig. 3), which resulted in that N_{ip} did not significantly affect methane uptake (Table 2).

3.3. Annual methane uptake and its drivers

This desert steppe can remove atmosphere methane at a native high uptake rate of 3.88 kg CH₄ ha⁻¹ yr⁻¹ (Fig. 3b). And N_{ip} tended to reduce annual methane uptake rate, especially at high N_{ip} (Fig. 3b). And found that this inhibitory effect of N_{ip} on methane uptake was significantly enhanced with the increase in soil moisture, especially in a wet year (Fig. 4). And found that the response of methane uptake in this desert steppe on N_{ip} was significantly related to soil water-filled pore space (WFPS) in a wet or dry year, the trend of change was exactly the same (Fig. 3 and 4). In addition, the *pmoA* gene abundance of methane-oxidizing bacteria, *mcrA* gene and soil inorganic N (NH₄⁺-N or NO₃⁻-N) also significantly affected methane uptake (Figure S3). The results of structural equation model (SEM) showed that methane uptake were the directly affected by the abundance ratio of *pmoA* to *mcrA* gene rather than soil NH₄⁺-N or NO₃⁻-N content (Fig. 5). And soil moisture had a key important indirect effect on methane uptake, mainly by affecting the abundance ratio of *pmoA* to *mcrA* gene (Fig. 5).

4. Discussion

4.1. The effect of nitrogen input on methane uptake

Methane flux in this desert steppe showed that there was a net uptake in a wet or dry year (Fig. 2), which was consistent with previous studies (Yue et al., 2019; 2022). This may be mainly due to the absolute dominance of methane-oxidizing bacteria (*pmoA* gene abundance) rather than methanogens (*mcrA* gene abundance) in the study area, which was >6 times that of methanogens (Fig. 1). It was well known that desert steppes were mainly limited by soil moisture, while an interesting finding was that methane uptake was much lower in a wet year (2019) than one in a dry year (2021, Fig. 3), which may be mainly mediated by WFPS (Fig. 4, Chen et al., 2011; Yue et al., 2022). In a wet year, higher WFPS reduced the diffusion rate of oxygen and methane in the soil,

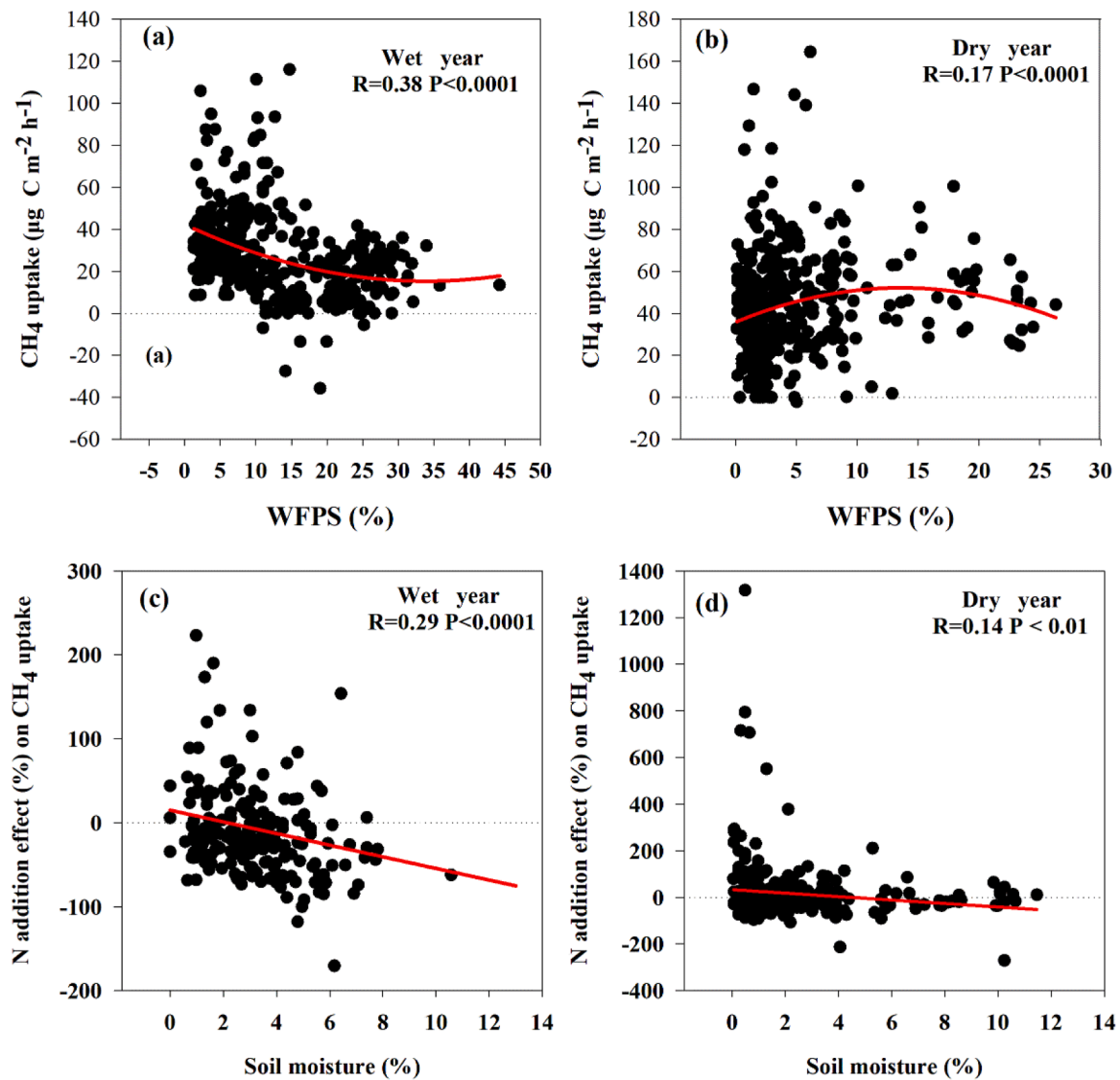


Fig. 4. Relationship between methane (CH_4) uptake and soil water-filled pore space (WFPS) in a wet year (a) and dry year (b). The effect of nitrogen (N) input on methane uptake with the variation in soil moisture. The positive value indicated enhancing effect, while the negative value indicated inhibiting effect in Figure c and d.

resulting in less methane being oxidized, while in a dry year, the relatively low WFPS reduced these negative effects, which in turn promoted methane uptake (Yue et al., 2022; Liu et al., 2019). In addition, it should be pointed out that we have found in the study area that too low soil WFPS could also significantly inhibit methane uptake, mainly due to inhibiting the activity of methane-oxidizing bacteria (Yue et al., 2022).

The methane uptake was not significantly affected by throughout the observation period N_{ip} , except at the highest rate (Table 1), support previous observation in a temperate degraded steppe (Chen et al., 2013). In contrast, this did not support the result a temperate desert soil (Yue et al., 2019) and a idea of meta-analyses suggested that low and medium N_{ip} promoted methane uptake in soil with good aeration conditions (Peng et al., 2019). This was also in contrast to the observation of Fang et al. (2014) in a meadow steppe of the Qinghai-Tibet Plateau where low N_{ip} inhibited methane uptake. This insignificant effect of N_{ip} in the study may be explained by (1) soil N loss was greater in a desert steppe than in other ecosystems due to drought, resulting in an insignificant increase in soil $\text{NH}_4^+\text{-N}$ or $\text{NO}_3^-\text{-N}$ content under low and medium N_{ip} (Table 1, Cui et al., 2017; Ullah et al., 2020); (2) Methane uptake was mainly mediated in our study area through soil WFPS (Fig. 4, Yue et al., 2022); (3) the effect of N_{ip} on methane uptake was variable (Fig. 2 and 4). As

observed, in periods of no precipitation, low N_{ip} tended to promoted methane uptake, espically in a dry year (Fig. 2c, d). In contrast, following precipitation, N_{ip} tended to inhibit methane uptake (Fig. 2c, d). This support the opinion of previous studies (Peng et al., 2019; Yue et al., 2019; 2022).

A significant inhibiting effect of N_{24} plots on methane uptake was observed (Fig. 2). Indeed, soil $\text{NH}_4^+\text{-N}$ content in the only N_{24} plots was significantly increased (Table 1). This may further support previous findings that the competitive relationship both methane and soil excess $\text{NH}_4^+\text{-N}$ (Schnell and King, 1994; King and Schnell, 1994). In contrast, enhancing methane uptake was occasionally facilitated by low N_{ip} (Fig. 2c, d), because low N_{ip} meets the requirement of methane oxidizing bacteria for N, thereby increasing the abundance of methane oxidizing bacteria, further enhancing methane uptake (Peng et al., 2019). And this inhibiting effect of N_{ip} on methane uptake was more pronounced in a wet year (2019, Fig. 2), supporting the existing results that the impact of N_{ip} on methane uptake was mediated though precipitation (Yue et al., 2019). As in a wet year, the methane uptake rate was decreased significantly with the increase in N_{ip} rate (Fig. 4), which was consistent with the result of previous studies (Cui et al., 2017; Yue et al., 2022). This may be mainly due to the increase in precipitation, reduced the loss

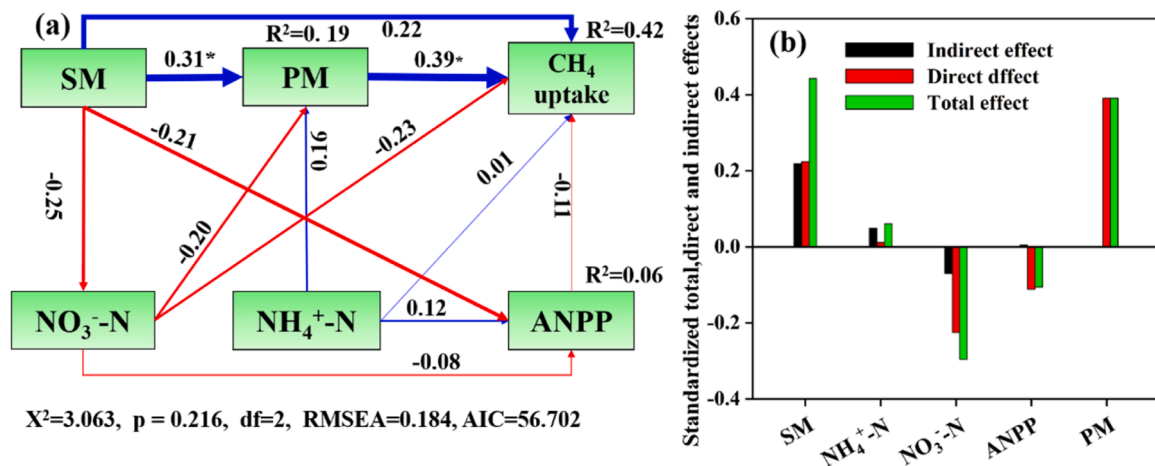


Fig. 5. The direct and indirect effects of soil moisture (SM), soil inorganic N (NH₄⁺-N and NO₃⁻-N) contents, above-ground primary productivity of plants (ANPP) and the abundance ratio of the *pmoA* to *mcrA* gene (PM) on methane (CH₄) uptake as analyzed using structural equation modeling. Blue arrows indicated enhancing CH₄ uptake and red arrows indicated an inhibition of CH₄ uptake. The thickness of the line represents the strength of the relationship between the two variables, and the value above the line represents the standardized path coefficient. Fitness parameters for model simulation were given at the bottom of the figure; * and *** represent levels of significance at $p < 0.05$ and $p < 0.001$, respectively.

of soil available N (NH₄⁺-N or NO₃⁻-N) and enhanced the activity of soil methane-oxidizing bacteria, which is consistent with the result of previous studies (Cui et al., 2017; Yue et al., 2022). In contrast, this inhibitory effect of N_{ip} on methane uptake was significantly weakened in a dry year (2021), which may be mainly due to the fact that the region was alkaline soil and drought exacerbated other loss paths such as soil ammonia volatilization and inhibited the activity of soil methane-oxidizing bacteria (Cui et al., 2017; Homyak et al., 2017; Yue et al., 2022).

4.2. Annual methane uptake and its controlling factors

Desert soils contribute significantly to mitigating climate change by removing methane from the atmosphere (Striegl et al., 1992; Yue et al., 2022). Our observation showed that methane uptake in this desert steppe was a relative high rate of 3.88 kg CH₄ ha⁻¹ yr⁻¹ (Fig. 2). This support previous observation in this study area (Yue et al., 2022), which mainly attribute to the soil sufficient O₂ content and a high *pmoA* / *mcrA* genes ratio (Fig. 1). The seasonal variation in methane uptake was observed, being lower in winter and higher in summer (Fig. 2), which also concurs with previous observation in the study area and other arid areas (Yue et al., 2019; 2022). The lower methane uptake in winter because of the soil freezing at low temperatures, greatly reducing the function of soil microorganisms. In contrast, higher soil temperature in the summer and higher precipitation than other seasons would promote methane oxidation functional microbial activities (Fig. 2).

The inhibiting effect of N_{ip} on methane uptake was much stronger with the increase in soil moisture in a wet year than that in a dry year (Fig. 4). This was probably mainly due to the fact that the wet year was better for retaining soil N than that in a dry year (Cui et al., 2017). In addition, the mediated pattern of soil WFPS on methane uptake in a wet and a dry year was also inconsistent (Fig. 4): In the wet year, soil WFPS tended to inhibit methane uptake, while in the dry year, it tended to increase first and then inhibit (Fig. 4a and b). This may be closely related to the inhibitory effect of N input on methane uptake, because more N remains in soil in wet years than in dry years (Cui et al., 2017).

The significantly relationship of methane uptake and the abundant of *pmoA*, *mcrA* genes, NH₄⁺-N, NO₃⁻-N or soil moisture were observed (Figure S3), supporting previous research (Yue et al., 2022). And found that the abundance of *pmoA* gene and *pmoA* / *mcrA* ratio corresponded well with the methane uptake rate (Figure S3). This further confirms that the methane uptake was a result of the trade-off between

methanotrophic bacteria and methanogens (Li et al., 2021). And our data also support this view, suggesting that *pmoA* / *mcrA* ratio was a critical directly control factor for methane uptake (Fig. 5), again, supported the finding of previous studies (Peng et al., 2019). Soil moisture not only directly promoted methane uptake, but also showed a key indirect effect, most likely through affecting the abundance ratio of *pmoA* and *mcrA* genes (Fig. 5). This may be attributed to (1) soil moisture regulated the content of oxygen in soil mainly by affecting the soil WFPS (Yue et al., 2022), and (2) soil moisture also affected the activity of methane-oxidizing bacteria and methanogens (Peng et al., 2019).

5. Conclusion

This Desert steppe can continue to consume large amounts of atmospheric methane (3.88 kg CH₄ ha⁻¹ yr⁻¹) even under conditions of increasing N_{ip} in a wet year or dry year. And found that the CH₄ uptake was higher in a dry year than that in a wet year, which was mainly mediated by soil WFPS. Methane uptake was significantly decreased with the increase in N_{ip} rate in a wet year, while N_{ip} did not significantly affect methane uptake in a dry year. It was found that the response of methane uptake to N_{ip} in wet and dry years was completely dependent on the change of soil WFPS. As N_{ip} also occasionally enhanced methane uptake, and this was more pronounced in a dry compared with wet year. And found that the inhibiting effect of N_{ip} on methane uptake was significantly enhanced with the increase in soil moisture in a wet year. The methane uptake directly depended on changes in the *pmoA* / *mcrA* gene ratio rather than soil N content. And soil moisture have an important indirectly effect on methane uptake by regulating the *pmoA* / *mcrA* ratio. Overall, the response of methane uptake was inconsistent to N_{ip} in a wet and a dry year, which may depend on changes in precipitation. Therefore, precise control experiments of precipitation gradient are recommended to further prove the regulatory role of precipitation change on N_{ip} effect on methane uptake in future.

CRedit authorship contribution statement

Ping Yue: Writing – review & editing, Writing – original draft, Conceptualization. **Kaihui Li:** Data curation. **Ya Hu:** Data curation. **Jingjuan Qiao:** Data curation. **Zhaobin Song:** Software. **Shaokun Wang:** Data curation, Conceptualization. **Tom Misselbrook:** Writing – review & editing. **Xiaolan Zuo:** Funding acquisition, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.agrformet.2024.110327](https://doi.org/10.1016/j.agrformet.2024.110327).

Data availability

Data will be made available on request.

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