



Annual methane uptake of an artificial grassland under different grazing strategies

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Abstract Increased grazing has led to the degradation of natural grasslands, which can be mitigated by implementing artificial grasslands. Continuous and rotational grazing are the most important and widely used grazing management strategies. However, our understanding of how diverse grazing management strategies affect methane (CH₄) flux from artificial grasslands remains limited. Using a static opaque chamber and gas chromatography technique, methane fluxes and environmental factors from three grazing strategies (continuous grazing (CG), rotational grazing (RG), and ungrazed (UG)) were compared over a three-year period in an artificial grassland in a temperate semi-arid area in northern China. Our results showed that artificial grasslands are a net sink for atmospheric CH₄. The sink strength was 2.0, 1.4, and

1.6 kg C ha⁻¹ yr⁻¹ for UG, CG, and RG, respectively. Grazing reduced CH₄ uptake by 28–42% for CG and 18–32% for RG, compared to UG. However, there was no significant difference in CH₄ uptake between CG and RG. CH₄ uptake during the non-growing season accounted for 32–34% of the annual CH₄ uptake, a significant proportion of the annual total. CH₄ uptake increased with soil temperature for CG, RG, and UG and was significantly correlated with water-filled pore space for CG and UG over the three-year period. Variations in soil ammonium and nitrate levels exhibited a slight influence on CH₄ flux for CG and RG. Our study provides long-term observations of grazing strategies affecting CH₄ uptake in grasslands, facilitating evaluation of the effects on grassland CH₄ uptake from rotational grazing and continuous grazing.

Shuai Li and Peng Chen contributed equally to this work.

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Introduction

An increase in the concentration of greenhouse gases in the atmosphere is one of the main causes of global warming. Besides carbon dioxide (CO₂), methane (CH₄) is the most important greenhouse gas, with a global warming potential 28 times that of CO₂ over a 100-year period and an atmospheric residence time of 9.1 years (IPCC, 2013). The concentration of atmospheric CH₄ has increased from 722 to 1803 ppb since pre-industrial times (IPCC, 2013). The predominant CH₄ sink is the reaction of CH₄ with hydroxyl radicals in the troposphere, which is the second largest global sink for atmospheric CH₄, estimated at 28–32 Tg yr⁻¹ (IPCC, 2013).

The consumption of atmospheric CH₄ by soils is mainly controlled by the activity of methanotrophic bacteria, which can be strongly affected by soil disturbance, such as what occurs in agricultural and animal husbandry practices (Le Mer and Roger 2001; Prem et al. 2014). Grazing can greatly alter CH₄ consumption by changing soil chemical and physical properties as well as microbial activities. Currently, research into the effect of grazing on CH₄ uptake has mainly focused on grazing intensity or seasonality (Chen et al. 2011; Liu et al. 2007, 2017). Continuous grazing and rotational grazing are the two most important and widely used grazing management strategies. Rotational grazing decreases soil bulk density, increases soil organic carbon, water infiltration, and water holding capacity and improves nutrient availability and forage production (Byrnes et al. 2018; Döbert et al. 2021; McDonald et al. 2019; Teague et al. 2011). The improvement of grassland soil health by rotational grazing is likely to increase the number and activity of methane-oxidizing bacteria in the soil (Li et al. 2020), thus affecting the CH₄ uptake rate of grasslands. However, whether rotational grazing enhances the CH₄ uptake in grasslands compared to continuous grazing remains uncertain (Baronti et al. 2022; Dowhower et al. 2020; Shrestha et al. 2020).

China's grassland area accounts for 6–8% of the world's grassland area, and China's northern semi-arid temperate grassland accounts for 78% of the

country's grassland area (Liu et al. 2017; Tang et al. 2019). Overgrazing and cultivation owing to population growth and food demand are the major causes of natural grassland degradation and desertification in these areas (Abril and Bucher 2001; Li and Chen 2021; Wei et al. 2012). Currently, establishing artificial grasslands on previous natural grasslands is a widely used restoration approach (Schwenke et al. 2013; Wang et al. 2013a). A few field studies have addressed the effects of grazing management on CH₄ fluxes in the natural grasslands of northern China (Chen et al. 2010, 2011; Liu et al. 2007). However, studies on the effects of grazing management on CH₄ flux from artificial grasslands are scarce.

Current estimates of annual CH₄ uptake in temperate grassland ecosystems are still highly uncertain, because most measurements correspond to the growing season (Holst et al. 2008; Liu et al. 2007; Wei et al. 2012). However, CH₄ oxidation still occurs even when the soil temperature is below the freezing point (Chen et al. 2010, 2011; Mosier et al. 1996; Wang et al. 2005). A few field measurements in natural grasslands have indicated that CH₄ uptake in the non-growing season account for 22–59% of annual CH₄ uptake (Chen et al. 2010, 2011; Rong et al. 2015). Thus, CH₄ uptake during the non-growing season cannot be ignored in the annual budget. Given that CH₄ fluxes in grasslands have been measured primarily during the growing season, a gap in knowledge exists around the contribution of non-growing seasonal fluxes to the annual budget of artificial grasslands. Therefore, the long-term effects of different grazing management strategies on soil CH₄ consumption in artificial grasslands require further investigation.

In this study, we conducted field observations of CH₄ flux for three years (from July 2012 to July 2015) in an artificial grassland with different grazing management strategies (continuous grazing (CG), rotational grazing (RG), and ungrazed (UG)) in the temperate semi-arid region of northern China. The effects of soil temperature, moisture, ammonium, and nitrate on CH₄ flux were also investigated. Our hypotheses were as follows: (1) rotational grazing may enhance CH₄ uptake by grasslands compared to continuous grazing and (2) CH₄ consumption in the non-growing season may significantly contribute to the annual uptake. The results of this study will improve our understanding of atmospheric CH₄ uptake levels in

artificial grasslands and help to evaluate the effects of different grazing management strategies on CH₄ consumption.

Materials and methods

Site description

The experimental site (40° 34' N, 111° 34' E, 1055 m above sea level) was located in Shaerqin town, Tumd Left Banner, Huhhot, Inner Mongolia, Northern China, which belongs to the agro-pastoral ecotone experimental demonstration base of the Institute of Grassland Research of the Chinese Academy of Agricultural Sciences. It has a typical semi-arid and temperate continental climate. The annual mean air temperature ranges from 5.8 to 6.3 °C. Approximately 130 days of the year are frost-free. The mean annual precipitation is 350–450 mm. The properties of the top 300 mm of the soil profile were pH=8.5, salt content=0.03%, organic matter=0.8%, and total N=0.035%. The region soil is Castanozems, according to the Food and Agriculture Organization of the United Nations (FAO) nomenclature. The artificial grassland was built and fenced in 2008 and was sown with a mixture of *Medicago sativa*, *Lespedeza floribunda*, *Leymus chinensis*, *Elmus dahuricus Turcz*, and *Bromus inermis Leyss* in 2009. Sheep grazing began in 2010.

Experimental design

The treatments applied to the artificial grasslands were continuous grazing (CG), rotational grazing (RG), and ungrazed (UG). There were 15 rotational grazing plots, 3 continuous grazing plots, and

3 ungrazed plots in total. The area of each plot was approximately 0.67 ha. We randomly chose 3 of the 15 rotational grazing plots as our sampling plots. Therefore, each treatment had three replicate plots for gas sampling.

Sheep grazed from June to October in 2010, and the sheep stayed in the winter sheepfold the rest of the year. The grazing treatments were set by the Institute of Grassland Research of the Chinese Academy of Agricultural Sciences, as they represent the traditionally used grazing practices in this region. During the grazing period, 30 sheep were rotationally grazed in five RG plots, with one plot for six days every month for five months. Therefore, there were three groups of rotational grazing in the 15 RG plots. Six sheep were continuously grazed in each CG plot for five months, whereas sheep were excluded from the UG plots during the three years (2012–2015). The grazing intensity was nine sheep per hectare per five grazing months per year in both the CG and RG plots. The management and soil characteristics of the artificial grasslands are summarized in Table 1.

CH₄ flux measurements

CH₄ fluxes were measured using a static opaque chamber and gas chromatography technique (Wang and Wang 2003). The sampling chamber was made of stainless steel (thickness=1 mm) consisting of two parts: a square-base frame (0.4 m×0.4 m, height 0.15 m) and a removable top lid (0.4 m×0.4 m, height 0.4 m) on which a gasket was placed to ensure air-tightness. The top lid was covered with a 30 mm thick layer of foam insulation to prevent heat exchange inside and outside the chamber. One side of the lid was fitted with a gas balance tube (120 mm polytetrafluoroethylene tube) to minimize pressure

Table 1 Soil characteristics and experimental treatment of artificial grassland

| Grazing treatment | Duration of grazing period (day/year) | Grazing rate (sheep/ha) | pH | SOC 0–200 mm (g/kg) | SAN 0–200 mm (g/kg) | SBD 0–100 mm (g/cm ³) |
|-------------------|---------------------------------------|-------------------------|-----------|---------------------|---------------------|-----------------------------------|
| RG | 30 | 9 | 8.34±0.07 | 7.16±0.52 | 0.07±0.003 | 1.49±0.03 |
| CG | 150 | 9 | 8.36±0.08 | 7.64±1.10 | 0.05±0.010 | 1.53±0.06 |
| UG | – | – | 8.55±0.01 | 7.40±1.06 | 0.07±0.008 | 1.47±0.05 |

Values are mean ± S.E. (standard error)

SOC: soil organic carbon; SAN: soil available nitrogen; SBD: soil bulk density. UG: ungrazed; CG: continuous grazing; RG: rotational grazing

perturbations when collecting gas samples, a digital display thermometer to measure the temperature in the chamber, and an interface to collect gas samples. The base frames were installed in three CG plots, three UG plots, and three RG plots. CH₄ fluxes were measured once a week during the growing season from May to October, twice a week during the spring thaw period from March to April, and twice a month during the frozen period from November to February. During each sampling time, chambers were closed for one hour, and five gas samples (60 mL per gas sample) were collected from the closed chambers at 15-min intervals using air-tight plastic syringes. The CH₄ concentrations of the gas samples stored in syringes were measured within 24 h after sampling with a flame ionization detector (FID) in a gas chromatograph (Agilent 7890A, Santa Clara, CA, USA). If the gas samples were not measured within 24 h, they were stored in gas bottles. The CH₄ flux was calculated jointly from the linear equation (Eqs. (1) and (2)) or non-linear (Eqs. (1) and (3)) changes in gas concentrations (Kroon et al. 2008; Liu et al. 2010, 2017; Wang et al. 2013b). Daily CH₄ fluxes were estimated using linear interpolation for non-sampling days. Seasonal and annual cumulative CH₄ fluxes were calculated from measured and interpolated values (Chen et al. 2011).

$$F_{\text{CH}_4} = dc/dt \times \rho \times H \quad (1)$$

$$C = a \times t + b(dc/dt = a) \quad (2)$$

$$C = k_1/k_2 + (C_0 - k_1/k_2) \times \exp(-k_2 \times t) \\ (dc/dt|_{t=0} = k_1 - k_2 \times C_0) \quad (3)$$

where F_{CH_4} is the hourly CH₄ flux ($\mu\text{g C m}^{-2} \text{ h}^{-1}$) (negative values indicate net CH₄ uptake by soils, whereas positive values indicate net CH₄ emission); ρ is the CH₄ gas density ($\mu\text{g m}^{-3}$); dc/dt is the rate of change in gas concentration inside the chamber (h^{-1}); H is the height of the chamber (m); C is the measured CH₄ concentration; C_0 is the concentration at the beginning of the enclosure; and a , b , k_1 , and k_2 are parameters derived by fitting linear or non-linear curves.

Auxiliary measurements

Soil temperature and moisture were measured near the chamber when the gas samples were collected. Soil temperature (50 mm depth) was measured using digital display thermocouples (JM624, Liwen Electronics LTD, Tianjin, China). The soil volumetric water content (0–60 mm) was measured using a portable moisture probe meter (MPKit, Ruidisheng Science and Technology LTD, Nanjing, China). The gravimetric water content of the soil was determined when the soil was frozen. Subsequently, both the volumetric water content and gravimetric water content were converted into water-filled pore space (WFPS, %) (Zhang and Han 2008).

volumetric water content = gravimetric water content \times soil bulk density

$$\text{WFPS}(\%) = \frac{\text{volumetric water content}(\%)}{1 - \text{soil bulk density}/2.65}$$

Soil samples at a depth of 0–150 mm were collected during the gas-sampling process and were taken back to the laboratory to measure NH₄⁺ and NO₃⁻ contents (1 mol/L KCL extraction), which were measured using a fully automated intermittent chemical analyzer (Smartchem140, AMS, Italy). Soil bulk density, organic carbon, and available nitrogen were measured once, and the soil properties are summarized in Table 1.

Statistical analyses

All statistical analyses were performed using SPSS 24.0 (IBM Corporation, Chicago, USA), and figures were drawn with Origin Pro 8 (Origin Lab Corporation, Northampton, MA, USA). MIXED models were used to analyze the differences in CH₄ fluxes between treatments and years. The grazing treatment was treated as a fixed effect, whereas grazing treatment, soil temperature, soil moisture, and their interactions were treated as random effects. The year was also treated as a fixed effect. Year, soil temperature, soil moisture, and their interactions were treated as random effects. The model was fitted using a restricted maximum likelihood procedure. The data were examined for normal distribution before analysis. Linear or non-linear regressions were used to analyze the relationship between CH₄ flux and environmental parameters.

Results

Environmental factors

Over the three-year period, the local annual mean air temperatures were 7.1, 8.6, and 8.3 °C for 2012–2013, 2013–2014, and 2014–2015, respectively. The precipitation in 2012–2013 (580.7 mm) was higher than the regional precipitation average of 400 mm;

however, the precipitation in 2013–2014 (476 mm) and 2014–2015 (417.5 mm) was similar to the regional average value. The precipitation was mainly concentrated during the growing season.

Over the course of this study, soils under the UG treatment were consistently colder than those under the CG and RG treatments (Fig. 1a, Table 2). Mean soil temperature over the three years was 12.7, 11.6, and 9.2 °C in the CG, RG, and UG plots,

Fig. 1 Seasonal variations of **a** soil temperature (50 mm depth, °C) and **b** soil moisture (0–60 mm, WFPS%) in the artificial grasslands. Values are presented as mean ± standard error (SE). CG: continuous grazing; RG: rotational grazing; UG: ungrazed

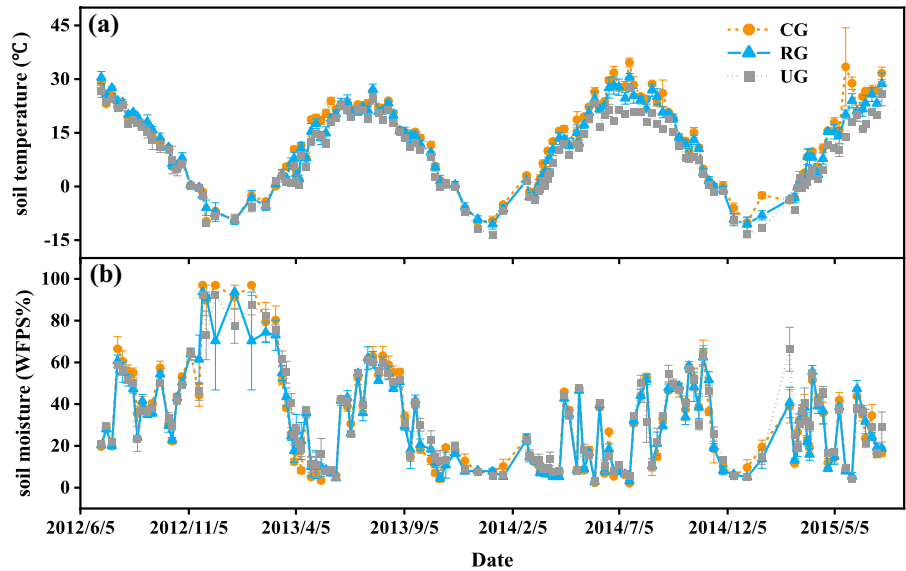


Table 2 Annual mean soil temperature, moisture, ammonium, and nitrate content in the artificial grasslands

| Grazing treatment | Soil temperature (50 mm, °C) | Soil moisture (0–60 mm, WFPS%) | NH ₄ ⁺ (mg N kg ⁻¹ dry soil) | NO ₃ ⁻ (mg N kg ⁻¹ dry soil) |
|----------------------|------------------------------|--------------------------------|---|---|
| <i>2012/7–2013/7</i> | | | | |
| UG | 9.8 ± 0.6bA | 42.6 ± 0.8aA | 4.9 ± 0.7aA | 7.5 ± 1.6aA |
| CG | 11.6 ± 0.3aB | 42.2 ± 1.2aA | 4.9 ± 0.7aA | 5.5 ± 0.5aA |
| RG | 11.3 ± 0.5abA | 41.2 ± 0.8aA | 7.3 ± 1.7aA | 12.7 ± 1.7bA |
| <i>2013/7–2014/7</i> | | | | |
| UG | 8.9 ± 0.2bA | 23.9 ± 1.0aC | 2.9 ± 0.3aB | 6.8 ± 1.6aA |
| CG | 12.5 ± 0.2aB | 23.3 ± 0.8aC | 3.6 ± 0.6aAB | 5.1 ± 0.5aA |
| RG | 11.3 ± 0.2aA | 22.2 ± 0.6aC | 3.4 ± 0.3aB | 13.7 ± 3.3bA |
| <i>2014/7–2015/7</i> | | | | |
| UG | 9.0 ± 0.2bA | 31.3 ± 1.2aB | 2.9 ± 0.3aB | 8.8 ± 1.0aA |
| CG | 14.1 ± 0.4aA | 29.4 ± 0.7aB | 3.2 ± 0.3aB | 9.8 ± 1.2abB |
| RG | 12.2 ± 1.0aA | 29.4 ± 0.7aB | 3.8 ± 0.5aB | 13.3 ± 1.6bA |
| <i>2012/7–2015/7</i> | | | | |
| UG | 9.2 ± 0.2b | 32.6 ± 0.9a | 3.5 ± 0.3a | 7.7 ± 0.8a |
| CG | 12.7 ± 0.3a | 31.6 ± 0.9a | 3.9 ± 0.3a | 6.9 ± 0.5a |
| RG | 11.6 ± 0.8a | 30.9 ± 0.6a | 4.8 ± 0.6a | 13.2 ± 1.3b |

Values are mean ± S.E. (standard error). A, B, and C represent significant differences between years (*P* < 0.05). a, b, and c represent significant differences between treatments (CG, RG, and UG) (*P* < 0.05). UG: ungrazed; CG: continuous grazing; RG: rotational grazing

respectively. At the annual scale, soil temperatures within treatments were similar during the study period, except for the CG treatment (Table 2). At the seasonal scale, soil temperature for all treatments was consistently higher during the growing season than during the frozen and freeze–thaw periods (Fig. 1a). Over the study period, the soil WFPS was similar among all three grazing treatments (Fig. 1b). The annual mean soil WFPS in the first year was the highest for all three treatments over the three-year period ($P < 0.05$, Table 2). During these three years, the mean soil WFPS was 31.6%, 30.9%, and 32.6%, ranging from 2.1 to 97.0%, 2.8 to 93.7%, and 3.1 to 92.1% for the CG, RG, and UG plots, respectively (Table 2, Fig. 1b).

Over the three-year period, the soil ammonium content of the three grazing treatments was similar, with higher values in the first year than in the other two years (Fig. 2a, Table 2). The three-year mean ammonium content was 3.9 ± 0.3 , 4.8 ± 0.6 , and 3.5 ± 0.3 mg N kg⁻¹ dry soil in the CG, RG and UG plots, respectively (Fig. 2a, Table 2). The soil nitrate content was always higher in RG than in CG and UG over the three-year period (Fig. 2b, Table 2). The three-year average nitrate content was 6.9 ± 0.5 , 13.2 ± 1.3 , and 7.7 ± 0.8 mg N kg⁻¹ dry soil in the CG, RG, and UG plots, respectively (Fig. 2b, Table 2).

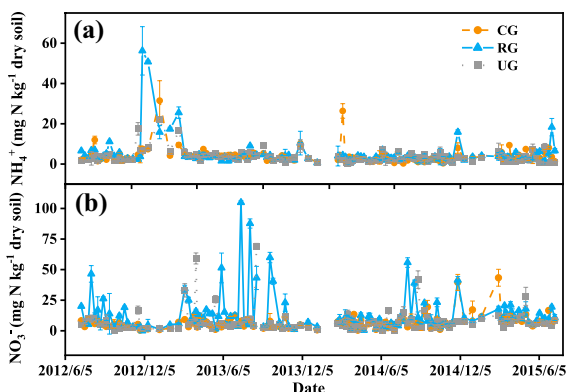


Fig. 2 Seasonal variations of **a** NH_4^+ and **b** NO_3^- (0–150 mm) in the artificial grasslands. Values are presented as mean \pm standard error (SE). CG: continuous grazing; RG: rotational grazing; UG: ungrazed

Effects of grazing strategies on CH_4 fluxes

The three-year mean CH_4 fluxes in UG were significantly lower than those in CG and RG ($P < 0.05$, Table 3), indicating that the CH_4 uptake rate in UG was higher than that in CG and RG. No differences in the net CH_4 uptake rate were observed between the CG and RG treatments ($P > 0.05$, Table 3). Soils in all treatments were net sinks of atmospheric CH_4 (Fig. 3, Table 3). The three-year mean CH_4 fluxes were -25.2 ± 0.9 , -17.7 ± 1.1 , and -18.5 ± 1.5 $\mu\text{g C m}^{-2} \text{h}^{-1}$ for the UG, CG, and RG plots, respectively (Table 3).

Seasonal and interannual variations of CH_4 fluxes

CH_4 fluxes in all treatments revealed distinct patterns of seasonal changes over the entire study period of 2012–2015, with high uptake rates during the growing season (from May to October) and low uptake rates during the frozen (from November to February of the following year) and freeze–thaw (from March to April) periods (Fig. 3, Table 3). At the annual scale, the CH_4 uptake rates within treatments differed for UG and RG during the study period, as opposed to the CG treatment (Table 3). The annual mean CH_4 uptake rate was highest in 2012–2013 and lowest in 2014–2015. However, interannual variations were not high and varied at best by 52% (e.g., at UG plot: 2012–2013, -29.1 ± 1.5 $\mu\text{g C m}^{-2} \text{h}^{-1}$; 2014–2015, -19.2 ± 2.1 $\mu\text{g C m}^{-2} \text{h}^{-1}$) (Table 3).

Seasonal and annual CH_4 uptake

The mean annual CH_4 uptake within the 2012–2015 period was 1.43 ± 0.07 , 1.56 ± 0.12 , and 1.98 ± 0.23 kg C ha⁻¹ yr⁻¹ for the CG, RG, and UG plots, respectively (Table 3). The growing-season CH_4 uptake dominated the total annual CH_4 uptake, accounting for 62%–74% of annual CH_4 uptake across all treatments (Table 3). Over the three-year period, the CH_4 uptake in the non-growing season accounted for 34%, 32%, and 33% of the annual CH_4 uptake in the CG, RG, and UG plots, respectively (Table 3).

Effects of soil temperature, moisture, ammonium, and nitrate on CH_4 fluxes

For all grazing treatments, CH_4 fluxes decreased (i.e., CH_4 uptake rates increased), with increasing top-soil

Table 3 Seasonal and annual mean CH₄ fluxes and the cumulative of CH₄ flux depending on different grazing treatments in the artificial grasslands

| Period | Grazing treatment | GS | | FP | | FTP | | NGS contribution (%) | | |
|---------------|-------------------|---|--|---|--|---|--|----------------------|--------------|----|
| | | Annual CH ₄ flux µg C m ⁻² h ⁻¹ | Cumulative CH ₄ flux kg C ha ⁻¹ | Annual CH ₄ flux µg C m ⁻² h ⁻¹ | Cumulative CH ₄ flux kg C ha ⁻¹ | Annual CH ₄ flux µg C m ⁻² h ⁻¹ | Cumulative CH ₄ flux kg C ha ⁻¹ | | | |
| 2012/7–2013/7 | UG | -29.1 ± 1.5aA | -2.17 ± 0.11 | -36.2 ± 2.7aA | -1.56 ± 0.11 | -11.0 ± 1.7aA | -0.28 ± 0.08 | -25.4 ± 2.2aA | -0.34 ± 0.03 | 28 |
| | CG | -20.2 ± 3.0bA | -1.56 ± 0.23 | -24.3 ± 4.4bA | -1.02 ± 0.17 | -11.2 ± 5.4aA | -0.25 ± 0.12 | -17.1 ± 2.6abA | -0.30 ± 0.05 | 35 |
| | RG | -21.1 ± 2.2bA | -1.78 ± 0.19 | -27.0 ± 2.3bA | -1.31 ± 0.13 | -9.4 ± 2.3aA | -0.24 ± 0.05 | -14.0 ± 3.3bA | -0.22 ± 0.05 | 26 |
| 2013/7–2014/7 | UG | -27.3 ± 1.6aA | -2.25 ± 0.12 | -31.3 ± 2.4aAB | -1.40 ± 0.11 | -19.0 ± 2.4aA | -0.51 ± 0.06 | -24.0 ± 3.0aA | -0.35 ± 0.05 | 38 |
| | CG | -16.4 ± 1.4bA | -1.34 ± 0.10 | -20.1 ± 1.6bA | -0.90 ± 0.06 | -9.8 ± 2.9bA | -0.23 ± 0.06 | -13.4 ± 1.2bAB | -0.20 ± 0.02 | 33 |
| | RG | -18.6 ± 1.8bAB | -1.53 ± 0.15 | -21.4 ± 1.8bA | -0.98 ± 0.07 | -15.5 ± 2.5abA | -0.36 ± 0.05 | -14.3 ± 1.7bA | -0.19 ± 0.03 | 36 |
| 2014/7–2015/7 | UG | -19.2 ± 2.1aB | -1.53 ± 0.18 | -25.1 ± 2.9aB | -1.05 ± 0.16 | -11.9 ± 3.9aA | -0.26 ± 0.09 | -11.2 ± 1.7aB | -0.22 ± 0.08 | 31 |
| | CG | -16.5 ± 1.0aA | -1.38 ± 0.14 | -20.4 ± 1.5aA | -0.89 ± 0.05 | -13.3 ± 2.9aA | -0.29 ± 0.07 | -10.2 ± 1.0aB | -0.20 ± 0.09 | 35 |
| | RG | -15.6 ± 2.4aB | -1.36 ± 0.24 | -20.2 ± 3.9aA | -0.87 ± 0.16 | -13.4 ± 2.6aA | -0.30 ± 0.06 | -8.1 ± 0.3aA | -0.19 ± 0.06 | 36 |
| 2012/7–2015/7 | UG | -25.2 ± 0.9a | -1.98 ± 0.23 | -30.9 ± 0.8a | -1.34 ± 0.15 | -14.0 ± 1.8a | -0.35 ± 0.08 | -20.2 ± 1.4a | -0.30 ± 0.04 | 33 |
| | CG | -17.7 ± 1.1b | -1.43 ± 0.07 | -21.6 ± 1.5b | -0.94 ± 0.04 | -11.4 ± 1.8a | -0.26 ± 0.02 | -13.5 ± 0.7b | -0.23 ± 0.03 | 34 |
| | RG | -18.5 ± 1.5b | -1.56 ± 0.12 | -22.9 ± 1.6b | -1.05 ± 0.13 | -12.8 ± 2.0a | -0.30 ± 0.03 | -12.1 ± 1.5b | -0.20 ± 0.01 | 32 |

Values are presented as mean ± standard error (SE). A, B, and C represent significant differences between years ($P < 0.05$). a, b, and c represent significant differences between treatments (CG, RG, and UG) ($P < 0.05$). UG: ungrazed; CG: continuous grazing; RG: rotational grazing. GS: growing season (from May to October); FP: frozen period (from November to February of the following year); FTP: freeze–thaw period (from March to April); NGS: non-growing season (from November to April of the following year)

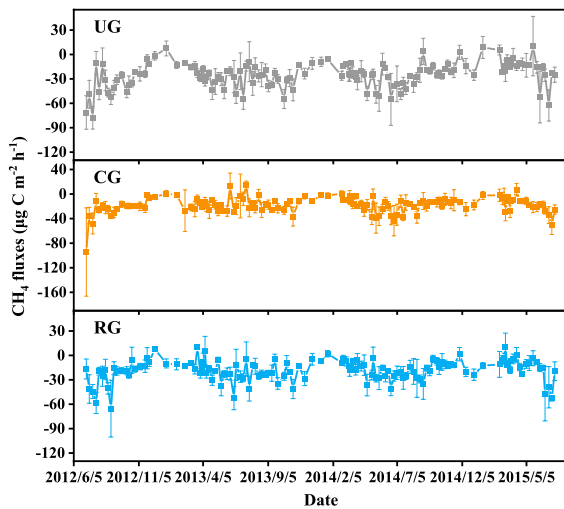


Fig. 3 Seasonal variations of CH_4 fluxes in the artificial grasslands. Values are presented as mean \pm standard error (SE). CG: continuous grazing; RG: rotational grazing; UG: ungrazed

(50 mm) temperature (correlation through the entire observed period, $R^2=0.20\text{--}0.27$, $P<0.01$; during the non-growing season, $R^2=0.07\text{--}0.24$, $P<0.01$ or 0.05 , Table 4, Fig. 4a, e). However, during the growing and freeze–thaw periods this correlation was not all significant (in the growing season, significant in CG and RG, $R^2=0.09$ and 0.13 , $P<0.01$; in the freeze–thaw period, significant in RG and UG, $R^2=0.29$ and 0.20 , $P<0.01$, Table 4, Fig. 4c, g). Soil temperature accounted for 20%, 27%, and 25% of the variation in CH_4 flux over the entire study period for the CG, RG, and UG treatments, respectively.

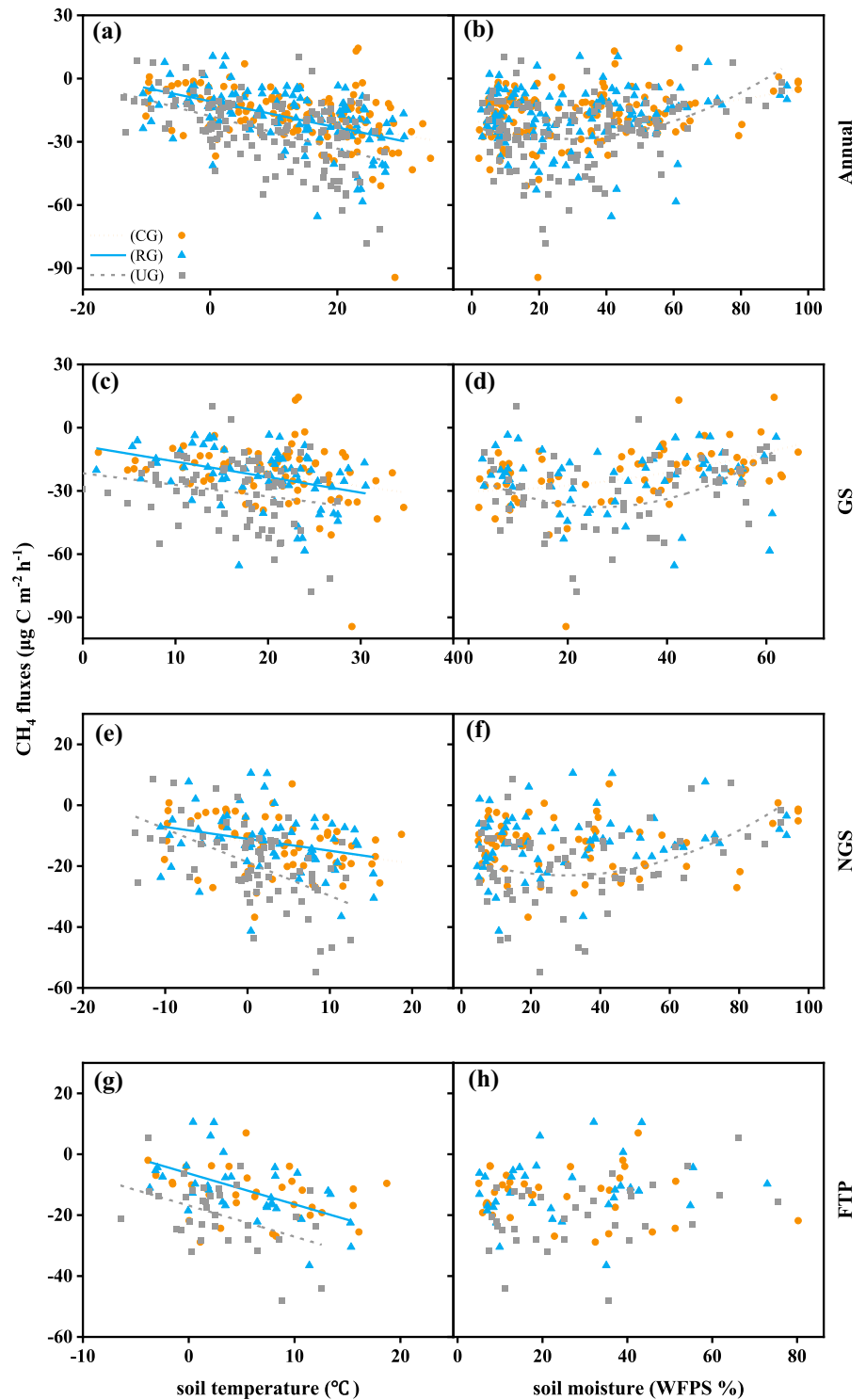
CH_4 fluxes were significantly correlated with soil WFPS for the CG and UG treatments during the entire observation period and growing season, described by binomial regression ($P<0.05$ or 0.01 , Table 4, Fig. 4b, d). Soil WFPS accounted for 6% and 14% of CH_4 fluxes for the CG and UG treatments, respectively, during the entire observation period and 16% and 17%, respectively, during the growing season. The optimum soil WFPS for CH_4 uptake

Table 4 Relationship between CH_4 fluxes with soil temperature (50 mm depth, $^{\circ}\text{C}$) and soil moisture (0–60 mm, WFPS%)

| Treatment | Period | | ST (50 mm, $^{\circ}\text{C}$) | SM (0–60 mm, WFPS%) |
|-----------|----------------------|-------|---------------------------------|------------------------------|
| CG | Annual (n = 131) | f(x) | $-11.31 - 0.51x$ | $-20.51 + 0.01x + 0.002x^2$ |
| | | R^2 | 0.20** | 0.06* |
| | GS (n = 75) (n = 75) | f(x) | $-9.3 - 0.62x$ | $-28.45 + 0.03x + 0.004x^2$ |
| | | R^2 | 0.09** | 0.16** |
| | NGS (n = 56) | f(x) | $-11.63 - 0.33x$ | $-9.88 - 0.24x + 0.003x^2$ |
| | | R^2 | 0.07* | 0.06 |
| | FTP (n = 34) | f(x) | $-10.84 - 0.39x$ | $-13.82 + 0.12x - 0.003x^2$ |
| | | R^2 | 0.08 | 0.03 |
| RG | Annual (n = 131) | f(x) | $-10.74 - 0.62x$ | $-16.39 - 0.22x + 0.004x^2$ |
| | | R^2 | 0.27** | 0.03 |
| | GS (n = 75) | f(x) | $-8.7 - 0.74x$ | $-20.48 - 0.30x + 0.005x^2$ |
| | | R^2 | 0.13** | 0.01 |
| | NGS (n = 56) | f(x) | $-10.90 - 0.42x$ | $-15.00 + 0.15x - 0.0008x^2$ |
| | | R^2 | 0.08* | 0.05 |
| | FTP (n = 34) | f(x) | $-6.31 - 1.01x$ | $-15.57 + 0.24x - 0.002x^2$ |
| | | R^2 | 0.29** | 0.04 |
| UG | Annual (n = 131) | f(x) | $-17.78 - 0.77x$ | $-22.64 - 0.44x + 0.008x^2$ |
| | | R^2 | 0.25** | 0.14** |
| | GS (n = 75) | f(x) | $-21.64 - 0.55x$ | $-22.31 - 1.14x + 0.02x^2$ |
| | | R^2 | 0.04 | 0.17** |
| | NGS (n = 56) | f(x) | $-17.52 - 0.91x$ | $-18.09 - 0.17x + 0.004x^2$ |
| | | R^2 | 0.24** | 0.15* |
| | FTP (n = 34) | f(x) | $-16.85 - 1.02x$ | $-24.31 + 0.06x + 0.002x^2$ |
| | | R^2 | 0.20** | 0.16 |

f(x): CH_4 flux; ST: soil temperature; SM: soil moisture. CG: continuous grazing; RG: rotational grazing; UG: ungrazed. GS: growing season (from May to October), NGS: non-growing season (from November to April of the following year), FTP: freeze–thaw period (from March to April). **: significance level of 0.01, *: significance level of 0.05, : low relevance

Fig. 4 Relationship between CH₄ fluxes with soil temperature (50 mm depth, °C) and soil moisture (0–60 mm, WFPS%) in the artificial grasslands. CG: continuous grazing; RG: rotational grazing; UG: ungrazed. GS: growing season (from May to October); FTP: freeze–thaw period (from March to April); NGS: non-growing season (from November to April of the following year)



was 27.5% in UG plots over the three years (Fig. 4b, Table 4). In the non-growing season, only the CH₄ flux of the UG treatment was significantly correlated with the soil WFPS, accounting for 15% of the CH₄ flux variation (Fig. 4f, Table 4).

Over the entire study period, CH₄ fluxes were positively correlated with soil ammonium content for the UG treatment ($P < 0.01$, Fig. 5a); that is, CH₄ uptake rates linearly decreased with soil ammonium content. For the RG and CG treatments, changes in CH₄ fluxes did not correlate with variations in soil ammonium content ($P > 0.05$, Fig. 5a). Regression analysis revealed no significant correlation between CH₄ fluxes and soil nitrate content for any of the three treatments ($P > 0.05$, Fig. 5b).

Discussion

Annual CH₄ uptake in artificial grassland

Regardless of grazing management, artificial grassland was a net CH₄ sink during the study period. The annual grassland CH₄ uptake for the three grazing treatments ranged from 1.43 to 1.98 kg C ha⁻¹ yr⁻¹ (Table 3), which was within the range of 0.57 to 2.12 kg C ha⁻¹ yr⁻¹, reported by Imer et al. (2013) in a managed grassland ecosystem in the Swiss Alps. The CH₄ uptake was also in the range of 1.02–2.72 kg C ha⁻¹ yr⁻¹, reported by Rong et al. (2015) in semi-arid natural grasslands in northern China. The mean annual CH₄ uptake in our artificial grasslands (1.66 kg C ha⁻¹ yr⁻¹) was slightly lower than that in other artificial/managed grasslands, e.g., artificial grasslands in northern China (3.28 kg C ha⁻¹ yr⁻¹

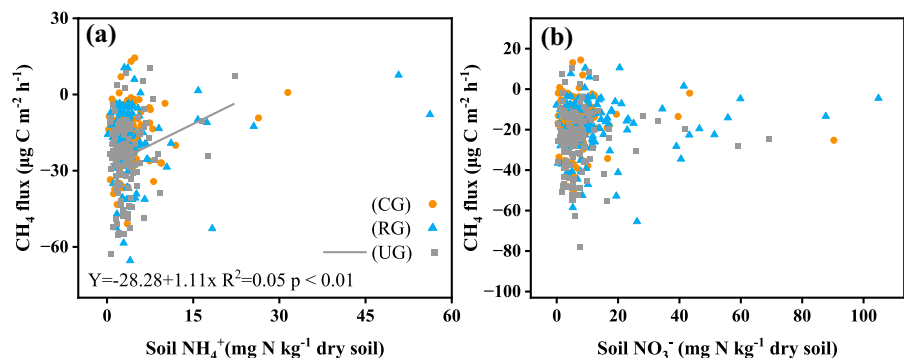
(Ma et al. 2020)) and managed grasslands in northern England (2.7–3.3 kg C ha⁻¹ yr⁻¹ (Eze et al. 2018)). In general, the annual CH₄ uptake from our artificial grasslands was comparable to that of other artificial or natural grasslands.

Effect of grazing on CH₄ uptake

Our results showed that grazing (CG and RG, 9 sheep ha⁻¹ yr⁻¹) significantly reduced CH₄ uptake by 18–42%, compared with UG (Table 3). This may be due to the soil compaction that results from animal treading, which reduces the diffusion of gas into the soil, thus limiting the utilization of CH₄ and O₂ in the oxidation process and, namely, reducing CH₄ uptake (Liu et al. 2007). In fact, the soil bulk densities of CG and RG were higher than those of UG (Table 1). Our results are in agreement with those of other studies of temperate semi-arid steppes. For example, Liu et al. (2007) reported that winter-grazing significantly reduced steppe CH₄ uptake during the growing season by 47%, and Chen et al. (2011) suggested that heavy grazing reduced annual CH₄ uptake by 24–31%.

The annual CH₄ uptake was similar between the CG and RG treatments, although both CG and RG decreased CH₄ uptake in the artificial grasslands, compared to the UG treatment (Table 3). This finding suggests that rotational grazing did not enhance the CH₄ uptake in grasslands compared to continuous grazing. To the best of our knowledge, few field studies have tested whether rotational grazing grasslands have advantages over continuous grazing grasslands in terms of enhancing CH₄ uptake. The few studies that have been conducted mostly support our findings. The results of Ma et al. (2021) (from two-year measurements, grazing strategy does not affect CH₄

Fig. 5 Relationship between CH₄ fluxes and soil ammonium and nitrate content during the study period in the artificial grasslands. Y: CH₄ flux in the UG treatment, x: soil NH₄⁺ content in the UG treatment. CG: continuous grazing; RG: rotational grazing; UG: ungrazed



uptake from temperate grasslands) were in agreement with our findings. Baronti et al. (2022) also reported similar CH₄ fluxes from continuous and rotational grazing treatments. Rotational grazing can improve soil health over that of continuous grazing (Byrnes et al. 2018; Döbert et al. 2021; McDonald et al. 2019; Teague et al. 2011); however, our results suggest that we should not expect major benefits in net CH₄ uptake if the treatment of grasslands is shifted from continuous grazing to rotational grazing.

Contribution of the non-growing season to annual CH₄ uptake

Regardless of the grazing strategy or year, the soil of the artificial grasslands in the temperate semi-arid area of northern China was a sink of atmospheric CH₄ throughout the non-growing season. Many studies have reported similar phenomena in grasslands, forests, and agricultural ecosystems (Butterbach-Bahl and Papen 2002; Chen et al. 2010, 2011; Dörsch et al. 2004; Mosier et al. 1996). Our results showed that non-growing season CH₄ uptake accounted for 34%, 32%, and 33% of the annual CH₄ uptake for CG, RG, and UG treatments, respectively (Table 3). Chen et al. (2011) reported that the contribution of the non-growing season to the annual CH₄ uptake ranged from 23 to 40% for temperate semi-arid grasslands in Inner Mongolia, which is in agreement with our results. Rong et al. (2015) observed that in temperate steppe, 22–59% of the annual CH₄ uptake took place in the non-growing season. Liu et al. (2017) observed that the non-growing season accounted for 28–43% of the annual CH₄ uptake in grasslands. These studies have highlighted that the significant contribution of the non-growing season to the annual CH₄ budget cannot be neglected.

Effects of soil temperature and moisture on CH₄ uptake

In this study, the CH₄ uptake of artificial grasslands was positively correlated with soil temperature over the entire observation period (Table 4, Fig. 4). This suggests that the lower CH₄ uptake in the non-growing season, compared to that in the growing season, was caused by the lower soil temperature. A similar phenomenon has been observed in many ecosystems (Chen et al. 2011; Liu et al. 2017; Maljanen et al.

2003; Wang et al. 2005). Our findings confirm those of Wu et al. (2010), which reported that the CH₄ uptake rate was mainly influenced by temperature. The dependence of CH₄ uptake on soil temperature can be attributed to the sensitivity of microbial activity to temperature, as the consumption of atmospheric CH₄ by soil is microbially mediated (Wu et al. 2020). Our results showed that the three-year mean soil temperatures of CG and RG were higher than that of UG, but the CH₄ uptake was lower. The main reason for this phenomenon might be soil compaction by grazing in CG and RG, rather than soil temperature.

Our results showed that CH₄ fluxes of the artificial grasslands (in UG and CG plots) were significantly correlated with the soil moisture during the entire study period, and 27.5% (WFPS%) was the optimum moisture for CH₄ uptake (Table 4, Fig. 4b). Dijkstra et al. (2011) observed that the optimum soil moisture (WFPS%) for CH₄ uptake in semi-arid grasslands is approximately 24%, which is in agreement with our results. A similar result was observed by Mosier et al. (1996) for Colorado grasslands, in which the soil moisture (WFPS%) was 20% at the peak of CH₄ uptake. The reason for this phenomenon might be that, at high soil wetness, the aeration of the soil decreased, the diffusion rate of CH₄ and O₂ was limited, and the ability of the soil to oxidize atmospheric CH₄ was suppressed; meanwhile, at low soil wetness, the methane-oxidizing bacteria faced severe physiological water stress and reduced soil microbial activity, limiting the soil uptake of atmospheric CH₄ (van den Pol-van Dasselaar et al. 1998; von Fischer et al. 2009).

Conclusions

Artificial grasslands were determined to be a sink for atmospheric CH₄, with a sink strength comparable to that of other types of grassland. The large contribution (one-third) to the annual CH₄ uptake in the non-growing season cannot be neglected in an annual inventory. Regardless of grazing strategy, grazing significantly reduced CH₄ uptake in grasslands compared to ungrazed pastures. However, rotational grazing had no advantage over continuous grazing in terms of enhancing the CH₄ uptake. Therefore, no major benefit in net CH₄ uptake should be expected from a shift from continuous to rotational grazing. In

addition, the seasonality of the annual CH₄ uptake is primarily regulated by soil temperature. Future efforts to develop optimal management strategies to enhance CH₄ uptake from grazing grasslands should consider environmental and soil conditions (e.g., soil temperature and bulk density) and interactions between grazing strategies and grazing intensity, given their significance in grassland methane uptake.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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