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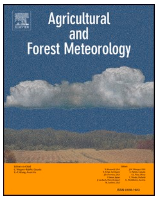

Grazing weakens N-addition effects on soil greenhouse gas emissions in a semi-arid grassland

Zhen Wang [a,b], Xiumin Zhang [c], Mengyuan Wang [d], Lan Li [a,b], An Hu [a,e], Xianjiang Chen [f], Shenghua Chang [a,b], Fujiang Hou [a,b,*]

[a] *State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China*
[b] *Key Laboratory of Grassland Agro-Ecosystem, Ministry of Agriculture, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China*
[c] *Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan 410125, China*
[d] *School of Computing, Ulster University, Belfast, United Kingdom*
[e] *Institute of Tropical Crop Genetic Resources, Chinese Academy of Tropical Agricultural Sciences, Haikou, China*
[f] *Sustainable Agri-Food Sciences Division, Agri-Food and Biosciences Institute, Hillsborough, County Down BT26 6DR, United Kingdom*

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ABSTRACT

Grazing and anthropogenic nitrogen (N) enrichment co-occur in most grassland ecosystems and may have substantial effects on production of soil greenhouse gases (GHGs). Although the individual effects of N addition and grazing on soil GHGs are well understood, their long-term interactive effects on grassland soil GHGs remain unclear. We conducted seven-year *in situ* measurement of three major GHGs in a long-term experiment comprising grazing (no, light, moderate, and heavy grazing intensity) and N-addition treatments (control, N addition: 10 g N m^{-2} $year^{-1}$) in a semi-arid grassland, to determine the effects of N addition and grazing on GHGs. We found that moderate grazing reduced cumulative CO_2 emissions by 10%–11% compared with no, light, and heavy grazing. Unusually, CH_4 emissions from soils and N_2O uptake were found in this semi-arid grassland. Soil CH_4 uptake was markedly inhibited by moderate and heavy grazing. Relative to no grazing, grazing significantly reduced 60%–88% N_2O uptake over seven years on average. Nitrogen addition alone increased cumulative CO_2 emissions by 16% relative to control. An antagonistic effect between grazing and N addition was found on cumulative CO_2 emissions, cumulative CH_4 uptake, and global warming potential (GWP). Light grazing on this semi-arid grassland could offset 14% of the soil GHG emissions induced by N addition. Soil NO_3^- -N was the most important factor controlling soil CO_2 emissions and CH_4 uptake, and soil pH was a major factor mediating soil N_2O uptake or consumption. Our study highlights the importance that adjusting the grazing intensity of grassland is one of efficient strategies to mitigate GHGs emissions in the context of climate change.

1. Introduction

Grasslands cover nearly 40% of the Earth's land surface, amounting to at least 10%–20% of global total soil carbon (C) stocks (Eswaran et al., 1993; Bardgett et al., 2021), and contribute a large proportion of the global budget of the three major greenhouse gases (GHGs): carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Livestock grazing is the most extensive and pervasive global land-use pattern in uncultivated grassland, which often affects the climate system in combination with other global change factors through the land–atmosphere exchanges of GHGs (Koncz et al., 2017). Increased atmospheric nitrogen (N) deposition and N fertilizer use from human activities strongly impacts GHG exchange at the interface of land and atmosphere (Pinder et al., 2012). In practice, grazing activity and N application on grassland not only co-occur but are also causally linked in the context of global change. However, little information is available about the simultaneous or interactive effects of grazing and N addition on grassland soil GHG emissions.

Previous studies have been conducted to investigate the isolated effects of N application or grazing on soil GHG emissions, but have

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* Corresponding author at: State Key Laboratory of Herbage Improvement and Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, No. 768 West Road Jiayuguan, Lanzhou 730020, China.
E-mail address: cyhoufj@lzu.edu.cn (F. Hou).

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shown inconsistent results (Lund et al., 2009; Wolf et al., 2010; Li et al., 2012; Louro et al., 2016; Cardoso et al., 2017; Moinet et al., 2017; Wang et al., 2017; Tang et al., 2018; Aronson et al., 2019; Tang et al., 2019b). The main reasons for these inconsistencies are as follows: (1) measurements of GHG fluxes are often conducted over short time periods, with low temporal resolution; (2) the response of GHGs to N addition depends on land-use pattern, N demand of the ecosystem, and the duration of N application; and (3) different grazing intensity, grazing regimes, grazing duration, and climate conditions lead to an uncertain response of GHGs to grazing. All of these issues emphasize the importance and necessity of long-term GHG observations under long-run global change manipulation experiments. Despite the extensive GHG emission measurements made in the past 20 years, there remains insufficient long-term data sets of field measurements to provide accurate information on how the three major GHGs respond to ongoing N enrichment interacting with grazing on grassland. Furthermore, the recent IPCC sixth assessment report revealed that there is growing evidence to support the notion that human influence is causing extreme climate events, but this evidence is still uncertain in many regions of the world because of a lack of observational data, particularly in arid and semi-arid areas (Zhou, 2021). Arid and semi-arid rangelands occupy about one third of the Earth's terrestrial surface, including the vast Central Asian steppes (over 10 million km^2) across the mid-latitudes of Eurasia (Morgan et al., 2011; Barbolini et al., 2020), and net primary production in these ecosystems is generally considered to be N-limited (LeBauer and Treseder, 2008). The Eurasian steppes have been subjected to intensive livestock grazing for a long period of time (Bai et al., 2012), and this is accompanied by the return of large amounts of fertilizer in the form of animal excrement to the soil. Furthermore, N deposition in this region continues to increase according to recent global estimates (Ackerman et al., 2019; Liu et al., 2022b). Consequently, this region is a critical area in which to assess climate change and human activities, but data from the *in situ* long-term observations of the three major GHGs remain very sparse.

In general, both grazing and N application could affect soil GHG emissions by altering plant growth, soil microbial community, and soil nutrients (Tang et al., 2019b; Deng et al., 2020). Grazing can reduce aboveground plant productivity and root biomass. This will reduce soil C and N availability, which may reduce soil GHG emissions by limiting soil microbial processes (Tang et al., 2019b). On the other hand, animals trampling could accelerate the decomposition of soil organic matter, and thus increasing soil CO_2 and CH_4 emissions (Piñeiro et al., 2010; AminiTabrizi et al., 2020). Moreover, grazing could reduce soil moisture and enhance topsoil compaction, inhibiting soil GHG emissions (Chen et al., 2011b; Tang et al., 2019b). However, N input seems to stimulate soil CO_2 and N_2O emissions (Chen et al., 2013; Aronson et al., 2019; Shi et al., 2021) and it seems to have a neutral effect on CH_4 uptake in dry grassland ecosystems (Chen et al., 2013; Aronson et al., 2019). In contrast, CO_2 released from topsoil organic C was shown to be the dominant contributor for GHG balance in Eurasian steppe (Schönbach et al., 2012), which implies that soil CH_4 and N_2O emissions likely contribute a negligible portion of the GHG balance.

Here, an N-addition experiment was embedded into a long-term (2001 to the present) grazing experimental platform that was set up with four grazing intensities (no, light, moderate, and heavy grazing) and three major GHGs were measured *in situ* for seven years in a semi-arid grassland. Based on previous findings, we hypothesize that (1) the contribution of soil CH_4 and N_2O emissions to the GHG balance is negligible in long-term timescales; (2) grazing and N input have interactive effects on soil GHG emissions and grazing can offset N-addition-induced GHG emissions in typical steppe; and (3) soil available N is the key driver for the production and consumption of soil GHGs in typical steppe. The objectives of this study were as follows: (1) to elucidate the individual and interactive effects of grazing and N application on soil GHG emissions; (2) to explore the mechanisms underlying grazing and N addition effects on soil GHGs; and (3) to propose management approaches toward the mitigation of grassland GHG emissions in the context of global change.

2. Materials and methods

2.1. Study site

The field experiment was carried out at the Grassland Agro-ecosystems Research Station of Lanzhou University, which is located in the core of the Loess Plateau, northeast of Gansu Province, China (37.12°N, 106.82°E, 1650 m a.s.l.). The region has a typical temperate continental monsoon climate with an average annual precipitation of 273.8 mm (2001–2018), and 60%–80% of rainfall occurs during the plant-growing season from May to late August (Fig. 1). The mean annual air temperature is 8.4°C, ranging from −6.1°C in January to 21.9°C in July. The precipitation fluctuated substantially both intra- and inter-annually from 2012 to 2017 (Fig. 1). Mean annual evaporation is close to 2,000 mm in this region. Annual air temperatures in 2013, 2015, 2016, 2017, and 2018 were warmer than the last 18-year mean (8.4°C), while the mean air temperature in 2012 was colder than the 18-year mean. The soil of this region is classified as sandy, free-draining loess, and the grassland is classified as typical temperate steppe. The main dominant species in the experimental plot are *Lespedeza davurica, Artemisia capillaris*, and *Stipa bungeana*; the main subdominant species are *Heteropappus altaicus, Potentilla bifurca*, and *Cleistogenes songorica*.

2.2. Experimental design

2.2.1. Long-term rotational grazing experiment

In 2001, 12 enclosed 0.5 ha plots with similar vegetation composition, slopes, and cover were established for a grazing trial. Prior to establishment of the grazing experiment, grazing was prohibited in the grassland of this region due to the government policy. A total of 0, 4, 8, and 13 sheep of similar liveweight (25 ± 1.2 kg) were rotationally grazed in three replicated 0.5 ha plots, representing stocking rates of 0 (no grazing), 2.7 (light grazing), 5.3 (moderate grazing), and 8.7 (heavy grazing) sheep ha^{-1}, respectively. All plots were arranged in a completely randomized design. The specific method used for rotational grazing involved allocating the sheep to three replicates for each of the four stocking rates, and then rotationally grazing them between each of the replicate plots allocated to that stocking rate. The plots were rotationally grazed from June to September (90 days), with a rotation cycle length of 30 days (10 days grazing and 20 days rest) and three rotations.

2.2.2. N-addition experiment

In April 2012, four N-addition experimental subplots (2 m × 2 m) were randomly assigned within each grazing plot. Subplots were separated from each other by 1-m-wide buffers to eliminate edge effects. Four levels of N addition treatments (0 (control), 5, 10, and 20 g N m^{-2} yr^{-1}) were applied to each subplot, for a total of 36 subplots (3 grazing intensity × 4 N-addition levels × 3 replicates). Within each fenced plot (no grazing), 12 subplots were laid out with four levels of N addition with three replicates, for a total of 36 subplots (4 N-addition levels × 3 plot × 3 replicates). Hence, there was an overall total of 72 experimental subplots. The N-addition subplots were uniformly sprayed with ammonium nitrate (NH_4NO_3) dissolved in 1 L of purified water, while the control plots were sprayed with the same amount of water without NH_4NO_3. N was applied to the subplots twice each year, with 60% added in May and 40% added in July. In the current study, soil GHGs were measured in the subplots treated with 0 (control) and 10 g N m^{-2} yr^{-1} because the N-addition rates were close to the median rates of N deposition and agricultural N input in this area.

2.3. Measurements of GHG fluxes

Soil GHG fluxes were measured using a static closed stainless steel chamber (50 cm length × 50 cm width × 50 cm height), which is an

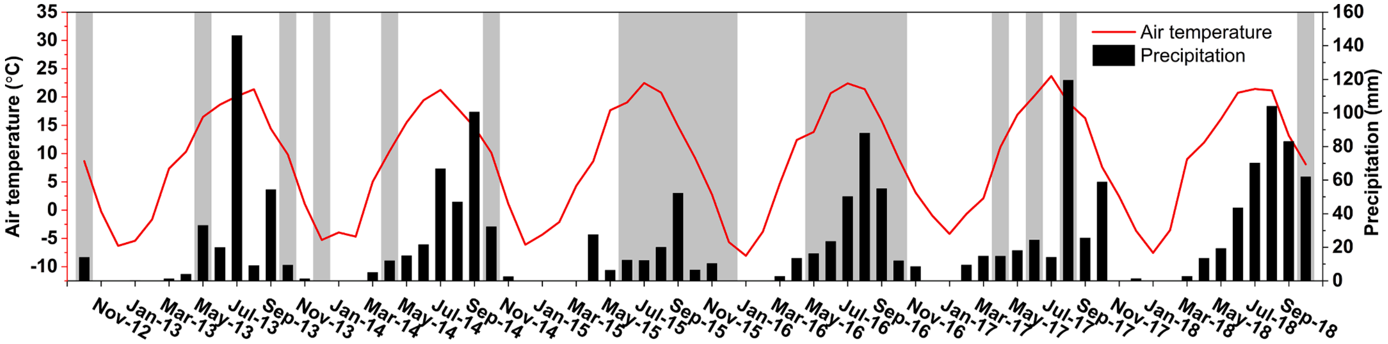

Fig. 1. Monthly precipitation and air temperature during 2012–2018 at the grazing experimental site. Gray-shaded bars indicate greenhouse gas measurement periods.

open-bottomed square box. A battery-operated fan was fixed to the top of the chamber to ensure sufficient gas mixing, and the box was wrapped with layers of formed plastic to reduce air temperature changes in the chamber. A stainless collar from the chamber (50 cm length × 50 cm width × 10 cm height) with a water groove was inserted 10 cm into the soil. A chamber was randomly placed in each of the subplots of the N application treatment for GHGs sampling. During the flux-measurement period, the chamber was attached to the seat and the groove was sealed with water to ensure air tightness. After the chamber had been closed for 30 min, gas samples were collected with 60-mL plastic syringes with three-way stopcocks via a tube connected to the headspace of each chamber at 0, 10, 20, and 30 min and then immediately transferred into 250-mL aluminum foil gas-collecting bags. All gas samples were taken from each chamber at a time between 05:00 to 17:00 (BST) on each sampling date with four times each day. We spent about three to five consecutive days for GHGs measurement each month as a measurement

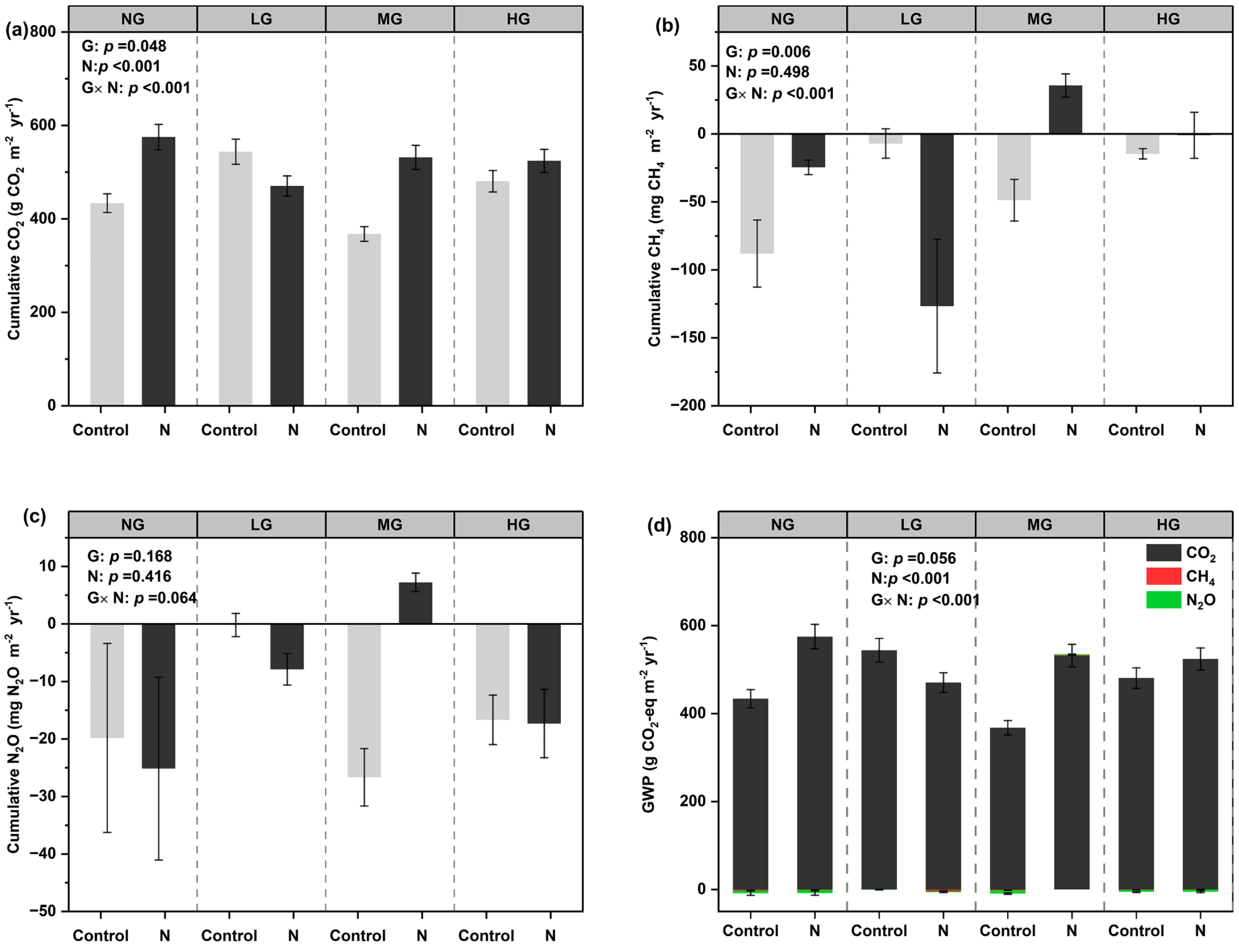

Fig. 2. Mean values (±SE) of the cumulative soil CO_2 (a), CH_4 (b), N_2O fluxes (c) and Global Warming Potential (GWP) (d) under grazing (G) and N-addition (N) treatments. NG: No grazing; LG: Light grazing; MG: Moderate grazing; HG: Heavy grazing.

event. The CO_2, CH_4 and N_2O flux of each measurement event reported in this study was mean value of each GHG flux of those consecutive days of sampling. Soil GHGs were measured from October 2012 through October 2018 for total of 24 measurement events (Fig. 3). When encountering a N application situation, GHGs measurement were carried out immediately as soon as N application is completed to avoid missing the peak of GHGs emissions.

Gas samples were analyzed using a gas chromatograph (Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA), which was equipped with an electron capture detector (ECD) and a flame ionization detector (FID). The ECD was used to determine N_2O concentrations, and the FID was used to determine CO_2 and CH_4 concentrations. We used certified CO_2 (398 and 2500 ppm), CH_4 (2.02 and 15 ppm), and N_2O (836 and 3000 ppb) standard gases to calibrate the gas chromatograph. Soil CO_2, CH_4, and N_2O fluxes were determined based on the slopes of the temporal changes (0, 10, 20, and 30 min after chamber closure) in gas concentrations within the chamber. Linear regression values were $R^2 > 0.90$ for CO_2 and CH_4 flux, and 0.70–0.80 for N_2O flux. Air temperature and soil temperature at 5 cm were simultaneously recorded by two digital thermometers in each chamber while gases were collected. Soil samples (at 5 cm depth) were collected synchronously near each chamber on the GHG sampling days to determine the soil gravimetric water content by drying soils at 105°C for 24 h.

2.4. Soil sampling and vegetation survey

Soil samples (at 10 cm depth) were taken near each static chamber on 15 October 2012 and 12 April, 15 June, and 12 August in 2017 for determining soil pH and soil organic carbon (SOC) contents. Soil samples (at 10 cm depth) were also collected on 20 May, 15 October, and 10 December in 2013; 15 April and 12 October in 2014; and 12 April, 15 June, and 12 August in 2017 near the chamber for determining soil nitrate (NO_3^--N) and ammonium (NH_4^+-N) contents. We used 50 mL of 1 M KCl solutions to extract soil NO_3^--N and NH_4^+-N, which were analyzed with a SmartChem 140 automatic chemical analyzer (AMS Alliance). The SOC contents were measured using the potassium dichromate method. Soil pH was determined by a pH meter with a glass electrode at a 1:5 soil-to-deionized water ratio (w/v). We investigated the aboveground biomass within a 0.5 × 0.5 m quadrat in each subplot in last August each year. Aboveground living plants were clipped, and dried and then weighed, to evaluate biomass and plant N content. Plant N content was measured using an elemental analyzer (Micro-cube, Elementar, Germany).

2.5. Cumulative GHGs fluxes and Global Warming Potential (GWP)

We multiplied the mean daily fluxes between the two consecutive sampling dates by the time interval to calculate the cumulative seasonal CO_2, CH_4, and N_2O fluxes and then summed up the daily fluxes for all time intervals over a seasonal or annual time period. Global warming potential (GWP), defined as time-integrated radiative forcing, was calculated to evaluate how the treatments and factors impacted the fluxes of different gases released from the ecosystem in the context of future climate change (Tian et al., 2016). To estimate the long-term climate impact of grazing and N addition-induced GHG balance, we adopted GWP metrics for a 100-year time horizon by using the following equation:

$$GWP = CO_2 + 25CH_4 + 296N_2O$$

where CO_2, CH_4 and N_2O are the annual cumulative fluxes of CO_2 CH_4 and N_2O, respectively. 25 and 296 are the conversion coefficients of CH_4 and N_2O to CO_2 equivalents, respectively.

2.6. Statistical analysis

Linear mixed effect models were used to test the main effects and interactions of grazing and N addition on soil CO_2, CH_4, and N_2O fluxes, as well as on soil variables (soil temperature, soil moisture, pH, SOC, NH_4^+-N, NO_3^--N), and plant variables (aboveground biomass, litter biomass and plant N content), in which grazing and N addition were treated as fixed factors, and year and subplot were treated as random factors. The effects of grazing, N addition, and their interactions on cumulative GHGs and GWP were also assessed using linear mixed effect models, wherein grazing, N addition were included as fixed effects, and subplot was treated as a random factor. Pairwise comparison with least significant difference was applied for all measured variables to identify sources of significant differences between factor levels. All analyses were conducted using SPSS statistical software (v. 26.0, IBM, NY, USA). All significance was defined at the $P < 0.05$ level. Structural equation modeling (SEM) was used to explore the direct and indirect effects of grazing and N addition on soil CO_2, CH_4, and N_2O fluxes through soil variables (soil moisture, temperature, pH, SOC, NH_4^+-N, and NO_3^--N). Based on the linear mixed effect analysis (Tables S1, S2), we constructed three models. The first and the second models examined the effects of grazing and N addition on cumulative soil CO_2 fluxes and CH_4 fluxes respectively, because the linear mixed models showed that grazing and N addition had an interactive effect on cumulative soil CO_2 emissions and CH_4 uptake. The third model only included the effect of grazing on soil N_2O fluxes since soil N_2O fluxes were only affected by grazing (Table S2). We treated soil moisture, pH, SOC, NH_4^+-N, and NO_3^--N as explanatory variables. The initial models included all possible pathways based on a priori known and theoretical knowledge of effects of grazing and N addition on cumulative soil CO_2 flxues or CH_4 fluxes, or N_2O fluxes via selected soil variables as well as direct or indirect pathways among these variables (Fig. S1) (Chen et al., 2011b; Schönbach et al., 2012; Aronson et al., 2019; Tang et al., 2019; Deng et al., 2020; Wang et al., 2020). Data were fitted to the model using the maximum likelihood estimation method. The chi-squared (χ^2) test, Akaike information criterion (AIC), and root mean square error of approximation (RMSEM) were used to test the adequacy of the model. When yielding a non-significant χ^2 ($P > 0.05$), a lower AIC value, and a lower RMSEM value, the model was deemed an adequate fit. The SEM analyses were conducted using AMOS 23.0 (IBM SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effects of grazing and N addition on cumulative GHG emissions

Cumulative CO_2 emissions were affected by grazing (Table S1, $P = 0.048$), N deposition (Table S1, $P < 0.001$), and their interactions (Table S1, $P < 0.001$). Relative to no grazing, moderate grazing decreased cumulative CO_2 emissions by 15%. Relative to control, N addition with no grazing, moderate grazing, and heavy grazing increased cumulative CO_2 emissions by 25%, 31%, and 8%, respectively, whereas N addition with light grazing reduced cumulative CO_2 emissions by 13% (Fig. 2a). Cumulative CH_4 uptake was greatly influenced by grazing and interactions between grazing and N addition (all $P < 0.001$, Table S2), whereas cumulative CH_4 uptake was not influenced by N addition ($P = 0.498$, Table S1, Fig. 2b). Grazing significantly inhibited cumulative CH_4 uptake by 2–12 times compared with no grazing ($P < 0.001$, Fig. 2b). Light grazing with N addition had the highest cumulative CH_4 uptake, but moderate grazing with N addition the highest cumulative CH_4 emissions (Fig. 2b). Grazing and N addition had no significant effect on cumulative N_2O fluxes (all $P > 0.15$, Table S1), but grazing × N addition had a marginal significant effect ($P = 0.06$, Fig. 2c).

Regardless of N addition or grazing treatment, cumulative soil CO_2 was the main contributor to the GWP at this study site (Fig. 2d). Grazing × N addition had a significant effect on GWP (Table S1, $P < 0.001$). Light grazing with N addition decreased GWP by 14% relative to light grazing without N addition (Fig. 2d).

3.2. Response of GHG fluxes to grazing and N addition

The dynamics of CO_2, CH_4 and N_2O fluxes showed smooth variation under all treatment during the experiment period, except for a few measurement events (Fig. 3). Specifically, we observed a peak emission of CO_2 under all treatment in May 2013, and a large variation in fluxes of CH_4 between the treatments in October 2018 (Fig. 3). In addition, the negative N_2O fluxes were observed in two measurement event, October 2013 and October 2018.

Grazing, N addition, and their interactions did not affect the CO_2 fluxes (Table S2, $P > 0.05$, Fig. 3a, d). The effect of grazing on soil CH_4 fluxes varied with N addition and year, yielding a grazing × N addition ($P < 0.001$, Table S2) and a grazing × year interaction ($P < 0.001$, Table S2), respectively. Compared with no grazing, light, moderate, and heavy grazing significantly inhibited the soil CH_4 uptake on seven-year average ($P < 0.001$, Fig. 3b). The minimum CH_4 fluxes (-0.006 mg m^{-2} min^{-1}) appeared in the N application treatment with light grazing, and the maximum CH_4 fluxes (0.002 mg m^{-2} min^{-1}) appeared in the N application treatment with heavy grazing (Fig. 3e). Nitrogen application alone had no effect on CH_4 fluxes ($P = 0.833$, Table S2, Fig. 3b).

The N_2O fluxes were significantly affected by grazing, year, and their interactions (Table S2, all $P < 0.001$), whereas they were not affected by N addition (Table S2, $P = 0.810$, Fig. 3c). Relative to no grazing, light, moderate, and heavy grazing significantly reduced N_2O uptake by 88%, 81%, and 60% (based on seven-year averages), respectively ($P < 0.001$, Fig. 3f).

3.3. Soil and vegetation characteristics

The SOC content was significantly affected by grazing ($P = 0.040$, Fig. 4a, Table S3), but it was not affected by N addition ($P = 0.283$, Table S3) and their interactions ($P = 0.246$, Table S3). Compared with no grazing, SOC content was reduced by 22% under moderate-grazing (Fig. 4a). Soil temperature and soil moisture were not affected by N application, grazing treatment, or interactions between grazing and N-application treatments (Table S3, Fig. S2, all $P > 0.05$). However, soil moisture in October 2018 was significantly higher than that of the same

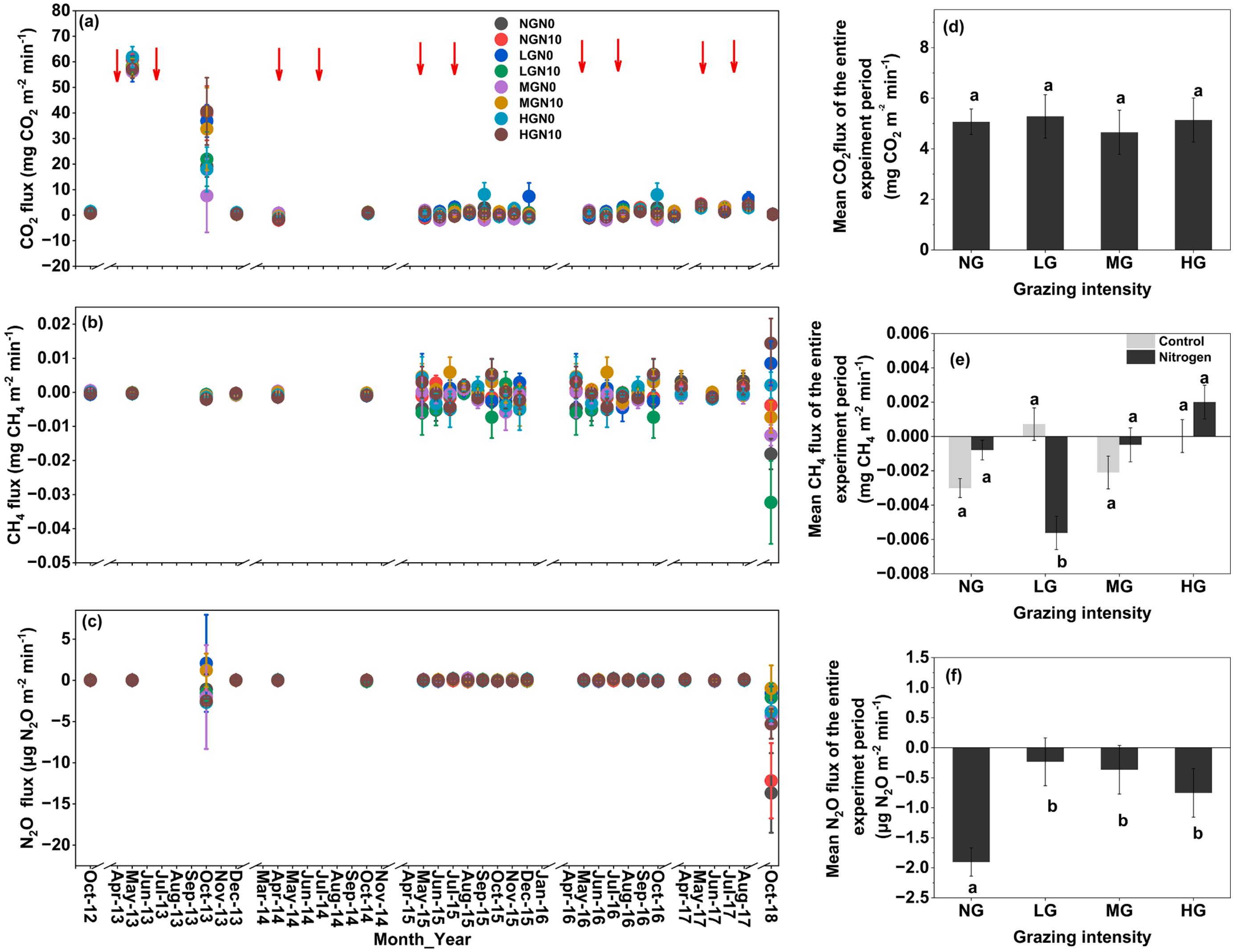

Fig. 3. Mean values (±SE) of soil CO_2 (a), CH_4 (b), and N_2O (c) fluxes of each measurement event (three to five consecutive days each month) under all treatment from 2012 to 2018, respectively. Mean values (±SE) of soil CO_2 (d), CH_4 (e), and N_2O (f) fluxes of the entire experiment period under grazing, grazing and N addition, and grazing treatment, respectively. Different lowercase letters on the top of the error bars indicate significant differences among grazing intensities (d and f), and indicate significant differences between N addition treatment at the same grazing intensity (e). G0N0: no grazing with no N addition; GLN0: light grazing with no N addition; GMN0: moderate grazing with no N addition; GHN0: heavy grazing with no N addition; G0N10: no grazing with N addition; GLN10: light grazing with N addition; GMN10: moderate grazing with N addition; GHN10: heavy grazing with N addition. The red arrows denote the time of N application.

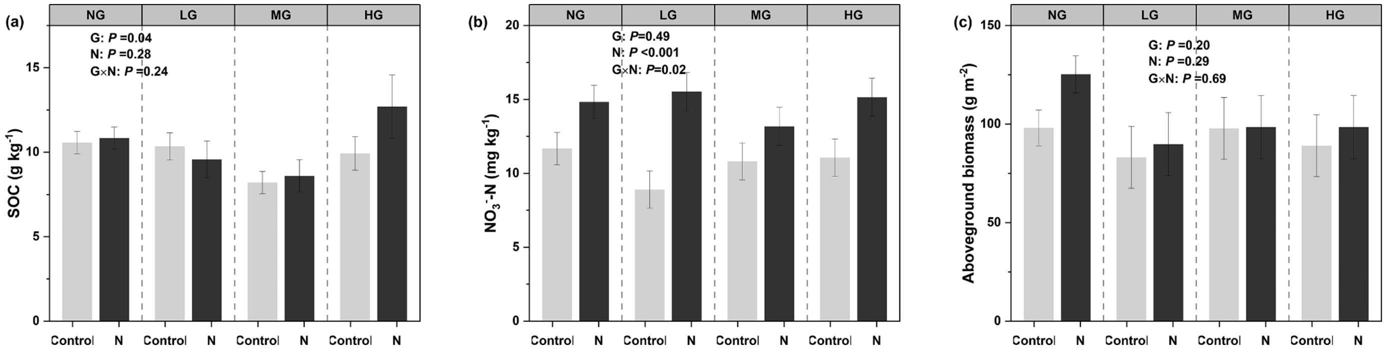

Fig. 4. Mean values of soil organic carbon (SOC), soil NO_3^--N content (b) and aboveground biomass and (c) under grazing (G) and N addition (N) treatments. NG: No grazing; LG: Light grazing; MG: Moderate grazing; HG: Heavy grazing.

month of the other years (Fig. S3). Grazing, N addition, and their interactions had no effect on soil NH_4^+-N content (all $P > 0.05$, Table S3, Fig. S4a). However, grazing, N addition and their interactions had a significant effect on soil NO_3^--N content (all $P < 0.05$, Table S3, Fig. 4b), with N addition increasing soil NO_3^--N content by 21% relative to control (Fig. 4b). Under N addition treatment, moderate grazing decreased soil NO_3^--N content by 11%, 15%, and 13% relative to no, light, and heavy grazing, respectively (Fig.4b). Grazing, N addition, and their interactions had no effect on soil pH (all $P > 0.05$, Table S3, Fig. S4b). Grazing did not affect plant N content ($P = 0.436$, Fig. S5a, Table S3), whereas N addition significantly increased plant N content by 21% relative to control ($P = 0.022$, Fig. S5 b). Grazing, N addition and their interactions had no significant effect on aboveground biomass (all $P > 0.05$, Table S3. Fig. 4c). Litter biomass was only affected by grazing ($P < 0.001$, Table S3), wherein light grazing and heavy grazing significantly increased it relative to no grazing (Fig. S6). While litter biomass under moderate grazing did not differ from the litter biomass under other grazing intensities (Fig. S6).

3.4. Grazing-induced relationship between environmental variables and soil GHG flux

The SEM analysis showed that grazing had a weak direct negative effect on cumulative soil CO_2 emissions, while N addition had an indirect positive effect on cumulative soil CO_2 emissions by increasing soil NO_3^--N content (Fig. 5a). In addition, cumulative soil CO_2 emissions were significantly affected by soil temperature, soil moisture, NH_4^+-N, and soil pH through direct or indirect pathways. Together, grazing, N addition, and these soil variables explained 70% of the variance of cumulative soil CO_2 emissions (Fig. 5a). Grazing had a direct positive effect on cumulative soil CH_4 fluxes, while N addition had indirect negative effects on cumulative soil CH_4 fluxes through a direct positive effect on soil NO_3^--N content, which had an indirect effect on cumulative soil CH_4 fluxes (Fig. 5b). Grazing, N addition, and soil variables together explained 27% of the variance of cumulative soil CH_4 fluxes (Fig. 5b). Grazing had a direct negative effect on soil N_2O fluxes, whereas it had an indirect positive effect on soil N_2O fluxes through elevating soil pH, but the positive effect was larger than the negative effect (Fig. 5c). In addition, soil N_2O fluxes were directly affected by soil moisture, SOC, soil NO_3^--N, and NH_4^+-N. Grazing and these soil variables explained 98% of the variance of soil N_2O fluxes (Fig. 5c). Standardized total effects revealed that soil available mineral N (NO_3^--N and NH_4^+-N) was the primary driving factor for soil CO_2 emissions and CH_4 uptake, followed by soil pH (Fig. 5d, e). For soil N_2O fluxes, SOC was the primary driving factor, followed by soil pH (Fig. 5f).

4. Discussion

4.1. GHG emission response to long-term grazing

Our results showed that grazing had a significant effect on cumulative soil CO_2 emissions, wherein moderate grazing significantly decreased cumulative soil CO_2 emissions relative to no grazing, light grazing, and heavy grazing (Fig. 2a). In line with our results, a previous study found that the negative effects of grazing on soil CO_2 emissions was largest with moderate grazing in a semi-arid steppe (Li et al., 2018; Wang et al., 2020). On the contrary, other studies showed that grazing increased soil CO_2 emissions in a tropical grassland of Brazil (Cardoso et al., 2017) and in a semi-arid grassland of China (Wang et al., 2020), and some cases showed that lack of effects of grazing on soil CO_2 emissions (Jia et al., 2007; Zhu et al., 2015; Gourlez de la Motte et al., 2018). Results from a global mate-analysis indicated that soil CO_2 emissions decline by grazing was due to grazing induced decrease in above- and belowground biomass, soil nutrients status and soil water content (Tang et al., 2019). Possible explanation for reduction of soil CO_2 emissions under moderate grazing in the current study related to decrease in SOC at this grazing intensity. It is documented that the fragile SOC pool is an essential substrate for potential soil CO_2 respiration, soils with less SOC content generally have less potential for soil respiration (Zheng et al., 2009; Ye et al., 2016). Another reason for the decreasing in soil CO_2 emissions under moderate grazing may be associated with grazing-induced changes in soil C: N: P stoichiometry, since the production of soil CO_2 would be generally constrained by nitrogen and phosphorus elements in soils (Finzi et al., 2011). A recent study conducted at our experimental site showed that moderate grazing reduced soil C:P ratio (Li et al., 2022), which resulted in a decrease in resistance of soil C cycling microbial groups (Luo et al., 2020), thereby reducing soil respiration.

However, we found grazing had no effects on soil CO_2 fluxes on the seven-year average (Fig. 3), which suggests that grazing have cumulative effects on soil CO_2 emissions. A possible explanation for the lack of effect of grazing on soil CO_2 fluxes the is that grazing did not change soil temperature and soil moisture (Fig. S2), which have been identified as the major controls on soil CO_2 fluxes (Oertel et al., 2016). Increased soil temperature and decreased soil moisture by grazing reported previously were often attributed to the removal of aboveground biomass, animal trampling as well as soil compaction (Tang et al., 2019; Yan et al., 2018). However, we adopted rotational grazing regime, which provides sufficient time and space for vegetation and soil to recover. The herbage will regrow quickly after grazing, so that there was no noticeable variation in aboveground biomass (Fig. 4c), which probably result in insignificant variations in soil temperature and soil moisture. In addition, effects of grazing on soil moisture did not differ with grazing intensities which could be attributed to extremely strong evaporation (more than 2,000 mm per year on 50 years average) in this semi-arid grassland, and lower

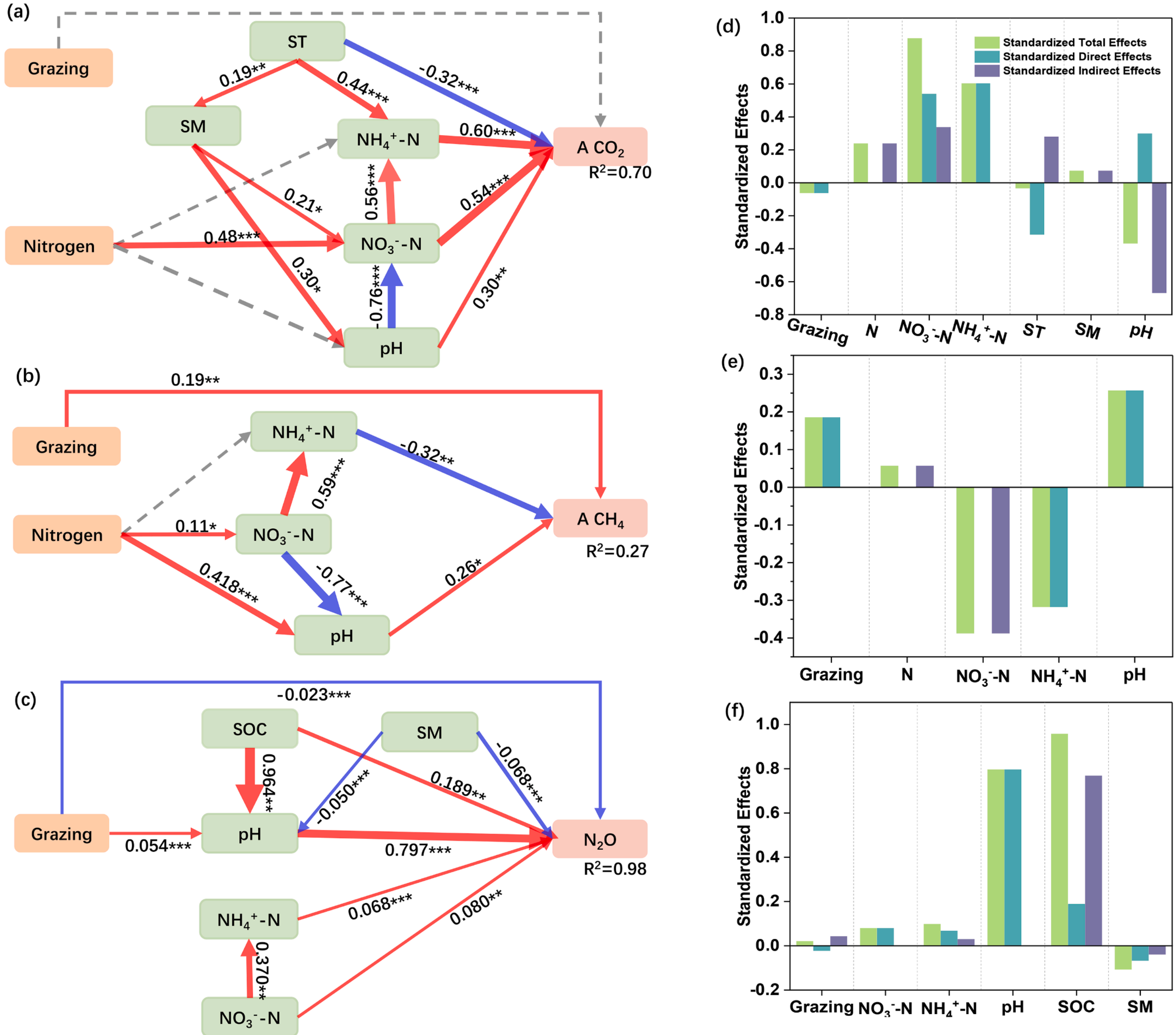

Fig. 5. The structural equation model (SEM) showing direct and indirect effects of grazing and N addition as well as soil variables (soil temperature (ST), soil moisture (SM), soil organic carbon (SOC), soil pH, soil NH_4^+-N, NO_3^--N) on cumulative soil CO_2 (A CO_2) emissions (a), cumulative soil CH_4 (A CH_4) fluxes (b), and direct and indirect effects of grazing and soil variables on soil N_2O fluxes (c). Standardized total, direct and indirect effects of grazing, N addition and soil variables on cumulative soil CO_2 fluxes (d) and cumulative CH_4 fluxes (e) analyzed by SEM, and standardized total, direct and indirect effects of grazing and soil variables on cumulative soil N_2O fluxes (f) analyzed by SEM. Blue and red solid arrows show negative and positive significant pathways, respectively, and dashed arrows indicate non-significant pathways. The width of the solid arrow is proportional to the strength of the relationship. Values adjacent to solid arrows represent standardized path coefficients. R^2 values near the response variables represent the proportion of variation explained by relationships with other variables. Significance levels: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Detailed statistics of model fitting: (a): $\chi^2 = 12.93$, $P = 0.45$, $df = 13$, RMSEA = 0.00, AIC = 74.93; (b) $\chi^2 = 2.8$, $P = 0.90$, $df = 7$, RMSEA = 0.00, AIC = 42.82; (c) $\chi^2 = 11.21$, $P = 0.35$, $df = 11$, RMSEA = 0.008, AIC = 60.12.

water holding of sandy soils, which probably moderated the effects of grazing on soil water content (Zhao et al., 2011).

We found that grazing significantly inhibited CH_4 uptake relative to no grazing, which indicates that over grazing can stimulate soil CH_4 emissions. The SEM further confirmed that grazing had a direct positive effect on cumulative soil CH_4 fluxes. Reduced soil CH_4 uptake under grazing could be explained as follows: first, long-term sheep grazing resulted in soil compaction, reducing air permeability and CH_4 diffusion (Chen et al., 2011b; Tang et al., 2019a). Second, a recent study at our experimental site showed that grazing tends to increase the abundance of soil methanotrophs (Wang et al., 2022), which implies that CH_4 entering the soils from the atmosphere is consumed by those microorganisms. Third, it is widely recognized that animal excreta patches are the source of CH_4 emissions. Long-term grazing allows more animal excreta to be deposited on grasslands, which may be more homogeneously distributed at grasslands with higher grazing intensities. Therefore, we speculated CH_4 released from animal excreta may be offset by CH_4 uptake by soils, thus reducing methane uptake in grazed areas (Chen et al., 2011a; Wang et al., 2013).

Interestingly, although numerous studies have suggested that grassland soils are generally N_2O sources (Wolf et al., 2010; Li et al., 2012; Schönbach et al., 2012; Luo et al., 2013; Shi et al., 2021), evidence from this study revealed that the soil of this typical steppe acted as a sink of N_2O (Fig. 2c). Soil N_2O sinks have been reported in various ecosystems (Chapuis-Lardy et al., 2007; Jones et al., 2014; Domeignoz-Horta et al., 2016), but the underlying mechanisms of soil N_2O uptake remain unclear, particularly in semi-arid areas. Denitrification is generally recognized as the only biological process for reducing N_2O to N_2 by N_2O reductase. However, another biological process has been reported through which non-denitrifying N_2O-reducing bacteria also could decrease soil N_2O production (Domeignoz-Horta et al., 2016), soil N_2O uptake occur when the N_2O reduction pathways in soil overwhelm the N_2O production pathways (Liu et al., 2022a). Either process is significantly affected by soil pH, SOC, soil moisture, and N availability (Dijkstra et al., 2013; Jones et al., 2014; Domeignoz-Horta et al., 2016; Liu et al., 2022a). Correspondingly, our SEM analysis showed that soil N_2O fluxes were directly affected by soil moisture, SOC, pH, soil NH_4^+-N, and NO_3^--N, and these variables together explained the overwhelming variation in N_2O fluxes (Fig. 5). Among these variables, SOC and pH were identified as the most important drivers for soil N_2O uptake. Moreover, we found that grazing significantly reduced N_2O uptake regardless of grazing intensity. The decrease in soil N_2O uptake under grazing is likely related to changes in soil pH caused by grazing. SEM analysis further revealed that grazing increased soil pH, which had a positive effect on soil N_2O fluxes (Fig. 5c). Although the biological process of soil N_2O uptake could not be verified in the current study, the results highlighted the key role of soil pH a in regulating soil N_2O uptake or consumption in a grazed grassland.

4.2. GHG emissions response to long-term N addition

In this study, we applied relatively moderate N addition rates to simulate atmospheric and agricultural N inputs. We found N addition increased cumulative soil CO_2 emissions relative to control on average across seven years. Increased soil CO_2 emissions can be caused by the elevation of soil NO_3^--N content under N application. In an N-limited system, soil available N typically stimulates root growth, soil enzyme activity, and soil microbial C, thus increasing soil microbiome respiration (Zhou et al., 2016; Zong et al., 2018). SEM confirmed that N addition had an indirect positive effect on cumulative soil CO_2 emissions by increasing the soil NO_3^--N content (Fig. 5a).

We found that N addition had little influence on soil CH_4 and N_2O fluxes over the experimental period. The non-significant effect of N application on soil CH_4 and N_2O fluxes could be explained by the following mechanisms: First, semi-arid grasslands are generally N-limited ecosystems (Zhu et al., 2020). As such, it is likely that most of the supplied N was consumed for plant growth, with little N available for the biogeochemical cycling processes in the soil (Chen et al., 2013, 2017). In support of this, we observed that N input led to a significant increase in plant total N content (Fig. S5). Second, N input did not alter SOC, NH_4^+-N content, and soil pH (Table S3), which are important controlling factors for soil CH_4 and N_2O production and consumption because the activity and abundance of soil methanotrophic bacteria, ammonia oxidizing archaea, and denitrifying bacteria are regulated by these variables (Chapuis-Lardy et al., 2007; Liu and Greaver, 2009; Song et al., 2020). It has also been reported that soil GHGs are well predicted by SOC because the labile pool of SOC provides substrates for methanogenesis, nitrification, and denitrification (Zheng et al., 2009; Ye et al., 2016). Third, the lack of a significant difference in CH_4 and N_2O fluxes under the N-addition treatments could be attributed to the lack of a significant variation in soil temperature and soil moisture induced by N addition (Fig. S2). This is because soil temperature combined with soil moisture can explain a substantial portion of the variation in soil GHGs (Schaufler et al., 2010; Luo et al., 2013; Tang et al., 2013). Last but not least, time lag between N application and measurement of N_2O fluxes could also results in insignificant variation in N_2O fluxes under N treatment. Indeed, we missed measurements of GHGs fluxes after nitrogen application in some measurement events in the current study, (Fig. 3), nevertheless, we captured the N_2O fluxes after N application in 2015 and 2016 with high-frequency measurements, and found that nitrogen addition still had no effect on N_2O fluxes. This indicated that the lack of effect of nitrogen addition on N_2O flux was not caused by a mismatch between the nitrogen application event and the time of GHGs measurement.

4.3. Interactive impacts of N addition and grazing on GHGs and GWP

We found significant interactive effects of grazing and N addition on cumulative soil CO_2 emissions and CH_4 uptake, in which light grazing with N addition reduced soil cumulative CO_2 emissions and increased soil CH_4 uptake (Fig. 2). A possible explanation for this result may be related to the reduction of SOC and soil NH_4^+-N in the light grazing with N-addition treatments (Figs. 4, S4), which would result in the inhibition of soil respiration (Zhou et al., 2016; Zong et al., 2018). The SEM further confirmed that N addition had an indirect positive effect on cumulative soil CO_2 emissions through an indirect positive effect on soil NH_4^+-N, whereas grazing had a weak negative effect on cumulative soil CO_2 emissions. Moreover, N input increased CH_4 uptake through an indirect positive effect on soil NH_4^+-N, while grazing decreased CH_4 uptake. These results suggest that N addition and grazing have antagonistic effects on soil CO_2 emissions and CH_4 uptake in this typical steppe and emphasize the importance of soil N availability in regulating key processes for the production or consumption of soil GHGs (Fig. 6). In addition, grazing interacted with N addition showed a marginally significant effect on soil N_2O emission or uptake (Fig. 2). We observed a opposite response of cumulative N_2O fluxes to N addition treatment under moderate grazing. This further supports that grazing has altered the magnitude and direction of the effect of N addition on soil N_2O emissions. Soil CH_4 uptake and N_2O consumption in this typical steppe could be negligible because the contribution of soil CH_4 and N_2O uptake or emission to total GWP relative to soil CO_2 emission was less than 3% (Fig. 2). The GWP was also significantly affected by interaction between grazing and N addition, where light grazing with N addition treatment reduced GHG emissions by 14% relative to light grazing without N application. Conversely, N addition increased GWP by 24%, 32%, and 8% relative to control under no, moderate, and heavy grazing, respectively. These results suggest that light grazing could offset N-addition-induced GHG emissions in this typical steppe.

4.4. Temporal dynamics of GHGs fluxes

Soil GHGs fluxes broadly exhibited smooth temporal dynamics during the large majority of measurement events in this study (Fig. 3). However, unusual variations in GHGs fluxes were observed during very few measurement events (e.g. a peak of soil CO_2 fluxes in May 2013, widely variable of CH_4 fluxes in October 2018, and negative N_2O fluxes in October 2013 and October 2018), even though the fluxes data generally fall in within the scope of the previously reports (Cardoso et al., 2017; Aronson et al., 2019). Semi-arid grasslands are water-limited ecosystem where precipitation is quite sparse at the early stage of the growing season (Fig. 1). Increased precipitation at this stage would stimulate soil microbial activity and also enhances plant root activity to ensure plant requirements for nutrient and water. As a result, both autotrophic and heterotrophic respiration are increased, which probably explains the appearance of peak CO_2 emissions in May 2013, as the precipitation in May 2013 (33 mm) was much higher than the precipitation in the same month of the other experimental years (less than 20 mm) (Fig. 1).

It has been reported that the large temporal variability in CH_4 fluxes is mainly attributed to climate induced changes in biotic or abiotic factors (Xu et al., 2010), such as precipitation induced soil water availability (Wei et al., 2015). In this study, soil moisture in October 2018 was higher than the same month in other experimental year (Fig. S3). This may account for the large variability in CH_4 fluxes observed in October 2018. Nevertheless, we did not observe noticeable variability in CO_2 fluxes in October 2018. This is due to the fact that

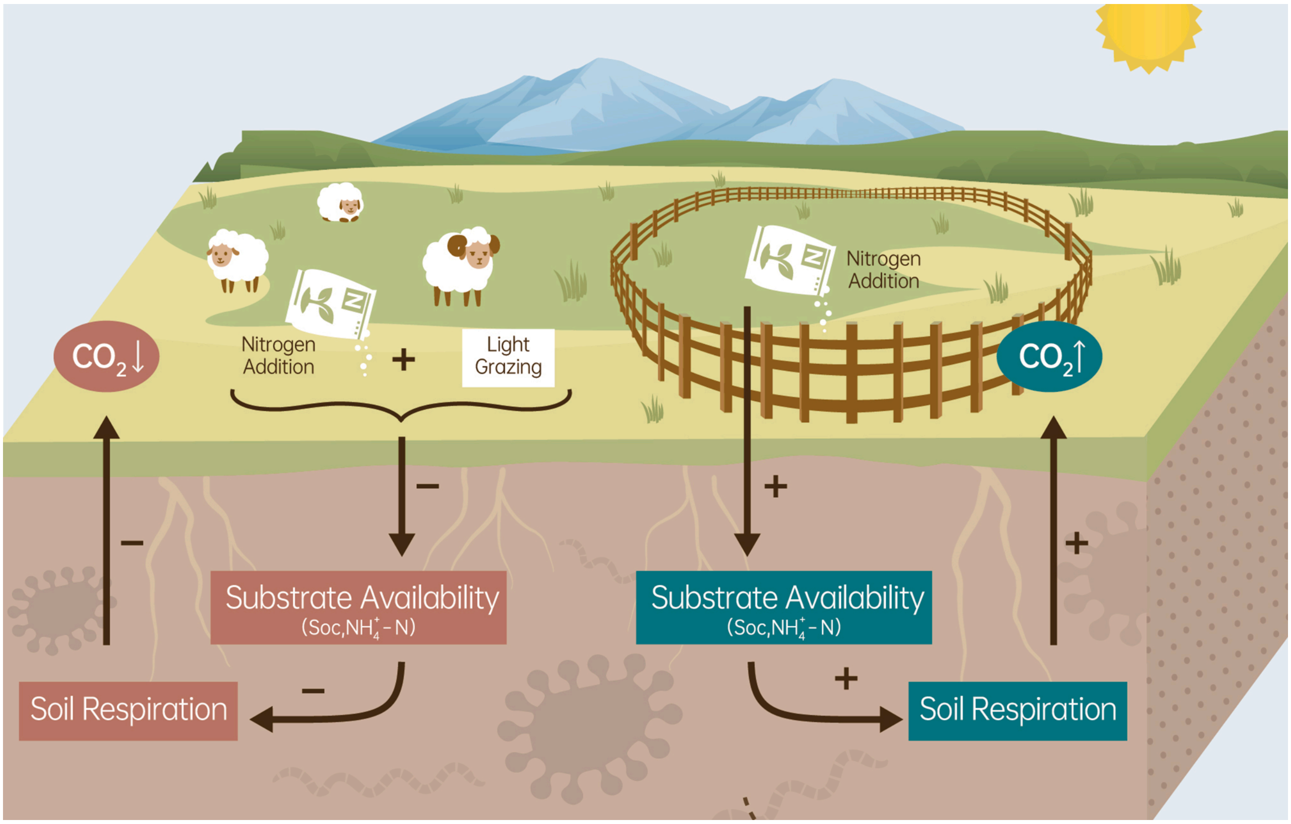

Fig. 6. Conceptual diagram showing the impacts of light grazing with N addition (left side) and N addition alone (right side) on soil CO_2 emissions by changing soil substrate availability. The "+" and "–" arrows indicate negative and positive effects, respectively. The up and down arrows adjacent to the CO_2 icon indicate an increase or decrease in emissions, respectively.

temporal dynamic of soil respiration is not only a function of soil temperature and moisture, but is also modulated by the coupled effects of canopy photosynthesis driven by climatic factors and plant growth (Vargas et al., 2012; Jia et al., 2018). During the non-growing season (e.g., October 2018 in this study), plant photosynthetic activity is suppressed, which results in a reduction in the supply of assimilate substrates to plant roots (Jia et al., 2018), thereby causing inhibition of the soil CO_2 production. As discussed above, semi-arid grasslands are usually N-limited ecosystems. N uptake by plants during the peak growing season and N immobilization by microorganisms later in the growing season are one of the mechanisms of N retention in N-limited ecosystems (Jaeger et al., 1999). The process of microbial N immobilization may stimulate the uptake of atmospheric N_2O due to the consumption of soil available N (Chapuis-Lardy et al., 2007; Audet et al., 2014). Thus, observed negative N_2O fluxes in October 2013 and October 2018 (non-growing season) in this study might be related to soil microbial N immobilization.

4.5. *Implications for grassland management*

To our knowledge, this is the first study to describe the antagonistic effect of long-term grazing and N addition on soil GHGs in semi-arid grassland ecosystem. Our results have important implications for grassland soil GHG mitigation potential under optimized grazing management (e.g., grazing intensity) in the context of global change. Grasslands threatened by global change, such as atmospheric N deposition (approximately 2.5 g N m^{-2} $year^{-1}$ in this region, Ackerman et al., 2019), not only provide key regulating ecosystem services but also must satisfy the increasing demand for livestock products (Hou et al., 2021). Thus, the trade-offs between the sustainable development goals related to grassland and the potential threats of climate change depend mainly on intelligent grassland management regimes. In this study, we found that light grazing intensity could offset 14% of the soil GHG emissions induced by N application. Our results suggest that improved grazing management is needed to achieve GHG mitigation, and light grazing is recommended for semi-arid grassland. Our results might also apply to the sustainable management of semiarid grassland more generally because almost all semi-arid grasslands in the world experience varying degrees of grazing and N enrichment. Furthermore, comprehensive standards of grazing management should be incorporated into land-surface models to improve the prediction of grassland GHG budgets in future global change scenarios.

5. Conclusions

Based on a seven-year field measurement, we found that grazing and N addition had antagonistic effects on soil GHGs emissions in this semi-arid grassland. Light grazing could offset the N addition-induced GHGs emissions by reducing the substrate availability of the soil (Fig. 6). Our results suggest that regulating the grazing intensity of grasslands in the context of climate change is one of the effective climate mitigation measures. Future studies need to consider the interaction of multi-global change factors and human activities on greenhouse gas emissions to provide more accurate information for climate mitigation in agricultural systems.

Data sharing and data accessibility

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Zhen Wang: Methodology, Investigation, Visualization, Data curation, Formal analysis, Software, Validation, Writing – original draft, Writing – review & editing. **Xiumin Zhang:** Investigation, Writing – review & editing. **Mengyuan Wang:** Investigation, Writing – review & editing. **Lan Li:** Investigation, Validation, Writing – review & editing. **An Hu:** Investigation, Writing – review & editing. **Xianjiang Chen:** Investigation, Writing – review & editing. **Shenghua Chang:** Investigation, Project administration. **Fujiang Hou:** Conceptualization, Supervision, Funding acquisition, 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.agrformet.2023.109423.

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