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Effects of grazing and nitrogen application on greenhouse gas emissions in alpine meadow

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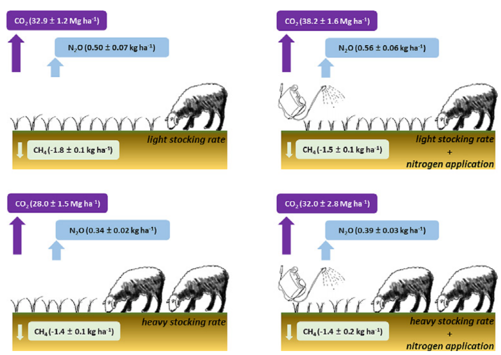
HIGHLIGHTS

- N application increased GHG emissions in alpine meadow.
- Light stocking rate led to an increase in cumulative CO_2 and N_2O emissions.
- Heavy stocking rate with N application showed lower GWP than light stocking rate.
- Nitrogen application and grazing had direct and indirect impacts on GHG fluxes.

GRAPHICAL ABSTRACT



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ABSTRACT

Overgrazing and injudicious nitrogen applications have increased emissions of greenhouse gases from grassland ecosystems. To explore the effects and potential mechanisms of grazing, nitrogen application, and their interaction with greenhouse gas (GHG) emissions, field experiments were conducted on the Qinghai-Tibet Plateau for three consecutive years. Alpine meadow plots were subjected to light (8 sheep ha^{-1}) and heavy (16 sheep ha^{-1}) stocking rates, with or without ammonium nitrate (NH_4NO_3) (90 kg N ha^{-1} yr^{-1}) treatment to simulate soil nitrogen deposition. During early warm growth season (May–June), peak growth season (July–September), and early cold season (October–November), static-chamber gas chromatography was used to analyze the soil's greenhouse gas emissions (CO_2, N_2O, and CH_4). Results indicated that light stocking rate (LG) led to an increase in cumulative CO_2 and N_2O emissions, while also promoting CH_4 uptake. Conversely, heavy stocking rate (HG) produced contrasting outcomes. Additionally, nitrogen applications significantly increased the short-term CO_2 and N_2O fluxes peaks. Combined treatment of nitrogen application and light stocking rate (LG + N) resulted in increased CO_2 and N_2O emissions while decreased CH_4 uptake, consequently leading to a significant increase in global warming potential. According to the structural equation model, we discovered that nitrogen application and grazing affected GHG fluxes both directly and indirectly through their impact on the environmental factors. Our findings suggest that in the context of increasing nitrogen deposition in the Qinghai-Tibet Plateau, a moderate increase in stocking rate is more effective than reducing grazing intensity for mitigating global warming potential in alpine meadow.

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1. Introduction

Global warming effects associated with the elevated emissions of greenhouse gases (GHG) have become a serious environmental concern in recent decades (IPCC, 2014; Kamran et al., 2023). Grazing and nitrogen application, being the most prevalent grassland utilization and management practices, profoundly influence greenhouse gas emissions from grassland ecosystems (Cai et al., 2017; Chen et al., 2014; Dong et al., 2020b). However, previous research has primarily focused on either grazing or nitrogen application, rather than examining their combined effects. Therefore, it is essential to conduct a comprehensive study to assess the impact of grazing, nitrogen application, and their interaction on GHG emissions from grasslands in order to achieve GHG reduction goals.

Grazing is an effective utilization measure of grassland resources (Dong et al., 2020a; Gao et al., 2018). Grazing livestock impacts soil moisture, porosity, and oxygen circulation through trampling (Zhao et al., 2011), alters vegetation biomass and community structure via feeding, and contributes a significant amount of nutrients to the soil (Cardenas et al., 2016; Dong et al., 2020a). Grazing influences the functional communities of plants and soil microorganisms, either directly or indirectly, thereby affecting GHG fluxes within the grassland ecosystem (Chen et al., 2016; Jarque-Bascuñana et al., 2022; Li et al., 2022). Studies have indicated that mild grazing rates results in less stable carbon for microorganisms compared to heavy grazing rates, leading to increased CO_2 emissions from grasslands (Cao et al., 2004; Zhang et al., 2022). However, heavy stocking rate decreases the soil moisture, and the dry soil inhibits microbial activities, decreasing soil respiration (Chen et al., 2015). Meanwhile, moderate grazing increases soil available nitrogen content and nitrification rate, resulting in the production and release of additional nitrous oxide (N_2O) (Rafique et al., 2011), a potent GHG (Hink et al., 2018). In grassland, CH_4 emissions primarily originate from enteric fermentation and dung patches of livestock (Cardenas et al., 2016; Tang et al., 2013), while microbial activity plays a pivotal role in CH_4 oxidation (Bian et al., 2019). In temperate grazing steppe systems, the seasonal or annual CH_4 budget increased with the increase in grazing rate, while the offset of soil CH_4 uptake to the total CH_4 emission from sheep grazing decreased exponentially (Ma et al., 2018b). In recent decades, the unprecedented intensification of grazing pressure has resulted in severe degradation of grasslands worldwide, significantly reducing soil CH_4 uptake (Bardgett et al., 2021; Deng et al., 2014; Li et al., 2023). These contradictions between human activities and ecological balance are primarily attributed to the variations in grassland carrying capacity and grazing system, which imbalance the GHG uptake and emission from grassland (Chang et al., 2021; Renou-Wilson et al., 2016).

Nitrogen is the primary yet limiting nutrient in most of the grassland ecosystems (Fay et al., 2015). In contrast, prolonged excessive nitrogen application increases nitrogen deposition, impeding microbial activity and reducing plant species richness, thereby diminishing soil nutrient sequestration (Li et al., 2015). Excessive nitrogen deposition exacerbates the emissions of GHG into the atmosphere (Zhao et al., 2017). The deposition of nitrogen has been reported to increase N_2O emission in grassland over the Tibetan Plateau because NH_4^+ deposition enhanced the microbial activity involved in soil nitrification and denitrification (Jiang et al., 2010). A long-term (13-years) in situ N addition (20–240 kg N ha^{-1}) experiment from a temperate grassland in Inner Mongolia revealed that soil CH_4 uptake was reduced, while N_2O emission was significantly enhanced (Chen et al., 2019). In alpine grassland of the Tianshan Mountains, CH_4 uptake was increased by N deposition during growing season, but no significant differences were found for all sites outside the growing season (Li et al., 2012). These variations are attributed to the differences in the climatic conditions of the referenced study locations, grazing managements, and nitrogen application levels.

Enhancing grassland productivity while mitigating greenhouse gas emissions are the two essential goals of sustainable grazing management (Gillingham and Stock, 2018). Overgrazing decreases the productivity of both plants and animals within the grassland ecosystem and depletes soil nutrients (Liu et al., 2021; Mysterud, 2006). However, the grazing exclusion reduces income for local herders and increases potential risks to ecosystems, such as wildfires (Cao et al., 2010; Papanastasis, 2009). Alpine meadows constitute a substantial portion of the global greenhouse gas budget and occupy vast expanses of the Tibetan plateau (Kato et al., 2004; Ma et al., 2018a). Recent studies have shown that nitrogen deposition in the region continues to increase (Lü and Tian, 2007; Liu et al., 2013; Chen et al., 2022). Despite increasing levels of nitrogen deposition, there is still a lack of comprehensive understanding regarding the effects of the interaction between stocking rate and nitrogen deposition on GHG emissions in alpine meadows on the Qinghai-Tibet Plateau. Therefore, we hypothesized that the interaction effects of grazing and nitrogen application to pasture would directly affect GHG emissions and indirectly by regulating environmental factors. In our present study, the interactive effects of nitrogen application to pastures and stocking rates were assessed to evaluate (i) the dynamics of seasonal greenhouse gases fluxes; (ii) changes in the environmental properties; (iii) identify the primary factors governing the changes in GHG fluxes; and (iv) the ecological significance of these changes in GHG fluxes.

2. Material and methods

2.1. Study site description

The study was conducted at Maqu Grassland Agriculture Experiment Station of Lanzhou University, Maqu, Gansu, China (101.8333°E, 33.9667°N; 3500 m above sea level) during 2012–2014. The annual average temperature at the experimental site in the last ten years was ~1.8 °C, while the average annual precipitation was about 620 mm. The soil of the study site is classified as Mat-Cryic Cambisols. During the study period (2012–2014), annual average precipitation was 570 mm (Fig. S1). There are typical alpine meadow plants in this area, including *Kobresia graminifolia*, *Elymus nutans*, *Poa pratensis*, *Saussurea hieracioides*, *Aster diplostephioides*, and *Anaphalis lactea*, *Anemone obtusiloba*, which are the dominant species (Yuan et al., 2016).

2.2. Experimental design

In April 2010, a flat alpine meadow with uniform vegetation distribution was selected as the test site. Eighteen rectangular plots, each with an area of 100 m × 100 m were fenced, and a separation border of 10 m was maintained between the adjacent plots. The experiment included six treatments, no-grazing without N application (control), no-grazing with N application (90 kg N ha^{-1} yr^{-1}, N90), light stocking rate without N application (LG), light stocking rate with N application (LG + N), heavy stocking rate without N application (HG), and heavy stocking rate with N application (HG + N). These treatments were arranged in a complete randomized design, each with three replicates. The stocking rate for LG was 8 sheep ha^{-1}, and for HG was 16 sheep ha^{-1}. The 6-month-old Tibetan sheep (40 ± 2 kg) were selected for grazing. Light stocking rate in our study was based on the local management practice. However, with the development of the livestock industry, there has been an increasing demand for grazing intensity. Therefore, we doubled the grazing intensity compared to the local rate. Approximately six times the rate of N deposition (12.3–-17.5 kg N ha^{-1} yr^{-1}) was applied and falls within the region's projected atmospheric deposition by 2050 (Galloway et al., 2004; Lü and Tian, 2007). An aqueous solution of Ammonium nitrate (NH_4NO_3) was applied to the soil on 11th of June and 11th of August each year at 50 % per application. A similar volume (450 L) of distilled water was used for control plots. Grazing was commenced from mid-May to mid-October 2010, with each plot grazing for ten days per month. From 2010 to 2014, the grazing and N applications for each treatment were the same each year.

2.3. Sampling and measurements

2.3.1. Biotic and abiotic factors

Data on air temperature and precipitation were collected from the local meteorological station. Each year, soil and plant samples were taken at the end of June, August, and October. In each plot, three quadrats (0.5 m × 0.5 m) were randomly selected for sample collection following a sigmoid transect. Plant above-ground parts of each quadrat were clipped at the soil surface with scissors. Two soil cores (0–20 cm) were sampled in each quadrat using a cylindrical corer (10.0 cm diameter) and mixed to form a composite sample. Gravel and plant debris were removed from soil samples by sieving (2 mm mesh). Soil samples were air-dried and used for further analysis. The measured environmental properties and methods are presented in Table S1.

2.3.2. Measurement of greenhouse gas fluxes

An optimized method utilizing static chamber gas chromatography was employed to quantify fluxes of greenhouse gases (GHGs) (Shrestha et al., 2009). The chamber base frame was 50 cm long, 50 cm wide, and 50 cm height. Chamber bases were interleaved into the soil at a depth of 10 cm. Static chambers were placed randomly in each plot for every measurement. Four air samples were collected from 9:00 a.m. to 11:00 a.m., representing an average flux over one day. The day on which annual GHG measurements are taken is designated as Day 1. The GHGs fluxes in 2012, 2013 and 2014 were measured on the 1st, 11th and 21st of May to November. In addition, after N application, greenhouse gases were measured every other day over a week.

By using a nylon stopcock fitted with a polypropylene syringe (50 mL), four gas samples were collected within 30 min at intervals of 10 min. LGR Gas Analyzer (908–0015–0000, Los Gatos Research, USA) was used to analyze N_2O, and LGR CH_4/CO_2 Analyzer (908–0011–0001, Los Gatos Research, USA) was used to analyze CO_2 and CH_4 simultaneously. GHG fluxes were calculated as follow:

$$G = \frac{dc \times M \times p \times T_0}{dt \times V_0 \times P_0 \times T} \times H \tag{1}$$

where G is CO_2 (mg m^{-2} h^{-1}), N_2O (μg m^{-2} h^{-1}), and CH_4 (μg m^{-2} h^{-1}) fluxes; change in gas concentration in chamber is measured by dc/dt; molar mass of the gas measured is represented by M (g mol^{-1}); absolute temperature is represented by T (K); atmospheric pressure is represented by P (Pa); chamber height is represented by H (cm). At standard conditions, V_0, P_0, and T_0 correspond to volume (mL), absolute temperature (K), and pressure (Pa).

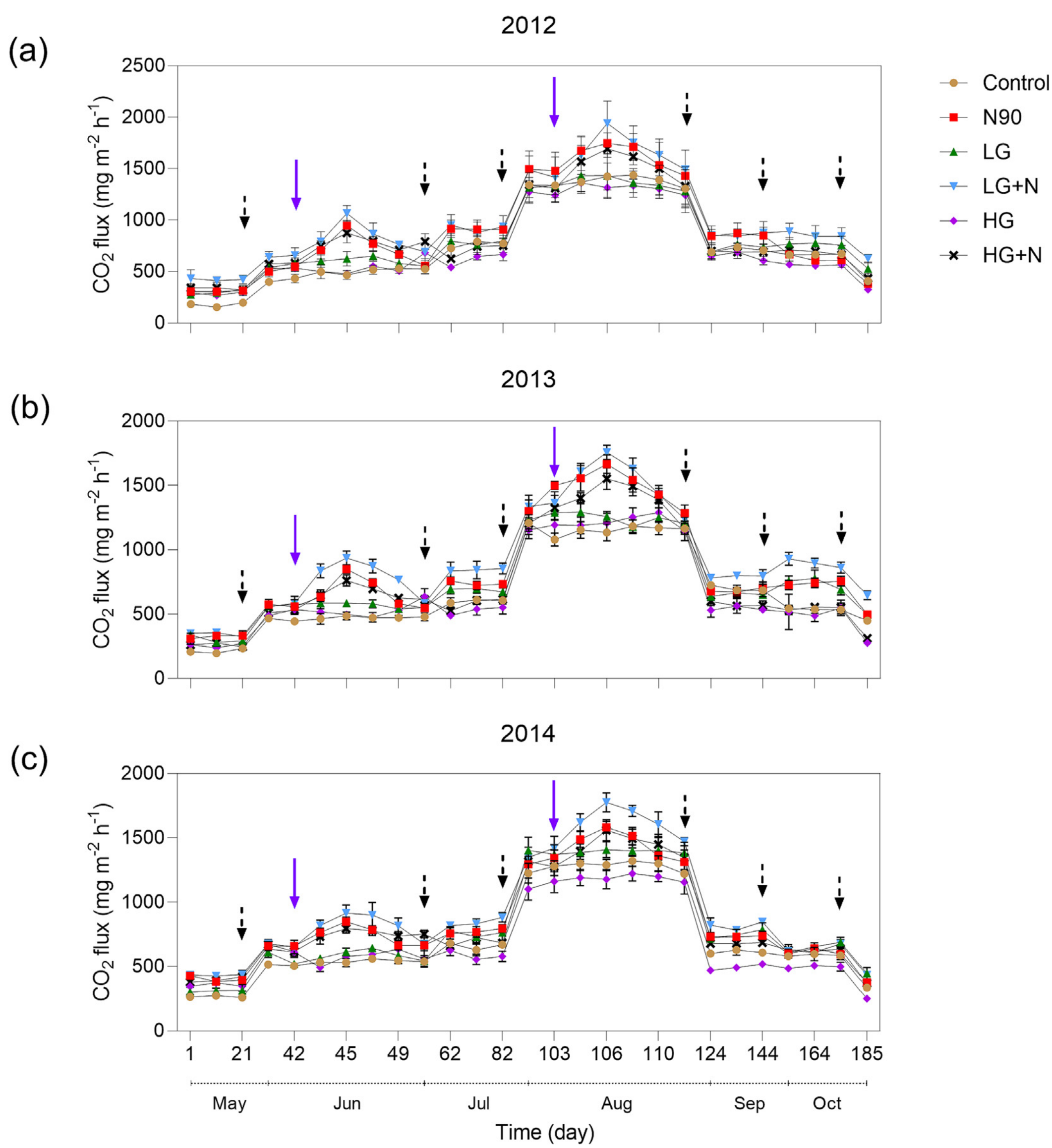

Fig. 1. Effects of nitrogen application and grazing treatments on CO_2 fluxes during 2012 to 2014 (a, b, c). Control: distilled water; N90: nitrogen application; LG: light stocking rate; LG + N: light stocking rate with N application; HG: heavy stocking rate; HG + N: heavy stocking rate with N application. Mean standard error of the data is indicated by the error bar. Solid and dotted arrows represent N application and grazing managements, respectively.

In order to estimate CO_2, N_2O, and CH_4 emissions, the following equation was used:

$$F = \Sigma\frac{(E_i + E_{i+1}) \times (d_{i+1} - d_i)}{2 \times 100} \times 24 \tag{2}$$

where accumulated GHG flux of CO_2, N_2O, and CH_4 is represented by F (kg ha^{-1}); current CO_2, N_2O, and CH_4 emissions flux (mg m^{-2} h^{-1}) is Ei, and the previous emissions flux is E_{i+1}, and d_{i+1}-d_i represents the number of days between two adjacent measurements.

2.3.3. Estimation of global warming potential

Global warming potential (GWP) was estimated using CO_2 as a reference gas, and N_2O and CH_4 emissions were converted into CO_2 equivalents (kg CO_2 eq. ha^{-1}). Over a 100-year period, the default GWP of CH_4 and N_2O emissions is 28 and 265 times that of CO_2 emissions, respectively (IPCC, 2014).

$$\text{GWP} = 28 \times Y_{CH4} + 265 \times Y_{N2O} \tag{3}$$

where the cumulative CH_4 emissions (kg ha^{-1}) and N_2O emissions (kg ha^{-1}) are represented by Y_{CH4} and Y_{N2O}, respectively.

2.4. Statistical analysis

According to the growth characteristics of alpine meadow plants at the experimental area, we divided the total study period (TSP) into three subperiods in each year; early warm growth season (May–June, EWS), peak growth season (July–September, PGS), and early cold season (October–November, ECS). The cumulative GHG emissions and GWP of different treatments in the same year were subjected to one-way ANOVA (Tukey HSD) analysis. The three-year average cumulative emissions of GHGs and GWP between different treatments were subjected to one-way ANOVA analysis. Three-way factorial ANOVA was used to evaluate the effect of the year, study period, and treatment on GHG emissions and GWP. To investigate the relationship between GHGs and environmental factors, the redundancy analysis (RDA) was performed with Canoco 5. Simple linear regression analysis was used to investigate the relationships between the major driving factors identified by RDA and greenhouse gas fluxes. Structural equation model (SEM) was used to estimate the effects of different stocking rates and nitrogen application on annual CO_2, N_2O, and CH_4 fluxes using AMOS (SPSS Inc.). STATA 17.0 (StataCorp LLC) was used to conduct these statistical analyses. Figures were constructed with Prism 9.5 (Graph Pad Inc.). Flow chart of statistical analysis is shown in Fig. S2.

3. Results

3.1. Treatment effects on greenhouse gas emissions fluxes

The CO_2 flux remained higher (1184.61–1923.08 mg m^{-2} h^{-1}) for more than three days after the first N application and later declined but increased again from 11th August, following the second N application event (Fig. 1). During the total study period (TSP), CO_2 fluxes were higher at N application and N application interaction with grazing compared to other treatments. In the case of cumulative CO_2, the effects of year, season, treatment, and their interactions were significant (Table S2). The cumulative CO_2 emissions for LG + N and HG + N treatments were greater than that for LG and HG treatments, respectively (Fig. 4). The three-year average results showed that cumulative CO_2 emissions for LG + N were significantly higher than those of control treatment during early warm growth season (EWS) (50.83 %), peak growth season (PGS) (22.14 %), early cold season (ECS) (34.06 %) and for the total study period (29.52 %) (Table 1). However, the CO_2 emissions for HG were significantly lower than that for control plots during PGS (10.75 %), ECS (16.18 %) and TSP (5.34 %).

Seasonal shift in N_2O fluxes followed a consistent pattern over the three years of the study (Fig. 2). In both EWS and ECS, N_2O fluxes for each treatment were lower than PGS. Nitrogen applications events significantly increased the short-term N_2O fluxes and N_2O fluxes peaks (4.92–14.91 μg m^{-1} h^{-1}, 10.88—27.63 μg m^{-1} h^{-1}), and LG + N had higher N_2O fluxes during this period. The ANOVA results showed significant effects of treatment, season, year, and their interactions on cumulative N_2O emissions (Table S2). Cumulative N_2O emission of LG + N was the highest among the three-year and the average values during 0.122, 0.38, and 0.56 kg ha^{-1} during EWS, PGS, and TSP, respectively (Fig. 5). The cumulative N_2O emission of LG + N during TSP was higher by 38.41 %, 36.38 %, and 31.39 % than that of control, and 11.24 %, 20.66 %, and 16.12 % than HG + N during the three consecutive

Table 1
Three-year average cumulative emissions of greenhouse gases and global warming potential.

		EWS	PGS	ECS	TSP
CO_2 (kg ha^{-1})	Control	5849.69 ± 554.82c	19,429.72 ± 1427.86bc	4180.24 ± 380.53ab	29,459.64 ± 1639.5 cd
	N90	7898.42 ± 643.03ab	22,129.33 ± 1909.11ab	4588.11 ± 543.84ab	34,615.86 ± 1358.15ab
	LG	7144.03 ± 139.62bc	20,727.52 ± 1158.53abc	5060.39 ± 390.04ab	32,931.94 ± 1198.78bc
	LG + N	8823.37 ± 646.9a	23,730.73 ± 911.03a	5602.17 ± 971.34a	38,156.26 ± 1630.22a
	HG	6825.26 ± 674.33bc	17,543.21 ± 1355.88c	3598.08 ± 237.04b	27,966.54 ± 1482.75d
	HG + N	7761.2 ± 996.88ab	19,906.6 ± 1498.47abc	4287.5 ± 489.97ab	31,955.3 ± 2847.85bcd
N_2O (kg ha^{-1})	Control	0.09 ± 0.02a	0.28 ± 0.03bcd	0.05 ± 0.02a	0.41 ± 0.05bc
	N90	0.10 ± 0.02a	0.31 ± 0.01abc	0.05 ± 0.01a	0.46 ± 0.05abc
	LG	0.11 ± 0.03a	0.34 ± 0.02ab	0.05 ± 0.02a	0.50 ± 0.07ab
	LG + N	0.12 ± 0.02a	0.38 ± 0.03a	0.06 ± 0.02a	0.56 ± 0.06a
	HG	0.08 ± 0.01a	0.22 ± 0.04d	0.04 ± 0.02a	0.34 ± 0.02c
	HG + N	0.09 ± 0.01a	0.26 ± 0.04 cd	0.05 ± 0.01a	0.39 ± 0.03bc
CH_4 (kg ha^{-1})	Control	−0.45 ± 0.05a	−1.00 ± 0.02ab	−0.25 ± 0.02ab	−1.70 ± 0.06bc
	N90	−0.43 ± 0.06a	−0.93 ± 0.07ab	−0.26 ± 0.01ab	−1.61 ± 0.11abc
	LG	−0.46 ± 0.04a	−1.07 ± 0.06b	−0.28 ± 0.02b	−1.81 ± 0.10c
	LG + N	−0.37 ± 0.03a	−0.85 ± 0.09a	−0.26 ± 0.00ab	−1.48 ± 0.12ab
	HG	−0.34 ± 0.01a	−0.80 ± 0.05a	−0.24 ± 0.00a	−1.37 ± 0.05a
	HG + N	−0.38 ± 0.06a	−0.81 ± 0.13a	−0.24 ± 0.01a	−1.43 ± 0.18ab
GWP (kg ha^{-1})	Control	11.42 ± 2.29a	45.03 ± 4.70bc	5.18 ± 2.23a	61.62 ± 7.11b
	N90	14.41 ± 2.26a	56.42 ± 1.91abc	6.56 ± 2.28a	77.39 ± 5.62ab
	LG	15.83 ± 4.98a	61.01 ± 4.41ab	5.73 ± 2.57a	82.57 ± 10.21ab
	LG + N	22.23 ± 3.41a	76.77 ± 3.54a	7.49 ± 2.46a	106.49 ± 7.85a
	HG	12.15 ± 1.15a	35.04 ± 6.43c	4.13 ± 2.41a	50.98 ± 3.48b
	HG + N	12.85 ± 1.23a	45.28 ± 7.83bc	5.81 ± 2.02a	63.93 ± 5.50b

Note: EWS: early warm season; PGS: peak growth season; ECS: early cold season; TSP: total study period. Control: distilled water; N90: nitrogen application; LG: light stocking rate; LG + N: light stocking rate with N application; HG: heavy stocking rate; HG + N: heavy stocking rate with N application. Significant differences at the 0.05 levels are indicated by different letters.

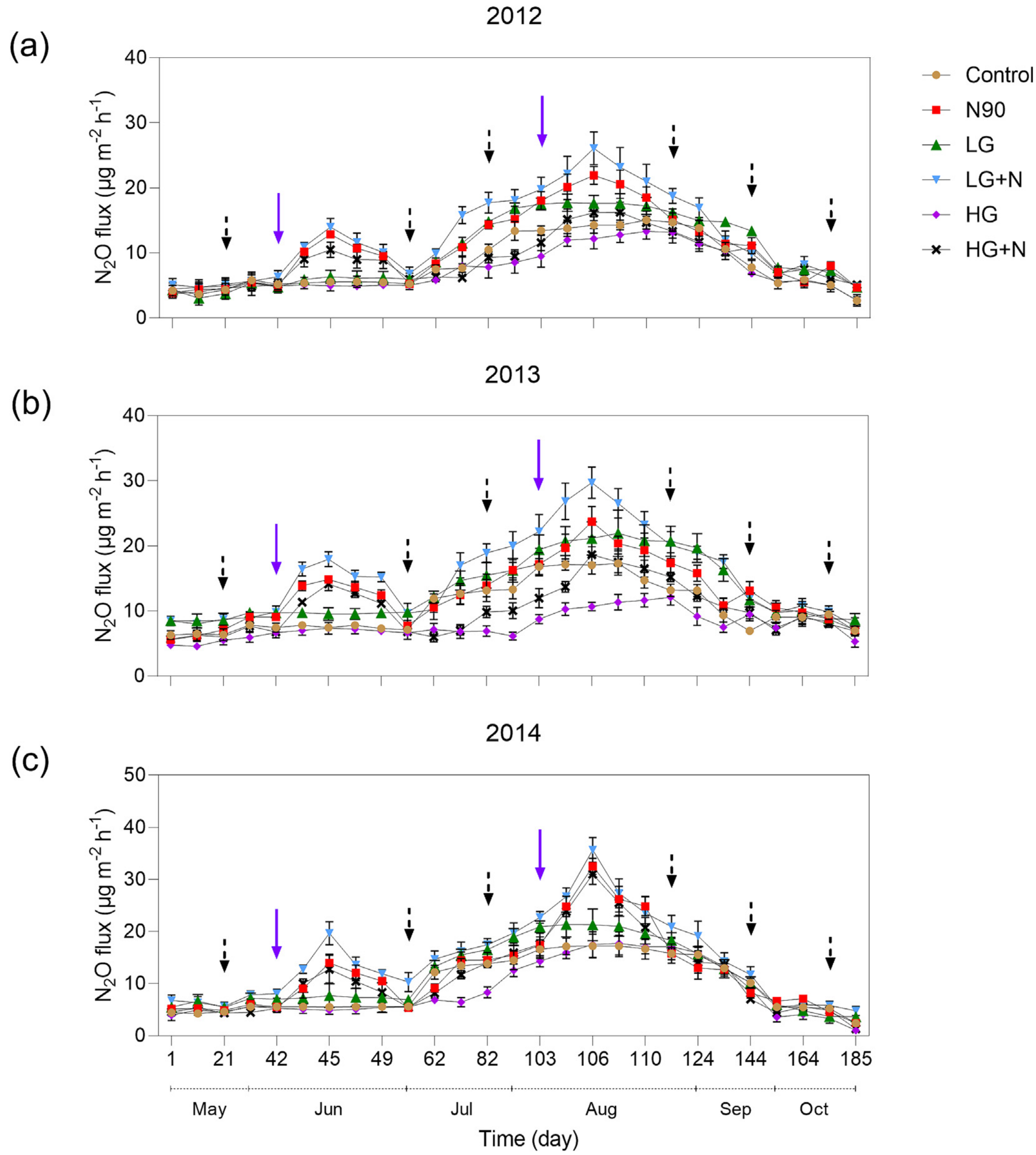

Fig. 2. Effects of nitrogen application and grazing treatments on N_2O fluxes during 2012 to 2014 (a, b, c). Treatment names are abbreviated as described in Fig. 1. Mean standard error of the data is indicated by the error bar. Solid and dotted arrows represent N application and grazing managements, respectively.

years (2012–2014), respectively. Three years' average data showed that the cumulative N_2O emission of LG + N was greater ($p < 0.05$) than that of HG and HG + N in PGS and TSP (Table 1).

Our results depicted negative CH_4 values with N90, indicating that the alpine meadow soils were CH_4 sinks throughout the study period (Fig. 3). Generally, CH_4 uptake was significantly affected by treatment, season, year, and interactions (Table S2). The CH_4 uptake was highest for LG than other treatments during TSP (−1.88, −1.86, and −1.70 kg ha^{-1}) from 2012 to 2014. LG + N significantly reduced CH_4 uptake during PGS and TSP in 2012 (37.91 % and 30.91 %), 2013 (11.66 % and 15.38 %), and 2014 (30.20 % and 23.12 %) compared to LG, respectively (Fig. 6). Three years' average data showed that the CH_4 uptake of LG was higher than that of HG ($p < 0.05$) in TSP (Table 1).

3.2. Treatment effects on global warming potential

Global warming potential (GWP) was significantly affected by treatment, year, season, and their interaction (Table S2). The data for the three seasons followed a similar trend, with the largest GWP values achieved for LG + N treatment. GWP values were significantly higher for N90 (45.53 %, 25.15 %, and 11.87 %) than those of control from 2012 to 2014, respectively (Fig. 7). The GWP of HG + N was significantly higher than HG (20.50 %, 26.03 %, and 28.83 %) during TSP in 2012, 2013, and 2014, respectively. The three-year average data depicted that LG + N had significantly higher GWP ($p < 0.05$) than that of the control (Table 1).

3.3. Relationship of greenhouse gas fluxes with environmental variables

The RDA results showed the relationship of the three GHGs emissions fluxes with biotic and abiotic factors during different growth periods (Fig. S3). During EWS, soil temperature (ST), NH_4^+-N, NO_3^--N, total nitrogen (ST), and below-ground biomass (BGB) were the major driving factors for GHGs fluxes (Fig. S3a). During PGS, ST, NH_4^+-N, and SMBN had the most significant correlation with three GHGs fluxes (Fig. S3b). In ECS, SM, SMBN and NH_4^+-N had the highest contribution to three GHGs fluxes (Fig. S3c). NH_4^+-N, SMBN and ST were the main driving factors for GHGs fluxes in TSP (Fig. S3d). The CO_2 fluxes showed strong and significant positive relationship with soil NH_4^+-N and NO_3^--N (Fig. S4a, d), and presented quadratic relationships with ST, SM, and SMBN ($p < 0.05$) (Fig. S4g, j, m).

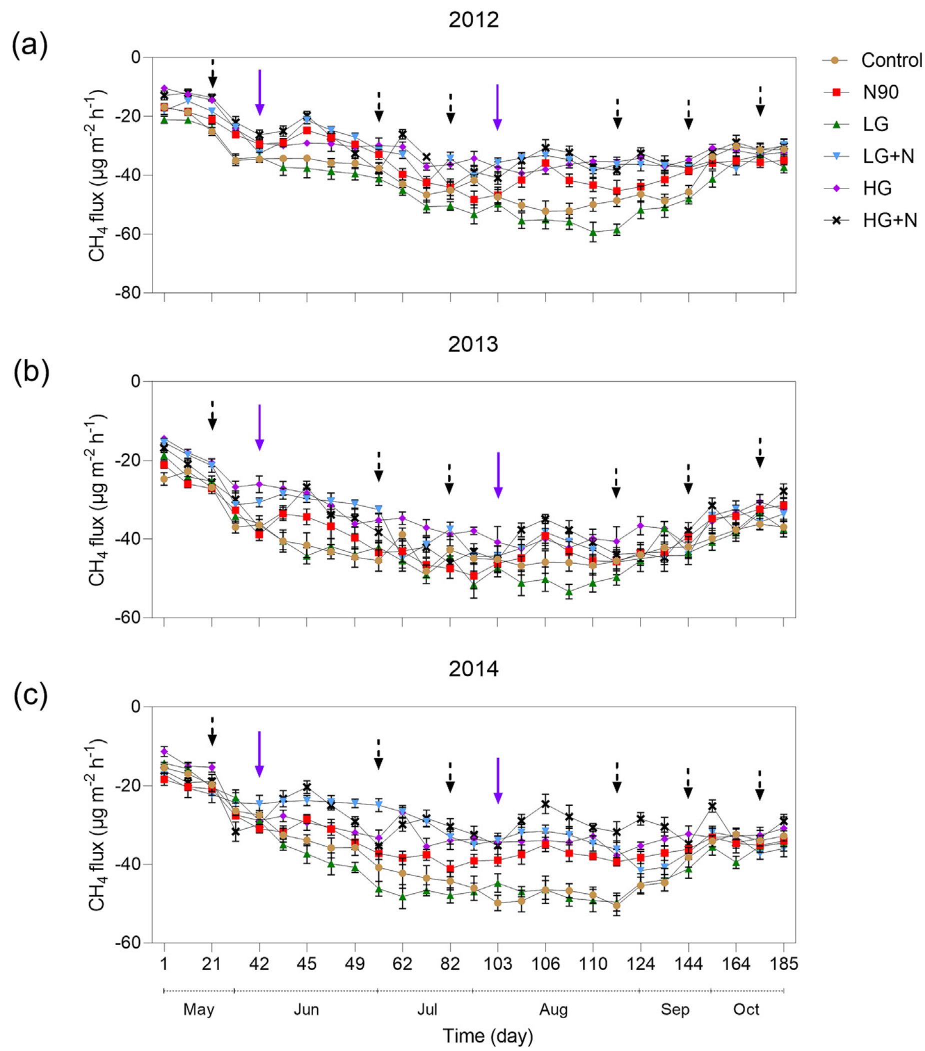

Fig. 3. Effects of nitrogen application and grazing treatments on CH_4 fluxes during 2012 to 2014 (a, b, c). Treatment names are abbreviated as described in Fig. 1. Mean standard error of the data is indicated by the error bar. Solid and dotted arrows represent N application and grazing managements, respectively.

N_2O expressed quadratic relationships with soil NH_4^+-N, NO_3^--N, ST, SM, and SMBN ($p < 0.05$) (Fig. S4b, e, h, k, n). CH_4 uptake presented quadratic relationships with soil NO_3^--N, ST, SM, SMBN ($p < 0.05$) (Fig. S4f, i, l, o), and weakly quadratic with soil NH_4^+-N, however (Fig. S4c).

Structural equation model (SEM) explained 39.2 % of the variance for CO_2 flux, 55.2 % for N_2O and 33.2 % for CH_4 (Fig. S5). Grazing showed positive effect on CH_4 flux directly, and enhanced the negative effects of soil environment, which inhibited CH_4 fluxes. Nitrogen application enhanced the positive effect of soil nutrients on CO_2 flux and negative effect of plant community on N_2O flux. As a result of grazing, the positive effect of soil environment on N_2O flux was diminished.

4. Discussion

4.1. Response of CO_2 emissions to nitrogen application and grazing

Soil microorganisms and root respiration are the primary sources of carbon dioxide emissions (Gao et al., 2018; Cai et al., 2017). In this study, N application stimulated a rapid increase in CO_2 fluxes in the short term, and the emissions were greater than those of the control. Furthermore, there was a positive correlation between CO_2 fluxes and soil NH_4^+-N and NO_3-$^-$N, further validating the achieved results. The possible mechanisms for the increase in CO_2 emissions are: (i) nitrogen application enhances the utilization rate of nitrogen by soil microorganisms (Johnson et al., 2002), and (ii) accelerated root autotrophic respiration pulses occur due to increased accumulation of soil nitrogen (Xiong et al., 2018). Generally, livestock impacts soil nutrient cycling and soil-atmosphere-mediated greenhouse gas fluxes through variations in stocking rates (Pan et al., 2021; Shrestha et al., 2020). This study demonstrates that a decrease in stocking rates leads to an increase in CO_2 emissions (Fig. 3), with soil microbial biomass nitrogen, soil temperature, and soil moisture being the primary drivers of CO_2 flux (Fig. S3, S4). Previous studies have shown that increased soil moisture and temperature regulates the exchange of organic matter and cations, thereby enhancing water holding capacity and nutrient availability (Teague et al., 2011). Increase in soil moisture facilitates dissolution of microbial cells or the release of microbial carbon, which is later mineralized by soil microorganisms, resulting in higher CO_2 emissions (Guenet et al., 2012; Schaufler et al., 2010). However, heavy grazing rate accelerated the removal of grassland plants, leaving behind minor residual biomass, which hinders soil carbon input, resulting in the rapid depletion of soil carbon stocks (Nishigaki et al., 2021) and reducing autotrophic respiration (Li et al., 2015). These effects in response to grazing and N application led to an enhancement in CO_2 emission compared to the single grazing treatment. However, combination of grazing and N application treatment exhibited an antagonistic effect on three-year average cumulative GHG emissions. This is because combined treatment emissions are lower than sum of the emissions from individual grazing and nitrogen application.

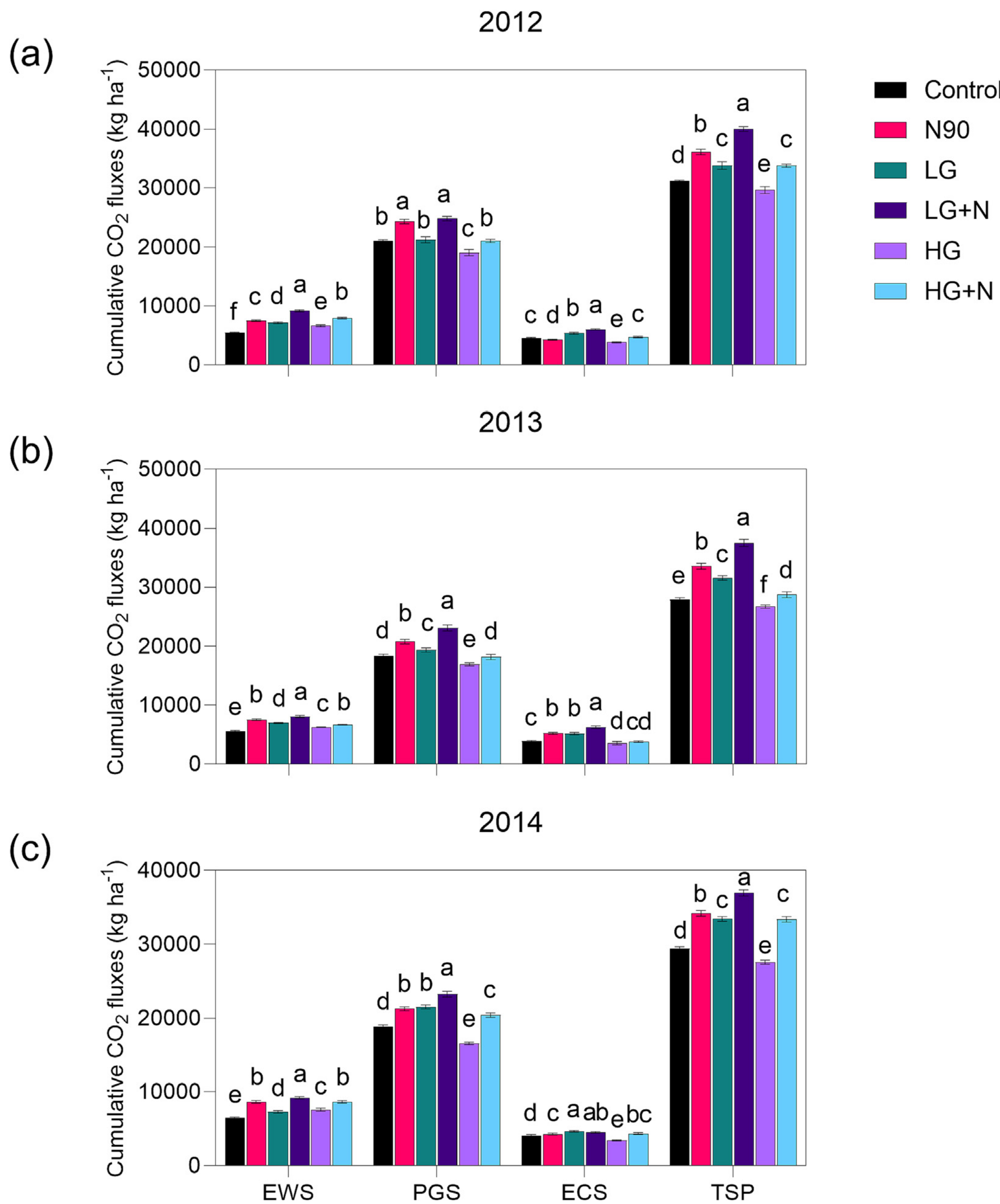

Fig. 4. Effects of nitrogen application and grazing on CO_2 (a, b, c) emission. Treatment names are abbreviated as described in Fig. 1. EWS: early warm season; PGS: peak growth season; ECS: early cold season; TSP: total study period. Mean standard error of the data is indicated by the error bar. Significant differences at 0.05 levels are indicated by different letters.

4.2. Response of N_2O emissions to nitrogen application and grazing

Organic and inorganic nitrogen are primarily derived from livestock excrement and nitrogen applied to grasslands (Xiong et al., 2018). The application of nitrogen to pastures significantly increased N_2O fluxes, resulting in higher cumulative N_2O emissions over the total study period (TSP) (Fig. 5), as a result of the rapid replenishment of soil nutrients and improved biological activity (Bai et al., 2012). Additionally, N_2O emissions exhibited an exponential growth beyond plant demands when nitrogen input surpasses the required amount (Kamran et al., 2023; Walter et al., 2015). A previous study demonstrated a positive correlation between cumulative N_2O emission and increasing nitrogen fertilizer application in alpine meadows (Zhu et al., 2015). NH_4^+-N and soil moisture were identified as primary factors influencing N_2O emissions in the present study (Figs. S3, S4). Specifically, nitrogen application promotes nitrification by stimulating soil nitrifying bacteria, and subsequently increases N_2O emissions (Liang et al., 2016; Tang et al., 2022). When soil nitrogen meets the demands of microorganisms, soil moisture becomes the primary controlling factor in regulating microbial nitrification and denitrification processes (Ma et al., 2020). The regulation of soil and plant water through nitrogen application is the medium that affects N_2O emissions in grassland (i) increasing plant biomass and transpiration; (ii) reducing surface water evaporation due to increased plant coverage and litter; and (iii) promoting oxygen-limited denitrification, which increases N_2O flux through higher soil moisture levels (Drury et al., 2006). However, reduced soil moisture levels can limit microbial denitrification and N_2O emissions due to insufficient nitrogen availability (Li et al., 2017). Grazing generally affects N_2O emissions through: (i) the uneven distribution of manure on grasslands, which provides additional nitrogen input; and (ii) trampling that reduces soil aeration and impacts the rate of soil N transformation (Zhan et al., 2021). Cumulative N_2O emissions were reduced by HG, probably due to reduced soil litter input, limiting microbial nitrification and

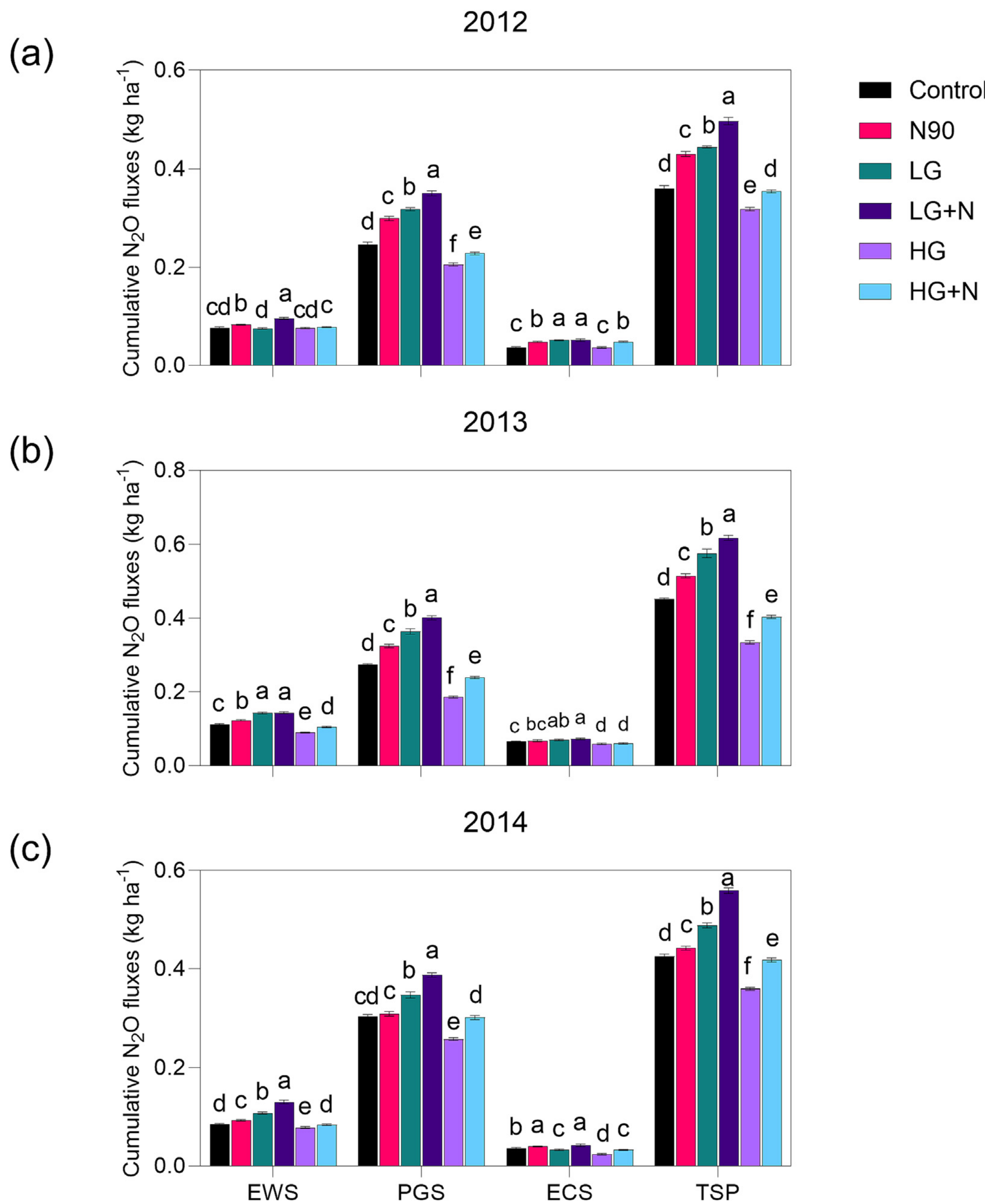

Fig. 5. Effects of nitrogen application and grazing on N_2O (a, b, c) emission. Treatment names are abbreviated as described in Fig. 1. EWS: early warm season; PGS: peak growth season; ECS: early cold season; TSP: total study period. Mean standard error of the data is indicated by the error bar. Significant differences at 0.05 levels are indicated by different letters.

denitrification. On the other hand, HG + N increased cumulative N_2O emissions, which is attributed to the compensatory effects of microorganisms in grassland effectively consuming soil nitrogen due to the nitrogen application (Fig. S5), and increased the accessible substrate pools in the process of N_2O production by soil nitrification and denitrification bacteria (Zhu et al., 2015).

4.3. Response of CH_4 emissions to nitrogen application and grazing

In northern China's temperate and semi-arid grasslands, continuous and rotational grazing reduced CH_4 uptake by 28–42 % and 18–32 %, respectively (Shrestha et al., 2020). In the Eurasian steppe, CH_4 uptake was 10.49 % higher for light stocking rates compared to grazing exclusion (Tang et al., 2019). These differences are due to multiple factors, including environmental differences, grazing rates, and nitrogen application rates and types (Dai et al., 2021). Our results showed that N application reduced CH_4 uptake in alpine meadows. The oxidation of CH_4 is impeded by elevated concentrations of NH_4^+ (Bodelier and Laanbroek, 2004). Adjusting the concentration of NH_4^+ can lead to a reduction in soil CH_4 emissions, thereby improving system performance (Amadori et al., 2022). The response of CH_4 uptake in grassland to nitrogen addition exhibited a non-linear pattern (Fig. S4), which could be attributed to the fluctuation in *pmoA* abundance, responsible for regulating CH_4 oxidation (Liao et al., 2023). Furthermore, nitrogen-induced plant community affects CH_4 uptake through plant rhizosphere effects (Chen et al., 2023a, 2023b). Our study showed that LG increased CH_4 uptake. The return of livestock manure and urine to grassland under LG conditions could alleviate the ammonia restriction of soil methanotrophs, and the increase in soil moisture was beneficial for improving the methanotrophs activity, thereby promoting CH_4 absorption (Li et al., 2020). In addition, trampling reduces soil porosity and permeability, limiting the abundance and diversity of soil methanogens (Li et al., 2020).

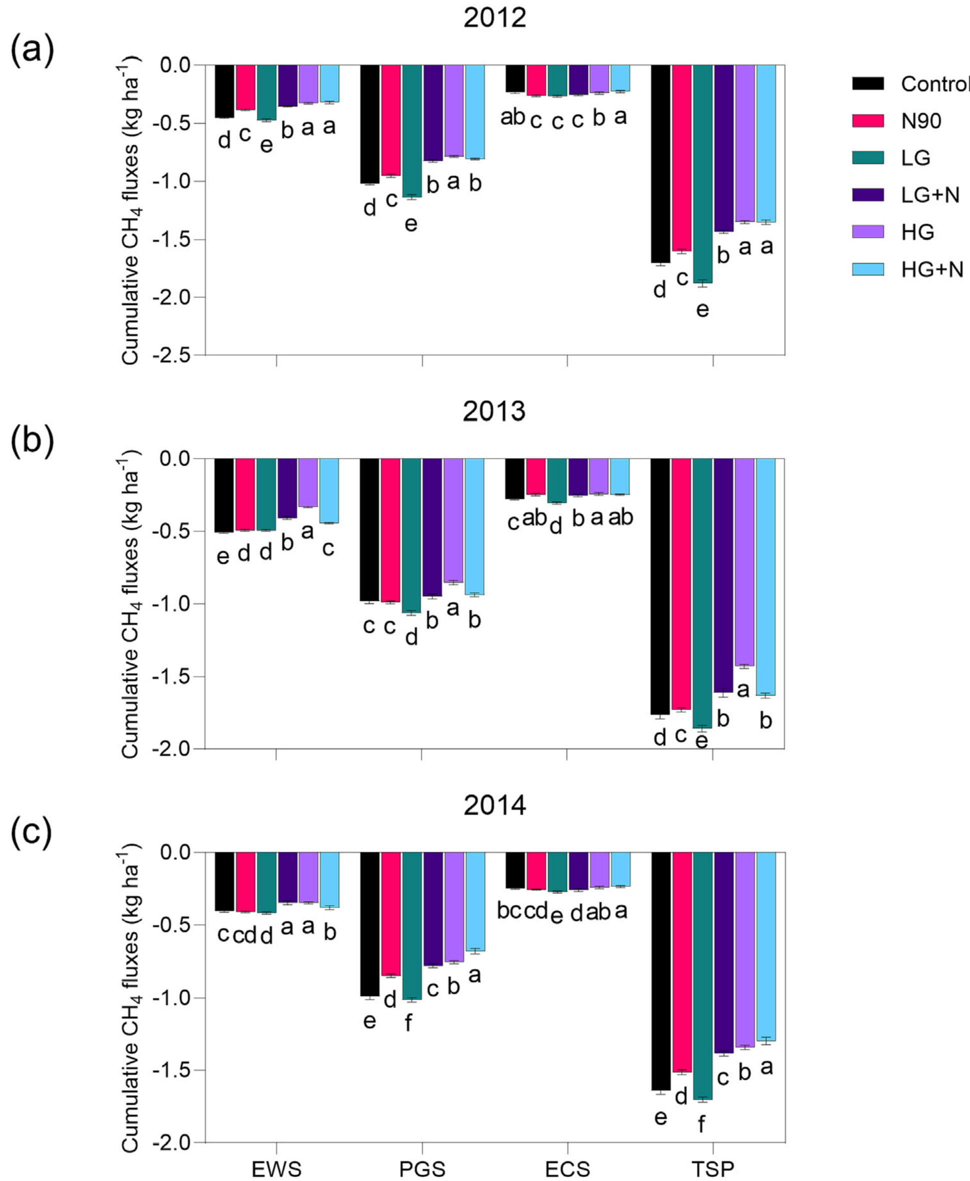

Fig. 6. Effects of nitrogen application and grazing on CH_4 (a, b, c) emissions. Treatment names are abbreviated as described in Fig. 1. EWS: early warm season; PGS: peak growth season; ECS: early cold season; TSP: total study period. Mean standard error of the data is indicated by the error bar. Significant differences at 0.05 levels are indicated by different letters.

4.4. Response of greenhouse gas emissions to plant growth seasons

Greenhouse gases emissions from grassland are closely related to seasons (Cardoso et al., 2017). During the spring soil thawing, the barrier to gas diffusion is disrupted, leading to the release of N_2O that was produced during winter and accumulated in deep unfrozen soil (Li et al., 2021). Moreover, the freeze-thaw cycle of soil generates an environment conducive to the denitrification process. Due to the abundant available carbon and nitrogen, it provides a suitable substrate for microbial activities and promotes the production and release of N_2O (Li et al., 2021). The cumulative N_2O emissions for HG + N and HG were not significantly different in 2013 ECS, because of the low temperature and low soil water content (Fig. S4) limiting the nitrification and denitrification processes (Zhu et al., 2015). GHG fluxes varied with seasons and were related to grazing intensity, temperature, precipitation, and soil properties (Bian et al., 2019; Li et al., 2012, 2020). At the experimental site, the temperature during the PGS was higher than in other seasons and precipitation was abundant. This resulted in an increase in soil microbial activity (Tang et al., 2013). During this period, CO_2 and N_2O emissions, and CH_4 uptake reached their maximum levels. As the non-growing season approached, CO_2 and N_2O emissions gradually decreased. In the later stages of maturity, as plants gradually age and soil temperature and moisture decrease, there was a corresponding reduction in soil microbial activity, vegetation growth, and microbial respiration (Li et al., 2023; Liu et al., 2007), which leads to a rapid decline in CH_4 uptake.

4.5. Response of global warming potential to nitrogen application and grazing

Determining Global Warming Potential (GWP) provides assessment for the trade-offs between greenhouse gas emissions resulting from different stocking rates and nitrogen application in alpine meadows. Our findings indicate that the GWP of LG + N was significantly higher than that of control, while there was no significant difference between HG + N and control (Fig. 7). High grazing rates enhanced the efficiency of net GWP reduction in crop-livestock systems located in southern Brazil, primarily by mitigating organic carbon accumulation (Ribeiro et al., 2020). Grazing in European salt marshes did not significantly impact GWP however, which was offset by a strong interaction with high soil moisture and low soil temperature in the experimental site (Ford et al., 2012). Our research has demonstrated

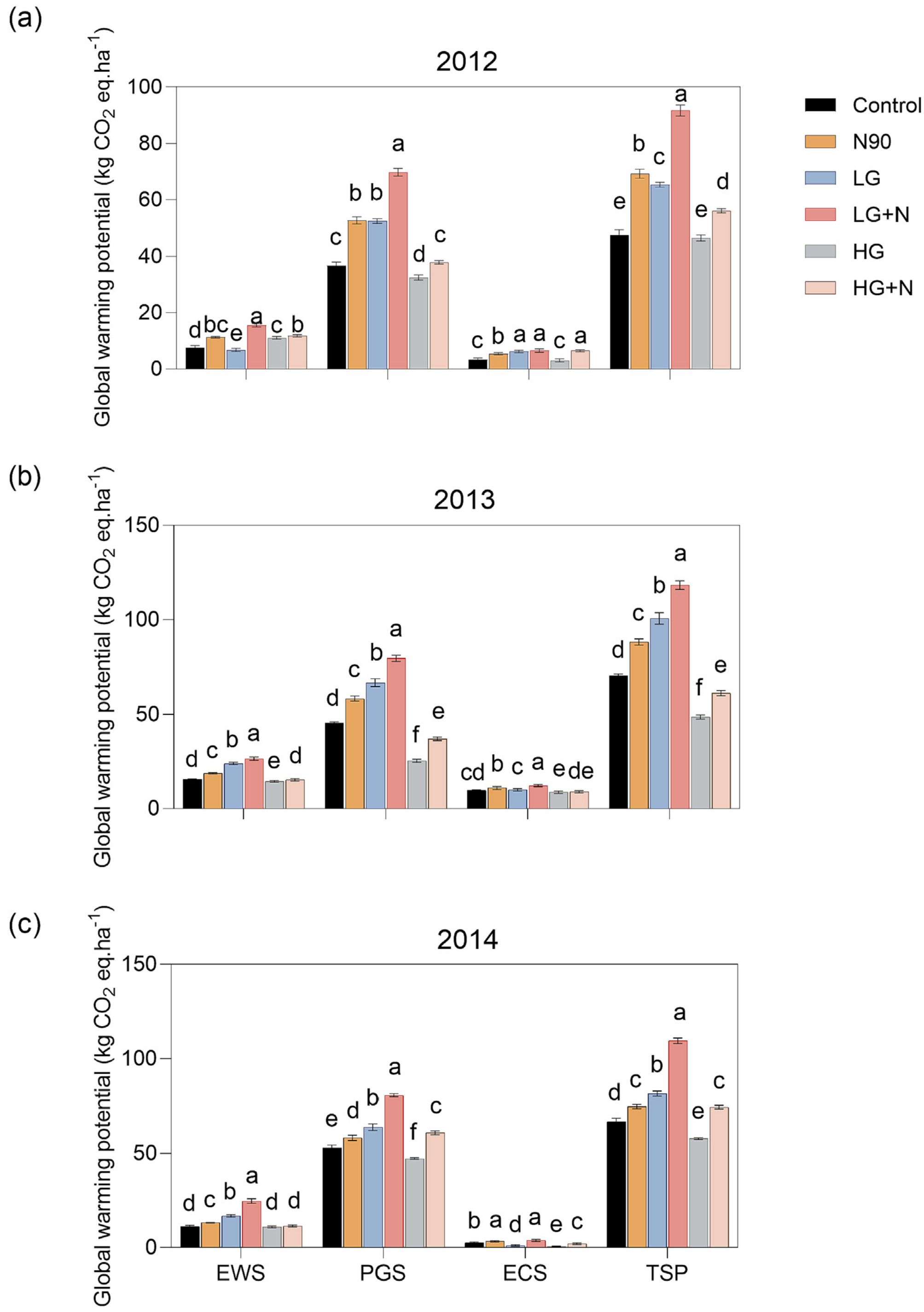

Fig. 7. Effects of N application and grazing on alpine meadow global warming potential during 2012 (a), 2013 (b), and 2014 (c). Treatment names are abbreviated as described in Fig. 1. EWS: early warm season; PGS: peak growth season; ECS: early cold season; TSP: total study period. Mean standard error of the data is indicated by the error bar. Significant differences at 0.05 levels are indicated by different letters.

that the primary cause of increased GWP in alpine meadow systems resulting from light grazing and nitrogen application is a reduction in net CH_4 sink intensity coupled with an increase in N_2O source intensity. On the other hand, grazing and nitrogen application exhibited antagonistic effects on global warming potential (GWP) since grazing could counterbalance greenhouse gas emissions caused by nitrogen addition through enhancing plant availability in soil substrate (Wang et al., 2023). Compared with reduced grazing rates, heavy (16 sheep ha^{-1}) stocking rates with ammonium nitrate (90 kg N ha^{-1} yr^{-1}) treatment would be beneficial to reduce GWP on the alpine meadow of our study sites.

5. Conclusion

The GHG fluxes in alpine meadow ecosystems are sensitive to nitrogen and grazing response. Light stocking rate increased the cumulative CO_2 and N_2O emissions, and increased CH_4 uptake. On the contrary, heavy grazing exhibited an inverse trend, and the highest GHG emissions were evident during the PGS period. Nitrogen application increased the CO_2 and N_2O fluxes but inhibited the CH_4 uptake in alpine meadow. Moreover, the combination of grazing and N application treatment exhibited an antagonistic effect on cumulative GHGs emissions and GWP. These results were the combined direct and indirect effects of nitrogen application and grazing on grassland ecological environment. The results indicate that, in the context of the continuous increase in nitrogen deposition on the alpine meadow of the Qinghai-Tibet Plateau, appropriately increasing the stocking rate is an effective strategy for mitigating greenhouse gas emissions. Although the present study has several limitations due to the use of pre-2014 data, it provides a foundation for greenhouse gas mitigation in light of the gradual increase in nitrogen deposition in the alpine meadows on the Qinghai-Tibet Plateau (Lü and Tian, 2007; Liu et al., 2013), which continues to this day (Ackerman et al., 2019; Liu et al., 2022; Chen et al., 2022). Meta-analysis and model-fitting techniques are recommended to validate scientific hypotheses more accurately by integrating previous experimental and predicted data (Tong et al., 2020; Chen et al., 2023a, 2023b), which will be the primary focus of our future research endeavors.

CRediT authorship contribution statement

Yang You: Writing – original draft, Conceptualization, Data curation, Validation, Formal analysis, Writing – review & editing. **Yang Liu:** Validation, Investigation. **Tianhao Xiao:** Investigation, Methodology. **Fujiang Hou:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Data availability

The authors do not have permission to share data.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164894.

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