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Legume-grass intercropping at low nitrogen input: Achieving the dual goals of lower greenhouse gas emissions and high quality forage productivity in the arid region

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ABSTRACT

Agriculture in the 21st century faces the dual challenges of increasing productivity while mitigating its impact on climate change. Nitrogen fertilizers are indispensable for increasing agricultural productivity, but hasten climate warming through greenhouse gas emissions. This study examined the effects of intercropping Cicer milkvetch (*Astragalus cicer* L.) with Smooth bromegrass (*Bromus inermis* Leyss.) and Ryegrass (*Lolium perenne*) at different nitrogen rates (300, 225, 150, and 75 kg ha^{-1}) on hay yield, forage quality, and greenhouse gas emissions. The decrease in nitrogen fertilizer resulted in a linear decline in the yield and quality of monoculture grasses. However, milkvetch+bromegrass and milkvetch+ryegrass intercrops with 150 kg nitrogen ha^{-1} achieved highest hay yield (9.05–9.12 and 8.17–8.88 t ha^{-1}), crude protein (1.39–1.43 and 1.18–1.23 t ha^{-1}), and relative feed value yield (20.70–20.73 and 19.63–21.33 t ha^{-1}) in 2021–2022, respectively. Legume-grass intercrops presented a good trade-off between environmental sustainability and yield benefits compared to monoculture pastures, indicated by lower greenhouse gas emissions, greenhouse gas intensity, and global warming potential. Milkvetch+bromegrass and milkvetch+ryegrass intercrops with 150 kg nitrogen ha^{-1} reduced cumulative nitrous oxide emissions by 59.11–62.92 % and 56.23–58.24 %, global warming potential by 59.83–64.02 % and 55.32–59.55 %, and greenhouse gas intensity based on hay yield by 70.96–78.47 % and 67.16–75.47 %, and based on crude protein yield by 83.14–88.12 % and 69.41–83.44 % compared to grass monocultures with nitrogen rate of 300 kg ha^{-1} in 2021 and 2022, respectively. Pasture fields acted as net methane sinks. Soil inorganic nitrogen and water contents were the primary regulating factors of greenhouse gas fluxes. Overall, legume-grass intercropping, specifically milkvetch+bromegrass with 150 kg nitrogen ha^{-1}, represents a climate-sustainable strategy for enhancing forage productivity in arid regions.

1. Introduction

The global demand for agricultural production (feed, food, and bioenergy) is projected to increase in the coming decades, primarily driven by the continuous rise in human population (Wang et al., 2021). Agricultural intensification is contributing to meeting the demands, but at the cost of increasing greenhouse gas (GHG) emissions (Tilman, 2020; Zhang et al., 2024). Agricultural production system accounts for one-third of global anthropogenic GHG emissions (Wang et al., 2024; Yang et al., 2024). Among the GHGs, N_2O (nitrous oxide) and CH_4 (methane) are the major contributors to global warming, with a warming potential of about 273 and 27 times greater than carbon dioxide, respectively (IPCC, 2021). In China, agriculture accounts for 17 % of the national total GHG emissions, with N_2O and CH_4 emissions representing about 92 % and 50 % of the total emissions, respectively, surpassing the global average (Raseduzzaman et al., 2024). Hence, agriculture is facing the dual challenges of mitigating GHG emissions while increasing yields. Fortunately, the agricultural sector has potential avenues for reducing GHG emissions associated with production practices (Millar et al., 2018; Wang et al., 2021).

Northwest China is an important agro-pastoral region, where crop production and livestock farming are significantly contributing to

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regional food security and economic development (Kamran et al., 2023a; Ning et al., 2022). The distinct climate and geography of the region make it suitable for livestock farming, which serves as a traditional livelihood for most inhabitants. The livestock production system in the region predominantly relies on monoculture pastures, and their multiple harvests deplete significant amounts of soil nutrients (Ghani et al., 2022; Xu et al., 2023). In recent years, the regional livestock sector has experienced a shortage of forage availability (Ning et al., 2022; Yang et al., 2023). Therefore, pasture growers use excessive amounts of nitrogen fertilizers to boost forage productivity and nutritive quality. However, studies have shown that a major portion of nitrogen fertilizer is lost to the environment due to low crop nitrogen use efficiency, which exacerbates environmental pollution, particularly the N_2O emissions (Lawrence et al., 2021; Yang et al., 2024). In the coming decades, nitrogen fertilizer inputs in developing countries are expected to further expand in line with the growing demands for agricultural products to support the global population (Pan et al., 2024). In parallel, their environmental impacts are projected to nearly double under 'business-as-usual' scenarios (Raseduzzaman et al., 2024; Zhang et al., 2024). Therefore, redesigning the structure of the pastoral production system is imperative to reduce reliance on nitrogen fertilizer while maintaining high forage productivity.

Legumes and grasses are essential sources of livestock feed due to the high protein content in legumes and fiber in grasses (Lithourgidis et al., 2006; Liu et al., 2023). Sole legumes or grasses do not provide sufficient nutritional value (Tahir et al., 2022; Xu et al., 2023). Instead, mixed cropping of legume pastures and grasses can enhance the balance of protein and energy levels by improving nutritional values (Muir et al., 2011; Suter et al., 2015; Xu et al., 2023), promoting optimal growth and development in ruminants (Khatiwada et al., 2020). In addition, legume-cereal intercropping has been reported to offer advantages of promoting soil fertility (Silva et al., 2022), acquisition and utilization of soil water and nutrients (Kermah et al., 2017) and productivity (Raseduzzaman and Jensen, 2017; Tahir et al., 2022; Yan et al., 2022). Legumes with nitrogen-fixation capability facilitate the companion plant species in temporal and spatial patterns (Li et al., 2021), thereby reducing dependence on chemical fertilizers (Jensen et al., 2020; Qiao et al., 2024; Xia et al., 2023), and lowering environmental impacts (Barneze et al., 2020; Pannu et al., 2019; Wang et al., 2021; Xu et al., 2022). Several studies have shown that legume-grass intercropping also enhances soil carbon and nutrients cycling by contributing organic matter from crop roots and foliage (Spohn et al., 2023; Wang et al., 2025). Moreover, intercropping introduces a planned diversity of plant species into the cropping systems, enhancing resilience to pests and diseases, thereby improving food security (Raseduzzaman and Jensen, 2017). However, not all intercropping systems are supposed to provide benefits in terms of all possible metrics (Brooker et al., 2015), and the beneficial effects may vary with the plant species, nitrogen inputs, and climatic conditions (Khatiwada et al., 2020; Li et al., 2020; Liu et al., 2023; Naudin et al., 2010; Suter et al., 2015; Thilakarathna et al., 2016). Therefore, effective nitrogen management and the selection of pasture legume and grass species that exhibit complementarity, rather than intense competition for growth and resource acquisition, are decisive for successful intercropping in a specific region.

Recent studies and meta-analyses on the intercropping of grain legumes with cereals crops indicate the potential for moderation of nitrogen fertilizers (Mudare et al., 2022; Raseduzzaman and Jensen, 2017; Wang et al., 2021; Xia et al., 2023; Zhang et al., 2024). As a result, intercropping of crops has received great attention from the global scientific community. However, the existing studies have mainly focused on grain crops, with limited research addressing the potential of intercropping legume pastures with grasses to mitigate GHG emissions by reducing chemical nitrogen fertilizers while simultaneously maintaining high forage yield and quality. We hypothesized that the intercropping of legume pasture with grasses at low nitrogen input would not only reduce global warming potential and yield-scale GHG emissions but would also maintain higher hay yield and nutritive quality, compared to the traditional cultivation of grass monocultures at high nitrogen inputs. To test this hypothesis, field experiments were carried out in the arid region of northwest China by using a forage legume (Cicer milkvetch) and two widely grown grass species (Smooth bromegrass and Ryegrass). The legume and grass pastures were sown as monocultures and intercrops under different nitrogen fertilizer rates to quantify their effects on forage yield, quality indices, GHG emissions, global warming potential, and GHG intensity based on hay yield ($GHGI_{HY}$), crude protein ($GHGI_{CP}$), and relative feed value ($GHGI_{RFV}$). This study's findings will provide new insights into the sustainable intensification of forage production systems, addressing both environmental and agricultural objectives in arid regions.

2. Materials and methods

2.1. Description of the experimental site

The two-year fixed site experiment was conducted in 2021 and 2022 at the Linze Pratacultural Research Station of Lanzhou University (39°15′N, 100°02′E; 1390 m above sea level), Zhangye City, Gansu, China. The research site is located in the northwest inland arid region of Hexi Corridor and represents a temperate continental arid climate with deficit precipitation and high evaporation rates. In 2021 and 2022, the annual mean temperature was 9.3 °C and 9.6 °C, while the annual precipitation was 140.5 and 142.2 mm, respectively. The annual evaporation rate reaches 2430 mm. Figure S1 presents the meteorological data for daily temperature, precipitation, and solar radiation. Soil at the research site is classified as Aridisols. The study site is flat, and the basic physicochemical properties of the top soil layer (0–20 cm) assessed before the experiment were as follows: pH, 7.8; soil organic carbon content, 9.52 g kg^{-1}; bulk density, 1.05 g cm^{-3}; and total nitrogen content, 1.12 g kg^{-1}.

2.2. Field management and experimental design

The experiment included five pasture plantings: Smooth bromegrass monoculture (BG), Ryegrass monoculture (RG), Cicer milkvetch monoculture (CM), Cicer milkvetch and Smooth bromegrass intercropping (CM+BG), Cicer milkvetch and Ryegrass intercropping (CM+RG). Furthermore, four nitrogen levels were used, including N300 (300 kg ha^{-1}), N225 (225 kg ha^{-1}), N150 (150 kg ha^{-1}), and N75 (75 kg ha^{-1}). The N300 treatment represents conventional fertilizer practice used by the local farmers for monoculture grasses. The treatments were organized in a randomized complete block design with four replications for each treatment. Overall, the experiment included 20 treatments with a total of 80 experimental plots, each with a size of 25 m^2 (5 m × 5 m). A separation ridge of 2.5 m was implemented between two adjacent plots to prevent potential infiltration of nutrients and water. A seeding rate of 18, 16, and 15 kg ha^{-1} was used for bromegrass, ryegrass, and milkvetch monocultures, respectively. The seeding rate for the legume-grass intercrops was half of that used for the corresponding monocultures. The pasture seeds were sown in early August 2020 at a depth of 2–3 cm, maintaining a row-to-row distance of 18 cm. In legume-grass intercrops, seeds for each pasture species were sown in separate rows.

The nitrogen fertilizer source in this experiment was Urea (46 % nitrogen) and applied in two equal split doses, i.e., half at the regreening stage of pastures (20 April in 2021 and 19 April in 2022) and the remaining half during the second irrigation (14 June in 2021 and 15 June in 2022). Three surface irrigations were provided during each pasture growing season, following the local farmers' management practice. In 2021 and 2022, the first irrigation was applied (120 mm) on 20 and 19 April, the second irrigation (120 mm) on 14 and 15 June, and the third irrigation (120 mm) on 15 and 17 August, respectively.

2.3. Measurement and calculation

2.3.1. Determination of precipitation and temperature

Data for precipitation and air temperature were obtained from a local meteorological station. Corresponding to the gas samplings, soil temperature at a depth of 0–10 cm within the gas chamber was measured using portable thermometers (JM624).

2.3.2. Measurement of seasonal fluxes and cumulative emissions of greenhouse gases

The closed static chamber method was used for measuring the fluxes of N_2O and CH_4 during the pasture growing period (Kamran et al., 2022b). Previous studies in the experimental region have shown that over 75 % of greenhouse gas emissions occurred within two weeks following nitrogen fertilizer application (Kamran et al., 2023a; Ning et al., 2022). Therefore, gas samples were collected at 2, 4, 6, 8, 10, 12, and 14 days after each nitrogen fertilizer with irrigation events. Additional samples were taken at 15-day intervals during the rest of the pasture growing period. Gas samples were collected at 10-minute intervals (0, 10, 20, and 30 min) using a 50 mL polypropylene syringe and were immediately injected into pre-evacuated aluminium foil bags (LB-101, Delin, Dalian, China). The bags were transported to the laboratory, where concentrations of N_2O and CH_4 were measured using the LGR gas Analyzers (Los Gatos Research, USA). GHG fluxes were determined using the linear regression slope of concentration over time.

Cumulative CH_4 and N_2O emissions throughout the pasture growing period were determined using a linear interpolation method according to the following equation (Raseduzzaman et al., 2024):

$$\mathrm{CE} = \sum_{i=1}^{n} (E_i + E_{i+1}) \div 2 \times (d_{i+1} - d_i) \times 24$$

Where CE represents cumulative emissions of N_2O and CH_4 throughout the pasture growing period (kg ha^{-1}), i is the ith measurement, while n indicates total sampling instances. E_i is the current N_2O/CH_4 emission flux (μg m^{-2} h^{-1}), E_{i+1} refers to the previous GHG flux measured, while (d_{i+1}-di) indicates the number of days between two consecutive measurements.

2.3.3. Determination of hay yield and forage quality

In each treatment plot, pastures were harvested manually in the central area (2 × 2 m) at 5 cm height. The samples were dried in an oven at 75 °C, and the hay yield was determined. Hay yield in this study represents the accumulated yield from five individual harvests of pastures during each growing season.

The dried samples were ground and sieved through a mesh (1 mm) for the evaluation of forage quality indices. Total nitrogen concentration in samples was measured using a semi-automatic analyzer (Kjeltec 2300, Foss Tecator, Sweden). Crude protein content (%CP) was calculated by multiplying nitrogen concentration by 6.25 (Lithourgidis et al., 2006). The content of acid detergent fiber and neutral detergent fiber was determined with an ANKOM 2000 fiber analyzer (ANKOM Technology, Macedon, NY) following the standard method (Van Soest et al., 1991). The relative feed value (RFV) was calculated from dry matter digestibility and dry matter intake using acid detergent fiber and neutral detergent fiber concentration, respectively (Kamran et al., 2023b, 2022b). The crude protein and relative feed value yield (t ha^{-1}) were determined as the product of dry matter yield and CP% and RFV%.

2.3.4. Calculation of global warming potential and yield-scale GHG emission

Using carbon dioxide as a reference gas, global warming potential (kg CO_{2eq} ha^{-1}) was calculated by converting cumulative greenhouse gas emissions (kg ha^{-1}) into carbon dioxide-equivalent (CO_2.eq.) over a 100-year time scale (IPCC, 2021):

$$\mathrm{GWP} = (273 \times G_{N_2O}) + (27 \times G_{CH_4})$$

Where GWP, G_{N2O}, and G_{CH4} refer to global warming potential, cumulative emissions of N_2O and CH_4, respectively.

The greenhouse gas intensity (GHGI) comprehensively assesses the balance between economic benefits and environmental impacts. The greenhouse gas intensity (t ha^{-1}) was calculated based on the total hay ($GHGI_{HY}$), crude protein ($GHGI_{CP}$), and relative feed value yield ($GHGI_{RFV}$) as follows:

$$GHGI_{HY} = \frac{\text{Global warming potential}}{\text{Hay } yield}$$

$$GHGI_{CP} = \frac{\text{Global warming potential}}{Crude\ \ protein\ \ yield}$$

$$GHGI_{RFV} = \frac{\text{Global warming potential}}{Relative\ \ feed\ \ value\ \ yield}$$

2.3.5. Analyzing soil moisture and mineral nitrogen content

Soil samples for moisture and mineral nitrogen contents were collected simultaneously with the greenhouse gas measurements. Soil cores were collected from three distinct locations within each treatment plot at a depth of 0–20 cm, and subsequently mixed to form a composite sample. Soil moisture was assessed gravimetrically by drying soil samples in an oven at 105 °C until a constant weight was achieved, and was expressed as water-filled pore space (Yang et al., 2024).

$$\mathrm{WFPS}(\%) = (\mathrm{SWC} \times \mathrm{BD}) \div (1 - (\frac{BD}{PD})) \times 100$$

Where WFPS is the water-filled pore space, SWC is the gravimetric water content (%), BD indicates soil bulk density (g cm^{-3}), and PD indicates theoretical soil particle density (2.65 g cm^{-3}).

Soil ammonium and nitrate concentrations were determined by collecting three samples from 0–20 cm soil layers in each treatment plot close to the gas chambers (~10 cm). Soil samples were transported to the lab, passed through a 2 mm mesh, and mixed to form a composite sample for each treatment plot. Soil samples were then extracted with potassium chloride solution (1 mol L^{-1}), and the extracts were analyzed for soil ammonium and nitrate concentration using a continuous flow analyzer (Seal Autoanalyzer 3, Seal Analytical, Norderstedt, Germany) (Xu et al., 2021).

2.4. Statistical analyses

The analysis of variance (ANOVA) was performed using Statistix 10.0 (Tallahassee, FL, USA) to evaluate the independent and interactive effects of pasture species, nitrogen treatments, and year on hay, crude protein, and relative feed value yield, along with cumulative greenhouse gas emissions, global warming potential, and greenhouse gas intensity. The LSD (Least Significant Difference) test was employed for the comparison of significant differences among treatment means at a significance level of 0.05. The normality and homogeneity of variance were checked before analysis. Regression analyses were performed to determine the relationship of greenhouse gas fluxes with soil inorganic nitrogen and moisture content. Figures were generated using Microsoft Excel 2010 and Origin 2024 software.

3. Results

3.1. Hay yield of pastures under reduced nitrogen levels

Results demonstrated significant effects ($P < 0.05$) of the year (Y), pasture species (P), nitrogen rates (N), and their interactions (except Y × N) on hay yield (Table S1, Fig. 1 and S1). The mean hay yield of all treatments in 2022 was higher by 5.75 % compared to that in 2021. The decrease in nitrogen rates led to a decline in hay yield of bromegrass and ryegrass monocultures (Fig. 1A-B). On the other hand, the hay yield of

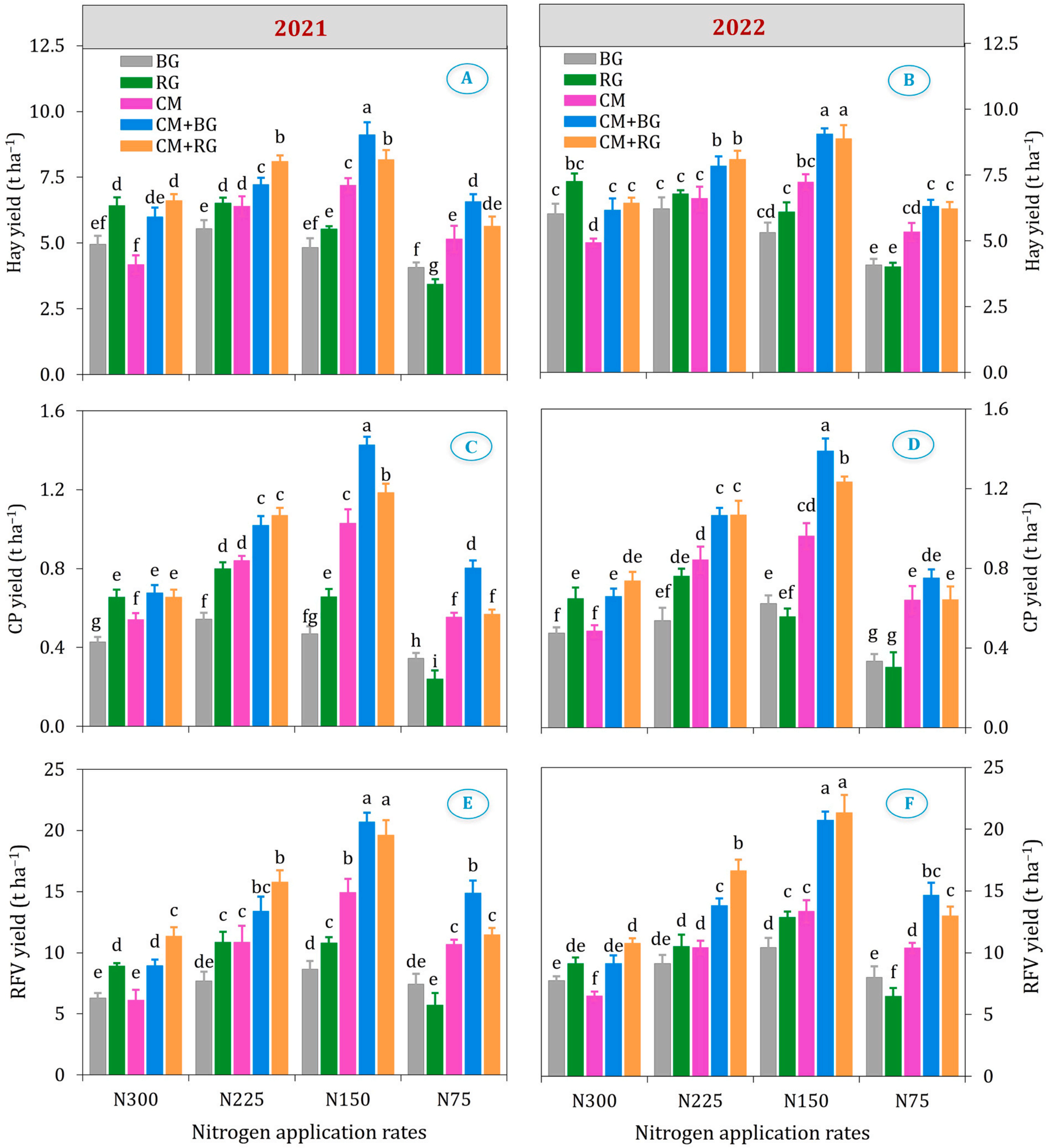

Fig. 1. Interactive effect of pastures and nitrogen rates on hay, crude protein (CP), and relative feed value (RFV) yield. Data are the means of four replicates ± SD. Different lowercase letters indicate significant differences among treatments based on the LSD test at $P < 0.05$. BG, smooth bromegrass; RG, ryegrass; CM, cicer milkvetch; CM+BG, cicer milkvetch+smooth bromegrass intercrop; CM+RG, cicer milkvetch+ryegrass intercrop. N300, N225, N150, and N75 represent nitrogen application rates of 300, 225, 150, and 75 kg ha^{-1}, respectively.

milkvetch, milkvetch+bromegrass, and milkvetch+ryegrass increased with the decrease in nitrogen rate from 300 (N300) to 150 kg ha^{-1} (N150). However, a further reduction in nitrogen (N75, 75 kg ha^{-1}) significantly decreased the hay yield. At each nitrogen treatment (except for N300 in 2022), the hay yield of milkvetch+bromegrass and milkvetch+ryegrass was significantly higher than that of monocultures in both years (Fig. 1A-B). Overall, the highest hay yield was achieved by milkvetch+bromegrass with N150 treatment (9.12 and 9.05 t ha^{-1}), followed by milkvetch+ryegrass (8.17 and 8.88 t ha^{-1}) in 2021 and 2022, respectively (Fig. 1A-B).

3.2. Crude protein and relative feed value of pastures under reduced nitrogen levels

The effect of year, pasture species, nitrogen rates, and their interactions (except Y × N) were significant ($P < 0.05$) for crude protein and relative feed value yield (Table S1, Fig. 1 and S1). The mean crude protein and relative feed value yield in 2022 were greater by 4.61 % and 4.82 % compared to those in 2021, respectively. In general, the crude protein and relative feed value yield of pastures increased with the decrease in nitrogen rate from 300 to 150 kg ha^{-1} (Fig. 1C-F). Nevertheless, too much reduction in nitrogen (75 kg ha^{-1}) significantly decreased the crude protein and relative feed value yield of pastures compared to that at 150 kg ha^{-1}. Milkvetch+bromegrass intercrop with N150 treatment achieved the highest crude protein yield (1.43 and 1.39 t ha^{-1}) and relative feed value yield (20.70 and 20.73 t ha^{-1}), followed by milkvetch+ryegrass with N150 treatment (CP, 1.18 and 1.23 t ha^{-1}; RFV, 19.63 and 21.33 t ha^{-1}) in 2021 and 2022, respectively (Fig. 1C-F).

3.3. Seasonal dynamics of GHG fluxes in pasture fields under different nitrogen levels

The seasonal pattern of N_2O fluxes from pasture fields varied significantly with nitrogen rates but presented similar trends in 2021 and 2022 (Figs. 2 and 3). Two peak fluxes of N_2O emissions were evident within each growing season, intrinsically linked to the timings of nitrogen fertilizer and irrigation (Figs. 2 and 3). The emission fluxes initially increased, reaching peak values (347.0 –356.6 μg N_2O-N m^{-2} h^{-1}) after eight days of the first fertilizer and irrigation event on 20 April 2021 and 19 April 2022 (Fig. 2). The fluxes then gradually decreased, reaching relatively low values within two weeks (< 70.0 μg N_2O-N m^{-2} h^{-1}). After the application of fertilizer and irrigation for the second time on 14 June 2021 and 15 June 2022, the emission fluxes elevated again, attaining a second peak (331.5 –344.7 μg N_2O-N m^{-2} h^{-1}) within one week (Figs. 2 and 3). Thereafter, the emission fluxes subsequently declined to lower levels (< 56 μg N_2O-N m^{-2} h^{-1}) and persisted until the growing season ended. The third irrigation event (with no fertilizer) on 15 August 2021 and 17 August 2022 showed no significant effects on N_2O fluxes (Fig. 3).

The CH_4 fluxes exhibited notable seasonal variations, closely linked to nitrogen fertilizer and irrigation events. Both negative and positive fluxes were evident in each growing season, indicating soil CH_4 uptake

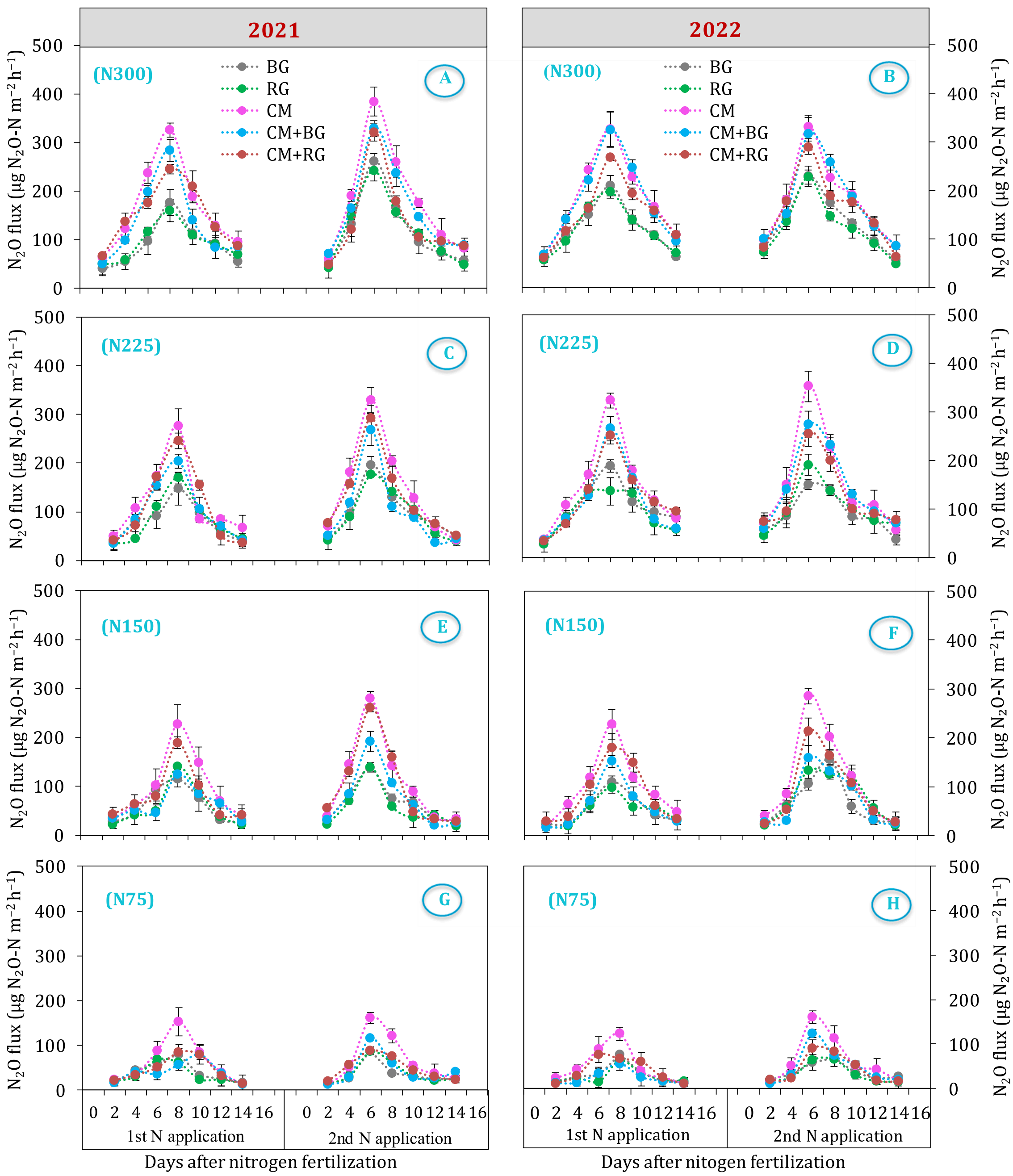

Fig. 2. Dynamics of daily N_2O fluxes over two weeks after nitrogen fertilizer application (followed by irrigation). Data are the means of four replicates ± SD. Treatment abbreviations are the same as explained in Fig. 1.

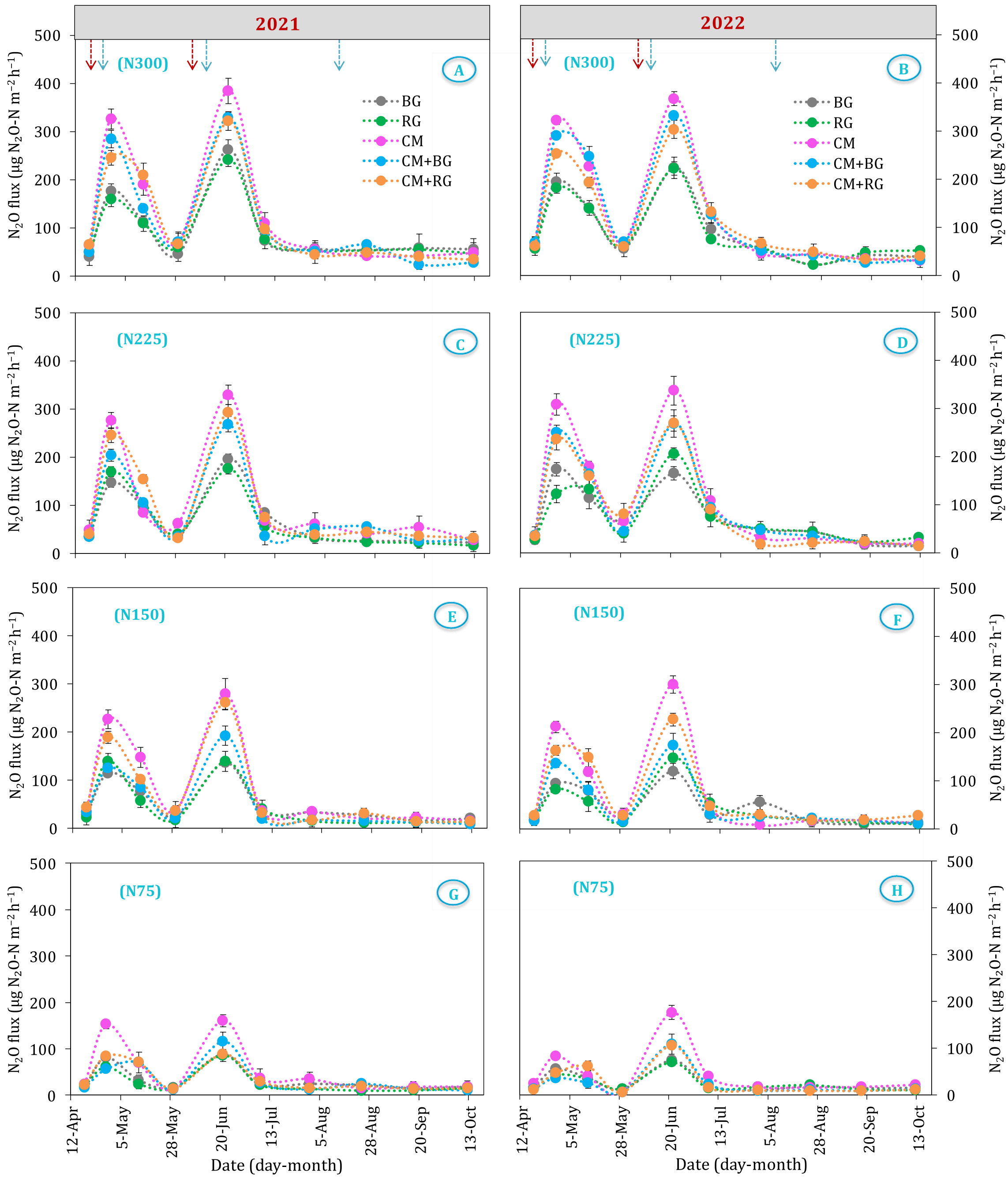

Fig. 3. The N_2O fluxes in pastures with different nitrogen rates. Data are the means of four replicates ± SD. Red and blue arrows indicate the timings of fertilizer and irrigation events, respectively. Treatment abbreviations are the same as explained in Fig. 1.

and emissions, respectively (Figs. 4 and 5). During the early growing season, CH_4 uptake fluxes were predominant, which gradually declined and were replaced by emission fluxes after two weeks of the first fertilizer application with irrigation on 20 April 2021 and 19 April 2022 (Fig. 4). Following the second nitrogen fertilization and irrigation on 14 June 2021 and 15th June 2022, CH_4 values tended to be more negative and CH_4 uptake reached peak values ranging (from −24.2 to −31.3 µg CH_4-C µg m^{-2} h^{-1}). A successive peak of CH_4 uptake (−30.7 to −34.3 µg CH_4-C m^{-2} h^{-1}) occurred shortly after the final irrigation on 15 August 2021 and 17 August 2022 (Fig. 5). Afterwards, the CH_4 uptake exhibited a gradual decline, with values approaching less negative or even positive at the end of the pasture growth period.

3.4. *Cumulative GHG emissions from pasture fields under different nitrogen levels*

Pastures, nitrogen rates, and year showed significant effects ($P < 0.05$) on cumulative N_2O emissions (Table S1, Fig. 6 and S3). The various interaction effects were also significant, except for Y × P and Y × P × N. Cumulative N_2O emission in 2022 (2.06 kg N_2O-N ha^{-1}) reflected a 20.47 % increase compared to that in 2021 (1.71 kg N_2O-N ha^{-1}). Cumulative N_2O emissions in all pastures exhibited a linear decrease corresponding to the reduction in nitrogen rates (Fig. 6A-B). At high nitrogen rates (300 and 225 kg ha^{-1}), cumulative N_2O emissions were greater in milkvetch, milkvetch+bromegrass, and milkvetch+ryegrass intercrops than in grass monocultures. However, no significant difference in N_2O emissions was observed between the

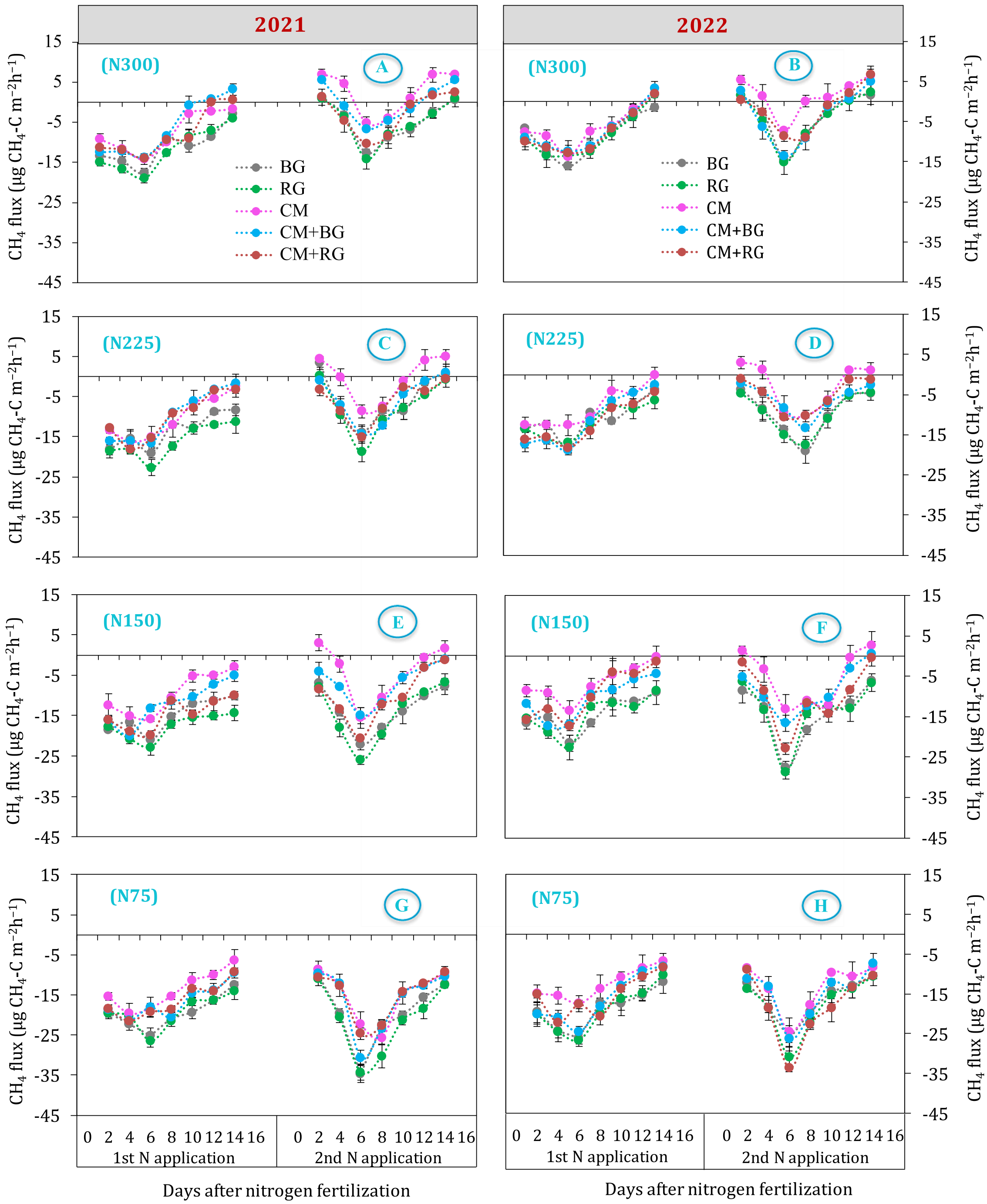

Fig. 4. Dynamics of daily CH_4 fluxes over two weeks after nitrogen fertilizer application (followed by irrigation). Data are the means of four replicates ± SD. Treatment abbreviations are the same as explained in Fig. 1.

legume-grass intercrops and grass monocultures at nitrogen rates of 150 and 75 kg ha^{-1}. Among all the treatments, highest cumulative N_2O emissions were observed in milkvetch (3.79 and 4.31 kg N_2O-N ha^{-1}), followed by milkvetch+bromegrass (3.27 and 3.74 kg N_2O-N ha^{-1}), and milkvetch+ryegrass (3.28 and 3.99 kg N_2O-N ha^{-1}) with N300 treatment in 2021 and 2022, respectively (Fig. 6A-B). The lowest N_2O emissions were achieved in bromegrass (0.56 and 0.55 kg N_2O-N ha^{-1}), ryegrass (0.44 and 0.50 kg N_2O-N ha^{-1}), and milkvetch+bromegrass (0.60 and 0.46 kg N_2O-N ha^{-1}) with N75 treatment in 2021 and 2022, respectively.

Pastures, nitrogen rates, year, and their interaction showed significant effects ($P < 0.05$) on the cumulative soil CH_4 uptake (Table S1, Fig. 6 and S3). The cumulative CH_4 uptake values fluctuated from −0.12 to −0.76 kg CH_4-C h^{-1} in 2021 and from −0.09 to −0.70 kg CH_4-C h^{-1} in 2022. The cumulative CH_4 uptake across all pastures exhibited a linear increase following the decrease in nitrogen rates. However, CH_4 uptake was higher in grass monocultures while the uptake was lower in legume monoculture plots at each nitrogen rate (Fig. 6C-D). Overall, the maximum CH_4 uptake was observed in bromegrass (−0.67 and −0.69 kg CH_4-C ha^{-1}) and ryegrass (−0.76 and −0.70 kg CH_4-C ha^{-1}) with N75 treatment in 2021 and 2022, respectively (Fig. 6C-D). On the other hand, the lowest uptake was achieved in milkvetch (−0.12 and −0.09 kg CH_4-C ha^{-1}), milkvetch+bromegrass (−0.12 and −0.17 kg CH_4-C ha^{-1}), and milkvetch+ryegrass (−0.19 and −0.17 kg CH_4-C ha^{-1}) with N300

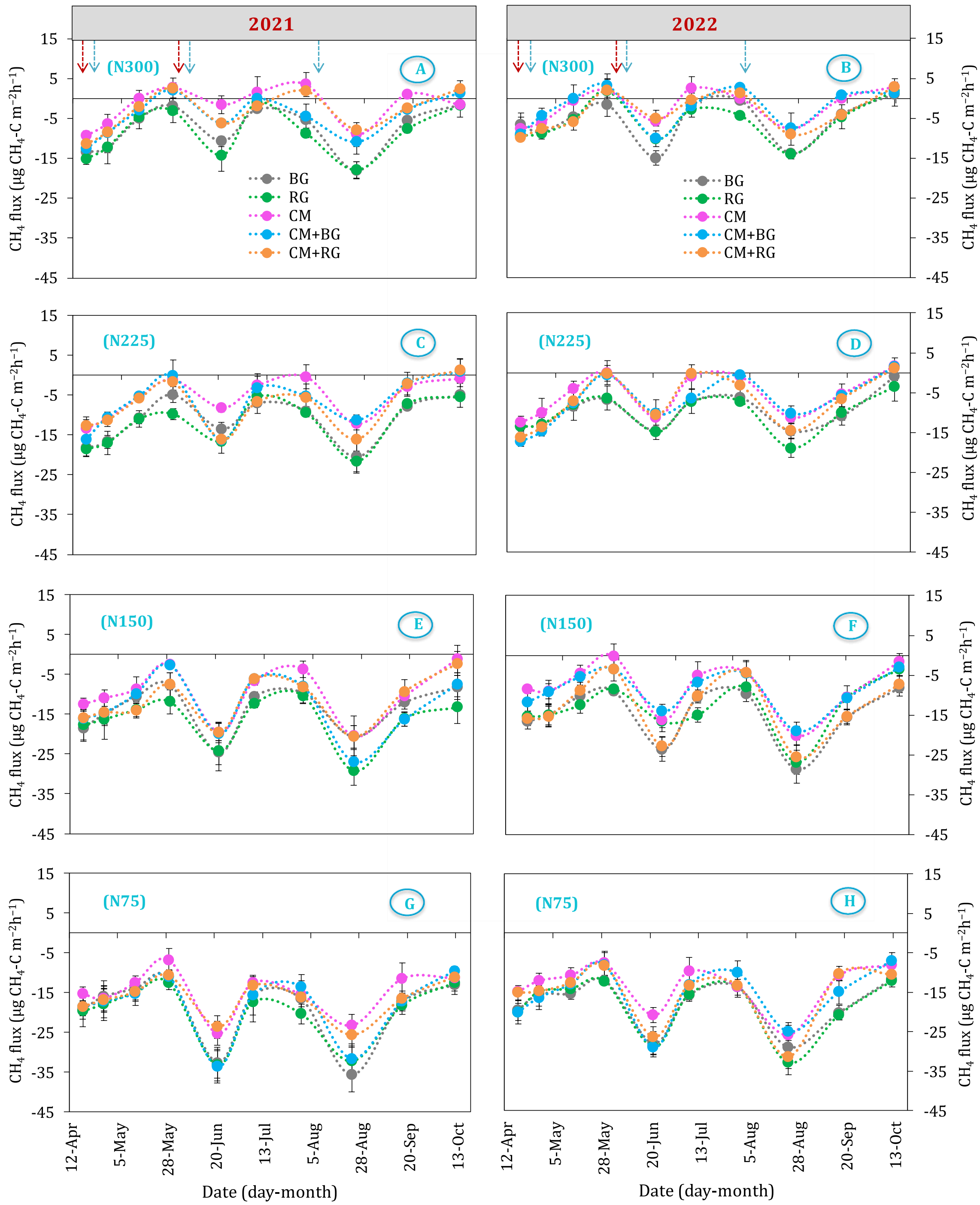

Fig. 5. The CH_4 fluxes in pastures with different nitrogen rates. Data are the means of four replicates ± SD. Red and blue arrows indicate the timings of fertilizer and irrigation events, respectively. Treatment abbreviations are the same as explained in Fig. 1.

treatment in 2021 and 2022, respectively.

3.5. *Effects of pastures and nitrogen inputs on global warming potential and yield-scale GHG emissions*

Pasture species, nitrogen treatments, year, and their interactions showed significant effects ($P < 0.01$) on annual global warming potential (Table S1, Fig. 6 and S3). The global warming potential in 2022 was greater by 16.89 % compared to that in 2021. At high nitrogen rates (300 and 225 kg ha^{-1}), the global warming potential values were greater for legume monoculture and legume-grass intercrops (Fig. 6E and F). However, no significant difference was observed between legume-grass intercrops and grass monocultures at nitrogen rates of 150 and 75 kg ha^{-1}. The highest values of global warming potential were observed for milkvetch (1032.32 and 1173.99 kg $CO_{2\text{-eq}}$ ha^{-1}), followed by milkvetch+bromegrass (889.23 and 1016.34 kg $CO_{2\text{-eq}}$ ha^{-1}), and milkvetch+ryegrass (891.18 and 1064.05 kg $CO_{2\text{-eq}}$ ha^{-1}) with N300 treatment in 2021 and 2022 (Fig. 6E and F). The lowest values of global warming potential were recorded for bromegrass (134.19 and 130.92 kg $CO_{2\text{-eq}}$ ha^{-1}), ryegrass (98.03 and 117.84 kg $CO_{2\text{-eq}}$ ha^{-1}), milkvetch+bromegrass (147.81 and 111.00 kg $CO_{2\text{-eq}}$ ha^{-1}), and milkvetch+ryegrass (158.40 and 140.82 kg $CO_{2\text{-eq}}$ ha^{-1}) with N75 treatment

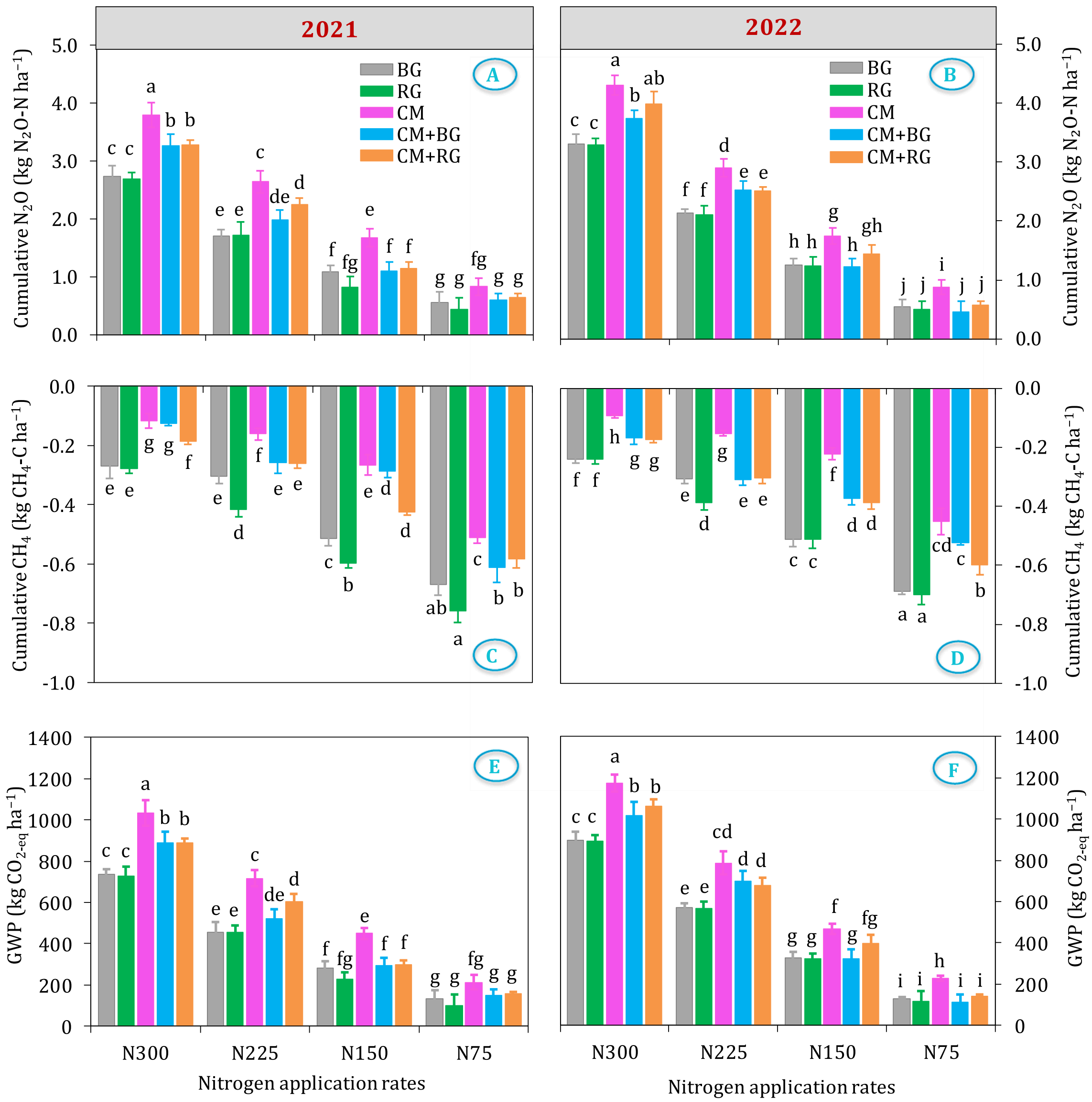

Fig. 6. Interactive effect of pastures and nitrogen rates on cumulative GHG emissions and global warming potential (GWP). Data are the means of four replicates ± SD. Different lowercase letters indicate significant differences among treatments based on the LSD test at $P < 0.05$. Treatment abbreviations are the same as explained in Fig. 1.

in 2021 and 2022, respectively.

In addition, pastures, nitrogen rates, year, and their interactions had a significant ($P < 0.01$) effect on $GHGI_{HY}$, $GHGI_{CP}$, and $GHGI_{RFV}$ (Table S1, Fig. 7 and S4). The GHGI values in all pastures were maximum at high nitrogen input and exhibited a linear decline with decreasing nitrogen rates (Fig. 7). The interactive effect of treatments depicted the highest $GHGI_{HY}$ (247.14 and 237.76 kg t^{-1}), $GHGI_{CP}$ (1907.99 and 2421.85 kg t^{-1}), and $GHGI_{RFV}$ (168.61 and 180.85 kg t^{-1}) for milkvetch with N300 treatment in 2021 and 2022, respectively. The lowest $GHGI_{HY}$, $GHGI_{CP}$, and $GHGI_{RFV}$ values were achieved for the milkvetch+bromegrass intercrop with N150 and N75 treatments.

3.6. Correlation analysis

The relationship of seasonal GHG fluxes with soil nitrogen contents (NO_3^- and NH_4^+) and soil moisture content (WFPS) was assessed and presented in Fig. 8. Soil N_2O fluxes in all pastures presented a significant ($P < 0.001$) positive correlation with soil nitrogen and moisture content. Moreover, a significant ($P < 0.001$) and linear relationship was observed between CH_4 fluxes and soil nitrogen contents. However, the CH_4 emission fluxes presented a significant ($P < 0.001$) but negative correlation with soil moisture content (Fig. 8).

4. Discussion

4.1. Legume-grass intercropping at low nitrogen input increases productivity

Different crops and pasture species demonstrate distinct responses to nitrogen fertilizer rates (Ning et al., 2022; Xia et al., 2023). Research highlighted a positive relationship between pasture growth and nitrogen fertilizers (Rostamza et al., 2011; Xu et al., 2023). The present study showed that grass monocultures achieved the highest hay yields at a nitrogen rate of 225 kg ha^{-1}, with no additional yield improvement observed at high nitrogen (300 kg ha^{-1}). This is because cereal monocultures with poor root growth and development in arid conditions are unable to effectively utilize high nitrogen inputs. Nevertheless, legume-grass intercrops (CM+BG and CM+RG) achieved maximum hay yield at a nitrogen rate of 150 kg ha^{-1}. These findings correspond with those of previous studies indicating an increase in productivity with the

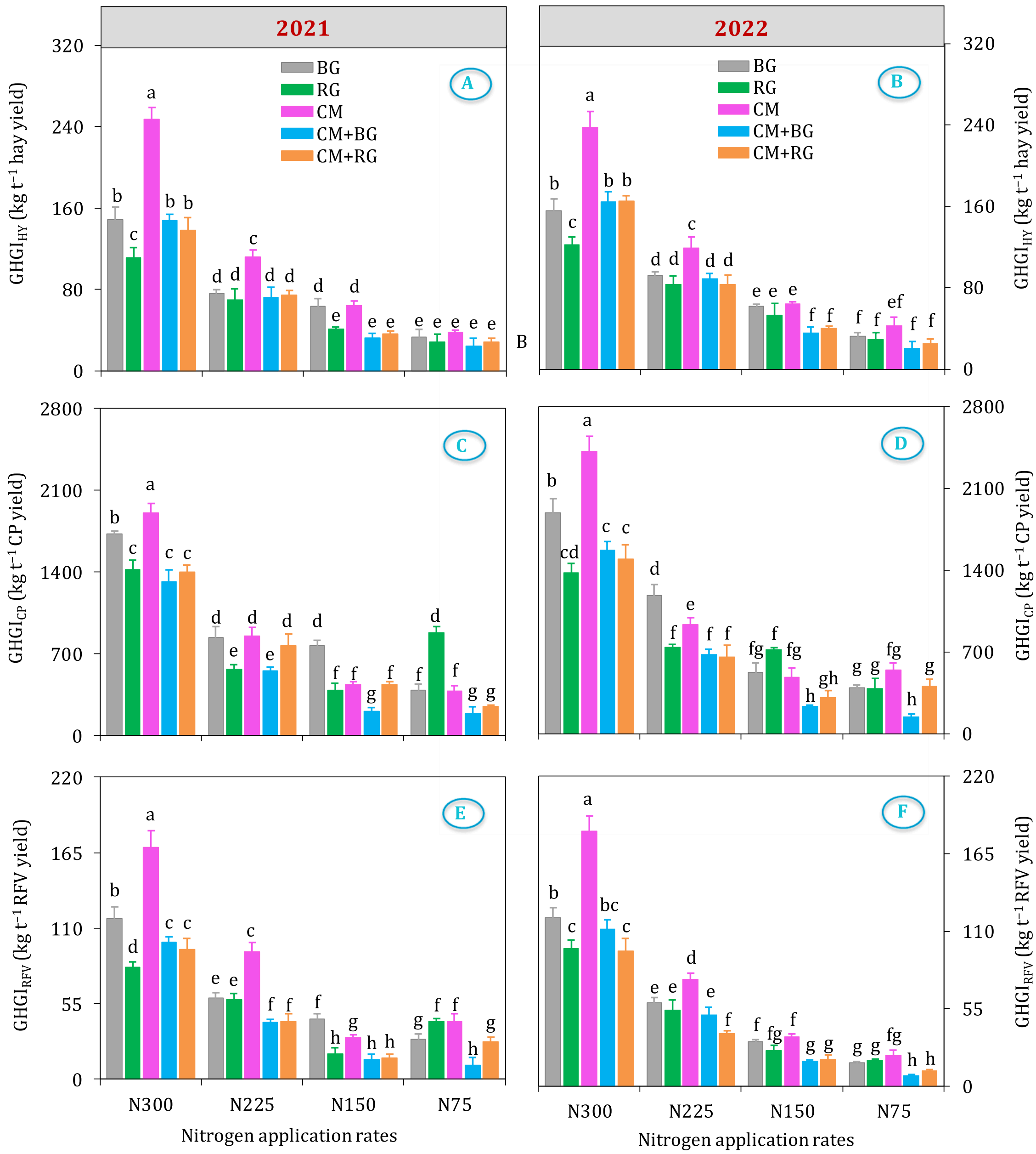

Fig. 7. Interactive effects of pastures and nitrogen rates on greenhouse gas intensity based on the hay yield ($GHGI_{HY}$), CP yield ($GHGI_{CP}$), and RFV yield ($GHGI_{RFV}$). Data are the means of four replicates ± SD. Different lowercase letters indicate significant differences among treatments based on the LSD test at $P < 0.05$. Treatment abbreviations are the same as explained in Fig. 1.

integration of legumes into cropping systems (Liu et al., 2022; Silva et al., 2022; Suter et al., 2015), attributed to the enhancement of soil fertility. Moreover, the hay yield of milkvetch+bromegrass intercrops was greater than that of milkvetch+ryegrass, suggesting a higher compatibility of cicer milkvetch with bromegrass than with ryegrass. Previous studies have indicated that the yield benefits of intercropping systems depend on the selection of suitable companion species (Liu et al., 2023; Muir et al., 2011; Thilakarathna et al., 2016). The potential mechanism includes balanced interspecific and intraspecific interactions, as well as complementarity among plant species, which enhances the effective acquisition and utilization of resources (Li et al., 2020; Mudare et al., 2022; Raseduzzaman and Jensen, 2017; Zhang et al., 2022a, 2022b). Moreover, the mineralization of legume residues and rhizodeposits in intercropping systems increases nitrogen availability to companion grasses at multiple points of demand during the growing season (Yang et al., 2019), which may account for the higher yields observed in legume-grass intercrops. Furthermore, the present study showed that legume-grass intercrops with nitrogen rates of 225 and 300 kg ha^{-1} produced lower hay yield as compared to 150 kg ha^{-1}. The observed effects are attributed to the detrimental effects of excessive fertilizer on root growth (Li et al., 2020, 2018), nodulation and nitrogen fixation capacity (Ledgard et al., 2001; Schipanski and Drinkwater, 2012), thereby reducing the intensity of facilitative effects (Zhu et al., 2023), and compromising the over-yielding benefits of intercrops (Xia et al., 2023; Zhu et al., 2023). Moreover, the decline in hay yield at very low nitrogen input (75 kg ha^{-1}) may have resulted from reduced expansion of stems and leaves, degradation of chlorophyll, and the premature onset of leaf senescence linked to nutrient deficiency. Since multiple harvests of pasture in arid regions significantly deplete topsoil nutrients, and nitrogen fixation by legumes may not be sufficient to meet the overall demands of the intercropping system (Xia et al., 2023; Xu et al., 2022; Yan et al., 2022). Therefore, an optimal nitrogen rate is

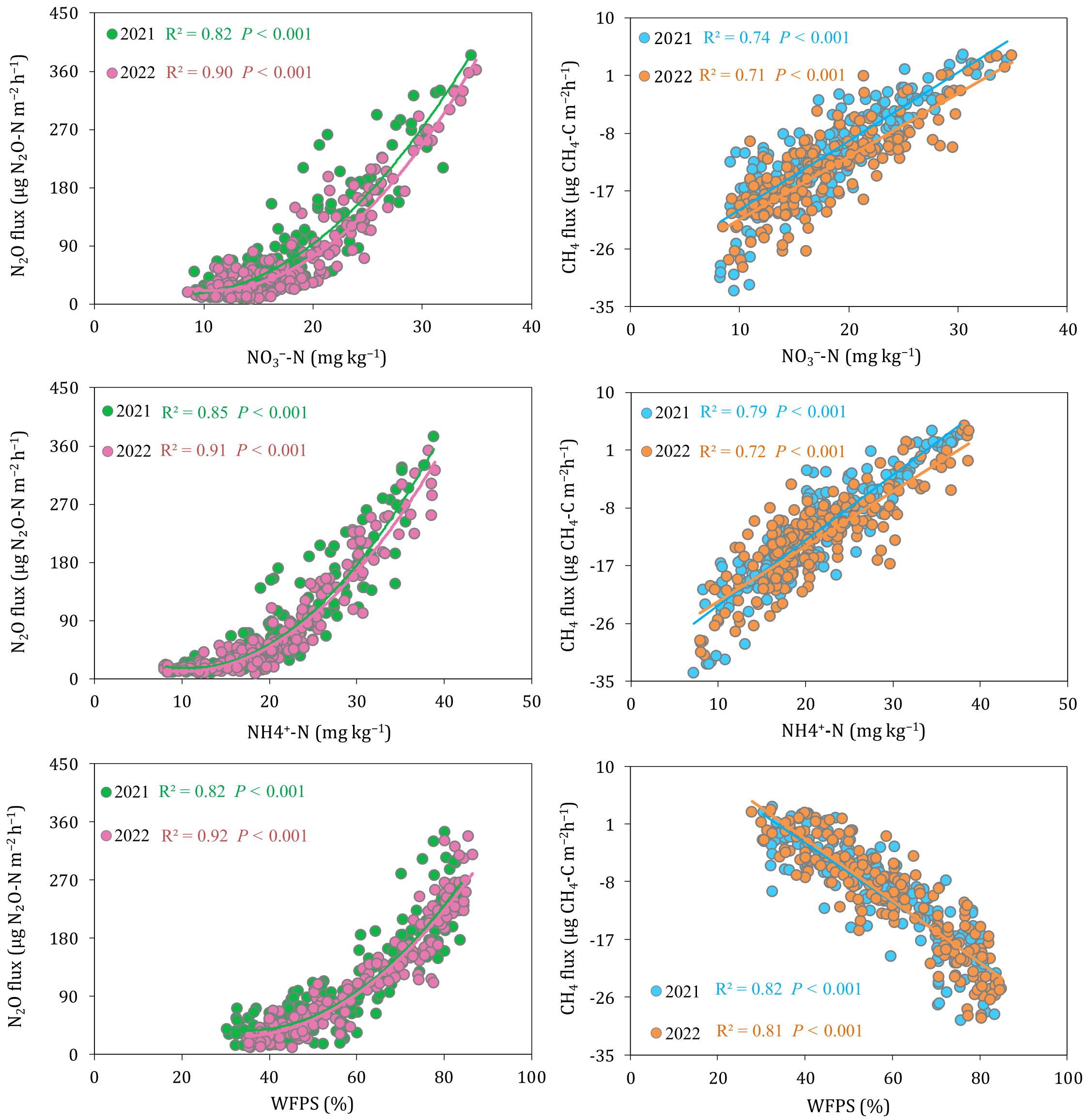

Fig. 8. Relationship of N_2O and CH_4 fluxes with soil NO_3^--N, NH_4^+-N, and WFPS.

essential for meeting the nutrient demands of legume-grass intercrops and improving forage productivity.

4.2. Legume-grass intercropping at low nitrogen input improves forage quality

The present study depicted higher crude protein and relative feed value yields in legume-grass intercrops, particularly for milkvetch+bromegrass, compared to monocultures. A similar trend of increase in crude protein and relative feed value yield was reported in alfalfa and brome mixture (Xu et al., 2023), and intercrops of forage maize with grass pea and hairy vetch (Javanmard et al., 2020). In the present study, the higher crude protein and relative feed value yields of milkvetch+bromegrass compared to milkvetch+ryegrass intercrops can be attributed to a greater ratio of legume to grass cover, as legumes have a higher crude protein concentration than grasses (Liu et al., 2022; Stoltz and Nadeau, 2014). Additionally, higher crude protein and relative feed value yield of legume-grass intercrops compared to monocultures were also associated with their greater dry matter production (Fig. 1 A-B). Few studies indicated that the crude protein content of intercrops was typically within the range of component sole crops, and greater crude protein yield was linked to an increase in plant biomass (Lithourgidis et al., 2006; Xu et al., 2023). The observed discrepancies in results may be related to differences in legume and grass species, along with the growing conditions, as indicated by previous studies (Liu et al., 2023; Tahir et al., 2022).

Studies have identified a positive relation between protein accumulation in forage crops with nitrogen fertilizer rates (Rostamza et al., 2011; Zhang et al., 2022a, 2022b). Indeed, our present study depicted an increase in crude protein and relative feed value yield of grasses with the increase in nitrogen rates. However, the crude protein and relative feed value yields in legume monoculture and legume-grass intercrops at nitrogen application rate of 150 kg ha^{-1} were higher than at 225 and 300 kg ha^{-1} (Fig. 1). Previous studies reported similar results, indicating enhanced forage quality in monoculture alfalfa (Kamran et al., 2022b) and alfalfa-bromegrass mixtures (Xu et al., 2023) with optimal nitrogen fertilizer rates rather than their excessive application. The proposed mechanism for enhanced forage quality involves the dense and vigorous root system of legume-grass mixtures (Yang et al., 2022), facilitating efficient acquisition and utilization of nutrients at the system level (Wei et al., 2022; Zhang et al., 2022a, 2022b). This enhances the biosynthesis of chlorophyll and enzymes, which improves photosynthetic activity and subsequently amino acids and protein synthesis (Liu et al., 2016). On the other hand, lower crude protein and relative feed value yields of legume monoculture and legume-grass intercrops with N300 and N225 treatments can be attributed to the negative impact of high nitrogen inputs on symbiotic rhizobium (Naudin et al., 2010; Schipanski and Drinkwater, 2012), reduced leaf to stem ratio and increased fiber accumulation in shoots (Kamran et al., 2022b; Rostamza et al., 2011), and lower biomass yields (Zhu et al., 2023).

4.3. Seasonal dynamic and cumulative N_2O emissions of pastures with different nitrogen levels

Nitrogen fertilizer and irrigation enhance soil microbial activity by facilitating substrate availability, thereby exponentially increasing N_2O fluxes (Mehmood et al., 2023; Millar et al., 2018; Smith et al., 2018). As expected, N_2O peak fluxes in this study were observed shortly after nitrogen fertilization followed by irrigation events during each growing season (Figs. 2–3). Similar patterns of N_2O emission fluxes have been documented across various crops and pastures in arid regions (Ghani et al., 2022; Kamran et al., 2023a, 2022a; Ning et al., 2022). Parallel to N_2O peak fluxes, an increase in soil nitrate (Figure S5) and ammonium content (Figure S6), along with higher values of water-filled pore space (Figure S7), was evident. These shifts facilitate the availability of substrate and oxygen for soil microbial activities, thereby accelerating nitrogen transformation and N_2O emissions (Pannu et al., 2019; Sainju et al., 2014). The third irrigation (without nitrogen fertilizer) during the later growth period of pastures had no significant impact on N_2O emissions, as the fluxes remained relatively stable. Two potential explanations account for the observed trend; Firstly, it can be attributed to low soil nitrogen content, as a major portion is either utilized by pastures or lost as GHG during the early to mid-growth period (Figure S5–6). Secondly, due to the arid conditions of the experimental site, the irrigation volumes applied are unlikely to result in anoxic microsites (Kamran et al., 2023b, 2022a). Instead, soil aeration is likely to be enhanced, which may reduce N_2O emissions by limiting the denitrification process (Zhang et al., 2020). Based on these findings, we conclude that N_2O fluxes in arid regions are triggered by the combination of irrigation with nitrogen fertilization, rather than irrigation alone.

Recent studies highlighted that legume-grass mixtures can reduce N_2O emissions than sole grasses (Johnson and Barbour, 2016; Pannu et al., 2019; Yang et al., 2019). For instance, Pannu et al. (2019) observed that alfalfa-switchgrass intercrop resulted in a 40 % reduction in N_2O emissions per Mg of biomass produced. Similarly, Johnson and Barbour (2016) reported a 30 % lower average yield-scaled N_2O emission when legumes were interseeded with switchgrass and big bluestem. In the present study, cumulative N_2O emissions in legume monoculture and legume-grass intercrops were higher by 12.95–31.77 % compared to grass monocultures at nitrogen rates of 225 and 300 kg ha^{-1}. This can largely be explained by the inhibiting effects of high nitrogen inputs on legume growth and productivity, resulting in surplus soil nitrogen, consequently increasing N_2O emissions. This observation closely aligns with the studies on red clover-grass intercropping (Barneze et al., 2020), alfalfa monoculture (Kamran et al., 2022a), sweet maize-soybean intercropping (Wang et al., 2021), and maize-soybean intercropping (Raseduzzaman et al., 2024). Furthermore, nitrogen release from the decomposition of legume residues contributes to N_2O emissions (Jensen et al., 2012; Yang et al., 2019), which could be another possible reason for higher N_2O emissions in legume monoculture and legume-grass intercrops compared to grasses. Under low nitrogen inputs, legume-grass intercrop and grass monoculture presented comparable seasonal N_2O emissions, while the emissions were higher in legume monoculture. The observed lower N_2O emission in legume-grass intercrops compared to legume monoculture can be attributed to enhanced synchrony between the timing of nitrogen mineralization by legume and demand of the companion grasses, supported by previous studies findings (Nyfeler et al., 2011; Suter et al., 2015). In addition, including grasses in the intercropping system replaces some rows of legumes, thereby reducing nitrogen-fixation potential compared to legume monoculture. This leads to lower soil nitrogen for nitrification and denitrification, as biologically fixed nitrogen may be used by the legume itself (Jensen et al., 2012; Ledgard et al., 2001).

4.4. Dynamic changes in seasonal fluxes and cumulative CH_4 of pastures with different nitrogen levels

In the present study, CH_4 emission fluxes in legume monoculture and legume-grass intercrops under high nitrogen inputs are attributed to elevated soil NH_4^+ and NO_3^- contents. Conversely, CH_4 uptake fluxes were evident in all pastures under low nitrogen input throughout the growing periods. Wang et al. (2024) demonstrated that nitrogen enrichment alters methane metabolism, particularly through the upregulation of methanogenesis genes (mch, ftr, and mcr). The present study also demonstrated that the fluxes were associated with irrigations, and peak fluxes appeared shortly after each irrigation event. These effects are attributed to enhanced activity of methanotrophs under increased soil moisture during the dry season, as reported in previous studies (Kamran et al., 2023b, 2022a; Zhang et al., 2020). Li et al. (2019) and Wang et al. (2024) indicated that irrigation may reduce CH_4 uptake fluxes or potentially induce CH_4 emission by resulting in soil anaerobic conditions. However, soil water content at our experimental site does not reach the saturation point due to high evaporation rates and irrigation intervals. Instead, irrigation in arid regions enhances soil gas diffusion and aeration, which accelerates methanotroph activity and increases CH_4 oxidation (Mehmood et al., 2023). The significant correlation ($P < 0.001$) between CH_4 uptake fluxes and water-filled pore space validates this observation (Fig. 8).

Experimental and meta-analytical studies have confirmed a negative relationship between CH_4 uptake and nitrogen application rates (Fang et al., 2014; Liu and Greaver, 2009; Sun et al., 2016). The present study demonstrated a steady decline in cumulative CH_4 uptake as nitrogen rates increased. Excessive nitrogen has been shown to inhibit the expression of genes involved in methane oxidation and formaldehyde assimilation (Wang et al., 2024), potentially explaining the reduced CH_4 uptake observed under high nitrogen inputs. Moreover, high soil ammonium concentrations (Liu and Greaver, 2009) and nitrite toxicity from ammonium nitrification (Bodelier and Laanbroek, 2004) inhibit methanotroph activity, which affects the net balance of CH_4 production and consumption, resulting in decreased CH_4 uptake. Yet, several studies suggest that nitrogen addition increases CH_4 uptake (Veldkamp et al., 2001; Xu et al., 2014). The observed discrepancies in results may be due to the dual effects of nitrogen on methanotrophs and variations in soil properties. Aronson and Helliker (2010) specified that low to moderate nitrogen rate promotes methanotroph growth in nitrogen-limited soils, thereby increasing CH_4 uptake. However, exceeding a critical threshold of nitrogen input (e.g., 10 g m^{-2} year^{-1}) is likely to reduce CH_4 uptake by inhibiting methanotrophy. Therefore, we assumed that the application rates of nitrogen fertilizer in our study were relatively high and surpassed the threshold, resulting in a steady decline in CH_4 uptake.

In addition, higher CH_4 uptake in grasses compared to legume and legume-grass intercrops in this study can be explained by several overlapping mechanisms. Firstly, interactions among species affect root architecture (Yang et al., 2022) and production of root exudates (Yan et al., 2022; Zhu et al., 2022), thereby changing soil aeration, spatio-temporal organic carbon deposition, and nitrogen utilization patterns (Smith et al., 2018; Wang et al., 2021). These factors are likely to increase plant growth and productivity, organic substrate availability, oxygen consumption, and soil diffusive conductance (Drinkwater et al., 2021; Smith et al., 2018). Subsequently, this changes the biomass and activity of soil methanogens and methanotrophs (Sun et al., 2016), and thereby CH_4 fluxes. Secondly, the low soil moisture content in intercrops resulting from their more efficient acquisition and utilization compared to monocultures limits the activity of methanotrophs (Ghani et al., 2022) and could explain the observed reduction in CH_4 uptake.

4.5. Legume-grass intercropping at low nitrogen input reduced global warming potential and yield-scale GHG emission

Studies have shown that even slight increase or decrease in GHG emissions with agronomic management practices can result in significant changes in global warming potential and greenhouse gas intensity (Guardia et al., 2016; Wang et al., 2021). Irrespective of nitrogen rates, global warming potential values for all pastures were positive, implying that the pastoral production system in the present study acted as a net source of atmospheric GHGs. Nevertheless, positive feedback of legume-grass intercrops significantly reduced global warming potential values by moderating nitrogen amount, thus corroborating our initial hypothesis. Legume-grass intercrops with low nitrogen inputs (75 and 150 kg ha^{-1}) presented 55.32 %-87.62 % reductions in global warming potential values compared to monocultures with high nitrogen input (300 kg ha^{-1}). The N_2O emissions were the primary contributor to increased global warming potential, as pasture fields were the net sinks of CH_4, consistent with previous studies conducted in arid regions (Ghani et al., 2022; Kamran et al., 2023a, 2022a; Raseduzzaman et al., 2024). These findings underscore the need for greater attention to the effects of agronomic practices on N_2O emissions for effectively mitigating the global warming potential of pasture production in arid regions. In addition, determining the yield-scale GHG emission (GHGI) is decisive for evaluating the balance between productivity and environmental sustainability. Previous studies have pointed out significant trade-offs among forage productivity, nutritional quality, and soil GHG emissions (Ghani et al., 2022; Ning et al., 2022). The values of GHGI based on hay, crude protein, and relative feed value yield of legume-grass intercrops at the nitrogen rate of 150 kg ha^{-1} were either lower or comparable to those of monoculture pastures (across any nitrogen rate). This is because the increased impact of GHG emissions was offset by their greater productivity and nutritional quality in intercrops. This indicated that legume-grass intercropping, particularly milkvetch+bromegrass with low nitrogen input, represents a win-win strategy for achieving the dual goals of high-quality forage productivity and lower GHG emissions.

5. Conclusions

Legume-grass intercrops resulted in greater hay, crude protein, and relative feed value yield at low nitrogen input compared to monoculture pastures across all nitrogen rates. The global warming potential and greenhouse gas intensity of legume-grass intercrops at 150 kg nitrogen ha^{-1} were either lower or comparable to those of monocultures at each nitrogen rate. Legume monoculture at low nitrogen input also improved forage yield and quality compared to grass monocultures, but increased the greenhouse gas intensity and global warming potential. Hence, compared to traditional highly fertilized grass monocultures, intercropping of cicer milkvetch and bromegrass with 150 kg nitrogen ha^{-1} represents a more suitable cultivation practice that balances forage productivity and environmental sustainability, while also delivering high-quality feed for local dairy farming in arid regions. Furthermore, the moderation of nitrogen input through legume-based cultivation may also reduce the indirect GHG emissions associated with fertilizer production, further contributing to the future goals for carbon neutrality. Climatic conditions, soil fertility, and pasture species can influence the response of legume-grass intercrops to nitrogen application rates, thereby affecting the productivity and GHG dynamics. Therefore, future studies should evaluate different legume and grass species across multiple environmental conditions to identify better intercrop companions, thus fostering the development of diverse pastoral cropping systems.

CRediT authorship contribution statement

Muhammad Kamran: Writing – original draft, Visualization, Software, Project administration, Investigation, Formal analysis, Conceptualization. **Min Zhang:** Visualization, Methodology, Investigation. **Qianmin Jia:** Visualization, Methodology, Investigation. **Muhammad Usman:** Writing – review & editing. **Moazma Waris:** Visualization, Methodology, Investigation. **Shenghua Chang:** Writing - review & editing, Resources. **Fujiang Hou:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109859.

Data availability

Data will be made available on request.

References

Aronson, E.L., Helliker, B.R., 2010. Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. Ecology 91, 3242–3251.
Barneze, A.S., Whitaker, J., McNamara, N.P., Ostle, N.J., 2020. Legumes increase grassland productivity with no effect on nitrous oxide emissions. Plant Soil 446, 163–177.
Bodelier, P.L.E., Laanbroek, H.J., 2004. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. FEMS Microbiol. Ecol. 47, 265–277.
Brooker, R.W., Bennett, A.E., Cong, W.F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L., Mckenzie, B.M., Pakeman, R.J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., White, P.J., 2015. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. N. Phytol. 206, 107–117.
Drinkwater, L.E., Midega, C.A.O., Awuor, R., Nyagol, D., Khan, Z.R., 2021. Perennial legume intercrops provide multiple belowground ecosystem services in smallholder farming systems. Agric. Ecosyst. Environ. 320, 107566.
Fang, H., Cheng, S., Yu, G., Cooch, J., Wang, Y., Xu, M., Li, L., Dang, X., Li, Y., 2014. Low-level nitrogen deposition significantly inhibits methane uptake from an alpine meadow soil on the Qinghai–Tibetan Plateau. Geoderma 213, 444–452.
Ghani, M.U., Kamran, M., Ahmad, I., Arshad, A., Zhang, C., Zhu, W., Lou, S., Hou, F., 2022. Alfalfa-grass mixtures reduce greenhouse gas emissions and net global warming potential while maintaining yield advantages over monocultures. Sci. Total Environ. 849, 157765.
Guardia, G., Tellez-Rio, A., García-Marco, S., Martin-Lammerding, D., Tenorio, J.L., Ibáñez, M.Á., Vallejo, A., 2016. Effect of tillage and crop (cereal versus legume) on greenhouse gas emissions and global warming potential in a non-irrigated Mediterranean field. Agric. Ecosyst. Environ. 221, 187–197.
IPCC, 2021. Summary for Policymakers. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Javanmard, A., Amani Machiani, M., Lithourgidis, A., Morshedloo, M.R., Ostadi, A., 2020. Intercropping of maize with legumes: a cleaner strategy for improving the quantity and quality of forage. Clean. Eng. Technol. 1, 100003.
Jensen, E.S., Peoples, M.B., Boddey, R.M., Gresshoff, P.M., Hauggaard-Nielsen, H., J.R. Alves, B., Morrison, M.J., 2012. Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. Agron. Sustain. Dev. 32, 329–364.
Jensen, E.S., Carlsson, G., Hauggaard-Nielsen, H., 2020. Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: a global-scale analysis. Agron. Sustain. Dev. 40, 5.
Johnson, J.M.F., Barbour, N.W., 2016. Nitrous oxide emission and soil carbon sequestration from herbaceous perennial biofuel feedstocks. Soil Sci. Soc. Am. J. 80, 1057–1070.
Kamran, M., Yan, Z., Chang, S., Chen, X., Ahmad, I., Jia, Q., Ghani, M.U., Nouman, M., Hou, F., 2022a. Enhancing resource use efficiency of alfalfa with appropriate irrigation and fertilization strategy mitigate greenhouse gases emissions in the arid region of Northwest China. Field Crops Res 289, 108715.
Kamran, M., Yan, Z., Jia, Q., Chang, S., Ahmad, I., Ghani, M.U., Hou, F., 2022b. Irrigation and nitrogen fertilization influence on alfalfa yield, nutritive value, and resource use efficiency in an arid environment. Field Crops Res 284, 108587.
Kamran, M., Yan, Z., Ahmad, I., Jia, Q., Ghani, M.U., Chen, X., Chang, S., Li, T., Siddique, K.H.M., Fahad, S., Hou, F., 2023a. Assessment of greenhouse gases emissions, global warming potential and net ecosystem economic benefits from wheat field with reduced irrigation and nitrogen management in an arid region of China. Agric. Ecosyst. Environ. 341, 108197.
Kamran, M., Yan, Z., Chang, S., Ning, J., Lou, S., Ahmad, I., Ghani, M.U., Arif, M., El Sabagh, A., Hou, F., 2023b. Interactive effects of reduced irrigation and nitrogen fertilization on resource use efficiency, forage nutritive quality, yield, and economic benefits of spring wheat in the arid region of Northwest China. Agric. Water Manag 275, 108000.
Kermah, M., Franke, A.C., Adjei-Nsiah, S., Ahiabor, B.D.K., Abaidoo, R.C., Giller, K.E., 2017. Maize-grain legume intercropping for enhanced resource use efficiency and crop productivity in the Guinea savanna of northern Ghana. Field Crops Res 213, 38–50.
Khatiwada, B., Acharya, S.N., Larney, F.J., Lupwayi, N.Z., Smith, E.G., Islam, M.A., Thomas, J.E., 2020. Benefits of mixed grass–legume pastures and pasture rejuvenation using bloat-free legumes in western Canada: a review. Can. J. Plant Sci. 100, 463–476.
Lawrence, N.C., Tenesaca, C.G., VanLoocke, A., Hall, S.J., 2021. Nitrous oxide emissions from agricultural soils challenge climate sustainability in the US Corn Belt. Proc. Natl. Acad. Sci. 118, e2112108118.
Ledgard, S.F., Sprosen, M.S., Penno, J.W., Rajendram, G.S., 2001. Nitrogen fixation by white clover in pastures grazed by dairy cows: Temporal variation and effects of nitrogen fertilization. Plant Soil 229, 177–187.
Li, C., Hoffland, E., Kuyper, T.W., Yu, Y., Li, H., Zhang, C., Zhang, F., van der Werf, W., 2020. Yield gain, complementarity and competitive dominance in intercropping in China: a meta-analysis of drivers of yield gain using additive partitioning. Eur. J. Agron. 113, 125987.
Li, J., Dong, W., Oenema, O., Chen, T., Hu, C., Yuan, H., Zhao, L., 2019. Irrigation reduces the negative effect of global warming on winter wheat yield and greenhouse gas intensity. Sci. Total Environ. 646, 290–299.
Li, M., Wang, Y., Adeli, A., Yan, H., 2018. Effects of application methods and urea rates on ammonia volatilization, yields and fine root biomass of alfalfa. Field Crops Res 218, 115–125.
Li, X.F., Wang, Z.G., Bao, X.G., Sun, J.H., Yang, S.C., Wang, P., Wang, C.B., Wu, J.P., Liu, X.R., Tian, X.L., Wang, Yu, Li, J.P., Wang, Yan, Xia, H.Y., Mei, P.P., Wang, X.F., Zhao, J.H., Yu, R.P., Zhang, W.P., Che, Z.X., Gui, L.G., Callaway, R.M., Tilman, D., Li, L., 2021. Long-term increased grain yield and soil fertility from intercropping. Nat. Sustain 4, 943–950.
Lithourgidis, A.S., Vasilakoglou, I.B., Dhima, K.V., Dordas, C.A., Yiakoulaki, M.D., 2006. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. Field Crops Res 99, 106–113.
Liu, H., Struik, P.C., Zhang, Y., Jing, J., Stomph, T.J., 2023. Forage quality in cereal/legume intercropping: a meta-analysis. Field Crops Res 304, 109174.
Liu, L., Greaver, T.L., 2009. A review of nitrogen enrichment effects on three biogenic GHGs: the CO_2 sink may be largely offset by stimulated N_2O and CH emission. Ecol. Lett. 12, 1103–1117.
Liu, M., Gong, J.R., Pan, Y., Luo, Q.P., Zhai, Z.W., Xu, S., Yang, L.L., 2016. Effects of grass–legume mixtures on the production and photosynthetic capacity of constructed grasslands in Inner Mongolia, China. Crop Pasture Sci. 67, 1188.
Liu, X., Tahir, M., Li, C., Chen, C., Xin, Y., Zhang, G., Cheng, M., Yan, Y., 2022. Mixture of Alfalfa, orchardgrass, and tall fescue produces greater biomass yield in Southwest China. Agronomy 12, 2425.
Mehmood, F., Wang, G., Abubakar, S.A., Zain, M., Rahman, S.U., Gao, Y., Duan, A., 2023. Optimizing irrigation management sustained grain yield, crop water productivity, and mitigated greenhouse gas emissions from the winter wheat field in North China Plain. Agric. Water Manag 290, 108599.
Millar, N., Urrea, A., Kahmark, K., Shcherbak, I., Robertson, G.P., Ortiz-Monasterio, I., 2018. Nitrous oxide (N_2O) flux responds exponentially to nitrogen fertilizer in irrigated wheat in the Yaqui Valley, Mexico. Agric. Ecosyst. Environ. 261, 125–132.
Mudare, S., Kanomanyanga, J., Jiao, X., Mabasa, S., Lamichhane, J.R., Jing, J., Cong, W. F., 2022. Yield and fertilizer benefits of maize/grain legume intercropping in China and Africa: a meta-analysis. Agron. Sustain. Dev. 42, 81.
Muir, J.P., Pitman, W.D., Foster, J.L., 2011. Sustainable, low-input, warm-season, grass-legume grassland mixtures: Mission (nearly) impossible? Grass Forage Sci. 66, 301–315.
Naudin, C., Corre-Hellou, G., Pineau, S., Crozat, Y., Jeuffroy, M.H., 2010. The effect of various dynamics of N availability on winter pea-wheat intercrops: Crop growth, N partitioning and symbiotic N_2 fixation. Field Crops Res 119, 2–11.
Ning, J., Lou, S., Guo, Y., Chang, S., Zhang, C., Zhu, W., Hou, F., 2022. Appropriate N fertilizer addition mitigates N2O emissions from forage crop fields. Sci. Total Environ. 829, 154628.
Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Lüscher, A., 2011. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. Agric. Ecosyst. Environ. 140, 155–163.
Pan, Z., Zhang, Z., Li, J., Zhang, Y., Zhai, M., Zhao, W., Wang, L., Li, A., Wang, K., Wang, Z., 2024. A global synthesis of nitrous oxide emissions across cotton-planted soils. Sustain. Prod. Consum 51, 315–326.
Pannu, M.W., Meinhardt, K.A., Bertagnolli, A., Fransen, S.C., Stahl, D.A., Strand, S.E., 2019. Nitrous oxide emissions associated with ammonia-oxidizing bacteria abundance in fields of switchgrass with and without intercropped alfalfa. Environ. Microbiol. Rep. 11, 727–735.
Qiao, M., Sun, R., Wang, Z., Dumack, K., Xie, X., Dai, C., Wang, E., Zhou, J., Sun, B., Peng, X., Bonkowski, M., Chen, Y., 2024. Legume rhizodeposition promotes nitrogen fixation by soil microbiota under crop diversification. Nat. Commun. 15, 2924.
Raseduzzaman, M., Jensen, E.S., 2017. Does intercropping enhance yield stability in arable crop production? A meta-analysis. Eur. J. Agron. 91, 25–33.
Raseduzzaman, M., Dong, W., Gaudel, G., Aluoch, S.O., Timilsina, A., Li, X., Hu, C., 2024. Maize-soybean intercropping reduces greenhouse gas emissions from the fertilized soil in the North China Plain. J. Soils Sediment. 24, 3115–3131.
Rostamza, M., Chaichi, M.R., Jahansouz, M.R., Alimadadi, A., 2011. Forage quality, water use and nitrogen utilization efficiencies of pearl millet (*Pennisetum americanum* L.) grown under different soil moisture and nitrogen levels. Agric. Water Manag 98, 1607–1614.
Sainju, U.M., Stevens, W.B., Caesar-TonThat, T., Liebig, M.A., Wang, J., 2014. Net global warming potential and greenhouse gas intensity influenced by irrigation, tillage, crop rotation, and nitrogen fertilization. J. Environ. Qual. 43, 777–788.
Schipanski, M.E., Drinkwater, L.E., 2012. Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. Plant Soil 357, 147–159.
Silva, L.S., Laroca, J.V., dos, S., Coelho, A.P., Gonçalves, E.C., Gomes, R.P., Pacheco, L.P., Carvalho, P.C., de, F., Pires, G.C., Oliveira, R.L., Souza, J.M.A., de, Freitas, C.M., Cabral, C.E.A., Wruck, F.J., Souza, E.D. de, 2022. Does grass-legume intercropping change soil quality and grain yield in integrated crop-livestock systems? Appl. Soil Ecol. 170, 104257.
Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A., 2018. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. Eur. J. Soil Sci. 69, 10–20.
Spohn, M., Bagchi, S., Biederman, L.A., Borer, E.T., Bråthen, K.A., Bugalho, M.N., Caldeira, M.C., Catford, J.A., Collins, S.L., Eisenhauer, N., Hagenah, N., Haider, S., Hautier, Y., Knops, J.M.H., Koerner, S.E., Laanisto, L., Lekberg, Y., Martina, J.P., Martinson, H., McCulley, R.L., Peri, P.L., Macek, P., Power, S.A., Risch, A.C., Roscher, C., Seabloom, E.W., Stevens, C., Veen, G.F. (Ciska, Virtanen, R., Yahdjian, L., 2023. The positive effect of plant diversity on soil carbon depends on climate. Nat. Commun. 14, 1–10.
Stoltz, E., Nadeau, E., 2014. Effects of intercropping on yield, weed incidence, forage quality and soil residual N in organically grown forage maize (*Zea mays* L.) and faba bean (*Vicia faba* L.). Field Crops Res 169, 21–29.
Sun, B.F., Zhao, H., Lü, Y.Z., Lu, F., Wang, X.K., 2016. The effects of nitrogen fertilizer application on methane and nitrous oxide emission/uptake in Chinese croplands. J. Integr. Agric. 15, 440–450.
Suter, M., Connolly, J., Finn, J.A., Loges, R., Kirwan, L., Sebastià, M.T., Lüscher, A., 2015. Nitrogen yield advantage from grass-legume mixtures is robust over a wide range of legume proportions and environmental conditions. Glob. Chang. Biol. 21, 2424–2438.
Tahir, M., Li, C., Zeng, T., Xin, Y., Chen, C., Javed, H.H., Yang, W., Yan, Y., 2022. Mixture composition influenced the biomass yield and nutritional quality of legume–grass pastures. Agronomy 12, 1449.
Thilakarathna, M.S., McElroy, M.S., Chapagain, T., Papadopoulos, Y.A., Raizada, M.N., 2016. Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. Agron. Sustain. Dev. 36, 58.
Tilman, D., 2020. Benefits of intensive agricultural intercropping. Nat. Plants 6, 604–605.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Veldkamp, E., Weitz, A.M., Keller, M., 2001. Management effects on methane fluxes in humid tropical pasture soils. Soil Biol. Biochem 33, 1493–1499.
Wang, W., Ye, Z., Li, J., Liu, G., Wu, Q., Wang, Z., He, G., Yan, W., Zhang, C., 2024. Intermediate irrigation with low fertilization promotes soil nutrient cycling and reduces CO2 and CH_4 emissions via regulating fungal communities in arid agroecosystems. J. Environ. Manag. 351, 119688.
Wang, W., Li, M.Y., Wang, Y., Li, J.M., Zhang, W., Wen, Q.H., Huang, S.J., Chen, G.R., Zhu, S.G., Wang, J., Ullah, F., Xiong, Y.C., 2025. Legume intercropping improves soil organic carbon stability in drylands: a 7-year experimental validation. Agric. Ecosyst. Environ. 381, 109456.
Wang, X., Chen, Y., Yang, K., Duan, F., Liu, P., Wang, Z., Wang, J., 2021. Effects of legume intercropping and nitrogen input on net greenhouse gas balances, intensity, carbon footprint and crop productivity in sweet maize cropland in South China. J. Clean. Prod. 314, 127997.

Wang, X., Wang, J., Zou, Y., Bie, Y., Mahmood, A., Zhang, L., Liao, L., Song, Z., Liu, G., Zhang, C., 2024. Urea fertilization increased CO_2 and CH_4 emissions by enhancing C-cycling genes in semi-arid grasslands. J. Environ. Manag. 356, 120718.
Wei, Z., Maxwell, T., Robinson, B., Dickinson, N., 2022. Grasses procure key soil nutrients for clovers. Nat. Plants 8, 923–929.
Xia, H., Qiao, Y., Li, X., Xue, Yanhui, Wang, N., Yan, W., Xue, Yanfang, Cui, Z., van der Werf, W., 2023. Moderation of nitrogen input and integration of legumes via intercropping enable sustainable intensification of wheat-maize double cropping in the North China Plain: a four-year rotation study. Agric. Syst. 204, 103540.
Xu, M., Cheng, S., Fang, H., Yu, G., Gao, W., Wang, Y., Dang, X., Li, L., 2014. Low-level nitrogen addition promotes net methane uptake in a boreal forest across the great Xing'an mountain region, China. For. Sci. 60, 973–981.
Xu, R., Zhao, H., Liu, G., You, Y., Ma, L., Liu, N., Zhang, Y., 2021. Effects of nitrogen and maize plant density on forage yield and nitrogen uptake in an alfalfa–silage maize relay intercropping system in the North China Plain. Field Crops Res 263, 108068.
Xu, R., Zhao, H., Liu, G., Li, Y., Li, S., Zhang, Y., Liu, N., Ma, L., 2022. Alfalfa and silage maize intercropping provides comparable productivity and profitability with lower environmental impacts than wheat–maize system in the North China plain. Agric. Syst. 195, 103305.
Xu, R., Shi, W., Kamran, M., Chang, S., Jia, Q., Hou, F., 2023. Grass-legume mixture and nitrogen application improve yield, quality, and water and nitrogen utilization efficiency of grazed pastures in the loess plateau. Front. Plant Sci. 14, 1088849.
Yan, H., Gu, S., Li, S., Shen, W., Zhou, X., Yu, H., Ma, K., Zhao, Y., Wang, Y., Zheng, H., Deng, Y., Lu, G., 2022. Grass-legume mixtures enhance forage production via the bacterial community. Agric. Ecosyst. Environ. 338, 108087.
Yang, H., Xu, H., Zhang, W., Li, Z., Fan, H., Lambers, H., Li, L., 2022. Overyielding is accounted for partly by plasticity and dissimilarity of crop root traits in maize/legume intercropping systems. Funct. Ecol. 36, 2163–2175.
Yang, T., Dong, J., Huang, L., Li, Y., Yan, H., Zhai, J., Wang, J., Jin, Z., Zhang, G., 2023. A large forage gap in forage availability in traditional pastoral regions in China. Fundam. Res. 3, 188–200.
Yang, X., Li, S., Du, T., Kang, S., Siddique, K.H.M., Butterbach-Bahl, K., 2024. Greenhouse gas emissions and crop-specific emission factors of eight upland crops based on a six-year field experiment in the North China Plain. Sustain. Prod. Consum 50, 416–430.
Yang, Y., Reilly, E.C., Jungers, J.M., Chen, J., Smith, T.M., 2019. Climate benefits of increasing plant diversity in perennial bioenergy crops. One Earth 1, 434–445.
Zhang, H., Shi, W., Ali, S., Chang, S., Jia, Q., Hou, F., 2022a. Legume/maize intercropping and n application for improved yield, quality, water and n utilization for forage production. Agronomy 12, 1777.
Zhang, W., Lu, J.S., Bai, J., Khan, A., Liu, S.T., Zhao, L., Wang, W., Zhu, S.G., Li, X.G., Tian, X.H., Li, S.Q., Xiong, Y.C., 2024. Introduction of soybean into maize field reduces N2O emission intensity via optimizing nitrogen source utilization. J. Clean. Prod. 442, 141052.
Zhang, X., Xiao, G., Li, H., Wang, L., Wu, S., Wu, W., Meng, F., 2020. Mitigation of greenhouse gas emissions through optimized irrigation and nitrogen fertilization in intensively managed wheat–maize production. Sci. Rep. 10, 5907.
Zhang, Y., Sun, Z., Su, Z., Du, G., Bai, W., Wang, Q., Wang, R., Nie, J., Sun, T., Feng, C., Zhang, Z., Yang, N., Zhang, X., Evers, J.B., van der Werf, W., Zhang, L., 2022b. Root plasticity and interspecific complementarity improve yields and water use efficiency of maize/soybean intercropping in a water-limited condition. Field Crops Res 282, 108523.
Zhu, S., Cheng, Z., Yin, H., Zhou, R., Yang, Y., Wang, J., Zhu, H., Wang, W., Wang, B., Li, W.-B., Tao, H., Xiong, Y., 2022. Transition in plant–plant facilitation in response to soil water and phosphorus availability in a legume-cereal intercropping system. BMC Plant Biol. 22, 311.
Zhu, S., Zhu, H., Zhou, R., Zhang, W., Wang, W., Zhou, Y., Wang, B., Yang, Y., Wang, J., Tao, H., Xiong, Y., 2023. Intercrop overyielding weakened by high inputs: global meta-analysis with experimental validation. Agric. Ecosyst. Environ. 342, 108239.