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Alfalfa-grass mixtures reduce greenhouse gas emissions and net global warming potential while maintaining yield advantages over monocultures

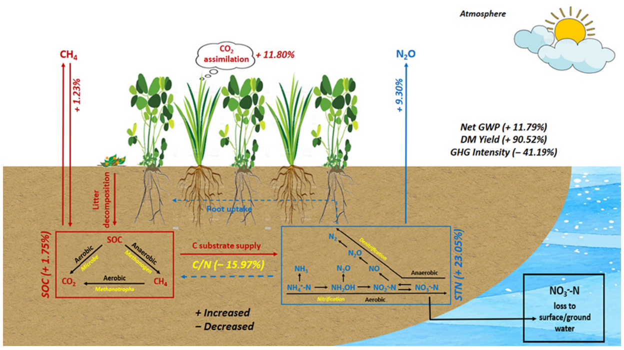
Muhammad Usman Ghani [a], Muhammad Kamran [a], Irshad Ahmad [a], Adnan Arshad [b], Cheng Zhang [a], Wanhe Zhu [a], Shanning Lou [a], Fujiang Hou [a,*]

[a] *State Key Laboratory of Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China*
[b] *College of Resources and Environmental Science, China Agricultural University, Beijing 100193, China*

HIGHLIGHTS

- Yield of perennial grasses with alfalfa mixture were greater than monocultures.
- Alfalfa and tall fescue mixture obtained greater yield than alfalfa and smooth bromegrass mixture.
- The soil organic carbon and total nitrogen contents were improved by the alfalfa and mixtures.
- Alfalfa monoculture emitted more greenhouse gas emissions compared with the mixtures.
- Alfalfa and tall fescue mixture resulted in lower global warming potential and greenhouse gas intensity.

GRAPHICAL ABSTRACT



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ABSTRACT

Improving forage productivity with lower greenhouse gas (GHG) emissions from limited grassland has been a hotspot of interest in global agricultural production. In this study, we analyzed the effects of grasses (tall fescue, smooth bromegrass), legume (alfalfa), and alfalfa-grass (alfalfa + smooth bromegrass and alfalfa + tall fescue) mixtures on GHG emissions, net global warming potential (Net GWP), yield-based greenhouse gas intensity (GHGI), soil chemical properties and forage productivity in cultivated grassland in northwest China during 2020–2021. Our results demonstrated that alfalfa-grass mixtures significantly improved forage productivity. The highest total dry matter yield (DMY) during 2020 and 2021 was obtained from alfalfa-tall fescue (11,311 and 13,338 kg ha^{-1}) and alfalfa-smooth bromegrass mixtures (10,781 and 12,467 kg ha^{-1}). The annual cumulative GHG emissions from mixtures were lower than alfalfa monoculture. Alfalfa-grass mixtures significantly reduced GHGI compared with the grass or alfalfa monocultures. Furthermore, results indicated that grass, alfalfa and alfalfa-grass mixtures differentially affected soil chemical properties. Lower soil pH and C/N ratio were recorded in alfalfa monoculture. Alfalfa and mixtures increased soil organic carbon (SOC) and soil total nitrogen (STN) contents. Importantly, alfalfa-grass mixtures are necessary for improving forage productivity and mitigating the GHG emissions in this region. In conclusion, the alfalfa-tall fescue mixture lowered net GWP and GHGI in cultivated grassland while maintaining high forage productivity. These advanced agricultural practices could contribute to the development of climate-sustainable grassland production in China.

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* Corresponding author.
E-mail address: cyhoufj@lzu.edu.cn (F. Hou).

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1. Introduction

The current agricultural situation is challenged by the necessity of feeding an ever-increasing world population with fewer resources (Alam et al., 2020; Godfray et al., 2010). A projected change in climate will further worsen the future food and nutrition security crisis around the world (IPCC, 2013; Malhi et al., 2021; Thornton et al., 2014). Therefore, grassland-based livestock agriculture can be highly profitable and plays an important role in sustaining animal husbandry and maintaining ecosystem functions across arid and semi-arid regions in China (Hou et al., 2021; Jiang and Wang, 2022; Wu et al., 2015; Zhao et al., 2020a). The majority of these kinds of production systems rely primarily on grass monocultures with generally high fertilizer inputs (Helgadottir et al., 2018). Mixed grass and legume pastures are proposed to improve forage productivity and sustainability with favorable environmental consequences, which is essential to maintain sustainable grassland ecosystems (Luscher et al., 2014; Suter et al., 2021).

Agro-diversity experiments have consistently shown that mixtures of multiple species have higher yields than their component monoculture species when grown under diverse pedo-climatic conditions in intensively managed grasslands (Aponte et al., 2019; Finn et al., 2013). These effects are mainly attributed to the better acquisition and management of resources in terms of space and time (niche complementarity), beneficial interactions between species (facilitation), and the selection of the best performing species (Brooker et al., 2015; Cardinale et al., 2007). In mixed pastures, increased biomass production may not only lead to overyielding but also to transgressive overyielding [mixtures produce more yield than their highest-yielding components if grown in monoculture (Bi et al., 2019)]. In the latter case, facilitation or improved resource acquisition may be required to achieve this goal (Cardinale et al., 2007; Meza et al., 2022). There is increasing evidence that high yields are related to the selection of high-yielding and best performing species rather than species richness and it is more important to select species for mixtures strategically rather than focus solely on complexity of the mixture (Klaus et al., 2020; Luscher et al., 2011; Sanderson et al., 2004; Storkey et al., 2015).

The goal of sustainable intensification of agro-ecosystems is to increase productivity while minimizing environmental impacts (Chen and Jin, 2019; Foley et al., 2011; Godfray et al., 2010; Luscher et al., 2014). At present, medium to high management intensity grassland livestock production is dependent mostly on highly productive pure grass stands that require high amounts of mineral N fertilizers (Weggler et al., 2019). The intensive usage of nitrogen fertilizers has been demonstrated to induce nutritional imbalances (Qiao et al., 2018; Sutton et al., 2011). Moreover, nitrogen fertilization is associated with excessive greenhouse gas (GHG) emissions (Gao et al., 2018; Schmeer et al., 2014), such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2), which are major causes of climate change (Liu et al., 2019b). The use of synthetic fertilizers in grassland ecosystems is a major source of N_2O emissions, as these fertilizers increase the availability of soil ammonium (NH_4^+) and nitrate (NO_3^-) to bacteria that are responsible for nitrification and denitrification (Jensen et al., 2012; Wang et al., 2022). Moreover, a decrease in CH_4 oxidation has been observed in fertilized soils, which has been attributed to the inhibition of methane monooxygenase activity by ammonia (NH_3) (Le Mer and Roger, 2001). Previous studies suggested that fertilizer-affected CO_2 emissions are mostly produced by changes in soil properties such as organic matter content, microbial abundance, and their activities (Smith et al., 2008; Tan et al., 2021; Ward et al., 2017; Zheng et al., 2021). Inputs of biologically fixed nitrogen (N) through legume-rhizobium symbiosis reduce synthetic nitrogen (N) fertilizer inputs and greenhouse gas emissions (Jensen et al., 2012; Shamseldin, 2022). In this context, switching from traditional fertilizer-based grass monocultures to grass-legume mixtures in grassland cropping systems is a potentially win-win strategy (Kaye and Quemada, 2017; Rosenstock et al., 2014). However, legumes are also known to contribute to the emission of N_2O, especially when their aboveground residues get incorporated into the soil (Jensen et al., 2012; Pappa et al., 2011). This is why it is extremely important to find a suitable grass-legume combination that minimizes environmental impacts without compromising yield.

Previous studies have demonstrated that the characteristics of plant communities and their composition can be associated with the mitigation of greenhouse gas emissions (Abalos et al., 2014, 2018; Chen et al., 2018), as a result of their interactions with carbon and nitrogen cycles (Niklaus et al., 2006; Schmidt et al., 2019; Ström et al., 2005). There is evidence that nitrogen (N) uptake by plants in communities (containing multiple plant species) is higher than in monocultures of the same type of plants (Finn et al., 2013; Meza et al., 2022). The grass-legume mixtures offer the benefit of symbiotic N_2 fixation by legumes, which can utilize atmospheric N_2 for their requirements (Niklaus et al., 2016; Peoples et al., 2019; Suter et al., 2015). Grown in mixtures with grasses, legumes receive over 80 % of their nitrogen (N) requirements from (living or decaying legume roots) symbiosis (Alemneh et al., 2020; Nyfeler et al., 2011; Rasmussen et al., 2012; Suter et al., 2015), consequently, there is an increase in the relative availability of soil nitrogen (N) for grasses (Temperton et al., 2007).

Previously, several studies have been conducted to evaluate the effects of mono-culture and mixed-culture pastures on yield and forage quality (Cong et al., 2018; Dehghanian et al., 2020; Komainda and Isselstein, 2020; Reiss and Drinkwater, 2020). Little attention has been focused on evaluating their impact on mitigating GHG emissions. Therefore, the present study was conducted to quantify the effects of various grass, alfalfa and alfalfa-grass mixtures swards on the mitigation of greenhouse gas emissions and improving soil fertility and forage productivity.

2. Materials and methods

2.1. Study site

In summer 2019, the field experiment was established at the Grassland Research Station of Lanzhou University, Linze (39°15′N, 100°02′E), Gansu province, China (Fig. 1). The research area has an arid temperate climate and is characterized by deficit precipitation and higher evaporation. The annual average temperature at the experimental site during the experiment was 9.6 °C. The long-term annual average precipitation was 110.7 mm, whereas evaporation was about 2643.9 mm, which is 24 times more than precipitation. The frost-free days account for about 175 days. Throughout the experimental period, the average wind speed was 2.21 m s^{-1}, with maximum speeds of 2.71 m s^{-1} and 1.91 m s^{-1} in the spring and fall, respectively. Soil of the experimental site is classified as Aridisols according to the USDA soil taxonomy. The basic soil nutrient status of 0 to 20 cm soil layer comprised of 9.34 soil organic carbon (SOC) g kg^{-1}, 1.07 soil total nitrogen (STN) g kg^{-1}, respectively. Soil pH of the experiment site was 8.5, salt contents were 0.6 to 0.9 %, and bulk density was 0.93 g cm^{-3}. Temperature and precipitation data during 2020 and 2021 were obtained from an automatic weather station (Fig. 2).

2.2. Experimental design and field management

A randomized complete block design with four replications was used for this study. Two perennial grass species, tall fescue (*Festuca arundinacea* Schreb.) and smooth bromegrass (*Bromus inermis* Leyss.), and the legume alfalfa (*Medicago sativa* L.) were sown as monocultures and binary mixtures on 15 August 2019. Data were collected from May to October in 2020 and 2021, respectively. The size of each plot in the experiment was 25 m^2 (5 m × 5 m). In this study, plots were kept at a distance of one meter from one another. The row-to-row space was maintained at 18 cm and the sowing depth was 2 to 3 cm. The seeding rate for monocultures of tall fescue, smooth bromegrass, and alfalfa were 16, 18, and 15 kg ha^{-1}, respectively. The seeding rates for alfalfa and smooth bromegrass in binary mixture were 7 and 9 kg ha^{-1}, respectively, while alfalfa and tall fescue were 7 and 8 kg ha^{-1}, respectively. Prior to sowing, alfalfa and smooth bromegrass as well as alfalfa and tall fescue seeds were mixed and sown in the same lines. In 2019 nitrogen (Urea, 46 % N), phosphorus (single super phosphate, 16 % P_2O_5), and potassium (potassium sulfate, 45 % K_2O) were applied at the recommended rate of 150 kg ha^{-1} at the time

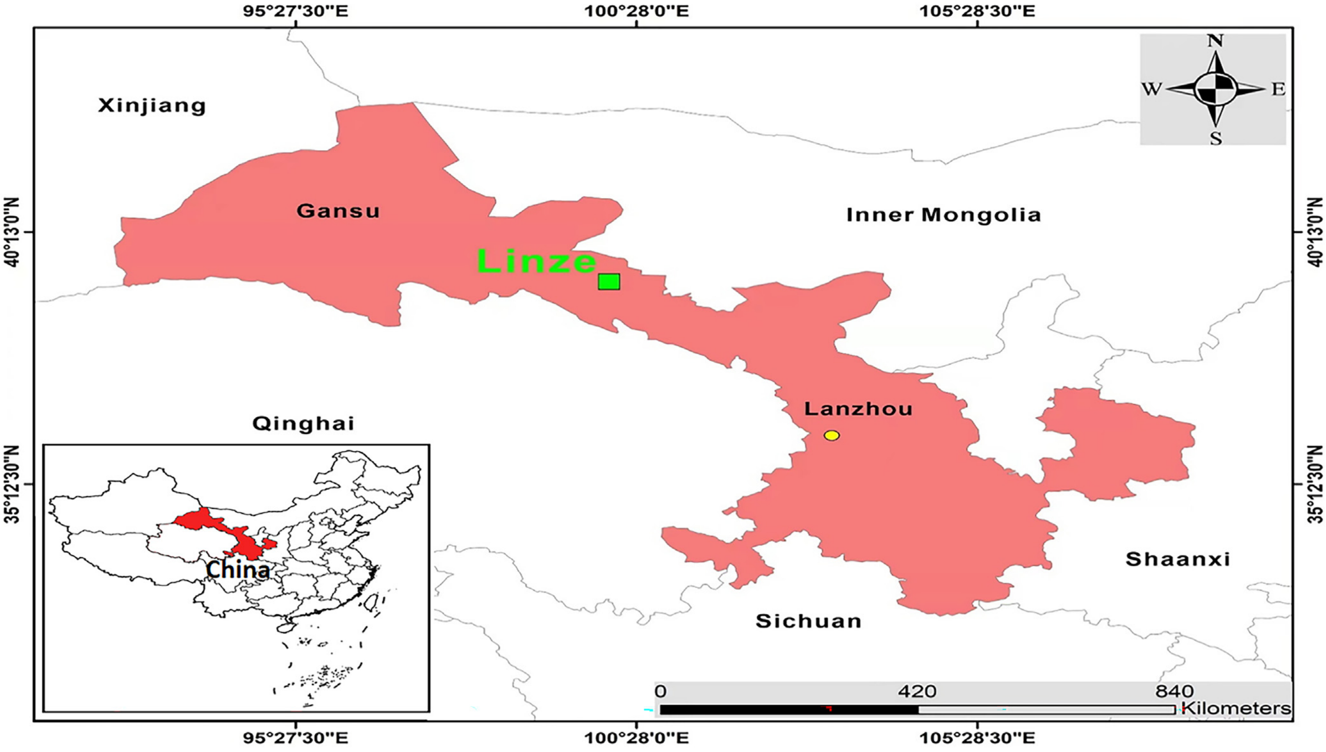

Fig. 1. The Lanzhou University experimental research site, Linze, Gansu province, China.

of sowing, whereas phosphorus and potassium were applied in May (start of re-greening stage) 2020 and 2021. In 2020 and 2021, two equal split doses of nitrogen were applied, with half of the nitrogen applied in May (start of re-greening stage) and the other half applied during the second irrigation. Three times, each year: In 2019, irrigation was applied on 22nd August. In 2020 irrigation was applied on 5th May (120 mm), 21st June (120 mm), and 18th August (120 mm). In 2021 irrigation was applied on 8th May (120 mm), 23rd June (120 mm), and 20th August (120 mm). All other agronomic practices were applied uniformly to all the treatments. These irrigation amounts and frequencies were based on the local farmers practice.

2.3. Samplings and measurements

2.3.1. Dry matter yield (kg ha^{-1})

Forage dry matter yield (DMY) was measured three times a year except in the year in which grass, alfalfa and alfalfa-grass mixtures were sown. The first DMY was measured in the first week of June (first harvest), the second was measured in the first week of August (second harvest), and the third was measured in the first week of October (third harvest) in each plot by randomly selecting three quadrats of 1 m^2 at three different locations. A constant weight was achieved by oven drying samples at 75 °C, and dry weight was determined with an electronic balance.

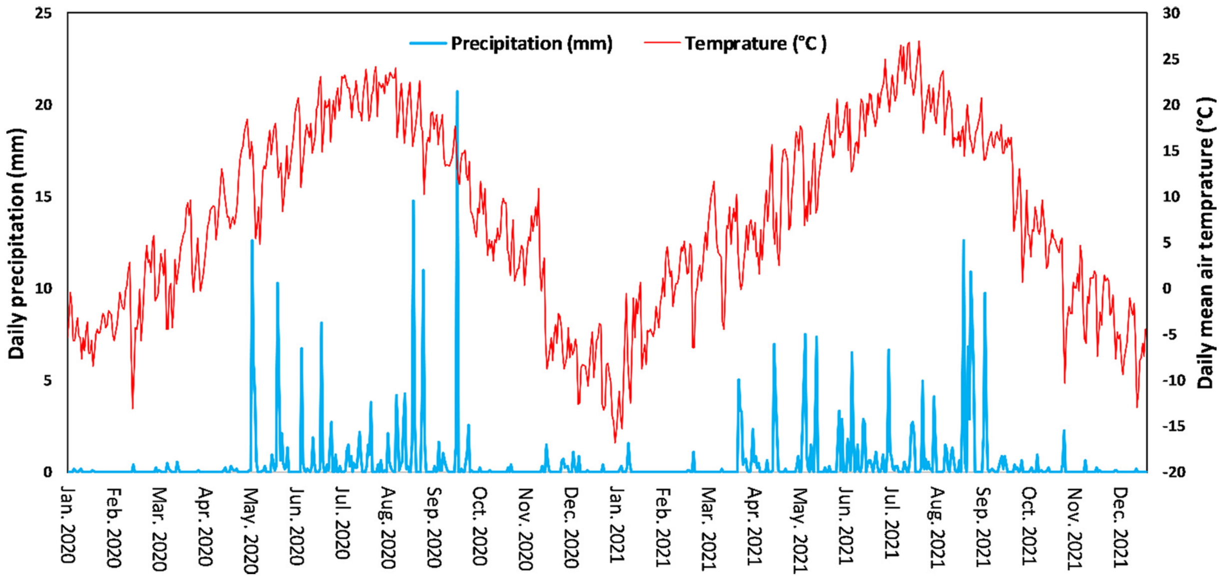

Fig. 2. Daily air temperature and precipitation at the study site in 2020 and 2021.

2.3.2. Soil physiochemical properties

At a depth of 0 to 20 cm, soil samples were collected in each plot at three random locations after GHG (N_2O, CH_4, CO_2) collection. To determine the soil moisture content (SM%), a gravimetric method was used. The soil samples were dried for 24 h at 105 °C, followed by 75 °C until constant weight (Ahmad et al., 2022).

In this experiment, the temperature of the soil was measured using a mercury-in-glass thermometer at a depth of 5 cm in the center of each plot. Soil temperature was recorded at 08:00, 10:00, 14:00, and 18:00 h daily. The samples of air-dried soil were ground into fine powder, then sieved through a mesh of 0.25 mm. Soil pH (SpH), soil organic carbon (SOC) content, and soil total nitrogen (STN) content were assayed according to Ning et al. (2020).

2.3.3. GHG sampling and analysis

Greenhouse gas emissions were measured three times per month from May until October in each plot. Greenhouse gas emissions sampling was carried out on 5th, 15th and 25th in May, June, July, August, September, and October. The average of three samplings in each month was used for data analysis. The measurements were carried out from 9:00 AM to 11:30 AM. A static opaque chamber was used to collect GHG samples. Static opaque stainless steel chambers (30 × 30 × 30 cm) were covered with a 2 cm thick polyurethane foam sheath to improve temperature stability. The chambers were equipped with battery-operated fans that mixed the air within the chamber as well as a silicone gel catheter (2 mm in diameter × 200 mm in length) attached to the top of each chamber for collecting gas samples. Greenhouse gasses samples were taken by using a stopcock and 50 mL plastic syringe. Samples were transferred into 300 mL plastic bags with aluminum foil and sealed with a screw cap. Greenhouse gas samples were collected at an intervals of 10 min (0, 10, 20, and 30 min), each containing approximately 300 mL of gas. The temperature inside the boxes was measured with an electronic thermometer at the time of sampling. Before and after gas sampling, soil temperatures were measured at 5 cm depth. The GHG concentrations were analyzed using a Los Gatos Research (LGR) analyzer (908–0011–0001, LGR, USA), for CH_4 and CO_2, and a different analyzer (908–0015–0000, LGR, USA) for N_2O concentration. To calculate the flux of gas emissions, the following formula was used (Hu et al., 2013).

$$\text{Flux} = \rho H \times \frac{P}{Po} \times \frac{273.15}{T} \times \frac{dc}{dt}$$

whereas ρ is the standard gas density (N_2O, CH_4, and CO_2), H is the height of the chamber, P atmospheric pressure at the site of sampling (85.48 kPa), P_0 pressure at standard atmospheric conditions (101.325 kPa), and T inside temperature of the chamber in Kelvin, and dc/dt is the rate of change in gas concentrations over time.

2.3.4. Cumulative GHG emissions, net GWP and GHGI

The cumulative emissions of N_2O, CH_4, and CO_2 were calculated based on Afreh et al. (2018).

$$\text{Cumulative emissions}\left(\text{kg ha}^{-1}\right) = \frac{\Sigma_{i=1}^{n}\left(F_i + F_{i+1}\right)}{2} \times \left(t_{i+1} - t_i\right) \times 24$$

where, F_i represents CO_2, N_2O and CH_4 gas emissions fluxes, F_{i+1} represents the next measured fluxes, (t_{i+1}—t_i) is the interval between two consecutive measurements, and n represents the total number of determinations.

Net GWP of CO_2, N_2O and CH_4 emissions were calculated using (IPCC) factors over a 100-year period (IPCC, 2013).

$$\text{Net GWP}\left(\text{kg CO}_2\ \text{eq.ha}^{-1}\right) = E_{CO_2} + 298E_{N_2O} + 25E_{CH_4}$$

where E represents the cumulative CO_2, N_2O and CH_4 fluxes in kg ha^{-1}. While 298 and 25 are the CO_2-equivalent conversion coefficients for N_2O and CH_4, respectively (Zhang et al., 2015).

According to the following equation, the GHGI was calculated (Lyu et al., 2019):

$$\text{GHGI}\left(\text{Kg CO}_2\ \text{eq.Kg}^{-1}\ \text{yield}\right) = \text{GWP/yield}$$

2.4. Statistical analysis

A two-way analysis of variance was performed for all the data using SPSS 17.0 (IBM Corporation) to evaluate the effect of species (grass, alfalfa and alfalfa-grass mixtures), year and their interaction on soil properties, DMY, GHG emissions, Net GWP and GHGI. Comparisons of means were performed with the least significant difference (LSD) test at $P < 0.05$. Pearson correlation analysis was used to determine the relationship of GHG fluxes with soil temperature, moisture and soil chemical properties. Graph Pad Prism 8 was used to develop the graphical presentation.

3. Results

3.1. Dry matter yield (kg ha^{-1})

Dry matter yield (DMY) was significantly affected by treatments and years (Table 1). The interaction between treatments and years for DMY was also highly significant. Maximum DMY of monoculture grasses was obtained in the first harvest, followed by the second harvest with the least yield at the last harvest. In contrast, the maximum DMY of alfalfa-grass mixtures was achieved during the second harvest, followed by the first harvest, and subsequently declined in the last harvest. The total DMY in 2021 was greater by 18 % compared with 2020. Forage yield varied among the grasses, alfalfa and the alfalfa-grass mixtures ($P < 0.01$), but there were no differences between grasses cultivated in monocultures ($P > 0.05$). Results indicated that alfalfa either in a mixture or as a monoculture crop produced more yield than grasses in both years. Specifically, binary mixtures of alfalfa with tall fescue produced the greatest total DMY (11,311 and 13,338 kg ha^{-1}), followed by alfalfa-smooth bromegrass mixtures (10,781 and 12,467 kg ha^{-1}), and alfalfa monoculture (9635.9 and 11,526 kg ha^{-1}) in 2020 and 2021, respectively. The yield of tall fescue or smooth bromegrass sown with alfalfa was greater by 96 and 85 % (mean of two years) compared to tall fescue and smooth bromegrass grass monocultures, respectively (Table 1). The ranking of DMY among different

Table 1
Dry matter yield (kg ha^{-1}) under grass, alfalfa, and alfalfa-grass mixtures throughout the experimental period in 2020 and 2021.

Year	Species	1st harvest (Kg ha^{-1})	2nd harvest (Kg ha^{-1})	3rd harvest (Kg ha^{-1})	Total DMY (Kg ha^{-1})
2020	Tall fescue	2599d	1634e	1611c	5845d
	Smooth bromegrass	2381e	1879d	1362d	5622d
	Alfalfa	3392c	3625c	2619b	9636c
	Alfalfa × S. bromegrass	3947a	4201b	2633b	10781b
	Alfalfa × T. fescue	3736b	4576a	2999a	11311a
	F	***104***	***300***	***239***	***714***
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***
2021	Tall fescue	2832c	2172d	1713c	6718d
	Smooth bromegrass	2987c	2476c	1492d	6955d
	Alfalfa	4032b	4642b	2851b	11526c
	Alfalfa × S. bromegrass	4336a	5183a	2947b	12467b
	Alfalfa × T. fescue	4538a	5400a	3399a	13338a
	F	***103***	***244***	***258***	***362***
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***
ANOVA analysis					
Species (S)		**	**	**	**
Years (Y)		**	**	**	**
S × Y		**	**	**	**

Different lowercase letters within the same column indicate significant differences ($P < 0.05$) between treatments. Stars indicate significance level, with *, $P < 0.05$, **, and $P < 0.01$.

species and species combinations was in the order of alfalfa-tall fescue > alfalfa-smooth bromegrass > alfalfa > tall fescue ≥ smooth bromegrass.

3.2. Soil physiochemical properties

3.2.1. Soil temperature (°C)

A higher soil temperature was observed during the growing season of 2021 compared to the growing season of 2020. Soil temperature gradually increased from May and reached to its maximum in July and then showed a decreasing trend until October (Fig. S1). Soil temperature was not significantly ($P < 0.05$) affected by species or species combinations.

3.2.2. Soil moisture content (%)

The soil moisture content reached its highest point in May and then declined until July, and again showed an increasing trend until October (Fig. S1). Soil moisture content was lower during 2021 growing season compared with 2020. Soil moisture was not significantly ($P < 0.05$) affected by species or species combinations.

3.2.3. Soil pH

Soil pH was significantly affected by species and year (Table 3). The interaction between species and year on soil pH was non-significant. Minimum soil pH was recorded in alfalfa during 2020 and 2021. The greater soil pH values (7.8 and 7.9) were observed in grasses monoculture plots, while the lowest pH values (7.5 and 7.6) were observed in alfalfa monoculture plots.

3.2.4. Soil organic carbon content

Species and year both significantly ($P < 0.05$) affected soil organic carbon content (Table 3). The interaction between species and year on SOC content was also significant. In comparison with grass monocultures, alfalfa and alfalfa-grass mixtures had a greater SOC. Soil organic carbon content was greater in 2021 compared with 2020. Alfalfa monoculture had a significantly greater soil organic carbon content (9.63 and 10.12 g kg^{-1}), followed by alfalfa-grass mixtures (9.56 and 9.85 g kg^{-1}), compared with grass monocultures (9.41 and 9.66 g kg^{-1}) in 2020 and 2021, respectively.

3.2.5. Soil total nitrogen content

The STN content was significantly affected by species and years, whereas the interaction between species and year was non-significant (Table 3). Soil total nitrogen content was greater in 2021 compared with 2020. Maximum soil total nitrogen content was recorded in alfalfa (0.898 and 1.200 g kg^{-1}) whereas minimum soil total nitrogen content was recorded in monoculture grasses (0.626 and 0.872 g kg^{-1}) in 2020 and 2021 respectively.

3.2.6. Soil C/N ratio

The C/N ratio was also significantly affected by species and year (Table 3). The interaction between species and year on C/N ratio was not significant. The soil C/N ratio was significantly lower in alfalfa (10.79 and 8.44) compared with grass monocultures (15.08 and 11.18) in 2020 and 2021 respectively.

3.3. GHG emissions

3.3.1. N_2O emission

The N_2O emission was significantly ($P < 0.05$) affected by plant species, years, as well as the interactive effects in an ANOVA analysis. Grass, alfalfa, mono-culture, and mix-culture were sources of N_2O emission. Both plant species and growing season significantly altered N_2O emission. Soil N_2O emissions reached its peak in the mid-growing season (July), then declined gradually at later stages in both growing seasons 2020–2021 (Fig. 3). The differences were substantial between N_2O emissions from alfalfa, grasses and alfalfa-grass mixtures. The highest cumulative N_2O emissions (0.31 and 0.39 kg ha^{-1}) was observed in alfalfa monoculture, followed by alfalfa-grass mixtures (0.26 and 0.34 kg ha^{-1}), while the lowest values (0.24 and 0.30 kg ha^{-1}) were observed in grasses monoculture plots in 2020 and 2021, respectively (Fig. 4). Seasonal N_2O emission was highest in alfalfa in each month (except May 2021) and cumulative N_2O emissions were ranked in the order of alfalfa > alfalfa-grass mixtures > grasses. The amount of N_2O emissions accumulated in 2021 was greater by 27.5 % than that in 2020 (Fig. 4).

3.3.2. CH_4 uptake

The CH_4 sink was measured in fields that contained grasses, alfalfa, and alfalfa-grass mixtures. The CH_4 uptake varied from −0.98 to −21.74 μg m^{-2} h^{-1} for grass, alfalfa, and alfalfa-grass mixtures. The CH_4 uptake tended to reach peak values from June (−8.11 and −12.55 μg m^{-2} h^{-1}) to July (−13.72 and −20.45 μg m^{-2} h^{-1}) and was lowest from September (−2.57 and −5.36 μg m^{-2} h^{-1}) to October (−1.32 and −2.83 μg m^{-2} h^{-1}), in 2020 and 2021 respectively (Fig. 3). Cumulative CH_4 uptake over all treatments was highest in 2021 (−0.422 kg h^{-1}) and lowest in 2020 (−0.280 kg h^{-1}), respectively (Fig. 4). Significant differences in the cumulative CH_4 uptake were observed between grass, alfalfa and alfalfa-grass mixtures. In 2020, maximum CH_4 uptake was observed in alfalfa monoculture (−0.302 kg ha^{-1}) whereas the lowest CH_4 uptake was achieved in monoculture tall fescue (−0.263 kg ha^{-1}). Contrary to the results presented in the first year, CH_4 uptake values in monoculture alfalfa (−0.395 kg ha^{-1}) declined in 2021 in comparison with the monoculture tall fescue plots (−0.443 kg ha^{-1}) (Fig. 4).

3.3.3. CO_2 emissions

Grass, alfalfa and alfalfa-grass mixtures significantly ($P < 0.05$) affected CO_2 emissions. The CO_2 emission fluctuated from month to month, with the highest emission observed in July (335.11 and 413.54 mg m^{-2} h^{-1}) and the lowest emission observed in October (82.80 and 125.86 mg m^{-2} h^{-1}) in all treatments (Fig. 3). As shown in Fig. 4, the CO_2 emission was significantly greater (12,098 kg ha^{-1} averaged across all treatments) in 2021 compared to 2020 (9354 kg ha^{-1}). In addition, significant differences were observed among the grass, alfalfa and alfalfa-grass mixtures. The highest CO_2 emission was observed in alfalfa monoculture (10,477 and 13,682 kg ha^{-1}), followed by alfalfa-grass mixtures (9488 and 12,446 kg ha^{-1}), while the lowest CO_2 emission (8660 and 10,958 kg ha^{-1}) was recorded in grasses during 2020 and 2021, respectively (Fig. 4).

3.4. Net GWP and GHGI

The Net GWP from alfalfa monoculture was 11,919 kg CO_2 eq. ha^{-1} (mean of 2020 and 2021), which was greater than tall fescue (by 23.84 %), smooth bromegrass (by 22.57 %), alfalfa-smooth bromegrass mixtures (by 5.51 %), and alfalfa-tall fescue mixtures (by 15.33 %). The GWP of N_2O emission from alfalfa monoculture during 2020 and 2021 was 103.55 kg CO_2 eq. ha^{-1}, which was greater than tall fescue (by 32.02 %), smooth bromegrass (by 25.29 %), alfalfa-smooth bromegrass mixtures (by 13.01 %), and alfalfa-tall fescue mixtures (by 22.69 %). During the growing season of 2020, alfalfa monoculture soil uptake more CH_4 than grasses and grass-legume mixtures; however, during the growing season of 2021, alfalfa monocultures absorbed a lower amount of CH_4 than grasses or alfalfa-grass mixtures. All cropping systems investigated were net contributors to global warming potential (Table 2).

GHGI was also significantly ($P < 0.05$) affected by grass, alfalfa and alfalfa-grass mixtures. Compared with the tall fescue, smooth bromegrass, and alfalfa monoculture, alfalfa-tall fescue and alfalfa-smooth bromegrass significantly ($P < 0.001$) decreased the GHGI (Table 2). Alfalfa-smooth bromegrass mixtures reduced GHGI by 14 and 59 % (mean of two years) compared with the alfalfa and smooth bromegrass monoculture. Alfalfa-tall fescue mixtures reduced GHGI by 33 and 82 % (mean of two years) compared with the alfalfa and tall fescue monoculture. Alfalfa-tall fescue mixtures reduced GHGI by 16 % (mean of two years) compared with the Alfalfa-smooth bromegrass mixture. Our results demonstrated that the

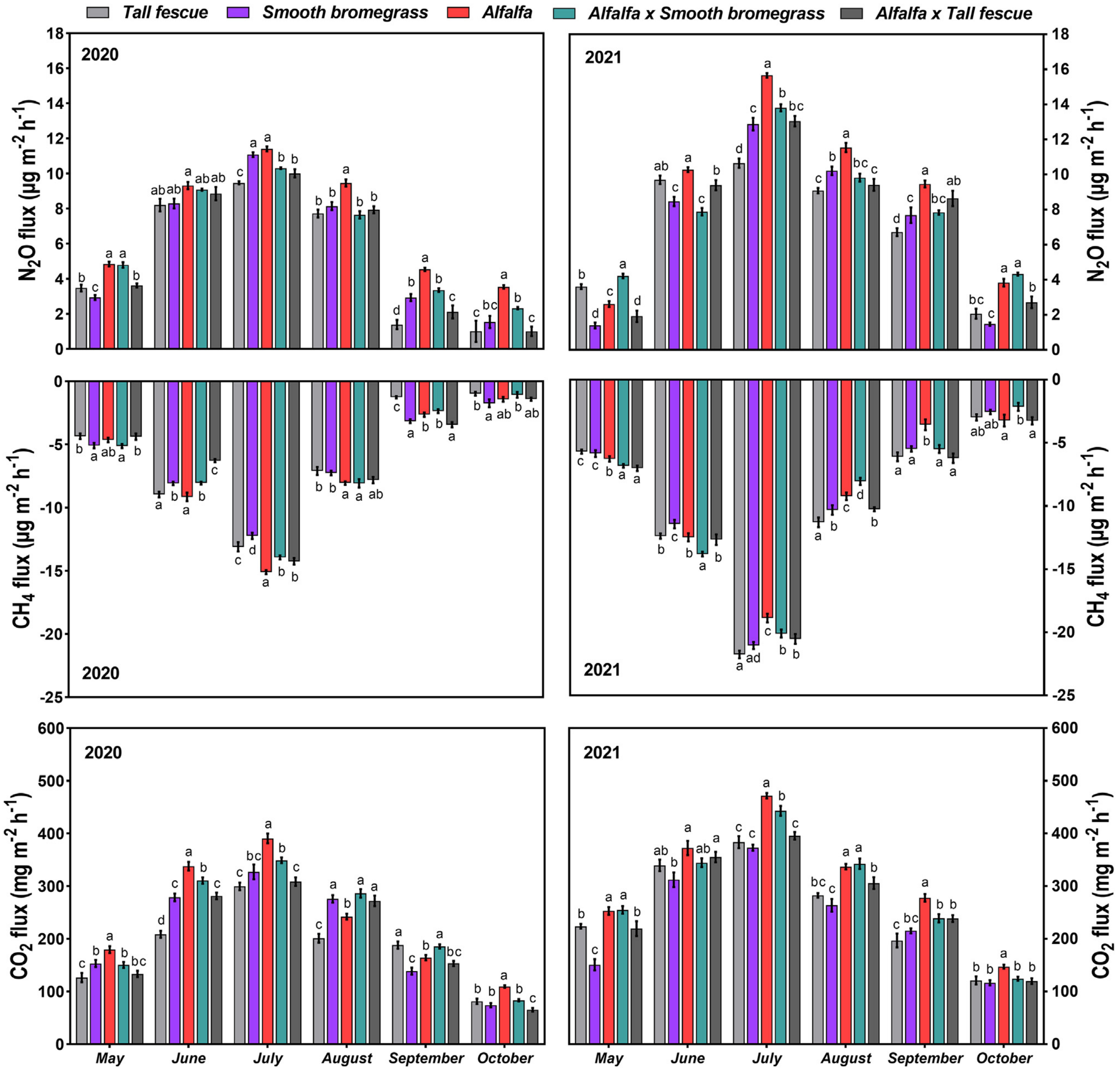

Fig. 3. The dynamics of soil N_2O, CH_4, and CO_2 emissions in 2020 and 2021 in the grass, alfalfa, and alfalfa-grass mixture species. Means followed by the same lowercase letters indicate non-significant ($P > 0.05$) differences among crop species in 2020–2021.

alfalfa-tall fescue mixture significantly reduced GHGI compared with other treatments.

3.5. Pearson correlation coefficient (r) of GHG emissions with soil temperature, moisture and soil chemical properties

Pearson correlation coefficient of soil GHG emissions with soil temperature, moisture, and soil chemical properties was statistically significant (Table 4). Soil N_2O and CO_2 emissions were significantly ($P < 0.05$) positively correlated with ST, SpH, SOC, and STN content, while negatively correlated with SM and C/N ratio in the grass, alfalfa, and alfalfa-grass mixtures. Furthermore, soil CH_4 emissions were significantly ($P < 0.05$) negatively correlated with soil ST, SpH, SOC, and STN content, but positively correlated with SM and C/N ration in the grass, alfalfa, and alfalfa-grass mixtures throughout the experimental period of 2020 and 2021.

4. Discussion

4.1. Dry matter yield as affected by grass, alfalfa and alfalfa-grass mixtures

Forage mixtures containing legumes are believed to produce greater yields than grass, legume monocultures (Ahmad et al., 2018; Gierus et al., 2012; Sturludóttir et al., 2014). The present study revealed that in both 2020 and 2021, alfalfa monoculture and alfalfa-grass mixtures yielded significantly greater dry matter yield than monoculture grasses. Grass-legume mixtures were previously reported to increase stand persistence and yield more than monocultures of alfalfa (Bélanger et al., 2014; Malhi et al., 2002). The niche complementarity effect contributes to the ability of mixtures to produce more forage biomass than monocultures (Finn et al., 2013; Nyfeler et al., 2009; Sanderson, 2010). Plant ecologists use niche complementarity as a theoretical concept to describe the effects of coexisting species on community productivity, where both species use

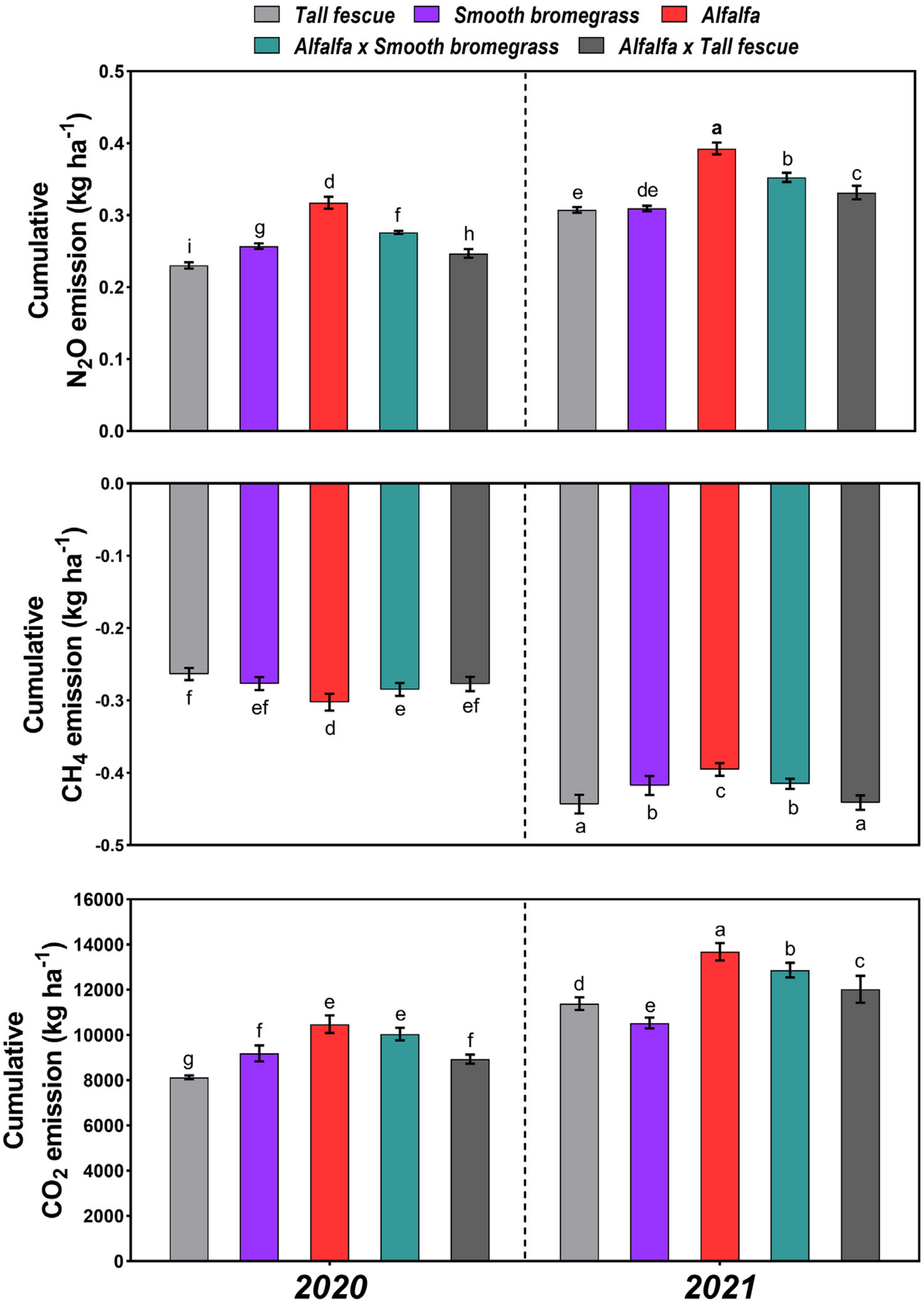

Fig. 4. Cumulative greenhouse gas (N_2O, CH_4, and CO_2) emissions from grass, alfalfa, and alfalfa-grass mixture species. Means followed by the same lowercase letters indicate non-significant ($P > 0.05$) differences among crop species in 2020–2021.

resources differently or use resources in different forms (Nyfeler et al., 2009). As an example, grasses and alfalfa have different root systems (fibrous roots vs. tap roots), which facilitate their utilization of nutrients at different depths (Aponte et al., 2019). A mixture of grass and alfalfa can also produce greater yields than monocultures due to their complementary seasonal growth cycles; legumes grow rapidly during mid growing season, on the other hand, grass production may slump (Baron and Belanger, 2007; Thompson, 2013). In agreement with the previous findings (Kamran et al., 2022), a greater production of alfalfa forage dry matter yield was observed in the second cut. Forage dry matter yields of tall fescue and smooth bromegrass decreased markedly from the first to the second and third cuts, respectively, and the second year was more productive than the first year in this study. Increasing forage dry matter yield from the first to second production year has been observed frequently for forage stands (Foster et al., 2014).

4.2. Relationship of GHG with soil temperature, moisture and soil chemical properties

Soil temperature is widely recognized as a contributing factor to GHG emissions (Lang et al., 2011). The N_2O fluxes response to soil temperature showed neutral (Li et al., 2016), positive (Cui et al., 2018), and even negative effects in previous studies (Hu et al., 2010). Such variations are possible due to the differences in biomes, climate manipulations, as well as experimental techniques, which may influence the composition of the microbial communities responsible for N_2O emissions (Li et al., 2020). A linear

Table 2

Global warming potentials (GWP, kg CO_2-eq ha^{-1}) and yield-based greenhouse gas emissions intensity (GHGI, kg CO_2 eq. kg^{-1} yield) throughout the experimental period in 2020 and 2021.

Year	Species	GWP_{N2O}	GWP_{CH4}	GWP_{CO2}	Net GWP	GHGI
2020	Tall fescue	67.088e	−6.434c	7963d	8024d	1.373b
	Smooth bromegrass	74.918c	−6.761bc	8988c	9056c	1.6073a
	Alfalfa	92.504a	−7.383a	10256a	10341a	1.0775c
	Alfalfa × S. bromegrass	80.472b	−6.951b	9836b	9909b	0.9315d
	Alfalfa × T. fescue	71.964d	−6.762bc	8745c	8810c	0.7778e
	F	***159.000***	***8.660***	***43.100***	***43.800***	***105.000***
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
2021	Tall fescue	89.78d	−10.833a	11145d	11224d	1.6858a
	Smooth Bromegrass	90.37d	−10.197b	10311e	10391e	1.5045b
	Alfalfa	114.6a	−9.657c	13392a	13497a	1.1632c
	Alfalfa × S. bromegrass	102.79b	−10.155b	12590b	12683b	1.0238d
	Alfalfa × T. fescue	96.83c	−10.785a	11773c	11859c	0.8958e
	F	***107.000***	***14.100***	***40.200***	***40.900***	***68.600***
	P	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
ANOVA analysis						
Species (S)		*	ns	**	**	**
Years (Y)		**	**	**	**	**
S × Y		ns	ns	ns	**	**

Different lowercase letters within the same column indicate significant differences ($P < 0.05$) between treatments. Stars indicate significance level, with *, $P < 0.05$, **, and $P < 0.01$.

Table 3

Soil pH (SpH), soil organic carbon (SOC), and soil total nitrogen (STN), under grass, alfalfa, and alfalfa-grass mixtures, throughout the experimental period in 2020 and 2021.

Year	Species	SpH	SOC g kg^{-1}	STN g kg^{-1}	C/N
2020	Tall fescue	7.8081a	9.3972c	0.6265c	15.07a
	Smooth bromegrass	7.8203a	9.4444c	0.6273c	15.10a
	Alfalfa	7.5219c	9.6367a	0.8988a	10.79d
	Alfalfa × S. bromegrass	7.665b	9.5849ab	0.8375ab	11.51c
	Alfalfa × T. fescue	7.6711b	9.5495b	0.7851b	12.25bc
	F	***32.1***	***21.4***	***12.7***	***13.8***
	P	***<0.001***	***<0.001***	***<0.01***	***<0.01***
2021	Tall fescue	7.9181a	9.645c	0.8675d	11.29a
	Smooth bromegrass	7.9303a	9.703c	0.8776 cd	11.07ab
	Alfalfa	7.6319c	10.12a	1.2008a	8.449c
	Alfalfa × S. bromegrass	7.7577b	9.876b	1.0438b	9.48bc
	Alfalfa × T. fescue	7.7811b	9.846b	1.024bc	9.82abc
	F	***31.4***	***16.2***	***7.3***	***3.98***
	P	***<0.05***	***<0.001***	***<0.01***	***<0.05***
ANOVA analysis					
Species (S)		**	**	**	**
Years (Y)		**	**	**	**
S × Y		ns	**	ns	ns

Different lowercase letters within the same column indicate significant differences ($P < 0.05$) between treatments. Stars indicate significance level, with *, $P < 0.05$, **, and $P < 0.01$.

increase in N_2O emission rate with an increase in soil temperature was evident in all treatments in this study. In general, the N_2O fluxes started to elevate and reached peak values in June and July, confirming that soil temperature has an impact on emissions by affecting the microorganisms and root activity (Schaufler et al., 2010). Similarly, soil moisture is essential for microbial growth and survival (Anthony et al., 2020; Banerjee et al., 2016; Lu et al., 2015; Pang et al., 2011; Trost et al., 2013). A negative correlation was found between soil moisture and soil N_2O emissions in this study. Greater soil moisture may be beneficial for microbes that regulate soil N_2O emissions (de Vries and Bardgett, 2016; Mommer et al., 2011), but, it also activates soluble salts in the soil, which adversely affects various processes associated with mineralization and nitrification (Ning et al., 2020). Furthermore, nitrification and denitrification are affected by soil pH (Wu et al., 2021). N_2O emission decreases as pH decreases due to decreased denitrification. Our results showed that soil pH is a moderate regulator of GHG emissions from experimental soils, and significant positive correlations between N_2O emissions and soil pH were observed. A positive correlation was also shown in previous studies between N_2O emissions and soil pH (Cuhel et al., 2010; Domeignoz-Horta et al., 2018). It is well known that increased SOC and STN emit more N_2O (Muhammad et al., 2019; Sanz-Cobena et al., 2014; Zhao et al., 2020b), our results confirm this, for SOC, and STN. The effect is most likely due to the increased N availability for microbial processes with soil total nitrogen, resulting in greater N_2O production (Butterbach-Bahl et al., 2002), while soils with a greater C/N ratio emitted less N_2O (He et al., 2019; Klemedtsson et al., 2005; Ming et al., 2018). There was moderately strong negative relationship between N_2O emissions and the C/N ratio, which is consistent with previous studies (Hu et al., 2019).

Although methanogenesis (production of CH_4) and methanotrophy (consumption of CH_4), are well known to increase with rising temperature (Nazaries et al., 2013). The rate of CH_4 uptake varied throughout the crop growth period, the uptake was greater in summer and lower in winter, and a significant correlation of CH_4 uptake with the soil temperature was observed in our study, which is consistent with a previous study (Liebig et al., 2019; Luo et al., 2013). It is expected that the microbial community structure changes when the temperature changes (Serrano-Silva et al., 2014). A negative relationship between soil moisture and CH_4 uptake rates was also observed in our study. Soil moisture adversely affects CH_4 uptake (Le Mer and Roger, 2001), because it stimulates methanogenesis (production of CH_4) and inhibits methanotrophy (consumption of CH_4). A possible explanation is that higher soil moisture leads to anaerobic conditions, which increased CH_4 production and at the same time reduced CH_4 consumption by reducing the availability of O_2 for methanotrophs (Nazaries et al., 2013). This study was consistent with previous studies (Hartmann et al., 2011; Price et al., 2004; Stiehl-Braun et al., 2011). Previous studies indicated that increased soil pH favors the growth of methanotrophy, thereby increasing the soil CH_4 uptake (Inubushi et al., 2005; Jeffery et al., 2016). Our results also demonstrate that the CH_4 uptake rate showed a clear increasing trend with soil pH. Previous studies found that the CH_4 uptake showed a positive response to soil pH in grassland soils (Yu et al., 2017). Similarly, significant positive correlations were observed between CH_4 uptake and soil nitrogen deposition. This study's findings are in agreement with those of previous studies (Zhao et al., 2017). One possible explanation is that the high nitrogen immobilization capacity in the grassland soils could protect methanotrophs from exposure to NH_4^+ (Steinkamp et al., 2001). In addition, soil organic carbon and CH_4 uptake showed a positive relationship. In contrast, a mire study in central Europe indicated that methane-oxidizing bacteria may preferentially utilize organic carbon over CH_4 as a substrate, resulting in lower overall rates of CH_4 oxidation (Wieczorek et al., 2011). We speculate that seasonal variations may influence the effect of organic carbon on CH_4 oxidation rates between these studies. In contrast, the uptake of CH_4 during the growing season was negatively correlated with the C/N ratio, consistent with previous studies (Shi et al., 2014).

Soil CO_2 emissions in cropland are mainly associated with heterotrophic (microbial) and autotrophic (roots) respiration (Kou et al., 2007; Kuzyakov, 2006), which otherwise depends on soil temperature and moisture (He et al., 2018). When soil moisture is not a limiting factor, temperature increases soil respiration (Jassal et al., 2008). Soil temperature controls CO_2 emissions by affecting organic matter decomposition, oxidation, microbial activity, and root growth, as well as the process of carbon mineralization (Jabro et al., 2008). We found a significant positive correlation between CO_2 emissions and soil temperatures in our study, which is consistent with findings from previous studies (Dong et al., 2017; Laganière et al., 2012; Shen et al., 2017; Tong et al., 2017; Yeboah et al., 2016). Similarly, changes in soil moisture alter soil physicochemical properties and microbial activity, which in turn affect soil CO_2 emissions (Hou et al., 2020). In low soil moisture conditions, microbial activity is limited, whereas in high soil moisture conditions, air porosity is reduced and limits gas diffusion,

Table. 4
Pearson Correlation coefficient (r) of GHG emissions with soil temperature, moisture and soil chemical properties in grass, alfalfa and alfalfa-grass mixtures, throughout the experimental period in 2020 and 2021.

GHG	Species	Factors					
		ST	SM	SpH	SOC	STN	C/N
N_2O	Tall fescue	0.910**	−0.432**	0.701**	0.663**	0.721**	−0.583**
	Smooth bromegrass	0.953**	−0.532**	0.738**	0.766**	0.869**	−0.827**
	Alfalfa	0.948**	−0.547**	0.828**	0.792**	0.897**	−0.706**
	Alfalfa × S. bromegrass	0.939**	−0.590**	0.836**	0.881**	0.803**	−0.653**
	Alfalfa × T. fescue	0.930**	−0.433**	0.825**	0.755**	0.817**	−0.761**
CH_4	Tall fescue	−0.902**	0.593**	−0.747**	−0.745**	−0.701**	0.519**
	Smooth bromegrass	−0.896**	0.664**	−0.704**	−0.816**	−0.775**	0.675**
	Alfalfa	−0.911**	0.725**	−0.883**	−0.849**	−0.812**	0.625**
	Alfalfa × S. bromegrass	−0.895**	0.535**	−0.904**	−0.889**	−0.696**	0.522**
	Alfalfa × T. fescue	−0.888**	0.546**	−0.855**	−0.791**	−0.802**	0.687**
CO_2	Tall fescue	0.863**	−0.579**	0.714**	0.757**	0.682**	−0.573**
	Smooth bromegrass	0.932**	−0.514**	0.762**	0.826**	0.822**	−0.804**
	Alfalfa	0.915**	−0.725**	0.840**	0.877**	0.835**	−0.713**
	Alfalfa × S. bromegrass	0.931**	−0.598**	0.899**	0.920**	0.777**	−0.652**
	Alfalfa × T. fescue	0.912**	−0.469**	0.878**	0.803**	0.790**	−0.807**

Stars indicate significance level, with *, $P < 0.05$, **, and $P < 0.01$.

affecting CO_2 emissions (Davidson et al., 2000; Zhang et al., 2011). According to the current study, CO_2 emissions and soil moisture were negatively correlated. This can be explained by the low diffusion rate of saturated soil at high moisture levels, leading to CO_2 and O_2 depletion within the soil, limiting both weathering and microbial activity (Cueva et al., 2019). In contrast, we found that CO_2 fluxes were positively correlated with soil pH, which is similar to previous research (Buragienė et al., 2019; Wanyama et al., 2019). In a similar manner, CO_2 emissions seem to be influenced by soil nitrogen, organic carbon, and the C/N ratio (Hashimoto et al., 2011). Soil nitrogen may increase (Contosta et al., 2011; Wang et al., 2015), decrease (Janssens et al., 2010; Jiang et al., 2010), or even have no significant effects on CO_2 emissions in previous studies (Krause et al., 2013; Li et al., 2012). In our study, soil nitrogen and soil organic carbon were positively correlated with CO_2 emissions. There is a possibility that soil microbes are stimulated by available carbon (Meijide et al., 2010; Whitaker et al., 2014). There is evidence that increases in soil organic carbon content can lead to an increase in soil CO_2 emissions (Badía et al., 2013; Liebig et al., 2013). Comparatively, the cumulative CO_2 emissions during the growing season were negatively correlated with C/N ratios, since low C/N ratios are more effective in minimizing CO_2 emissions (Guo et al., 2012; Kim et al., 2013).

4.3. GHG fluxes as affected by grass, alfalfa and alfalfa-grass mixtures

Overall, results showed lower N_2O emissions in alfalfa-grass mixtures than that of alfalfa monoculture stands, suggesting that grass addition to alfalfa monocultures can be an effective means to reduce soil N_2O emissions. The results of this study are similar to those of a previous study, which concluded that legume crops reduce N_2O losses when grown in legume/non-legume mix cropping systems (Luo et al., 2018; Pappa et al., 2011). There is no surprise that legume crops emit more N_2O than non-legume crop. This is because legumes fix nitrogen from the atmosphere and provide more nitrogen to the succeeding crops than non-legume crops (Muhammad et al., 2019). Moreover, the grasses also use part of the N fixed reducing the N available to be emitted as N_2O (Byers et al., 2021; Cummins et al., 2021; Meza et al., 2022). In response to the increase in soil N availability, more N_2O is produced through the process of nitrification and denitrification, resulting from increased microbial activity (Basche et al., 2014; Chirinda et al., 2010; Komatsuzaki et al., 2008; McSwiney et al., 2010). The results of our study are consistent with the findings of a previous study, which indicated that legume crops increase N_2O emissions through increased nitrogen input in comparison with non-legumes (Garland et al., 2011; Hwang et al., 2017; Sanz-Cobena et al., 2014). In contrast to legume crops, non-legume crops can reduce soil profile NO_3^-N and nitrogen leaching more effectively (Aguilera et al., 2013; Kashif et al., 2018; Moore et al., 2014; Zhao et al., 2015) resulting in reduced soil N_2O emissions (Abdalla et al., 2014; Mitchell et al., 2013; Sanz-Cobena et al., 2014; Snyder et al., 2009).

Grassland ecosystems normally act as net sinks for atmospheric CH_4 (Megonigal and Guenther, 2008). The CH_4 uptake rates in all plant species types (grasses, alfalfa and mixture) in our study were negative, suggesting that soil acts as sink for atmospheric CH_4 when these crops are grown. These results are consistent with findings of other studies on upland ecosystems (Liu et al., 2017a; Martins et al., 2017; Rong et al., 2015; Tate, 2015). In contrast, the uptake of CH_4 by alfalfa-grass mixtures and monoculture grasses increased with time during the second experimental year of 2021. The seasonal variation in soil CH_4 uptake in our study could be attributed to differences in the structure and function of soil microbial communities, and these microbial communities may differ among plant species (Aronson et al., 2013; Nazaries et al., 2011). Similarly, previous studies have shown that monocultures of legumes are a smaller sink for atmospheric CH_4 than monocultures of grasses or grass-legume mixtures (Niklaus et al., 2006). It is possible that the addition of N to the soil system via legume fixation of atmospheric N_2 would increase soil N pools and suppress CH_4 uptake in alfalfa plots (Aronson and Helliker, 2010). However, several studies indicated that soil NH_4^+ N inhibits CH_4 uptake (Liu et al., 2017b; Wu et al., 2020), and similar to NH_4^+ N, soil NO_3^- N also inhibits the uptake of CH_4 (Hu et al., 2020). This could be contributing to the accumulation of NH_4^+-N and NO_3^--N and, further, affecting CH_4 uptake by modifying the activity and composition of the methanotrophic microbial community (Mohanty et al., 2006). Furthermore, short-term and long-term N addition stimulates CH_4 uptake in soil, however, studies have also reported no effects or inhibiting effects (Chen et al., 2013; Fang et al., 2014; Jang et al., 2011).

Elevated CO_2 is a major factor influencing global climate change (Malhi et al., 2021; Pugnaire et al., 2019; Xiao et al., 2018). Different crop species may contribute to the increase in CO_2 emissions due to the increased carbon inputs into the soil, increasing the carbon substrate availability and microbial activity (Poeplau and Don, 2015; Steenwerth and Belina, 2008a, 2008b). Similar findings have been reported from other research indicating that different crops increase soil respiration and metabolic activity, which increases CO_2 emissions (Sanz-Cobena et al., 2014). Furthermore, CO_2 emissions are indirectly increased by crops because they increase soil organic matter, soil porosity, soil moisture, and subsequent vegetation root growth (Al-Kaisi and Yin, 2005; Bhattacharyya et al., 2012; Hubbard et al., 2013; Olesen et al., 2007; Parkin et al., 2016). Assessing the impacts of forage crop species and combinations on CO_2 balance is key for developing land management strategies that mitigate climate change, as well as optimize productivity (Ibañez et al., 2021). According to our findings, CO_2 fluxes significantly increased by the presence of alfalfa in all cases, although grass monocultures and mixtures with alfalfa produced significant differences compared to alfalfa monocultures. These results are consistent with those reported in other studies (Asgedom and Kebreab, 2011; Huang et al., 2004; Rochette and Janzen, 2005), who found that legumes emit more CO_2 than cereals and grasses. There is a possibility that high CO_2 emissions are associated with legume plots due to their high microbial activity in the rhizosphere, which increases soil organic matter mineralization and subsequently increases CO_2 emissions (Chapela et al., 2001; Sapkota et al., 2012).

4.4. Net GWP and GHGI

The net exchange of nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) in cropping systems is measured as the net global warming potential (Net GWP) of crop production (Sainju et al., 2014; Wolff et al., 2018). Furthermore, over 100 years, the global warming potential of N_2O and CH_4 is 298 and 25 times greater, respectively, than that of CO_2 (IPCC, 2013; Mander et al., 2014; Sun et al., 2018). In consequence, even very small amounts of N_2O and CH_4 emissions could result in significant CO_2 equivalents (CO_2-eq) and pose a significant risk to the environment (Chen et al., 2020). Based on net global warming potential (GWP), monoculture grasses showed the lowest value. Comparing alfalfa-grass mixtures with alfalfa monoculture, the alfalfa-grass mixture showed a significant reduction in N_2O emissions, which was attributed to lowering net global warming potential. Similarly, in a previous study, intercropping legume (*Trifolium ambiguum* M. Bieb.) with grass (*Spartina pectinata* Link) decreased N_2O flux and resulted in low net GWP (Abagandura et al., 2020). The overall positive global warming potential observed in this study is consistent with previous research on managed grasslands (Merbold et al., 2014; Van de Riet et al., 2013), and alpine steppes, and meadow grasslands (Cai et al., 2013).

An environmental indicator of greenhouse gas intensity (GHGI) is the global warming potential divided by crop yield. It is used to measure agricultural production in relation to greenhouse gases emissions (Zhang et al., 2012). A lower GHGI value means that less GWP is generated to produce the same crop yield, while a higher value indicates that more GWP is generated to produce the same crop yield (Liu et al., 2019a). Hence, to address climate change, GHGI can be used to determine the most appropriate farming practices for mitigating the effects of climate change and ensuring food security (Huang et al., 2013; Van Groenigen et al., 2013). Our results showed that GHGI can be substantially decreased with the alfalfa-grass mixed cropping. In other words, the mixed cropping strategy resulted in lower greenhouse gas emissions when producing the same amount of outputs (Wang et al., 2021).

5. Conclusions

In conclusion, our findings indicated that alfalfa-perennial grass mixtures are a promising strategy for enhancing grassland production while minimizing GHG emissions. Alfalfa mixture with tall fescue and smooth bromegrass was more productive than alfalfa and grass monoculture. Alfalfa-tall fescue mixtures resulted in greater yield than alfalfa-smooth bromegrass mixtures. Alfalfa monoculture and alfalfa-grass mixtures increased the SOC and STN contents compared to grasses monoculture. At the same time, the GHG emissions from alfalfa-grass mixtures were lower than alfalfa monoculture. Alfalfa emitted more N_2O than grasses monoculture or alfalfa-grass mixtures. An increase in N_2O emissions can result in changes in global warming potential, and alfalfa-grass mixtures mitigated GHG emissions. Ultimately, our research contributes to the knowledge of forage yield stability and feed security, and reducing global warming potential and help to design optimal forage crop management strategies for fields and farms.

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CRediT authorship contribution statement

Fujiang Hou conceived and designed the experiment. Muhammad Usman Ghani and Muhammad Kamran performed the experiment. Muhammad Usman Ghani wrote the manuscript. Irshad Ahmad and Shanning Lou contributed to reagents/materials/analysis tools. Cheng Zhang, Wanhe Zhu contributed to samplings and field management. Muhamad Kamran and Adnan Arshad assisted in data analysis and English language revision. All authors read and approved the final manuscript.

Data availability

Data will be made available on request.

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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