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C3 and C4 Grass Species: Who Can Reduce Soil Nitrous Oxide Emissions in a Continental Arid Region?

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Abstract: In order to relieve grazing pressure, drought-tolerant grass species are widely cultivated in arid regions. However, soil N emission is largely neglected while pursuing forage yield. We carried out a randomized block study to investigate whether and how the C3 and C4 grass species differ in soil N emission in a typical salinized field with temperate continental arid climate in the northwest inland regions, China. We quantified soil N_2O flux from two C3 (barley and rye) and two C4 grass species [corngrass and sorghum hybrid sudangrass (SHS)] in fields during the growing season (from May to September) by using the static box method, and then determined the relationships between soil N_2O fluxes and forage yield and soil properties. Results show that soil available nitrogen, soil temperature, pH, soil organic carbon, and total nitrogen were correlated, but soil water was anti-correlated with soil N_2O fluxes. In addition, N_2O flux increased significantly faster with soil temperature in C4 than in C3 grass fields. Although the lower total N_2O emission fluxes were detected for C3 species, the lower yield-scaled N_2O was detected for C4 species. Our study provided insights into the determination of grass species and the understanding of mechanisms regulating N_2O fluxes in C3 and C4 species in the continental arid regions.

Keywords: greenhouse gas; denitrification; soil organic carbon; hay yield; crude protein yield; pasture

1. Introduction

Crops and livestock are the core components of an agricultural system, and their interaction drives the continuous evolution of agricultural systems. Forage crops sustain more than 70% of sheep and goat, as well as 50% of meat products in the world [1]. In China, more than 60% of cultivated land is located in arid and semi-arid areas [2], where stall-feeding rather than grazing is the main model of livestock production due to the serious seasonal drought [3]. Therefore, in recent years crop forages have been widely planted to meet the increasing demand for meat products and release grazing pressure in these areas. Gramineae grass is one kind of the most important forage crops, and some annual grass species are very productive even under arid conditions [4–6]. Compared to C3 species, C4 species are more suitable for growing in an arid region due to shrinking pore diameter and then reducing transpiration and water loss under drought. Land-use in which intensified C3 species are strategically diversified with C4 species may lead to benefits for soil microbiological diversity [7], soil water content and N use efficiencies [8], soil carbon sequestration [9], and other ecosystem services including saline-alkali land improvement [10]. Despite considerable recent research try to better understand these factors, the differences between both species of grass system in term of N_2O emission

remain poorly understood, even though grassland represents a great anthropogenic source of these emissions [11,12]. As a potent greenhouse gras (GHG), N_2O has a global warming potential 298 times of carbon dioxide and is the most important ozone-depleting emission [13,14].

Soil N_2O fluxes are driven by nitrification (oxidation of NH_4C to NO_3^- via NO_2^-) under aerobic conditions and denitrification (reduction of NO_3^- to N_2O and N_2) under anaerobic conditions, which are mainly related to the balance between soil NO_3^--N and NH_4^+-N concentrations [15]. These two processes affecting soil N_2O emission are mediated by soil physic-chemical properties, such as soil temperature, soil water content, soil organic carbon, total nitrogen, and pH [16–18]. Therefore, the difference of N_2O emission between C3 and C4 grass soils is inevitable because they regulate the biotic and abiotic factors driving N_2O emissions in diverging ways [7–10]. In addition, the forage productivity may also differ between both types, affecting the use efficiency of plants for soil water and nutrient resources [19]. At present, although the plant types have been found to be one of the key factors governing N_2O emission [20], whether the changes of soil properties caused by C3 and C4 species will significantly influence N_2O emission flux is still unknown.

Previous studies regarding GHG emissions in healthy C3 and C4 grass lands have focused on CO_2 [21–23], while few pieces of research have explored the mechanism of N_2O emission. Salinization is an enormous challenge to environmental resources, and the area of salinization land accounts for more than 50% of the arable land around the world [24]. Soluble salts in soil negatively affect the mineralization and nitrification processes, imposing various effects on N_2O emission of soils [25,26]. In the present study, two drought- and salt-tolerant C3 (i.e., barley and rye) and C4 species (i.e., sorghum hybrid sudangrass and corngrass) were selected and planted in salinization fields of an inland arid region, and soil properties, soil N_2O flux, and forage productivity of these grass fields were measured. Greenhouse gas emission intensity (GEI, GHG flux per unit crop yield) has been used to compare the GHG emissions to produce the same crop yield [27–29]. A number of researchers agree that GEI can assist in solving the global challenges of increasing crop production and concomitantly identifying the main targets for GHG mitigations in different cropping systems, which is important when seeking ways to decrease total GHG emissions associated with agricultural production, especially in China [30]. Based on the above statement, we hypothesize that the grass species with higher N uptake for soil (i.e., crude protein yield) emits lower N_2O by decreasing the soil available N concentration. The purposes of our study are to clarify the mechanisms of soil N_2O emission in C3 and C4 grass fields, and to determine the appropriate grass species with low yield-scaled N_2O emission in salinization fields of arid region. Our study will contribute to the achievement of sustainable herbivore agriculture and the transfer of arid farm system toward lower N emissions.

2. Materials and Methods

2.1. Climate and Soil

The field experiments were performed in 2016 and 2017 at the Linze Grassland Agricultural Trial Station of Lanzhou University, Linze County, Zhangye City and Gansu Province, China. The experimental site is located at latitude 39°15′ and longitude 100°02′ E at an elevation of 1390 m above sea level. The northwest inland arid area of the research station is a secondary salinization meadow with temperate continental climate. The average annual temperature is 7.6 °C and the average hour of sunshine is 3042 h/yr. There is a frost-free period of approximately 180 days/year and an average annual mean rainfall of 121 mm yr^{-1}, with over 60% of the rainfall occurring in summer and fall. The average annual free water evaporation is 2430 mm. The amount of precipitation was 50 mm in 2016 and 87 mm in 2017. The monthly precipitation during the experimental years is shown in Figure 1. The soil in the research site is classified as Aquisalids according to USDA soil taxonomy (salt 0.7–0.9%). Before experiment, the initial soil properties measured at 30 cm soil depth were: soil organ carbon 9.34 g/kg, total N 1.07 g/kg, pH 8.5, bulk density 0. 93 g/cm^3.

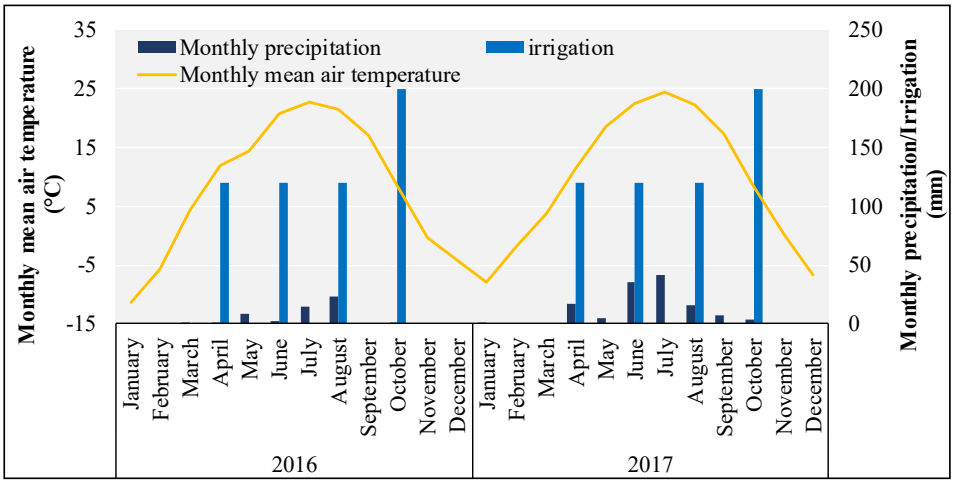

Figure 1. The air temperature, precipitation, and irrigation of study site in 2016 and 2017.

2.2. Experimental Design

Two C4 grass species, sorghum hybrid sudangrass (SHS, *Sorghum bicolor* × *S. sudanenese*) and corngrass I (*Zea mays* × *Zea mexicana*), and two C3 grass species, barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.) were chosen to establish plots for measuring N_2O emission and forage yield. This study was conducted with a randomized complete block design with three replications (plots) for each grass species. The length and width of each plot were 15 m and 6 m, respectively (area = 90 m^2), and a 1.5-m-wide isolation belt and a ridge were placed between each plot to prevent nutrient and water leakage. Traditional flat planting of 50 cm in width was used for the two C4 species with different seeding spacing (25 cm for corngrass and 20 cm for SHS) and evaluated density (82,500 plants/ha corngrass and 10,500 plants/ha for SHS). Broadcasting sowing was used for barley and rye with a seeding rate of 375 kg/ha and 300 kg/ha, respectively.

Forages were sown on 20 May 2016 and 24 May 2017 due to the local unstable weather condition (e.g., air temperature declining suddenly) in spring and no cold tolerance of chosen C4 species, using a hole-sowing machine with a seeding depth of 3–4 cm and manual broadcasting sower. A base fertilizer containing 150 kg/ha P_2O_5 was spread evenly over the furrow. Irrigation (about 120 mm) was applied twice during growing season (Table 1). The corngrass and SHS were harvested three times per year at the stubble height of 15 cm, while the plant heights were 100–130 cm and 130–160 cm for corngrass and SHS, respectively. Barley and rye also were harvested three times per year at the stubble height of 5 cm, while the plant grew to 25–30 cm. The schedule for harvests was listed in Table 1. Weeds were controlled manually during each growing season.

Table 1. The schematic timeline of the N_2O flux measurements, irrigation events and harvests for C3 and C4 grass species.

Items	2016		2017	
	C3	C4	C3	C4
N_2O flux measurement	21–23 May 20–22 June 18–20 July 21–23 August 25–27 September	21–23 May 20–22 June 18–20 July 21–23 August 25–27 September	25–27 May 26–28 June 19–21 July 21–23 August 27–29 September	25–27 May 26–28 June 19–21 July 21–23 August 27–29 September
Irrigation	28 June 10 August	28 June 10 August	30 June 12 August	30 June 12 August
Harvest	26 July 24 August 26 September	20 July 17 August 20 September	28 July 25 August 28 September	23 July 20 August 22 September

2.3. Sampling and Measurements

2.3.1. Nitrous Oxide (N_2O)

Three successive sunny days avoiding the irrigation and harvest events in each month during growing season were chosen for measuring the N_2O emission, and the specific times were listed in Table 1.

Measurement of N_2O was carried out once at 9:00–11:00 of each chosen day in each plot. A static opaque chamber was used to sample N_2O in each plot. The static opaque chamber (30 cm × 30 cm × 30 cm) was constructed from stainless steel and sheathed with 2-cm-thick foam plastic for improving temperature stability. It was fitted with an internal battery-operated fan to mix the air and with a silica gel catheter (2 mm diameter × 200 mm length) on the top of chamber for gas sampling. Gas samples were drawn through a three-way stopcock, using a 50-mL plastic syringe, and then transferred into 300-mL aluminum foil gas-collecting bags. For each sampling event, four gas samples of approximately 300 mL were taken at time interval of 10 min (i.e., 0, 10, 20, and 30 min). The chamber was also equipped with an electronic thermometer. The temperature inside the chamber was recorded during gas sampling and applied to calculate gas flux; soil temperature (ST) was also measured by a mercurial thermometer inserted 5 cm into the soil at the sampling site before and after gas sampling and the mean temperature was applied to detect its effect on GHG emissions.

Samples were brought back to the laboratory, and gas concentration was measured within 24 h by a N_2O analyzer (Model No. 908-0015-0000, Los Gatos Research, San Jose, CA, USA). The exchange flux of N_2O describes the change of gases in unit time in the sampling box, which was calculated according to Liu et al. [31] and Ning et al. [32]. The total N_2O flux in growing season was calculated according to Ning et al. (2020) [32]: total N_2O flux = mean daily N_2O flux × test days. N_2O emission intensity (NEI) measuring the N_2O flux per unit hay yield (NEI_{hay}) and per unit crude protein yield (NEI_{CP}) was estimated according to Dyer et al. [33].

2.3.2. Forage Yield and Soil Property

To determine the forage yield, three quadrats (50 cm × 50 cm) were set up in each plot for each C3 species, and eight typical plants of each C4 species were randomly selected in each plot, and then these fresh grasses mowed were oven dry for a minimum of 48 h. After weighting, the forage samples were smashed and extracted by passing through a 0.25-mm sieve, then the CP was measured by Kjeldahl method [34].

Three sites in each plot were randomly selected for collecting soil at a 0–15 cm depth using the bucket auger (5 cm diameter) after each N_2O collection. The soil water content (SWC) was estimated as: (original wet weight − soil dry weight)/original dry weight × 100%. Part of fresh soil was used to measure soil available nitrogen (SAN) (NH_4^+-N and NO_3^--N) by colorimetric method [35]. The other part of soil samples was air-dried then ground through a 0.25-mm sieve. Soil organic carbon (SOC) was measured by chromic acid REDOX titration [36], soil pH (SpH) was measured by potential method [37], and soil total nitrogen (STN) was determined by following the methods of Bremner and Mulvaney [34].

2.4. Statistical Analysis

All other statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Results of a Shapiro–Wilk test (UNIVARIATE Procedure) indicated that data collected from this study were normally distributed. As the interactions between year and species were not significantly different, a one-way ANOVA (GLM Procedure) was applied to compare the mean difference in soil N_2O flux between months or between species (Figures 2 and 3), and that in forage production between species (Figure 5), with a least significant difference (LSD) test for multiple comparisons. N_2O flux in relation to each soil property (Figure 4) was determined for each species using a linear regression (GLM Procedure). As there was no significant difference in soil properties and N_2O flux between 2016 and 2017, data were pooled before analyses. The regression slopes of different species were

compared using an analysis of covariance (ANCOVA, GLM Procedure) with a CONTRAST statement for pairwise comparison. As there was no significant difference in slope between the two C3 or C4 species, we also determined the relationships between N_2O flux and soil properties for the C3 and C4 species (Figure 4; Tables 2 and 3). A multiple variate linear regression was used to test the soil properties affecting the N_2O flux, while only the significant factors (properties) remained in the final optimal model (Table 4).

3. Results

3.1. Soil N_2O Emission in Barley, Rye, Corngrass, and SHS

For each species, soil N_2O fluxes significantly increased from early growing season (May) to mid-growing season (July), then significantly decreased ($P < 0.05$) (Figure 2). Soil N_2O flux from SHS field (N_2O flux = 22.3 and 16.2 μg/m^2/h, respectively for June and September) was significantly greater than that from barley field (N_2O flux = 16.4 and 8.3, respectively for June and September) in June and September in 2016 ($P < 0.05$) (Figure 2A), and soil N_2O fluxes from corngrass (22.8 μg/m^2/h) and SHS (22.3 μg/m^2/h) were significantly greater than that from rye (16.8 μg/m^2/h) in June of 2017 ($P < 0.05$) (Figure 2B).

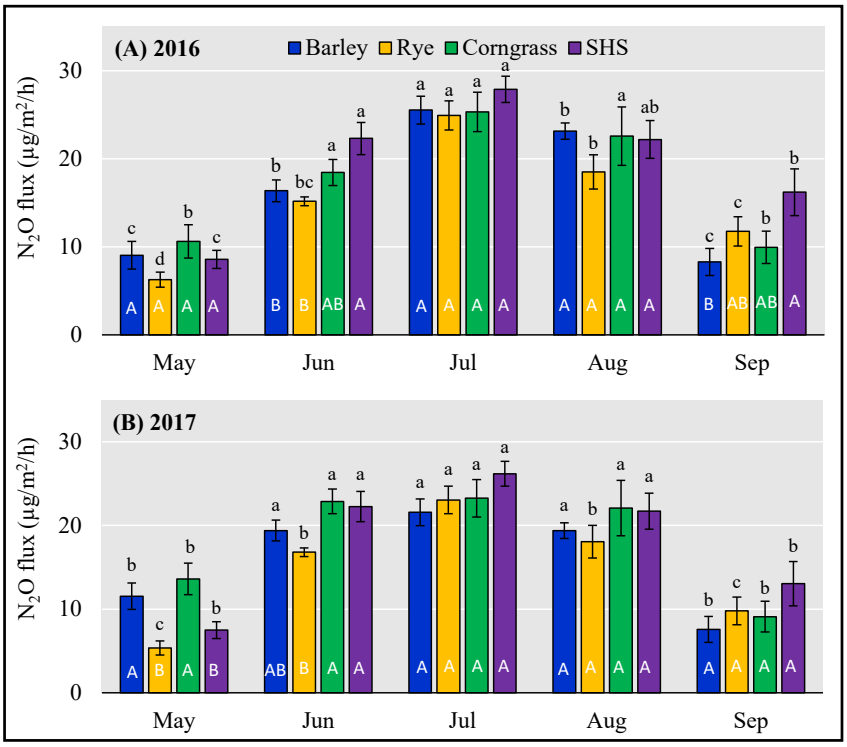

Figure 2. The dynamics of soil N_2O emission in 2016 (**A**) and 2017 (**B**) in barley, rye, sorghum hybrid sudangrass (SHS), and corngrass fields. For each species, means with the same lowercase letters are not significantly different between different months ($P > 0.05$); for each month, means with the same uppercase letters are not significantly different between different grass species ($P > 0.05$).

The total soil N_2O quantities during the growing seasons were significantly greater from SHS than from rye in 2016 ($P < 0.05$) and significantly greater from SHS and corngrass than from rye in 2017 ($P < 0.05$) (Figure 3). The total soil N_2O quantity in C3 fields during the growing seasons was 15.08% lower compared to that in C4 fields.

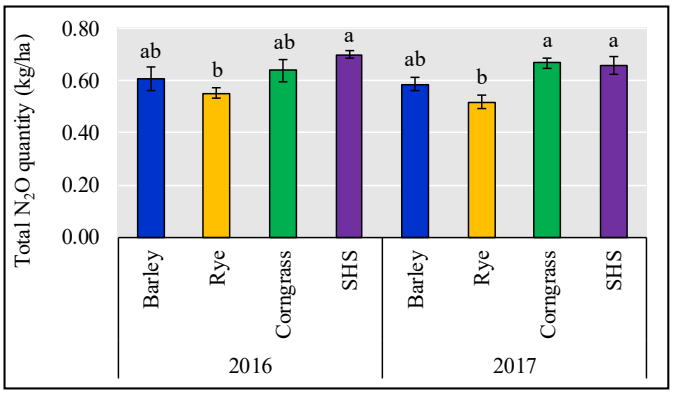

Figure 3. The total soil N_2O flux from barley, rye, SHS, and corngrass fields during growing seasons in 2016 and 2017. Means with the same letters are not significantly different ($P > 0.05$).

3.2. Soil N_2O Emission in Relation to Soil Property

Increasing soil temperature, organic carbon, pH, available nitrogen, and total nitrogen was significantly promoted, but increasing soil water content significantly decreased N_2O emissions ($P < 0.05$), except that soil organic carbon did not have a significant effect on N_2O emission from barley (Figure 4, Table 2). For a given soil property, the regression slope was not significantly different between barley and rye or between corngrass and SHS ($P > 0.05$). The soil temperature, water content, organic carbon, pH, available nitrogen, and total nitrogen respectively explained 69.37%, 76.03%, 22.54%, 70.26%, 74.00%, and 35.55% variation of N_2O emission from C3 species and 74.48%, 66.09%, 38.10%, 66.45%, 70.26%, and 18.78% variation of N_2O emission from C4 species (Figure 4, Table 3). N_2O emission increased significantly faster with increasing ST in C4 fields than in C3 fields ($P = 0.0083$).

Table 2. Summary of linear regressions (Figure 3) between soil N_2O fluxes and soil properties: soil temperature (ST), soil water content (SWC), soil organic carbon (SOC), soil pH (SpH), soil available nitrogen (SAT), and soil total nitrogen (STN) in barley, rye, corngrass, and SHS fields. For each soil property, slopes with the same letters are not significantly different between species ($P > 0.05$).

Species	Intercept	Slope	R^2	$F_{(1,28)}$	P	Intercept	Slope	R^2	$F_{(1,28)}$	P
	ST					**SWC**				
Barley	−0.15	0.91 a	0.6856	61.05	<0.0001	45.00	−0.94 a	0.8154	123.71	<0.0001
Rye	−1.24	0.98 a	0.7377	78.75	<0.0001	42.73	−0.89 a	0.7083	68.00	<0.0001
Corngrass	−2.17	1.17 a	0.7522	85.00	<0.0001	42.83	−0.86 a	0.7434	81.11	<0.0001
SHS	−3.03	1.23 a	0.7375	78.67	<0.0001	44.86	−0.88 a	0.6087	43.56	<0.0001
	SOC					**SpH**				
Barley	−98.78	12.30 a	0.1203	3.83	0.0604	−164.39	23.46 a	0.6209	45.68	<0.0001
Rye	−150.96	17.56 a	0.4184	20.14	0.0001	−159.61	22.74 a	0.7868	103.30	<0.0001
Corngrass	−197.85	23.03 a	0.2747	10.60	0.0030	−164.64	23.67 a	0.6291	47.49	<0.0001
SHS	−63.57	19.30 a	0.4734	25.17	<0.0001	−162.71	23.61 a	0.7095	68.39	<0.0001
	SAN					**STN**				
Barley	−7.90	0.56 b	0.8181	125.96	<0.0001	−15.90	24.78 a	0.3444	14.71	0.0007
Rye	−13.02	0.71 ab	0.6806	59.66	<0.0001	−21.17	30.36 a	0.3899	17.90	0.0002
Corngrass	−9.60	0.61 ab	0.8651	79.31	<0.0001	−10.32	22.07 a	0.2064	7.20	0.0121
SHS	−15.64	0.83 a	0.7490	83.56	<0.0001	−20.39	33.08 a	0.2845	11.13	0.0024

Table 3. Summary of linear regressions (Figure 3) between soil N_2O fluxes and soil properties: soil temperature (ST), soil water content (SWC), soil organic carbon (SOC), soil pH (SpH), soil available nitrogen (SAT), and soil total nitrogen (STN) in C3 and C4 species. For each soil property, slopes with the same letters are not significantly different between C3 and C4 species ($P > 0.05$).

Species	Intercept	Slope	R^2	$F_{(1,28)}$	P	Intercept	Slope	R^2	$F_{(1,58)}$	P
	ST					**SWC**				
C3	−0.51	0.88 b	0.6937	131.33	<0.0001	43.96	−0.92 a	0.7604	194.01	<0.0001
C4	−2.63	1.20 a	0.7448	169.30	<0.0001	43.70	−0.86 a	0.6609	113.03	<0.0001
	SOC					**SpH**				
C3	−117.47	14.15 a	0.2254	16.88	<0.0001	−162.10	23.11 a	0.7026	137.00	<0.0001
C4	−169.18	19.93 a	0.3810	35.70	<0.0001	−162.86	23.51 a	0.6645	114.87	<0.0001
	SAN					**STN**				
C3	−9.40	0.61 a	0.7400	165.08	<0.0001	−16.03	25.42 a	0.3555	31.99	<0.0001
C4	−10.06	0.66 a	0.7315	158.04	<0.0001	−9.91	22.39 a	0.1878	19.41	0.0005

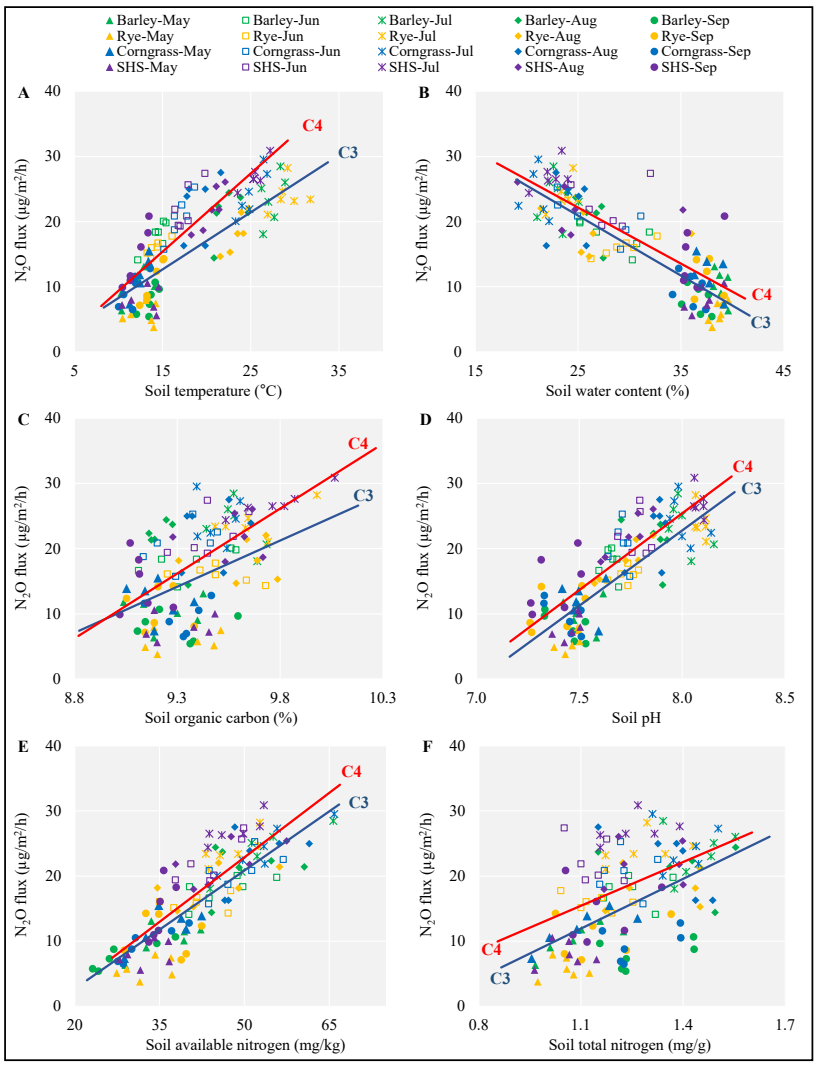

Figure 4. The linear regressions between soil N_2O fluxes and soil properties of soil temperature (**A**), soil water content (**B**), soil organic carbon (**C**), soil pH (**D**), soil available nitrogen (**E**), and soil total nitrogen (**F**) in barley, rye, corngrass, and SHS fields. The statistical results are summarized in Table 2 for each species and in Table 3 for C3 and C4 species.

Amount soil properties, soil available nitrogen, soil water content, and soil temperature were the main factors that significantly affected N_2O fluxes and explained 88.23% variation of N_2O fluxes in C3 fields (Table 1); while only two factors (i.e., soil available nitrogen and temperature) significantly affected N_2O flux and explained 82.55% variation of N_2O flux in C4 field. Soil-available nitrogen accounted for ≥ 70% variation of N_2O fluxes for both field types (Table 4).

Table 4. The most parsimonious multiple linear regression model including soil available nitrogen (SAN, mg/kg), soil temperature (ST, °C) or soil water content (SWC, g/cm^3), and their contributions to N_2O flux (μg/m^2/h) during growing seasons in C3 and C4 fields.

Species	Factor	df	Type I SS	Contribution (%)	*F*	*P*
C3	SAN	1	1971.21	74.00	352.00	<0.0001
	SWC	1	325.30	12.21	58.09	<0.0001
	ST	1	53.90	2.02	9.59	0.0031
	Error	56	313.60	11.77		
Final model: N_2O flux = 10.06 + 0.30 AN − 0.38 SWC + 0.26 ST (R^2 = 0.8823)						
C4	SAN	1	2157.26	73.15	238.93	<0.0001
	ST	1	277.07	9.40	30.69	<0.0001
	Error	57	514.64	17.45		
Final model: N_2O flux = −9.11 + 0.36 AN + 0.69 ST (R^2 = 0.8255)						

3.3. Soil N_2O Emission and Forage Production

The hay and crude protein yields of SHS were significantly greater than that of corngrass in 2016 and 2017, and both were significantly greater than that of barley and rye ($P < 0.05$) (Figure 5A,B). The total hay and crude protein yields of C4 species were, respectively, 1.61 and 1.37 times greater than that of C3 species. The NEI_{hay} and NEI_{CP} were significantly lower in corngrass and SHS than in barley in 2016 and 2017, and significantly lower in SHS than in corngrass and in rye than in barley in 2017 ($P < 0.05$) (Figure 5C,D). The NEI_{hay} and NEI_{CP} of C4 species were, respectively, 26.50% and 13.44% lower than that of C3 species.

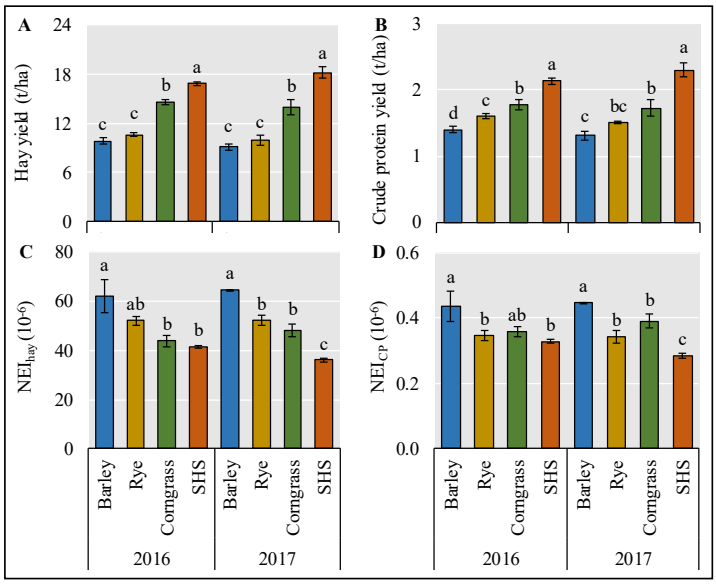

Figure 5. Mean (±SE) hay yield (**A**), crude protein yield (**B**), NEI_{hay} (**C**), and NEI_{CP} (**D**) (N_2O emission intensity, i.e., yield-scaled N_2O emission) for barley, rye, corngrass, and SHS in 2016 and 2017. For each category and each year, means with the same letters are not significantly different ($P > 0.05$).

4. Discussion

When soil-available N is large, a great plant N capture will reduce soil mineral availability, substrate for denitrifiers, and the abundance of nitrate-reducing microorganisms, which will increase soil N_2O emission [20,38,39]. Therefore, the productivity of biomass and crude protein are the critical factors regulating plant–soil interactions in terms of N_2O emission [19]. However, there were contrasting results between C3 and C4 grasses, i.e., C4 grass species had a relatively higher soil N_2O flux and higher crude protein and biomass yields than C3 species (Figures 3 and 5B). These discrepancies were probably because of species particularities and the limited soil N concentration. C4 grass species (such as the corngrass and SHS in the study) require more water, which may cause soil temperature to be more sensitive to the change of sunshine, while soil temperature is positively correlated with N_2O emission [40,41]. Meanwhile, the competitions of soil microbial communities in high-N soil are more effective for N source than that in in low-N soil [42,43]. Therefore, it is likely that grass species with great water requirement moderate N_2O emission by decreasing soil temperature and enhancing the N competitions of soil microbes instead of controlling N uptake and then diminishing the abundance or activity of soil microbes in low-available-N or no-N-addition soils. Our results show that soil-available N explained > 70% variance of soil N_2O emission, and the sensitivities of soil N_2O emission to available N between C3 and C4 species was not significantly different (Table 3). Soil-available N contributed greater impact to N_2O emission than soil temperature and soil water content (Table 4). Fertilizing N significantly increases soil N_2O emission because it actually increases the soil-available N as a substrate for nitrification and denitrification [29]. Our results prove again that available N is the most important factor that directly reflects the soil N_2O emission no matter what type of grass species is used in the land [15,29].

The most parsimonious multiple linear regression model unraveled the two most important factors, beside available N, as the key predictors of N_2O emission, the soil temperature and soil water content (Table 4). Soil temperature is the major driver regulating soil N_2O fluxes mainly via soil respiration and microbial activity [16–18]. As reported in studies on N_2O fluxes in sorghum and wheat fields in arid region [40,41], we found that increasing soil temperature elevated N_2O fluxes (Figure 4). These results agree with the general conclusions of previous studies [16–18,44]. Therefore, it may be prevalent that N_2O fluxes start to increase in spring (May) and peak in summer (July) (Figure 2A,B), because the soil warming promotes microbial activity [16,17]. In the inland arid region of northwest China, the optimum water requirement for wheat is 414 mm, which is less than that for corn (e.g., 570 mm) [42]. Grass species have different water requirements, and nutrient use efficiency is likely to have an effect on soil properties including soil temperature [4,5], and then to vary the richness and diversity of microbes regulating soil N_2O emission [43,44]. The greater richness and diversity of microbes in C4 grass fields is likely to result in the higher sensitivity of soil N_2O emission to soil temperature in C4 than in C3 grass fields (Table 3).

Contrary to other findings [45,46], a significantly negative impact of soil water content on soil N_2O emission was detected in the study (Figure 4). A greater soil water content may be beneficial for microbe-regulating soil N_2O emission [38,39]. However, it also activates soluble salts in soil that negatively affects the mineralization and nitrification. In fact, soil water content influences N_2O flux via changing gas diffusion rate and oxygen availability or regulating microbial communities because they require water for physiological activities [17]. However, different soil types may have specific soil moisture to optimize N_2O flux [47]. N_2O transport is restricted when moisture exceeds the optimum level [48,49] leading to anaerobic conditions, whereas suboptimal moisture levels will limit N_2O flux due to water stress of soil microbes [16]. In addition, the high soil water content may restrict the soil temperature, which may significantly suppress soil N_2O emission. Therefore, depending on the region, the change of soil water content may make the soil N_2O emission absolutely different.

N_2O emission intensity (NEI, yield-scaled N_2O emissions, i.e., cumulative emissions divided by hay yield or crude protein yield [28]) gives an effective measure to balance potential trade-offs between food production and environmental sustainability [50]. We found that C4 grass species may

respectively lead to 26.5% and 13.4% reductions on N_2O emission per unit of hay and crude protein production (Figure 5C,D). In arid regions, traditional annual C3 grass species are planted more in order to release grazing pressure in cool seasons due to its short growing season and abundant biomass. Our study shows that in the current context of widespread C3 grass species use, C4 species should be promoted to develop animal husbandry that retain N more efficiently and contribute optimally to climate change mitigation. Therefore, improving cultivation of C4 species will be a huge challenge or an opportunity for future agriculture.

5. Conclusions

Our results indicated that in the arid regions with higher soil salt, soil in C4 fields emitted more N_2O than that in C3 fields; however, C4 grass species produced greater hay and crude protein yield with lower hay/crude protein yield-scaled N_2O emission. Therefore, we may conclude that C4 species contribute optimally to climate change mitigation in arid regions. We revealed that soil-available N was the most important factor regulating soil N_2O flux in both C3 and C4 grass fields, and soil temperature and soil water content were the other two key factors. Our results assume that C4 species with great water requirement moderate N_2O emission by decreasing soil temperature and enhancing the N competitions of soil microbes instead of controlling N uptake and then diminishing the abundance or activity of soil microbes in low available N or no N addition soils. Further investigations into this assumption should be conducted in the similar environmental region.

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