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ECOSYSTEMS

Impact of Grazing Intensity and Seasons on Greenhouse Gas Emissions in Tropical Grassland

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ABSTRACT

Greenhouse gases (GHG) can be affected by grazing intensity, soil, and climate variables. This study aimed at assessing GHG emissions from a tropical pasture of Brazil to evaluate (i) how the grazing intensity affects the magnitude of GHG emissions; (ii) how season influences GHG production and consumption; and (iii) what are the key driving variables associated with GHG emissions. We measured under field conditions, during two years in a palisade-grass pasture managed with 3 grazing intensities: heavy (15 cm height), moderate (25 cm height), and light (35 cm height) N_2O, CH_4 and CO_2 fluxes using static closed chambers and chromatographic quantification. The greater emissions occurred in the summer and the lower in the winter. N_2O, CH_4, and CO_2 fluxes varied according to the season and were correlated with pasture grazing intensity, temperature, precipitation, % WFPS (water-filled pores space), and soil inorganic N. The explanatory variables differ according to the gas and season. Grazing intensity had a negative linear effect on annual cumulative N_2O emissions and a positive linear effect on annual cumulative CO_2 emissions. Grazing intensity, season, and year affected N_2O, CH_4, and CO_2 emissions. Tropical grassland can be a large sink of N_2O and CH_4. GHG emissions were explained for different key driving variables according to the season.

Key words: grassland management; grazing height; CH_4 from grassland soil; soil respiration; water-filled pore space; nitrous oxide.

INTRODUCTION

Livestock production accounts for 70% of all agricultural land and 30% of the land surface of the planet. The sector is responsible for 18% of global greenhouse gas emissions (GHG) measured in CO_2 equivalents. It accounts for 9% of anthropogenic CO_2, 37% of methane (CH_4), and 65% of nitrous oxide (N_2O) emissions as well as 64% of ammonia (NH_3) release, which contributes indirectly to N_2O

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emissions and acidification of ecosystems (Steinfeld and others 2006). Livestock is an important sector in tropical countries.

Grassland management strategies affect animal intake, forage residue amount and quality, and pasture structure (Shariff and others 1994; Apolinário and others 2014). Nutrients from grazing areas return through the deposition of dung and urine (Haynes and Williams 1993). Different defoliation frequencies and intensities, which influence the proportions of leaf, stem, and dead material, affect C and N cycling due to their effects on the biochemical composition of residues (Liu and others 2011), N concentrations in plant tissues (Boddey and others 2004), and soil microbial population and diversity (Zhou and others 2010). We hypothesized that grazing intensity can affect CO_2, CH_4, and N_2O emission from soil.

The edaphoclimatic characteristics of the northwestern region of the Brazilian state of São Paulo, which is the region of our research interest, are similar to those of the Cerrado biome (Miranda and Fonseca 2013). These characteristics include annual rainfall ranging between 1200 and 1600 mm and occurring mostly during the warm summer season (October to April), thereby contrasting with temperate and subtropical regions (Lopes 1996). It is known that high levels of precipitation and temperature result in the rapid formation of anoxic conditions in the soil, although rapid water infiltration and high evapotranspiration rates suggest that these conditions may be temporary (Skiba and Ball 2002; Lessa and others 2014). Anoxic microsites in the soil can favor N_2O production (Smith and others 2003).

The winters in this Brazilian region are severely dry, frequently having more than 90 days without rain, and have a temperature range from 15 to 25°C, conditions that do not favor CH_4 and N_2O production (Lessa and others 2014). The dry winter season also has a short photoperiod and low soil moisture. The low rainfall compromises grass growth, thereby leading to seasonality of grass production (de Pinho Costa and others 2006). The climatic characteristics described above led us to hypothesize that season affects GHG production and consumption in a grassland soil.

The importance of GHG emissions resulting from livestock production in tropical regions is increasing. Understanding the effects of grassland management and season on GHG production and consumption may help develop strategies to mitigate GHG. Here, we assessed GHG emissions from a tropical pasture in Brazil to determine (i) how grazing intensity (light, moderate, and heavy) affects the magnitude of GHG emissions; (ii) how season influences GHG production and consumption; and (iii) how the key driving variables are associated with GHG emissions.

MATERIALS AND METHODS

Site Description

The experiment was conducted at the Forages and Grasslands division of São Paulo State University ''Julio de Mesquita Filho'' (UNESP), in Jaboticabal, SP, Brazil (21°15′22″S, 48°18′58″W), at an altitude of 595 m. The local climate is tropical, with a rainy season (October to March), during which more than 80% of annual precipitation normally occurs, and a dry season (April to September). Mean annual rainfall is 1424 mm and mean air temperature is 22.3°C. The soil is a Rhodic Ferralsol (IUSS 2006) derived from basalt. The pastures used in the study site were seeded in 2001 with *Brachiaria brizantha* (Hockst ex A. Rich) Stapf cv. Marandu (palisadegrass). Soil chemical composition and physical characteristic are presented in Table 1.

Experimental Design and Grassland Management

The cultivar of *Brachiaria brizantha* Marandu was realized by Embrapa (Brazilian Agricultural Research Corporation) in 1984, and studies found that the best nutritional quality and vigor of the cultivar are observed at 40-cm plant height, whereas the minimal height that does not prejudice plant regrowth or sustentation of pasture perennials is 15 cm height (Euclides and others 2014). Thus, three pasture management heights were chosen, 15, 25, and 35 cm that consist of three grazing intensity treatments: heavy, moderate, and light, respectively, with six replications (paddocks) in a completely randomized design. Paddocks were managed under continuous stocking, with paddock areas of 0.7, 1.0, and 1.3 ha for the 15 cm (heavy grazing intensity), 25 cm (moderate grazing intensity), and 35 cm (light grazing intensity) pasture heights, respectively. The variation in paddock size was implemented to maintain the same number of animals per paddock. Six Nellore yearling bulls were utilized per paddock. Additional animals were used to maintain pre-determined grazing intensity, using the put-and-take methodology (Mott and Lucas 1952).

The experiment was conducted over 2 years, from 11/21/2012 to 11/28/2014. Maintenance fertilizer was applied to all paddocks on December 10, 2012, at a rate of 180 kg ha^{-1} of a mixture

Table 1. Soil Chemical Composition and Texture in the Palisade-Grass Pasture Paddocks Under Light (35 cm height), Moderate (25 cm height), and Heavy (15 cm height) Grazing Intensities

Treatments	Soil chemical composition								Soil texture[4]			SBD[5] ($g\ cm^{-3}$)
	pH[1]	OM[2] ($g\ dm^{-3}$)	P (mg dm^{-3})	K (mmolc dm^{-3})	Ca (mmolc dm^{-3})	Mg (mmolc dm^{-3})	H + Al (mmolc dm^{-3})	V[3] (%)	Clay ($g\ kg^{-1}$)	Lime ($g\ kg^{-1}$)	Sand ($g\ kg^{-1}$)	
Light	4.7	27	6	1.4	16	10	38	42	269	98	633	1.59
Moderate	4.3	25	8	2.3	11	8	58	27	291	86	623	1.57
Heavy	4.7	26	7	0.8	15	10	38	40	312	107	581	1.61

[1] *Soil pH in $CaCl_2$.*
[2] *Soil organic matter.*
[3] *Percent of base saturation.*
[4] *Soil texture was sandy-clay-loam.*
[5] *SBD—Soil bulk density.*

containing 4% N, 14% P_2O_5, and 8% K_2O. N maintenance fertilizer was split among three applications. During the first year of the experiment, a total of 160 kg N ha^{-1} as urea was applied according to the precipitation schedule on December 27, 2012, January 22, 2013, and February 26, 2013. During the second year, a total of 180 kg N ha^{-1} as urea was applied on November 22, 2013, January 8, 2014, and February 28, 2014.

Greenhouse Gas Flux Measurements

We used the closed static chamber technique (Mosier 1989) to collect air samples. Polyurethane vented chambers 38 cm in height were covered with thermal insulation mantles. Chamber headspace was 0.025 m^3. Chambers were deployed on round metal bases at the beginning of each 9:00–10:00 am sampling event, as suggested by Alves and others (2012). The chambers were equipped with a rubber belt to seal the chamber base and an output valve for sample removal. The linearity of gas accumulation in the chamber was successfully tested in a preliminary experiment with intensive sampling every 10 min for 1 h. Air samples were taken with 50-ml polypropylene syringes. Air temperatures outside and inside of the chambers were recorded using a digital thermometer. The air sample was transferred to into 20-ml pre-evacuated vials (Shimadzu flasks). Two sampling schemes were adopted. One for the rainy season when GHG production was favored due to rain, plant growth, higher N deposition, most soil moisture and higher temperatures. In this period, samples were collected 3 times a week during the rainy season (from January 10 to February 23, 2013, and from January 8 to February 26, 2014), and every 14 days during the rest of the experimental period. There were a total of 72 sampling events.

Samples were analyzed by gas chromatography (Shimadzu Greenhouse 2014) under the following conditions: (1) N_2O measurement: injector at 250°C, column at 80°C, carrier gas was N_2 (30 ml min^{-1}), electron capture detector at 325°C; (2) CH_4 measurement: flame gas was H_2 (30 ml min^{-1}), flame ionization detector at 280°C. (3) CO_2 measurement: thermal conductivity detector at 250°C.

Fluxes of N_2O ($\mu g\ m^{-2}\ h^{-1}$), CH_4 ($\mu g\ m^{-2}\ h^{-1}$), and CO_2 ($mg\ m^{-2}\ h^{-1}$) were calculated taking into account the linear increase of gas concentration during the incubation period, air temperature and pressure, chamber volume, and area of the metal bases (Cardoso and others 2016). Cumulative emissions ($g\ m^{-2}$) in each season were calculated by integrating the hourly fluxes over time.

Soil and Meteorological Parameters

Soil samples of the 0–10 cm layer were collected at each air sampling event to quantify inorganic N, gravimetric water content (by drying soil at 105°C), and percent water filled pore space (WFPS). Soil bulk density in the 0–10 cm layer was measured using a cylinder 50 mm in diameter and 50 mm in height. WFPS was calculated from the gravimetric water content and bulk density using a particle density of 2.65 g cm^{-3}.

For mineral N analysis, extraction with 2 M KCl was performed on field moist samples with correction for water content. Ammonium-N was determined using the Berthelot reaction with spectrometry at 647 nm (Kempers and Zweers 1986). Nitrate-N quantification was carried out by ultraviolet absorption spectrometry at 220 nm (Miyazawa and others 1985; Olsen 2008). Daily maximum, mean, and minimum temperatures and daily rainfall precipitation were obtained at a meteorological station located 1.5 km from the experimental site.

Statistical Analysis

N_2O, CH_4, and CO_2 fluxes were reported as means and standard error of the mean. Integrated data for each season were submitted to ANOVA after testing for normality and equal variance using R version 3.1.2 (2014), and, when significance was found, orthogonal polynomial contrast analysis was done at 5% probability.

Pearson correlation analysis was run to test for relationships between transformed GHG fluxes and pasture height, temperature, precipitation, % WFPS, NO_3-N, and NH_4-N using data from each sampling event within season.

RESULTS

Environmental Conditions

A total of 1468 mm rain fell at the study site in the first year of sampling, of which 438, 731, 229, and 69 fell during the spring, summer, autumn, and winter, respectively. In the second year, 966.5 mm rain fell, of which 452, 359, 88, and 57 mm fell during the spring, summer, autumn, and winter, respectively. The amount of rain that fell during the rainy season was 65.8% of the 30-year period average from 1971 to 2010 (FCAV 2014). Historically, the region studied is characterized by warm rainy summers and mild dry winters; however, during the second year of this study, the summer and autumn were markedly dry (Fig. 1A).

All data analyzed in this study were obtained on the sampling days while the chambers were closed between 9:00 and 10:00 am. Mean air temperature was 25.3°C. The lowest temperature recorded was 13.7°C in July 2013, whereas the highest was 34.7°C in September 2014. During times in which the chambers were closed, temperatures inside ranged from −5.1 to 6.3°C. Usually, the temperature increased during the 1-h sampling period. However, the temperature tended to fall when the skies changed from sunny to cloudy during the sampling period.

WFPS of the surface soil (0–10 cm) varied seasonally in response to precipitation and grass growth (Fig. 1B). Soil WFPS tended to be greater in the summer. In both years, soil WFPS increased following summer rainfall; however, saturation of soil pores was reached only in January 2013. Isolated rainfall, which occurred in June and August 2013, also increased soil WFPS (Fig. 1B). A long period of low soil WFPS (around 30%) occurred from April to September of 2014, probably because of low rainfall.

N_2O Flux

Nitrous oxide emissions were greatest following rainfall events and application of urea fertilizer (Figs. 1A, 2A). N_2O emissions were highest in the summers (Fig. 2A), whereas the other seasons had lower fluxes associated with frequent instances of N_2O consumption. The maximum rate of N_2O emissions was recorded in the second week of December 2013, when mean fluxes were 279, 394, and 469 µg N_2O-N m^{-2} h^{-1} for light, moderate and heavy grazing intensity, respectively.

Negative fluxes were observed in approximately 60% of all N_2O sampling events independently of grazing intensity and especially in the spring and autumn of the second year (Fig. 2A), when lower values of WFPS were also recorded (Fig. 1B). The highest rate of N_2O consumption was measured on May 23, 2014 (autumn season), when mean fluxes were −287, −235, and −299 µg N_2O-N m^{-2} h^{-1} for light, moderate, and heavy grazing intensity, respectively.

Pasture height was significantly correlated with N_2O flux during the summer. In the spring, N_2O flux was strongly correlated with inorganic-N and temperature, and mildly correlated with % WFPS. In the autumn, N_2O flux was strongly correlated with % WFPS and temperature, and mildly with NH_4^+ (Table 2).

CH_4 Flux

The highest levels of CH_4 production and consumption (oxidation) occurred during the rainy season of 2013, followed by the rainy season of 2014. Indeed, CH_4 flux fell by 50% in January 2014 (Fig. 2B), closely following the pattern of precipitation (Fig. 1A).

The highest variation in CH_4 flux occurred in January 2013. The highest mean CH_4 fluxes were 380, 423, and 504 µg CH_4-C m^{-2} h^{-1} for light, moderate, and heavy grazing intensity, respectively. The highest mean CH_4 oxidation values were −544, −203, and −411 µg CH_4-C for light, moderate, and heavy grazing intensity, respectively (Fig. 2B). The CH_4 fluxes reported here had large daily standard errors, except during the rainy season. In the summer, CH_4 flux was strongly correlated with NH_4^+ content and mildly with temperature and precipitation. In the autumn and winter, CH_4 fluxes were correlated only with temperature (Table 2).

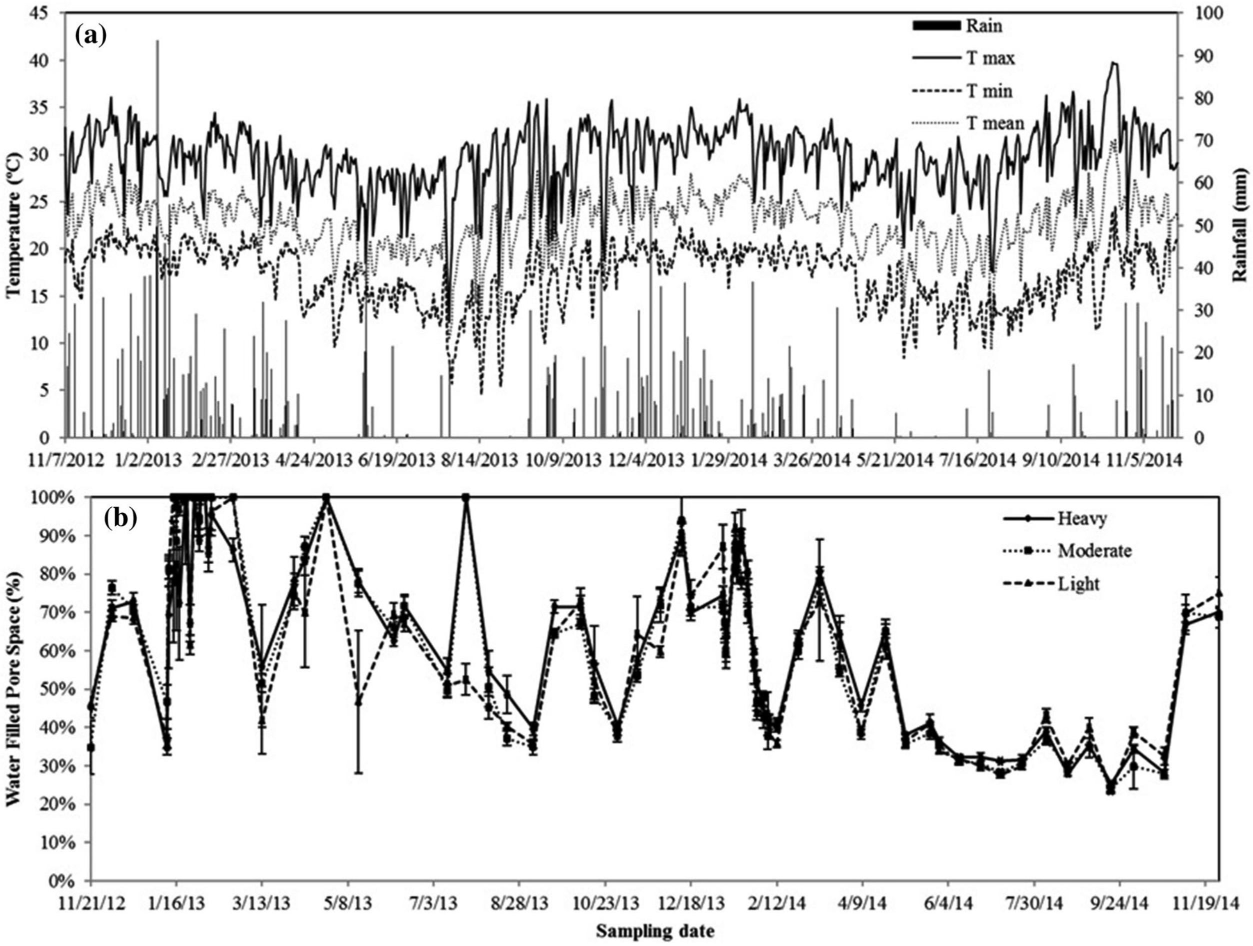

Figure 1. **A** Daily temperature (maximum, mean, and minimum; °C) and precipitation (mm) and **B** % WFPS for grazing intensity (light, moderate and heavy) during two years of evaluation (November 21, 2012 to November 26, 2014).

CO_2 Flux

The highest levels of CO_2 emissions occurred during the rainy summer season, when grass growth is favored, whereas the lowest levels were measured during the dry winter season. Intermediate levels of CO_2 flux occurred in the spring and autumn (Figs. 1A, 2C). Indeed, the highest CO_2 emission level (1 g m^{-2} h^{-1}), which occurred in the light grazing intensity treatment, was measured on December 5, 2012, and again on February 26, 2014. The highest emission observed for the moderate grazing intensity occurred on February 1, 2013 (885 mg CO_2 m^{-2} h^{-1}), and for the heavy grazing intensity on December 5, 2012 (630 mg CO_2 m^{-2} h^{-1}). Minimal emissions were observed in the period of July to September of 2014.

Percent WFPS was strongly correlated with CO_2 emissions in the autumn and winter. CO_2 emissions were also correlated with soil NH_4^+ in the autumn and with precipitation events in the winter (Table 2).

Inorganic-N

Inorganic-N was low throughout the study period. The sum of NH_4-N and NO_3-N totaled approximately 0.1–0.3% of total N, which is the lowest level usually reported for inorganic-N in soils. The concentrations of soil NH_4-N and NO_3-N were similar and ranged from 5 to 25 mg N kg^{-1} dry soil (Fig. 3A, B). As mentioned above, NH_4-N was significantly correlated with N_2O flux in the spring and autumn, with CH_4 flux in the summer, and with CO_2 emissions in the autumn. Soil NO_3-N was correlated with N_2O emissions in the spring (Table 2).

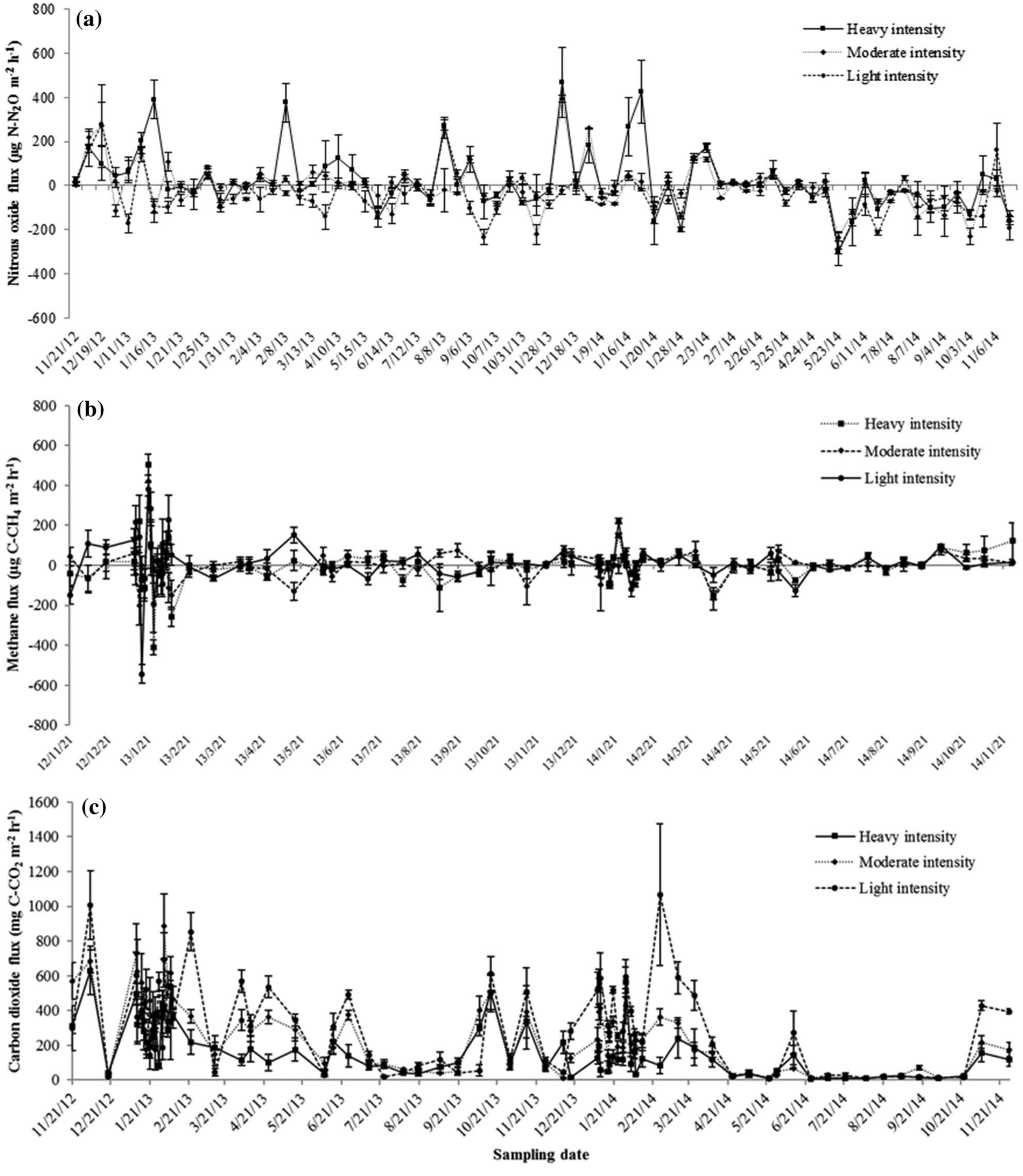

Figure 2. **A** N_2O flux (µg N_2O-N m^{-2} h^{-1}) and **B** fluxes of CH_4 (µg CH_4-C m^{-2} h^{-1}) and **C** CO_2 (mg CO_2-C m^{-2} h^{-1}) for the grazing intensities light, moderate, and heavy (respectively, 35, 25, and 15 cm pasture height) during two years of evaluation (November 21, 2012 to November 26, 2014).

Cumulative Greenhouse Gas Emissions

Table 3 shows a linear reduction in annual cumulative N_2O emissions ($P < 0.05$) with increasing pasture height for both years, in other words, the highest emissions occurred in the heavy grazing intensity decreasing to the light. Light, moderate,

Table 2. Pearson Correlation Coefficients (r) for Assessing the Relation Between Fluxes of N_2O, CH_4, and CO_2 from Palisade-Grass Pastureland and Daily Explanatory Variables

	Pasture height	T_i	T_f	Prec	%WFPS	NH_4^+	NO_3^-	CO_2
N_2O								
Spring		0.64**	0.50*		0.41.	0.66**	0.50*	
Summer	−0.24*							
Autumn			0.35*		0.42**	0.27#		0.27
CH_4								
Summer		−0.17		0.22#	0.21#	0.35**		
Autumn			0.39**	0.28#				
Winter			−0.26					
CO_2								
Autumn					0.52**	0.36*		
Winter				0.38*	0.41**			

Explanatory variables: Pasture height; T_i, initial temperature; T_f, final temperature; Prec, precipitation, % WFPS, percent water-filled pore space; NH_4^+, soil ammonium content; NO_3^-, soil nitrate content; CO_2, CO_2 flux.
*Significance codes: #$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*

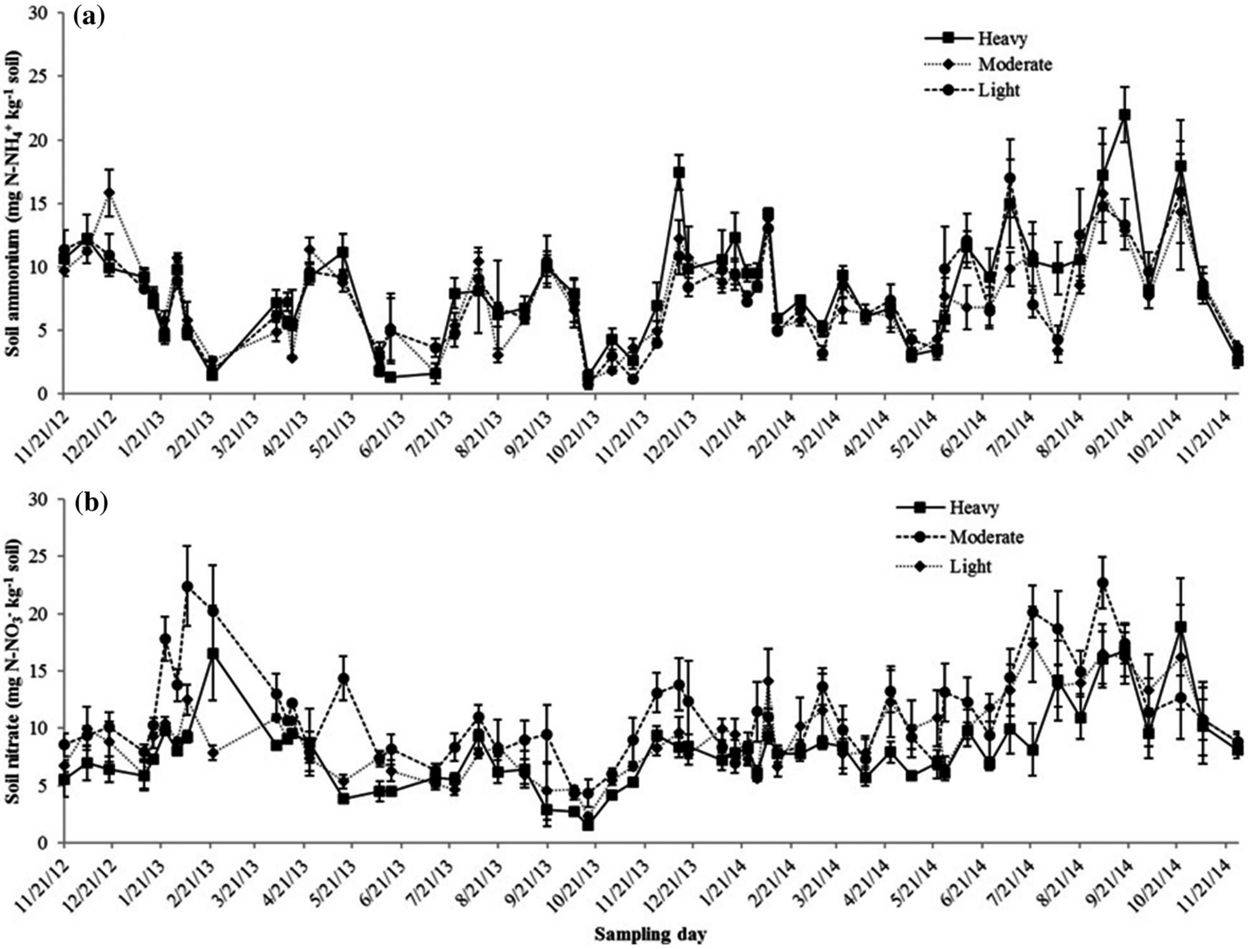

Figure 3. **A** Soil ammonium content (mg NH_4^+-N kg^{-1} dry soil) and **B** soil nitrate content (mg NO_3-N kg^{-1} dry soil) for three grazing intensities light, moderate, and heavy (respectively, 35, 25, and 15 cm pasture height) during two years of evaluation (November 21, 2012 to November 26, 2014).

Table 3. Cumulative N_2O Emissions (mg N_2O-N m^{-2}) from Palisade-Grass Pastureland Managed at Light, Moderate, and Heavy Grazing Intensity (Respectively, 35, 25, and 15 cm Pasture Height) Across Seasons[1] and Years

	Spring	Summer	Autumn	Winter	Total year
Year 1					
Light	76.5 (42.5)	−11.7 (39.5)	−102.3 (19.5)	−10.8 (45.6)	−48.3 (118.9)
Moderate	80.5 (44.2)	−28.8 (51.0)	−1.3 (34.7)	−8.8 (34.2)	41.6 (127.4)
Heavy	46.3 (14.1)	128.4 (20.5)	33.2 (39.2)	92.3 (25.0)	300.1 (49.3)
Mean	67.8 (20.0)	29.3 (27.2)	−23.5 (22.4)	24.2 (22.7)	97.81 (67.1)
Effect[2]	ns	L*	L***	ns	L*
R^2		0.51	0.60		0.52
Year 2					
Light	−101.4 (56.5)	10.54 (14.5)	−221.8 (37.2)	−166.6 (32.4)	−479.2 (91.1)
Moderate	−48.1 (26.3)	61.02 (15.6)	−127.1 (10.8)	−149.5 (76.2)	−263.7 (90.8)
Heavy	9.2 (51.1)	91.64 (32.8)	−107.1 (12.4)	−147.0 (55.3)	−153.2 (88.7)
Mean	−46.7 (27.5)	54.40 (14.7)	−152.0 (17.6)	−154.3 (31.7)	−298.7 (58.9)
Effect[2]	ns	L*	L***	ns	L*
R^2		0.55	0.66		0.55

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[1] *Rainy summer, dry winter, transitional spring, and autumn. Within parentheses: ± standard error of the mean (SEM). Anova significance: year, $P < 0.01$; season $P < 0.01$; pasture height, $P < 0.01$; spring, $n = 7$; summer, $n = 15$; autumn, $n = 7$; winter, $n = 7$.*

[2] *Effect for different grazing intensities (15, 25, and 35 pasture heights) on accumulated N_2O. L = linear. Significance codes: $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*

Table 4. Cumulative CH_4 Emissions (mg CH_4-C m^{-2}) from Palisade-Grass Pastureland Managed at Light, Moderate, and Heavy Grazing Intensity (respectively, 35, 25, and 15 cm pasture height) Across Seasons[1] and Years

	Spring	Summer	Autumn	Winter	Total year
Year 1					
Light	34.2 (32.1)	2.7 (1.5)	80.4 (36.8)	−4.7 (24.6)	112.5 (66.8)
Moderate	−9.6 (21.3)	−0.8 (1.0)	−72.1 (35.3)	42.0 (25.4)	−40.5 (60.0)
Heavy	−21.5 (30.8)	−0.5 (1.4)	−21.3 (24.6)	14.7 (20.6)	−28.6 (60.9)
Mean	1.0 (16.5)	0.5 (0.8)	−4.3 (23.5)	17.3 (13.6)	14.5 (37.9)
Effect[2]	ns	ns	L.	ns	ns
R^2			0.43		
Year 2					
Light	54.2 (16.4)	45.0 (16.4)	−45.2 (25.8)	2.8 (12.5)	56.8 (35.7)
Moderate	81.4 (15.2)	5.1 (14.9)	−25.2 (36.0)	1.3 (20.0)	62.2 (41.5)
Heavy	119.1 (72.4)	19.6 (8.1)	−68.5 (12.2)	7.4 (12.3)	70.6 (70.3)
Mean	84.8 (24.3)	23.4 (8.4)	−43.3 (8.4)	3.9 (8.4)	65.6 (28.0)
Effect[2]	L*	ns	ns	ns	ns
R^2	0.46				

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[1] *Rainy summer, dry winter, transitional spring, and autumn. Within parentheses: ± standard error of the mean (SEM). Anova significance: year, $P = 0.23$; season, $P < 0.01$; pasture height, $P < 0.01$; spring, $n = 7$; summer, $n = 15$; autumn, $n = 7$; winter, $n = 7$.*

[2] *Effect for different grazing intensities (15, 25, and 35 pasture heights) on accumulated CH_4 emitted. L = linear. Significance codes: $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*

and heavy grazing had total N_2O emissions of -48.3, 41.6, and 300.1 mg N_2O-N m^{-2} in year 1 and totals of −298.7, −263.7, and −153.2 mg N_2O-N m^{-2} in year 2, respectively. The heavy grazing intensity had the highest levels of N_2O emissions in all seasons except for in the spring of year 1. The light and moderate grazing intensity had cumulative N_2O emissions only in the spring of year 1 and in the summer of year 2. The effect of pasture height on cumulative N_2O emissions was negatively linear in the annual analysis ($P < 0.05$), driven by the negative associations observed in the summer ($P < 0.05$) and even more so in autumn ($P < 0.001$).

Annual cumulative CH_4 emissions were not affected by grazing intensity treatment (Table 4). In

Table 5. Cumulative CO_2 Emissions (Mg CO_2 ha^{-1}) from Palisade-Grass Pastureland Managed at Light, Moderate, and Heavy Grazing Intensity (respectively, 35, 25, and 15 cm pasture height) Across Seasons[1] and Years

	Spring	Summer	Autumn	Winter	Total year
Year 1					
Light	26.8 (2.9)	31.8 (1.8)	28.3 (1.8)	10.9 (1.0)	97.8 (4.7)
Moderate	23.0 (2.7)	29.1 (2.94)	22.7 (1.7)	9.0 (0.7)	83.7 (4.0)
Heavy	25.0 (3.8)	20.6 (1.6)	11.8 (2.7)	6.9 (0.7)	64.3 (2.9)
Mean	24.9 (1.8)	27.2 (1.7)	20.9 (2.0)	8.9 (0.6)	81.9 (3.9)
Effect[2]	ns	L***	L***	L***	L***
R^2		0.67	0.82	0.67	0.84
Proportion					
Light	27.4%	32.5%	29.0%	11.1%	
Moderate	27.4%	34.8%	27.1%	10.%	
Heavy	38.8%	32.1%	18.4%	10.7%	
Year 2					
Light	12.1 (2.3)	37.8 (7.0)	10.5 (1.4)	1.9 (0.5)	62.3 (7.4)
Moderate	7.2 (0.5)	20.7 (1.9)	5.8 (0.6)	1.9 (0.3)	35.1 (1.9)
Heavy	6.8 (0.8)	10.0 (2.0)	6.0 (1.6)	1.3 (0.1)	24.1 (3.3)
Mean	8.7 (1.0)	22.7 (3.7)	7.4 (0.9)	1.7 (0.2)	40.5 (4.7)
Effect[c]	L*	L***	L*	ns	L***
R^2	0.55	0.75	0.51		0.81
Proportion					
Light	19.5%	60.7%	16.8%	3.0%	
Moderate	20.6%	57.4%	16.6%	5.4%	
Heavy	28.1%	41.6%	25.0%	5.4%	

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[1] *Rainy summer, dry winter, transitional spring, and autumn. Within parentheses: ±standard error of the mean (SEM). Anova significance: year, $P < 0.01$; season, $P < 0.01$; pasture height, $P < 0.01$; spring, $n = 7$; summer, $n = 15$; autumn, $n = 7$; winter, $n = 7$.*

[2] *Effect for different grazing intensities (15, 25, and 35 pasture heights) on accumulated CO_2 emitted: L = linear. Significance codes: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.*

year 1, net oxidation occurred in the moderate and heavy grazing intensity, totaling −40.5 and −28.6 mg CH_4-C m^{-2}, respectively. In year 2, net production for the for light, moderate, and heavy grazing intensity was 56.8, 62.2, and 70.6 µg CH_4-C m^{-2}, respectively. Cumulative CH_4 emissions were negatively associated with pasture height only in the autumn of year 1 and in the spring of year 2 ($P < 0.05$).

Table 5 shows that annual cumulative CO_2 emissions were positively associated with pasture height in both years of study ($P < 0.001$). Indeed, for light, moderate, and heavy grazing intensity, cumulative CO_2 emissions totaled 81.9, 83.7, and 64.3 Mg CO_2 ha^{-1} in year 1 and 62.3, 35.1, and 24.1 Mg CO_2 ha^{-1} in year 2, respectively. The reduction of 35% in the amount of precipitation that occurred in year 2 was probably responsible for the sharp drop in the cumulative CO_2 emissions in that year.

Pasture height was positively associated with cumulative CO_2 emissions in all seasons, except for spring of year 1 and winter of year 2. The summers of 2013 and 2014 accounted for approximately 35% and 50% of annual cumulative CO_2 emissions, respectively. In contrast, the winters of 2013 and 2014 accounted for just 10% and 5% of annual cumulative CO_2 emissions, respectively. Spring and autumn accounted for approximately 27% and 20% of annual cumulative CO_2 emissions, respectively, in both years (Table 5). The contribution of each season to annual cumulative CO_2 emissions was probably affected by the amount of precipitation in each season.

DISCUSSION

N_2O Flux

Once fertilizer was applied, soil water content and its consequent effect on WFPS were the key driving variables for N_2O emission from grassland in the summer months. Soil water content depends on the amount of rainfall and how rapidly rain infiltrates into the soil (Dobbie and others 1999). In our study, the highest N_2O fluxes occurred in the summer and following rainfall events. Rainfall events increased WFPS levels to above 60-70%, which create good conditions for anaerobic denitrification (Smith and others 2003). Rainfall can stimulate N_2O emissions, as has been observed in other studies (van der Weerden and others 2011; Sordi and others 2013). Previous study showed that grazing significantly increased N_2O flux during the growing season (Hu and others 2010; Zhu and others 2015) and may have contributed to the higher fluxes found in the present study in the summer, which correspond with the growing season in the Cerrado region. Most studies of temperate regions have reported that N_2O emissions increase in the cooler/wetter compared with warmer/drier periods of the year (Allen and others 1996; Zaman and Nguyen 2012; Rochette and others 2014). In contrast, the seasonal variability of N_2O emissions has a different pattern in tropical grasslands, where the warm and wet summer has the highest fluxes. Lessa and others (2014) found large differences in N_2O emissions between seasons, with the lowest N_2O emissions in the dry winter season, a result similar to ours. Here, we show that the dry winter season had lower values of % WFPS (30–40%) and lower temperatures, both of which may help explain the low level of N_2O release. Variations in soil moisture drove the seasonal variations in N_2O fluxes in line with Zhang and others (2015), who observed a similar effect in temperate grasslands in Inner Mongolia, Northern China.

Mechanisms of N_2O consumption in tropical grasslands need to be studied. A plausible explanation for the negative N_2O fluxes found in all seasons depends on the occurrence of an equilibrium between the concentration of N_2O in soil pores and that in the atmosphere at the surface of the grassland (Gas diffusion). According to Mazzetto and others (2014), one possible mechanism for N_2O uptake is a lack of available nitrate in the soil, leading denitrifying bacteria to use N_2O as an electron acceptor, which in turn contributes to net N_2O uptake. Low values of mineral N and high values of % WFPS have been shown to favor N_2O consumption (Chapuis-Lardy and others 2007; Mazzetto and others 2014). The flux of -299 µg N_2O-N m^{-2} h^{-1} reported here may be the lowest value of N_2O flux ever reported, exceeding the uptake rate of -207 µg N_2O-N m^{-2} h^{-1} reported by Blicher-Mathiesen and Hoffmann (1999). Schlesinger (2013) suggested that uptake rates greater than 20 µg N_2O-N m^{-2} h^{-1} are related to wet soil, which is inconsistent with our findings.

CH_4 Flux

Seasonal variation in CH_4 emissions has been associated with temperature and insolation (Sorrell and Boon 1992) as well as with air temperature and moisture (Mazzetto and others 2014). We observed the highest levels of both production and oxidation of CH_4 in the rainy summer season. The high rate of evapotranspiration that occurred at our site may explain the rapid variation between CH_4 production and oxidation. Indeed, various environmental conditions allow methanotroph expression in soils subjected to moisture variation, during which their methanotrophic potential is maintained because they remain viable in anaerobiosis (Roslev and King 1994; Le Mer and Roger 2001).

During the autumn and winter, temperature was correlated with CH_4 flux, and thus temperature appears to control CH_4 production and oxidation during these seasons. However, an explanation for this observation is hindered by the lack of consistent results among several other publications. Indeed, Chiavegato and others (2015) found no association between CH_4 flux and either soil water content or soil or air temperature. Likewise, Dengel and others (2011), measuring CH_4 in a grassland site, found no relationship with air temperature. On the other hand, and in accordance with our results, Chamberlain and others (2015) found an association between CH_4 emissions and soil temperature. We found that emissions during the warm season (end of spring and summer) were much higher than in winter (mild and dry). Other studies of CH_4 emissions have also reported that emissions were higher in the summer and lower in the winter (Holter 1997; Mazzetto and others 2014). Contrarily, Zhu and others (2015) showed in an alpine meadow on the Tibetan Plateau that warming significantly increased the CH_4 oxidation rate in line with Carter and others (2012), who studied CH_4 uptake in European peatlands and shrublands.

Differences of CH_4 emissions/oxidation could be due to differences in grazing intensity and climate conditions (Zhu and others 2015). We observed a significant effect of grazing intensity on CH_4 emissions. Grazing can affect CH_4 flux due to soil compaction caused by animal trampling which may decrease O_2 diffusion into the soil and does not favor CH_4 oxidation because of the reduction in O_2 availability (Saggar and others 2007; Liu and others 2007). Our findings differ from those of others (Chen and others 2011; Zhu and others 2015), who found that grazing did not significantly affect CH_4 fluxes.

CO_2 Flux

CO_2 flux has been reported to be influenced by season, soil, and climatic variables (Xu and Baldocchi 2004; Wohlfahrt and others 2008; Thomas and others 2014). In our study, the highest CO_2 fluxes occurred at the end of spring and in the summer. During these periods, we observed increased grass growth and higher rainfall, which may have favored root and soil microorganism respiration. We observed low fluxes in the winter, when temperature and precipitation are lower. However, no explanatory variable was associated with CO_2 flux in the summer and spring, whereas it was significantly correlated with precipitation and % WFPS in the autumn and winter (Table 1). We suggest that soil water controlled CO_2 flux in the latter two seasons in line with Gong and others (2014), who measured CO_2 emissions in cold and arid environments and found that soil water content played a critical role in soil respiration. Brito and others (2015) found a positive linear association between CO_2 flux and soil temperature in summer and autumn. They proposed that seasonal variation of CO_2 was directly related to variations in precipitation and soil temperature. Immediately following re-wetting, soil CO_2 efflux rates can be very high (Thomas and Hoon 2010). We also found high CO_2 fluxes in the spring, after the rainy season began (Fig. 2) and % WFPS increased (Fig. 1B).

Cumulative Greenhouse Gas Emissions

The differences in N_2O emissions between seasons are difficult to interpret. Van Groenigen and others (2005) proposed that such variations are more likely to occur due to fertilization schedules than to urine deposition. In the present study, we found higher amounts of cumulative N_2O emissions in the spring and summer, when fertilizer was applied to the soil.

Grassland management affected N_2O emission accumulation in the summer, autumn, and annually, whereas in the spring and winter it did not (Table 2). The amount of N_2O emitted/consumed decreased from the heavy grazing intensity to the light in line with Klumpp and others (2011). Pastures with heavy grazing intensity have a lower demand for nitrogen for growth. Moreover, the higher stocking rate in the heavy grazing intensity treatment resulted in more nitrogen being returned to the soil, although deposition of feces and urine favor N losses (Haynes and Williams 1993). Under light grazing intensity, more litter is produced with a high C/N ratio, which in turn, requires more N for decomposition and N mineralization (Shariff and others 1994), thereby resulting in less N available to be lost as well as this is a low N system; thus N uptake by grass is perhaps more likely than N_2O emissions. However, in other cases, for instance, Zou and others (2005) established a positive linear relationship between aboveground plant biomass and N_2O emissions in line with Zhang and others (2015). They suggest that reducing plant litter and aboveground biomass might result in decreases in the substrates supplied to microbes, which are involved in nitrification and denitrification process. These differences in N_2O emissions cannot be interpreted without considering nitrogen recycling from animal excreta. In some cases, as observed in the higher grazing intensity, the main source of N is animal excreta which might augment N_2O production compared to that with higher pasture height (higher aboveground biomass) but with lower animal stocks and lower N returning from animal excreta. Some soil properties have an important roles in N_2O production. When soil properties like texture, chemical composition and bulk density were similar in the different paddocks (Table 1) we were not able to attribute effects of this variable on cumulative N_2O emissions.

Cumulative N_2O emissions were lower in winter than in summer in a subtropical pastureland of the southern Brazilian state Paraná (Sordi and others 2013), almost zero in the Cerrado (Lessa and others 2014), and negative in a grassland of the Brazilian state Rondônia in the Amazon region (Mazzetto and others 2014), consistent with the current study. Decreases in N_2O production in drier weather similar to our winter probably occur due to reduced denitrification (Carter and others 2012). Our data agree with studies conducted in tropical regions and differ from those conducted in temperate regions. In temperate regions, winters are usually wet, thereby favoring anaerobiosis and low N uptake by plants, which in turn augment N availability for N_2O emission (Allen and others 1996; Luo and others 2008). In these regions, higher soil water content in cooler periods of the year favors N_2O production by denitrification (Zaman and Nguyen 2012), and, in areas where pasture is irrigated, small differences in emissions between seasons are observed (Kelly and others 2008).

In our study, we did not observe a clear pattern of CH_4 flux variation within each season. However, we found significant effects on CH_4 production and oxidation between seasons. Similar effects were reported by Chamberlain and others (2015), whereas the opposite was shown by Chiavegato and others (2015). Net CH_4 production occurred in all seasons except autumn. At the study site, rainfall diminished during autumn, which reduced soil moisture content and thus may have favored CH_4 oxidation. Conversely, higher rainfall during spring coupled with the rotting of the accumulated grass litter probably contributed to the increase in CH_4 emissions observed in the spring of 2014 (Table 4).

Dung is the main source of CH_4 released to the atmosphere from grassland soils (Saggar and others 2004). In the present study, CH_4 production and oxidation were affected by pasture grazing intensity; however, we did not observe a clear trend, because in year 1, the heavy grazing intensity had lower CH_4 production whereas in year 2 it had the highest cumulative emissions. Soil parameters and climate variables affect CH_4 dynamics, as reviewed by Le Mer and Roger (2001) and Saggar and others (2004). Indeed, although soil and climate variables affected CH_4 production in our study, we cannot exclude the role of other variables not investigated here.

We observed that CH_4 emissions were higher in the heavy grazing intensity, decreased in the light in the spring and in autumn was lower in the heavy grazing intensity increasing to the light. Heavy grazing intensity implies high deposition of excreta, which favors CH_4 production in the spring. The pattern of urination and defecation in paddocks varies widely (Haynes and Williams 1993; Gusmao and others 2015). Cumulative CH_4 production and oxidation had higher standard errors than did other GHG (Table 4), suggesting that spatial variation in CH_4 flux may be associated with the spatial variability of excreta deposition.

Seasonal variability of CO_2 emissions has been attributed to precipitation and soil temperature in a tropical pasture (Brito and others 2015). Thus, in the present study, the observed variability in precipitation may explain the interannual differences in CO_2 emissions. From year 1 to year 2, precipitation declined by 35% whereas cumulative CO_2 emissions decreased by 50%. The warm and rainy summer season contributed 35% and 50% of total CO_2 emissions in year 1 and year 2, respectively. In the region studied, there is seasonality in grass growth and biomass production, which occurs mainly in the summer (de Pinho Costa and others 2006), consequently CO_2 emissions probably varied with grass growth.

Grazing intensity had a positive linear effect on cumulative CO_2 flux, both annually and by season. A higher stocking rate was necessary to maintain a heavy grazing intensity (Barbero and others 2015). Wang and others (2009) observed that CO_2 fluxes were decreased significantly at a high stocking rate, which might have been related to the low soil moisture and biomass at the study site. A high pasture height under light grazing intensity implies high aboveground biomass and litter deposition (Shariff and others 1994), resulting in a greater amount of C that can be released through decomposition. Laporte and others (2002) found a positive effect of aboveground plant biomass on soil surface CO_2 efflux at a grassland site in line with Cao and others (2004) who showed that increasing aboveground and belowground biomass augmented plant autotrophic respiration. Bremer and others (1998) reported reduced CO_2 fluxes in grazed versus ungrazed tallgrass prairie. They suggested that the reduction in photosynthetic surface area and available carbohydrates was the dominant factor driving lowered respiration rates. In contrast, Gong and others (2014) found no significant change in CO_2 fluxes despite detecting a tendency of plant cover and above- or belowground biomass, and that was in line with the observations by Liebig and others (2013) in the semiarid northern Great Plains of North America.

Optimal pasture management aims to mitigate GHG emissions. Here we presented some of the first research in which GHG production was measured for different grazing intensities. We observed that grazing intensity affected GHG emissions. Higher N_2O emissions were found in the heavy grazing intensity. Managing pastures for light grazing intensity and avoiding overgrazing can reduce N_2O emissions as well as improve C sequestration in the soil, which may compensate in part for GHG emissions during animal production.

CONCLUSIONS

Fluxes of N_2O, CH_4, and CO_2 varied among seasons and between years. The magnitude of fluxes observed in this study can be explained by seasonal variations in temperature, precipitation, % WFPS, and inorganic N content.

Temperature was associated with N_2O fluxes in the spring and autumn and with CH_4 fluxes in summer, autumn and winter. %WFPS and ammonium content were correlated with N_2O fluxes in the spring and autumn, with CH_4 fluxes in the summer and CO_2 in autumn. Nitrate was correlated with N_2O emissions in spring and %WFPS with CO_2 efflux in the winter. Cumulative N_2O and CO_2 emissions were influenced by year and season, whereas CH_4 was affected by season.

Grazing intensity had a negative linear effect on annual, summer, and autumn cumulative N_2O

emissions/consumption and a positive linear effect on annual cumulative CO_2 emissions.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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