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Strategic grazing management and nitrous oxide fluxes from pasture soils in tropical dairy systems

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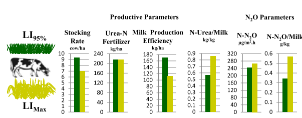
HIGHLIGHTS

- The study regards N_2O fluxes from pasture soils as influenced by grazing strategies.
- More frequent defoliation ($LI_{95\%}$) does not increase N_2O fluxes from pasture soils.
- Strategic grazing decreases 34% the N-urea applied per unit of milk production.
- Strategic grazing decreases 40% the N_2O emission per unit of milk production.
- Strategic grazing is a non-cost and provides intensification of systems resources.

GRAPHICAL ABSTRACT



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ABSTRACT

Greenhouse gases emissions are considered one of the most important environmental issues of dairy farming systems. Nitrous oxide (N_2O) has particular importance owing to its global warming potential and stratospheric ozone depletion. The objective of this study was to investigate the influence of two rotational grazing strategies characterized by two pre-grazing targets (95% and maximum canopy light interception; $LI_{95\%}$ and LI_{Max}, respectively) on milk production efficiency and N_2O fluxes from soil in a tropical dairy farming system based on elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon). Results indicated that $LI_{95\%}$ pre-grazing target provided more frequent defoliations than LI_{Max}. Water-filled pore space, soil and chamber temperatures were affected by sampling periods (P_1 and P_2). There was a significant pre-grazing target treatment × sampling period interaction effect on soil NH_4^+ concentration, which was most likely associated with urinary-N discharge. During P_1, there was a greater urinary-N discharge for $LI_{95\%}$ than LI_{Max} (26.3 vs. 20.9 kg of urinary-N/paddock) caused by higher stocking rate, which resulted in greater N_2O fluxes for $LI_{95\%}$. Inversely, during P_2, the soil NH_4^+ and N_2O fluxes were greater for LI_{Max} than $LI_{95\%}$. During this period, the greater urinary-N discharge (46.8 vs. 44.8 kg of urinary-N/paddock) was likely associated with longer stocking period for LI_{Max} relative to $LI_{95\%}$, since both treatments had similar stocking rate. Converting hourly N_2O fluxes to daily basis and relating to milk production efficiency, $LI_{95\%}$ was 40% more efficient than LI_{Max} (0.34 vs. 0.57 g $N\text{-}N_2O$/kg milk·ha). In addition, $LI_{95\%}$ pre-grazing target decreased urea-N loading per milk production by 34%. Strategic grazing management represented by the $LI_{95\%}$ pre-grazing target allows for intensification of tropical pasture-based dairy systems, enhanced milk production efficiency and decreased $N\text{-}N_2O$ emission intensity.

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1. Introduction

Dairy farming systems provide essential high-quality protein, a major component of human diet (O'Brien et al., 2012; Aguirre-Villegas et al., 2017). Pasture-based systems are important milk suppliers to dairy industry in temperate (Chapman, 2016; Macdonald et al., 2017) and tropical climate regions (Santos et al., 2014) and thereby will play relevant role to meet world's growing demand (Godfray et al., 2010; Conforti, 2011; Alexandratos and Bruinsma, 2012).

The key to understanding the principles used to conceive grazing management strategies is to comprehend that the harvestable components are photosynthetic organs – predominantly leaves (Parsons et al., 2011). Studies have reported that grazing management strategies based on canopy critical leaf area index (i.e. LAI that allows for the interception of 95% of the incident light) minimize stem elongation and prioritize leaf rather than other plant component accumulation, corresponding to an useful tool for planning and managing efficient pasture-based systems in the tropics (Da Silva et al., 2017; Sbrissia et al., 2018). Leafy swards mean high herbage quality, since it provides high rates of herbage intake by grazing animals, as leaves require less strength to be harvested, and because they have greater nutritive value than stems and dead material (Trindade et al., 2007). In this sense, the development of efficient pasture-based systems of animal production with perennial tropical grasses usually focuses on the control of stem elongation and excessive senescence and dead material accumulation through adequate grazing management strategies (Da Silva et al., 2015).

Greenhouse gases (GHG) emissions are considered one of the most important environmental issues of dairy farming systems (O'Brien et al., 2012; Guerci et al., 2013; Gregorini et al., 2016) and nitrous oxide (N_2O) is the second most representative among all GHG, ranging from 15 to 25% of total GHG emissions in those systems (Aguirre-Villegas et al., 2017). Nitrous oxide is formed through soil microbial transformation of nitrogen (N) compounds, typically by incomplete denitrification or by nitrification (Wrage et al., 2001; Saggar et al., 2013). Nitrous oxide fluxes are affected by a wide range of proximal and distal regulators, making its regulation a very complex process (de Klein et al., 2008; Luo et al., 2017). Proximal soil factors include mineral N (NH_4^+ and NO_3^-) and organic carbon availabilities, moisture, pH, temperature, and texture that, in turn, are affected by distal regulators such as rainfall or irrigation, soil compaction, organic matter and N inputs (de Klein et al., 2008; Luo et al., 2017). Periods when soil characteristics coincide for favorable N_2O production are called "hot moments" (Luo et al., 2017). In tropical conditions, "hot moments" usually occur during spring and summer when pastures are intensively growing owing to the abundance of solar radiation, rainfall, and N inputs.

Grazing management strategies can strongly affect the majority of distal regulators. It determines ecophysiological plant processes such as herbage growth, senescence and decay (Da Silva et al., 2009; Pereira et al., 2014; Pereira et al., 2015; Da Silva et al., 2015; Congio et al., 2018) that strongly affect animal responses such as herbage intake (Congio et al., 2018), herbage losses by cattle trampling (Carnevalli et al., 2006; Silveira et al., 2013; Congio et al., 2018), stocking rate (Voltolini et al., 2010; Gimenes et al., 2011; Congio et al., 2018), excreta spatial distribution (White et al., 2001; Auerswald et al., 2010) and N load into pastures (Vibart et al., 2017). These factors, in turn, modify soil properties (i.e. bulk density, moisture, temperature, pH, aeration) (Warren et al., 1986; Silva et al., 2003; Schmalz et al., 2013) that affect microbial community growth and activity (Bardgett et al., 1996; Bardgett et al., 2001; Bardgett and Wardle, 2003) determining the intensity of processes associated with N_2O fluxes from soils (de Klein et al., 2008; Levine et al., 2011; Luo et al., 2017).

The majority of studies involving N_2O fluxes from pasture soils have been addressed to assess the effects of proximal factors on processes and emission factors in temperate climate conditions (Saggar et al., 2013; de Klein et al., 2014; Barneze et al., 2015; Venterea et al., 2015; Gardiner et al., 2016; Samad et al., 2016; Clough et al., 2017; Gardiner et al., 2017; van der Weerden et al., 2017; Luo et al., 2018; Rex et al., 2018). The little information available for tropical pastures has also been focused on nitrous oxide fluxes related to proximal factors within urine patches (Barneze et al., 2014; Lessa et al., 2014; Mazzetto et al., 2014; Mazzetto et al., 2015). There is no information available regarding the influence of grazing management strategies on N_2O fluxes from soils of tropical pasture-based dairy farming systems. In fact, farming scale studies are scarce even in temperate climate conditions. Experimental approaches have shown that intensively managed grasslands are stronger sources of N_2O than extensively managed grasslands owing to greater inputs of N fertilizer and excreta (Smith et al., 2001; Flechard et al., 2007; Rafique et al., 2011). However, they have not accounted for milk production efficiency that is usually greater in intensively managed systems and could decrease the intensity of N_2O emission (i.e. g N-N_2O/kg milk·ha·day).

The objective of this study was to investigate the influence of two rotational grazing strategies characterized by two pre-grazing targets (95% and maximum canopy light interception during sward regrowth; $LI_{95\%}$ and LI_{Max}, respectively) on milk production efficiency and N_2O fluxes from soil in a tropical dairy farming system based on elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon). The general hypothesis was that frequent defoliations generated by the $LI_{95\%}$ pre-grazing target would increase milk production efficiency and decrease N_2O emission intensity.

2. Material and methods

All procedures for this study were approved by the Animal (15.5.1246.11.2) and Environment Ethics Committees (17.5.999.11.9) at the University of São Paulo, College of Agriculture "Luiz de Queiroz" (USP/ESALQ).

2.1. Study site

The experiment was conducted in Piracicaba, SP, Brazil (22°42′S, 47°38′W and 546 a.s.l.) on a rainfed, non-irrigated elephant grass (*Pennisetum purpureum* Schum. cv. Cameroon) pasture established in 1972 in a Eutroferric Red Nitossol soil (NVef; EMBRAPA, 2018) (Table 1). The climate is sub-tropical with dry winters and 1328 mm average annual rainfall (CEPAGRI, 2012). The lowest and highest mean air temperatures were recorded in July (19.7 °C) and December (27.1 °C), respectively. The greatest accumulated rainfall was observed from late spring to summer (1090 mm from November 2015 to March 2016), and the lowest from winter to early spring (356 mm from June to October 2015).

2.2. Treatments and experimental design

The two treatments were pre-grazing targets of either 95% or maximum canopy light interception during regrowth ($LI_{95\%}$ and LI_{Max}, respectively). The 2.5 ha experimental area was comprised of 12 adjacent elephant grass paddocks (2100 m^2 on average). Paddocks were assigned to treatments according to a randomized complete block design (slope and chemical soil characteristics were considered as blocking criteria), with six replications. Paddocks were then divided into three sub-paddocks (686 m^2 on average) in order to create two farmlets of 18 sub-paddocks each where two groups of dairy cows were allocated as grazing herd throughout the experimental period (detailed information in Congio et al., 2018).

Treatments based on canopy light interception resulted in contrasting sward structures and determined pre-grazing sward surface heights (SSH) of 100 cm ($LI_{95\%}$) and 135 cm (LI_{Max}). For both pre-grazing SSH, the herbage depletion level (post-grazing height) corresponded to 50% of the pre-grazing SSH to maintain high short-term rates of herbage intake (Fonseca et al., 2012; Carvalho, 2013). Treatments were allocated

Table 1
Soil properties (0–10 cm layer) at the beginning of the experiment.

Clay	Sand	Silt	Bulk density	pH	OM	P	K	Ca	Mg	S	H + Al	CEC	BS
g/kg			g/cm^3	$CaCl_2$	g/dm^3	mg/dm^3	mmol$_c$/cm^3						%
482	328	190	1.34	4.8	55.2	49.8	3.3	40.2	22.7	8.6	55.5	121.7	54.4

OM = organic matter; P = phosphorus - ion-exchange resin extraction method; K = potassium; Ca = calcium; Mg = magnesium; S = sulphur; H + Al = hydrogen + aluminum; CEC = cation exchange capacity; BS = base saturation; the units are expressed in units of soil.

to farmlets in mid-January 2015 after grazing and mowing at 45-cm for standardization. During the 11-months prior to field measurements (mid-January to mid-December 2015), each farmlet was adapted to its respective grazing management strategy. Paddocks were rotationally grazed by 10–13 dairy cows in order to keep grazing management targets, as specified. The adaptation period was necessary to adapt sward structure to treatments and to identify the corresponding pre-grazing SSH for the LI pre-grazing targets used ($LI_{95\%}$ and LI_{Max}) (Congio et al., 2018).

Measurements were performed after the adaptation period during the second rainy season from mid-December 2015 to mid-April 2016 (experimental period). A total of 215 kg N/ha (as urea, 45% of N) was applied throughout the experimental period. Because grazing interval was not constant (as a consequence of treatments specification), the total amount of N to be applied was divided throughout the experimental period (119 days) and a daily rate of N fertilizer was calculated. The amount of N applied per paddock after each grazing was proportional to the length of the corresponding rest period (daily rate × rest period), ensuring similar N fertilizer application to both treatments at the end of the experimental period (Da Silva et al., 2017). On average, a total of 215 kg N/ha was divided in 3.5 and 5.6 installments for LIMax and LI95%, respectively. Therefore, N inputs from urea fertilizer immediately before N_2O sampling were greater for LI_{Max} than $LI_{95\%}$ during P_1 (75 vs. 44 kg N/ha) and P_2 (111 vs. 57 kg N/ha).

Milk yield of twenty-six Holstein × Jersey dairy cows (n = 13) was recorded daily. An additional herd of dry-cows (n = 10–13) was maintained in an adjacent area of elephant grass and was used to adjust stocking rate and keep grazing management targets constant, as needed. The stocking rate was calculated based on the number of cows used daily for each treatment, considering experimental cows and the additional herd. Milk productivity was calculated using stocking rate and milk yield for each treatment (Congio et al., 2018).

2.3. Soil flux measurements, analysis and flux calculation

Soil gaseous fluxes were measured using the non-ventilated closed static chamber methodology updated by the Global Research Alliance on Agricultural Greenhouse Gases (de Klein and Harvey, 2015). Gas samples, from both treatments, were collected during two sampling periods throughout the experimental period (P_1 = 01/08/2016 to 01/22/2016 and P_2 = 02/25/2016 to 03/10/2016). Measurements were made at post-grazing, immediately after N fertilization, with ten chambers randomly placed 5-cm into bare ground in each paddock (n = 10).

Chambers of 17.67 L were made of PVC, composed of a base (30 cm diameter and 20 cm height) plus cap (30 cm diameter and 10 cm height), and were insulated with thermal blanket to avoid heating during sampling (de Klein et al., 2014; Di et al., 2016). Chamber base and cap were sealed with rubber. Gas samples were collected immediately after chamber closing, and at 30 and 60 min. Samples were collected from a cap sampling port using 20 mL plastic syringes (Becton Dickinson, Franklin Lakes, NJ, EUA) and precision glide needles (0.8 × 40 mm; BD), and injected into sealed and evacuated 10 mL glass sample vials. Gas sampling started 24 h after chamber placement to allow soil microbial community to stabilize and minimize overestimation or underestimation of emissions (Chiavegato et al., 2015). Sampling was performed over five consecutive days, and then every five days until the 15th day after N fertilizer was applied. Chambers were removed after P_1 evaluation and re-placed following the same procedures described for P_1 at the beginning of P_2. All samples were collected from 8 to 9:15 am (Alves et al., 2012) and analyzed using gas chromatography at the Laboratory of Analytical Chemistry (Embrapa Pecuária Sudeste, São Carlos, SP, BRA).

The chromatograph GC-2014 (Shimadzu, Columbia, MD, EUA) was equipped with electron capture detectors (ECD) at 325 °C (column HayeSep T 80/100) for N_2O and flame ionization detectors (FID) at 250 °C for CO_2 (column HayeSep T 80/100). Calibration curves were established using standard certified gases for CO_2 (260.2 ± 1.77; 508.3 ± 3.10, 1058 ± 14.49 and 1995 ± 10.78 ppm) and N_2O (257.3 ± 1.95; 502.8 ± 3.47, 999.5 ± 17.79 and 2328 ± 112.67 ppt). Gas chromatography outputs were analyzed to determine linearity from 0 to 60 min. A strong linear relationship was observed for N_2O ($r^2 = 0.88$) and the hourly gas fluxes were calculated according to the increase of gas concentration into the headspace over sampling time (de Klein et al., 2014; Luo et al., 2018):

$$Gas\ flux = \frac{\delta Gas}{\delta T} \times \frac{M}{Vm} \times \frac{V}{A} \quad (1)$$

where δGas is the increase in headspace gas concentration overtime (μL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O; V_m is the molar volume of gas at the sampling temperature (L/mol); V is the headspace volume (m^3); and A is the area covered (m^2). Fluxes were corrected for chamber bias to account for suppression of the surface-atmosphere concentration gradient using numerical technique (Venterea, 2010) and hourly fluxes were assumed to represent mean daily fluxes (de Klein et al., 2014).

2.4. Weather and ancillary measurements

Atmospheric pressure, ambient temperature, and rainfall were monitored daily at the weather station located 50 m from the experimental area. Soil and headspace temperatures were recorded for each chamber at each time point with a digital thermometer (TE-300, Instrutherm, São Paulo, SP, BRA). Soil chemical and physical properties presented in Table 1 (except bulk density) were characterized using fifteen randomized soil subsamples per paddock collected at a 10 cm depth. Soil bulk density and particle density were determined using four soil cores per paddock at a 10 cm depth at the first day of each sampling period and calculated according to Grossman and Reinsch (2002) and Flint and Flint (2002). During the first day of sampling, additional soil samples were taken at 0–5 cm depth adjacent to each chamber in order to determine soil nitrate (NO_3^-) and ammonium (NH_4^+). Soil N was extracted for 1 h with 2 M KCl, filtered (Whatman 42) and samples were analyzed for mineral N concentration by flow injection analysis (ASIA; Ismatec, Zürich, Switzerland). At each sampling day prior to gas collection, soil samples were taken at 0–5 cm depth from the adjacent area of each chamber for soil gravimetric moisture determination (24 h at 105 °C). Volumetric water contents were calculated by multiplying gravimetric water contents by soil bulk density, and soil water-filled pore-space (WFPS) was calculated by dividing volumetric water content by total soil porosity (de Klein et al., 2014; Luo et al., 2018). Soil porosity was calculated according to Selbie (2013).

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed using the Mixed Procedure of SAS (SAS 9.3; SAS Institute Inc., Cary, NC). Different structures of the variance–covariance matrices were tested, and variance components matrix was chosen as the best fit for the majority of variables based on the Bayesian Information Criterion. The model included fixed effects of treatment, sampling period, and their interaction, and random effect of chamber. Chambers were considered experimental units and sampling periods were treated as repeated measures. All data were checked for normality and homogeneity of variances and log-transformed when they did not meet the assumptions. Soil temperature, air temperature, WFPS, soil NH_4^+ and soil NO_3^- were tested as explanatory variables. Means were calculated using the Least-Squares Means statement, compared using the Student's *t*-test and differences were declared significant at $P \le 0.05$. For N_2O fluxes, WFPS was used as a covariate. To better understand the relations among dependent variables, a principal component analysis (PCA) was performed using a data set comprised of N_2O fluxes, soil NH_4^+, soil NO_3^-, soil temperature, chamber temperature, and WFPS. Principal components scores were submitted to ANOVA to describe and interpret the effects of treatments and periods (Jolliffe, 2002).

3. Results

3.1. Weather conditions

Weather conditions during the two sampling periods are presented in Fig. 1. Air temperature ranged from 16.6 to 35.2 °C with average of 25.7 °C during P_1 (Fig. 1A). Similarly, during P_2, air temperature ranged from 18.4 to 33.3 °C with average of 24.9 °C (Fig. 1B). Average soil temperatures were 22.7 and 24.7 °C for P_1 and P_2, respectively. Accumulated rainfall was 199 mm during P_1 and 106 during P_2 (Fig. 1A and B, respectively).

3.2. Soil parameters

Water-filled pore space, soil and chamber temperatures varied with sampling period ($P < 0.01$) being greater for P_2 than P_1 (Table 2). Both soil NH_4^+ and NO_3^- concentrations were not affected by treatment or sampling period ($P > 0.05$), however there was a significant interaction between treatments and sampling period for soil NH_4^+ ($P = 0.0006$) and a trend for soil NO_3^- ($P = 0.0725$). During P_1, there was an effect of LI pre-grazing targets on soil NH_4^+, with greater values observed for $LI_{95\%}$ than LI_{Max}; however, during P_2 soil NH_4^+ was greater for LI_{Max} than $LI_{95\%}$ ($P < 0.05$). Water-filled pore space and rainfall patterns for both periods are presented in Fig. 2. Days with no rainfall markedly decreased WFPS during the beginning and the end of P_1 (Fig. 2A). There was no effect of LI pre-grazing target on WFPS during P_1 ($P = 0.9967$; Fig. 2A), but the effect was significant during P_2 ($P = 0.05$; Fig. 2B).

3.3. Nitrous oxide fluxes and milk production efficiency

Nitrous oxide fluxes were strongly affected by sampling period and WFPS ($P < 0.01$; Table 3). On average, N_2O fluxes were greater during P_1 than P_2 (312.8 vs. 197.7 μg $N\text{-}N_2O/m^2 \cdot hr$; $P < 0.01$). There was a significant interaction between treatments and sampling period ($P < 0.0001$). During P_1, N_2O fluxes were greater for $LI_{95\%}$ ($P = 0.0171$) and during P_2, fluxes were greater for LI_{Max} ($P = 0.0011$). Nitrous oxide fluxes across sampling periods and days are shown in Fig. 3. During P_1, difference in N_2O fluxes occurred on one day only (1/10/2016), ($P > 0.05$; Fig. 3A). During P_2, two out of seven days had greater N_2O fluxes for LI_{Max} than $LI_{95\%}$ ($P < 0.05$; Fig. 3B). Milk production efficiency was 52% greater for $LI_{95\%}$ than LI_{Max} (170 and 112 kg/ha·day for $LI_{95\%}$ and LI_{Max}, respectively; $P = 0.0012$).

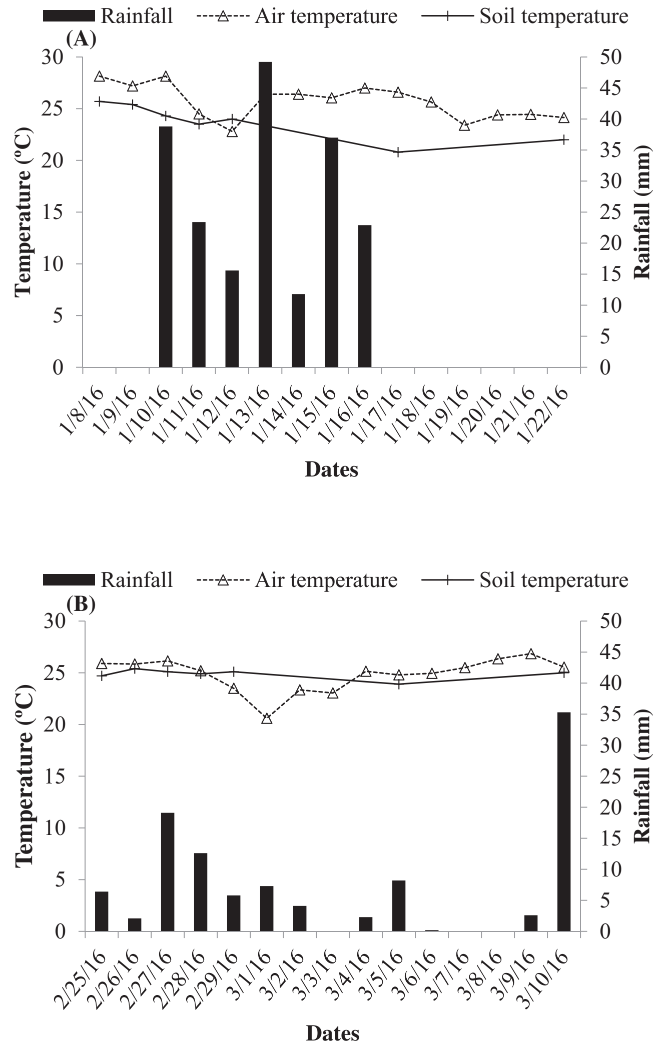

Fig. 1. Daily air and soil (0-5 cm depth) temperatures (°C) and rainfall (mm) during sampling periods P_1 (A) and P_2 (B) at the study site (Jan-Mar 2016).

3.4. Principal component analysis

Principal component analysis generated six principal components, however, only the first two were explored because these had eigenvalues >1 (Kaiser criterion; Jolliffe, 2002) and accounted for 71.8% of the total variance in N_2O fluxes (Table 4). The first principal component (PC1) explained 49% of the total variance and indicated high positive scores for N_2O fluxes and WFPS, and high negative scores for soil and chamber temperatures. Analysis of variance on PC1 scores showed a significant effect of sampling period ($P < 0.01$). The second principal component (PC2) accounted for 22.8% of the total variance and showed high positive score for soil NH_4^+ and high negative score for soil NO_3^- contents. Analysis of variance on PC2 scores showed a significant effect of treatment × sampling period interaction ($P = 0.0015$).

4. Discussion

The objective of this study was to investigate the influence of two rotational grazing strategies characterized by two pre-grazing targets on milk production efficiency and N_2O fluxes from the soil in a tropical dairy farming system during a “hot moment”. In tropical conditions, “hot moments” usually occur during spring and summer (~180 days) when pastures are intensively growing owing to the abundance of

Table 2
Water-filled pore space (WFPS), soil temperature, chamber temperature, ammonium and nitrate concentrations from soil growing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during sampling periods P_1 (01/08/2016 to 01/22/2016) and P_2 (02/25/2016 to 03/10/2016) (n = 10).

	Period		SEM[a]	*P*-value		
	1	2		Trt[b]	Per[c]	Trt × Per
	WFPS, %					
$LI_{95\%}$	77.8	94.5	1.57	0.1654	<0.0001	0.1672
LI_{Max}						
	Soil temp., °C					
$LI_{95\%}$	23.7	24.9	0.11	0.4125	<0.0001	0.4631
LI_{Max}						
	Chamber temp., °C					
$LI_{95\%}$	22.6	23.7	0.14	0.7344	<0.0001	0.8221
LI_{Max}						
	NH_4^+, mg/kg dry soil					
$LI_{95\%}$	283.4 Aa	76.6 Bb	69.44	0.8771	0.4915	0.0006
LI_{Max}	21.4 Bb	318.4 Aa				
	NO_3^-, mg/kg dry soil					
$LI_{95\%}$	5.0 Aa	20.6 Aa	6.18	0.2218	0.5126	0.0725
LI_{Max}	8.8 Aa	1.4 Ba				

Means followed by the same upper case letter in columns and lower case letter in rows do not differ ($P > 0.05$).
[a] Standard error of the mean.
[b] Treatment effect.
[c] Sampling period effect.

Table 3
Nitrous oxide fluxes (μg $N\text{-}N_2O/m^2\cdot hr$) from soil growing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during sampling periods P_1 (01/08/2016 to 01/22/2016) and P_2 (02/25/2016 to 03/10/2016) (n = 10).

	N-N_2O, μg N-$N_2O/m^2\cdot$hr		SEM[a]	*P*-value			
	P_1	P_2		Trt[b]	Per[c]	Trt × Per	WFPS[d]
$LI_{95\%}$	369.6 Aa	117.5 Bb	40.10	0.4907	0.011	<0.0001	<0.0001
LI_{Max}	256.0 Ba	277.9 Aa					

Means followed by the same upper case letter in columns and the lower case letter in rows do not differ ($P > 0.05$).
[a] Standard error of the mean.
[b] Treatment effect.
[c] Sampling period effect.
[d] Water-filled pore space effect.

solar radiation, rainfall, and N inputs. Prior to the beginning of measurements, all paddocks were subjected to an 11-month adaptation period to grazing treatments (horizontal and vertical structure and dynamics pattern of plant growth and herbage accumulation) to ensure that any observed effects would be a direct consequence of the dynamics associated with each grazing strategy. Samples for N_2O determination were taken as snapshots during 2 grazing cycles (sampling periods P_1 and P_2) during periods of active and intensive plant growth and development ("hot moments") from a total of 3.5 and 5.6 grazing cycles for

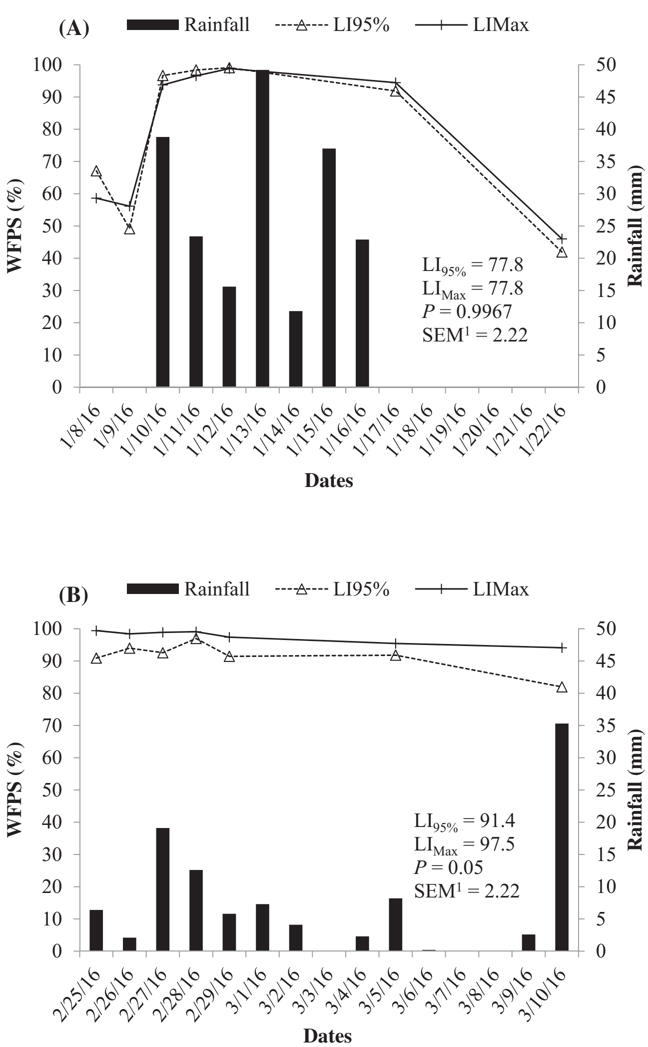

Fig. 2. Water-filled pore space (WFPS) and rainfall (mm) during sampling periods P_1 (A) and P_2 (B) at the study site (Jan-Mar 2016). [1]Standard error of the mean.

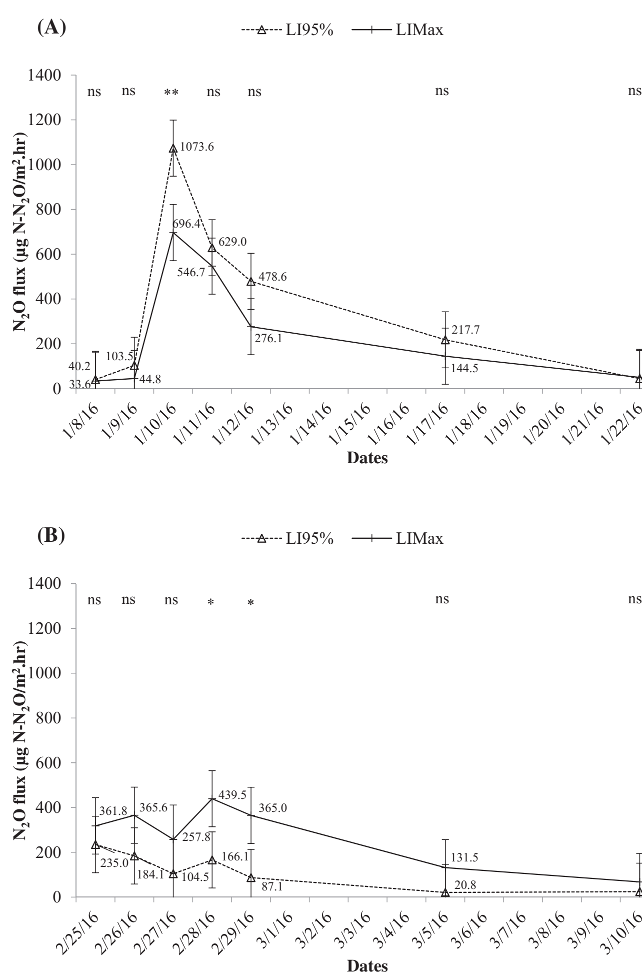

Fig. 3. Nitrous oxide fluxes (μg $N\text{-}N_2O/m^2\cdot hr$) derived from soil growing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during sampling periods P_1 (A) and P_2 (B). ns ($P > 0.05$), * ($P \le 0.05$), ** ($P \le 0.01$)

Table 4
Coefficients of principal components based on the correlation matrix for N_2O fluxes, soil NH_4^+ and NO_3^-, soil and chamber temperatures, and water-filled pore space from soil growing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}).

Variables	PC1	PC2
N_2O fluxes	0.49	-0.08
Soil NH_4^+	0.13	0.67
Soil NO_3^-	0.00	-0.70
Soil temperature	-0.48	0.19
Chamber temperature	-0.49	0.07
Water-filled pore space	0.52	0.15
Eigenvalue	2.94	1.37
% of variation explained	49.0	22.8
ANOVA	***P*-value**	
Trt[1]	0.1149	0.6239
Per[2]	<0.0001	0.2950
Trt×Per	0.6934	0.0015

[1] Treatment effect
[2] Sampling period effect

LI_{Max} and $LI_{95\%}$, which represented 57 and 36% of the entire growing periods, respectively.

The grazing management strategies used in this study provided contrasting pre- and post-grazing SSH that affected grazing interval and ultimately the number of grazing cycles. For LI_{Max}, pre- and post-grazing SSH were 135 and 64 cm, respectively, which resulted in an average grazing interval of 32 days and 3.5 grazing cycles during the experimental period (Congio et al., 2018). On the other hand, for $LI_{95\%}$, pre- and post-grazing SSH were 100 and 50 cm, respectively, which resulted in an average grazing interval of 21 days and 5.6 grazing cycles (Congio et al., 2018). Considering adaptation and experimental periods (from January 2015 to April 2016) there were 9.3 grazing cycles for LI_{Max} and 14.1 for $LI_{95\%}$, indicating greater frequency of defoliation on paddocks managed with the $LI_{95\%}$ target relative to those managed with the LI_{Max} target. To keep the pre- and post-grazing targets, the average stocking rate during the entire experimental period was 33% greater for $LI_{95\%}$ than LI_{Max} (9.3 vs. 7.0 cows/ha; Congio et al., 2018). These grazing conditions resulted in different scenarios of intensification, solely by changing pre-grazing targets ($LI_{95\%}$ or LI_{Max}). It is worthwhile to mention that the greater stocking rate obtained in $LI_{95\%}$ was supported by greater leaf accumulation and greater grazing efficiency rather than increased N fertilizer input, usually applied in intensive temperate pasture-based systems (Ramsbottom et al., 2015; Macdonald et al., 2017; Congio et al., 2018).

In grazed pastoral soils, the factors pointed out as key drivers of N_2O fluxes are N inputs (i.e. urine patches and fertilizer) and WFPS (de Klein et al., 2008; Luo et al., 2017). Nitrous oxide fluxes and soil NH_4^+ varied with LI pre-grazing target × sampling period interaction, while a trend was observed for NO_3^-. On the other hand, the variables related to weather (i.e. WFPS, soil and chamber temperatures) varied only with sampling period. Most studies have indicated that high N_2O emissions are usually associated with anaerobic soils with enough NO_3^- supply suggesting that denitrification is the main process responsible for N_2O emissions (de Klein and Eckard, 2008; de Klein et al., 2008). However, on excessively saturated soils with higher WFPS (i.e. optimal conditions for denitrification), as observed in P_2, denitrification is complete and results in a greater $N_2:N_2O$ ratio (Bolan et al., 2004; de Klein et al., 2008).

Although the accumulated rainfall was greater during P_1 (199 mm) than P_2 (106 mm), the WFPS was constantly greater throughout P_2 than P_1. These results are likely associated with better rainfall distribution during P_2, where there were 80% of rainy days, while during P_1 there were just 47% of rainy days. Studies have reported that peak N_2O emissions occurred at WFPS values around 60–80%, when simultaneous nitrification and denitrification were at maximum levels (Davidson, 1992; Rafique et al., 2011). Above this WFPS range, denitrification is the main source of N_2O and under excessively anaerobic conditions, $N_2:N_2O$ ratio remains greater (Bolan et al., 2004; de Klein et al., 2008; Rafique et al., 2011). The results of PCA pointed to an interaction among the driving factors regulating N_2O fluxes from soil. The first PCA indicated that environmental factors (i.e. WFPS, soil and chamber temperatures) were determinants of N_2O emissions and explained 49% of the whole dataset variability. Principal component analysis two showed that factors related to LI pre-grazing targets (i.e. soil NH_4^+ and NO_3^-) had the highest scores and accounted for 22.8% of total variance. Flechard et al. (2007) also reported that weather factors explained half of the total variability in their N_2O flux dataset of ten sites for three years across Europe. Analysis of variance on PC1 and PC2 scores corroborated the results from the analysis of variance, where environmental factors showed significant effect for sampling period, as observed in PC1, and treatment related factors showed a significant LI pre-grazing target × sampling period interaction effect, as observed in PC2.

Both soil NH_4^+ and NO_3^- represented the concentration immediately after urea fertilization at day one, and therefore indicate N availability at the beginning of each sampling period. For both LI pre-grazing targets, a total of 215 kg N/ha was applied throughout the experimental period. However, this amount was divided in 3.5 and 5.6 installments for LI_{Max} and $LI_{95\%}$, respectively. Therefore, the N inputs from urea fertilizer immediately before N_2O sampling were greater for LI_{Max} than $LI_{95\%}$ during P_1 and P_2 (Table 5). However, there was a significant LI pre-grazing target × sampling period interaction on soil NH_4^+ concentration, most likely associated with urinary-N discharge. During P_1, there was a greater urinary-N discharge for $LI_{95\%}$ than LI_{Max} caused by higher stocking rate (Table 5), which resulted in greater N_2O fluxes for $LI_{95\%}$. Inversely, during P_2, the soil NH_4^+ and N_2O fluxes were greater for LI_{Max} than $LI_{95\%}$. During this period, the greater urinary-N discharge was likely associated with greater stocking period for LI_{Max} relative to $LI_{95\%}$, since both treatments had similar stocking rate (Table 5). These results are in agreement with most studies that have reported urine patches as the main source of N_2O from grazed pasture soil mainly by providing highly localized concentrations of available N, ranging from 200 to 2000 kg N/ha, associated with increased moisture and temperature conditions (Selbie et al., 2015; Luo et al., 2018).

Dairy farming systems based in temperate pastures are usually more intensive than tropical pasture-based dairy systems (Macdonald et al., 2017; Congio et al., 2018). Temperate forage crops have been studied in depth and the understanding of their ecophysiology allowed for better use by farmers through adoption of adequate grazing management strategies, ensuring high milk production efficiency (Chapman, 2016). The intensification of such systems is usually coupled with extra inputs of N fertilizer to boost forage growth or with external supplementary feed, both aiming at increased stocking rate (Ramsbottom et al., 2015; Macdonald et al., 2017). In the tropics, dairy farming systems usually have low N inputs and adopt inadequate grazing management strategies resulting in low milk productivity (Santos et al., 2014). Therefore, the intensification of tropical pasture-based dairy systems is possible through adoption of adequate grazing strategies rather than extra N inputs or additional supplements, provided that minimum soil fertility to meet plant nutritional demand is ensured. The results indicated the opportunity to increase milk productivity in 52% only with adoption of strategic grazing management (i.e. $LI_{95\%}$ pre-grazing target).

Table 5
Nitrogen (N) fertilization rate, urinary-N discharge, stocking rate and stocking period from Holstein × Jersey dairy cows grazing elephant grass subjected to strategies of rotational stocking management ($LI_{95\%}$ or LI_{Max}) during sampling periods P_1 and P_2.

	P_1		P_2	
	$LI_{95\%}$	LI_{Max}	$LI_{95\%}$	LI_{Max}
N-fertilization rate, kg N/ha	44	75	57	111
Urinary-N discharge, kg N/paddock	26.3	20.9	44.8	46.8
Stocking rate[a], cows/ha	10.0	8.3	9.9	9.5
Stocking period, days	0.88	0.88	1.46	1.88

[a] Represents the specific stocking rates immediately before sampling periods P_1 and P_2.

Experimental approaches have shown that intensively managed pastures are greater sources of N_2O than extensively managed pastures (Flechard et al., 2007; Rafique et al., 2011). Rafique et al. (2011) reported that frequently grazed sites that applied 400 kg of N/ha emitted two times more N_2O compared to less frequently grazed sites that used around 300 kg of N/ha. However, in their study, intensively managed systems were generated through greater inputs of N fertilizer. Although urinary-N excretion increased soil NH_4^+ and ultimately N_2O fluxes during P_1 for $LI_{95\%}$, the urinary-N excretion and N_2O fluxes during P_2 were greater for LI_{Max} counterbalancing the emissions for the entire experimental period (255.3 μg N-N_2O/m^2·hr; $P = 0.4907$). Converting hourly N_2O fluxes to hectare and daily basis (g N-N_2O/ha·day) and relating to milk production efficiency (kg milk/ha·day), $LI_{95\%}$ was 40% more efficient than LI_{Max} considering emissions for the entire period (0.34 vs. 0.57 g N-N_2O/kg milk·ha·day). In addition, strategic grazing management decreased urea-N loading per milk production efficiency by 34% (0.57 vs. 0.86 g urea-N/kg milk·ha·day).

In the context of growing concern about the intensification of temperate pasture-based dairy systems through greater N fertilizer inputs (Di and Cameron, 2016; Gregorini et al., 2016; OECD, 2017), these findings highlight an opportunity to improve the efficiency of tropical pasture-based dairy systems through optimization of natural ecological processes. Strategic grazing allows for intensification that is not coupled with increases in inputs of external resources (i.e. additional fertilizer, external supplements) but rather with efficient use of existing resources (i.e. solar radiation, rainwater, pasture, fertilizer, supplement). Strategic grazing management is a non-cost and readily available practice with easy adoption that enhances profitability of tropical pasture-based systems.

5. Conclusions

Nitrous oxide fluxes from grazed pastoral soils in moist-warm conditions are a very complex process regulated by environmental conditions and soil nitrogen availability. The central hypothesis that frequent defoliation provided by the $LI_{95\%}$ pre-grazing target would result in lower N_2O emission intensity from soil than less frequent defoliation (i.e. LI_{Max}) was confirmed. These results highlight that it is possible to intensify tropical pasture-based dairy systems through the adoption of adequate grazing strategies before using extra N fertilizer or supplemental feed, as is usual for temperate grazing systems. This indicates the opportunity to significantly enhance milk production efficiency from tropical pasture-based systems using strategic grazing management (i.e. $LI_{95\%}$) and decrease N-N_2O emission intensity by 40%.

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