

GAS EXCHANGE IN MASSAI GRASS FERTILIZED WITH NITROGEN AND GRAZED BY SHEEP

TROCAS GASOSAS EM CAPIM-MASSAI ADUBADO COM NITROGÊNIO E PASTEJADO POR OVINOS

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ABSTRACT: The knowledge of gas exchanges in forage plants is essential for a better understanding of the process of forage biomass production in pasture. This study evaluated the gas exchange in massai grass fertilized with increasing levels of nitrogen fertilizer (control - without nitrogen fertilizer; 400; 800 and 1200 kg ha⁻¹ year⁻¹) and under rotational grazing by sheep, in a completely randomized design with repeated measures in time. The rest period was approximately 1.5 new leaves per tiller, as determined in the pre-test at the beginning of the experiment, providing interval of 22; 18; 16 and 13 days for the levels 0.0 - control; 400; 800 and 1200 kg ha⁻¹ year⁻¹ nitrogen, respectively. The animals used to lower the sward height to the recommended residual height were sheep (½ Morada Nova x ½ undefined breed), placed in paddocks of 42.3 m². As the animals grazed, the height of the sward was monitored with a ruler until the canopy reached the recommended residual height of approximately 15 cm, corresponding to the residual LAI of exit of the animals from the paddock at approximately 1.5, as determined in a pre-test to set up the experiment. The variables stomatal conductance, leaf photosynthesis rate, leaf carbon dioxide concentration, photosynthesis/transpiration ratio, chlorophyll relative index and nitrogen sufficiency index revealed a positive linear response to nitrogen fertilization. Nitrogen fertilization level at 1200 kg ha⁻¹ year⁻¹ caused an increment of 92.3% in leaf photosynthesis rate in relation to the control. The leaf temperature and photosynthesis/conductance ratio were reduced with increasing nitrogen levels. The leaf transpiration showed a quadratic response with maximum point with increasing nitrogen levels. Nitrogen fertilization favor the gas exchange in massai grass up to the last level tested.

KEYWORDS: Nitrogen fertilization. Chlorophyll relative index. Leaf photosynthesis. *Panicum maximum*.

INTRODUCTION

Maximization of biomass production in forages reflects the appropriate conditions of medium and management, the latter includes soil fertility, especially the nitrogen supply, given the relevance of this nutrient for plant growth. The nitrogen demand by the plant for maximum yield in forage biomass production reflects the participation of this nutrient as an essential constituent of proteins and pigments, with a great influence on the physiological processes of the plant, participating directly in numerous stages of photosynthesis (capture of light, fixation of carbon dioxide, etc.), as

well as innumerable other metabolic processes of the plant (CABRERA-BOSQUET et al., 2009).

The intensification of ruminant production in pastures prioritizes the use of forages with morphophysiological characteristics capable of responding efficiently to the management adopted, especially the use of fertilizers, which contribute notably to the increase of forage biomass. In this way, massai grass, a cultivar of *Panicum maximum*, reveals important characteristics regarding the productive parameters, standing out the low stem production, high tillering, high regrowth capacity after grazing, high leaf biomass production, besides promoting good soil cover.

Studies evaluating gas exchanges in forages make it possible to quantify plant responses in terms of internal CO₂ concentration in the leaf, stomatal conductance, leaf photosynthesis among others, due to the availability of abiotic factors, being of fundamental importance for the expression of the production potential of forage plants. Such studies allow a joint evaluation of the physiological processes that are occurring in the plant in response to environmental factors and management.

Studying gas exchange in *Panicum maximum* cv. Aruana under four levels of nitrogen fertilizer (0, 125, 250 and 375 mg dm⁻³ soil), Pompeu et al. (2010) observed an increasing linear response for leaf transpiration, stomatal conductance, leaf temperature and leaf photosynthesis rate.

The chlorophyll content in the leaf is used to predict the nitrogen requirements of the plants, and the readings can be carried out in a non-destructive manner and even in the field with the aid of a portable chlorophyll meter, which calculates the amount of light transmitted by the leaf, based on two wavelengths, with different absorbances of chlorophyll (MINOLTA, 1989). Studies have shown the positive influence of nitrogen fertilization on the relative index of chlorophyll in forages.

In *Brachiaria*, Lavres Júnior and Monteiro (2006) showed an increase in the leaf chlorophyll content with the increase in nitrogen levels, with positive correlations between chlorophyll content and N concentration in the leaf. In a study with *B. brizantha* cv. Marandu subjected to different levels (0, 100, 200 and 300 kg ha⁻¹ year⁻¹) and sources of N (ammonium sulfate and urea), Costa et al. (2008) found that the level of 300 kg N ha⁻¹ year⁻¹ provided

the highest levels of chlorophyll in the leaf for all the years of the study, with values of 44.23; 45.03 and 46.14 SPAD units for the pastures supplied with 300 kg N ha⁻¹ year⁻¹ in the years of 2004; 2005 and 2006, respectively.

Researches evaluating the gas exchange in intensively managed forage plants with nitrogen fertilization and under grazing are essential for a better understanding of how the biomass production process occurs in the pasture. Further, they are also essential for the definition of levels of fertilization that signal the maximum biological efficiency of the forage, given the relevance of the physiological processes for the growth and consequent accumulation of forage in the pasture. However, studies of this nature are still incipient for many forage grasses. This study aimed to evaluate the gas exchange in massai grass subjected to nitrogen fertilization and grazed by sheep in rotational stocking.

CONTENTS

The experiment was conducted on pasture of *Panicum maximum* cv. Massai, belonging to the Center for Education and Studies on Forage Crops, in Fortaleza, State of Ceará. The city of Fortaleza is located at an average altitude of 21 meters, at the following geographic coordinates: 03°45'47" South latitude and 38°31'23" West longitude with Aw' climate, tropical rainy, according to Köppen climate classification. The monthly average temperature (mean maximum, average and mean minimum temperatures), rainfall and insolation for the experimental period are presented in Figure 1.

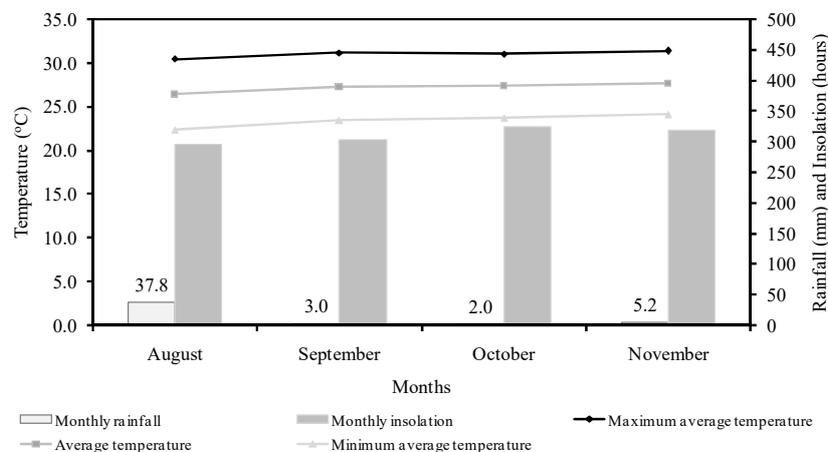


Figure 1. Climatic data referring to the experimental period, in Fortaleza, State of Ceará, in 2009.

The soil of the experimental area is classified as yellow podzolic soil, whose source

material are sandy-clayey sediments of the barrier formation (EMBRAPA, 1999). The chemical

characteristics of the soil revealed by the analysis (0 - 20 cm depth), performed at the beginning of the experiment, are listed in Table 1. From the results of the soil analysis, fertilization was carried out,

according to CFSEMG recommendation (1999), for fertility levels suggested for grasses with high productive potential and with high level of production.

Table 1. Soil chemical properties of the experimental area, at 0 to 20 cm deep, at the beginning of the experimental setup.

P	K	Ca ²⁺	Mg ²⁺	Fe ²⁺	Cu ²⁺	Zn ²⁺	Mn	pH	Al ³⁺	Na ⁺	SB	CTCt	MO
								H ₂ O	cmol _c dm ⁻³			g kg ⁻¹	
9.0	15.64	260.51	145.84	10.9	0.4	8.3	11.9	5.7	0.35	0.10	2.64	2.99	18.62

The experimental was a split plot completely randomized design with measures repeated in time. In the mentioned experiment (gas exchange), we analyzed the effect of nitrogen fertilizer (control - without nitrogen fertilization, 400, 800 and 1200 kg ha⁻¹ year⁻¹ nitrogen) during four successive grazing cycles.

Massai grass was managed under low-pressure fixed sprinkler irrigation (working pressure <2.0 kgf cm⁻²), with liquid rate of 7.0 mm day⁻¹ at irrigation schedule of 3 days and time of irrigation (Ti) of 8 hours, at night, seeking a better uniformization of the applied water volume. To determine the parameters mentioned above, the irrigation system was evaluated initially, as it would have been during the experimental period.

Phosphate (single superphosphate), potassium (potassium chloride) and micronutrients (FTE BR-12) fertilization were carried out according to the results of the soil analysis. The applications of nitrogen (urea) and potassium were splitted. The nitrogen level was divided for each treatment in two applications, the first half applied immediately after removing the animals from the paddock and the second half applied in the middle of the rest period, according to each level evaluated. In all nitrogen applications, the urea was diluted in water (with posterior irrigation), seeking a better uniformity of application, due to the small amount of fertilizer per plot, making it difficult to apply it in solid form. In the application, a backpack sprayer with spray volume standardized was used according to the field test previously performed.

Potassium was made available in three applications, the first one (160 kg ha⁻¹ K₂O) carried out at the beginning of the experiment, together with the first application of nitrogen. The second and third applications of potassium (160 and 160 kg ha⁻¹ K₂O, respectively) occurred with the first level of nitrogen shortly after removing the animals in each of the subsequent grazing cycles. Phosphorus (250 kg ha⁻¹ P₂O₅) was applied at once, together with the first applications of potassium and nitrogen, at the beginning of the experimental setup. At this time,

the micronutrients (50 kg ha⁻¹ FTE BR-12) were applied.

The rest period was approximately 1.5 new leaves per tiller, as determined in the pre-test at the beginning of the experiment, providing interval of 22; 18; 16 and 13 days for the levels 0.0 - control; 400; 800 and 1200 kg ha⁻¹ year⁻¹ nitrogen, respectively.

The animals used to lower the sward height to the recommended residual height were sheep (½ Morada Nova x ½ undefined breed), placed in paddocks of 42.3 m². The “mob-grazing” technique (GILDERSLEEVE et al., 1987) was used for grazing, using groups of animals for rapid defoliation (duration of 7 to 11 hours), simulating a rotational stocking. As the animals grazed, the height of the sward was monitored with a ruler until the canopy reached the recommended residual height of approximately 15 cm, corresponding to the residual LAI of exit of the animals from the paddock at approximately 1.5, as determined in a pre-test to set up the experiment.

For evaluation of gas exchange in the pre-grazing of the forage, an infrared CO₂ analyzer (Infrared Gas Analyzer - IRGA, LCI BioScientific) was used. In each of the experimental units (42.3 m² paddock), newly expanded leaves were selected in five tillers, and the readings were made in the middle part of the leaf, always between 9h and 11h. At the time of the readings, soil presented moisture close to the field capacity.

The variables analyzed were leaf transpiration rate (E, mmol m⁻² s⁻¹), leaf temperature (TFOL, in °C), leaf photosynthesis rate (A, μmol m⁻² s⁻¹), leaf carbon dioxide concentration (C_i, ppm), stomatal conductance (g_s, mol m⁻² s⁻¹), photosynthesis/transpiration ratio (A/E, water efficient use) and photosynthesis/conductance ratio (A/g_s, intrinsic water efficient use).

The relative chlorophyll index (RCI) was measured with a chlorophyll meter (Chlorophyll Meter SPAD-502) on the day prior to the animals entering the paddocks, always at the same time and on the same leaf of the other physiological

evaluations, and five readings were performed on the newly expanded leaf, in the morning between 9 and 11 a.m.

The nitrogen sufficiency index (NSI) was calculated by the relationship between the mean value of chlorophyllometer measurements in the leaves of the treatments (MCT) and the mean in the plants that received the highest level (MCR), considered as a reference area ($NSI = (MCT/MCR) \times 100$), because it is more likely to have no nitrogen deficiency and to provide the maximum concentration of chlorophyll in the leaves.

Data were tested by analysis of variance and regression analysis. The selection of the models was

based on the significance of the linear and quadratic coefficients, using Student's t-test ($P < 0.05$) and the coefficient of determination. As a tool to aid statistical analysis, we adopted the MIXED and GLM procedure of SAS software (SAS INSTITUTE, 2003).

There was an increasing linear response ($P < 0.05$) for the stomatal conductance (g_s) of massai grass with increasing levels of nitrogen fertilization, showing an estimate of 0,192 and 0.677 $\text{mol m}^{-2} \text{s}^{-1}$ at the levels 0 and 1200 $\text{kg ha}^{-1} \text{ year}^{-1}$, respectively (Figure 2A).

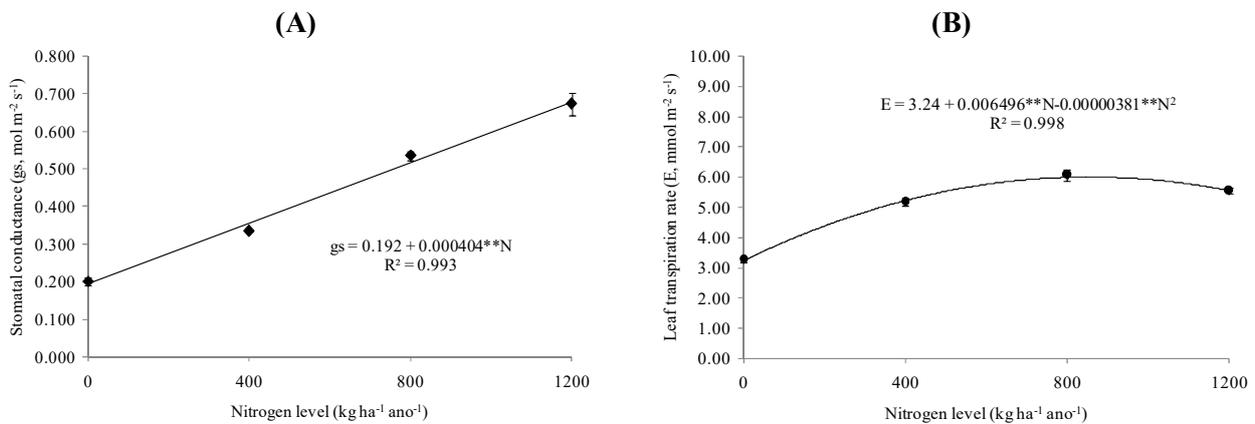


Figure 2. Stomatal conductance (A) and leaf transpiration rate (B) in Massai grass pastures according to nitrogen fertilization, grazed by sheep under rotational stocking.

N = nitrogen level; significant at 1% (**) probability.

This superiority observed in the g_s values reflects the larger stomatal opening (POMPEU et al., 2010) as a mechanism to meet CO_2 absorption from the external environment (KUWAHARA; SOUZA, 2009) and to regulate leaf temperature through transpiration (SLATYER, 1967).

The leaf transpiration rate (E) revealed a quadratic response ($P < 0.05$) as the nitrogen levels were increased, with values estimated at 3.24 and 5.55 $\text{mmol m}^{-2} \text{s}^{-1}$ at levels 0 and 1200 $\text{kg ha}^{-1} \text{ year}^{-1}$, respectively, reaching a maximum value of 6.01 $\text{mmol m}^{-2} \text{s}^{-1}$ at the nitrogen level equivalent to 852.5 $\text{kg ha}^{-1} \text{ year}^{-1}$ (Figure 2B).

The increase observed in leaf transpiration up to the maximization in the nitrogen level occurred as a function of the higher enzymatic activity, increasing the water uptake by the roots (YIN et al., 2009) and stomatal opening and, consequently, increasing the photosynthetic rate

(POMPEU et al., 2010), with a positive response on leaf biomass production.

The leaf photosynthesis rate (A) increased ($P < 0.05$) with increasing nitrogen levels, showing an estimate of 19.64 and 37.76 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at nitrogen levels of 0 and 1200 $\text{kg ha}^{-1} \text{ year}^{-1}$, respectively (Figure 3A), with a 92.3% increase for the level equivalent to 1200 $\text{kg ha}^{-1} \text{ year}^{-1}$ in relation to the absence of nitrogen fertilization.

The leaf photosynthesis rate in massai grass fertilized with increasing levels of nitrogen corroborates the behavior of this variable in studies conducted by Pompeu et al. (2010) with aruana grass, under greenhouse conditions and fertilized with nitrogen. This increase in photosynthesis ratifies the relevance of this nutrient for the growth and vigor of regrowth in intensively managed pastures.

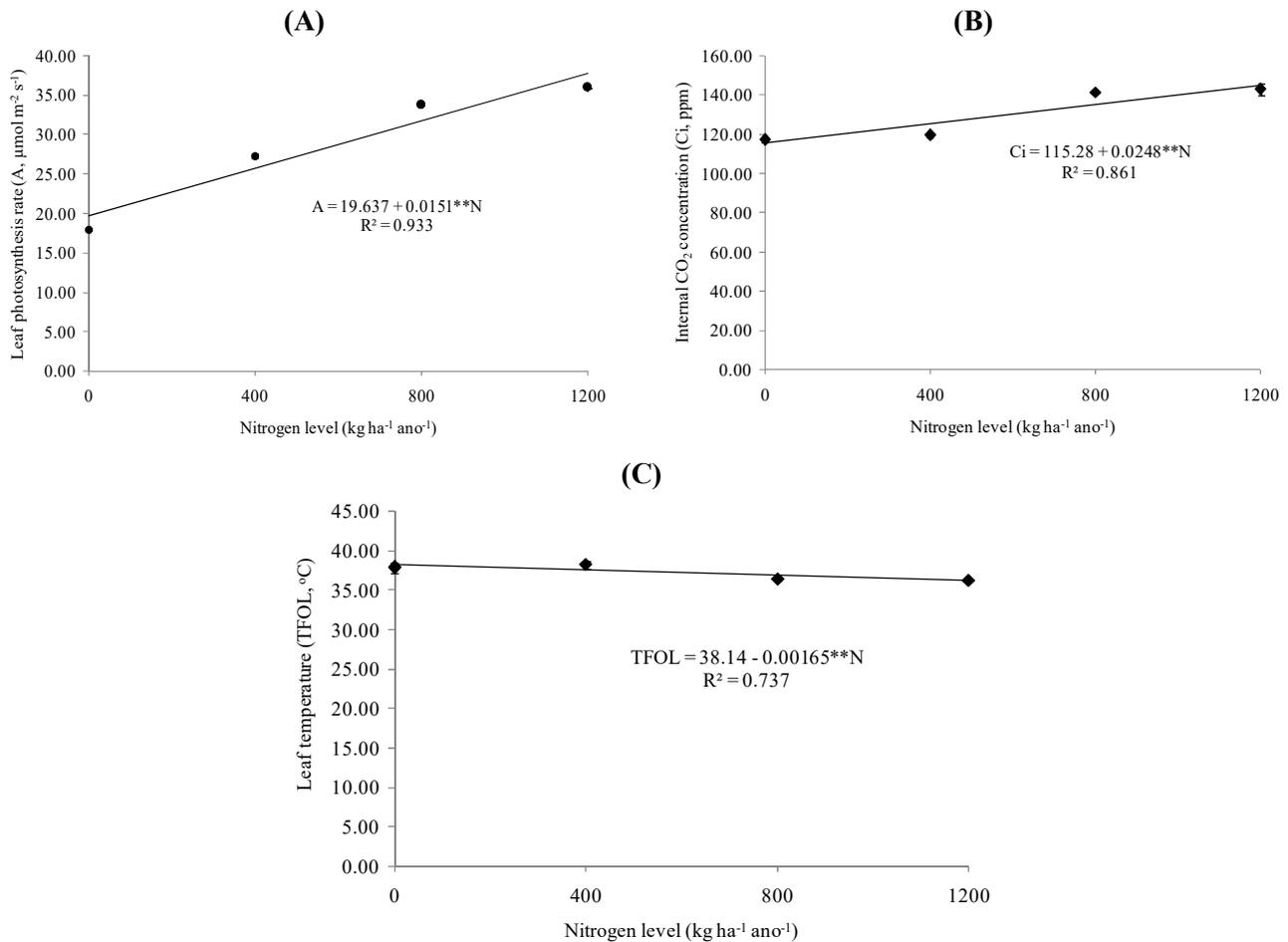


Figure 3. Leaf photosynthesis rate (A), internal CO₂ concentration (B) and leaf temperature (C) in Massai grass pastures according to nitrogen fertilization, grazed by sheep under rotational stocking. N = nitrogen level; significant at 1% (**) probability.

The positive effect of nitrogen fertilization on the photosynthetic rate is due to the greater stimulus to enzymatic activity and to the greater synthesis of the ribulose-1,5-bisphosphate-carboxylase-oxygenase enzyme (RUBISCO), responsible for photosynthesis, among others, associated with the effect on leaf transpiration, which favors photosynthesis (CABRERA-BOSQUET et al., 2009). In addition, the higher photosynthesis rate observed at higher levels of nitrogen is also a reflection of increases, both in the photochemical phase and in the biochemical phase. In the photochemical phase, there was an increase in the light harvesting apparatus, which is evidenced by the higher relative chlorophyll content in the higher nitrogen levels (Figure 9). In the biochemical phase, higher levels of nitrogen may have favored greater biosynthesis of proteins and enzymes linked to photosynthesis (TAIZ; ZEIGER, 2009). According to Bolton and Brown (1980), in the evaluation of the photosynthesis of C₃ and C₄ grasses receiving increasing levels of nitrogen,

higher levels of nitrogen fertilization reflect in a greater leaf thickness, which, in turn, may be related to higher concentration of carboxylic enzymes per unit area of leaf.

The internal CO₂ concentration (C_i) and the leaf temperature (TFOL) were influenced (P < 0.05) by nitrogen fertilization, with the C_i showing increase (P < 0.05) (Figure 3B) and the inverse occurring for TFOL (Figure 3C). The values estimated were 115.28 and 145.04 ppm (C_i) and 38.14 and 36.16°C (TFOL) at levels 0 and 1200 kg ha⁻¹ year⁻¹, respectively.

The elevation in C_i found in the present study is in agreement with that observed by Pompeu et al. (2010), who evaluated gas exchange in aruana grass under nitrogen fertilization, where the C_i was reduced with increasing levels of nitrogen, justified by the increased activity of carboxylic enzymes at higher levels of said nutrient, favoring the carboxylation of organic molecules and reducing the concentration of free carbon dioxide in the mesophyll (PAN et al., 2004). Nevertheless, such an

increase in C_i for the massai grass pastures supplied with the highest nitrogen levels can be attributed to the increase in stomatal conductance (Figure 2A), due to the fact that the greater stomatal opening favors the higher CO_2 absorption from the external environment (LAMBERS et al., 1998), demonstrating that stomatal resistance was not a limiting factor for the influx of CO_2 .

The reduction in TFOL possibly occurred in response to the behavior revealed by leaf transpiration (Figure 2B) at higher nitrogen levels, increasing the water demand by the plants (BONFIM-SILVA et al., 2007; LOPES et al.,

2011a) with higher absorption of water by the roots. This contributes to the reduction of leaf heating since transpiration is the primary mechanism for regulating leaf temperature, dissipating part of the energy from solar radiation (SLATYER, 1967; HOPKINS, 1999).

The photosynthesis/transpiration ratio (A/E), which represents the instantaneous water use efficiency and quantitatively expresses the momentary behavior of the gas exchanges in the leaf, revealed an increasing linear response ($P < 0.05$) with elevation in nitrogen fertilization (Figure 4A).

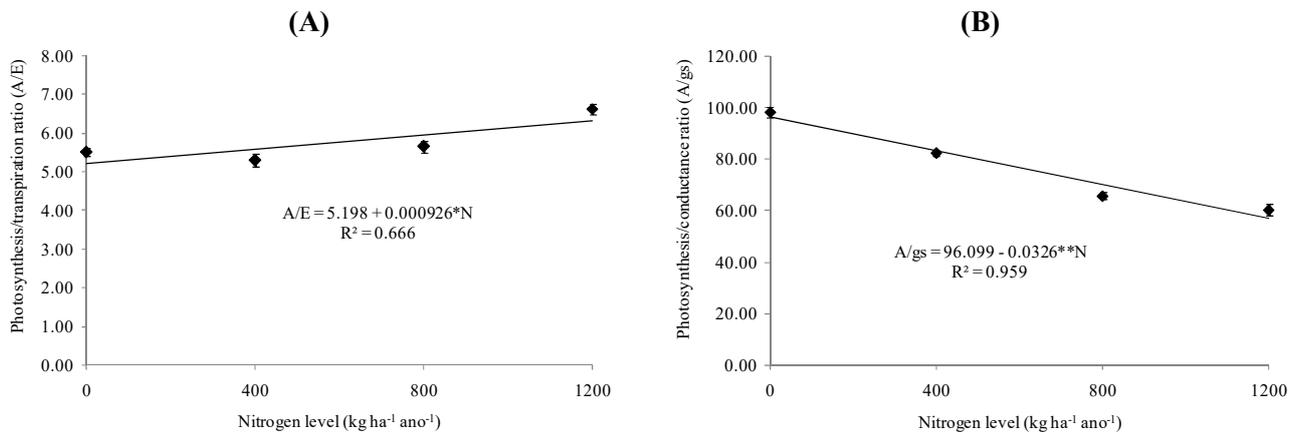


Figure 4. Photosynthesis/transpiration ratio (A) and photosynthesis/conductance ratio (B) in Massai grass pastures according to nitrogen fertilization, grazed by sheep under rotational stocking. N = nitrogen level; significant at 1% (**) and 5% (*) probability.

The A/E ratio was increased by 0.000926 for each kilogram of $N\ ha^{-1}\ year^{-1}$ added, with values for the referred ratio estimated at 5.20 and 6.31 at levels 0 and 1200 $kg\ ha^{-1}\ year^{-1}$, respectively, as a result of the greater increase in the photosynthetic rate when compared to the leaf transpiration with the elevation of the nitrogen levels, demonstrating a more efficient use of water (instantaneous EUA) in nitrogen fertilized pastures. As a result, it provides better development of the plant, with a more consolidated root system (LOPES et al., 2011), and thus, responding more efficiently to the environmental factors (fertilization, radiation, temperature, air humidity).

The photosynthesis/conductance ratio (A/g_s) showed a linear decreasing response ($P < 0.05$) to the increase in nitrogen fertilizer levels, with estimates of 96.10 and 56.98 for the mentioned ratio at levels 0 and 1200 $kg\ ha^{-1}\ year^{-1}$, respectively (Figure 4B), with a reduction of 40.7% in A/g_s for the pastures supplied with 1200 $kg\ ha^{-1}\ year^{-1}$ in relation to the absence of nitrogen fertilization.

The reduction observed in the A/g_s ratio with the increase in nitrogen levels was due to the

higher increase in g_s for each kilogram of $N\ ha^{-1}\ year^{-1}$ applied, compared to the increase in the photosynthetic rate for each unit of the fertilizer added ($kg\ N\ ha^{-1}\ year^{-1}$), that is, an increase of 0.21% in g_s was observed, against a 0.076% increase in the photosynthesis rate, when analyzing the absence of nitrogen and the application of 1 $kg\ N\ ha^{-1}\ year^{-1}$. This justifies the narrowing and consequent reduction in the A/g_s ratio with the increase in nitrogen fertilizer levels. It should be pointed out that the best ratio between CO_2 absorption and water loss is reached when there is a high number of partially closed stomata, with the A/E ratio reaching higher values and A/g_s ratio reaching lower values (LARCHER, 2006).

The relative chlorophyll index (RCI) and the nitrogen sufficiency index (NSI, evaluated from the RCI and calculated by the relationship between the measurement of the chlorophyll meter in the plants of massai grass and the plants of the reference area, which were those plants that receiving the highest level of nitrogen without presenting nitrogen deficiency; constituting a non-destructive method to predict N deficiency in the plant) responded in an

increasing linear manner ($P < 0.05$) to nitrogen levels (Figure 5A and B), with values estimated at 24.07 and 37.70 SPAD units (RCI) and 66.66 and 104.46% (NSI) for the levels 0 and 1200 kg ha^{-1} year⁻¹, respectively, accounting for increments of

56.6 and 56.7% for the RCI and NSI, respectively, at the level of nitrogen equivalent to 1200 kg ha^{-1} year⁻¹ in relation to the absence of nitrogen fertilization.

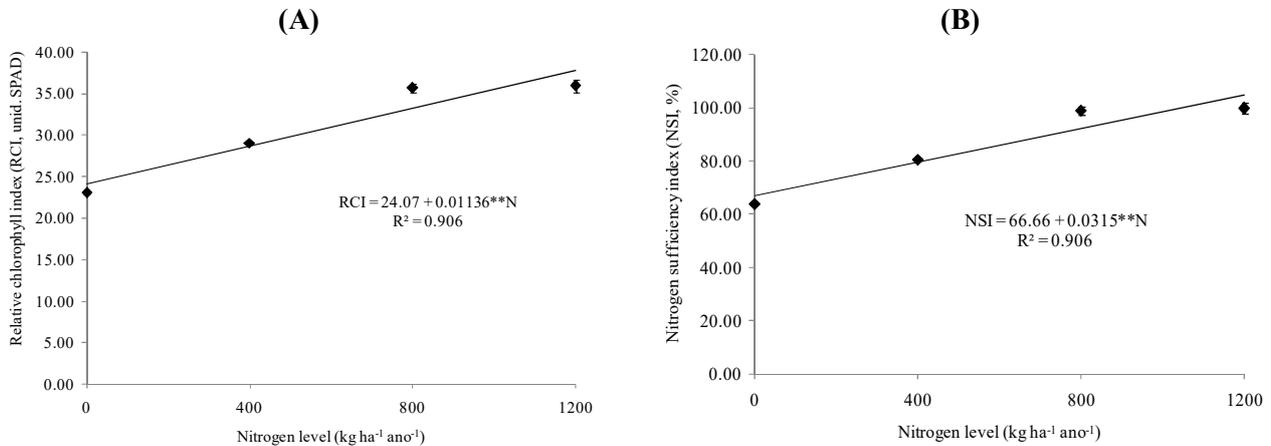


Figure 5. Relative chlorophyll index (A) and nitrogen sufficiency index (B) in Massai grass pastures according to nitrogen fertilization, grazed by sheep under rotational stocking. N = nitrogen level; significant at 1% (**) probability.

The increase observed for RCI at the highest nitrogen levels can be attributed to improved structuring of the plant with a more consolidated root system, reflecting the increase in root biomass (ALVES et al., 2008; LOPES et al., 2011), exploring a larger volume of soil and with a higher water and nutrient absorption capacity, reflecting a higher content of chlorophyll in the leaves of the massai grass, due to the better response to environmental factors, mainly nitrogen fertilization.

The increasing readings for RCI at higher nitrogen levels corroborate the pattern of response revealed by this variable in a study conducted by Alves et al. (2008), which showed higher content of this pigment in the leaves of this grass, justifying the use of devices with this purpose as a relevant tool to predict the initial appearance of nitrogen deficiency.

It is also emphasized that more chlorophyll is synthesized with the increased availability of nitrogen to the plant, resulting in an increase in leaf green intensity. However, such elevation in chlorophyll content reaches a plateau called the photosynthetic maturity, which remains unchanged, even with increased nitrogen content in the plant tissue (COSTA et al., 2001). This was not verified in this study, since the chlorophyll content responded linearly to the nitrogen fertilization up to the maximum level examined (equivalent to 1200

kg ha^{-1} year⁻¹ nitrogen), that is, no stabilization was observed in the values of chlorophyll with the highest levels of nitrogen fertilization, thus demonstrating the positive genetics of the forage studied in relation to chlorophyll synthesis from the availability of nitrogen, due to the fact that it only reached the photosynthetic maturity (COSTA et al., 2001) at high levels of nitrogen fertilizer.

The superiority found for the NSI in pastures fertilized with the highest nitrogen levels in relation to the pastures not supplied with this nutrient makes evident the difference in the index between both managements of massai grass (with and without nitrogen fertilization). Therefore, it indicates the deficiency of this nutrient in the absence of fertilization and the adequate supply at higher levels of nitrogen.

CONCLUSION

Nitrogen fertilization is a relevant practice for improving photosynthesis and favors the other variables of gas exchange in massai grass up to the last level tested. It is important to emphasize the importance of carrying out additional studies regarding the economic and environmental aspects of intensive production systems.

RESUMO: O conhecimento das trocas gasosas em plantas forrageiras é essencial para melhor entendimento de como ocorre o processo de produção de biomassa de forragem na pastagem. Objetivou-se avaliar as trocas gasosas no capim-massai submetido a crescentes doses de nitrogênio (controle - sem nitrogênio; 400; 800 e 1200 kg ha⁻¹ ano⁻¹) e sob lotação rotativa com ovinos, num delineamento inteiramente casualizado com medidas repetidas no tempo. O período de descanso adotado foi de aproximadamente 1,5 novas folhas por perfilho, conforme determinação em pré-ensaio quando do início da instalação do experimento, propiciando um intervalo de 22; 18; 16 e 13 dias para as doses 0,0 – controle; 400; 800 e 1200 kg ha⁻¹ ano⁻¹ de nitrogênio, respectivamente. Os animais utilizados para rebaixamento do pasto até a altura residual preconizada foram ovinos (½ Morada Nova x ½ SPRD), alocados em piquetes de 42,3 m². À medida que os animais pastejavam, a altura do pasto foi monitorada com auxílio de uma régua, até que o dossel atingisse a altura residual preconizada de aproximadamente 15 cm, correspondendo ao IAF residual de saída dos animais do piquete de aproximadamente 1,5, conforme determinação em pré-ensaio para instalação do experimento. As variáveis: condutância estomática, taxa de fotossíntese foliar, concentração interna de CO₂, relação fotossíntese/transpiração, índice relativo de clorofila e índice de suficiência de nitrogênio responderam de forma linear crescente ao incremento nas doses de nitrogênio. Verificou-se aumento de 92,3% na taxa de fotossíntese para a dose de N de 1200 kg ha⁻¹ ano⁻¹ em relação à ausência de nitrogênio. A temperatura da folha e a relação fotossíntese/condutância foram reduzidas com o aumento das doses de nitrogênio. A adubação nitrogenada proporcionou resposta quadrática com ponto de máximo sobre a taxa de transpiração foliar. A adubação nitrogenada favorece as trocas gasosas em capim-massai até a última dose estudada.

PALAVRAS-CHAVE: Adubação nitrogenada. Índice relativo de clorofila. Fotossíntese foliar. *Panicum maximum*.

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