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Universidade Estadual de Londrina  
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de Souza, Igor Alexandre; Guimarães Ribeiro, Karina; Rocha, Wellington Willian; do  
Carmo Araújo, Saulo Alberto; Gomes Pereira, Odilon; Cecon, Paulo Roberto  
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## Forage mass, chemical composition and leaf chlorophyll index of signal grass and organic matter in soil under increasing levels of nitrogen

## Massa de forragem, composição química e índice de clorofila foliar do capim-braquiária e matéria orgânica no solo sob doses crescentes de nitrogênio

Igor Alexandre de Souza<sup>1\*</sup>; Karina Guimarães Ribeiro<sup>2</sup>; Wellington Willian Rocha<sup>3</sup>; Saulo Alberto do Carmo Araújo<sup>3</sup>; Odilon Gomes Pereira<sup>2</sup>; Paulo Roberto Cecon<sup>4</sup>

### Abstract

This study evaluated the forage mass, plant height, chemical composition and chlorophyll index of signal grass (*Brachiaria decumbens* cv. Basilisk), as well as the content of organic matter (OM) in soil under increasing levels of nitrogen. The experiment was conducted in the Rio Manso Farm, experimental unit of the Federal University of the Jequitinhonha and Mucuri Valleys - UFVJM in the municipality of Couto de Magalhães de Minas, state of Minas Gerais. For plant evaluation, we use a split plot randomized block design with four replications. Nitrogen levels (0, 25, 50, 75, 100 kg ha<sup>-1</sup> N) were assigned to plots and the cuts made in plants, to subplots. For the assessment of soil organic matter, we used a split-split-plot, with nitrogen levels (0, 25, 50, 75 and 100 kg ha<sup>-1</sup> by cut) in the plots, collection depth (0-10 and 10-20 cm) in the subplots and three sampling times (before the onset of the experiment, at the end of the 1<sup>st</sup> crop year and at the end of the 2<sup>nd</sup> crop year) in sub-subplots in a randomized block design with four replications. It can be concluded that nitrogen fertilization linearly increases forage mass, plant height and crude protein content of signal grass, which are positively correlated with chlorophyll index. At the end of two agricultural years, the content of organic matter in the soil decreases, which can be attributed to management of cutting and removal of the cut material.

**Key words:** Height. *Brachiaria decumbens*. Neutral detergent fiber. Crude protein.

### Resumo

Objetivaram-se avaliar a massa de forragem, a altura de plantas, a composição química e o índice de clorofila do capim-braquiária (*Brachiaria decumbens* cv. Basilisk), assim como, o teor de matéria orgânica (MO) no solo sob níveis crescentes de nitrogênio. O experimento foi conduzido na Fazenda Rio Manso, unidade experimental da Universidade Federal dos Vales do Jequitinhonha e Mucuri/UFVJM, na cidade de Couto de Magalhães de Minas, em Minas Gerais. Para avaliação da planta, utilizou-se o esquema de parcelas subdivididas no delineamento em blocos casualizados, com quatro repetições.

<sup>1</sup> Zootecnista, Discente de Doutorado, Universidade Estadual do Sudoeste da Bahia, UESB, Itapetinga, BA, Brasil. E-mail: igordadim@hotmail.com

<sup>2</sup> Profs., Departamento de Zootecnia, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brasil. E-mail: karinaribeiro@ufv.br; odilon@ufv.br

<sup>3</sup> Profs., Departamento de Agronomia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, UFVJM, Diamantina, MG, Brasil. E-mail: wwillian@ufvjm.edu.br; sauloaraujo.ufvjm@gmail.com

<sup>4</sup> Prof., Departamento de Informática, UFV, Viçosa, MG, Brasil. E-mail: cecon@ufv.br

\* Author for correspondence

Nas parcelas distribuíram-se as doses de nitrogênio (0; 25; 50; 75; 100 kg ha<sup>-1</sup> de N) e nas subparcelas os cortes efetuados nas plantas. Para a avaliação da matéria orgânica do solo, utilizou-se o esquema de parcelas subsubdivididas, com as doses de nitrogênio (0; 25; 50; 75 e 100 kg ha<sup>-1</sup> por corte) nas parcelas, profundidade de coleta (0-10 e 10-20 cm) nas subparcelas e os três momentos de amostragem (antes do início do experimento, ao final do 1º ano agrícola e ao final do 2º ano agrícola) nas subsubparcelas, no delineamento em blocos casualizados, com quatro repetições. Com base nos resultados obtidos, conclui-se que a aplicação de nitrogênio aumenta linearmente a massa de forragem, a altura de plantas e o teor de proteína bruta do capim-braquiária, os quais apresentam correlação positiva com o índice de clorofila. Ao final de dois anos agrícolas, o teor de matéria orgânica no solo decresce, o que pode ser atribuído ao manejo aplicado, de corte e remoção do material cortado.

**Palavras-chave:** Altura. *Brachiaria decumbens*. Fibra em detergente neutro. Proteína bruta.

## Introduction

Nutrient availability has a strong influence on plant nutrition, which is reflected in plant development and physiological recovery (BONFIM-SILVA; MONTEIRO, 2010). Among the nutrients, nitrogen is the main limiting macronutrient for pasture productivity, as it actively participates in the synthesis of organic compounds that form the structure of the plant, such as amines, amides, vitamins, pigments, amino acids, proteins, nucleic acids (MALAVOLTA, 1980).

The strategic use of nitrogen fertilizer can improve the accumulation of forage, especially in the period of increased rainfall, since the nitrogen increases the growth rate of grasses (TEIXEIRA et al., 2011). Nitrogen also has a strong influence on the nutritional value of forage, and hence on stocking rate and weight gain per animal and per hectare (VITOR et al., 2009).

Monitoring the nitrogen content of the forage plant is very important to determine the crude protein content. The relative chlorophyll index in leaves is a parameter that can be used to assess the nutritional level of nitrogen in plants, since the amount of such pigment positively correlates with the amount of this element in the plant (ARGENTA et al., 2001). In addition, nitrogen interferes directly with the photosynthetic process, given its participation in the chlorophyll molecule (MACEDO et al., 2012), increasing the photosynthetic efficiency and forage mass production.

It has been proposed the use of portable gauges, called chlorophyll meter, allowing instant readings in a non-destructive way. This reading is directly related to evaluation of nitrogen nutrition, since there is a positive correlation between the reading of leaf chlorophyll index and nitrogen concentration in grass leaves (ZOTARELLI et al., 2003).

Another factor of great importance in the study of productivity and recovery of pastures in Brazil is the content of organic matter in the soil. The stock of organic matter is a combination of processes related to the addition and loss of organic material. The main process of adding organic matter to the soil has the plant as active component, while the main process of organic material loss is the mineralization promoted by fungi and bacteria (DICK et al., 2009).

The accumulation of organic matter from appropriate soil management and crop residues is usually associated with an improvement of soil physical conditions (CAMPOS et al., 1995; ARVIDSSON, 1998). Organic matter can bring several benefits to soil structure, improving water penetration rate, lowering the pressure applied by animals, increasing porosity, aeration and water retention in the soil (BAUMGARTL; HORN, 1991).

Given the above, this study aimed to evaluate the forage mass, plant height, leaf chlorophyll index and chemical composition of a pasture of signal grass cv. Basilisk, as well as the content of organic matter in soil under increasing levels of nitrogen.

## Material and Methods

The experiment was conducted at Rio Manso farm, experimental unit of the Federal University of the Jequitinhonha and Mucuri Valleys - UFVJM, in the municipality of Couto de Magalhães de Minas,

state of Minas Gerais, located at 18° 4' 25" S and 43° 28' 16" W, 756 meters altitude. The climate is humid subtropical, Cwa, according to the climatic classification of Köppen. The annual average temperature is 23.8°C and the average rainfall is 1.404 mm/year (Table 1).

**Table 1.** Data of cumulative rainfall and temperature in the experimental period.

Cut	Regrowth period	Cumulative rainfall (mm)	Average maximum temperature (°C)	Average minimum temperature (°C)
1 <sup>st</sup>	Dec 13 <sup>rd</sup> , 2010 to Jan 23 <sup>rd</sup> , 2011	326.7	24.5	16.0
2 <sup>nd</sup>	Jan 24 <sup>th</sup> to March 13 <sup>rd</sup> , 2011	350.2	26.8	16.9
3 <sup>rd</sup>	Dec 09 <sup>th</sup> , 2011 to Jan 16 <sup>th</sup> , 2012	532.1	23.6	16.2
4 <sup>th</sup>	Jan 17 <sup>th</sup> to Feb 27 <sup>th</sup> , 2012	99.0	25.7	16.0

**Source:** National Institute of Meteorology INMET, Diamantina, state of Minas Gerais.

Physical and chemical analyses of soil were performed in the layers of 0-10 and 10-20 cm for due characterization (Table 2). The soil was classified as Dystrophic Red-Yellow Latosol, clayey, deep, with

little differentiation between horizons, for being quite weathered, with low natural fertility, gently undulating relief, with slope between 3 and 6% (EMBRAPA, 2006).

**Table 2.** Chemical and physical characteristics of soil samples taken at 0-10 and 10-20 cm depth layers, Laboratory of Soil fertility and Physics/ UFVJM.

Depth	pH	H+Al	Al	Ca	Mg	K	P	OM	V	M	SB	t	T
- cm -		-----	cmolc dm <sup>-3</sup>	-----		mg dm <sup>-3</sup>		Dag kg <sup>-1</sup>	-- % --		----	cmolc dm <sup>-3</sup>	----
0 - 10	5.4	7.3	0.5	1.34	0.48	49	1.63	1.9	21	20	1.95	2.45	9.25
10 - 20	5.5	6.5	0.4	1.02	0.60	30	1.03	1.3	21	19	1.7	2.10	8.2
Depth	Sand	Silt	Clay										
- cm -	-----	%	-----										
0 - 10	43	15	42										
10 - 20	43	13	44										

pH in water - Ratio 1: 2.5; H + Al - Calcium Acetate extractor 0.5 mol/L; Al, Ca and Mg - Extractor KCl 1 mol/L; K and P - Extrator Extractor -1; OM. - Organic matter = C. org x 1.724; V - Base saturation; m Aluminum saturation; SB sum of bases; t effective cation exchange capacity; T cation exchange capacity at pH 7.0.

The experiment was established on degraded pasture (*Brachiaria decumbens* cv. Basilisk), formed about ten years ago and that had low forage mass production and considerable weed infestation. The pasture over that time was managed without

grazing pressure control and no fertilization.

In a representative area of the pasture, we delimited four blocks spaced 2 meters apart, and each block was divided into five plots, totaling 20 experimental units. The whole area was fenced

to prevent invasion by animals grazing in the surroundings. Weed control was carried out by manual weeding.

For the variables related to the plant, data were analyzed in a split-plot arrangement, including 1/3 of total nitrogen levels (0; 25; 50; 75 and 100 kg ha<sup>-1</sup> per cut) in the plots and cuts in subplots in time, in a randomized block design with four replications. Four cuts were used to evaluate the forage mass (FM), content of crude protein (CP) and neutral detergent fiber (NDF); three cuts to evaluate the plant height and two cuts to evaluate the leaf chlorophyll content.

On December 13<sup>th</sup>, 2010, in the first agricultural year, it was held the uniformity cut of plants in all plots to the height of 10 cm from ground level, with a motorized backpack brush cutter. The cut material was removed from the area with the aid of a rake, and then fertilization was carried out with 100 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 75 kg ha<sup>-1</sup> K<sub>2</sub>O, in all plots, based on the chemical analysis of the soil along with 1/3 of total nitrogen levels according to the treatments (0; 25; 50; 75 and 100 kg ha<sup>-1</sup> per cut), using single superphosphate, potassium chloride and ammonium sulfate, respectively.

On January 24<sup>th</sup>, 2011, the first cut was done in plants and reapplied the levels of nitrogen, along with 75 kg ha<sup>-1</sup> K<sub>2</sub>O; on March 14<sup>th</sup>, 2011, the second cut was made in plants and reapplied nitrogen and potassium, and, on April 30<sup>th</sup>, 2014, the third cut was done in plants without fertilization. After all the cuts, the material was removed from the plots. In this year, samples of the third cut were not evaluated.

On October 31<sup>st</sup>, 2011, the second agricultural year, the uniformity cut was performed in plants of all plots as in the first agricultural year. The first cut was made on December 08<sup>th</sup>, 2011 the second, on January 16<sup>th</sup>, 2012 and the third, on February 27<sup>th</sup>, 2012. Fertilization management was the same as in the first year. After all the cuts, the material was removed from the plots. In this year, samples of the first cut were not evaluated.

For forage sampling, we used a frame made of PVC pipes with an area of 1.0 m<sup>2</sup>, placed at the center of each plot, in which, plants were manually cut with a cleaver, at a height of 10 cm from ground level. Before the cuts, heights of the plants were recorded in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> cuts, and chlorophyll index was measured in plant leaves, in the 3<sup>rd</sup> and 4<sup>th</sup> cuts.

For height measurement, we used a metal measuring tape to take three measurements from the base to the horizon of curvature of the leaves in each plot. To estimate the chlorophyll index, we used a portable chlorophyll meter, model ClorofiLOG<sup>®</sup> CFL 1030, operated according to manufacturer's instructions (FALKER, 2008). Total chlorophyll (a + b) indexes were determined in the middle third of fully expanded leaves, taking three measurements on each leaf, to obtain an average value. These measurements were performed in 10 leaves/plot at random, calculating the averages. In this equipment, the measurement units are called Falker Chlorophyll Index (FCI).

For measuring the forage mass, samples were placed in bags and sent to the Forage Laboratory of the Federal University of the Jequitinhonha e Mucuri Valleys - UFVJM, where they were weighed. Then, representative forage samples were taken from each treatment, weighed and oven-dried under forced air circulation at 55°C to constant weight, and then weighed and ground in a Wiley mill with a sieve of 1 mm, and stored in sealed plastic containers, for analysis of chemical composition. Next, sub-samples were taken for analysis of dry matter (DM), crude protein (CP) and neutral detergent fiber (NDF), following the methodology described by Detmann et al. (2012).

For the assessment of soil organic matter, we used a split split plot arrangement, with nitrogen levels (0; 25; 50; 75 and 100 kg ha<sup>-1</sup> per cut) assigned to plots, depth layers (0- 10 and 10-20 cm) to subplots and the three sample times, before the onset of the experiment (Dec10<sup>th</sup>, 2010), at the end of the 1<sup>st</sup>

year (May 14<sup>th</sup> 2011) and at the end of the 2<sup>nd</sup> year (March 03<sup>rd</sup> 2012) to sub-subplots, in a randomized block design with four replications.

Samples for OM analysis were collected with the aid of a cutting spade, two samples in each of 20 plots, one at a depth of 0-10 cm and another at 10-20 cm, totaling 40 samples per collection. Samples were analyzed by the colorimetric method described by RAIJ et al. (2001).

Data were subjected to analysis of variance and regression analysis. For qualitative variables, we used the Tukey's test and F-test and for quantitative variables, we applied regression analysis, at 5% probability, using the statistical software SAEG 9.1 (SAEG-UFV, 2007).

## Results and Discussion

There was no effect of the interaction between nitrogen levels and cuts for forage mass (FM), but rather independent effects of cuts and nitrogen levels for this variable (Tables 3 and 4). There

was an increase of 60% in forage mass produced in the last cut compared to the first cut. The FM of signal grass also responded positively and linearly to levels of nitrogen fertilizer, ranging from 1,394 to 2,599 kg ha<sup>-1</sup> cut at the levels of 0 and 100 kg ha<sup>-1</sup> per cut, respectively. Similarly, Viana et al. (2011) investigated the influence of 0, 100, 200 and 300 kg ha<sup>-1</sup> nitrogen in the rainy season on signal grass pasture (*Brachiaria decumbens* cv. Basilisk) and reported 56% increase in forage mass with the maximum level used.

For plant height, no effect of the interaction between nitrogen levels and cuts was detected, but rather independent effects of cuts and nitrogen. Table 3 shows that in the third and fourth cuts, in the second year of the study, plant height exceeded the average height recommended for 95% radiation interception, which is 25 cm, according to Silva et al. (2008), which can be attributed to residual effect of nitrogen and greater rainfall recorded (Table 1), especially in the third cut, because the plants were harvested at the same age.

**Table 3.** Forage mass (FM), plant height and dry matter content (DM) of *Brachiaria decumbens* cv. Basilisk, per cut (average of five nitrogen levels).

	Cut/Year			
	1 <sup>st</sup> /2011	2 <sup>nd</sup> /2011	3 <sup>rd</sup> /2012	4 <sup>th</sup> /2012
FM (kg ha <sup>-1</sup> )	1789.14c	940.68d	2382.13b	2875.67a
Height (cm)	NA	21.41c	33.46a	28.76b
DM (%)	24.40a	17.39c	20.10b	24.13a

NA = non-evaluated; cut 1 = Jan 24<sup>th</sup> 2011; cut 2 = March 14<sup>th</sup> 2011; cut 3 = Jan 16<sup>th</sup> 2012 and cut 4 = March 21<sup>st</sup> 2012. Means followed by different letters in the same row are significantly different by Tukey's test at 1% probability.

With the increase of nitrogen levels, plant height varied between 22.1 cm, in the absence of nitrogen fertilizer, and 33.67 cm in plants that received the highest level of nitrogen (100 kg ha<sup>-1</sup> per cut) (Table 4). These results are consistent with increased forage mass as a function of nitrogen levels.

In an experiment evaluating height of signal grass plants (*Brachiaria decumbens* cv Basilisk.) in three periods of deferment (73, 95 and 116 days) and four nitrogen levels (0, 40, 80 and 120 kg ha<sup>-1</sup>), Santos et al. (2009) observed height values ranging from 23 to 73 cm, for the shorter period, in the absence of nitrogen, and the longer period, with the highest level of nitrogen, respectively.



**Table 4.** Forage mass (FM), plant height and leaf chlorophyll index of *Brachiaria decumbens* cv. Basilisk fertilized with increasing levels of nitrogen (0; 25; 50; 75 and 100 kg ha<sup>-1</sup> per cut), respective regression equations, coefficients of determination (r<sup>2</sup>) and coefficients of variation (CV).

N (kg ha <sup>-1</sup> )					r <sup>2</sup>	CV (%)
0	25	50	75	100		
FM (kg ha <sup>-1</sup> per cut)						
1.297.0	1.721.3	2.141.8	2.319.2	2.504.9		
$\hat{Y} = 1394.17 + 12.0547^{**}N$					0.96	26.4
Plant height (cm)						
20.89	24.62	30.32	31.89	35.20		
$\hat{Y} = 22.1047 + 0.115507^{**}N$					0.89	20.62
Leaf chlorophyll index						
35.26	37.65	40.60	42.7	49.1		
$\hat{Y} = 34.5125 + 0.13101^{**}N$					0.95	6.28
DM (%)						
23.29	22.09	21.43	20.34	20.38		
$\hat{Y} = 23.015 - 0.0301993^{**}N$					0.93	4.77

Chlorophyll index values (FCI) were not affected by the interaction between nitrogen levels and cuts, as well as there was no independent effect of cuts, only nitrogen levels (Table 4). Thus, FCI varied from 34.51 to 47.61, increasing linearly and positively with the levels of nitrogen, from 0 to 100 kg ha<sup>-1</sup> per cut, respectively. Reading the chlorophyll index can optimize the management of pastures, allowing an instant estimate of crude protein content, and indicates the nutritional status of forage grasses. Silveira (2009) employed equipment similar to that used herein and noticed an FCI values range of 27-44, using nitrogen levels of 0-100 kg ha<sup>-1</sup> per cut, in *Brachiaria decumbens*.

The dry matter content of plants was not influenced by the interaction between nitrogen levels and cuts, but was affected by nitrogen levels and the cuts separately (Tables 3 and 4). In the analysis of DM per cut, DM content values were higher in the 1<sup>st</sup> and 4<sup>th</sup> cuts, close to 24%, while in the 2<sup>nd</sup> cut, we observed the lowest DM content (17.4%). The dry matter content ranged from 23 to

20% DM, with levels varying between 0 and 100 kg ha<sup>-1</sup> per cut, respectively, consistent with increasing levels of nitrogen fertilizer.

There was a significant interaction between nitrogen levels and cuts for crude protein, whose results are listed in Table 5. In general, the crude protein content increased linearly in response to increasing levels of nitrogen, ranging from 5.74% (4<sup>th</sup> cut) to 16.59% (2<sup>nd</sup> cut), respectively, with increasing levels of nitrogen from 0 to 100 kg ha<sup>-1</sup> per cut. There were much higher values of CP in the 2<sup>nd</sup> cut than in the other cuts, which can be attributed to lower forage mass (940.680 kg ha<sup>-1</sup>), due to a water stress for 29 days, which contributed to increased crude protein content. In the 4<sup>th</sup> cut, the crude protein content of unfertilized plants was quite below the lower limit of 7%, recommended by the NRC (1996), to meet the minimum requirements of rumen microorganisms, indicating worse chemical composition in plants not fertilized with nitrogen.

**Table 5.** Crude protein content of *Brachiaria decumbens* cv. Basilisk fertilized with increasing nitrogen levels (0; 25; 50; 75 and 100 kg ha<sup>-1</sup>), in four cuts, respective regression equations and coefficients of determination (r<sup>2</sup>) (Coefficient of variation = 7.45%).

Cut/Year	N (kg ha <sup>-1</sup> )					r <sup>2</sup>
	0	25	50	75	100	
Crude protein (% DM)						
1 <sup>st</sup> /2011	7.88	8.07	8.72	9.39	10.48	0.94
$\hat{Y} = 7.60962 + 0.0259958^{**}N$						
2 <sup>nd</sup> /2011	11.25	12.06	14.26	15.33	16.43	0.98
$\hat{Y} = 11.1349 + 0.05461^{**}N$						
3 <sup>rd</sup> /2012	9.47	8.06	9.46	10.40	10.93	0.58
$\hat{Y} = 8.6102 + 0.02107^{**}N$						
4 <sup>th</sup> /2012	6.34	6.13	8.66	9.89	11.25	0.93
$\hat{Y} = 5.74134 + 0.0542711^{**}N$						

\*\*1% probability level.

Regarding the neutral detergent fiber content (NDF), there was effect of the interaction between nitrogen and cuts, however, the data obtained in the 1<sup>st</sup>, 3<sup>rd</sup> and 4<sup>th</sup> cuts did not fit to any regression equation to explain the biological phenomenon, whose mean values of treatments were 70.7; 71.2 and 71.7%, respectively (Table 6). In the 2<sup>nd</sup> cut,

NDF values were estimated as varying from 62.8 and 59.5% in the absence of nitrogen and the maximum level, respectively. These values are relatively lower than those obtained in other cuts, which can be explained by climatic limitation during regrowth of plants, which grew more slowly and have not reached physiological maturity.

**Table 6.** Neutral detergent fiber content of *Brachiaria decumbens* cv. Basilisk fertilized with increasing nitrogen levels (0; 25; 50; 75 and 100 kg ha<sup>-1</sup>), in four cuts, regression equation and coefficient of determination (r<sup>2</sup>) (coefficient of variation = 2.25%).

Cut	N (kg ha <sup>-1</sup> )					r <sup>2</sup>
	0	25	50	75	100	
Neutral detergent fiber (% DM)						
1°/2011	70.99	70.39	70.86	70.96	70.52	
Mean = 70.7%						-
2°/2011	63.10	62.24	59.98	60.68	59.80	
Ŷ = 62.7924 - 0.0326289**N						0.79
3°/2012	70.38	69.55	72.01	73.87	70.06	
Mean = 71.2%						-
4°/2012	71.53	71.33	72.46	69.28	74.09	
Mean = 71.7%						-

\*\*1% probability level. OBS.: Equations obtained for cuts 1, 3 and 4 showed no good fit to the data, choosing the mean of treatments

Correlations were tested between all the variables studied and chlorophyll index. Table 7 shows significant correlations between the Falker chlorophyll index and forage mass (r = 0.54), plant



height ( $r = 0.66$ ) and crude protein content ( $r = 0.63$ ), which is explained by the close relationship of chlorophyll content, and hence, the photosynthetic activity, with the nitrogen content in leaf biomass (BLACKBURN, 2007).

**Table 7.** Correlation between leaf Falker Chlorophyll Index (FCI) and forage mass (FM), plant height and crude protein content (CP) in signal grass (*Brachiaria decumbens*).

	FCI x FM	FCI x height	FCI x CP
Correlation	0.5411	0.6613	0.6392

The linear increase in protein content with increasing nitrogen levels, in all cuts, supports the estimation analysis of chlorophyll index through portable equipment. Maranhão et al. (2009) observed linear correlation ( $r = 0.96$ ) between the chlorophyll values and crude protein content in two cultivars of signal grass (*Brachiaria brizantha* cv. Marandu and *B. decumbens* cv. Basilisk), fertilized with annual levels of 0; 50; 100 and 150 kg ha<sup>-1</sup> N

per cut. Moreover, Costa et al. (2012) found that levels of 0, 50, 100, 150 and 200 kg ha<sup>-1</sup> N applied to *Brachiaria brizantha* cv. Xaraés and *Brachiaria ruziziensis* influenced the FCI values, which were positively correlated ( $r = 0.75$ ) with crude protein content. Silva et al. (2011) found that chlorophyll index values were correlated ( $r = 0.95$ ) with the total chlorophyll content extracted from leaf blades of forages, and chlorophyll index close to 30 were related to protein content  $\leq 7\%$ . In this sense, *Brachiaria* showed chlorophyll content above the critical, regardless of the level of nitrogen fertilizer.

The levels of organic matter (OM) in the soil were influenced by the three factors studied (Tables 8 and 9). In general, OM content between depths did not change or was higher at smaller depth (0-10 cm). At the end of the second year, the organic matter content was reduced in both depths and for all nitrogen fertilizer levels. As the grazing was stopped in the study area and that all the cut material was removed from the area, litter accumulation on the soil was reduced, which consequently may have contributed to the decrease in soil organic matter.

**Table 8.** Organic matter content (dag kg<sup>-1</sup>) in five nitrogen levels (0; 25; 50; 75 and 100 kg ha<sup>-1</sup> per cut), at two soil depths P1 (0-10 cm) and P2 (10-20 cm) and in three sampling times.

Times	N 0 kg ha <sup>-1</sup> cut		N 25 kg ha <sup>-1</sup> cut		N 50 kg ha <sup>-1</sup> cut	
	P1	P2	P1	P2	P1	P2
M1	2.2Aa	1.8Aa	2.57Aa	1.67Ab	2.62Aa	1.65Ab
M2	2.3Aa	1.32Ab	1.82Ba	1.52Aa	1.55Ba	1.62Aa
M3	0.72Ba	0.55Ba	0.85Ca	0.60Ba	0.95Ca	0.75Ba
	N 75 kg ha <sup>-1</sup> cut		N 100 kg ha <sup>-1</sup> cut			
	P1	P2	P1	P2		
M1	2.10Aa	1.67Aa	2.45Aa	1.82Aa		
M2	1.75Aa	1.32Aa	2.15Aa	1.35ABb		
M3	0.97Ba	0.62Ba	1.07Ba	0.92Ba		

Different lower case letters in the same row indicate significant differences by F-test and different upper case letters in the same column indicate significant differences by Tukey's test at 5% probability level.

N = nitrogen; M1= time of collection before treatments; M2= time of collection at the end of the first year; M3= time of collection at the end of the second year.

In Table 9, the behavior of OM content according to nitrogen levels was not consistent. In the depth of 0-10 cm, the OM content presented a quadratic effect and reached a minimum of 1.60 dag kg<sup>-1</sup> soil at a level of 50 kg ha<sup>-1</sup> N per cut, at the end of the first year (M2), while at the end of the second year (M3) it showed a positive linear effect and the values varied between 0.75 and 1.08 dag kg<sup>-1</sup> soil at

levels from 0 to 100 kg ha<sup>-1</sup> N per cut, respectively. In the 10-20 cm depth, we found a square root fit at the end of the first year (M2) and the minimum content of OM was 1.31 kg dag<sup>-1</sup> soil with 100 kg ha<sup>-1</sup> N per cut; at the end of the second year (M3), we observed a positive linear effect, ranging from 0.75 to 1.08 dag kg<sup>-1</sup> soil for levels of 0-100 kg ha<sup>-1</sup> N per cut.

**Table 9.** Regression equations of organic matter (dag kg<sup>-1</sup>) according to five levels of nitrogen (0; 25; 50; 75 and 100 kg ha<sup>-1</sup> cut), at two soil depth layers (0-10 cm and 10-20 cm) and in three sampling times.

Time	Equations fit	r <sup>2</sup> /R <sup>2</sup>
Depth = 0-10 cm		
M1	$Y = 2.17571 + 0.0416429N - 0.0010314N^2 + 0.0000064N^3$	0.81
M2	$Y = 2.30786 - 0.0269286N + 0.000254286N^2$	0.99
M3	$Y = 0.75 + 0.0033N$	0.96
Depth = 10-20 cm		
M1	$Y = 1.80071 - 0.00665714N + 0.0000685714N^2$	0.99
M2	$Y = 1.32249 + 0.0972343N^{1/2} - 0.00980236N$	0.67
M3	$Y = 0.535 + 0.0031N$	0.66

N = nitrogen; M1=time of collection before treatments; M2= time of collection at the end of the first year; M3= time of collection at the end of the second year.

## Conclusions

Nitrogen fertilization linearly increases the forage mass, plant height, dry matter content and chlorophyll index of signal grass. Leaf chlorophyll index is positively correlated with forage mass, plant height and crude protein content. At the end of two growing seasons, the content of organic matter in the soil decreases, which can be attributed to management of cutting and removal of the cut material.

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