University of São Paulo
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Carbon assimilation, herbage accumulation, nutritive value, and grazing efficiency of Mulato II brachiariagrass under continuous stocking

Valdson José da Silva

Thesis presented to obtain the degree of Doctor in Science. Area: Animal Science and Pastures

Piracicaba
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"The only way to do great work is to love what you do."

-Steve Jobs

CONTENTS

RESUMO

Assimilação de carbono, acúmulo de forragem e eficiência de pastejo do Capim Mulato II sob lotação contínua

As pastagens são o elemento central da pecuária brasileira, sendo as gramíneas do gênero *Brachiaria* (sin. *Urochloa*) as plantas forrageiras tropicais mais utilizadas. O capim Mulato II (Convert HD 364, Dow AgroSciences, São Paulo, Brazil) (*B. brizantha* × *B. ruziziensis* × *B. decumbens*) foi lançado como uma opção para diversas condições ambientais e de manejo. Entretanto não existem informações de práticas de manejo específicas para o capim Mulato II sob lotação contínua no Brasil. Os objetivos desse estudo foram descrever e explicar variações na assimilação de carbono, acúmulo de forragem (AF), acúmulo de componentes morfológicos no dossel, valor nutritivo e eficiência de pastejo (EP) do capim Mulato II em resposta a alturas do dossel mantidas constantes e taxas de crescimento impostas por doses de nitrogênio sob lotação contínua. Um experimento foi conduzido em Piracicaba-SP, durante dois verões agrostológicos, utilizando o delineamento experimental de blocos completos casualizados com arranjo fatorial 3 × 2, correspondendo a três alturas (10, 25 e 40 cm) e duas doses de N (50 e 250 kg N ha^{-1} ano^{-1}), com três repetições. A maior parte das variáveis estudadas não foram afetadas pela interação altura × dose de N. O AF do capim Mulato II aumentou linearmente (de 8640 para 13400 kg MS ha^{-1} ano^{-1}), a digestibilidade in vitro da matéria orgânica (DIVMO) reduziu linearmente (de 652 para 586 g kg^{-1}), e a EP foi reduzida (efeito linear e quadrático) de 65 para 44% com o aumento da altura do dossel. Com isso, embora a EP e a DIVMO tenham sido maiores em dosséis mantidos a 10 cm, o AF foi reduzido em 36% em comparação com aquele a 40 cm. As taxas de assimilação de carbono de folhas foi maior nos dosséis mantidos a 10 cm, mas a assimilação do dossel foi maior nos dosséis mais altos devido ao maior índice de área foliar (IAF). A redução do AF, do acúmulo de componentes morfológicos e do IAF não foram associados com outros sinais de deterioração do dossel. Folha foi o principal componente morfológico acumulado e a taxa de acúmulo aumentou linearmente de 70 para 100 kg DM ha^{-1} dia^{-1} quando a altura de manejo aumentou de 10 para 40 cm. O capim Mulato II foi menos produtivo (7940 vs. 13380 kg ha^{-1} ano^{-1}) e apresentou menor DIVMO (581 vs. 652 g kg^{-1}) na menor dose de N. O aumento na dose de N afetou o crescimento da planta, resultando em aumentos na assimilação de carbono, IAF, acúmulo de componentes morfológicos e AF. Os resultados indicam que o aumento nas taxas de acúmulo de material morto devido a maior dose de N foi resultado do aumento nas taxas de acúmulo de todos os componentes morfológicos do dossel. A manutenção do dossel mais alto (25 ou 40 cm) pode ser vantajosa devido ao aumento no AF do capim Mulato II, embora o valor nutritivo e EP tenha sido maior a 25 cm, sugerindo que esse capim deve ser mantido na altura de ~25-cm quando manejado sob lotação contínua.

Palavras-chave: Altura do dossel; CONVERT HD 364; Híbrido de *Brachiaria;* Intensidade de pastejo; Manejo do pastejo; Taxas de crescimento; Índice de area foliar; Doses de nitrogênio; Fotossíntese; *Urochloa* spp

ABSTRACT

Carbon assimilation, herbage accumulation, nutritive value, and grazing efficiency of Mulato II brachiariagrass under continuous stocking

Grazed pastures are the backbone of the Brazilian livestock industry and grasses of the genus *Brachiaria* (syn. *Urochloa*) are some of most used tropical forages in the country. Although the dependence on the forage resource is high, grazing management is often empirical and based on broad and non-specific guidelines. Mulato II brachiariagrass (Convert HD 364, Dow AgroSciences, São Paulo, Brazil) (*B. brizantha* × *B. ruziziensis* × *B. decumbens*), a new *Brachiaria* hybrid, was released as an option for a broad range of environmental conditions. There is no scientific information on specific management practices for Mulato II under continuous stocking in Brazil. The objectives of this research were to describe and explain variations in carbon assimilation, herbage accumulation (HA), plant-part accumulation, nutritive value, and grazing efficiency (GE) of Mulato II brachiariagrass as affected by canopy height and growth rate, the latter imposed by N fertilization rate, under continuous stocking. An experiment was carried out in Piracicaba, SP, Brazil, during two summer grazing seasons. The experimental design was a randomized complete block, with a 3 x 2 factorial arrangement, corresponding to three steady-state canopy heights (10, 25 and 40 cm) maintained by mimicked continuous stocking and two growth rates (imposed as 50 and 250 kg N ha^{-1} yr^{-1}), with three replications. There were no height × N rate interactions for most of the responses studied. The HA of Mulato II increased linearly (8640 to 13400 kg DM ha^{-1} yr^{-1}), the in vitro digestible organic matter (IVDOM) decreased linearly (652 to 586 g kg^{-1}), and the GE decreased (65 to 44%) as canopy height increased. Thus, although GE and IVDOM were greatest at 10 cm height, HA was 36% less for the 10- than for the 40-cm height. The leaf carbon assimilation was greater for the shortest canopy (10 cm), but canopy assimilation was less than in taller canopies, likely a result of less leaf area index (LAI). The reductions in HA, plant-part accumulation, and LAI, were not associated with other signs of stand deterioration. Leaf was the main plant-part accumulated, at a rate that increased from 70 to 100 kg DM ha^{-1} d^{-1} as canopy height increased from 10 to 40 cm. Mulato II was less productive (7940 vs. 13380 kg ha^{-1} yr^{-1}) and had lesser IVDOM (581 vs. 652 g kg^{-1}) at the lower N rate. The increase in N rate affected plant growth, increasing carbon assimilation, LAI, rates of plant-part accumulation (leaf, stem, and dead), and HA. The results indicate that the increase in the rate of dead material accumulation due to more N applied is a result of overall increase in the accumulation rates of all plant-parts. Taller canopies (25 or 40 cm) are advantageous for herbage accumulation of Mulato II, but nutritive value and GE was greater for 25 cm, suggesting that maintaining ~25-cm canopy height is optimal for continuously stocked Mulato II.

Keywords: Canopy height; CONVERT HD 364; *Brachiaria* hybrid; Defoliation; Grazing intensity; Grazing management; Growth rates; Leaf; Leaf area index; Nitrogen rates; Photosynthesis; *Urochloa* spp

1 INTRODUCTION

Grazed pastures are key for the Brazilian livestock-industry. The country has the world's largest commercial cattle (*Bos* spp.) herd (~ 212 million head) (FAO, 2013) and approximately 90% are maintained exclusively on grazed pastures. Since 2004, the country has been the world's first or second biggest beef exporter. In 2015, the beef industry exported approximately 1.39 million tons, corresponding to more than U$$ 5.9 billion in exports (ASSOCIATION OF BEEF EXPORTERS - ABIEC, 2016). Grasses of the genus *Brachiaria* (syn. *Urochloa*) are widely used for grazing in these beef production systems, comprising about 80% of the 100 Mha of improved pastures in Brazil. 'Marandu' palisadegrass [*B. brizantha* (Hochst. ex A. Rich.) Stapf.] is the most widely grown, covering about 45 Mha, and has been considered the largest monoculture in the country on an area basis (JANK et al., 2014).

Despite the importance of gazed pastures in Brazil, most of the pasture area is not exploited efficiently. The forage-livestock systems are characterized by low adoption of grazing management technologies by producers, who rarely see the pasture as a crop that demands technical inputs, and this contributes to reduce the beef industry profitability. It also contributes negatively to the international visibility of the beef industry, which has been frequently associated with deforestation to increase pasture areas for beef production (MARTHA Jr.; ALVES; CONTINI, 2012). In addition, there is an increased interest of pasture areas for other agricultural activities considered more profitable, such as sugar-cane and grain crops (ALKIMIM; SPAROVEK; CLARKE, 2015). If well managed, however, the actual pasture area could support a much greater number of animals, contribute to carbon sequestration, and help to mitigate greenhouse gas emissions (SILVA et al., 2016).

Reversing this scenario should be a priority, increasing pasture production (quantitatively and qualitatively), not only to increase animal productivity, but also to increase the efficiency of utilization (SILVA; PEDREIRA, 1997). In most grazing systems, the importance of the fundamental principles of biology and plant growth are not recognized when implementing management strategies, which often contributes to making wrong management decisions. Another problem, however, has emerged along with newly released forage genotypes, namely, the limited knowledge of optimal management guidelines, especially under continuous stocking, a grazing method that has been the subject of much less research than the intermittent defoliation methods, although it is the most common grazing method in Brazil.

The search for new forage materials in Brazil has been regarded as priority in recent years, mainly to avoid issues arising from monoculture and the decline of large pasture areas. One example is the area occupied by Marandu palisadegrass which has been widely planted to many States, and where persistence problems have been reported (ANDRADE; VALENTIM, 2007; CAETANO; DIAS-FILHO, 2008). Among these problems, the "Marandu death syndrome" and the decreased resistance to species of spittlebugs (e.g. *Zulia entreriana* [Berg], *Deois incompleta* [Walker], *Deois flavopicta* [Stall], and *Aeneolamia selecta selecta* [Walker]) (BARBOSA, 2006; ANDRADE; VALENTIM, 2007) have been listed. The "Marandu death syndrome" has contributed to the decline in productivity and persistence of this grass in Central and Northern Brazil (CAETANO; DIAS-FILHO, 2008). The causes of this syndrome have not yet been well established, but are thought to be the combined result poor soil drainage and low soil fertility, and possibly pests and diseases (spittlebugs and diseases caused by fungi such as *Rhizoctonia solani, Pythium* sp., and *Fusarium* sp.) (PEQUENO et al., 2015). Research efforts have been employed in search for improvements in pasture management, aiming at the intensification of these pasture-based animal production systems. New and more productive materials that are persistent and easy to manage and that can contribute to the sustainability of farming systems have been sought after.

Mulato II brachiariagrass, a new *Brachiaria* hybrid, was developed from three generations of hybridization between ruzigrass [*B. ruziziensis* R. Germ & Evrard (clone 44-6)] and signalgrass [*B. decumbens* (Stapf) R. D. Webster (cv. Basilisk)], where the first generation was exposed to open pollination from lines of *B. brizantha*, including cv. Marandu (ARGEL et al., 2007). This genotype was subsequently identified as *Brachiaria* hybrid accession CIAT 36087 and it was released in 2005 as cv. Mulato II. 'Mulato II' was developed to have a broad range of adaptation (including acidic soils of low fertility and moderate moisture saturation), high nutritive value and forage production, and good-quality seed (ARGEL et al., 2007). It has been comercialized in Brazil since 2009 as Convert HD 364 (DowAgrosciences, São Paulo, Brazil) and it is advertised as an option to be used for pasture diversification in Brazil.

Mulato II has been evaluated under a range of harvest frequencies and intermittent stocking in Brazil (TEODORO, 2011; DEMSKI, 2013; LEAL, 2014; PEQUENO et al., 2015), but not under continuous stocking. In part, this reflects the reduced interest in grazing research using the continuous stocking method in Brazil, due to possible advantages of using intermittent methods such as rotational stocking. Continuous stocking, however, is the most used grazing method in the country, and can show similar potential of animal production when compared to rotational stocking (PARSONS; PENNING, 1988). Vendramini et al. (2012) compared Mulato II to 'Tifleaf 3' pearl millet [*Pennisetum glaucum* (L.) R. Br.] and 'Hayday' sorghum sudangrassgrass [*Sorghum bicolor* (L.) Moench] pastures maintained at 30 cm under continuous stocking during one year in Florida. They reported that Mulato II had greater herbage allowance (2.0 vs. 0.7 kg DM kg^{-1} LW) and average daily gain (0.78 vs. 0.41 kg d^{-1}) than Tifleaf 3 and Hayday and similar gain per hectare (302 kg). The scarcity of management recommendations for tropical grasses under continuous stocking can compromise the success of adoption of recently released cultivars.

Under continuous stocking, it has been suggested that the adoption of management targets to maintain specific sward conditions may be advantageous (SOLLENBERGER et al., 2012), including characteristics that favor plant growth and persistence, as well as greater forage utilization and animal production. For several temperate pasture species it has been reported that the use of canopy height as a management target improved pasture and animal performance in continuously grazed canopies (BAKER; LE DU; ALVAREZ, 1981; WRIGHT; WHYTE, 1989). Studies conducted with tropical grasses show that, similar to temperate pastures, the use of a specific range of canopy heights can result in greater herbage accumulation, as well as contribute to grass persistence and improve animal productivity (FLORES et al., 2008; CARLOTO et al., 2011; PAULA et al., 2012; HERNANDEZ-GARAY et al., 2014).

The recommended range of canopy heights varies across tropical forage genotypes under continuous stocking. Hernandez-Garay et al. (2014) studied the canopy height of 15, 30 and 45-cm on 'Xaraes' palisadegrass [*B. brizantha* (Hochst. ex A. Rich.) Stapf.] and reported greater animal production on continuously stocked at 30 cm (1170 kg ha^{-1}), compared to 15 and 45 cm (~ 978 kg ha^{-1}). Paula et al. (2012) described an increase on forage intake on Marandu palisadegrass managed at 30 cm canopy height, compared to 15 and 45 cm. The HA of 'Basilisk' signalgrass (*B. decumbens* Stapf.) pastures was increased when a 10 and 17 cm height was used compared to 25cm height (FERREIRA, 2010), and 'Tifton 85' bemudagrass (*Cynodon* spp.) also had greater HA at 15 and 20 cm height, compared to 5 and 10 cm (PINTO et al., 2001). The use of specific steady-state canopy height as a target level of grazing intensity can help improve pasture and animal productivity, as it can affect herbage accumulation, nutritive value and persistence of grazed pastures (SOLLENBERGER et al., 2012).

In grazed pastures, the lack of nitrogen fertilization has been considered an important cause of pasture decline and reduced forage and animal productivity in tropical areas

(BODDEY et al., 2004). Low N also contributes to reduce pasture carrying capacity, and decrease animal output from grazed areas, which may reduce the competitiveness of the forage-beef cattle industry. Nitrogen fertilization usually results in increased herbage accumulation, which may allow increasing stocking rates on grazed pastures. N fertilization can also result in changes in plant morphological (PEREIRA et al., 2014) and physiological characteristics (ONODA; HIKOSAKA; HIROSE, 2004), such as plant-part composition, leaf area, and carbon assimilation, due to its effects on partitioning and on the dynamics of herbage accumulation.

Pasture management practices, such as fertilization and grazing to maintain a target canopy height can impact the patterns of accumulation of herbage components, and also the root mass (SILVA et al., 2015), and stored reserves. The study and understanding of morphological and physiological characteristics of forage plants is key for optimizing management strategies for each grass under each environmental condition, according to soil and climate conditions, and considering specific objectives of the forage-livestock system. It also allows for the identification of management specificities in terms of ecophysiological limits of forage plants and making for increased efficiency of pasture utilization. Therefore, the study of the effects of grazing management on canopy carbon assimilation, morphogenetic and morphologic characteristics, herbage accumulation, nutritive value, and grazing efficiency can help identify optimal management strategies for Mulato II brachiariagrass. Understanding the process of herbage accumulation associated with different grazing managements, such as different targets of canopy height and N rates may contribute to improve pasture production and to the development of more efficient and sustainable grazing systems.

1.1 Hypothesis

The hypothesis of the study was that the carbon assimilation, herbage accumulation, nutritive value, grazing efficiency, dynamic of plant growth, and persistency of Mulato II brachiariagrass are affected by nitrogen rates and canopy heights maintained under continuous stocking with variable stocking rate.

1.2 Objectives

The general objectives of this study were to describe and explain the effects of three steady-state canopy heights (10, 25 and 40-cm), and two growth rates, the former imposed by grazing and latter imposed by the application nitrogen rates (50 and 250 kg ha^{-1} $year^{-1}$) on leaf canopy carbon assimilation, plant-part dynamics, herbage accumulation and nutritive value, grazing efficiency, and root organic reserves of Mulato II brachiariagrass.

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2 HERBAGE ACCUMULATION, NUTRITIVE VALUE, AND GRAZING EFFICIENCY OF 'MULATO II' BRACHIARIAGRASS UNDER CONTINUOUS STOCKING

Abstract

Brachiaria sp. (syn. *Urochloa* sp.) grasses are widely used in planted pastures in Brazil. 'Mulato II' is a productive *Brachiaria* hybrid (*B. brizantha* × *B. decumbens* × *B. ruziziensis*) with high nutritive value, but it has not been evaluated under continuous stocking in Brazil, despite this method being widely used by producers. The objectives of this research were to quantify the effects of three canopy heights (10, 25, and 40 cm), maintained by mimicking continuous stocking, and two N rates (50 and 250 kg ha^{-1} yr^{-1}) on herbage accumulation (HA), grazing efficiency (GE), and nutritive value of Mulato II during two summer rainy seasons in Piracicaba, Brazil. The N rates were chosen so that canopy height effects could be evaluated under distinctly different grass growth rates. Grass HA increased linearly (8640 to 13 400 kg DM ha^{-1} yr^{-1}), in vitro digestible organic matter (IVDOM) decreased linearly (652 to 586 g kg^{-1}), and GE decreased (linear and quadratic; 65 to 44%) as canopy height increased. There were no height × N rate interactions for these responses. Mulato II was less productive (7940 vs. 13 380 kg ha^{-1} yr^{-1}) and had lesser IVDOM (581 vs. 652 g kg^{-1}) at the lower N rate. Thus, although GE and IVDOM were greatest for 10 cm, HA was 36% less for the 10- than the 40-cm height, and despite greatest HA occurring at 40 cm, both GE and IVDOM were greater at shorter heights. These data suggest that ~25-cm canopy height is optimal for continuously stocked Mulato II.

Keywords: Convert HD 364; Grazing management; Grazing intensity; Hybrid; Nitrogen; *Urochloa* sp

2.1 Introduction

Grasses of the genus *Brachiaria* (syn. *Urochloa*) are widely used for grazing in beef production systems, comprising about 80% of the 100 million ha of improved pastures in Brazil, where 'Marandu' palisadegrass [*Brachiaria brizantha* (Hochst. ex A. Rich.) Stapf.] is grown on about 45 million ha. In recent years the "Marandu death syndrome" has contributed to the decline in productivity and persistence of this grass in central and northern Brazil (CAETANO; DIAS-FILHO, 2008). Alternatives to Marandu are needed in these pasture-based systems. Mulato II brachiariagrass was the second *Brachiaria* hybrid released by CIAT. In Brazil, this grass has been commercialized as Convert HD 364 (Dow AgroSciences, São Paulo, Brazil) since 2009, and it is an option for increasing diversity of pasture grass species used in Brazil due to its high forage accumulation and nutritive value when well fertilized and well managed (PEQUENO et al., 2015).

Despite the great importance of pastures for the livestock industry, grazing management in Brazil is often inadequate. This limitation along with low nutrient replacement (lack of fertilization), and, in some cases, the use of non-adapted genotypes, contributes to pasture degradation (EUCLIDES et al., 2010). As a result, it is estimated that around 50% of planted pastures in Brazil show some degree of degradation, resulting in reduced animal production (BODDEY et al., 2004). Studies on grazing management are important to generate reliable information for producers, especially for new forage cultivars as they are released.

Mulato II has been evaluated under various frequencies of defoliation and using rotational stocking (TEODORO, 2011; DEMSKI, 2013; LEAL, 2014; PEQUENO et al., 2015), but it has not been tested under continuous stocking in Brazil. In part, this reflects limited interest among Brazilian researchers in continuous stocking due to perceived advantages of using rotational stocking. Continuous stocking, however, is the most commonly used grazing method in the country and can result in similar animal production to rotational stocking (PARSONS; PENNING, 1988). The scarcity of grazing management recommendations under continuous stocking reduces the likelihood of successful adoption of recently released cultivars. There has been very limited grazing evaluation of Mulato II under continuous stocking outside of Brazil. Vendramini et al. (2012) compared Mulato II with 'Tifleaf 3' pearl millet [*Pennisetum glaucum* (L.) R. Br.] and 'Hayday' sorghum sudangrassgrass [*Sorghum bicolor* (L.) Moench] pastures maintained at 30 cm under continuous stocking during 1 yr in Florida. They reported that Mulato II had greater herbage allowance (2.0 vs. 0.7 kg DM kg^{-1} LW) and average daily gain (0.78 vs. 0.41 kg d^{-1}) than Tifleaf 3 and Hayday, but gain per hectare (302 kg) was similar among grasses.

It has been suggested that the adoption of management targets to maintain specific sward conditions may be advantageous under continuous stocking (SOLLENBERGER et al., 2012). For several temperate pasture species it has been reported that the use of a target canopy height can improve pasture and animal performance in continuously stocked pastures (BAKER et al., 1981; WRIGHT; WHYTE, 1989). Studies conducted with tropical grasses have also shown that use of a specific range of canopy heights can result in greater HA and contribute to grass persistence and improved animal productivity (CARVALHO et al., 2000; CARLOTO et al., 2011; PAULA et al., 2012; HERNANDEZ-GARAY et al., 2014). Continuous stocking canopy heights of 15, 30, and 45 cm were studied on 'Xaraes' palisadegrass (*Brachiaria brizantha* Stapf.) (HERNANDEZ-GARAY et al., 2014). Animal production was greater on pastures grazed at 30 cm (1170 kg ha^{-1}) compared with 15 and 45 cm (~ 980 kg ha^{-1}). Paula et al. (2012) reported an increase in forage intake on Marandu palisadegrass managed at a 30-cm canopy height compared with 15 and 45 cm. The HA of 'Basilisk' signalgrass (*Brachiaria decumbens* Stapf.) pastures was increased when 10- and 17-cm heights were used compared with a 25-cm height (FERREIRA, 2010). Similarly, 'Tifton 85' bermudagrass (*Cynodon* spp.) HA was greater at 15- and 20-cm heights compared with 5 and 10 cm (PINTO et al., 2001). Thus, the use of specific steady-state target canopy heights can help improve pasture and animal productivity through its effects on HA, nutritive value, and persistence (SOLLENBERGER et al., 2012).

Nitrogen fertilization accelerates the dynamics of pasture growth (LEMAIRE et al., 2009). This can contribute to improvement of overall system productivity, resulting in increased HA, and sometimes nutritive value. Considering that canopy height can impact morphological and structural characteristics of grazed pasture canopies and N can change the dynamics of pasture growth, the objective of this study was to describe and explain differences in HA, GE, and nutritive value of Mulato II brachiariagrass in response to combinations of N fertilization rates and various canopy heights mimicking continuous stocking.

2.2 Materials and Methods

2.2.1 Site description, treatments, and experimental design

The study was carried out in Piracicaba, São Paulo, Brazil (22º42' S, 47º37' W, 546 m a.s.l.) at the "Luiz de Queiroz" College of Agriculture, University of São Paulo (USP-ESALQ). The soil at the experimental site is a highly fertile Kandiudalfic Eutrudox. Average soil chemical characteristics were: P = 18.2 mg dm^{-3} (ion-exchange resin extraction method); organic matter (OM) = 37 g dm^{-3}; pH (0.01 mol $L^{-1}CaCl_2$) = 6.0; K = 5.7 $mmol_c$ dm^{-3}; Ca = 57 $mmol_c$ dm^{-3}; Mg = 21 $mmol_c$ dm^{-3}; H+Al = 31 $mmol_c$ dm^{-3}; sum of bases = 83.8 $mmol_c$ dm^{-3}; cation ex-change capacity = 115 $mmol_c$ dm^{-3}; base saturation = 73%. The proportions of clay, silt, and sand were 431, 199, and 370 g kg^{-1}, respectively. Weather data for the experimental period (Table 1) were recorded at a weather station located 2 km from the experimental site.

Table 1 - Monthly weather data at the experimental site during 2 yr of evaluation in Piracicaba, SP, Brazil

Weather variable	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
				2012-2013				
Max. temperature (ºC)	32.6	30.6	32.9	29.6	31.7	30.6	28.5	27.1
Min. temperature (ºC)	17.9	18.0	21.1	19.2	19.9	19.3	16.3	14.3
Rainfall (mm)	76	100	193	232	114	142	201	84
				2013-2014				
Max. temperature (ºC)	29.9	30.5	32.5	33.4	33.7	31.4	29.2	26.9
Min. temperature (ºC)	16.8	18.6	20.2	20.2	20.5	19.3	17.2	13.6
Rainfall (mm)	154	110	121	83	62	109	54	40
				Historic average†				
Max. temperature (ºC)	29.1	29.6	29.7	30.0	30.4	30.1	28.5	26.1
Min. temperature (ºC)	15.8	16.9	18.3	19.0	19.1	18.3	15.6	12.2
Rainfall (mm)	110	130	199	230	180	142	66	54

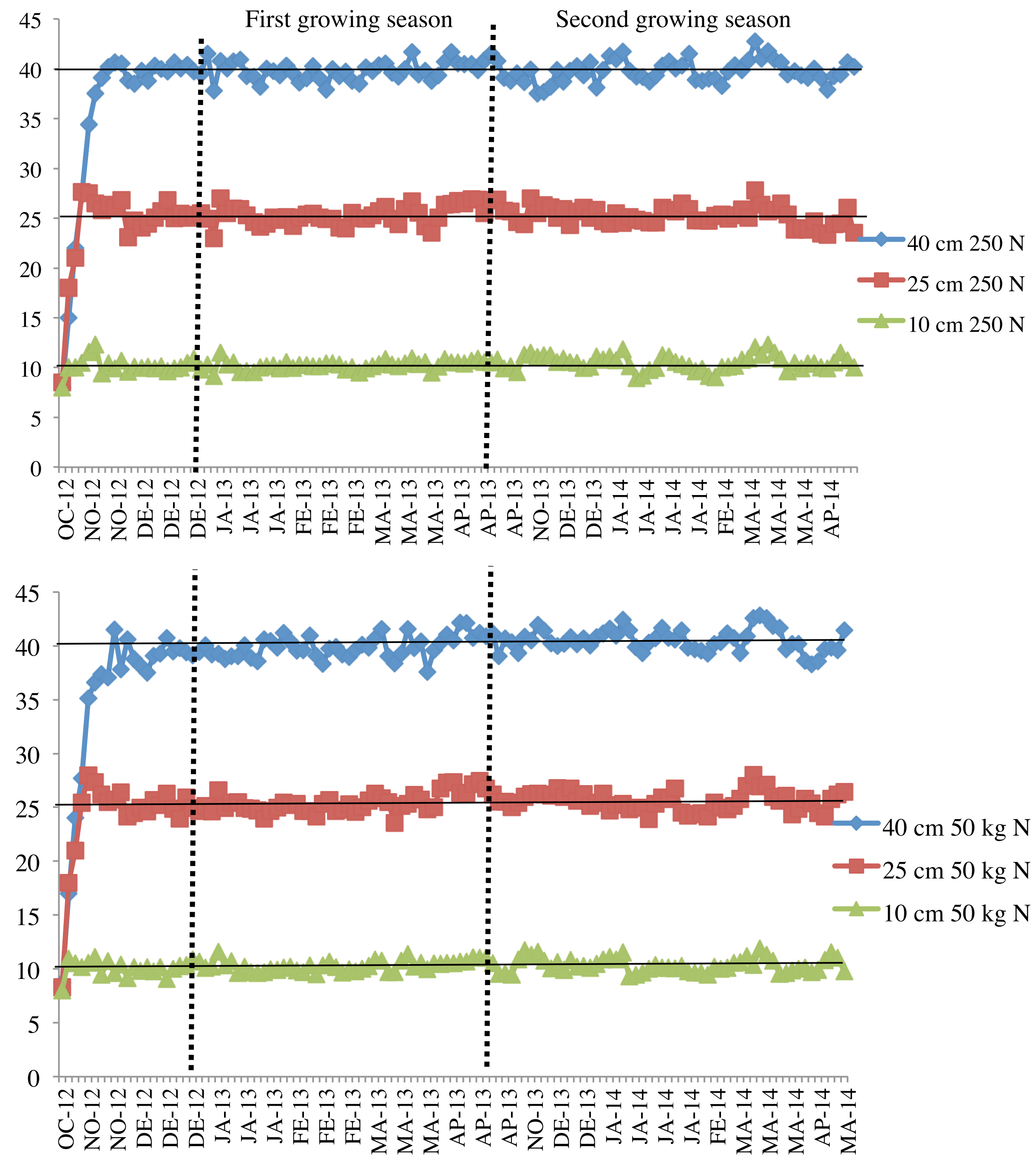
† Historic average weather data from 1917 to 2012.

Mulato II brachiariagrass was planted in January 2012 using 8 kg ha^{-1} of pure-live seed. At sowing, 16 kg P ha^{-1} was applied. Grazing was initiated in May 2012 to stimulate tillering and ensure good establishment of the pasture. On 29 Aug. 2012, after an intense grazing by cattle, paddocks were mechanically staged to 8-cm height and 50 kg N ha^{-1} was applied to stimulate growth. From September to December 2012 the paddocks were maintained at their designated treatment canopy heights, and this was considered an adaptation period.

Data were collected from 21 Dec. 2012 through 10 May 2013 and 10 Dec. 2013 to 16 Apr. 2014. Treatments were the factorial combinations of three canopy heights (10, 25, and 40 cm; intense, moderate, and lenient grazing, respectively) and two growth rates (slow and fast). Growth rates were achieved by using two N rates (50 and 250 kg N yr^{-1} for slow and fast growth rates, respectively). The experimental design was a randomized complete block with three replications. Area of each pasture (experimental unit) was 200 m^2 (Figure 1). Between the end of the first and the beginning of the second grazing seasons canopy height was measured weekly, and when necessary paddocks were grazed to maintain the height targets in each experimental unit. No measurements other than height were made outside of the evaluation periods mentioned above.

Figure 1 - General view of the experimental area

The grazing protocol was intended to mimic continuous stocking using frequent grazing events with the objective of keeping canopy height constant (Figure 2). The average non-extended and non-compressed canopy height was measured using a light polyethylene sheet and a graduated measuring stick at 40 sites in each paddock three times a week. Dry crossbred dairy cows (~ 450 kg BW) were brought onto the paddocks when the average canopy height reached 11, 27, and 42 cm, and taken off when the height reached 9, 23, and 38 cm, for the 10-, 25-, and 40-cm treatments, respectively. Grazing events lasted from 0.5 to 1.5 h, usually in the morning hours.



Figue 2 - Control of canopy height of grazed Mulato II under continuous stoking

Each pasture received 208 kg K ha^{-1} yr^{-1}, and the treatment N rates and the K fertilization were split applied in four equal applications of NH_4NO_3 and KCl fertilizers, respectively, per growing season. Application dates were 21 Dec. 2012, 28 Jan. 2013, 21 Feb. 2013, and 1 Apr. 2013 in the first year, and 10 Dec. 2013, 7 Jan. 2014, 4 Feb. 2014, and 1 Apr. 2014 in the second year.

2.2.2 Herbage mass and herbage accumulation

A double sampling technique was used to determine herbage mass (HM). The indirect sampling method was a rising plate meter (Ashgrove Pastoral Products, Hamilton, New Zealand), measuring 35.5 cm in diameter and weighing 480 g, and the direct measurement involved clipping all HM inside 0.25-m^2 quadrats to 2 cm above soil level using electric clippers. The forage harvested was dried at 60^{o}C in a forced-draft oven to constant weight. Double sampling for calibration occurred on 2 Feb. and 29 Mar. 2013 in the first growing season and 18 Dec. 2013, and 9 Jan., 29 Jan., and 25 Mar. 2014 in the second growing season. At each double sampling date, two sites were chosen per experimental unit representing the range of present HM (maximum and minimum) for a total of 36 double sampling sites on each date. Data from these samplings (72 and 144 sites in Years 1 and 2, respectively) were used to develop a regression equation to estimate HM. The average r^2 of the equations used to predict HM were 0.78 and 0.86 for the first and second growing seasons, respectively.

Exclosure cages were used to estimate HA. Three circular 0.9-m^2 cages were placed in each pasture at the beginning of the experimental period at sites where the plate reading was the same (±1) as the pasture average. After 21 d, a plate reading was taken inside the caged areas, and the cages were moved to new locations in the pasture where the current plate reading was the same as the pasture average. The regression equations from double sampling data were used to estimate HM at the time when cages were first placed and when they were moved from a sampling site. Herbage accumulation was calculated as the HM inside cages at the end of each 21-d period minus the HM in the paddock at the time of cage placement (DAVIES; FOTHERRGILL; MORGAN, 1993), and daily herbage accumulation rate (HAR) was calculated by dividing HA by 21. The total annual HA was calculated as the sum across 21-d periods within a growing season.

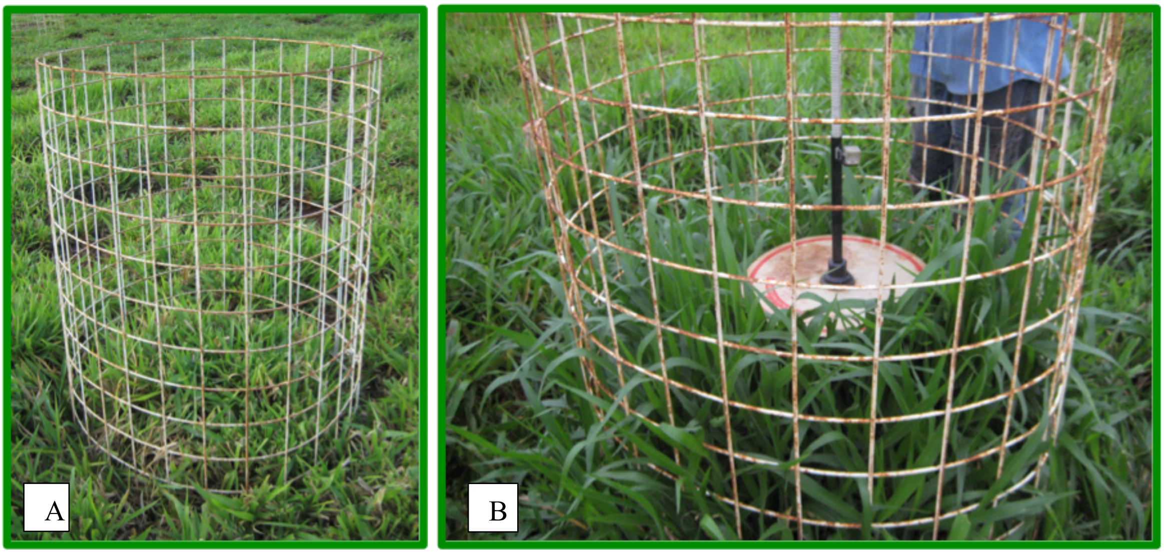


Figure 3 - Exclosure cage placed in a paddock at the beginning (A) and after 21 d of exclosure (B)

Forage losses were quantified and used to calculate GE. Three representative sites (mean of vegetation condition by visual assessment) measuring 0.7 × 1.25 m were selected per pasture and permanently marked. The soil surface within these sites was cleared of all trampled plant material and litter at the beginning of the experimental period and every 24 d thereafter. On each sampling occasion, unutilized forage was considered to be all plant material that was mechanically damaged (even if green and still attached to the tillers at the time of sampling), trampled, or fouled upon, plus all forage material on the soil surface (litter). This material was collected, dried in a forced-air oven at 60°C to constant weight and weighed. Tissue senescence was also considered forage loss during each period. In order to quantify tissue senescence, tiller population density was assessed by counting the number of tillers inside two 1.2 × 0.35 m quadrats every 28 d. In addition, a total of 30 tillers were marked per paddock and these were evaluated twice a week. The mean leaf length of each leaf category (expanding leaves, fully expanded leaves, and senescing leaves) was measured. Every 24 d evaluated tillers were harvested and new tillers marked. The sample of harvested tillers was separated into leaf blade, stem plus sheath, and dead material. The various categories of leaf blades were scanned using a model LI 3100 leaf area meter (Li-Cor, Lincoln, NE) to determine the leaf area and then dried separately. All samples were dried in a forced-draft oven at 60ºC to constant weight and weighed. After weighing, a conversion factor between length and weight (g cm^{-1}) was calculated by dividing the total mass of fully-expanded leaves by their total length. The daily senescence rates were obtained from marked tillers and dry weight relations and multiplied by the corresponding average tiller population density (BIRCHAM; HODGSON, 1983). Forage losses were calculated by summing the forage loss and senescence during the period. Grazing efficiency was calculated as the percentage of the mean HA measured in the cages (assuming that the difference in HM inside and outside the cages was the forage that disappeared due to consumption plus losses) that was damaged during grazing (due to trampling) plus litter deposition (during grazing), plus senescence, and this was expressed as: $GE = [(HA - GL)/HA] \times 100$, where HA is HA during a 21-d period and GL is the grazing losses (trampling, litter deposition, and senescence) during that period.

2.2.3 Herbage bulk density, plant-part composition and leaf area index

For the specific purpose of assessing bulk density, plant part composition, and canopy leaf area index (LAI), HM samples were taken every 21 d by clipping all herbage inside two representative 0.75- × 0.35-m quadrats per paddock to a 2-cm height above soil level using electric clippers. The forage inside each of the quadrats was weighed fresh in the field and a fresh subsample was taken and separated into leaf, stem + sheath, and dead material. Each fraction was then dried separately at 60^{o}C to constant weight and weighed. From the fresh leaf fraction, approximately 30 leaf blades were scanned in a model LI 3100 leaf area meter (Li-Cor, Lincoln, NE) to determine the leaf area, and then dried separately at 60^{o}C to constant weight and weighed. The dry weight of each fraction was used to determine the relative proportion of leaf, stem, and dead material in the HM. The relation of leaf area and leaf weight was used to obtain specific leaf weight and to estimate the total leaf area of the sample, which in turn was used to calculate the LAI (m^2 m^{-2}). The sum of all fractions was used to determine the total HM. Bulk density was calculated by dividing HM and mass of plant-part components by canopy height minus the sampling stubble height (2 cm).

2.2.4 Forage nutritive value

Nutritive value was assessed using hand-plucked samples that were taken every 21 d following observation of the grazing behavior of the animals in each pasture. Samples were dried at 60°C in a forced-air oven to constant weight and ground in a Wiley mill to pass a 1-mm screen. Nitrogen concentration was measured using a micro-Kjeldahl technique (GALLAHER; WELDON; FUTRAL, 1975). Nitrogen concentration in the digestate was determined by semi-automated colorimetry (HAMBLETON, 1977), and the crude protein (CP) concentration in the herbage calculated as N × 6.25. Herbage IVDOM concentration was determined using the two-stage procedure of Tilley and Terry (1963) as modified by Moore and Mott (1974). The neutral detergent fiber (NDF) concentration was determined according to the A2000 Filter Bag Technique - Method 13 (Ankom Technology, Macedon, NY) (ANKOM, 2014). Herbage CP, NDF, and IVDOM data reported are the weighted means across sampling dates within the growing season each year.

2.2.5 Statistical Methods

Data were analyzed using PROC MIXED of SAS (SAS Institute, 2013) with a model including effects of canopy height, N rate, and their interaction. Year and block were considered random effects to allow for broader inference (LITTELL et al., 2006). The covariance structure was chosen based on Akaike's Information Criterion (AIC) (WOLFINGER, 1993). Quantile-quantile plots were used to check normality of the model residuals, and when necessary data transformations were performed. Lognormal transformation was used for LAI and plant-part composition variables, but data presented are non-transformed data. Single degree of freedom polynomial contrasts were used (linear and quadratic) to determine the nature of responses to canopy height. Treatment means were compared using PDIFF by Student test ($P < 0.05$), and least squares means are reported.

2.3 Results and Discussion

2.3.1 Herbage mass, herbage accumulation and grazing efficiency

Herbage mass was affected only by canopy height ($P < 0.0001$), and it increased linearly from 6800 to 16680 kg ha^{-1} as canopy height increased from 10 to 40 cm (Table 2). Herbage accumulation and GE were affected by canopy height ($P < 0.0001$) and N rate ($P < 0.0001$ and $P = 0.0124$, for HA and GE, respectively), but the canopy height × N rate interaction was not significant ($P > 0.50$ for both variables). The HA was less when pastures were managed at shorter height (Table 2). Contrasting with this result, the HA and HAR of Xaraes palisadegrass were not affected by canopy heights of 15, 30, and 45 cm under continuous stocking (PEQUENO, 2010). Marandu palisadegrass did not show differences in HAR when managed at different grazing intensities (10, 20, 30, and 40 cm) although cattle liveweight gain ha^{-1} was greater for canopies maintained at 30 to 40 cm (SILVA et al., 2013).

Sbrissia et al. (2009) reported that maintaining Marandu palisadegrass at 10-cm canopy height was detrimental to persistence and productivity. In the current study, the HA of 10-cm canopies was reduced by 36% compared with that of the 40-cm canopies. This suggests that continuously stocked Mulato II is affected by grazing intensity to a greater extent than other brachiariagrasses, resulting in the observed reduction in HA. A large reduction in HA can result in reduced carrying capacity, and over the long term affect grass persistence (SOLLENBERGER et al., 2012). Despite differences in canopy height effects, the HA of Mulato II is comparable to that reported for other *Brachiaria* grasses under continuous stocking (PEQUENO, 2010; CARLOTO et al., 2011; FERREIRA, 2010; PAULA et al., 2012).

Table 2 - Herbage mass, herbage accumulation, forage losses and grazing efficiency of Mulato II brachiariagrass as affected by canopy height under continuous stocking in Piracicaba, SP, Brazil

Canopy height	Herbage mass	Herbage accumulation	Forage losses	Grazing efficiency
cm	kg DM ha^{-1}	-----------kg DM ha^{-1} yr^{-1}----------		%
10	6800	8460	2730	65
25	13 350	10 120	3780	62
40	16 680	13 400	5750	54
Polynomial contrast†	L*	L*	L*	L*Q*
SE‡	403	415	454	3.0

* Significant at the 0.05 probability level.
†Polynomial contrast for the effect of canopy height: L = linear, Q = quadratic.
‡SE= standard error.

Despite the lesser HA, pastures grazed at 10 cm showed lesser forage losses and greater GE (Table 2). This probably occurred due to taller canopies having greater HM (Table 4), contributing to greater senescence and forage losses during grazing. Despite the increase in forage losses with increased canopy height, there was little difference in GE of pastures managed at 10 and 25 cm, resulting in linear and quadratic effects of height on GE (Table 2). Pastures grazed at 40 cm lodged in some areas of the paddock, which may have increased forage losses. In contrast, for shorter canopies the HM was less, and GE was greater. Greater GE may not result in greater animal productivity (BRAGA et al., 2007) due to differences in efficiency of conversion of forage to animal product. Additionally, the greatest GE was obtained when the pasture was maintained at 10 cm, a treatment for which HA was reduced by 36% compared with the 40-cm height. Reduction in GE with lesser grazing intensity has been reported for tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.], wheatgrass [*Thinopyrum ponticum* (Podp.) Barkw. & Dewey], palisadegrass [*Brachiaria brizantha* (Hochst ex A. Rich.) Stapf, cv. Marandu], and 'Tanzania' guineagrass (*Panicum maximum* Jacq.) (AGNUSDEI et al., 2007; BRAGA et al., 2007; SMART et al., 2010; PENATI et al., 2014).

Mean annual HA of Mulato II increased by 69% for the 250 vs. the 50 kg N ha^{-1} treatment (Table 3). The application of N in grazed pastures increases leaf elongation rate, an important component of grass growth (GASTAL; NELSON, 1994). Additionally, greater N rate can increase tiller number (CRUZ; BOVAL, 2000; SILVA, 2015). Greater tiller density and greater leaf elongation rate together contribute to greater HA, which increases pasture carrying capacity.

Table 3 - Herbage accumulation, forage losses, and grazing efficiency of Mulato II brachiariagrass as affected by N rate under continuous stocking in Piracicaba, SP, Brazil

N rate	Herbage accumulation	Forage losses	Grazing efficiency
kg ha^{-1} yr^{-1}	----------kg DM ha^{-1} yr^{-1}--------		%
50	7940	3730	54
250	13 380	4440	63
$P^{\dagger}$	<0.0001	<0.0001	0.0007
SE‡	339	447	3.1

†*P* value for N rate.
‡SE= standard error.

The HA and grazing losses were greatest when Mulato II grew most rapidly due to receiving 250 kg N ha^{-1} yr^{-1} (Table 3). Despite this, GE was greater for 250 compared with 50 kg N ha^{-1} yr^{-1}. This was due to the greater relative increase in annual HA than in forage losses associated with application of the greater N rate.

2.3.2 Plant-part composition, leaf area index and canopy bulk density

The proportions of leaf, stem, and dead material, the LAI, leaf:stem ratio, and total canopy and leaf bulk density were affected by canopy height ($P < 0.0001$ for all variables), but only the proportions of leaf and dead, LAI, and leaf bulk density were affected by N rate ($P < 0.0001$ for all variables). As HM increased with canopy height (Table 2), the total and leaf bulk density were reduced (Table 4), suggesting differences in plant-part distribution in different vertical strata of the canopy. Intensively grazed tropical grass pastures have been reported to have less total forage biomass but greater herbage bulk density than taller pastures (SOLLENBERGER; BURNS, 2001), which often results in less opportunity for livestock to prehend leaf, negatively affecting intake and animal performance (CHACON; STOBBS, 1976). This suggests that the vertical herbage distribution may have an important impact on animal intake, and greater bulk density may not result in greater animal intake and performance. For example, there was a linear decline in total and leaf bulk density as canopy height of continuously stocked limpograss [*Hemarthria altissima* (Poir.) Stapf & Hubb.] pastures increased from 20 to 60 cm, and cattle average daily gain was greater for the intermediate bulk density 40-cm canopy height, than the greater bulk density 20-cm height (NEWMAN et al., 2002).

Table 4 - Herbage morphological characteristics of Mulato II brachiariagrass as affected by canopy height under continuous stocking in Piracicaba, SP, Brazil

Canopy height	Leaf	Stem	Dead	LAI	Leaf:stem	Total bulk density	Leaf bulk density
cm	---------------%---------------			$m^2\ m^{-2}$		-------kg cm^{-3}------	
10	26	21	53	2.5	1.3	63	16
25	23	31	46	4.5	0.7	56	12
40	22	36	41	5.8	0.6	42	9
Polynomial contrast†	L*Q*	L*Q*	L*Q*	L*	L*	L*	L*
SE‡	1.3	2.9	1.8	0.3	0.13	3.5	0.61

* Significant at the 0.05 probability level.
†Polynomial contrast for the effect of canopy height: L = linear, Q = quadratic.
‡SE= standard error.

Despite the linear effect of canopy height on HM, the proportion of leaf, stem, and dead material changed little above 25 cm, resulting in both linear and quadratic effects of height on plant-part composition (Table 4). Canopies grazed at 10 cm had the greatest proportion of leaf and greater leaf:stem ratio, but also greater proportion of dead material compared with taller canopies. This is a result of greater tiller death due to increased grazing intensity (Silva, 2015), and also to dead leaf sheaths contributing significantly to an observed greater dead proportion in the HM. Despite this, pastures grazed at 10 cm had less stem proportion and increased leaf bulk density compared with taller canopies (Table 4). The greater bulk density associated with shorter canopy height can result in limitations to bite volume, bite weight, and forage intake, which may negatively impact animal performance (SOLLENBERGER; BURNS, 2001; NEWMAN et al., 2002). In taller canopies, even though the leaf bulk density and leaf proportion decreased, differences in vertical distribution of leaf in the canopy were greater, with most of the green leaf in the top vs. the bottom layers of the canopy. In addition, most leaf production occurs from the apical growing points (BURNS et al., 1991), which may increase the difference in herbage component distribution in the canopy, especially in taller canopies.

Nitrogen fertilization increased LAI, leaf proportion, and leaf bulk density of Mulato II (Table 5). In a Marandu palisadegrass pasture maintained at 30 cm under continuous stocking and fertilized with 0, 150, 300, and 450 kg N ha^{-1} yr^{-1}, the LAI was increased by 100% when N rate increased from 0 to 450 kg ha^{-1} yr^{-1} (Pereira et al., 2014). Greater proportion of leaves in the HM, especially newly expanded leaves, can contribute to greater carbon assimilation rates (PARSONS; JOHNSON; HARVEY, 1988), as well as to greater forage nutritive value (BIDLACK; BUXTON, 1992). The increased leaf bulk density in the HM can provide greater opportunity for selection of leaf in grazed pastures, potentially enhancing animal production (NEWMAN et al., 2002).

Table 5 - Plant-part composition of Mulato II brachiariagrass as affected by N rate under continuous stocking in Piracicaba, SP, Brazil

N rate	Leaf	Dead	LAI	Leaf bulk density
kg ha^{-1} yr^{-1}	---------------%-------------		m^2 m^{-2}	kg cm^{-3}
50	22	49	3.7	12
250	25	45	4.8	13
$P^{\dagger}$	0.0002	0.0010	<0.0001	0.0007
SE‡	1.3	1.7	0.4	0.39

†*P* value for N rate.
‡SE= standard error.

2.3.3 Nutritive value

Concentrations of CP, NDF, and IVDOM were affected by canopy height ($P < 0.0001$ for all variables) and N rate ($P < 0.0001$ for all variables), but there was no canopy height × N rate interaction for any measure of nutritive value ($P > 0.54$). In general, nutritive value was greater when canopy height was maintained at 10 cm (Table 6). When grass canopies are grazed more intensively the leaf proportion in the HM is greater and leaves are generally younger because of shorter intervals between animal visits to individual patches (SOLLENBERGER et al., 2012). In the current study, there was a linear decrease in forage IVDOM concentration with increasing canopy height, but only small changes occurred for CP and NDF concentrations between 25- and 40-cm canopy heights. Despite greater nutritive value under greater grazing intensity (10 cm), the HA was reduced by 36% compared with the 40-cm height, suggesting that this nutritional advantage may not always be an attractive trade-off. It is well documented that both forage mass and nutritive value affect animal performance in grazed pastures, but across a wide range of grazing intensities, forage mass generally explains a greater proportion of the animal response (SOLLENBERGER; VANZANT, 2011).

Table 6 - Crude protein (CP), neutral detergent fiber (NDF), and in vitro digestible organic matter (IVDOM) concentrations in Mulato II brachiariagrass forage as affected by canopy height under continuous stocking

Canopy height	CP	NDF	IVDOM
cm	---------------------g kg^{-1}---------------------		
10	150	542	652
25	137	555	607
40	121	557	586
Polynomial contrast†	L*Q*	L*Q*	L*
SE‡	4.7	2.9	4.7

* Significant at the 0.05 probability level.
†Polynomial contrast for the effect of canopy height within a grass: L = linear, Q = quadratic.
‡SE= standard error.

Nitrogen fertilization resulted in greater forage nutritive value (Table 7). The increase in nutritive value and HA (Table 3) with N fertilization can contribute to increased daily animal performance and/or allow for greater stocking rates. Johnson et al. (2001) studied the effect of N rate on bahiagrass (*Paspalum notatum* Flugge), bermudagrass [*Cynodon dactylon* (L.) Pers.] and stargrass (*Cynodon nlemfuensis* Vanderyst) pastures and reported increasing CP and IVDOM concentrations and decreasing NDF with increasing N rates. The lesser nutritive value in pastures under the lower N rate is probably a result of slower tissue turnover, resulting in increased cell-wall deposition (BIDLACK; BUXTON, 1992) and greater NDF concentration (Table 7).

Table 7 - Crude protein (CP), neutral detergent fiber (NDF), and in vitro digestible organic matter (IVDOM) concentrations in Mulato II brachiariagrass forage as affected by N rate under continuous stocking

N rate	CP	NDF	IVDOM
kg ha^{-1} yr^{-1}	---------------------g kg^{-1}-------------------		
50	106	560	581
250	164	543	652
P†	<0.0001	<0.0001	<0.0001
SE‡	3.8	2.6	3.8

† *P* value for N rate.
‡SE= standard error.

2.4 Summary and Conclusions

Our data mimicking continuous stocking suggest that it is a viable grazing method for utilization of Mulato II, resulting in similar HA but greater nutritive value than that of other brachiariagrasses currently used in Brazil. The nutritive value of Mulato II was greatest when it was maintained at a 10-cm height, but HA was 36% less compared with the HA of the 40-cm canopies. Canopies maintained at a 40-cm height had greater HA (13400 kg DM ha^{-1}) but concurrently had greater forage losses (5550 kg DM ha^{-1}), resulting in reduced GE (54%). Moderate grazing intensity (25 cm) resulted in intermediate levels of HA (10120 kg DM ha^{-1}), but greater GE (62%) and nutritive value than pastures maintained at 40 cm, suggesting that 25 cm height favors efficient utilization of high quality forage. When Mulato II pastures are growing rapidly as a result of greater N rate, the HA, LAI, leaf proportion, leaf bulk density, and nutritive value increase resulting in more productive and higher nutritive value pastures that can contribute to greater animal productivity.

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3 CARBON ASSIMILATION, HERBAGE PLANT-PART ACCUMULATION, AND ORGANIC RESERVES OF GRAZED 'MULATO II' BRACHIARIAGRASS

Abstract

Pasture management including the control of grazing intensity and fertilization can trigger plant physiological and morphological responses that affect plant growth and impact stand persistence. The objective of this research was to quantify the effects of three canopy heights (10, 25, and 40 cm), maintained by mimicking continuous stocking, and two N rates (50 and 250 kg ha^{-1} yr^{-1}) on canopy carbon exchange rate (CER), plant-part accumulation, and organic reserves of 'Mulato II' brachiariagrass hybrid (*Brachiaria brizantha* × *B. decumbens* × *B. ruziziensis*), also known as Convert HD 364 (Dow AgroSciences, São Paulo, Brazil) during two summer rainy seasons in Piracicaba, Brazil. Leaf CER was greater for the 10-cm canopy, but canopy CER, plant-part accumulation, root mass, and root reserves were less in 10- than 40-cm canopies, although this was not associated with stand deterioration after 2 yr. Leaf was the main plant part accumulated, and it increased linearly from 70 to 100 kg DM ha^{-1} d^{-1} as canopy height increased from 10 to 40 cm. Increasing N rate from 50 to 250 kg N ha^{-1} affected plant growth, increasing CER, leaf area index (LAI), and the rates of leaf, stem, and dead herbage accumulation. The senescence-to-growth ratio (S:G) was not affected by N rate, indicating that the increase in rate of dead material accumulation with greater N rate was a result of an increase in the accumulation rates of all plant parts. Taller canopy heights (25 or 40 cm) are recommended for Mulato II under continuous stocking.

Keywords: *Brachiaria* hybrid; Continuous stocking; CONVERT HD 364; Grazing management; Nitrogen; *Urochloa* sp

3.1 Introduction

Carbon assimilation is the fundamental basis of crop growth and is affected by many environmental factors (e.g., solar radiation, temperature, water, nutrients), as well as by management practices such as fertilization and defoliation. In response to defoliation, plants may modify both growth patterns and use of available resources (LEMAIRE; CHAPMAN, 1996), including mechanisms for restoring photosynthetic capacity and changes in carbon assimilation and partitioning. This may impact tissue turnover in the sward, ultimately affecting net herbage accumulation rates (HAR).

Generally the most important grazing management decision is the choice of grazing intensity because it is an important determinant of herbage accumulation (HA) and plant persistence (SOLLENBERGER et al., 2012). Under continuous stocking, maintaining an optimal canopy height has been reported to improve pasture production and persistence, as well as animal productivity. Carloto et al. (2011) studied pasture and animal performance on grazed ‘Xaraes’ palisadegrass [*B. brizantha* (Hochst. ex A. Rich.) Stapf.] pastures subjected to three steady-state canopy heights (15, 30, and 45 cm). They reported a decrease in HA with increased grazing intensity, but animal average daily gain was similar across heights, with 15-cm canopies resulting in greater weight gain per unit area due to increased stocking rate. Herbage accumulation rates of ‘Marandu’ palisadegrass pastures were similar when canopy height was 10, 20, 30, and 40 cm under continuous stocking (SILVA et al., 2015b), although canopy heights of 10 cm or less reduced persistence (SBRISSIA et al., 2009). For continuously stocked ‘Mulato I’ brachiariagrass (*B. brizantha* × *B. ruziziensis*), when stocking rate was high and canopy height was ~10 cm, the leaf area and HAR were reduced compared with lower stocking rate treatments where canopy height was 30 cm or greater (INYANG et al., 2010b). Ground cover of Mulato II brachiariagrass decreased linearly from 89 to 78% as clipping stubble height decreased from 12.7 to 2.5 cm (INYANG et al., 2010a). Thus, the effects of canopy height on plant and animal response are quite significant, and it is important to identify the range of canopy heights under which plants perform optimally.

In perennial grass pastures, insufficient N has been considered an important cause of stand decline and reduced animal productivity in tropical areas (BODDEY et al., 2004). Nitrogen-deficient pastures are characterized by low carrying capacity and animal output, which diminishes the economic competitiveness of forage-livestock enterprises. Nitrogen fertilization changes forage morphological (LEMAIRE et al., 2009) and physiological characteristics (ONODA; HIKOSAKA; HIROSE, 2004), including leaf area and carbon assimilation, photosynthate partitioning, and the dynamics of herbage accumulation. Thus, both N fertilization and grazing intensity are major drivers of plant response in pastures (Silva et al., 2015a), and understanding the nature of their effects and interaction is important for new grasses being introduced into forage-livestock systems.

Knowledge of optimal canopy heights for forages growing under different levels of N fertilizer input contributes to the development of more efficient and sustainable grazing systems. The objective of this study was to describe and explain variations in the dynamics of HA of the novel Mulato II brachiariagrass in response to combinations of three steady-state canopy heights (10, 25, and 40 cm) and two N fertilization rates (50 and 250 kg N ha^{-1} yr^{-1}) during two summer growing seasons in Brazil.

3.2 Materials and Methods

3.2.1 Site description, treatments, and experimental design

The study was carried out in Piracicaba, São Paulo, Brazil (22º42' S, 47º37' W, 546 m a.s.l.) at the "Luiz de Queiroz" College of Agriculture, University of São Paulo (USP-ESALQ). The soil at the experimental site is a highly fertile Kandiudalfic Eutrudox. Average soil chemical characteristics were: P = 18 mg dm^{-3} (ion-exchange resin extraction method); organic matter (OM) = 37 g dm^{-3}; pH (0.01 mol $L^{-1}CaCl_2$) = 6.0; K = 5.7 $mmol_c$ dm^{-3}; Ca = 57 $mmol_c$ dm^{-3}; Mg = 21 $mmol_c$ dm^{-3}; H+Al = 31 $mmol_c$ dm^{-3}; sum of bases = 84 $mmol_c$ dm^{-3}; cation exchange capacity = 115 $mmol_c$ dm^{-3}; base saturation = 73%. The proportions of clay, silt, and sand were 431, 199, and 370 g kg^{-1}, respectively. Weather data for the experimental period (Table 1) were recorded at a weather station located 2 km from the research site.

Table 1 - Monthly weather data at the experimental site during 2 yr of evaluation in Piracicaba, SP, Brazil

Weather variable	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
				2012-2013				
Max. temperature (ºC)	32.6	30.6	32.9	29.6	31.7	30.6	28.5	27.1
Min. temperature (ºC)	17.9	18.0	21.1	19.2	19.9	19.3	16.3	14.3
Rainfall (mm)	76	100	193	232	114	142	201	84
				2013-2014				
Max. temperature (ºC)	29.9	30.5	32.5	33.4	33.7	31.4	29.2	26.9
Min. temperature (ºC)	16.8	18.6	20.2	20.2	20.5	19.3	17.2	13.6
Rainfall (mm)	154	110	121	83	62	109	54	40
				Historic average†				
Max. temperature (ºC)	29.1	29.6	29.7	30.0	30.4	30.1	28.5	26.1
Min. temperature (ºC)	15.8	16.9	18.3	19.0	19.1	18.3	15.6	12.2
Rainfall (mm)	110	130	199	230	180	142	66	54

† Historic average weather data from 1917 to 2012.

Mulato II brachiariagrass, was planted in January 2012 using 8 kg ha^{-1} of pure-live seed. At sowing, 16 kg P ha^{-1} was applied. Grazing was initiated in May 2012 to stimulate tillering and ensure good stand establishment. On 29 Aug. 2012, after an intense grazing by cattle, paddocks were mechanically staged to 8-cm height and 50 kg N ha^{-1} was applied to stimulate growth. From September to December 2012 the paddocks were maintained at their designated treatment canopy heights, and this was considered an adaptation period.

Data were collected from 21 Dec. 2012 through 10 May 2013 and from 10 Dec. 2013 to 16 Apr. 2014. Treatments were the factorial combinations of three canopy heights (10, 25, and 40 cm; intense, moderate, and lenient grazing, respectively) and two growth rates which were achieved by applying two N rates (50 and 250 kg N yr^{-1} for slow and fast growth rates, respectively). The experimental design was a randomized complete block with three replications. The area of each pasture (experimental unit) was 200 m^2. Between the end of the first and the beginning of the second grazing season canopy height was measured weekly, and when necessary paddocks were grazed to maintain the height targets in each experimental unit. No measurements other than height were made during this time.

The grazing protocol was intended to mimic continuous stocking using frequent grazing events with the objective of keeping canopy height constant. The average non-extended and non-compressed canopy height was measured using a light polyethylene sheet and a graduated measuring stick at 40 sites in each paddock three times a week. Dry crossbred dairy cows (~ 450 kg BW) were brought onto the paddocks when the average canopy height reached 11, 27, and 42 cm, and taken off when the height reached 9, 23, and 38 cm, for the 10-, 25-, and 40-cm treatments, respectively. Grazing occurred in the morning and events lasted from 0.5 to 1.5 h.

Each pasture received 208 kg K ha^{-1} yr^{-1}, and the treatment N rate and K were split applied in four equal applications of NH_4NO_3 and KCl fertilizers, respectively, in each growing season. Application dates were 21 Dec. 2012, 28 Jan. 2013, 21 Feb. 2013, and 1 Apr. 2013 in the first year, and 10 Dec. 2013, 7 Jan. 2014, 4 Feb. 2014, and 1 Apr. 2014 in the second year.

3.2.2 Leaf area index, leaf and canopy net assimilation rates

For the determination of leaf area index (LAI), samples were collected every 21 d by harvesting all the plant material inside two representative (visual assessment) 0.75- × 0.35-m quadrats per paddock to 2-cm height above soil level using electric clippers. The forage inside each of the quadrats was weighed fresh in the field and a fresh subsample (400 g approx.) was taken to the lab and separated into leaf, stem + sheath, and dead material. Each fraction was then dried separately in a forced-draft oven at 60°C to constant weight and weighed. From the fresh leaf fraction, approximately 30 leaf blades were scanned in a model LI 3100 leaf area meter (Li-Cor, Lincoln, NE) to determine the leaf area, and then dried separately in a forced-draft oven at 60°C to constant weight and weighed. The dry weight of each fraction was used to determine the relative proportion of leaf, stem, and dead material in the herbage mass. The relation of leaf area and leaf weight was used to obtain specific leaf weight and to estimate the total leaf area of the sample, which in turn was used to calculate the LAI.

Leaf net carbon exchange rate (CER) was measured with a model LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) (Figure 1), under a continuously controlled light intensity of 2000 µmol m^{-2} s^{-1} PPFD. The CER was measured during the 2013/2014 growing season on 9 and 28 Jan., 19 Feb., and 17 Mar. 2014. On each date, measurements were taken on the youngest fully expanded leaf of four vegetative tillers per paddock, between 900 h and 1200 h using a CO_2 concentration of 400 ± 1 µmol CO_2 mol^{-1} inside the chamber.

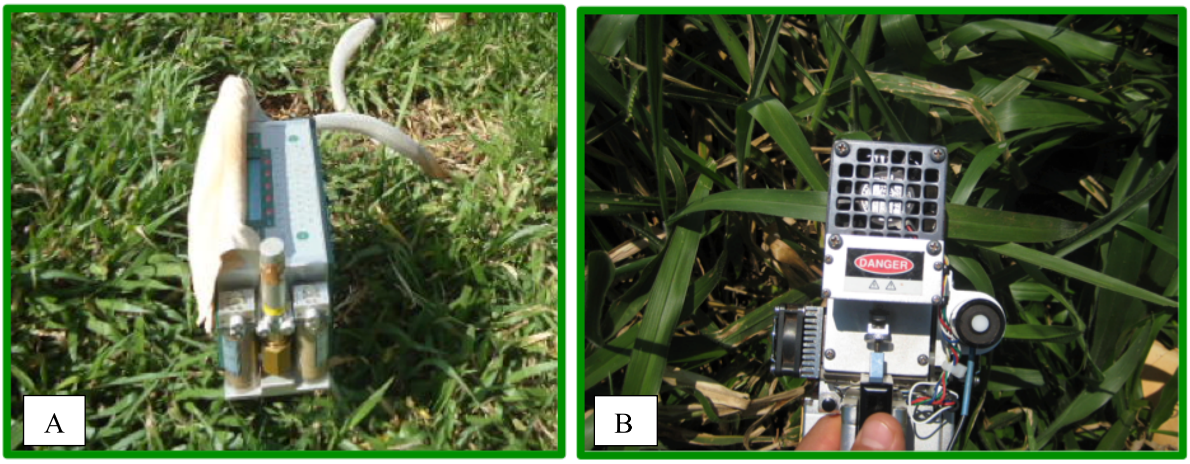

Figure 1 - Li-6400 (Li-Cor, Lincoln, NE) equipment (A) and measurement of leaf net carbon exchange rate of grazed Mulato II brachiariagrass

Canopy net carbon assimilation rates were estimated using the “sunlit and shaded leaves” model (BOOTE; JONES, 1987), that uses quantum efficiency, maximum leaf rate, light extinction (k), LAI, and photon flux density (equations presented in Appendix A). The sunlit LAI was calculated using the destructive LAI (previously described) values and k, and shaded LAI calculated as the difference between the total LAI and sunlit LAI. The adopted constants were: PAR = 2000 µmol m^{-2} s^{-1} (PEDREIRA; PEDREIRA, 2007); $k = -[\log_e (I/I_o)] / IAF$, where I and I_o correspond to the irradiance at the bottom and the top of the canopy, respectively; Q_e = 0,054 for C_4 plants (DIAS-FILHO, 2002). It was assumed that the coefficient of light reflection and transmission (σ) inside the canopy was 20%. The I and I_o values for calculating k were obtained from measurements taken every 21 d using a model LAI-2000 canopy analyzer (Li-Cor, Lincoln, NE) (Figure 2).

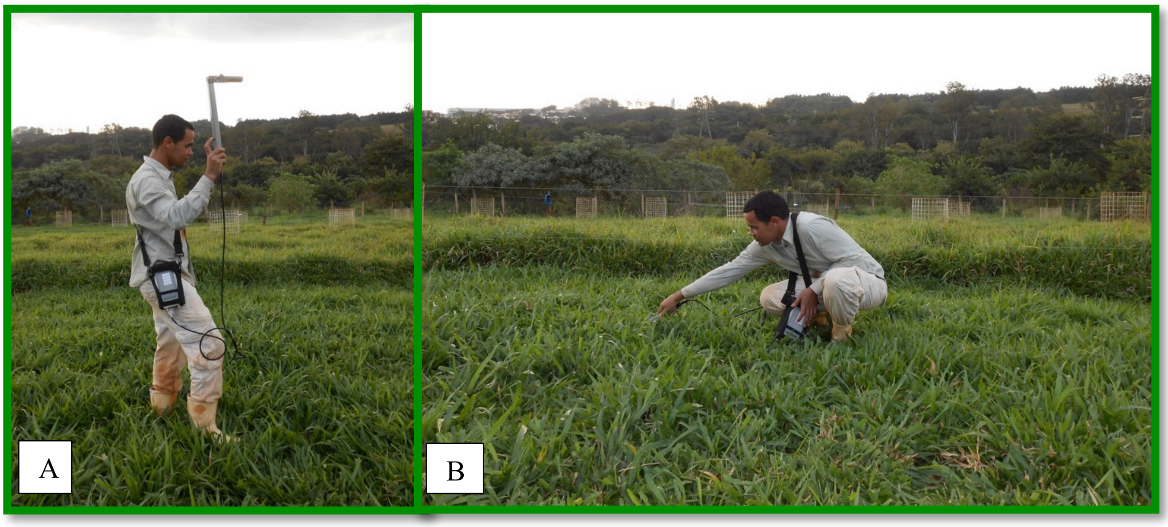

Figure 4 - Measurement using LAI-2000 canopy analyzer (Li-Cor, Lincoln, NE) above (A) and below (B) grazed Mulato II brachiariagrass canopy

3.2.3 Accumulation rates of herbage plant-part components

Accumulation rates of herbage components were calculated based on the results of the evaluation of the morphogenetic and structural characteristics of individual tillers. For the evaluation of morphogenetic characteristics, 30 tillers per paddock were marked at sites approximately 20 cm distant from each other along three transects, chosen to represent the average condition of each paddock. Measurements of different leaf categories (expanding, mature, and senescing/dead) and stem were taken twice a week during the two growing seasons (Figure 5). The leaf was considered mature after the complete leaf collar was visible, and senescing when reduction in the length of the lamina green area was detected, until the lamina become completely yellow or brown.

Figure 5 - Marked tillers (A) and leaf measurement (B) for the determination of morphogenetic characteristics of grazed Mulato II brachiariagrass

The marked tillers were harvested and replaced by new tillers every 24 d, and the lengths of the leaf laminae (expanding and mature leaves) and of the stems of basal tillers were measured. The plant part components were then hand separated and dried in a forced-draft oven at 60°C until constant weight. After weighing, a conversion factor between length and weight (g cm^{-1}) was established. Tiller population density was determined by counting the number of tillers within three 0.35- × 1.2-m quadrats placed at sites representative (visual assessment) of the average canopy condition at the time of evaluation.

Accumulation of herbage components was calculated based on the variation in the lengths of the leaf laminae and stems over the evaluation periods. Variations in both leaf and stem length were used to calculate the rates of leaf and stem elongation. The resulting rates of elongation and senescence per tiller (cm $tiller^{-1}d^{-1}$) were used to calculate the rates of growth and senescence (kg $ha^{-1}d^{-1}$) using the conversion factor generated for each tiller component, i.e., the conversion factor of expanding leaves was used for the calculation of leaf growth, the factor for mature leaves was used for the calculation of senescence, and the factor for stems was used for the calculation of stem growth, along with the corresponding average value of tiller population density (BIRCHAM; HODGSON, 1983). The rates of net HA were calculated as the difference between the rates of leaf + stem growth and senescence. The senescence-to-growth ratio was calculated as the ratio between leaf senescence and stem and leaf accumulation rates.

3.2.4 Herbage and root mass, and organic reserves

Root samples were collected at the end of the first (April 2013) and second (April 2014) growing seasons. In this evaluation, herbage mass data used were those from the samples collected to determine LAI (as previously described) because the root and shoot sampling dates were close to each other. At each root sampling event, a representative site was selected in each paddock, and a 0.2- × 0.5-m soil core sample was collected to a 0.2-m depth (Figure 6). The sample was immediately washed on sieves, and the roots taken to a forced-draft oven at 100°C for 1h to inactivate respiratory enzymes, followed by drying in a forced-draft oven at 60°C to constant weight, and weighed.

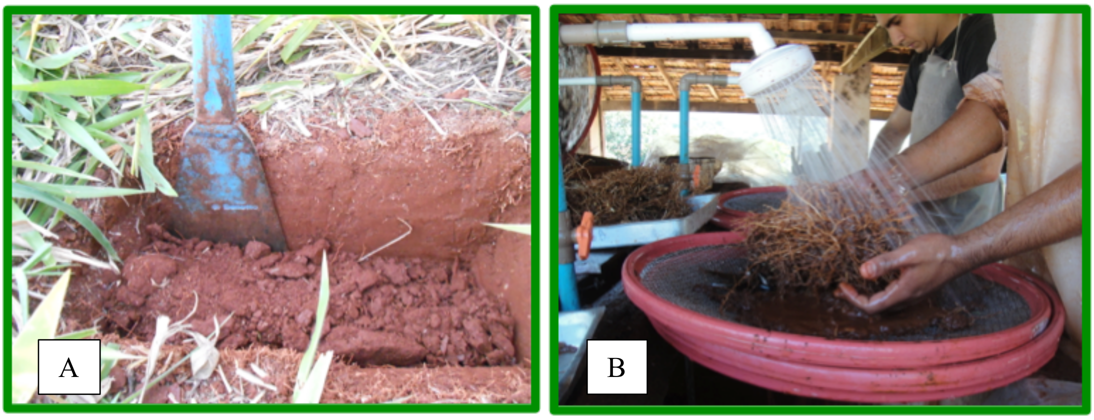

Figure 6 - Collecting (A) and washing root samples (B) of grazed Mulato II brachiariagrass

The root samples were ground in a Wiley mill to pass 1-mm screen, and used to determine concentration of N and total non-structural carbohydrates (TNC). Nitrogen concentration was determined using a modification of the block digestion technique (GALLAHER; WELDON; FUTRAL, 1975). Nitrogen in the digestate was determined by semi-automated colorimetry (HAMBLETON, 1977). The TNC concentration was determined following a modification of procedure of Smith (1981), as described by Christiansen et al. (1988), in which samples were incubated with invertase and amyloglucosidase to hydrolyze sucrose and starch, respectively, and then the resulting total hexoses were analyzed by a reducing sugar assay.

The dry root weight was used to estimate the root mass per area of soil, and root reserve pools were determined by multiplying the concentration by the respective root mass at each sampling event. Root-shoot ratio was obtained by dividing the root mass collected to a 20-cm depth by the average shoot mass. All root data are expressed on an organic matter (OM) basis.

3.2.4 Statistical methods

Data were analyzed using PROC MIXED of SAS (SAS - INSTITUTE, 2013) with a model including effects of canopy height, N rate, and their interactions. Year and block were considered random effects to allow for broader inference (LITTELL et al., 2006). In the model used for root mass and reserve pools, year was considered as a fixed effect to assess possible changes in reserve pools across years. The covariance structure was chosen based on Akaike's Information Criterion (AIC) (WOLFINGER, 1993). Quantile-quantile plots were used to check normality of the model residuals, and when necessary data transformations were performed. Lognormal transformation was used for LAI and root mass, but data presented are non-transformed data. Single degree of freedom orthogonal polynomial contrasts were used (linear and quadratic) to determine the response of variables to canopy height. Treatment means were compared using PDIFF by the Student test ($P < 0.05$), and least squares means are reported.

3.3 Results and Discussion

3.3.1 Leaf area index, leaf and canopy net assimilation rates

Canopy height affected LAI and leaf and canopy CER ($P < 0.0001$ for all variables). These responses were also affected by N rate ($P < 0.0001$; $P < 0.0001$; $P < 0.03$, respectively). The LAI decreased as canopy height decreased (Table 2), but leaf CER was greatest for 10-cm canopies (Table 2). The greater leaf CER with shorter canopy height is probably a result of lesser mean leaf age and increased tissue turnover that occur in these closely grazed swards because greater grazing intensity increases the frequency at which tillers are defoliated (SOLLENBERGER et al., 2012). In addition, there is less shade effect, since the LAI is also reduced, contributing to a greater leaf CER.

Table 2 - Leaf area index (LAI) and leaf ($Leaf_{CER}$) and canopy ($Canopy_{CER}$) carbon exchange rates in Mulato II brachiariagrass as affected by canopy height under continuous stocking in Piracicaba, SP, Brazil

Canopy height	LAI	$Leaf_{CER}$	$Canopy_{CER}$
cm	m^2 m^{-2}	-----mg CO_2 m^{-2} s^{-1}-----	
10	2.6	1.04	1.46
25	4.4	0.96	1.96
40	5.5	0.94	1.94
OPC†	L*	L*Q*	L*Q*
SE‡	0.25	0.02	0.19

* Significant at the 0.05 probability level.
†Orthogonal polynomial contrast for the effect of canopy height within a grass: L = linear, Q = quadratic.
‡SE = standard error.

Differently from leaf CER, canopy CER increased from 10- to 40-cm canopies (Table 2), but the response changed little for treatments taller than 25 cm. Even though leaf photosynthesis was generally greater in shorter canopies, canopy CER increased with increasing canopy height. The greater canopy CER of taller canopies is a result of increased LAI, which appears to be a trade-off for the reduction in individual leaf assimilation.

Contrasting results of leaf and canopy CER suggest that the reduction in LAI is probably more limiting to canopy CO_2 uptake than is leaf CER, and this contributed to lesser HA in shorter canopies. Lara and Pedreira (2011) suggested that LAI had a significant impact on calculated canopy CER of five brachiariagrasses (*Brachiaria* sp.). They reported small changes in leaf CER during the regrowth, but canopy CER differed and increased with increasing LAI.

Under full light conditions, canopy CER is maximized at a high LAI (HIROSE, 2005), which may contribute to increased radiation use-efficiency (KINIRY; TISCHLER; VAN ESBROECK, 1999). Our study supports that conclusion as greater canopy CER was achieved in taller canopies (Table 2). Parsons et al. (1983) reported a greater gross CO_2 uptake in a leniently grazed than heavily-grazed canopy, although a larger part of CO_2 was lost by respiration or by partitioning to non-harvested plant parts and eventually lost to senescence. This suggests that the greater CER and growth in taller canopies may not be associated with larger amounts of harvested forage in grazed pastures.

The greater N rate resulted in increased LAI and greater leaf and canopy CER compared with the lesser N rate (Table 3). Most N is accumulated in leaves, and about half of the total leaf N is used for photosynthetic activities (ONODA; HIKOSAKA; HIROSE, 2004), with lesser N concentration contributing to reduced leaf CER. During vegetative growth, N is used for the construction of either large leaf area or large leaf N concentration (SINCLAIR; HORIE, 1989). Bolton and Brown (1980) reported a linear increase in apparent leaf photosynthesis of *Lolium arundinaceum* (Schreb.) Darbysh., *Panicum maximum* (Jacq.), and *Panicum milioides* (Nees) as leaf N increased from 10 to 50 g kg^{-1} in the DM. The reduced canopy CER observed at lower N rates in the current study is likely a result of lesser leaf CER and reduced LAI. Thus, the combination of increased LAI and leaf CER due to N fertilization contributed to increase the canopy CER, which resulted in greater HA. In fact, in a companion study the total annual HA of Mulato II was increased by 69% with 250 compared with 50 kg N ha^{-1} (SILVA et al., 2016).

Table 3 - Leaf area index (LAI) and leaf ($Leaf_{CER}$) and canopy carbon exchange rates ($Canopy_{CER}$) in Mulato II brachiariagrass as affected by N rate under continuous stocking in Piracicaba, SP, Brazil

N rate	LAI	$Leaf_{CER}$	$Canopy_{CER}$
kg ha^{-1} yr^{-1}	m^2 m^{-2}	---mg CO_2 m^{-2} s^{-1}---	
50	3.7	0.85	1.54
250	4.8	1.12	2.03
$P^{\dagger}$	< 0.0001	0.023	0.031
SE‡	0.24	0.03	0.18

†*P* value for growth rates.
‡SE = standard error.

3.3.2 Rate of accumulation of plant-part components, net total herbage accumulation, and S:G ratio

The rates of plant-part component accumulation, net total HA and senescence-to-growth ratio (S:G) were affected by canopy height (P = 0.0002 for the stem component and P < 0.0001 for all other responses), but only the rates of leaf, dead material, and net total HA were affected by N rate (P < 0.0001 for all variables). The rates of leaf and dead material accumulation, net total HA rate, and the S:G increased linearly with increasing canopy height (Table 4). The rates of stem accumulation, on the other hand, changed little from 25 to 40 cm, resulting in linear and quadratic effects of canopy height.

Table 4 - Rate of plant-part accumulation (leaf, stem, and dead material), net total herbage accumulation rate (THAR), and senescence to growth ratio (S:G) in Mulato II brachiariagrass as affected by canopy height under continuous stocking in Piracicaba, SP, Brazil

Height	Leaf	Stem	Dead	THAR	S:G
cm	---------------------kg DM ha^{-1} d^{-1}------------------				
10	70	5	6	69	0.08
25	83	10	10	83	0.11
40	100	12	17	98	0.15
OPC†	L*	L*Q*	L*	L*	L*
SE‡	4	1	1	4	0.011

* Significant at the 0.05 probability level.
†Orthogonal polynomial contrast for the effect of canopy height within a grass: L = linear, Q = quadratic.
‡SE = standard error.

Leaf was the main component of HA of Mulato II under continuous stocking and had the greatest contribution to net total accumulation rate (Table 4). This is likely a result of the steady-state canopy height, where grazing happens at the top of the canopy, which in the vegetative phase is comprised mostly of leaves. Even though the stem accumulation rates increased with canopy height, the contribution of stem to total HA was relatively small. The greater rates of plant part accumulation in taller canopies contributed to a greater net total HAR, likely a result of increased canopy CER (Table 2), and this has been associated with higher HA (PARSONS et al., 1983). In a companion study, Silva et al. (in press) reported that the annual HA of Mulato II was greater for 40-cm, compared with 10- and 25-cm canopy heights. Increased HA for taller canopies under continuous stocking has been reported for other brachiariagrasses such as 'Xaraes' (CARLOTO et al., 2011) and 'Marandu' (FLORES et al., 2008) palisadegrasses.

The rate of dead material accumulation was greater in taller canopies, probably due to a self-shading effect and greater LAI. Net total HAR, however, was also increased indicating that the increase in senescence was not great enough to compromise the potential of HA in taller canopies. The combination of high senescence rates and herbage mass, however, can contribute to lesser grazing efficiency (SILVA et al., 2016). Additionally, increased senescence rates in taller canopies may be detrimental to the canopy structure in the long term. Even though dead material proportion in the grazed forage is small, an increase may occur in dead material proportion in the total herbage mass, compromising tillering dynamics due to low light intensity at the base of the canopy.

The increase in N rate from 50 to 250 kg ha^{-1} increased the HAR of leaf and net total herbage, but also of dead material (Table 5). This is likely a result of increased plant growth due to N fertilization, first affecting canopy CER and the main component of HA (leaf), but also increasing the accumulation of stem and dead material. Despite the increased dead material HAR, S:G was not affected by N rate (P = 0.28) suggesting that the increase in proportion of dead material is likely a result of more rapid plant growth, and that increased harvest efficiency may be achieved through more efficient grazing.

Table 5 - Accumulation rate of plant part components and net total herbage accumulation rate (THAR) in Mulato II brachiariagrass as affected by N rate under continuous stocking in Piracicaba, SP, Brazil

N rate	Leaf	Stem	Dead	THAR
kg ha^{-1} yr^{-1}	------------------------------kg DM ha^{-1} d^{-1}--------------------------------			
50	70	6	9	66
250	99	13	13	100
$P^{\dagger}$	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SE‡	3.7	1	0.7	3.4

†*P* value for growth rates.
‡SE= standard error.

3.3.3 Herbage and root mass, and organic reserves

Herbage mass increased (*P* < 0.0001) linearly from 0.7 to 1.6 kg DM m^2 as canopy height increased from 10 to 40 cm, but was not affected by N rate or by the height × interaction (*P* > 0.05). Root mass was affected by canopy height × N rate interaction (*P* = 0.0023). The root mass was less when canopies where kept at 10 cm and increased with increasing canopy height, but it changed little from 25 to 40 cm for pastures receiving 50 kg N ha^{-1}, resulting in linear and quadratic effects (Table 6). For pastures receiving 250 kg N ha^{-1}, on the other hand, root mass increased linearly with increasing canopy height. Canopies grazed at 25 cm and receiving 250 kg N were associated with less root mass than those receiving 50 kg N (*P* = 0.0001).

Table 6 - Root mass as affected by the interaction of canopy height and N rate for Mulato II brachiariagrass under continuous stocking during two growing seasons in Piracicaba, SP, Brazil

N rate	Height (cm)			OPC†	SE‡
	10	25	40		
kg ha^{-1} yr^{-1}	---------kg OM m^2--------				
50	1.2	1.6	1.7	L*Q*	0.09
250	1.1	1.4	1.6	L*	0.09
$P^{\S}$	0.0636	0.0001	0.2537		

* Significant at the 0.05 probability level.
†Orthogonal polynomial contrast for the effect of canopy height within a grass: L = linear, Q = quadratic.
‡SE = standard error.
§*P* value for growth rates.

Root TNC concentration at the end of the grazing season was not affected by year (*P* = 0.0555), height (*P* = 0.6766), N rate (*P* = 0.3142), or any interactions (*P* > 0.05), averaging 32.2 g kg^{-1}. The root TNC pool, on the other hand, was affected by canopy height (*P* =

0.0021) and year (P = 0.003). Root TNC pool increased linearly from 34.7 to 46.6 to 55.8 g m^2 for 10, 25, and 40-cm heights, respectively (data not presented), and was greater at the end of the first year compared with the second (52.8 vs. 38.6 g m^{-2}, respectively). The root TNC pool is strongly affected by the total root mass. Thus, more intensively grazed pastures (10 cm) that experienced a reduction in root mass also had a smaller root TNC pool (Table 6). Close and frequent defoliation reduced root mass and TNC pools of *Pennisetum* spp., affecting grass persistence (CHAPARRO; SOLLENBERGER; QUESENBERRY, 1996). Carvalho et al. (2001) studied HAR, root mass, and root reserves of three *Cynodon* sp. grasses ('Tifton 85', 'Florakirk', and 'Coastcross') under continuous stocking and with canopy heights of 5, 10, 15, and 20 cm. They reported that root mass was affected only by grass, and root TNC pools followed the same trend as root mass. In the present study, both shoot mass and root mass decreased as canopy height decreased, resulting in a reduced TNC pool in shorter canopies. Although the canopy CER, accumulation of herbage plant-part components, LAI, shoot mass, root mass, and reserve pools were all reduced in the shorter canopy height (10 cm), there was no visible sign of reduction in persistence in any of the paddocks at the end of the experimental period (e.g., no visually evident areas with bare soil, low tiller/stand density, or weed encroachment). Two summer grazing seasons may not be enough to assess Mulato II persistence under the conditions studied and our findings do not support the trade-off between reduced LAI of shorter canopies and a larger population density of young tillers as suggested by Silva et al. (2015b) for continuously stocked Marandu palisadegrass. Greater tiller appearance rate and greater tiller population density was reported by (SILVA, 2015) for 10-cm canopies in a companion study. Even though leaf CER was greater in 10-cm pastures, canopy CER and TNC were reduced. In addition, in the same companion study the HA was reduced by 36% at a 10- vs. 40-cm canopy height.

Root N concentration was affected only by N rate (P = 0.0012). The N pool, on the other hand, was affected by year (P = 0.0076) and N rate (P = 0.0032). The root N concentration and pool was greater for 250 kg N compared to 50 kg N (Table 7). This change in N pool is likely a result of overall N availability due to more N applied, since root N concentration was also increased. The ranges in the N concentration and pool, however, were much less than the range in the N rates applied. The N pool was slightly higher for the high N rate at the end of the first growing season compared with the second (11.3 vs. 8.78 g m^{-2}, respectively), which may be a result of changes in root mass. According to Pedreira et al. (2000), N compounds do not seem to be used and stored in the same way as C compounds, resulting in more evident responses of TNC to grazing management than that of N.

Table 7 - Nitrogen concentration and pool in the roots of grazed Mulato II brachiariagrass as affected by N rate under continuous stocking during two growing seasons in Piracicaba, SP, Brazil

N rate	N concentration	N pool
kg ha^{-1} yr^{-1}	------g kg^{-1}-------	-------g m^{-2}--------
50	9.1	8.6
250	10.6	11.5
P†	0.0012	0.0032
SE‡	0.2735	0.6097

†*P* value for N rate.
‡SE= standard error.

3.4 Summary and Conclusions

Leaf CER increased when Mulato II pastures were grazed to maintain a 10-cm canopy height, but canopy CER was greater in taller canopies (25 and 40 cm) which had greater LAI. The HAR of all plant parts was greater in taller canopies, resulting in greater net total HAR. Herbage accumulation of Mulato II under continuous stocking is achieved primarily by high leaf accumulation rates, increasing from 70 to 100 kg DM ha^{-1} d^{-1} as canopy height increased from 10 to 40 cm. The greater canopy CER of taller canopies likely contributed to increased plant-part HAR, as well as to maintain greater root mass and TNC pools. After two years of grazing, even though there was no sign of decreased persistence of Mulato II in shorter canopies, the canopy CER, LAI, shoot and root mass, TNC pools, leaf and total net accumulation rates were significantly reduced, suggesting that taller canopies (25 or 40 cm) are advantageous for Mulato II under continuous stocking. The increase in N rate from 50 to 250 kg N ha^{-1} affected plant growth, increasing CER, LAI, and the HAR of plant-part components, as well as dead material and net HAR, but the S:G was not affected. The increase in rates of dead material accumulation due to more N applied is a result of overall increase in plant-part accumulation, and increased harvest efficiency may be achieved through improved grazing management.

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4 GENERAL COMMENTS AND CONCLUSIONS

Mulato II brachiariagrass, although recently released, is already considered a good forage option due to comparable herbage accumulation (HA) and greater nutritive value compared to the widely used Marandu palisadegrass (TEODORO, 2011; DEMSKI, 2013; PEQUENO et al., 2015) and other brachiariagrass hybrids genotypes (PIZARRO et al., 2013; HARE et al., 2015b). Studies have been conducted in Brazil, Mexico, Honduras, Colombia, Australia, Unganda, Vietnan, Thayland, United States, and other countries (DEMSKI, 2013; VENDRAMINI et al., 2013; HARE et al., 2015a; PEQUENO et al., 2015), but most of them evaluating harvest frequency or intensity of defoliation under clipping conditions or intermittent stocking. The objective in this study was to assess the agronomic performance of Mulato II brachiariagrass maintained at three constant canopy heights by grazing, receiving two N fertilization rates. The results indicate that the HA and forage nutritive value of Mulato II are impacted by canopy height and N rate.

Difficulties in the quantification of management effects under continuous stocking conditions exist. The most commonly used method for determination HA studies under continuous stocking is the exclosure cages. It has been argued that this technique may either overestimate or underestimate HA, and there is evidence to the fact that this may depend on canopy condition at the time of placement of cages. Under low forage mass conditions the use of exclosure cages may overestimate HAR by 33 to 55% (BARKER et al., 2010). According to Barker et al. (2010), the error in estimating herbage mass may have a greater effect on HAR at low than at high herbage mass. This may contribute to hide differences between grazing intensities, resulting in similar HA for a wide range of grazing intensities (e.g. canopy heights). In this study the HA obtained using exclosure cages was 36% less when Mulato II brachiariagrass was maintained at 10 cm compared to 40 cm, and increased linearly with canopy height. This differences, however, could be higher. When the accumulation of plant-part components was calculated using morphogenetic characteristics, the accumulation of leaf - the main component of HA - was 30% less when pastures were maintained at 10 cm compared to 40 cm.

Despite the reduction in HA, plant-part accumulation, root mass, and TNC pools, canopies maintained at 10 cm did not show sign of stand degradation, suggesting that two summer grazing seasons may not be enough time to assess detrimental effects of high grazing intensities on Mulato II brachiariagrass. This may indicate considerable flexibility of this grass, but also that under high grazing intensity Mulato II pasture is less productive and may result in cumulative negative impact in a long-term.

Short canopy height (10 cm) resulted in greater leaf carbon assimilation rates than in taller canopies (40 cm), but canopy photosynthesis was reduced, contributing to a reduced rate of plant-part components accumulation and HA. The grazing efficiency, however, was increased as canopy height decreased. When pastures were maintained at 25 cm, the HA and forage nutritive value were intermediatecompared to those maintained at 40 and 10 cm, but grazing efficiency was similar to that of pastures maintained at 10 cm, suggesting that canopy heights around 25 cm are optimal for continuously stocked Mulato II.

Nitrogen fertilization affects positively the HA of Mulato II brachiariagrass. Compared to 50 kg N ha^{-1} the use of 250 kg N ha^{-1} increased leaf carbon assimilation rates and leaf area index, which contributed to a greater HAR and greater rates of accumulation of plant-part components. This resulted in greater accumulation rates of dead material, and net HAR, and ultimately in greater HA. The increase in HA contributes to improve carrying capacity of tropical pastures and to reduce the need for land area to increase beef cattle production to meet the demand for food production.

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APPENDIX

Appendix A

Equations of "sunlit and shaded leaves" model (BOOTE; JONES, 1987)

$$LAI_{sun} = (1/k)[1 - \exp(- k \times LAI_{total})] \quad \text{(Eq.1)}$$

$$LAI_{shade} = LAI_{total} - LAI_{sun} \quad \text{(Eq.2)}$$

$$k = -[\log_e(I/I_o)]/IAF \quad \text{(Eq.3)}$$

$$P_{sun} = P_{max}\{1- \exp[- Q_e \times k(1 - \sigma)\, PAR/P_{max}]\} \quad \text{(Eq.4)}$$

$$PAR_{shade} = \sigma \times PAR\,[1- \exp(- k \times LAI_{shade})]/LAI_{shade} \quad \text{(Eq.5)}$$

$$P_{shade} = P_{max}[1- \exp(- Q_e \times PAR_{shade}/P_{max})] \quad \text{(Eq.6)}$$

$$P_{canopy} = P_{sun} \times LAI_{sun} + P_{shade} \times LAI_{shade} \quad \text{(Eq.7)}$$

Where:

LAI_{sun} = leaf area index of sunlit leaves;

LAI_{shade} = leaf area index of shaded leaves;

LAI_{total} = total leaf area index;

k = light extinction coefficient;

I and I_o = irradiance at the bottom and the top of the canopy, respectively;

P_{sun} = carbon assimilation by sunlight leaves;

P_{max} = maximum leaf carbon assimilation;

Q_e = coefficient of light use efficiency;

σ = coefficient of light reflection and transmission;

PAR = photosynthetic active radiation; and

P_{canopy} = estimated canopy CO_2 assimilation.