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Critical concentration and management of nitrogen fertilization in the establishment of *Brachiaria* hybrid Mavuno[1]

Concentração crítica e manejo da adubação nitrogenada no estabelecimento de *Brachiaria* híbrida Mavuno

Lilian Elgalise Techio Pereira[2]*, Luan Jardim de Sousa[2], Liandra Maria Abaker Bertipaglia[3], Valdo Rodrigues Herling[2], Adriano Rogério Bruno Tech[4]

ABSTRACT - The critical range of leaf nitrogen ($\%N_{Leaf}$) concentration can be used to monitor the plant's nitrogen (N) status, being an essential tool to define strategic fertilization protocols, where the time and amounts of fertilizers are applied according to pasture's demand. The productivity parameters and two methods to determine N sufficiency – (i) the N nutrition index (NNI) and (ii) the normalized N sufficiency index (NSI) – were evaluated in *Brachiaria* hybrid Mavuno (*Brachiaria* spp. Syn. *Urochloa* spp.) along the regrowth cycles of the first growth season after seeding (establishment phase). The aims of the study were to estimate the critical $\%N_{Leaf}$ for Mavuno grass pastures through productivity parameters, considering the sampling of the youngest expanded leaf as reference. Fertilization rates were defined as follow: no-nitrogen (N0), 15 (N15), 30 (N30) and 45 (N45) kg ha^{-1} of N and were applied after each cutting. The Falker chlorophyll index (FCI) was obtained from a portable chlorophyll meter Falker ClorofiLOG® CFL1030, and results were used to calculate the NSI. The regression analysis, on a regrowth cycle basis, provided the parameters to estimate the critical range of $\%N_{Leaf}$ and the fertilization rates to attain the maximum forage accumulation. Population density of tiller was the main response contributing to increasing leaf and stem mass, leaf area index and forage accumulation as N fertilization increased. The FCI and NSI were unable to capture differences in $\%N_{Leaf}$, but NNI was a suitable tool to detect N status in Mavuno grass. The critical $\%N_{Leaf}$ range varied from 2.75 to 3.07% and may be indicated to monitor pasture's N status. The strategic N fertilization protocol suggested for the growth season after seeding is to apply 42.0 to 46.7 kg ha^{-1} of N at the first regrowth, 27.0 to 32.5 kg ha^{-1} of N during the two following regrowth cycles, whereas at the end of the season (April to May regrowth) only 22.2 to 24.7 kg ha^{-1} of N would be enough to sustain a maximum growth.

Key words: *Brachiaria* spp. Best management practices. Nitrogen sufficiency. Tropical pastures.

RESUMO - A amplitude crítica na concentração foliar de nitrogênio (N_{Foliar}) pode ser utilizada para monitorar o status de N das plantas, sendo ferramenta essencial para definir protocolos de fertilização estratégica, onde o período e quantidades de fertilizantes são aplicados de acordo com a demanda da pastagem. Foram avaliados parâmetros de produtividade e dois métodos para determinação da suficiência em N – (i) o índice de nutrição nitrogenada (INN) e (ii) o índice normalizado de suficiência em N (INS) – em *Brachiaria* híbrida Mavuno (*Brachiaria* spp. Syn. *Urochloa* spp.) ao longo dos ciclos de rebrotação da primeira estação de crescimento após a semeadura (fase de estabelecimento). Os objetivos do estudo foram estimar amplitude crítica do N_{Foliar} para o capim Mavuno através de seus parâmetros de produtividade, considerando a amostragem da folha mais jovem completamente expandida como referência. Os níveis de fertilização adotados foram: 0 (N0), 15 (N15), 30 (N30) e 45 (N45) kg ha^{-1} de N, e foram aplicados após cada corte. O medidor de clorofila portátil Falker ClorofiLOG® CFL1030 foi utilizado para obter o Índice de Clorofila Falker (ICF), e os resultados foram utilizados para calcular o INS. Análises de regressão, tomando por base cada ciclo de rebrotação, proveram os parâmetros para estimar de amplitude crítica do N_{Foliar} e os níveis de fertilização requeridos para atingir o máximo acúmulo de forragem (FAc). A densidade populacional de perfilhos foi a principal resposta que contribuiu para aumentos em massa de folhas e colmos, índice de área foliar e FAc com aumentos nos níveis de fertilização. O índice ICF e o método NSI não foram capazes de detectar diferenças em N_{Foliar}, mas o NNI foi considerado adequado para detecção do status de N. A amplitude crítica do N_{Foliar} variou de 2,75 a 3,07%, e pode ser indicada para monitorar o status de N da pastagem. O protocolo de fertilização estratégica sugerido para a estação de crescimento sucessiva ao estabelecimento da pastagem corresponde à aplicação de 42,0 a 46,7 kg ha^{-1} de N no primeiro ciclo de rebrotação, 27,0 a 32,5 kg ha^{-1} de N durante os dois ciclos subsequentes, mas para ciclos de rebrotação do final da estação de crescimento (abril a maio) apenas 22,2 a 24,7 kg ha^{-1} de N seriam suficientes para sustentar o máximo acúmulo de forragem.

Palavras-chave: *Brachiaria* spp. Melhores práticas de manejo. Suficiência em N. Pastagens tropicais.

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INTRODUCTION

Nitrogen (N) fertilization is well known to act maximizing tillering, leaves renewal and photosynthesis (YASUOKA *et al*., 2018), thus sustaining pasture's growth, productivity and long-term persistency. However, once insufficient N leads to lower chlorophyll content and photosynthetic rates, less biomass and poor soil covering, but excessive N fertilization can lead to the soil and water pollution, increased greenhouse gases (GHG) emissions and economic losses (CARDOSO *et al*., 2016), best management practices for N fertilization has been recognized as the main options towards the establishment of sustainable pastured-based systems (SILVA *et al*., 2017).

Strategic fertilization, modifying the timing and rates of fertilizer applied, have potential to be more efficient in N use (VILLAR *et al*., 2015), decreasing both N inputs and losses (WILSON *et al*., 2020) with little impact on pasture yield, compared to approaches based on the traditional fertilization strategies, where N amount is set to a fixed rate applied following grazing (SMITH *et al*., 2018). For this purpose, the knowledge and monitoring of plant's N status are important to define the time and amount of fertilizers according to demand (CORRÊDO *et al*., 2019).

The Nitrogen Nutrition Index (NNI) is an effective tool for determination and monitoring plant's N status (FLETCHER; CHAKWIZIRA, 2012), once is an indicator well connected with the physiological regulation of N uptake at canopy level (LEMAIRE; JEUFFLOY; GASTAL, 2008). Despite the NNI be considered a practical and adequate tool for in-season plant N diagnosis, the method requires that whole plant shoots or all leaves at the top of canopy be sampled (FARRUGGIA; GASTAL; SCHOLEFIELD, 2004). However, the metabolic activities during the pasture establishment after sowing mainly rely on metabolic components (leaves) rather the structural component (stems), and at this stage the N allocation is optimized to satisfy the growth demand of leaves (YAO *et al*., 2014; ZHAO *et al*., 2017). Once forage accumulation during the pasture's establishment phase depends heavily on the leaves (WANG *et al.*, 2017), it has been proposed that the determination of critical N based on the actual leaves N concentration might be appropriate for N diagnosis (ATA-UL-KARIM *et al*., 2017; ZHAO *et al*., 2017). Lavres Júnior, Santos Júnior and Monteiro (2010) pointed out that the youngest expanded leaf is a morphological component recommended for evaluating N status in tropical grasses. The use of this leaf in nutritional diagnosis is of interest once would offer new prospects in real time field-based evaluations, also through spectral methods based on image analysis and computer vision applications, which are amenable to rapid and relatively inexpensive screening (SADRAS; LEMAIRE, 2014). Other parameters have also been described as a way to make nutritional diagnosis faster and practical, such as the normalized Nitrogen Sufficiency Index (NSI), obtained from chlorophyll meters, being considered a convenient alternative to laboratory analyses (CORRÊDO *et al*., 2019; COSTA *et al*., 2015), due to a strong relationship between chlorophyll and N content in several crops (SAMBORSKI; TREMBLAY; FALLON, 2009).

Based on this background, we evaluated productivity parameters, leaf N concentration and the potential for using the NNI and NSI for detecting N status of *Brachiaria* hybrid Mavuno (*Brachiaria* spp. Syn. *Urochloa* spp.) during the phase of establishment, which included the regrowth cycles throughout the first growth season following seeding. Considering the productivity parameters, the main aims of the study were: i) to determine a critical N range concentration for Mavuno pastures during the phase of establishment, considering the sampling of the youngest expanded leaf as reference, and ii) to suggest a strategic N fertilization protocol to this grass focusing the definition of best management practices for the establishment phase.

MATERIAL AND METHODS

Experimental area and treatments

The experiment was carried out at Faculty of Animal Science and Food Engineering (FZEA), University of São Paulo, Pirassununga, SP, Brazil (21o57'31'' S, 47o27'07'' W 21º36' N, 47º15' W, 620 m a.s.l.). The climate in the region is Cwa, sub-tropical with dry winter (ALVARES *et al.*, 2013), and the annual average rainfall is 1,238 mm. In the experimental area, the slope is moderately undulating and the soil is classified as Rhodic Hapludox (SOIL SURVEY STAFF, 2015).

Soil samples were collected at 0-20 cm soil depth in July 2018 (ten subsamples were grouped into a composed sample), and results were used to establish the need of nutrients correction. Soil chemical analysis revealed the following chemical characteristics: pH ($CaCl_2$) = 5.2; P (resine) = 9.0 mg dm^{-3}; S (calcium phosphate turbidimetric method) = 23.0 mg dm^{-3}; K (resine) = 4.4 $mmol_c$ dm^{-3}; Ca (resine) = 25.0 $mmol_c$ dm^{-3}; Mg (resine) = 6.0 $mmol_c$ dm^{-3}; Organic matter (colorimetric method), (YEOMANS; BREMNER, 1988) = 16.0 g kg^{-1}, soil base saturation (V) = 51.0%. The micronutrients corresponded to: B (hot water extraction) = 0.48; Cu (DTPA - diethylenetriaminepentaacetic acid - micronutrient extraction method) = 3.60; Fe (DTPA)= 14.06, Mn (DTPA) = 8.79 and Zn (DTPA) = 1.04 mg dm^{-3}.

The experimental area was comprised of 16 plots of 20 m² (5 m x 4 m) each. The studied species was *Brachiaria* hybrid Mavuno, registered in the Ministry of

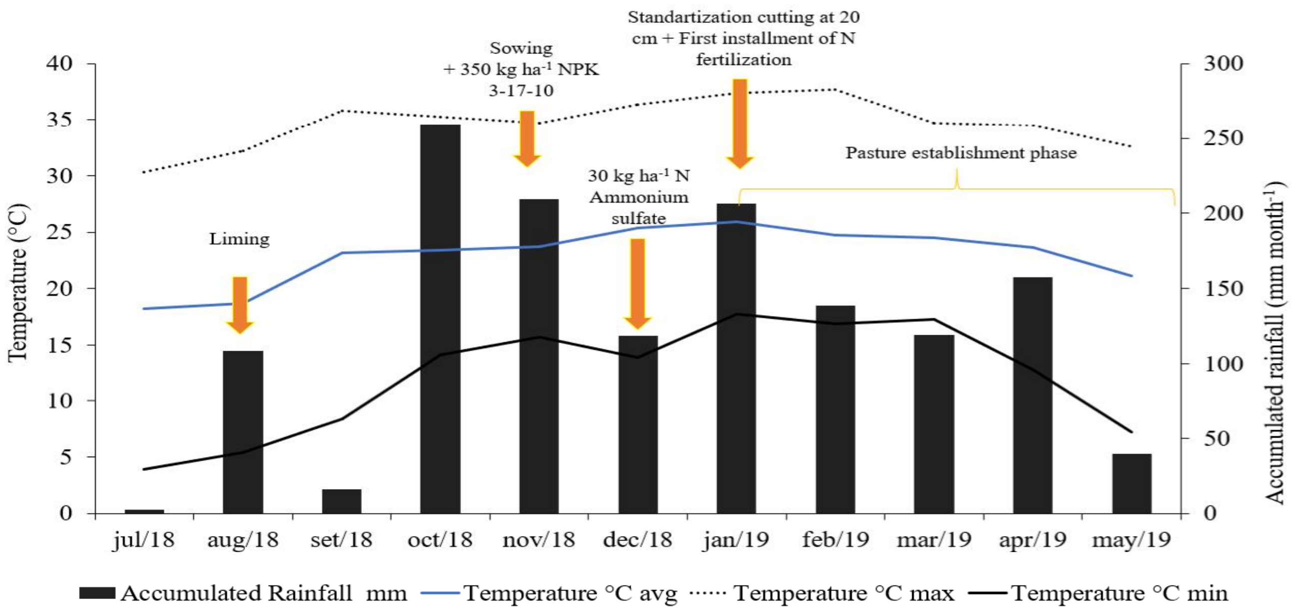
Agriculture, Livestock and Food Supply as MIXE DRWN 12, nº. 30488, and originated from crossing between a maternal line of *Brachiaria ruziziensis* x *Brachiaria brizantha* and a male line of *Brachiaria brizantha* x *Brachiaria brizantha*. It is tropical perennial grass with a tussock-forming architecture, able to form roots at the nodes when their stems are in contact with soil. However, there is no scientific research estimating productivity responses and N parameters for guide fertilization strategies for this grass until the present. Liming was manually performed in August 2018, using dolomitic limestone aiming a soil base saturation of 60% (VAN RAIJ *et al.*, 1997). Mavuno grass was sown in 08 of November 2018, using the equivalent to 15 kg ha^{-1} of commercial seeds. Seeds were manually distributed each 40 cm, in lines spaced 25 cm each other. A first instalment of fertilization was applied in the day of sowing, using the equivalent to 350 kg ha^{-1} of a commercial formula 03-17-10 (N-P-K, corresponding to 10 kg ha^{-1} of N, 60 kg ha^{-1} of P_2O_5 and 35 kg ha^{-1} K_2O). Around 45 days after sowing were manually applied onto the soil surface without incorporation in all plots the equivalent to 30 kg ha^{-1} of N using ammonium sulfate. A standardization cutting was made at a 20 cm residual height (Figure 1) in January and, the regrowth cycles afterwards were established on a 30-day basis, on average, leaving a residual height of 20 cm in all treatments.

The first evaluation cutting was made on 20 February 2019, corresponding to the regrowth cycle from January to February, and subsequent cuttings were made at 20 March 2019 (February to March), 18 April 2019 (March to April) and 22 May 2019 (April to May). Due to the low forage accumulation rates and low rainfall verified at May, the establishment phase was finished after the fourth cutting, and no fertilization was made afterwards. In order to generate gradients of N concentration in the plants, four nitrogen rates were defined and applied at the beginning of regrowth and after each cutting procedure in January, February, March and April: no-nitrogen (N0), 15 (N15), 30 (N30) and 45 (N45) kg ha^{-1} of N using urea. The treatments were distributed in a randomized complete blocks design with four replications.

Measurements

Measurements of sward height were taken each seven days after cutting until the plots have reached the defined cutting target. The height was defined as the average of 10 systematic readings along two transect lines into the plot, using a graduated measuring stick. For the determination of forage mass (FM) and morphological composition at the pre-harvest, two samples were collected at a residual height (20 cm from soil level), each one within the area of a metal frame measuring 0.50 x 0.50 m (0.25 m^2). Subsequently, the samples were weighed and separated into two subsamples: one for the determination of the dry matter (DM) content, and the other to hand-separate into leaf (leaf laminae), stem (leaf sheath + stems) and dead material components. Morphological components were

Figure 1 - Monthly accumulated rainfall (mm month^{-1}), averages of minimum, maximum and mean temperatures (ºC) along the experimental period. Arrows represent the periods in which the pre-establishment procedures related to liming, sowing and standardization cut were performed. Pirassununga, São Paulo, Brazil



dried up to constant mass in a forced-air oven at 65 °C, and the data were used to calculate the FM and the proportion of the morphological components, leaves, stems (leaf sheath + stems) and dead material, as well as their mass. Total herbage mass above the residue, estimated from the total weight of the sample taken within the metal frame and the respective dry matter content of the sub-sample (%DM), was considered the forage accumulation during each regrowth cycle (kg ha^{-1} of DM).

The tiller population density (TPD, tiller m^{-2}) was determined by counting all tillers within two metallic frame measuring 0.50 x 0.50 m (0.25 m^2) always the plots reached the defined pre-cutting target. For determination of average tiller weight (TW, g $tiller^{-1}$) and the specific leaf area (SLA, cm^2 g^{-1} of leaves), ten tillers were randomly chosen into the plot, and their morphological components were hand-separate into leaf (leaf laminae), stems (leaf sheath + stems) and dead material. The leaves were immediately placed side by side on an image collecting table. The image collecting table was built using a white matte background (to avoid the interference of brightness on the image), with a fixed support for the cellphone acquire three images at a height of 23 cm from the base. A black square measuring 1 cm^2 (1 x 1 cm) was placed at the left side in the collecting table, which is used as the reference for the software USPLeaf® to measure the leaf area of the samples (TECH *et al*., 2018). The digital images were stored as 24-bit color images with resolution of 800 x 600 pixels and saved in RGB color space in JPEG format. Morphological components were dried up to constant mass in a forced-air oven at 65 °C. The TW was then determined as the sum of dry weight of the morphological components divided by the number of tillers collected, and the SLA was determined by the relationship among the leaf area of the sample (cm^2), determined by the images processed in the USPLeaf® software, and the dry weight of the leaves (g). The leaf area index (LAI) was then obtained as follow: LAI = [FM (g m^{-2}) × LP (%)] × SLA (cm^2 g^{-1}), where FM means total forage mass; LP: leaf proportion in the total forage mass, obtained from the subsamples of FM; and SLA: the specific leaf area.

At the time of cutting, the younger completely expanded leaf (diagnostic leaf) from 20 tillers randomly chosen into the plot was detached from the plant and the total N concentration ($\%N_{Leaf}$) was then determined (FARRUGGIA; GASTAL; SCHOLEFIELD, 2004; LAVRES JÚNIOR, SANTOS JÚNIOR; MONTEIRO, 2010; LEMAIRE; JEUFFLOY; GASTAL, 2008), using the Kjeldahl method (NOGUEIRA; SOUZA, 2005). The critical N concentration (%Nc) was calculated according Lemaire, Jeuffloy and Gastal (2008), as follow: $\%Nc = a_c W^{-b}$, where %Nc represents the critical plant N concentration required for the maximum growth rate; a_c is the critical plant N concentration for W = 1 ton ha^{-1} DM whose value for tropical species is 3.6; and b is a tabulated value, suggested by the authors, of 0.34. Nitrogen nutrition index (NNI) was determined by the relationship between the $\%N_{Leaf}$ of the samples determined in the laboratory and the calculated value of %Nc.

The chlorophyll index was determined in 10 tillers randomly selected per plot, considering the younger completely expanded leaf (diagnostic leaf). Readings were taken at left and right side of the central nervure at the medial portion and in the adaxial face of the leaf, using a portable meter (Falker ClorofiLOG®, Model CFL1030). The chlorophyll meter emits light at three wavelengths (λ), 635 and 660 nm (red) and 880 nm (infrared), and the measurement units are called Falker Chlorophyll Index (FCI), providing instantaneous indices varying from 0 to 100 (SCHLICHTING *et al*., 2015). To avoid differences in crop growth stages and compare the N status from the different N fertilization rates it was calculated the index called normalized Nitrogen sufficiency index (NSI), according to Samborski, Tremblay and Fallon (2009), by dividing the averages of total chlorophyll reading in each N rate by the corresponding average total chlorophyll reading from the highest N rate (COSTA *et al*., 2015; SAMBORSKI; TREMBLAY; FALLON, 2009; VILLAR *et al*., 2015).

Statistical analysis

Analysis of variance was carried out using mixed models in the software SAS®, version 9.3 for Windows®. The fertilization rates, regrowth cycles and its interactions, and blocks were considered fixed effects. The regrowth cycle was considered a repeated measurement, and the covariance matrices were selected using the Bayesian Information Criterion (BIC). For all variables, correction for degrees of freedom was applied according to the Kenward and Roger (1997) method. When appropriate, means comparisons were made using the Tukey test, and significant differences were declared when $P < 0.05$. Regression analysis were carried in the software SAS®, version 9.3 for Windows®, considering the relationship between N fertilization rates applied in the field and the productivity parameters. For the N status parameters, when detected significant effects of fertilization rates and regrowth cycles, the regression analysis was carried out on a regrowth cycle basis (REYES *et al*., 2015). The parameters of the regression equations allowed to estimate the point of maximum forage accumulation and also provided the models to estimate the required N rates to attain this level (GASTAL *et al*., 2015).

RESULTS AND DISCUSSION

Parameters of productivity along the growth season

There were no significant interactions between regrowth cycles and fertilization rates for the parameters of productivity evaluated (P > 0.05), whereas a significant isolated effect of the regrowth cycle was detected (Table 1).

The highest values of forage accumulation (FAc), leaf (LM) and stems mass (SM) were registered during the first regrowth cycle, for which was also observed a higher sward height (SH). Parallelly, the dry matter content (%DM) of the total forage mass was lower during the first and second regrowth cycles. The growth's potential of the pasture was higher due to the favorable climatic conditions during the first regrowth cycle, with no restrictions of rainfall and temperatures, and the presence of a young tiller population (up to 90 days old), which is typical of the establishment phase of the pasture. Tillers at this age are proven to express higher leaf appearance and elongation rates, lower senescence rates and stem length, and a higher number of live leaves (MONTAGNER *et al.*, 2012; PAIVA *et al.*, 2012), which also contributes to the lower dry matter content. As successive regrowth cycles advanced from summer to autumn the values of FAc, LM, SM and SH decreased, reaching the lowest means in the last month of evaluation (April/May) but associated to a higher %DM. These changes on morphological components are an expected response as the growth season advances, as previously reported to other tropical perennial grasses such as marandu palisade grass (*Brachiaria brizantha* Stapf. Syn. *Urochloa brizantha* 'Marandu') and Mombaça grass (*Panicum maximum* 'Mombaça') (ANJOS *et al.*, 2016; DA SILVA *et al.*, 2019), since from end summer to autumn climatic factors became to be more restrictive to growth (Table 1).

During the first regrowth cycle was observed the highest value of tiller weight (TW) and the lowest tiller population density (TPD). The inverse relationship between these two population responses in known as tiller size/density compensation mechanism, being widely recognized in the literature as a way of adjusting to the light availability into the canopy (SBRISSIA; DA SILVA, 2008). However, along the growth season, TW decreased by approximately 13.7%, 8.2% and 11.6%, respectively, during February/March, March/April and April/May compared with January/February. Thus, the adjustments in the pasture along successive regrowth cycles were mainly observed by increasing TPD by approximately 50% during February/March and March/April, and approximately 90% of increasing on number of tillers was detected during the last regrowth cycle, during April/May, compared with January/February (Table 1).

The number of tillers in a grass sward is driven by a dynamic process of tiller appearance, survival of individual generations and tillers death. The increasing of the number of tillers in the population may be a result of different processes, which are widely responsive to the targets of management adopted, fertilization practices and the climatic conditions (CAMINHA *et al.,* 2010). During late spring and summer (active growth period) normally the tiller appearance rates are high, but the rates of mortality are also high, resulting in tiller's generation productive but with low longevity (ROCHA *et al.*, 2019). The population stability is normally high, because the rates

Table 1 - Parameters of productivity (mean ± standard error) throughout the regrowth cycles (RC) of Mavuno grass pastures

Canopy parameters²	Regrowth cycles (RC)				F_{value} ¹
	Jan/Feb	Feb/Mar	Mar/Apr	Apr/May	
Sward height	58.0±0.42 A	44.7±0.40 B	42.9±1.12 B	38.9±0.55 C	310.33**
%DM	26.2±0.37 C	25.1±0.37 C	27.8±0.37 B	32.1±0.39 A	57.97**
FAc	6154±228.3 A	4017±218.8 B	4026±218.8 B	3087±218.8 C	33.29**
Leaf mass	3696±197.0 A	2524±48.9 B	2530±131.2 B	1998±133.2 C	21.59**
Stem mass	2462±109.9 A	1494±94.8 B	1496±94.8 B	1089±94.8 C	26.96**
TPD	543±29.6 C	790±29.6 B	831±29.6 B	1034±29.6 A	46.25**
TW	2.83±0.030 A	2.53±0.027 C	2.69±0.027 B	2.59±0.027 C	29.93**
SLA	62.5±2.60 A	54.5±2.49AB	58.3±2.49 AB	49.0±2.49 B	5.13*
LAI	2.22±0.110 A	1.36±0.101 B	1.82±0.101 A	0.96±0.101 C	32.29**
ChloT	35.5±1.01 C	47.5±1.01 A	42.8±1.01 B	40.0±1.01 B	25.26**

Uppercase letters compare means in rows (Tukey test at P < 0.05). ¹Anova Fvalue for the effect of regrowth cycle, *P < 0.05 and **P < 0.0001. ²Sward height, in cm; Dry matter (%DM) content, in %; Forage accumulation (FAc), in kg ha^{-1} DM; Leaf and Stem mass, in kg ha^{-1} DM; Tiller population density (TPD), in tiller m^{-2}; Tiller weight (TW), in g $tiller^{-1}$; Specific leaf area (SLA), in cm^2 g^{-1}; Leaf area index (LAI); ChloT represents the Falker chlorophyll index for total chlorophyll (Falker ClorofiLOG®, Model CFL1030) of appearance are higher than mortality, being possible to observe an increasing in TPD (SBRISSIA *et al*., 2010). As climatic conditions become unfavorable to growth, from summer to autumn and autumn to winter (may include early spring in regions experiencing extreme shortage on rainfall from late September and early October) rates of appearance decrease, and the population triggers resource conservation strategies, maintaining high tiller survival rates to ensure population persistency (ROCHA *et al*., 2019; SBRISSIA *et al*., 2010). This strategy of maximize tillers survival occurs independently of the grazing strategy and fertilization rates adopted (CAMINHA *et al*., 2010), and explains the increasing in TPD in Mavuno grass along the regrowth cycles. A similar pattern was observed in Rocha *et al.* (2019) in *Brachiaria brizantha* ‘Piatã’, where TPD increased by approximately 33% from summer to autumn, regardless the frequency or severity of defoliation.

However, even increasing population density, the FAc, LM and SM decreased from summer to autumn, and this response can be explained by adjustments in growth components of individual tillers, evaluated in the present experiment through TW and SLA. The specific leaf area (SLA, cm^2 g^{-1}) decreased by approximately 22%, 16.7% and 30%, respectively during February/March, March/April and April/May compared with January/February, but mean values were statistically different only between the first and the last regrowth (Table 1). The TW and SLA can be view as parameters representing the prevailing specie’s growth strategy, and their changes express the plant’s plasticity for ecological adjustments at individual tiller level. For a given species, changes in SLA represents a well-documented acclimation response triggered by intraspecific competition, in which light capture, growth and competitive ability of individual plants are maximized through a higher investment on leaf area than in leaf dry weight (BARUCH; GUENNI, 2007), and are normally associated to a response to shading. As previously reported, during the first regrowth, pastures reached the highest sward height and, according to Sbrissia and Da Silva (2008), taller canopies are characterized by a low light availability reaching the sward base. In this way, individual tillers maximized their size and, at leaf level, increased the leaf surface area, showing high SLA.

However, according to Dwyer, Hobbs and Mayfield (2014), changes in SLA can also be seen as an opportunistic response, more influenced by local environmental variation than other leaf traits, reflecting local-scale spectrums of leaf investment strategies and varying temporally, especially in drier climates or seasons, and on nutrient deficient soils. Confirming this pattern, Sbrissia and Da Silva (2008) reported that the decreasing in SLA of marandu palisade represents a resource saving strategy, and Wellstein *et al.* (2017) also showed that lower SLA can be seen as a strategy of phenotypic adjustment, being associated with enhanced water use efficiency under water stress. Thus, the decreasing on TW and SLA at the end of growth season are pointing out a change in resource allocation strategy, in which plants are investing less in components of growth in individual tillers and expressing a more conservative behavior at leaf and tiller level, as a result of the variability in climatic conditions (low rainfall and shorter days) from summer (February/March) to autumn (April/May), similarly to the pattern observed in Sbrissia *et al.* (2001).

It is also important to point out that, as observed by Castagnara *et al.* (2014), when considering the different morphological types of the tropical perennial grasses, tall tussock-forming species normally retain weightier tillers at low population densities compared with intermediate or short-tussock forming grasses. The average TW of Mavuno grass found in the present experiment, varying from 2.53 ± 0.038 to 2.93 ± 0.038 g $tiller^{-1}$, is similar to the values described for tall tussock-forming *Panicum* cultivars, but much higher than their short-tussock forming *Brachiaria brizantha* and *Brachiaria ruziziensis* progenitor lines (SBRISSIA; DA SILVA, 2008). In this way, differences between species related to their TW, associated to the TPD, can be considered one of the descriptors of the growth habit and management requirements of grasses, and Mavuno grass expressed intermediary traits between the *Panicum* cultivars and those of the *Brachiaria* genus.

The LAI was high at the first regrowth cycle at the same time in which the Falker Chlorophyll Index for total chlorophyll (ChloT) reached its lowest value (Table 1). During this regrowth, pastures were taller than in the subsequent regrowth cycles. As observed by Yasuoka *et al.* (2018), in pastures of ‘Mulato II’ *Brachiaria* hybrid, taller canopies showed a higher LAI than the shorter ones, but were characterized by a lower proportion of young leaves and higher contribution of mature leaves to the total LAI. According to the same authors, mature leaves are located at lower levels in the vertical canopy profile and subjected to shade, making their photosynthetic rates significantly lower than young leaves.

Moreover, at the end of the regrowth cycle of January/February, the cutting procedure removed around 65% of the canopy height, allowing light to reach the sward base, and the pastures to produce new tillers and leaves. As a result, swards attained lower heights at the end of the second regrowth, LAI decreased by approximately 38.7%, whereas ChloT increased 33.8% compared to the previous regrowth. However, from March/April onwards, plants changed their growth strategy as a response to the transition from summer to autumn. Despite the LAI increased during this period, probably as a result of increasing tiller population density, the ChloT decreased and then remained stable up to the last regrowth of the growth season. The lowest LAI was registered at April/May, when values were reduced in approximately 61% compared with the values found in January/February, and this results also express the change in plant's resource allocation strategy previously described.

Impacts of N fertilization on canopy parameters

The primary responses of pastures to N fertilizer application at early regrowth aim to maximize leaf area (YAO *et al*., 2014; ZHAO *et al*., 2017), through maximization of tiller number (LOPES *et al*., 2016), at population level, and leaf appearance and elongation rates at individual tiller level (CASTAGNARA *et al*., 2014; LOPES *et al*., 2016). As regrowth progress, the incident light is predominantly intercepted by the upper layer, and the environment of low light reaching the sward base triggers shade avoidance responses, particularly stem elongation, which also results in increased sward height (DA SILVA *et al.*, 2019). From a management point of view, then, increased stem elongation and sward height are typical responses of pastures subjected to long regrowth periods (DA SILVA *et al*., 2019), but can also be triggered when management procedures result in high residual herbage mass at the beginning of regrowth cycle. In the present experiment, the results showed that N fertilization rates did not affect the SH and %DM (P > 0.05), whereas a significant effect was registered on FAc, LM and SM as well as in LAI (Table 2). All these parameters followed a quadratic response to N fertilization, similarly to the report of Castagnara *et al*. (2014) for other tropical perennial grasses. Pastures receiving N15 increased around 40.6%, 38.1% and 44.9%, respectively, the FAc, LM and SM compared with non-fertilized swards. When applied N30, values increased by approximately 68.0%, 65.4% and 72.4%, respectively, for the FAc, LM and SM and, for the same variables, values were 49.7%, 47.0% and 54.1% higher in N45 compared to N0. Despite the increased FAc, LM and SM with N fertilization, the proportion of leaves and stems in the total forage accumulation was similar for all N fertilization rates and corresponded to approximately 62% of leaves and 38% of stems.

The TPD was the more plastic response of Mavuno grass during the establishment phase, being this the main component contributing to the increasing on leaf and stem mass and forage accumulation as N fertilization increased. For a similar canopy height and DM content, there was a linear increasing on TPD from non-fertilized swards to the highest N rate applied (Table 2). These results support the previous view that pastures, which were predominantly composed by young tillers, were able to maximize tissue flows and tillering during the regrowth process in response to fertilization, increasing then TPD, LM and SM, and resulting in higher FAc and LAI. Considering the average pattern for all the regrowth periods of the growth season, the fertilization rates at points of maximum FAc, LM, SM and LAI were, respectively, 29.1, 31.3, 29.9 and 28.6 kg ha^{-1} of N, highlighting that using a higher N rate would be questionable.

It is important to point out that the low determination coefficients, which is associated to the amount or proportion of variance in the dependent variable (parameters of productivity) explained by the predictors included in the model (in the present experiment the fertilization rates), are related to the

Table 2 - Parameters of productivity of Mavuno grass pastures during the establishment phase, in response to nitrogen fertilization rates (FR). Fertilization rates were defined as follow: no-nitrogen (N0), 15 (N15), 30 (N30) and 45 (N45) kg ha^{-1} of N applied after each cutting

Canopy parameters²	Fertilization rates (FR)				SEM	F_{value} ¹	Descriptive statistics of regression models³
	N0	N15	N30	N45			
Sward height	46.0	46.0	45.9	46.6	± 0.68	0.32^{ns}	$\hat{Y}$= 46.1
%DM	28.2	27.8	27.2	27.4	± 0.37	1.38^{ns}	$\hat{Y}$= 27.5
FAc	3,123	4,392	5,246	4,523	± 218.8	16.25**	Quadratic, F_{Value}=8.23*; R^2=21.5
Leaf mass	1,952	2,696	3,228	2,870	± 163.9	10.75*	Quadratic, F_{Value}=9.14*; R^2=23.1
Stem mass	1,170	1,696	1,930	1,744	± 94.74	13.54**	Quadratic, F_{Value}=7.04*; R^2=19.5
TPD	682	727	851	938	± 29.6	15.56**	Linear, F_{Value}=11.98*; R^2=16.2
TW	2.66	2.65	2.67	2.66	± 0.026	0.02^{ns}	$\hat{Y}$= 2.65
SLA	56.8	55.5	56.1	56.0	± 2.49	0.04^{ns}	$\hat{Y}$= 56.0
LAI	1.22	1.63	1.89	1.63	± 0.105	10.90**	Quadratic, F_{Value}=3.98**; R^2=15.4
ChloT	40.9	39.6	41.8	43.4	± 1.01	2.48^{ns}	$\hat{Y}$= 41.4

¹Anova Fvalue for the effect of fertilization rates, nsnon-significant effect, *P < 0.05 and **P < 0.0001. ²Sward height, in cm; Dry matter (%DM) content, in %; Forage accumulation (FAc), in kg ha^{-1} DM; Leaf and Stem mass, in kg ha^{-1} DM; Tiller population density (TPD), in tiller m^{-2}; Tiller weight (TW), in g tiller^{-1}; Specific leaf area (SLA), in cm^2 g^{-1}; Leaf area index (LAI); ChloT represents the Falker chlorophyll index for total chlorophyll (Falker ClorofiLOG®, Model CFL1030). R^2 represent the determination coefficient of the regression models

fact that regression analysis were based on the pooled data of all regrowth cycles (Table 2). As previously discussed, natural variations in climatic growth factors between regrowth cycles were determinants of changes in plant's resource allocation strategy, being expected a lower impact of nitrogen fertilization on pasture responses as regrowth cycles progress from summer to autumn. Moreover, according to Barret (1974), the coefficient of determination actually partially measures goodness of fit in the sense of how close data points fit the regression surface and, then, only partially measures the usefulness of a regression equation.

There were non-significant effects of N fertilization on TW and SLA. For a given season of the year, the tiller size is one of the individual plant parameters triggered in response to shading, and weightier tillers are particularly observed on pastures maintained under lax or lenient grazing, following the size/density compensation mechanism as pastures are kept taller (SBRISSIA *et al*., 2001; SBRISSIA; DA SILVA, 2008). However, Lopes *et al.* (2016) reported that under high fertilization levels (more than 400 kg ha^{-1} of N), plants need to invest proportionally more into supporting structures, notably on length and dry weight of stems, to support longer leaves in response to nitrogen. Different plant species express relevant differences on their plasticity to adjust TW and SLA in response to gradients of nitrogen and, for a given species, the low plasticity for changes on these traits are normally compensated by other morphological and physiological mechanisms, such as adjustments on leaf tissue flows, leaf properties within the canopy profile (WANG *et al*., 2017), leaf lifespan and leaf thickness (DWYER; HOBBS; MAYFIELD, 2014). Moreover, Dwyer, Hobbs and Mayfield (2014) argued that the magnitude of the changes on SLA associated soil nutrient additions can be contingent on light levels and may reflect more an opportunistic response to local variations and intraspecific competition. In this way, the lack of response to N fertilization are indicatives that the range of N applied in the present study was not enough to impose a level of shading that trigger changes on SLA or a higher investment on stem length at individual plants.

Thus, the overall growth responses of Mavuno grass pastures are indicating that management practices able to maximize the number of tillers in the population may exert a positive impact for the pasture's establishment, since at this time a higher plasticity to adjust TPD was observed compared with growth attributes of individual plants.

Parameters of N status

There were no significant interactions between regrowth cycles and fertilization rates for the parameters of N status evaluated (P > 0.05), whereas the actual leaf nitrogen concentration (%N_{Leaf}) and the nitrogen nutrition index (NNI) varied with the regrowth cycles and N fertilization rates. The average values for both responses were statistically similar between the regrowth cycles from January/February to March/April, but decreased in the last regrowth. On the other hand, there were no statistically significant effects of regrowth cycles, N fertilization rates or significant interactions (Table 3) for the normalized N sufficiency index (NSI).

An accurate diagnosis of N status is required for precise N fertilizer management, particularly at the early stage of pasture development (YAO *et al*., 2014). The synchrony between N supply and the demand is essential once tillering and leaf area development at individual tillers take the priority in the plant's growth resources use at this stage. Villar *et al*. (2015) applied the NSI method

Table 3 - Parameters of N status throughout the regrowth cycles (RC) in Mavuno grass pastures subjected to nitrogen fertilization rates. Fertilization rates were defined as follow: no-nitrogen (N0), 15 (N15), 30 (N30) and 45 (N45) kg ha^{-1} of N applied after each cutting

Parameters of N status[3]	Regrowth cycles (RC)				SEM[2]	F_{value}[1]	Descriptive statistics of regression models[4]
	Jan/Feb	Feb/Mar	Mar/Apr	Apr/May			
%N_{Leaf}	2.82 AB	3.02 A	2.99 A	2.75 B	± 0.056	5.38*	-
NNI	1.45 A	1.34 A	1.33 A	1.10 B	± 0.033	18.87**	-
NSI	0.95 A	0.98 A	0.96 A	0.94 A	± 0.018	0.84^{ns}	-
	Fertilization rates (FR)						
	N0	N15	N30	N45			
%N_{Leaf}	2.70	2.88	2.90	3.10	± 0.056	8.35*	Linear F_{Value}=13.18*; R^2=18.5
NNI	1.09	1.31	1.40	1.42	± 0.033	20.07**	Quadratic F_{Value}=12.67**; R^2=30.4
NSI	0.93	0.93	0.96	1.00	± 0.023	3.41^{ns}	$\hat{Y}$= 0.96

[1]Anova F_{value} for the effect of regrowth cycles and fertilization rates, ^{ns} non-significant effect, *P < 0.05 and **P < 0.0001. [2] SEM represent the standard error of the means. [3] % N_{Leaf} represents the leaves N concentration, NNI represents the N nutrition index and NSI the N sufficiency index. Uppercase letters compare means in the rows (Tukey test at P < 0.05). [4] Descriptive statistics of regression models, R^2 represent the determination coefficient of the regression models

based on measurements from Minolta SPAD 502 portable chlorophyll meter (Konica Minolta Sensing Americas Inc., Ramsey, MI, USA) to recommend variable rates of N fertilization in *Brachiaria decumbens* pastures. The authors observed that, to attain a similar yield, the total amount of fertilizers applied was lower when using the NSI method from the portable chlorophyll meter readings compared with a fixed rate or using other spectral readings for applying N fertilization at variable rates. It is important to highlight that from this method the N fertilization would be required when NSI values are below 0.95, which requires that pastures be monitored on a regrowth cycle basis. From the pooled data from the present experiment, it can be seen that non-fertilized pastures or those receiving 15 kg ha^{-1} of N expressed average NSI values lower than 0.95 (Table 3), suggesting that more N would be required to sustain their N nutritional requirements. However, in a general way, from the methods of N nutritional diagnosis, the total chlorophyll measurements (Table 2) and NSI were unable to capture differences in N content generated by the range of fertilization rates of the present study, whereas NNI was more sensible (Table 3).

Samborski, Tremblay and Fallon (2009) pointed out that for some species, chlorophyll meter measurements may not accurately detect suboptimal N status in plants that received moderate levels of fertilizer, whereas it could be used to identify crops that are extremely N deficient. This occurs because chlorophyll meters are based on the optical properties of the leaves, and were developed to provide *proxy* measurements of photosynthetic pigments and, indirectly, the plant N content. In fact, Costa *et al*. (2015) evaluating N nutritional parameters in Xaraés palisadegrass (*U. brizantha* cv. Xaraés Syn. *Brachiaria brizantha*) observed that measurements of chlorophyll's relative rate, using a chlorophyll meter Minolta SPAD-502 Plus (Konica Minolta Sensing Americas Inc., Ramsey, MI, USA), reach a plateau at an NSI of 0.85 indicating that, from this point onwards, the measured values would not be sensible even if plant N content be higher.

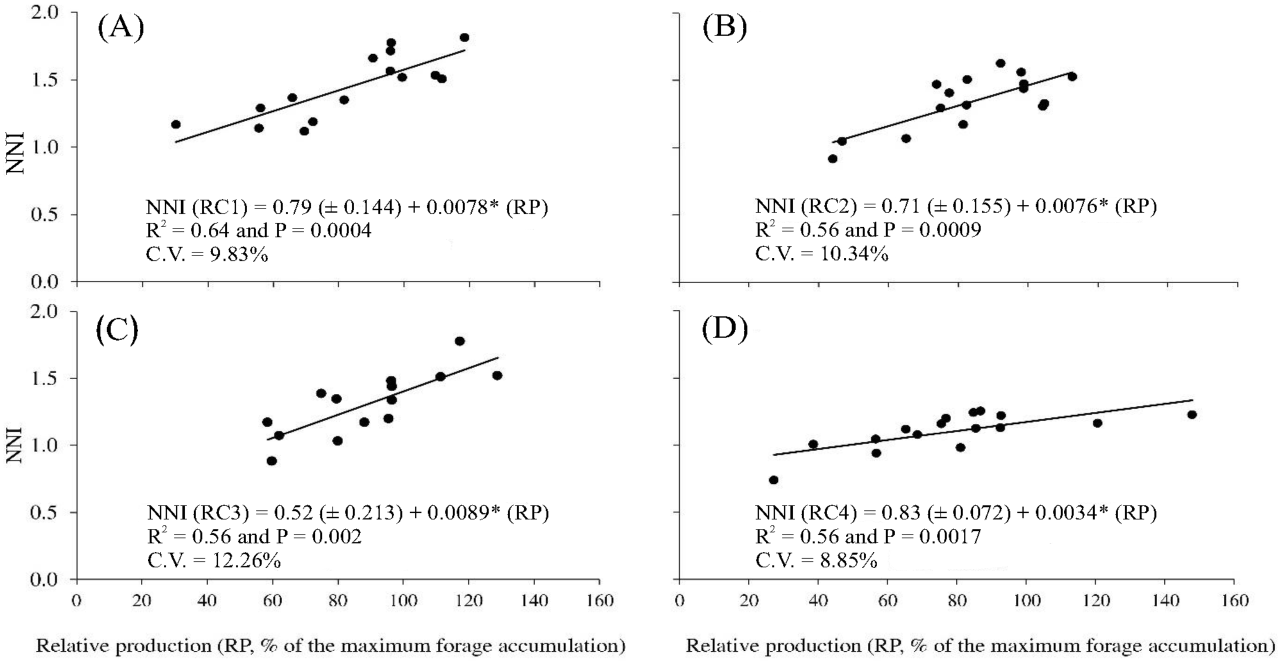
It has been observed that N deficient plants may to degrade chlorophyll molecules as a way to recycle N to regions of active growth within the plant. However, the forms of N in leaves include other soluble components such as nitrates, amino acids, and insoluble components in cell walls, membranes and other structures (MU *et al*., 2016). Thus, a significant portion of the N can be driven to form non pigments and compounds other than chlorophylls, such as storage proteins, free amino acids or to be stored as nitrate ions. Many plant species accumulate amino acids and amides in their vegetative tissues in large quantities, not only in leaves but also in rhizomes, roots, leaf sheaths and stem bases (DIERKING *et al*., 2017; GLOSER, 2002), being all these plant's N compounds not measured by chlorophyll meters. Thus, in pastures, it would be expected higher correlations between the chlorophyll indices and the leaf N content with the measurements provided by the chlorophyll meters as higher is the plant's N nutritional deficiency. In this way, and for the experimental conditions of the present experiment, the NSI method was unable to detect changes in $\%N_{Leaf}$ in response to N fertilization rates and along the regrowth cycles (Table 3).

On the other hand, the NNI was more sensible to the nitrogen fertilization rates, being considered a suitable tool to detect N status in Mavuno grass pastures within the range of leaf nitrogen content registered in the present experiment. In this sense, for the present experiment the critical levels were calculated only to the NNI. The NNI is based on the concept of critical N concentration (Nc), corresponding to the minimum shoot N concentration which is needed for the crop to grow at its maximum growth rate. Originally, the NNI is calculated as the ratio of the actual N concentration of the sward and the N concentration it would have to be, at a similar biomass, to sustain a maximum biomass accumulation (FARRUGGIA; GASTAL; SCHOLEFIELD, 2004). However, recent studies on winter wheat (YAO *et al.*, 2014), japonica and indica rice (ATA-UL-KARIM *et al.*, 2017) and summer maize (ZHAO *et al.*, 2017), have pointing out that the development of nutritional parameters based on leaves N concentration can provide a better understanding of the physiological and functional N use, being also crucial for finding environmental differences of N dynamics and its use efficiency for leaves production (ATA-UL-KARIM *et al.*, 2017; ZHAO *et al.*, 2017).

Statistically significant linear relationships between the relative production (as a proportion of the maximum forage accumulation) and the NNI based on the $\%N_{Leaf}$ were observed for all regrowth cycles (Figure 2).

According to Ziadi *et al*. (2008) values of NNI $\geq$ 1.0 indicate that the crop is in a situation of nonlimiting supply of N, whereas values of NNI smaller than 1.0 indicate N deficiency. However, the values corresponding to at least 90% of the maximum forage accumulation were equivalent to an NNI= 1.50 in January/February, 1.39 in February/March, 1.33 in March/April and 1.11 in April/May, indicating that values of reference should be adjusted when using the sampling of the youngest expanded leaf as reference for N concentration. However, it is important to highlight that the values mentioned above should be applied only to the regrowth cycles of the first growth season after seeding (establishment phase). Through the relationship of $\%N_{Leaf}$ and NNI reference previously identified, it was possible to estimate the critical leaf N concentration ($Nc_{Leaf,}$ in %) for Mavuno pastures during the phase of establishment (first growth season after sowing), which represents the N concentration required to plants attain at

Figure 2 - Relationship between the NNI and the relative production (RP, in % of the maximum) of four successive regrowth cycles (RC) in Mavuno grass pastures subjected to N fertilization rates (n= 16, four N fertilization rates x four blocks). The regrowth cycles correspond to RC1 (A)=January/February, RC2 (B) = February/March, RC3 (C)= March/April and RC4 (D)= April/May



least 90% of their maximum forage accumulation. Values corresponded to 2.81, 3.07, 2.96 and 2.75, respectively, for the regrowth cycles of January/February, February/March, March/April and April/May (Figure 3).

This critical range of leaf nitrogen concentration (2.75 to 3.07%), then, may be indicated to monitor plant's N nutritional status of Mavuno grass pastures during the regrowth cycles of the first season after seeding. According to Ata-Ul-Karim *et al.* (2017), the N parameters based on leaf sampling are crop specific, but are precise and biologically sound in relation to actual crop growth, which make them practical and useful tools to make corrective decisions on time and amount of N fertilization during the growth season. However, it is important to point out that the NSI and NNI indicate the current plant N status, but neither can predict its future one nor indicates how much N fertilizer should be applied (SAMBORSKI; TREMBLAY; FALLON, 2009). The traditional fertilizer strategies have been based on a "recipe" approach where N fertilizer is applied to a set rate following grazing. However, it has been increasingly proposed that there is a need to move to approaches were the time and amount of fertilizers can be defined according to the pasture demand (VILLAR *et al.*, 2015). Thus, once is identified the NNI reference, the fertilization rates required to plants attain their maximum forage accumulation can be defined and strategic fertilization may be applied.

In the present research, we were able to define a strategic N fertilization protocol for Mavuno grass pastures during the establishment phase (Table 4 and Figure 4) for the region of the study and considering that there are no restrictions of other soil nutrients.

The N fertilization rate, estimated by the equations parameters, required to maintain a range of 90% to 100% of the maximum forage accumulation for the first regrowth cycle varied from 42.0 to 46.7 kg ha^{-1} of N (Table 4). During the two following regrowth cycles, the N fertilization rates required were relatively stable and lower than the first regrowth, following the decreasing in forage accumulation, and varied from 27.0 to 32.5 kg ha^{-1} of N. A lower fertilization requirement was observed in the last regrowth, and 22.2 to 24.7 kg ha^{-1} of N would be enough for plants to sustain a maximum growth. The lower demand of fertilization during this last regrowth cycle, which represents the entrance in the autumn, is related with the decreasing on plant's growth processes, since was also observed the lowest LAI and forage accumulation (Figure 4).

It is important to point out that by using this method, the amount of fertilizers would be defined at each regrowth cycle and, as a consequence, the annual amount of fertilizers will be variable between places, seasons as well as soil and climatic conditions. In the same way, this methodology offers the opportunity of mapping and identification of the variability of the nutritional requirements of the plants in

Figure 3 - Relationship between the NNI and the actual leaf N concentration (N_{Leaf}, in %) in Mavuno grass pastures (n= 16, four N fertilization rates x four blocks) during the regrowth cycles of January/February (A), February/March (B), March/April (C) and April/May (D). The critical leaf N content (Nc_{Leaf}) is the value required to attain at least 90% of the maximum forage accumulation, and values corresponded to an NNI= 1.50 in January/February, 1.39 in February/March, 1.33 in March/April and 1.11 in April/May

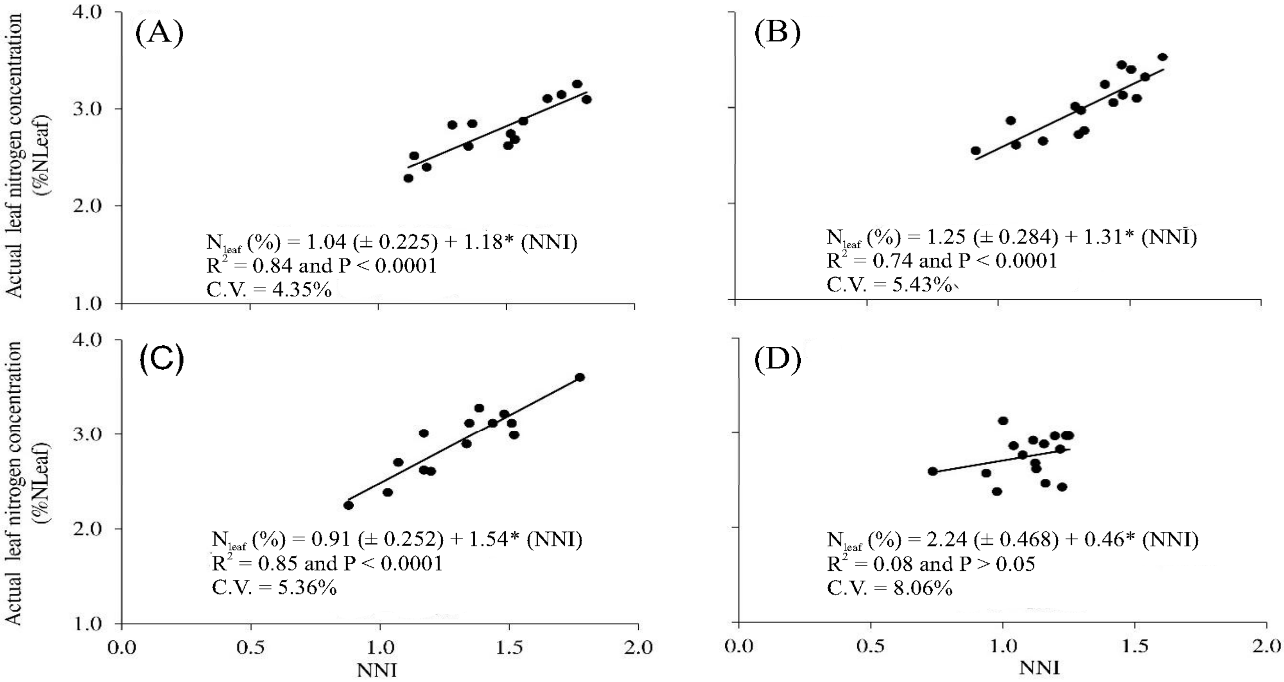
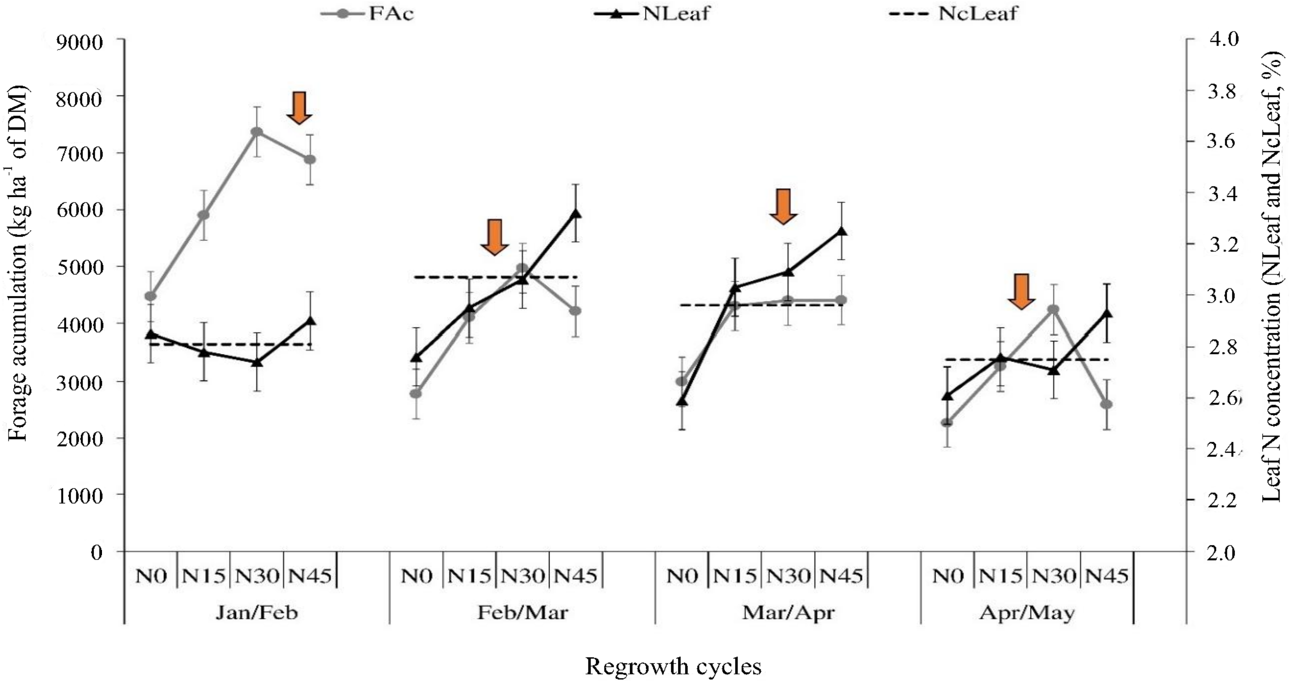


Table 4 - Average values of forage accumulation and parameters of the regression equations ($\hat{Y}= ax^2+bx+c$) of the relationship of forage accumulation (FAc) and fertilization rates (FR) of Mavuno grass pastures throughout the regrowth cycles during the establishment phase. Fertilization rates were defined as follow: no-nitrogen (N0), 15 (N15), 30 (N30) and 45 (N45) kg ha^{-1} of N applied after each cutting

Fertilization rates (FR)	Regrowth cycles (RC)			
	Jan/Feb	Feb/Mar	Mar/Apr	Apr/May
	Forage accumulation (FAc, in kg ha^{-1} of DM)			
N0	4472.1	2772.5	2984.3	2261.3
N15	5898.6	4105.8	4307.2	3255.9
N30	7367.0	4973.7	4401.2	4244.0
N45	7438.6	4216.1	4409.7	2587.1
	Parameters of the regression equations ($\hat{Y}$=FAc)			
c	4403.2 (±672.94)	2714.5 (±267.85)	3041.4 (±398.13)	2129.4 (±461.30)
b	135.5 (±72.04)	139.2 (±28.68)	94.9 (±42.62)	145.7 (±49.38)
a	-1.45 (±1.53)	-2.32 (±0.61)	-1.46 (±0.91)	-2.95 (±1.05)
R^2	0.49	0.71	0.39	0.40
[1]F_{value}	6.23*	16.18*	4.16*	4.35*
[2]FAc at $P_{Máx}$	7566.9	4802.5	4582.3	3927.9
[2]FR at $P_{Máx}$	46.7	30.0	32.5	24.7
[3]FR at $P_{90\%}$	42.0	27.0	29.2	22.2

R^2 represent the determination coefficient of the regression models. [1]F_{value} of the regression models, [ns]non-significant effect, *P < 0.05 and **P < 0.0001. [2]FAc at $P_{Máx}$ and FR at $P_{Máx}$ represent the point of maximum forage accumulation and the fertilization rate required to attain this point. [3]FR at $P_{90\%}$ represents the fertilization rate required to attain 90% of the maximum forage accumulation

Figure 4 - Plot of the variation in forage accumulation (FAc, in kg ha^{-1} of DM), the actual leaf N concentration (N_{Leaf}, in %) and critical leaf N content (Nc_{Leaf}, obtained from NNI=1.5, 1.39, 1.33 and 1.11 along the regrowth cycles) in Mavuno grass pastures in response to N fertilization rates (applied after each cutting). Regrowth cycles corresponded to January/February, February/March, March/April and April/May. Arrows represent the N fertilization rate (FR) required to attain 90% of the maximum growth (FR at $P_{90\%}$, equations are described in Table 4)



the field, enabling the adoption of precision agriculture techniques and variable N fertilization rates. The strategic N fertilization have the potential to improve the N use efficiency, and lower both N inputs and N losses with little impact on pasture dry matter production (SMITH *et al*., 2018; VILLAR *et al*., 2015). By applying this strategic N fertilization protocol, Mavuno grass pastures would be able to produce approximately 20.8 ton ha^{-1} of effectively harvested dry matter, which indicates a substantial productivity considering around 120 days of growth along the establishment phase. A total amount of 150 to 184 kg ha^{-1} of N (also considering the first N application at 45 days after sowing) in installments according the plant's demand would be required (Figure 4) for Mavuno grass pastures.

CONCLUSIONS

1. Tiller population density is a plastic response during the establishment phase of Mavuno grass pastures, contributing to increasing leaf and stem mass, leaf area index and forage accumulation as N fertilization increases. A conservative investment strategy is triggered on individual tillers at the end of growth season, and plants decrease the specific leaf area, tiller weight and the Falker total chlorophyll index;
2. The total chlorophyll measurement and NSI were unable to capture differences in the leaf N content in response to N fertilization rates and along the regrowth cycles, but NNI was a suitable tool to detect N status in Mavuno grass pastures;
3. The youngest expanded leaf can be used to monitor plant's N status through the NNI, being considered a practical and faster alternative to the sampling of the total herbage mass. However, the NNI used as reference to the regrowth cycles of the first growth season after seeding corresponded to values above 1 (varying from 1.1 up to 1.5);
4. The critical range of leaf nitrogen (%N_{Leaf}) concentration varied from 2.75 to 3.07%, and this range may be indicated to monitor plant's N status along the regrowth cycles of the first growth season after seeding;
5. A strategic fertilization protocol was identified, based on the amounts required by plants to sustain their maximum forage accumulation rates. Therefore, the strategic N fertilization suggested for the growth season after seeding is to apply 42.0 to 46.7 kg ha^{-1} of N at the first regrowth, 27.0 to 32.5 kg ha^{-1} of N during the two following regrowth cycles, whereas at the end of the season (April to May regrowth) only 22.2 to 24.7 kg ha^{-1} of N would be enough to sustain a maximum growth.

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