**UNIVERSIDADE ESTADUAL PAULISTA – UNESP**

**CÂMPUS DE JABOTICABAL**

# EFFICIENCY OF NITROGEN SOURCES AND RATES ON GROWTH AND DRY MATTER YIELD OF TIFTON 85

**Bernardo Melo Montes Nogueira Borges**

**Engenheiro Agrônomo**

**2016**

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

# EFFICIENCY OF NITROGEN SOURCES AND RATES ON GROWTH AND DRY MATTER YIELD OF TIFTON 85

**Bernardo Melo Montes Nogueira Borges**
**Orientador: Prof. Dr. Edson Luiz Mendes Coutinho**
**Coorientador: Prof. Dr. Takashi Muraoka**

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Agronomia (Produção Vegetal).

**2016**

B732e

Borges, Bernardo Melo Montes Nogueira

Efficiency of nitrogen sources and rates on growth and dry matter yield of Tifton 85 / Bernardo Melo Montes Nogueira Borges. – – Jaboticabal, 2016

xi, 48 p. : il. ; 28 cm

Tese (doutorado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, 2016

Orientador: Edson Luiz Mendes Coutinho

Coorientador: Takashi Muraoka

Banca examinadora: Carolina Fernandes, José Eduardo Corá, Henrique Coutinho Junqueira Franco, Valdeci Orioli Júnior

Bibliografia

1. Eficiência de uso. 2. Forragem. 3. Índice de área foliar. 4.Nitrato de amônio. 5.Radiação fotossinteticamente ativa. 6. Ureia. I. Título. II. Jaboticabal-Faculdade de Ciências Agrárias e Veterinárias.

CDU 631.84;633.3

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação – Serviço Técnico de Biblioteca e Documentação - UNESP, Câmpus de Jaboticabal.

UNIVERSIDADE ESTADUAL PAULISTA

**Câmpus de Jaboticabal**

## CERTIFICADO DE APROVAÇÃO

TÍTULO: EFFICIENCY OF NITROGEN SOURCES AND RATES ON GROWTH AND DRY MATTER YIELD OF TIFTON 85

**AUTOR: BERNARDO MELO MONTES NOGUEIRA BORGES**
**ORIENTADOR: EDSON LUIZ MENDES COUTINHO**
**CO-ORIENTADOR: TAKASHI MURAOKA**

Aprovado como parte das exigências para obtenção do Título de Doutor em AGRONOMIA (PRODUÇÃO VEGETAL), pela Comissão Examinadora:

Prof. Dr. EDSON LUIZ MENDES COUTINHO
Departamento de Solos e Adubos / FCAV / UNESP - Jaboticabal

Pesquisador Dr. HENRIQUE COUTINHO JUNQUEIRA FRANCO
Centro Nacional de Pesquisas em Energia e Materias / CNPEM - Campinas/SP

Prof. Dr. VALDECI ORIOLI JÚNIOR
Departamento de Agronomia / IFTM - Uberaba/MG

Prof. Dr. JOSÉ EDUARDO CORÁ
Departamento de Solos e Adubos / FCAV / UNESP - Jaboticabal

Profa. Dra. CAROLINA FERNANDES
Departamento de Solos e Adubos / FCAV / UNESP - Jaboticabal

Jaboticabal, 31 de março de 2016.

## DADOS CURRICULARES DO AUTOR

**Bernardo Melo Montes Nogueira Borges –** Nascido em 10 de Junho de 1987, na cidade de Uberaba – MG, é Engenheiro Agrônomo formado em 2010, pelas Faculdades Associadas de Uberaba – FAZU. No mesmo ano iniciou o curso de Mestrado no Programa de Agronomia (Produção Vegetal) na Universidade Estadual Paulista – Faculdade de Ciências Agrárias e Veterinárias – Câmpus de Jaboticabal – SP. Foi bolsista CAPES no período de Agosto de 2010 até Fevereiro de 2012 quando concluiu o curso. Iniciou em Março de 2012 o curso de Doutorado em Agronomia (Produção Vegetal) na mesma instituição sendo bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES no período de Março de 2012 até Agosto de 2015. Durante o curso de doutorado foi bolsista do Programa de Doutorado Sanduíche no Exterior – PDSE/CAPES do programa de doutorado sanduíche na University of Florida entre os meses de Maio de 2014 à Fevereiro de 2015. Atuou como Professor Substituto no curso de Agronomia e Zootecnia na Universidade Estadual Paulista – Faculdade de Ciências Agrárias e Veterinárias – Câmpus de Jaboticabal – SP nas disciplinas de Fertilizantes e Corretivos, Adubação de Culturas e Adubos e Adubação de Plantas Forrageiras durante o segundo semestre de 2015.

“Os dois dias mais importantes da vida de uma pessoa são: o dia em que ela nasce, e o dia em que ela descobre o porquê.”

**-Mark Twain**

Aos meus pais, Solange e Guilherme, aos meus irmãos Mariana e Guilherme Filho, pelo apoio incondicional, amizade, carinho e cumplicidade.

***Ofereço e Dedico***

## AGRADECIMENTOS

À Deus pela oportunidade das conquistas.

À minha família pelo interminável apoio, carinho e por acreditar.

À minha namorada Luisa Carneiro pela paciência, companheirismo, amor e carinho irrestritos.

Ao Professor Dr. Edson Luiz Mendes Coutinho pela orientação e ensinamentos, pela confiança depositada em mim, pelo apoio em todas as horas e sobretudo, pela amizade ilibada, cultivada durante esses anos.

Ao Professor Dr. Takashi Muraoka pela coorientação e por todo auxílio, disponibilidade e paciência de sempre.

À Professora Dra. Maria Lucia Silveira pela orientação durante o período de doutorado sanduíche, pela confiança depositada em mim, pela disposição, ajuda e amizade criada.

À Unesp Câmpus de Jaboticabal, ao Programa de Pós-Graduação em Agronomia (Produção Vegetal) e à University of Florida pelo suporte à realização dos trabalhos.

Aos membros da banca do exame geral de qualificação Prof. Dr. Arthur Bernardes Cecílio Filho, Profa. Dra. Carolina Fernandes, Prof. Dr. José Carlos Barbosa e Profa. Dra. Mara Cristina Pessoa da Cruz pelas sugestões apresentadas.

Aos membros da banca de defesa de tese Profa. Dra. Carolina Fernandes, Dr. Henrique Coutinho Junqueira Franco, Prof. Dr. José Eduardo Corá e Prof. Dr. Valdeci Orioli Júnior pelas sugestões apresentadas.

Ao Professor Dr. José Carlos Barbosa pelo auxílio nas análises estatísticas.

Ao Professor Dr. Pedro Luiz da Costa Aguiar Alves pela ajuda e disponibilidade de sempre.

Ao coordenador do curso de Pós-graduação em Agronomia (Produção Vegetal) Prof. Dr. Rouverson Pereira da Silva pela compreensão e ajuda durante todo o doutoramento.

Aos funcionários do Departamento Solos e Adubos Cláudia Campos Dela Marta e Dejair Lúcio da Silva, pelo auxílio e amizade.

Aos funcionários da Fazenda de Ensino, Pesquisa e Extensão, em especial ao Marcelo Scatolin pela ajuda na condução do experimento e pelas boas risadas.

Aos amigos André Coutinho Neto, André França, Bárbara Brunozzi, Carlos Eduardo Junqueira, Ederlon Flávio, Fábio Paranhos, Fábio Teixeira, Fábio Tiraboschi, Fernanda Peixoto, Gabriel Damasceno, Guilherme Castro, João Vítor Pasquetto, Marco Antonio Ribeiro Filho, Marilena Braga, Rafael Tomicioli e Saulo Strazeio pelo auxílio durante todo o experimento.

Aos funcionários da Range Cattle and Education Center, em especial à Cindy Holley for all the good time.

Aos amigos de velha data, mesmo que distantes sempre presentes.

À CAPES pelo apoio financeiro nas bolsas de doutorado e doutorado sanduíche.

A todos que tornaram essa caminhada mais prazerosa e possível, minha gratidão e disponibilidade eterna.

*Muito obrigado por tudo, sempre!*

# SUMMARY

# EFICIÊNCIA DE FONTES E DOSES DE NITROGÊNIO NO CRESCIMENTO E PRODUÇÃO DE MATÉRIA SECA DO CAPIM-TIFTON 85

**RESUMO** – Forrageiras do gênero *Cynodon* são conhecidas por sua capacidade de resposta a altas doses de nitrogênio (N). Em condições tropicais o N pode se tornar um problema ambiental e financeiro devido à sua baixa eficiência de uso pelas plantas, principalmente como resultado de perdas por volatilização e/ou lixiviação. Portanto, objetivou-se com este trabalho avaliar fontes e doses de N no crescimento e produção de matéria seca do Tifton 85, bem como mudanças nas frações do carbono (C) no solo. Um experimento foi conduzido de 2012 a 2014, e constituiu de um fatorial em um delineamento de blocos ao acaso, onde os tratamentos foram a associação de duas fontes de N (nitrato de amônio [NA] e ureia), e cinco doses do nutriente (0, 60, 120, 180 e 240 kg $ha^{-1}$ N por corte) aplicadas a cada 30 dias. Neste experimento foram utilizados NA e ureia enriquecidos com $^{15}N$ como uma ferramenta para quantificar a recuperação do N advindo do fertilizante, nas plantas e no sistema solo-planta. As plantas foram cortadas para avaliar a produção de matéria seca, concentração de N na parte aérea e recuperação. O índice de área foliar (LAI), radiação fotossinteticamente ativa interceptada (PARi) e índice de clorofila (CI) foram mensurados no dia anterior ao corte. As amostras de solo foram coletadas nas parcelas que receberam NA e foram fracionadas em carbono orgânico particulado (POC), fração leve livre (FLF) e fração mineral (C-min); O teor de C nas frações foi quantificado. Os resultados demonstraram que o NA pode ser uma fonte mais eficiente somente quando a quantidade de precipitação é insuficiente para incorporar o fertilizante ao solo, resultando em maior produção de forragem, na somatória dos dois anos ambas as fontes produziram a mesma quantidade de matéria seca onde a maior produtividade 37,2 Mg $ha^{-1}$, foi atingida com a dose de 210 kg $ha^{-1}$ N por corte. Por outro lado, a quantidade de N recuperado pelo sistema de planta+solo foi maior quando a ureia foi utilizada, com destaque para a quantidade de N no solo, onde a ureia foi capaz de manter 10% mais N que o NA, a recuperação do nutriente diminuiu à medida que as doses foram elevadas. Nenhuma alteração no conteúdo de C foi notada devido às diferentes doses de N utilizadas, no entanto o POC e o C-min foram mais sensíveis às mudanças na camada de 0-0,1 m do que na camada de 0,1-0,2 m.

**Palavras-chave:** Eficiência de uso; Forragem; Índice de área foliar; Nitrato de amônio; Radiação fotossinteticamente ativa interceptada; Ureia

# EFFICIENCY OF NITROGEN SOURCES AND RATES ON GROWTH AND DRY MATTER YIELD OF TIFTON 85

**ABSTRACT** – *Cynodon* hybrids are known by their ability to respond to high rates of nitrogen (N). In tropical conditions, N may become an environmental and financial issue due to its low efficiency of use by plants, mainly as a result of losses by volatilization and/or leaching. Therefore, the aims with this work were to evaluate N sources and rates on growth and dry matter yield of Tifton 85 and to determine changes in soil carbon (C) fractions in response to N fertilization. The 2-yr field experiment was conducted from 2012 to 2014, and consisted of a factorial design. Treatments were a combination of two sources of N (ammonium nitrate [AN] and urea) and five rates of the nutrient (0, 60, 120, 180 and 240 kg ha$^{-1}$ N per cut) applied broadcast every 30 days. This study used AN and urea enriched with $^{15}$N as a tool to quantify the recovery of N derived from fertilizer in plants and the soil-plant system. Forage was cut at 30-d intervals for dry matter yield, shoot N concentration determinations and N recovery. The leaf area index (LAI), photosynthetic active radiation intercepted (PARi) and chlorophyll index (CI) were evaluated on the day before clipping. Soil samples were collected in plots receiving AN and they were fractionated in to particulate organic carbon (POC), free light fraction (FLF) and mineral fraction (C-min); The C concentration of the various fractions was determined. Results showed that AN was a more efficient source only when the amount of precipitation is insufficient to incorporate the fertilizer to the soil, resulting in increased production, in the sum of the two years both sources produced the same amount of dry matter in which the highest productivity, 37.2 t ha$^{-1}$, was achieved at the rate of 210 kg ha$^{-1}$ N per cutting. On the other hand, the amount of N recovered by the plant+soil system was higher when urea was used, especially the amount of N in the soil, where urea was able to maintain 10% more N than AN, the nutrient recovery decreased as the rates were increased. No change in soil C concentration was detected in response to the different N rates used, however the POC and the C-min were more sensitive to changes in the layer of 0-0.1 m than the layer from 0.1-0.2 m.

**Keywords:** Use efficiency; Forage; Leaf Area Index; Ammonium nitrate; Photosynthetically Active Radiation intercepted; Urea

# 1 INTRODUCTION

Brazil is a country with continental proportions and has in its territory a large variety of climate, ecosystems, edaphic characteristics and production systems. The majority of the forage production areas in Brazil have been established in low fertility soils or degraded areas resulting from intensive agriculture.

Improper soil management can often result in serious production and environmental problems, including poor soil fertility conditions. Once soil reserves are exhausted, considerable amount of investment and time are necessary to restore the soil quality.

Adequate nitrogen (N) supply is essential to maintain high yields in intensive forage production systems (GEISSELER et al., 2012). However, because the relatively high cost and environmental issues associated with N losses (CONNEL et al., 2011), N fertilizer management has become an important global issue that has been receiving increased public attention. Fertilizer recommendation in Sao Paulo State are based on the concentration of N in shoot and the expected dry matter yield (WERNER et al., 1996). However, N use-efficiency is often very poor, with a typical recovery of less than 50% of total applied N (IMPITHUKSA; BLUE, 1985). Urea is the most commonly used N fertilizer source due to its lower cost, but potentially is also the source most susceptible to losses, mainly by volatilization.

Nitrogen losses can be harmful to the environment and, depending on how the nutrient is managed in agricultural systems, N fertilization can result in unintended detrimental effects on soil, water and air quality. Water quality problems are typically associated with nitrate leaching (SILVEIRA; HABY; LEONARD., 2007), while ammonia volatilization and nitrous oxide emissions are the primary sources of air contamination (CABRERA; KISSEL; BOCK, 1991), still losses can reach up to 20% by volatilization when utilized urea as nitrogen source (MASSEY et al., 2011). Nitrogen losses may have negative impacts on forage production and subsequently reduced soil organic matter levels.

Improper fertilization management can be harmful to the environment and also affect soil quality. For instance, intensively managed production system receiving low N input may result in a depletion of soil C levels (MAIA et al., 2009), in which management can influence changes in the soil more than texture itself (NEIL et al., 1997). Although total soil carbon is often suggested as an indicator of changes in response to management, specific soil fractions are generally more sensitive to different soil management, particularly in the short term (DUBEUX et al., 2006). Promoting soil C sequestration in intensive forage production ecosystems can be beneficial to forage production by improving soil quality. From a broader perspective, increasing soil C sequestration can also have societal benefits by removing $CO_2$ from the atmosphere and storing it in more stable below ground pools.

Tifton 85 is known by its capacity to responds to high N fertilization regimens. However, information about best management practices for N fertilization in pastures are still scarce, especially, in tropical areas inserted in low fertility soils associated with periods of intensive rainfall in which it may promote poor N utilization and subsequent effects on forage production and soil quality.

The hypothesis of this study was that N sources and rates will have significant impact on forage production, particularly in tropical conditions where N losses can be exacerbated.

Thus, the objective was to evaluate nitrogen sources and rates, their influence on growth, dry matter yield, nitrogen recovery and changes on soil organic carbon fractions.

## 2 LITERATURE REVIEW

Tifton 85 (*Cynodon spp. L.)* is a perennial bermudagrass hybrid, resulted from the crossing of Tifton 68 (*Cynodon nlemfuënsis* L.) and PI 290884 (*Cynodon dactlon* L.) released in 1992 by the USDA-ARS at Tifton, Georgia. This variety has a prostrate growth being stoloniferous and rhizomatous. It is taller, and has larger stems and leaves than most commercially available bermudagrasses, being responsive to N fertilization, capable of producing a large amount of forage for either hay or grazing, moreover, *Cynodon* plants are known by its great management flexibility (SOHM et al., 2014).

Haying has as purposes the high quality forage production and conservation, by a rapid dehydration (REIS, 1996). Compared to other perennial $C_4$ grass species, tifton 85 exhibits relatively high nutritive value, but it also requires high fertilizers inputs, especially nitrogen.

Nitrogen fertilization is essential to sustain adequate forage performance. However, due to this excessive use in agriculture, N fertilizers are often perceived as a cause of major environmental problems, such as soil, water and air pollution (CHIEN; PROCHNOW; CANTARELLA, 2009).

In order to reach a productive, technified and sustainable environment, several factors have to be covered, such as the quality of the forage, its capacity to respond fertilization, the utilization of more efficient sources and a well-planned management in grasslands. One of the most important facts above mentioned is to improve soil fertility, once forage lands in Brazil are, at the majority, inserted in low fertility soil areas, in which nitrogen is a major limiting factor.

Nitrogen promotes forage dry matter (DM) yield and quality. It is important, however, to manage it adequately, in order to avoid losses, especially, by volatilization and leaching (MASSEY et al., 2011). Nitrogen added via ammonium nitrate, for example, can be lost by leaching. The extent that N losses occurs depends on the various factors such as application rate and soil texture, where clay soils are less suitable to this kind of loss when compared to sandy soils that have larger pores allowing the nutrient to be carried by water in a facilitated way (BOWEN et al., 1993; GUERTAL; HOWE, 2012).

Compared to other warm-season forages, Tifton 85 can produce relatively high yields when fertilized with N. For example, application of up to 240 kg $ha^{-1}$ N resulted in a linear increase in DM yield with an estimated efficiency of 22.67 kg of DM per kg of N, and maximum shoot N content of 17 g $kg^{-1}$ (QUARESMA et al., 2011). In a study conducted by Ribeiro and Pereira (2011), Tifton 85 DM yield increased linearly up to rates of 400 kg $ha^{-1}$ N per year, with efficiency of 36.8 kg of DM per kg of N. Sohm et al. (2014) observed a quadratic response up to 672 kg $ha^{-1}$ N per year.

Increases in the forage DM yield with N fertilization can be explained by the increase of tiller density (FAGUNDES et al., 2011), height (QUARESMA et al., 2011), leaf area index (PEREIRA et al., 2012 and GÓMEZ; GUENNI; GUENNI, 2013) and intercepted photosynthetically active radiation (SILVA et al., 2012; MATTERA et al., 2013), but not by the number of leaves per tiller since it is determined by a genetic factor. According to Oliveira, Pereira and Huaman. (2000) it corresponds to a maximum of 9.5 leaves/tiller. Therefore, it is necessary to increase tillering and expand leaf area to be able to enhance production.

Photosynthetically active radiation (PARi) measures capacity of plants to absorb light by its canopy. According to Silva et al. (2012), when the forage is able to intercept 95% of the PAR, it is capable to express its maximum potential of production. Humphreys (1991) mentioned that leaf area index for forages should range between 3 to 5, what in fact means that in this range the crop should reach the 95% of canopy light interception.

Increasing light interception would be useless if the plant is not able to convert radiation into chemical energy by the photosynthesis process. Nitrogen is one of the nutrients that constitutes the chlorophyll molecule and this is the pigment responsible by photosynthesis (TAIZ; ZEIGER, 2010). Whenever there is a decrease of N in the systems, plants present a lighter green coloration due to the lower amount of the pigment (MARSCHNER, 2012), leading to a status of deficiency, diminishing production.

Plant growth is conditioned primarily to light absorption (NABINGER; PONTES, 2001), compromising also cell expansion and plant development, generated by an insufficient amount of water and nutrient (ANDRADE et al., 2002; KUNZ et al., 2007; SANGOI et al., 2011). Therefore, the rate of leaf expansion on a plant can be limited by the production of assimilates, controlling cell division and creating a demand for C and N to provide energy and material for leaf tissue expansion (BEN-HAJ-SALAH; TARDIEU, 1995).

According to Marschner (2012), adequate N supply is necessary to sustain root growth, in which, it intensifies the synthesis and export of cytokinins to the shoots. The presence of this hormone will start up cell division and expansion, delay leaf senescence, increase leaf area index, protein synthesis and delay protein degradation, improving forage quality (TAIZ; ZEIGER, 2010).

Due to the high demand of N by tifton 85, especially in hay production, it is recommended the application of N after every cutting (WERNER et al., 1996). Although the surface applied urea might become a risky option, that might be increased when applied over the litter, that contain 20 times more urease than soil (HARGROVE, 1988). This could lead to an accentuated $NH_3$ loss by volatilization, up to 20% according to Massey et al. (2011), therefore this may decrease DM yield, production efficiency, N uptake, the efficiency of the N fertilizer source and forage quality (CONNELL et al., 2011).

According to Alderman, Boote and Sollenberger (2011a), forage quality is affected by the shoot N concentration, therefore, N fertilization can also promote forage intake. Forage quality not only influence the plant itself but can also affect animal intake. Milford and Minson (1966) suggested that values inferior to 11 g $kg^{-1}$ of N are detrimental to hay consumption, in this case a large volume of forage with low amount of protein have to be ingested. According to Kelling and Matocha (1990) and Werner et al. (1996) bermudagrass is considered to be in a satisfactory level of nutrition when in a range of 21 to 26 g $kg^{-1}$ of N, above this value response on DM yield are reduced where plants reach a luxury consumption status (MENGEL; KIRKBY, 2001) and since hay production demands a large amount of investment this is a scenario that is undesirable when aiming a profitable exploit of the crop.

The intense exploration of the area is one of the main characteristics of hay production, and it can lead to a soil exhaustion if not managed properly. Given such a problem, the utilization of different N sources can be a plausible and simple solution. In Brazil the main N source utilized is urea, followed by ammonium nitrate (AN) and ammonium sulfate (AS) each one with its own advantages and disadvantages.

In a study performed at the Texas A&M University in a loamy fine sand and fine sandy loam soil textures with pH of 5.0 and 5.1, respectively, utilizing a *Cynodon* hybrid, Silveira, Haby and Leonard (2007), the authors showed the different efficiencies of each N source, in which it varies with the amount of rainfall. This way at the first two years the uptake efficiency of AN was superior than urea, independently of the rate utilized. At the third year a drought period, could have interfered, in all the rates utilized, consequently, both fertilizers presented the same response. This is dependent on the volume of rainfall after urea is applied. If it is not enough to incorporate the fertilizer into the soil, before the process of hydrolysis is completed, losses by volatilization might be increased, especially when this fertilizer is applied over straw (FOX; KERN; PIEKIELEK, 1986).

Nitrogen recovery by plant above ground tissue is generally considered to be about 50% of the total N applied as fertilizer. Impithuksa and Blue (1985) studying a *Cynodon* hybrid reported N recoveries ranging from 15 to 35% when N was applied as AN. In a 3-yr experiment evaluating different N sources in coastal bermudagrass (*Cynodon dactylon* L.) grown on a sandy soil, Burton and Jackson (1962) reported that surface application of AN resulted in 18.7% greater production efficiency compared to urea. When evaluated N recovery, AN resulted in 26% greater recovery than urea (BURTON; JACKSON, 1962). Conversely, Picchioni and Quiroga-Garza (1999) reported N losses, measured as $^{15}N$, were not influenced by source. These latter authors reported that regardless of the source, increased N rate increased N losses. Similar pattern was also reported by Dillard et al. (2015).

Relative to N losses by volatilization, Massey et al. (2011) observed that ammonia volatilization in no-till systems was 20% greater when urea was surface applied than AN. Knight, Guertal and Wood (2007) also reported that N losses via ammonia volatilization can be as high as 35% of applied N.

Proper forage production can have positive impacts on increased food demand, especially animal protein, and it can also be a viable alternative to increase soil carbon sequestration, removing it from the atmosphere and inserting into the soil. Because most forage species produce large quantities of below- and above-ground plant material, and pastures and hayfields can promote soil C accumulation and protection against mineralization.

According to the Food and Agricultural Organization of the United Nations (FAO, 2015), grazing lands occupy 3.4 billion hectares and account for about one-fourth of potential carbon sequestration in the world. According to the Brazilian Institute of Geography and Statistics (IBGE, 2012), grazings occupy 170 million hectares in Brazil, which represent 5% of global potential C sequestration. Management of grasslands in the Cerrado biome in Brazil, with their below-ground C storage, seasonal burning and regrowth, is a key component in the global C cycle (SCURLOCK; HALL, 1998). In addition to the societal benefits, carbon sequestration can also have important implications in terms of forage production and sustainability of grazingland ecosystems, it is an important factor from the point of view of both, the individual farmer and the community, for their mutual benefit (FISHER et al., 1994).

Histroically, grazing lands in Brazil are often extensive production systems that utilize low stocking rate or cutting interval combined with a low (or no) inputs of fertilizer or soil amendments. In most cases, forage production areas occur primarly in low fertility soils that have been depleted previously by intensive agriculture. Therefore, continuation of this trend without proper soil conservation practices can degrade grazinglands even further with major impacts on soil quality and C content in an almost undetectable rate.

Proper soil management can have major impacts on soil C stocks more so than texture (Neil et al. (1997). Soil C stocks is determined by the balance between C inputs to the soil via plant fixation, and C losses to the atmosphere via decomposition. High inputs can be achieved by improving soil fertility or grazing management in order to enhance plant productivity (FOLLETT; REED, 2010).

Soil management affects soil C stocks and its characteristics. For instance, Lal (2002) demonstrated that about 60 to 70% of the C can be resequestered through adoption of different practices, such as conversion from conventional plow till to no till, fertilization, enhanced fertilizer use-efficiency, and the use of improved varieties with the ability to produce a large amount of root biomass with high content of lignin and suberin.

Nitrogen fertilization can affect plant growth (shoot and root system) with subsequent impacts on soil organic carbon (SOC), particularly in the labile fractions that are more sensitive to management.

Proper grassland management can also change the composition and the characteristics of C stored in the soil. Neil et al. (1997) reported a decay of C derived from $C_3$ plants up to 60% in an 8-yr-old pasture, in which it was replaced by the C derived from the $C_4$ vegetation.

High grazing intensity associated with low N inputs may result in a significant decline in soil organic C levels. Although soil C is often used as an indicator of modifications in the system, it is generally not sensitive to short-term changes (DUBEUX et al., 2006). Conversely, labile C can be used as a sensible indicator of changes in soil C levels in response to management (MAIA et al., 2009; SILVEIRA et al., 2013).

According to Six et al. (1998), accessing soil fractions that were functionally meaningful are important challenges for research and are necessary to a better understanding of soil C dynamics. According to the same authors, C associated to mineral fractions responded in a slower rate than for example free light fraction, that is decomposed more quickly than when they are protected within aggregate. Mineral fractions, according to Silveira et al. (2013), represent an important mechanism of C protection, particularly in coarse-textured soils.

Grasslands are important biome that affect the global C cycle. In addition to producing food,they can also act as a functional component for regulating cycles and the dynamics of biodiversity (LEMAIRE, 2007). A rapid population growth will decrease the land designated to grassland, forcing producers to intensify their agricultural systems. Intensification is often associated with high fertilizer inputs to enhance forage and animal production (VENDRAMINI et al. 2007). Given the actual scenario, there is a clear need to better understand the impacts of intensification on forage production and the impacts of management practices intended to increase forage and animal production on grassland sustainability and productivity (SBRISSIA; SILVA, 2001).

## 3 MATERIAL AND METHODS

### 3.1 Experimental area

The experiments were conducted in an established Tifton 85 hayfield at the Sao Paulo State University Campus of Jaboticabal, SP, Brazil (21°15’22” S, 48°15’18” W, 600 m asl). Soil was classified as a Typic Haplustox clay soil, utilizing the criteria of the USDA Soil Taxonomy (2014). Initial soil characterization to a depth of 0-0.1 m showed the following values; pH 5.8 ($CaCl_2$); O.M. 29 g $dm^{-3}$; P (resin) 10 mg $dm^{-3}$; K, Ca, Mg, CEC of 0.4; 2.5; 1.4; 9.5 $cmol_c$ $dm^{-3}$, respectively; 580, 140 and 280 g $kg^{-1}$ of clay, silt and sand, respectively.

Daily temperature and rainfall data were collected during the experimental period, utilizing the agrometeorological station at the University, located approximately 2,000 m from the experimental area (Figure 1 and 2).

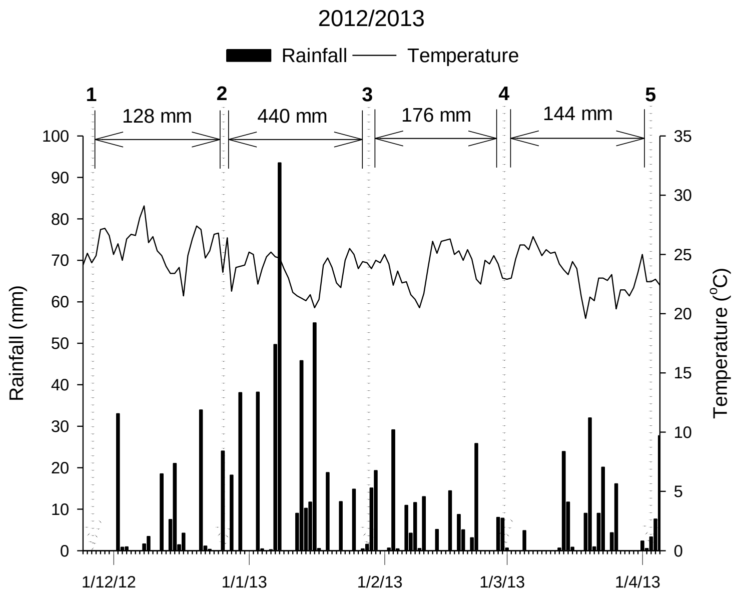


**Figure 1.** Daily rainfall and average temperature at the experimental area during 2012/2013. 1) First N fertilizers application; 2) First cutting and second N fertilizers application; 3) Second cutting and third N fertilizers application; 4) Third cutting and fourth N fertilizers application; 5) Fourth cutting.

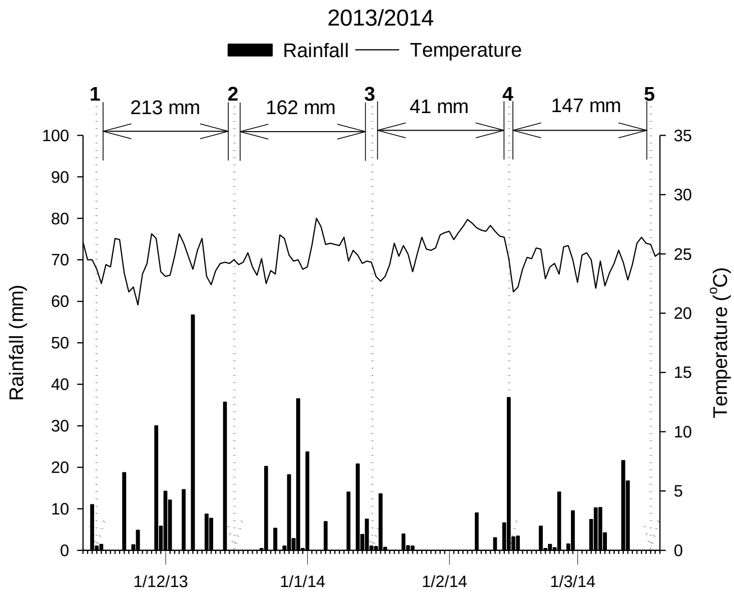


**Figure 2.** Daily rainfall and average temperature at the experimental area during 2013/2014. 1) First N fertilizers application; 2) First cutting and second N fertilizers application; 3) Second cutting and third N fertilizers application; 4) Third cutting and fourth N fertilizers application; 5) Fourth cutting.

### 3.2 Experiment 1: Effects of N sources and rates on Tifton 85 responses

A field experiment was conducted from November 26th 2012 to March 26th 2013 and November 16th 2013 to March 17th 2014 in order to evaluate Tifton 85 response to rates and N sources.

The experiment was arranged in a complete randomized block design with three replicates in a factorial scheme 5 x 2 [five N rates: 0, 60, 120, 180 and 240 kg $ha^{-1}$ N per cutting and two N sources: urea and ammonium nitrate (AN)], treatments were applied in November and on the day of every cutting.

Plots were 25 $m^2$ (5 x 5 m) with a 2 m alley. The entire experimental area was fertilized with 117 kg $ha^{-1}$ $P_2O_5$ and 52 kg $ha^{-1}$ S on October 2012 and 40 kg $ha^{-1}$ $P_2O_5$ and 18 kg $ha^{-1}$ S on October 2013, both utilizing ordinary superphosphate. Plots received 80 kg $ha^{-1}$ $K_2O$ as potassium chloride on November of 2012 and 2013 and on the day of each cutting. All fertilizers were manually applied at the surface of each plot. The forage was cut to a 7 cm stubble height at 30 day intervals for a total of eight cutting events, four in each year.

Tiller density was performed using the colored wires technique (DAVIES, 1981) utilizing one microplot (0.3 x 0.4 m) per regular plot. Tillers were marked with a single and different color for each cutting and after the fourth cutting of each year the wires were collected and separated by color, representing the number of new tillers per square meter per cutting. The same procedure was adopted on the following year. Fertilization followed the same procedure as the regular plots.

On the day before cutting event growth variables were analyzed. Chlorophyll Index (CI) was quantified using the clorofiLOG Falker CFL1030, readings per plot were performed on the middle third of the +2 leaf (second leaf totally developed with a visible ligule) as indicated by the manufacturer and the average reading was analyzed. There were also performance Leaf Area Index (LAI) and Intercepted Photosynthetically Active Radiation (PARi) readings, utilizing the AccuPAR LP-80 with three readings per plot. Readings were taken above the canopy and on the soil surface between 11h 30min and 12h 30min. Readings were utilized to calculate the percentage of light intercepted by the forage canopy utilizing equation 1:

$$\text{PARi} = \left(1 - \frac{\text{PAR soil surface}}{\text{PAR above canopy}}\right) \times 100 \text{ (eq. 1)}$$

After cutting 13 $m^2$ of the total plot, fresh material of each plot was weighted. In order to quantify dry matter yield, evaluation of the moisture of the forage was carried out, in other words a sample of each plot was weighted on the field and then it was taken to the laboratory, weighted and oven dried at 65 $^oC$ for 72h, and then reweighted. Dry samples were ground in a Wiley mill through a 1 mm sieve and material was analyzed for total Kjeldahl N.

To evaluate N recovery from the fertilizer, a microplot (0.3 x 0.4 m) was installed within each plot and received urea and ammonium nitrate with $^{15}N$. Fertilization procedure followed the same procedure as the regular plots. Urea enriched with 1.366% of $^{15}N$ atoms and ammonium nitrate (doubly leballed) with 2.000% of $^{15}N$ atoms were applied in the same way as in the regular plots. Tissue and soil samples were analyzed at CENA/USP in order to determine total N content and $^{15}N$ abundance. Analyses were performed in a mass spectrometer with automatic analyzer as mentioned in Barrier and Prosser (1996).

Microplots were also cut at a 30-day intervals at a 7 cm stubble height using scissors. After the fourth cutting inside microplots were also collected roots from 0 – 0.4 m depth in 0.1 m layers utilizing the method of the modified auger as described in Caires et al. (2008). Plant material was separated from the soil by gently washing the samples with deionized water through 2.0 mm, 1.0 mm and 0.3 mm mesh sieves. Samples were oven dried at 65 $^oC$ for 72h for dry matter determination. Root samples were composited by depth and analyzed for N recovery. Results were reported as root DM by sampling volume (dry matter root density – DMRD).

In order to quantify the N remained in the system, litter material (below cutting height and dead plant material on the soil surface) was also collected by hand and washed with deionized water and detergent followed by a HCl 0.1 mol $L^{-1}$ solution and deionized water. Samples were oven dried at 65 $^oC$ for 72 h for dry matter determination.

Soil samples (0 – 0.4 m) in 0.1 m layers utilizing an auger, were collected, air-dried and sieved through a 2 mm sieve.

It was utilized the system balance between input and output of $^{15}N$ (GAVA et al., 2006). With the results of isotopic enrichment (shoot, litter and root) nitrogen in the plant derived from the fertilizer (Npdf) and nitrogen recovery were calculated according to equations 2, 3 and 4:

$$\text{Npdf (\%)} = \left(\frac{\text{\% of excess atoms of } ^{15}\text{N in the plant}}{\text{\% of excess atoms of } ^{15}\text{N in the fertilizer}}\right) \text{ x 100 (eq. 2)}$$

$$\text{Npdf (kg ha}^{-1}) = \left[\frac{\text{Npdf(\%)}}{100}\right] \text{ x total N on plant tissue (eq. 3)}$$

$$\text{Recovery (\%)} = \left[\frac{\text{Npdf (kg ha}^{-1})}{\text{N rate (kg ha}^{-1})}\right] \text{ x 100 (eq. 4)}$$

The % of N on the soil derived from the fertilizer (Nsdf) and N recovery on soil (NRS) by the following equations, 5, 6 and 7:

$$\text{Nsdf (\%)} = \left(\frac{\text{\% of excess atoms of } ^{15}\text{N in the soil}}{\text{\% of excess atoms of } ^{15}\text{N in the fertilizer}}\right) \text{ x 100 (eq. 5)}$$

$$\text{Nsdf (kg ha}^{-1}) = \frac{\left[\text{N in the soil}\left(\text{kg ha}^{-1}\right) \text{ x Nsdf(\%)}\right]}{100} \text{ (eq. 6)}$$

$$\text{NRS (\%)} = \left[\frac{\text{Nsdf}\left(\text{kg ha}^{-1}\right)}{\text{N rate}\left(\text{kg ha}^{-1}\right)}\right] \text{ x 100 (eq. 7)}$$

Statistical analyses were performed using AgroEstat (BARBOSA; MALDONADO, 2015) software. Treatments and interaction were considered different when F-test P values were $<0.05$. Regressions were adjusted utilizing the statistical package SigmaPlot version 11.0 (Systat Software, San Jose, CA).

### 3.3 Experiment 2: Soil organic carbon fractions

Thirty soil samples mixed in one composed sample were collected from random location at the experimental area prior to the beginning of the experiment in 2012. The experimental area has been cultivated with forage since 1985. Soil samples were also collected from an area of native forest located adjacent to the experimental area. Samples were collected at the end of the 2-yr study (March of 2014) from the treatments receiving ammonium nitrate. Ten sub-samples samples were collected (0-0.1 and 0.1-0.2 m depth) from each site, mixed well, and combined into a composed sample.  Samples were air-dried, and sieved through a 2-mm screen. Soil analysis was performed as a split-plot design where N rates were the primary treatment and soil depth the secondary treatment, with three replicates. Undisturbed soil cores were also collected in order to determine soil bulk density. Soil samples were fractionated in to particulate organic carbon (POC), free light fraction (FLF) and mineral fraction associated with silt plus clay (C-min) (CAMBARELLA; ELLIOTT, 1992). Carbon (C) was quantified in each fraction by dry combustion.

POC was determined with a modified procedure from Cambarella and Elliott (1992) described by Silveira et al. (2013). A 10-g subsample of soil (<2 mm) was shaken in 30 mL of 5 g $L^{-1}$ sodium hexametaphosphate solution on a reciprocal shaker (200 rpm) for 15 h. The suspension (FLF) was gently washed with distilled deionized water and the remaining was filtered through a 53-µm sieve. Fractions retained 53 µm sieves and the remaining slurry was transferred to drying dishes, and weighed after dried at 55 $^{o}$C for 72 h. Material retained in the 53 µm sieve corresponded to POC whereas the fractions <53 µm were assumed to be associated with silt plus clay (C-min). Total C for all samples were quantified by dry combustion using a Flash EA 1112-NC elemental analyzer (CE Elantech, Lakewood, NJ). Natural abundance stable isotopes ratios ($\delta^{13}$C) was quantified on a Thermo-Finnigan MAT DeltaPlus XL Isotope Ratio Mass Spectrometer (IRMS) interfaced via a Conflo-III device to a Costech ECS 4010 elemental analyzer (Costech, Valencia, CA). Values were expressed in relation to $\delta^{13}$C standard (BOUTTON, 1991).

The difference between initial soil C and that determined 2 yr after the treatments were imposed was calculated. Negative values represent C losses while positive values represent inserted gains.

Data were subjected to an ANOVA test Statistical analyses using AgroEstat (BARBOSA; MALDONADO, 2015) software.

# 4 RESULTS AND DISCUSSION

## 4.1 Climatological data

Cumulative rainfall during the period 2012/2013 (Figure 1) was 17% greater than the 30-year average. Despite the relatively high rainfall amount, rainfall pattern was not evenly distributed throughout the growing season. For instance, while rainfall during January 2013 was 60% above the 30-yr average; in February 2013 the rainfall recorded was 30% (57 mm) below average. Inadequate rainfall during February may have affected forage yields during this period. Minimum (average 19 $^{o}$C) and maximum (average 30 $^{o}$C) temperature were typical for the region; average temperature was above 20$^{o}$C.

On the second year 2013/20114 during experimental period, the cumulative rainfall (Figure 2) was lower than then 30-yr average. At the second and third cuttings the difference reached 33% and 79% less rainfall than the average for the region. This insufficient amount of water could have affected the efficiency of the N sources as well as the capacity of the culture to respond to an increase in nitrogen fertilization.

## 4.2 Experiment 1: Effects of N sources and rates on Tifton 85 responses

### 4.2.1 Shoot N concentration and Chlorophyll Index

Nitrogen shoot concentration was affected by source in three cuttings. In these cases, AN provided higher shoot N concentration than urea. Nitrogen rates influenced N concentration represented by a linear adjust in all cuts (Table 1). When interaction was significant, it presented a linear regression with AN promoting the highest shoot N concentration (Figure 3).

**Table 1.** Effect of rates and N sources on Tifton 85 shoot N concentration (g kg$^{-1}$).

| Treatments | Cuttings First Year (2012/2013) | | | | Cuttings Second Year (2013/2014) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1$^{st}$ | 2$^{nd}$ | 3$^{rd}$ | 4$^{th}$ | 1$^{st}$ | 2$^{nd}$ | 3$^{rd}$ | 4$^{th}$ |
| **Source (S)** | ------------------------------------------g kg$^{-1}$------------------------------------------ | | | | | | | |
| **AN** | 16.1 | 20.0 | 21.3 | 23.4 | 19.3 | 22.6 | 23.7 | 22.2 |
| **Urea** | 14.8 | 19.0 | 21.5 | 23.5 | 20.0 | 21.8 | 21.1 | 22.3 |
| **F test** | 9.1** | 8.4** | 0.1$^{ns}$ | 0.1$^{ns}$ | 2.3$^{ns}$ | 1.8$^{ns}$ | 37.4** | 0.1$^{ns}$ |
| **N (kg ha$^{-1}$)** | | | | | | | | |
| **0** | 11.1 | 15.5 | 17.1 | 20.1 | 17.0 | 18.4 | 16.3 | 16.3 |
| **60** | 14.1 | 17.4 | 17.4 | 20.7 | 17.2 | 19.5 | 19.4 | 18.5 |
| **120** | 15.4 | 18.8 | 19.4 | 21.9 | 18.8 | 22.0 | 22.7 | 22.5 |
| **180** | 17.5 | 22.7 | 26.3 | 28.1 | 20.1 | 24.6 | 25.8 | 26.5 |
| **240** | 19.2 | 23.2 | 26.8 | 27.0 | 24.3 | 26.5 | 28.0 | 27.5 |
| **F test** | 154.7** | 257.5** | 38.7** | 22.2** | 11.4** | 90.0** | 420.3** | 408.1** |
| **Regression** | (L) | (L) | (L) | (L) | (L) | (L) | (L) | (L) |
| **Interaction** | ------------------------------------------F test------------------------------------------ | | | | | | | |
| **S x N** | 1.4$^{ns}$ | 3.9** | 0.3$^{ns}$ | 0.8$^{ns}$ | 2.5$^{ns}$ | 2.5$^{ns}$ | 3.9* | 1.1$^{ns}$ |
| **CV%** | 7.9 | 5.1 | 16.4 | 14.4 | 6.7 | 7.9 | 5.0 | 5.2 |

**, *, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (L) Linear adjust; CV coefficient of variation.

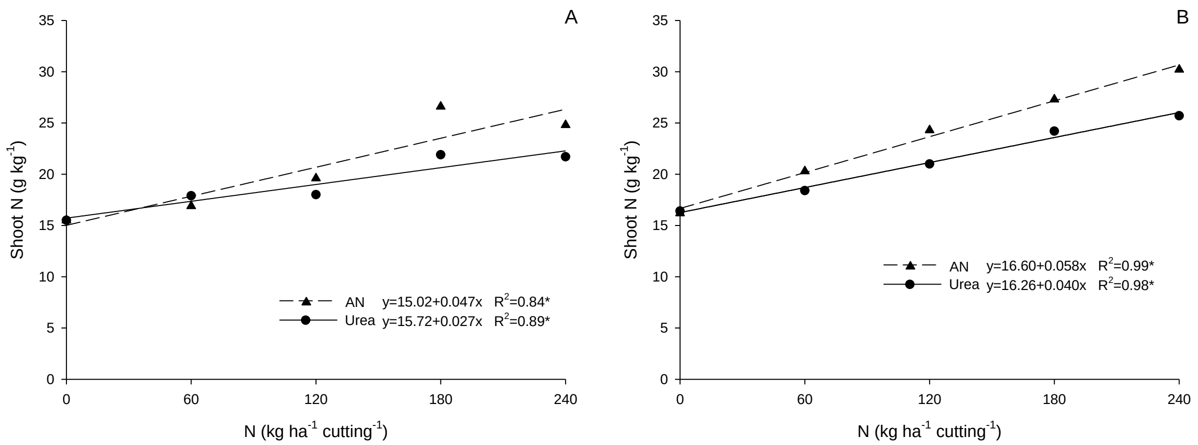


**Figure 3.** Shoot N concentration on Tifton 85 due to rates and N sources. **A)** Second cutting 2012/2013 and **B)** third cutting 2013/2014. **Significantly different (P<0.01); *Significantly different (P<0.05).

Along with shoot N concentration, Chlorophyll Index (CI) measured *in situ*, also showed an increasing pattern whenever N supply was increased. When there was a difference in source treatments AN highlights as a more efficient source than Urea presenting higher CI values (Table 2). Interaction was only reported in one cutting, both sources presented a quadratic regression, AN presented a higher peak compared to urea whenever N was applied to the system (Figure 4).

Urea is known to be a less efficient source when compared to ammonium nitrate, especially when the amount of water is not sufficient to incorporate the fertilizer to the soil. Some authors claim that this decrease in efficiency is due to more suitable losses of ammonia by volatilization (LARA CABEZAS, KORNDORFER; CANTARELLA, 1997; KNIGHT; GUERTAL; WOOD, 2007; MASSEY et al., 2011). That usually happen when the amount of rainfall after fertilization is not sufficient to incorporate urea into the soil before the process of hydrolysis is completed (FOX; KERN; PIEKIELEK, 1986), and this way ammonia is lost and becomes not available, decreasing N uptake by the plants (CONNELL et al., 2011), what can lead to low values of shoot N content and consequently CI.

**Table 2.** Effect of rates and N sources on Tifton 85 Chlorophyll Index (CI).

| Treatments | Cuttings First Year (2012/2013) | | | | Cuttings Second Year (2013/2014) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 1st | 2nd | 3rd | 4th |
| **Source (S)** | | | | | | | | |
| **AN** | 26.3 | 25.4 | 25.4 | 21.5 | 22.6 | 25.8 | 27.3 | 21.9 |
| **Urea** | 22.5 | 24.0 | 22.7 | 18.4 | 23.1 | 24.6 | 25.9 | 20.9 |
| **F test** | 4.6* | 2.3$^{ns}$ | 6.6* | 7.2* | 0.2$^{ns}$ | 1.9$^{ns}$ | 1.2$^{ns}$ | 1.6$^{ns}$ |
| **N (kg ha$^{-1}$)** | | | | | | | | |
| **0** | 12.2 | 10.3 | 13.8 | 8.5 | 10.2 | 11.8 | 17.7 | 11.9 |
| **60** | 21.4 | 20.8 | 21.1 | 18.2 | 20.0 | 21.5 | 20.5 | 19.6 |
| **120** | 25.0 | 26.9 | 23.8 | 20.0 | 24.4 | 26.3 | 29.5 | 23.0 |
| **180** | 29.3 | 31.0 | 31.6 | 25.8 | 28.7 | 32.3 | 31.8 | 26.6 |
| **240** | 34.1 | 34.7 | 29.9 | 27.3 | 31.1 | 34.0 | 33.4 | 25.9 |
| **F test** | 69.9 ** | 15.3 ** | 9.1 ** | 6.6 * | 8.6 ** | 14.9 ** | 88.0 ** | 26.2 ** |
| **Regression** | (L) | (Q) | (Q) | (Q) | (Q) | (Q) | (L) | (Q) |
| | **----------F test----------** | | | | | | | |
| **S x N** | 1.5$^{ns}$ | 4.3* | 1.9$^{ns}$ | 1.1$^{ns}$ | 1.1$^{ns}$ | 1.0$^{ns}$ | 1.0$^{ns}$ | 2.2$^{ns}$ |
| **CV%** | 19.6 | 10.5 | 11.7 | 15.8 | 14.3 | 10.0 | 13.3 | 10.0 |

**, *, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (Q), (L) Quadratic and Linear adjust, respectively; CV coefficient of variation.

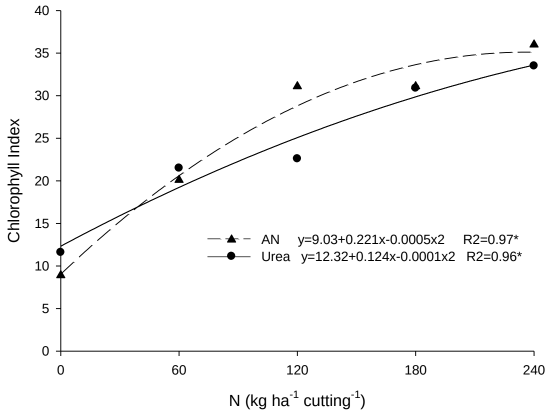


**Figure 4.** Chlorophyll Index on Tifton 85 due to rates and N sources. Second cutting 2012/2013. **Significantly different ($P<0.01$); *Significantly different ($P<0.05$).

Nitrogen present in ammonium nitrate is also a subjected to losses, particularly via leaching. Bowen et al. (1993) and Guertal and Howe (2012) indicated that the extent that N present in AN can be lost by leaching is dependent on soil texture, with clay soils generally resulting in less N losses.

Shoot N concentration in forages is an important tool to monitor forage quality. In-situ alternatives to estimate forage nutritive value can be valuable tools for producers. In this experiment, when comparing shoot N concentration and chlorophyll index the same pattern and response were observed, presenting a linear increasing regression (Figure 5). In this case it is possible to infer that CI might be a valued and practical alternative method to evaluate Tifton 85 nutritional status *in situ*.

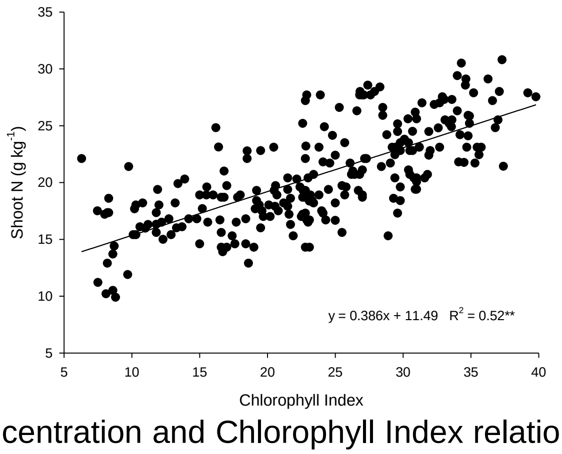


**Figure 5.** Shoot N concentration and Chlorophyll Index relation on Tifton 85 (n=240). **Significantly different ($P<0.01$).

Similar to shoot N, chlorophyll Index responded to N fertilization. This response may be explained by the increase in sometimes more than 100%. The equipment measures the intensity of green in the leaf to generate a value that correspond to the amount of N present in plant tissue. Since nitrogen is one of the nutrients that constitutes the chlorophyll molecule and chlorophyll is the pigment that gives the green color to plant tissue (TAIZ; ZEIGER, 2010), whenever there is a decrease of N in the systems the plants present a lighter green coloration due to the lower amount of the pigment, or the opposite (MARSCHNER, 2012). The equipment was able to quantify these differences in green tones and state if the plant was or was not well nourished. These deficiency symptoms could be noticed among the treatments in this experiment, older leaves became light green followed by yellowish colored tones and in some cases senescence of elder leaves, being more accentuated when N supply was omitted. Nitrogen concentrations inferior to 18 g kg$^{-1}$ were associated with symptoms of N deficiency on the forage.

Nitrogen is also constituting many other compounds such as proteins. Shoot N concentration and chlorophyll index are important factors to discuss forage quality, that is a limiting factor for animal production, especially when commercializing hay. According to Alderman et al. (2011a) since forage quality is determined directly from shoot N concentration, it follows that N fertilization is able to increase plant uptake, as observed in the present work (Table 1), and consequently increase quality.

Shoot N concentration was close to the ones obtained by Quaresma et al. (2011) and Silveira et al. (2015), since in this experiment there were utilized higher N rates than the ones mentioned by the authors, higher shoot N concentration was also recorded. According to Kelling and Matocha (1990) bermudagrass was considered to be in a satisfactory level of nutrition when in a range of 22 to 30 g kg$^{-1}$ of N.

Forage quality can also affect animal consumption. Although Kelling and Matocha (1990) considered the forage in a deficient nutritional status when shoot N concentration was below 15 g kg$^{-1}$, Milford and Minson (1966) suggested that only values inferior to 11 g kg$^{-1}$ of N were detrimental to hay consumption by animals; although, such values were not founded in this study, even in plots that did not received N. What might have happened in the control plots was a concentration effect, the plant absorbs a certain amount of N nevertheless this is not enough for plant development and this is stocked in the tissues simulating a false well-nourished plant status (MARSCHNER, 2012).

### 4.2.2 Tiller density, Leaf Area Index and Intercepted Photosynthetically Active Radiation

N source effect on tiller density was not as pronounce as the N rate effect. N sources shown effect in two cuttings but results were controversial. In each cutting, a different source presented the highest tiller density (Table 3). Increased N rates tended to increase tiller density, but whenever there was an insufficient amount of rainfall tillering was compromised as observed in the third cutting of 2013/2014 (Table 3).

**Table 3.** Effect of rates and N sources on Tifton 85 tillering.

| Treatments | Cuttings First Year (2012/2013) | | | | Cuttings Second Year (2013/2014) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 1st | 2nd | 3rd | 4th |
| **Source (S)** | tillers $m^{-2}$ | | | | | | | |
| **AN** | 322 | 157 | 290 | 375 | 505 | 632 | 363 | 553 |
| **Urea** | 299 | 124 | 311 | 303 | 545 | 739 | 439 | 610 |
| **F test** | $1.2^{ns}$ | $3.2^{ns}$ | $1.0^{ns}$ | 19.5** | $1.4^{ns}$ | 5.0* | $3.4^{ns}$ | $1.1^{ns}$ |
| **N (kg $ha^{-1}$)** | | | | | | | | |
| **0** | 343 | 53 | 164 | 120 | 312 | 626 | 381 | 296 |
| **60** | 245 | 113 | 219 | 258 | 565 | 635 | 460 | 647 |
| **120** | 322 | 128 | 282 | 375 | 540 | 818 | 340 | 685 |
| **180** | 283 | 185 | 395 | 390 | 697 | 688 | 449 | 749 |
| **240** | 359 | 224 | 443 | 550 | 518 | 660 | 375 | 532 |
| **F test** | 3.8* | 10.3* | 24.9** | 78.1** | 11.5** | $2.1^{ns}$ | $1.2^{ns}$ | 8.4** |
| **Regression** | Q | L | L | L | Q | - | - | Q |
| **Interaction** | F test | | | | | | | |
| **S x N** | 4.6* | $1.4^{ns}$ | 3.1** | 8.2** | $1.1^{ns}$ | 4.4* | $1.3^{ns}$ | $0.6^{ns}$ |
| **CV%** | 18.8 | 36.0 | 19.1 | 13.1 | 19.0 | 19.1 | 28.3 | 25.9 |

**, *, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (Q), (L) Quadratic and Linear adjust, respectively; CV coefficient of variation.

The source x rate interaction was significant in both years at the majority of cuttings, and a curious pattern was observed, on the first year (Figure 6.1). Whenever there was a significance for the regression a linear increase was noted, and, on the second year (Figure 6.2) significant responses were represented by quadratic adjust. A fact that might explain these responses was that as tillers grew, in higher N rates, they achieved apical dominance and continued to grow, while nondominant tillers senesced (PREMAZZI; MONTEIRO; CORRENTE, 2003; ALDERMAN; BOOTE; SOLLENBERGER, 2011b), thereby reducing tiller density and tending to a stabilization number. It is also relevant to mention that no drought sever limitations were observed in the first year (Figure 1) but on the second year the amount of rainfall was 60% lower than the usual for the area (Figure 2).

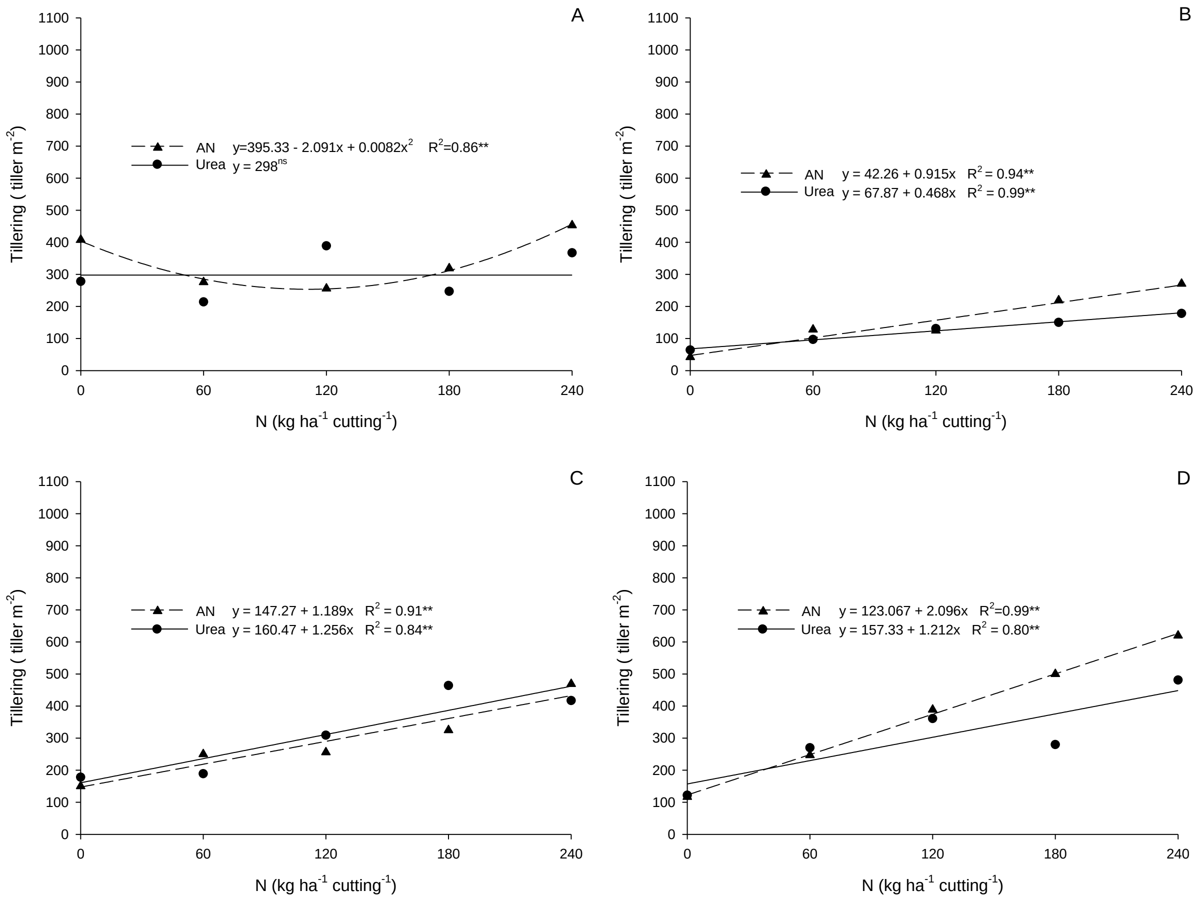


**Figure 6.1.** Tillering on Tifton 85 due to rates and N Sources. **A)** First; **B)** second; **C)** third and **D)** fourth cutting 2012/2013. **Significantly different ($P<0.01$); $^{ns}$ Non-significant.

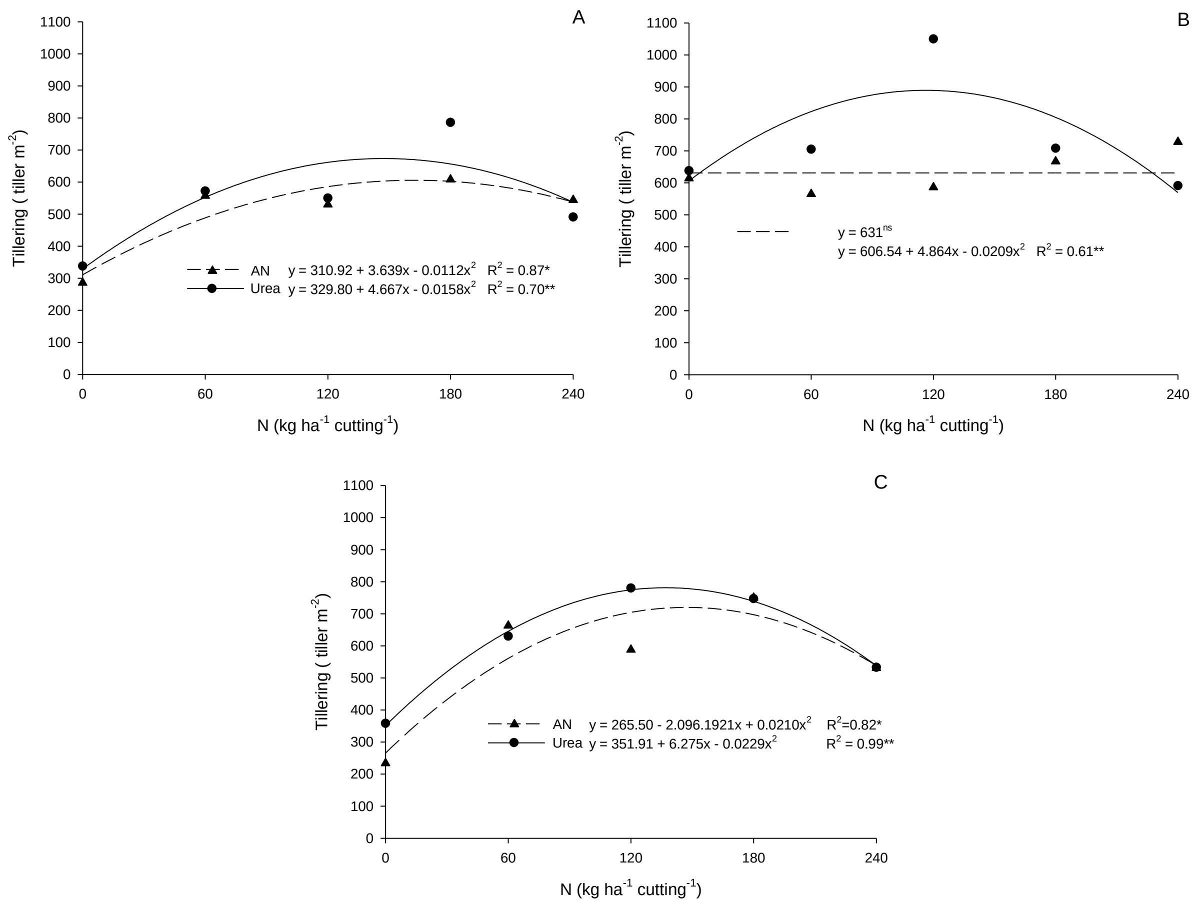


**Figure 6.2.** Tillering on Tifton 85 due to rates and N sources. **A)** First; **B)** second and **C)** fourth cutting 2013/2014. **Significantly different (P<0.01); *Significantly different (P<0.05); $^{ns}$ Non-significant.

Leaf area index was affected by N sources, rates and their interaction. Ammonium nitrate generally resulted in higher LAI values compared to urea (Table 4). The N rate effect on LAI can be noticed throughout the cuttings presenting a linear or quadratic adjust as N rate increased (Figure 7). Linear adjusts were only found in the first cuts of both years where the crop was able to respond to an increase in N fertilization rate up to the highest rate utilized. The following cuttings were represented by quadratic regressions, those indicate that a plateau of response was reached and the forage would not respond to an increase on N supply. Significant interaction was only reported in two cuttings; first cutting on 2012/2013 where the system was still under development tending to a stabilization and on the third cutting 2013/2014 where a drought period was observed. Lower LAI at this cutting might be attributed to an inferior cell expansion rate and an intense process of leaf senescence generated by an insufficient amount of water and nutrient (ANDRADE et al., 2002; KUNZ et al., 2007; SANGOI et al., 2011). Only AN presented quadratic regression, since urea adjusts were linear that means that an increase in N rates, when utilizing urea, might still be responsive. What in fact, this was probably due to its higher losses by volatilization, as mentioned before, thus a larger amount of fertilizer has to be applied in order to reach crops maximum response.

**Table 4.** Effect of rates and N sources on Tifton 85 Leaf Area Index (LAI).

| Treatments | Cuttings First Year (2012/2013) | | | | Cuttings Second Year (2013/2014) | | | |
|---|---|---|---|---|---|---|---|---|
| | **1st** | **2nd** | **3rd** | **4th** | **1st** | **2nd** | **3rd** | **4th** |
| **Source (S)** | | | | | | | | |
| **AN** | 3.5 | 2.8 | 4.2 | 4.2 | 3.8 | 4.3 | 2.9 | 3.7 |
| **Urea** | 2.5 | 2.6 | 4.0 | 3.6 | 3.5 | 3.7 | 2.0 | 3.5 |
| **F test** | 14.5** | 2.1$^{ns}$ | 0.5$^{ns}$ | 14.2** | 2.5$^{ns}$ | 5.3* | 28.7** | 1.1$^{ns}$ |
| **N (kg ha$^{-1}$)** | | | | | | | | |
| **0** | 1.3 | 0.4 | 0.5 | 0.7 | 0.3 | 0.4 | 0.3 | 0.2 |
| **60** | 2.1 | 1.5 | 2.7 | 3.0 | 1.6 | 2.7 | 1.7 | 2.6 |
| **120** | 3.1 | 3.0 | 4.7 | 4.6 | 3.5 | 4.9 | 2.8 | 4.8 |
| **180** | 3.8 | 4.3 | 6.3 | 5.7 | 5.8 | 6.1 | 3.5 | 5.2 |
| **240** | 4.9 | 4.4 | 6.3 | 5.5 | 7.3 | 6.1 | 3.9 | 5.2 |
| **F test** | 87.2** | 13.6 ** | 8.9** | 68.6** | 661.9 ** | 27.4 ** | 12.8 ** | 137.4 ** |
| **Regression** | (L) | (Q) | (Q) | (Q) | (L) | (Q) | (Q) | (Q) |
| **Interaction** | ----------------------------------------------**F test**---------------------------------------------------- | | | | | | | |
| **S x N** | 6.3** | 3.7* | 0.8$^{ns}$ | 2.2$^{ns}$ | 2.3$^{ns}$ | 0.6$^{ns}$ | 4.7** | 2.1$^{ns}$ |
| **CV%** | 24.2 | 14.4 | 25.6 | 10.8 | 14.9 | 17.2 | 18.3 | 9.9 |

**, *, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (Q), (L) Quadratic and Linear adjust, respectively; CV coefficient of variation.

Leaf area index was affected by the size and number of leaves. Since number of leaves per tiller in forages are limited by a genetic factor (OLIVEIRA; PEREIRA; HUAMAN, 2000) thus, an increase in LAI can only be achieved increasing tiller density and leaf area. In order to increase both, N supply plays an important role being part of carbohydrates, amino acids, enzymes and phytohormones such as cytokinins. According to Marschner (2012) the enhance of N supply increased root growth, therefore, it enhanced the synthesis and exportation of cytokinins to the shoots. The presence of this hormone will increase cell division and expansion, increasing leaf area index, protein synthesis and delay in protein degradation, aggregate forage quality, and also delay leaf senescence (TAIZ; ZEIGER, 2010).

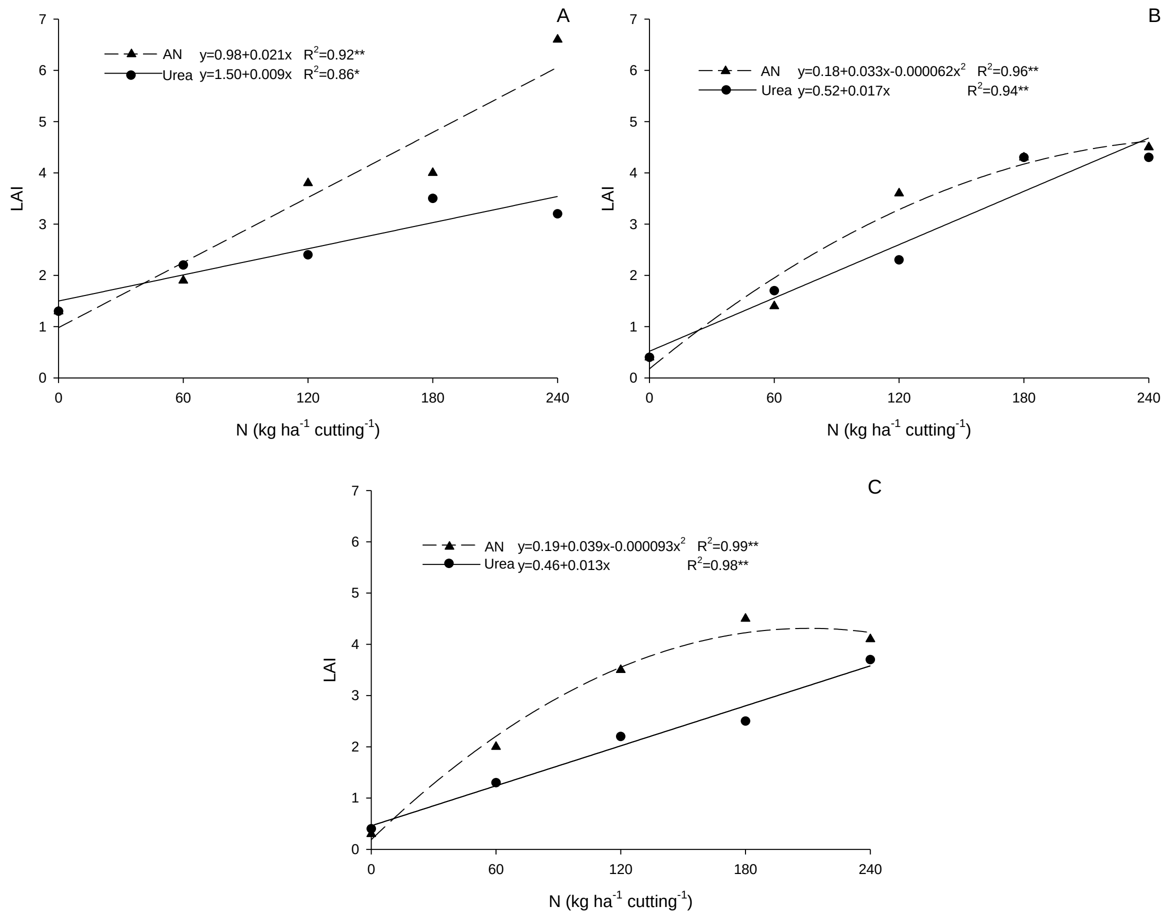


**Figure 7.** Leaf Area Index (LAI) on Tifton 85 due to rates and N sources. **A)** First and **B)** second cuttings 2012/2013 and **C)** third cutting 2013/2014. **Significantly different (P<0.01); *Significantly different (P<0.05).

Plant growth is conditioned primarily to light absorption derived from solar radiation, by its interception and utilization in the process of photosynthesis (NABINGER; PONTES, 2001). Enhancing tiller density and leaf area can increase LAI, and subsequently promote a larger leaf surface available for photosynthesis. The light that is absorbed by the plant is called intercepted photosynthetically active radiation, that in this experiment, as well as LAI, presented a significant effect on N source at the third cutting 2013/2014, whereupon water was a limiting factor (Table 5).

**Table 5.** Effect of rates and N sources on Tifton 85 intercepted Photosynthetically Active Radiation (%PARi).

| Treatments | Cuttings First Year (2012/2013) | | | | Cuttings Second Year (2013/2014) | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 1st | 2nd | 3rd | 4th |
| **Source (S)** | ------------------------%------------------------ | | | | | | | |
| **AN** | 77.6 | 71.2 | 78.2 | 84.1 | 68.6 | 76.9 | 65.6 | 78.9 |
| **Urea** | 72.0 | 67.0 | 77.9 | 80.2 | 63.0 | 73.8 | 55.1 | 79.4 |
| **F test** | 3.1ns | 2.0ns | 0.02ns | 4.3ns | 3.9ns | 4.0ns | 33.9** | 0.2ns |
| **N (kg ha$^{-1}$)** | | | | | | | | |
| **0** | 49.6 | 24.0 | 25.5 | 37.9 | 11.3 | 18.0 | 14.1 | 16.1 |
| **60** | 69.0 | 63.0 | 77.9 | 84.1 | 52.1 | 74.8 | 52.2 | 86.1 |
| **120** | 78.9 | 72.7 | 92.9 | 94.8 | 77.2 | 92.2 | 71.5 | 97.2 |
| **180** | 86.2 | 93.2 | 97.3 | 97.8 | 92.3 | 95.8 | 81.0 | 98.1 |
| **240** | 90.3 | 92.6 | 96.8 | 97.6 | 96.2 | 95.8 | 82.9 | 98.1 |
| **F test** | 6.3* | 29.8** | 177.1** | 231.5** | 50.2 ** | 363.5** | 119.6** | 1042.3** |
| **Regression** | (Q) | (Q) | (Q) | (Q) | (Q) | (Q) | (Q) | (Q) |
| **Interaction** | ------------------------F test------------------------ | | | | | | | |
| **S x N** | 1.2ns | 2.3ns | 0.7ns | 2.0ns | 1.0ns | 0.9ns | 4.6** | 0.8ns |
| **CV%** | 11.6 | 11.9 | 7.3 | 5.2 | 11.8 | 5.8 | 8.2 | 3.9 |

**, *, ns Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (Q) Quadratic adjust; CV coefficient of variation.

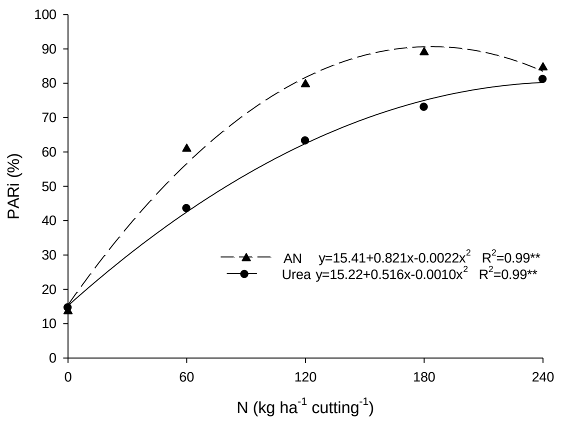


**Figure 8.** Intercepted Photosynthetically Active Radiation (PARi) on Tifton 85 due to rates and N sources. Third cutting 2013/2014. **Significantly different (P<0.01); *Significantly different (P<0.05).

The N rate effect was noticed in all cutting events with quadratic regressions which values superior to 95% were only reached at rates higher than 180 kg ha$^{-1}$ per cutting. According to Silva et al. (2012) when the forage is able to intercept 95% of the PAR is when the crop expresses its maximum potential.

A significant interaction was only observed in the third cutting (2013/2014). Both sources presenting quadratic adjusts and as reported on the source effect (Figure 8), AN showed higher results compared to urea on the 120 and 180 kg ha$^{-1}$ N rates.

Since photosynthetically active radiation measures the ability of plants to absorb light by its canopy and forage canopy is mainly constituted by leaves. Due to this, LAI and PARi were close related in the present study (Figure 9), which is consistent with Madakadze et al. (1998), Alderman, Boote and Sollenberger (2011b) and Borges et al. (2011).

Humphreys (1991) suggested that LAI for forages should range between 3 to 5, which correspond to 95% of canopy light interception, called critical LAI. In the present study, critical LAI was reached at 5.2, above this PARi value stabilized and even though increasing LAI plants were not able to intercept more light (Figure 9).

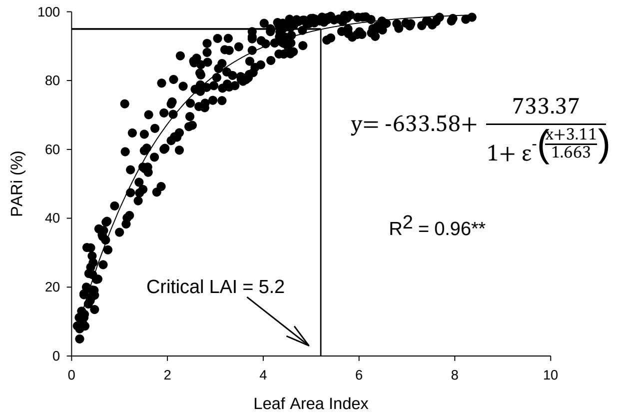


**Figure 9.** Photosynthetically Active Radiation intercepted and Leaf Area Index relation on Tifton 85 (n=240). **Significantly different (P<0.01).

It is also important to report that for control treatment, after a few cuts, Tifton 85 sward completely change its structure compared to fertilized plots. Visual N deficiency was observed in the control treatments. Plants were prostrated and tillers were thin and woody, leaves were small and there were also failures in the stand. The poor performance of control treatments is consistent with low PARi and LAI values (11%

and 0.2, respectively) while at some plots plants were able to absorb 98% of the incident light and LAI was 7.3 (Table 5). According to Alderman, Boote and Sollenberger (2011b), N supply was not sufficient to maintain forage production in the control treatments.

### 4.2.3 Dry Matter Yield

Nitrogen source effect was only noticed on DM yield of the third cutting 2013/2014 and on cumulative production of both years. Ammonium nitrate was generally a more efficient source compared to urea. While N source presented difference only in one cut, N rates presented significance in all cuttings and cumulative production in which a linear regression was only noticed on the first cut, all the others presented quadratic adjusts (Table 6). Cumulative production was not influenced by N sources and a maximum DM yield was reached at the rate of 210 kg ha$^{-1}$ per cutting producing 37.2 Mg ha$^{-1}$ ($R^2$=0.99). Nitrogen fertilization increased yield, on average, by 85% when compared to the control treatment. The difference from the plots that received fertilizer to the ones that did not receive was visible. Insufficient amount of rainfall may have likely affected N sources and rate efficiency, due to that the third cutting of 2013/2014 was the only one that presented a significant interaction where AN was a more efficient source than urea and regression followed a quadratic adjust (Figure 10).

Dry matter yield on Tifton 85 should be explained over a series of; shoot N concentration, chlorophyll index, tillering, leaf area, leaf area index, photosynthetically active radiation intercepted, rainfall and temperature, all those mentioned before. The efficiency of the sources or the response to nitrogen rates were all influenced by those factors.

**Table 6.** Effect of rates and nitrogen sources, on Tifton 85 dry matter yield.

| Treatments | Cutting First Year (2012/2013) | | | | | Cutting Second Year (2013/2014) | | | | | Cumulative |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | Sum | 1st | 2nd | 3rd | 4th | Sum | Total |
| **Source (S)** | Mg ha$^{-1}$ | | | | | | | | | | |
| **AN** | 3.4 | 2.7 | 3.1 | 3.1 | 12.3 | 4.5 | 3.6 | 2.2 | 3.7 | 14.0 | 26.3 |
| **Urea** | 3.1 | 2.6 | 2.9 | 2.8 | 11.5 | 4.6 | 3.7 | 1.6 | 3.8 | 13.7 | 25.2 |
| **F test** | 2.3ns | 0.5ns | 1.6ns | 2.6ns | 4.3ns | 0.1ns | 0.4ns | 59.4** | 0.2ns | 0.6ns | 3.4ns |
| **N (kg ha$^{-1}$)** | | | | | | | | | | | |
| **0** | 1.7 | 0.1 | 0.2 | 0.2 | 2.2 | 0.8 | 0.9 | 0.1 | 0.8 | 2.7 | 4.9 |
| **60** | 2.6 | 1.6 | 2.0 | 2.4 | 8.6 | 3.5 | 3.0 | 1.3 | 4.1 | 12.0 | 20.6 |
| **120** | 3.5 | 3.1 | 3.8 | 3.7 | 14.1 | 6.0 | 4.3 | 2.4 | 4.6 | 17.2 | 31.3 |
| **180** | 4.1 | 4.2 | 4.4 | 4.1 | 17.0 | 6.1 | 4.9 | 2.7 | 4.5 | 18.2 | 35.1 |
| **240** | 4.5 | 4.3 | 4.5 | 4.3 | 17.6 | 6.6 | 5.1 | 2.8 | 4.8 | 19.3 | 36.9 |
| **F test** | 114.6** | 26.0** | 39.5** | 61.4** | 67.1** | 38.6** | 39.6** | 78.6** | 50.4** | 141.0** | 226.0** |
| **Regression** | L | Q | Q | Q | Q | Q | Q | Q | Q | Q | Q |
| **Interaction** | F test | | | | | | | | | | |
| **S x N** | 1.5ns | 2.4ns | 0.6ns | 1.0ns | 1.9ns | 1.7ns | 0.9ns | 4.7** | 1.0ns | 0.6ns | 0.5ns |
| **CV%** | 15.6 | 15.1 | 15.7 | 14.6 | 9.5 | 15.5 | 12.5 | 11.7 | 16.3 | 8.2 | 5.9 |

**, *, ns Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (Q), (L) Quadratic and Linear adjust, respectively; CV coefficient of variation.

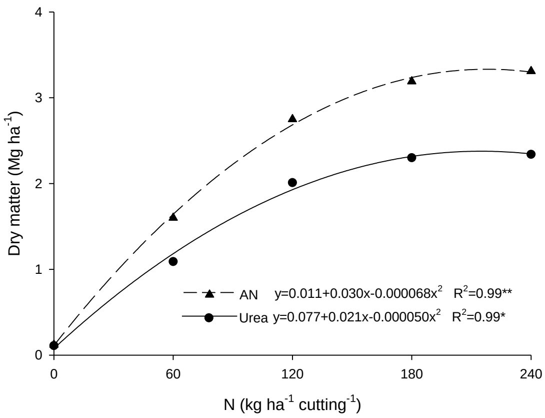


**Figure 10.** Dry matter yield on Tifton 85 due to rates and N sources. Third cutting 2013/2014. **Significantly different (P<0.01); *Significantly different (P<0.05).

When rainfall was limited (third cut in 2013/2014 growing season) (Figure 2), AN resulted in great forage production than urea. Similar results were also observed for shoot N concentration, LAI and PARi. Poor urea performance during the drought period could be related to poor ability of urea to be able to incorporate into the soil before hydrolysis was completed (FOX; KERN; PIEKIELEK, 1986). Under this circunstances, N losses via volatilization were likely promoted (LARA CABEZAS; KORNDORFER; CANTARELLA, 1997; KNIGHT; GUERTAL; WOOD, 2007; MASSEY et al., 2011; SOHM et al., 2014) leading to a lower plant uptake and consequently low shoot N concentration and overall poor forage performance (CONNELL et al., 2011).

From an animal performance standpoint, Tifton 85 is considered satisfactory when shoot N concentrations range from 21 to 26 g $kg^{-1}$ (KELLING; MATOCHA, 1990; WERNER et al., 1996). In this present experiment, 90% of the relative production was observed when plants presented N concentration of 23.7 g $kg^{-1}$ (Figure 11), above this value DM yield was not responsive and plants reached a luxury consumption status according to Mengel and Kirkby (2001).

Chlorophyll index is as representative as shoot N concentration in order to validate well nourish status of Tifton 85 in relation to its relative production (Figure 11). According to the calculated in the present experiment, both variables were positively related and as mentioned before one should well represent the other. Equivalency for both methods were noted, transforming the shoot N concentration interval to CI values are in a range of 25 to 38. Relative production was associated with a CI of 37.8 also in the range considered ideal for the Sao Paulo State WERNER et al., 1996).

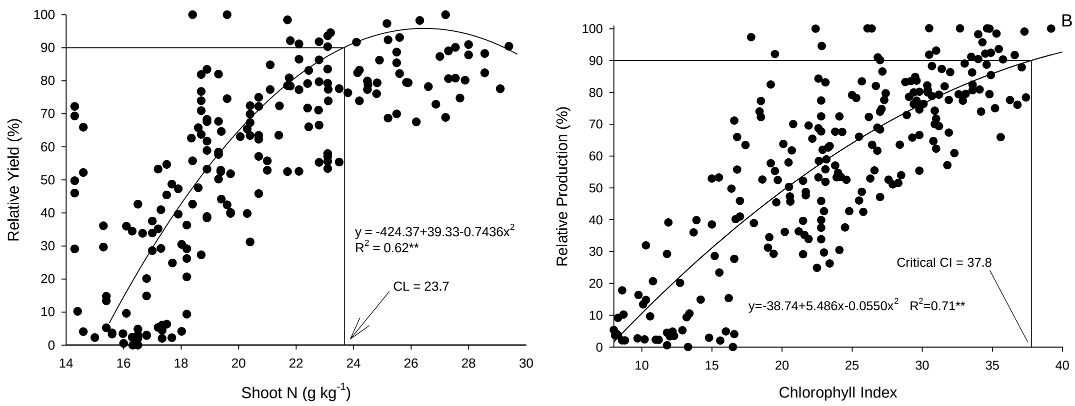


**Figure 11.** Dry matter relative production of Tifton 85 due to **A)** shoot N concentration and **B)** Chlorophyll Index (n=240). **Significantly different (P<0.01).

Nitrogen is a nutrient that constitutes many compounds such as amino acids, proteins, chlorophyll, carbohydrates, enzymes and phytohormones such as cytokinins, all these, are known to be fundamental in order to obtain and maintain an optimal plant development. Consequently, whenever N along with water became a limiting factor this could compromise the whole cycle.

As shown on Table 3, tillering was affected by source and as reported by Oliveira, Pereira and Huaman (2000), treatments showed no significant effect on the number of leaves per tiller, which is limited by a genetic factor (9.5 leaves per tiller). Therefore, in order to increase forage production, it is necessary to increase the length and width of leaves.

Nitrogen sources and application rates showed significant effects on Tifton 85 responses. As mentioned previously, increasing leaf area is an important factor that determine forage production potential. Within this context, adequate N supply can promote root system growth and increase cytokinins production and transport to the shoots (MARSCHNER, 2012). This hormone mainly promotes cell division and expansion and can play a major role on increasing LAI and the ability of the plant absorb light.

In the present study, data indicated that the relation of PARi to relative production were concomitant. Each and every time PARi was higher than 71% plants were considered to absorb enough light to express its maximum productive potential, as presented in figure 12. Differing from Humphreys (1991) which proposed that this potential would only be expressed when 95% of PAR were intercepted. According to Mattera et al. (2013), DM yield and PARi were related ($R^2$=0.76), and one should well represent the other. Leaf area index values above 4.7 showed no relation with DM yields (Figure 12). According to Strieder et al. (2008), this response occurs due to an overlap of leaves and consequently an over shading, and the photosynthetic process of basal leaves was diminished and they become drains instead of sources of energy.

Absorbing more light typically means that plant is able to increase PARi and photosynthesis, thus, light energy is converted in chemical energy oxidizing water, releasing oxygen and reducing carbon dioxide, forming large carbon compounds, and consequently affecting plant growth (TAIZ; ZEIGER, 2010).

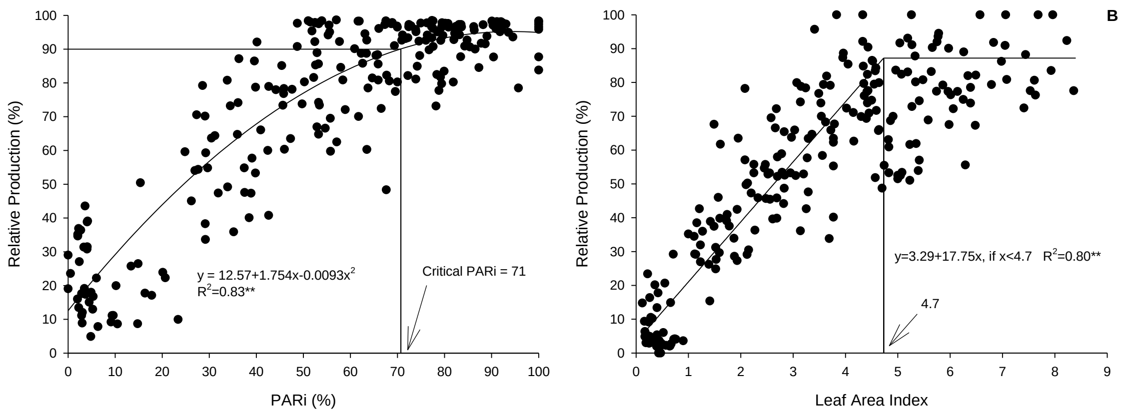


**Figure 12.** Dry matter relative production of Tifton 85 due to **A)** intercepted photosynthetically active radiation (PARi) and **B)** leaf area index (LAI) (n=240). **Significantly different (P<0.01).

### 4.2.4 Nitrogen recovery and total nitrogen on Plant-Soil system

Recovery of nitrogen fertilizers by above-ground plant tissue is generally considered to be about 50% of the total applied (IMPITHUKSA; BLUE, 1985). In this current study, nitrogen recovery quantified by $^{15}N$ isotope was significantly lower that reported values (average of 22% of applied N).

Nitrogen recovery for root and litter presented a significant source x rate interaction (Table 7). In this case urea was a more efficient source when outspreading data. Though, whenever there was significance on the regression, both sources were represented by a decrescent linear pattern.

According to Martha Junior, Trivelin and Corsi (2009), increased N supply can promote volatilization of ammonia and, consequently, reduce its utilization by the plants. Conversely, Hargrove (1988) reported that volatilization of $NH_3$ can respond linearly or exponentially depending on the N application rate and, in some circumstances, relative losses (%N) may be constant regardless of the N rate. In a 3-yr study, Huckaby, Wood and Guertal (2012) evaluating surface applied urea on turfgrass reported an average of 16% of N losses by volatilization, when utilized urea.

**Table 7** Effect of rates and $^{15}N$ sources on Tifton 85 nitrogen derived from fertilizer recovery on shoot (average of four cuttings), root, litter, soil and in the plant-soil system.

| Treatments | Shoot (average) | Root | Litter | Soil | Plant + Soil |
|---|---|---|---|---|---|
| **Source (S)** | ---------------------- | ---------- | % | ---------- | ---------------------- |
| **AN** | 21.7 | 4.3 | 1.8 | 13.0 | 40.8 |
| **Urea** | 21.4 | 5.9 | 2.9 | 21.8 | 52.0 |
| **F test** | 0.2$^{ns}$ | 6.4* | 56.0** | 23.1** | 21.0** |
| **N (kg ha$^{-1}$)** | | | | | |
| **60** | 23.8 | 5.4 | 3.2 | 23.6 | 56.0 |
| **120** | 21.2 | 7.3 | 2.6 | 15.4 | 46.7 |
| **180** | 20.1 | 3.4 | 1.9 | 16.6 | 42.0 |
| **240** | 21.2 | 4.3 | 1.5 | 14.0 | 41.0 |
| **F test** | 4.1* | 6.5** | 26.6** | 5.4* | 7.7** |
| **Regression** | (Q) | (L) | (L) | (L) | (L) |
| | ---------------------- | ---------- | **F test** | ---------- | ---------------------- |
| **S x N** | 0.7$^{ns}$ | 5.5** | 3.8** | 0.1$^{ns}$ | 0.6$^{ns}$ |
| **CV%** | 8.7 | 31.8 | 15.5 | 25.9 | 13.0 |

**, *, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; (Q), (L) Quadratic and Linear adjust, respectively; CV coefficient of variation.

Similar pattern was observed for nitrogen derived from fertilizer (Ndff) on soil (Table 7); however, the interaction between N source and rate was not significant. Results indicated that urea was a more efficient source than AN and increasing N rates decrease the amount of N retained in a 0-0.4 m soil layer (Figure 13). In addition to better plant utilization, another plausible explanation for this response is that AN was likely subjected to losses (more so than urea) during the periods of intensive rainfall (Figure 1). According to Raij (2011), since nitrate has a negative charge, it is repulsed by the negative surface of soil particles, remaining in solution, being mobile and susceptible to leaching which is accentuated in heavy rainfall periods, and since 50% of AN is composed by this ion, a great part of the fertilizer is liable to be lost.

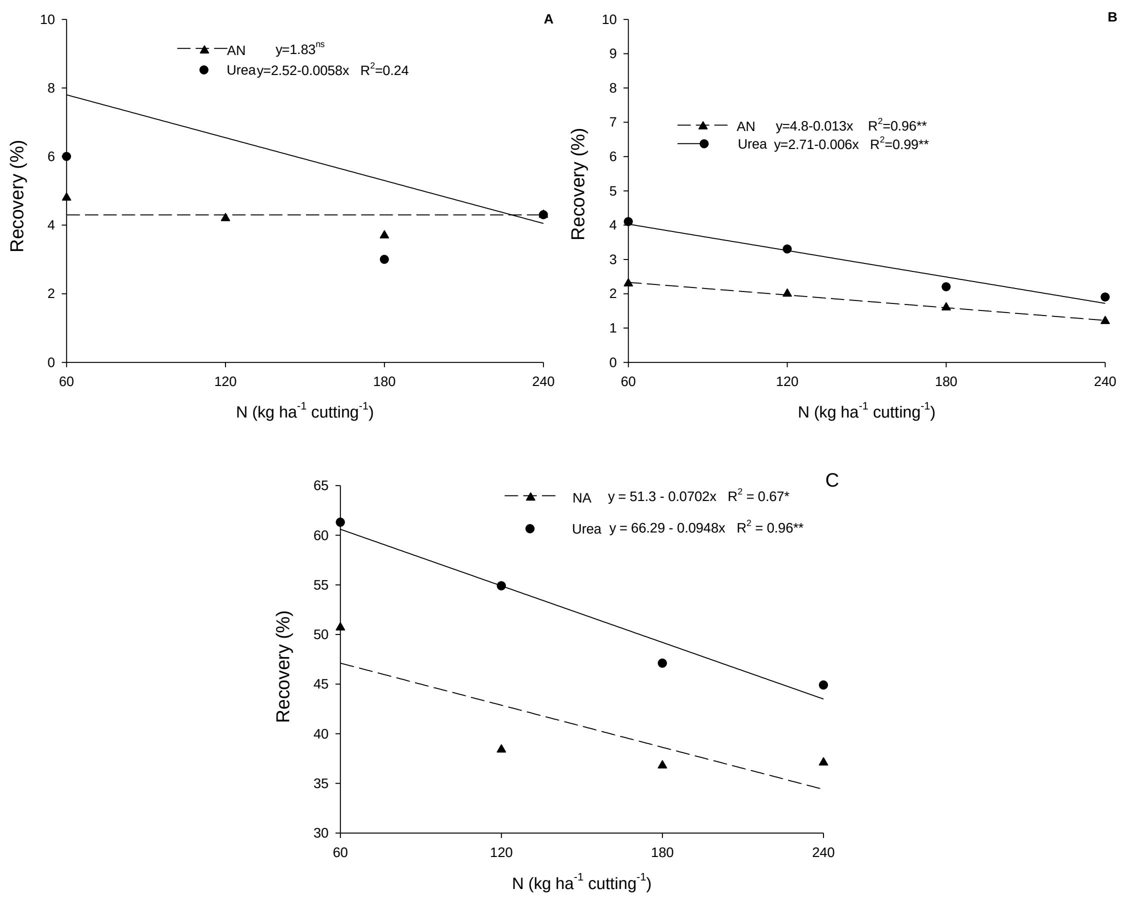


**Figure 13.** Nitrogen use-efficiency on Tifton 85 **A)** root, **B)** litter and **C)** Plant+Soil of rates and $^{15}N$ sources. **Significantly different (P<0.01); $^{NS}$ Non-significant.

The whole plant-soil system was able to retain 40.8 and 52% of the N applied, AN and urea respectively (Table 8). These values highlight the fact that a great proportion of the N derived from the fertilizers was stored in the soil. The data in the present work are consistent with the values reported by Gava et al. (2006). Figure 14 indicated that regardless of the N rate, higher levels of soil N were observed when urea was utilized as compared to AN. These data contradict the hypothesis that urea results in higher losses by volatilization as evidenced (BURTON; JACKSON 1962; LARA CABEZAS; KORNDORFER; CANTARELLA, 1997; KNIGHT; GUERTAL; WOOD, 2007). The relatively high soil N in the treatments receiving urea is likely due to the fact that urea was less subject to leaching during the periods of intensive rainfall.

**Table 8.** Distribution of N from Ammonium Nitrate and Urea on the Plant-soil system of Tifton-85 production.

| **Balance** | **Ammonium Nitrate** | **Urea** |
|---|---|---|
| | ------------------------------ % | ------------------------------- |
| **Plant** | 27.8 | 30.2 |
| **Soil** | 13.0 | 21.8 |
| **Plant+Soil** | 40.8 | 52.0 |
| **NRN*** | 59.2 | 48.0 |

*Non-recovered Nitrogen by the system.

Although plant+soil recovery values seem to be low, other authors reported and supported similar results. Impithuksa and Blue (1985) studying a *Cynodon* hybrid observed a 35% recovery utilizing AN as source in a sandy soil. Picchioni and Quiroga-Garza (1999) also reported, in a *Cynodon* hybrid, a range from 17 to 34% and 17 to 27% of recovery on a greenhouse study utilizing AN and urea, therefore not only sources control uptake efficiency but also external factors might limit its use by the plants.

### 4.3 Experiment 2: Soil organic carbon fractions

During the 2-yr study, application of N showed no significant effects on soil C responses. Particulate organic matter (POC), and free light f (FLF) and mineral fraction (C-min) fractions were not affected by either N source or application rate.

Lack of response was likely due to soil characteristics (fine texture, initial soil C levels), and climatic conditions (high temperature and moisture conditions that favor C decomposition). In addition, the relatively short-duration of the study may have also masked changes in soil C. These results contradict Maia et al. (2009) and Stewart et al. (2012), who observed a reduction on soil C levels in response to a low N input system.

Thus, N supply had no significant impact on soil of C stocks and distribution among the various pools. On the hay field area carbon derived from $C_4$ plants was 70% of the total $\delta^{13}C$ meaning that only 30% of total carbon was still derived from the vegetative material from the native forest (data not shown).

As indicated by Lal et al. (2002), increases up to 60 to 70% in soil C may occur in response to the adoption of proper management practices, including fertilization. According to Neil et al. (1997), significant increases in C input and subsequent C accumulation in the soil may occur in response to more productive plant species. Although the treatments had only been imposed for 2 yr, the experimental area has been continuously cultivated with perennial forage species for more than 20 yr, which may limit our ability to detect changes in soil C in the short-term.

In this study, POC decreased as soil depth increased. C associated with POC fraction in the surface (0-10 cm) was 45% higher than in deeper layer (Table 9). Similar trend was observed for the C-min fraction (Table 11). Soil C associated with POC and mineral-min fraction tended to increase as compared to the samples collected at the beginning of the experiment (1.3 to 3.6 Mg $ha^{-1}$ for POC and C-min, respectively). When comparing these data with the values from a native forest values close to the ones reported were removed from the system and always, either removing or adding, the superficial layer presented higher inputs and lower outputs. What could be deduced is that fertilization and the maintenance of crop helped not to add but to conserve the C in the soil.

Although soil C is an indicator of changes in the system, Dubeux et al. (2006) mentioned that different soil fractions could respond differently to soil management. The free light fraction presented results oppose to the other fractions, resulting in higher carbon concentration in the 0.1-0.2 m layer (Table 10). Since FLF was composed by larger particles that were recently added to the system or yet have not been decomposed and the experiment was conducted in a tropical condition submitted to high temperatures and heavy and condensed rainfall period this could contributed to an increase on microbial activity decomposing the major part of the FLF transforming it into POC or C-min fractions.

According to Six et al. (1998), C associated to mineral fractions respond in a slower rate than C associated to the FLF, which is decomposed quicker than the ones associated to C-min since it has few protections from environmental conditions. This fractions, according to Silveira et al. (2013), represent an important mechanism of C protection, on their study, even occupying only 3% of total soil mass, C-min accounted for 20% of total carbon, in this present study C-min fraction represented more than 70% of total soil mass which in this case this fraction occupied more than 80% of total C (Table 11) suggesting a greater accumulation associated to the fine particle offering more physical protection than bigger ones.

**Table 9.** Particulate Organic C (POC) as affected by N fertilization and soil depth.

| Treatments | Mg ha$^{-1}$ | g kg$^{-1}$ Fraction | g kg$^{-1}$ Soil | %total | Initial (Mg ha$^{-1}$) | Forest (Mg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| **N (kg ha$^{-1}$)** | | | | | | |
| **0** | 1.6 | 4.2 | 1.4 | 7.7 | 0.8 | -2.0 |
| **60** | 1.6 | 4.2 | 1.4 | 7.6 | 0.7 | -2.1 |
| **120** | 2.0 | 5.0 | 1.7 | 9.1 | 1.1 | -1.7 |
| **180** | 1.6 | 4.0 | 1.4 | 7.9 | 0.7 | -2.0 |
| **240** | 1.8 | 4.5 | 1.5 | 8.3 | 0.9 | -1.8 |
| **F test** | 1.0$^{ns}$ | 2.2$^{ns}$ | 1.1$^{ns}$ | 0.7$^{ns}$ | 1.0$^{ns}$ | 0.9$^{ns}$ |
| **Depth (m)** | | | | | | |
| **0-0.1** | 2.2 | 55 | 1.9 | 10.0 | 1.3 | -1.4 |
| **0.1-0.2** | 1.2 | 3.2 | 1.0 | 6.3 | 0.3 | -2.4 |
| **F test** | 14.5** | 18.5** | 16.3** | 11.0** | 14.5** | 14.5** |
| | **------------------------------------------F test-----------------------------------------------** | | | | | |
| **N x D** | 0.6$^{ns}$ | 0.6$^{ns}$ | 0.5$^{ns}$ | 0.4$^{ns}$ | 0.6$^{ns}$ | 0.6$^{ns}$ |
| **CV%** | 40.4 | 33.4 | 39.5 | 36.7 | 82.2 | 36.2 |

**, $^{ns}$ Significantly different at: P<0.01 and Non-significantly different; respectively; CV coefficient of variation.

**Table 10.** Free Light fraction (FLF) as affected by N fertilization and soil depth.

| Treatments | Mg ha$^{-1}$ | g kg$^{-1}$ Fraction | g kg$^{-1}$ Soil | %total | Initial (Mg ha$^{-1}$) | Forest (Mg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| **N (kg ha$^{-1}$)** | | | | | | |
| **0** | 1.9 | 269.6 | 1.6 | 9.1 | 0.6 | 0.1 |
| **60** | 1.9 | 287.1 | 1.6 | 9.4 | 0.5 | 0.1 |
| **120** | 1.5 | 262.2 | 1.3 | 7.4 | 0.2 | -0.3 |
| **180** | 1.8 | 250.6 | 1.5 | 9.1 | 0.4 | 0.0 |
| **240** | 1.7 | 270.1 | 1.4 | 8.4 | 0.4 | -0.1 |
| **F test** | 1.5$^{ns}$ | 1.1$^{ns}$ | 1.6$^{ns}$ | 1.9$^{ns}$ | 1.5$^{ns}$ | 1.5$^{ns}$ |
| **Depth (m)** | | | | | | |
| **0-0.1** | 1.7 | 255.1 | 1.4 | 7.7 | 0.3 | -0.1 |
| **0.1-0.2** | 1.9 | 280.7 | 1.6 | 9.6 | 0.5 | 0.1 |
| **F test** | 3.3$^{ns}$ | 6.1* | 1.9$^{ns}$ | 20.7** | 3.3$^{ns}$ | 3.3$^{ns}$ |
| | **F test** | | | | | |
| **N x D** | 0.6$^{ns}$ | 2.5$^{ns}$ | 0.5$^{ns}$ | 0.7$^{ns}$ | 0.6$^{ns}$ | 0.6$^{ns}$ |
| **CV%** | 14.6 | 10.6 | 14.7 | 12.7 | 63.1 | 76.1 |

**, *, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; CV coefficient of variation.

**Table 11.** Mineral fraction (C-min) as affected by N fertilization and soil depth.

| Treatments | Mg ha$^{-1}$ | g kg$^{-1}$ Fraction | g kg$^{-1}$ Soil | %total | Initial (Mg ha$^{-1}$) | Forest (Mg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| **N (kg ha$^{-1}$)** | | | | | | |
| **0** | 17.3 | 22.5 | 14.9 | 83.2 | 3.2 | -4.4 |
| **60** | 16.7 | 21.6 | 14.4 | 83.0 | 2.3 | -5.4 |
| **120** | 17.3 | 22.6 | 15.0 | 83.5 | 3.1 | -4.6 |
| **180** | 16.2 | 20.9 | 13.9 | 83.0 | 1.9 | -5.8 |
| **240** | 17.7 | 22.0 | 14.7 | 83.4 | 3.3 | -4.4 |
| **F test** | 2.0$^{ns}$ | 1.1$^{ns}$ | 1.5$^{ns}$ | 0.1$^{ns}$ | 2.0$^{ns}$ | 2.0$^{ns}$ |
| **Depth (m)** | | | | | | |
| **0-0.1** | 18.0 | 23.5 | 15.5 | 82.3 | 3.6 | -4.1 |
| **0.1-0.2** | 16.3 | 20.3 | 13.7 | 84.1 | 1.9 | -5.8 |
| **F test** | 41.3** | 339.7** | 98.4** | 3.0$^{ns}$ | 41.3** | 41.4** |
| | **F test** | | | | | |
| **N x D** | 1.5$^{ns}$ | 1.2$^{ns}$ | 0.8$^{ns}$ | 0.6$^{ns}$ | 1.5$^{ns}$ | 1.5$^{ns}$ |
| **CV%** | 4.2 | 2.2 | 3.4 | 3.4 | 25.9 | 14.1 |

**, $^{ns}$ Significantly different at: P<0.01, P<0.05 and Non-significantly different; respectively; CV coefficient of variation.

Particulate organic carbon accounted for 10% of total C mass close to the reported on FLF, 7.7%, according to Fiedler et al. (2008), the POC including FLF part on total C ranged from 15 to 30%, even though the experiment from these authors was conducted in different environment (temperate climate) from the present, results corroborated. Regardless of the amount of clay influenced the quantity of carbon on the C-min fraction, Neil et al. (1997) believe that management can influence soil C stocks more than soil texture itself. The management might influence balance between C inputs to the soil via plant fixation sequestering from the atmosphere, C losses from the soil to the atmosphere via decomposition, in this case higher inputs could be achieved by improving soil fertility and animal production (FOLLET; REED, 2010).

## 4.4 Final considerations

Nitrogen application rate increased Tifton 85 shoot N concentration, chlorophyll index, LAI, PARi, and dry matter yield. During periods of limited rainfall, differences in N source were observed, with AN generally outperforming urea. However, cumulative annual production during the 2-yr study was not significantly affected by N sources.

Nitrogen recovery by the plant was affected by N source. Urea showed the highest N recovery in the plant-soil system with a great proportion of the N retained in the soil.

Regardless of the N source, N recovery decreased as N rates increased.

Nitrogen rates showed no effect on soil C responses, nevertheless POC and C-min on the 0-0.1 m depth were more sensible to changes than the ones in the 0.1-0.2 m layer.

Soil C levels measured at the end of the 2-yr study were generally greater than the values obtained before the N fertilization treatments were imposed; however, soil C stocks were lower than those in the native forest.

## 5 CONCLUSIONS

Nitrogen sources resulted in similar Tifton 85 annual cumulative dry matter yield. Optimum N rate was established at the 210 kg $ha^{-1}$ N per cutting, with a maximum annual cumulative production of 37.2 Mg $ha^{-1}$.

Recovery of N derived from the fertilizer was higher in the plots fertilized with urea compared to ammonium nitrate. Regardless of the N source, N recovery decreased as N rates increased.

Soil carbon fractions were not influenced by N rates but the treatments affected soil C distribution among the various fractions, particularly at the 0-0.1 m layer.

**REFERENCES**

ALDERMAN, P. D.; BOOTE, K. J.; SOLLENBERGER, J. E. Carbohydrate and nitrogen reserves relative to regrowth dynamics of ‘tifton 85’ bermudagrass as affected by nitrogen fertilization. **Crop Science**, v. 51, p. 1727-1738, 2011a.

ALDERMAN, P. D.; BOOTE, K. J.; SOLLENBERGER, J. E. Regrowth dynamics of ’tifton 85’ bermudagrass as affected by nitrogen fertilization. **Crop Science**, v. 51, p. 1716-1726, 2011b.

ANDRADE, F. H.; CALVIÑO, P.; CIRILO, A.; BARBIERI, P. Yield responses to narrow rows dependo n increased radiation interception. **Agronomy Journal,** v.94, p. 975-980, 2002.

BARBOSA, J. C.; MALDONADO Jr., W. Experimentação agronômica & AgroEstat: Sistema para análises estatísticas de ensaios agronômicos. **Multipress,** v.1, 396p., 2015.

BARRIE, A.; PROSSER, S. J. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry. p.1-46. In: BOUTTON, T. W.; YAMASAKI, S., eds. **Mass spectrometry of soils**. 1996. p.1-46.

BEN-HAJ-SALAH, M.; TARDIEU F. Temperature affects expansion rate of maize leaves without change in spatial distribution of cell length. **Plant Physiology,** v.109, p. 861-870, 1995.

BORGES, B. M. M. N., SILVA JUNIOR, L. C.; LUCAS, F. T.; SILVA, W. J. Relationship between luminous flux intercepted at different times in leaf area index of different forages. **Semina,** v.32, p.1589-1594, 2011.

BOWEN, W.T.; JONES, R.J.; CARSKY, R.J.; QUINTANA, J.O. Evaluation of the nitrogen submodel of CERES-maize following legume Green manure incorporation. **Agronomy Journal**, v.85, p.153-158, 1993.

BOUTTON, T. W. Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. In: Coleman, D.C., Fry, B. (Eds.), **Carbon Isotope Techniques**, p. 155-171, 1991.

BURTON, G. W.; JACKSON, J. E. Effect of rate and frequency of applying six nitrogen sources on coastal bermudagrass. **Agronomy Journal**, v. 54, p. 40-43, 1962.

CABRERA, M. L.; KISSEL, D. E.; BOCK, B. R. Urea hydrolysis in soil – Effects of urea concentration and soil – pH. **Soil Biology and Biochemistry**, v. 23, p. 1121-1124, 1991.

CAIRES, E. F.; GARBUIO, F. J.; CHURKA, S.; BARTH, G.; CORRÊA, J. C. L. Effects of soil acidity amelioration by surface liming on no-till corn, soybean, and wheat root growth and yield. **European Journal of Agronomy**, v.28, p. 57-64, 2008.

CAMBARDELLA, C. A.; ELLIOTT, E. T. Particulate soil organic matter changes across a grassland cultivation sequence. **Soil Science Society of America Journal**, v. 56, p. 777-783, 1992.

CHIEN, S. H.; PROCHNOW, L. I.; CANTARELLA, H. Recent developments of fertilizers production and use to improve nutrient efficiency and minimize environmental impacts. In Sparks DL (ed) **Advances in Agronomy**, v. 102, p. 267-322, 2009.

CONNELL, J. A.; HANCOCK, D. W.; DURHAM, R. G.; CABRERA, M. L.; HARRIS, G.H. Comparison of enhanced-efficiency nitrogen fertilizers of reducing ammonia loss and improving Bermuda grass forage production. **Crop Science**, v. 51, p. 2237-2248, 2011.

DAVIES, A. Tissue turnover in the sward. In: HOGDSON, J.; BAKER, R.D.; DAVIES, A.; LAIDLAW, A.S.; LEAVER, J.D. (Ed.) Sward measurement handbook. Hurley: **The British Grassland Society**, p.197-209, 1981.

DILLARD, S. L.; WOOD, C. W.; WOOD, B. H.; FENG, Y.; OWSLEY, W. F.; MUNTIFERING, R. B. Effects of nitrogen fertilization on soil nutrient concentration and phosphatase activity and forage nutrient uptake from a grazed pasture system. **Journal of Environmental Management**, v. 154, p. 208-215, 2015.

DUBEUX, J. C. B.; SOLLENBERGER, L. E.; COMERFORD, N. B.; SCHOLBERG, J. M.; RUGGIERI, A. C.; VENDRAMINI, J. M. B.; INTERRANTE, S. M.; PORTIER, K. M. Management intensity affects density fractions of soil organic matter from grazed bahiagrass swards. **Soil Biology and Biochemistry**, v. 38, p. 2705-2711, 2006.

FAGUNDES, J.L.; MOREIRA, A.L.; FREITAS, A.W.P.; ZONTA, A.; HENRICHS, R.; ROCHA, F.C.; BACKES, A.A. e VIEIRA, J.S. Capacidade de suporte de capim-tifton 85 adubado com nitrogênio manejadas em lotação continua com ovinos. **Revista Brasileira de Zootecnia**, v. 40, p. 2651-2657, 2011.

FAO Food and Agriculture Organization of the United Nations (2012) **Statistical Division.** Available at: http://faostat3.fao.org/browse/R/RL/E. Accessed 7 August 2015.

FIEDLER, S.; HOLL, B. S.; FREIBAUER, A.; STAHR, K.; DROSLER, M.; SCHLOTER, M.; JUNGKNUST, H. F. Particulate organic carbon (POC) in relation to other pore water carbon fractions in drained and rewetted fens in Southern Germany. **Biogeoscience**, v.5, p.1615-1623, 2008.

FISHER, M.J.; RAO, I.M.; AYARZA, M.A.; LASCANO, C.E.; SANZ, J.I.; THOMAS, R.J.; VERA, R.R. Carbon storage by introduced deep-rooted grasses in the South American savannas. **Nature**, v. 371, p. 236-238, 1994.

FOLLET, R.F.; REED, D.A. Soil carbon sequestration in grazing lands: Societal benefits and policy implications. **Rangeland Ecology and Management**, v. 63, p. 4-15, 2010.

FOX, R. H.; KERN, J. M.; PIEKIELEK, W. P. Nitrogen fertilizer source and method and time of application effects on no-till corn yields and N uptake. **Agronomy Journal**, v.78, p.741-746, 1986.

GAVA, G. J. C.; TRIVELIN, P. C. O.; OLIVEIRA, M. W.; HEINRICHS, R.; SILVA, M. A. Balanço do nitrogênio da uréia ($^{15}N$) no sistema solo-planta na implantação da semeadura direta na cultura do milho. **Bragantia**, v.65, p.477-486, 2006.

GEISSELER, D.; LAZICKI, P. A.; PETTYGROVE, G. S.; LUDWIG, B.; BACHAND, P. A. M.; HORWATH, W. R. Nitrogen dynamics in irrigated forage systems fertilized with liquid dairy manure. **Agronomy Journal**, v. 104, p. 897-907, 2012.

GÓMEZ, S.; GUENNI, O.; de GUENNI, L.B. (2013) Growth, leaf photosynthesis and canopy light use efficiency under differing irradiance and soil N supplies in the forage grass Brachiaria decumbens Stapf. **Grass and Forage Science**, v. 68, p. 395-407, 2013.

GUERTAL, E. A.; HOWE, J. A. Nitrate, ammonuium and urea leaching in hybrid bermudagrass as affected by nitrogen sources. **Agronomy Journal**, v. 104, p. 344-352, 2012.

HARGROVE, W.L. Soil environmental and management factors influencing ammonia volatilization under field conditions. In: BOCK, B.R. & KISSEL, D.E., eds. **Ammonia volatilization from urea fertilizers.** Muscle Schoals, Tenessee Valley Authority, 1988. p.17-36.

HUCKABY, E.C.K.; WOOD, C.W.; GUERTAL, E.A. (2012) Nitrogen Source Eff ects on Ammonia Volatilization from Warm-Season Sod. **Crop Science**, v. 52, p. 1379-1384, 2012.

HUMPHREYS, L. R. **Tropical pasture utilization***.* Cambridge: Cambridge University Press, 1991, 206 p.

IBGE (Instituto Brasileiro de Geografia e Estatística). **Pesquisa pecuária municipal**: PPM 2012. Rio de Janeiro, 2013.

IMPITHUKSA, V.; BLUE, W. G. Fertilizer Nitrogen and Nitrogen-15 in Three Warm-season Grasses Grown on a Florida Spodosol. **Soil Science Society of America Journal,** v. 49, p. 1201-1204, 1985.

KELLING, K. A.; MATOCHA, J. E. Plant Analysis as an Aid in Fertilizing Forage Crops. In. WESTERMAN, R. L. (Ed). **Soil Testing and Plant Analysis,** n. 3, p. 603-643, 1990.

KNIGHT, E.C.; GUERTAL, E.A.; WOOD, C.W. Mowing and nitrogen source effect on ammonia volatilization from turfgrass. **Crop Science**, v. 47, p. 1628-1634, 2007.

KUNZ, J. H.; BERGONCI, J. I.; BERGAMASCHI, H.; DALMAGO, G. A.; HECLKER, B. M. M.; COMIRAN, F. Uso da radiação solar pelo milho sob diferentes preparos do solo, espaçamento e disponibilidade hídrica. **Pesquisa agropecuária brasileira,** v. 42, p.1511-1520, 2007.

LAL, R. Soil carbon dynamics in cropland and rangeland. **Environmental Pollution**, v. 116, p. 353-362, 2002.

LARA CABEZAS, W.A.R.; KORNDORFER, G.H.; MOTTA, S.A. Volatilização de N-$NH_3$ na cultura de milho: II. Avaliação de fontes sólidas e fluidas em sistema de plantio direto e convencional. **Revista Brasileira de Ciência do Solo**, v.21, p.489-496, 1997.

LEMAIRE, G. Research priorities for grassland Science: the need of long term integrated experiments networks. **Revista Brasileira de Zootecnia.** v.36, suplemento especial, p. 93-100, 2007.

MADAKADZE, I. C.; COULMAN, B. E.; PETERSON, P.; STEWART, K. A.; SAMSON, R.; SMITH, D. L. Leaf area development, light interception, and yield among switchgrass populations in a short-season area. **Crop Science**, v.38, p.827-834, 1998.

MAIA, S. M. F.; OGLE, S. M.; CERRI, C. E. P.; CERRI, C. C. Effect of grasslands management on soil carbon sequestration in Rondonia and Mato Grosso state, Brazil. **Geoderma,** v.149, p.84-91, 2009.

MARSCHNER, H. **Mineral nutrition of higher plants**. 3.ed London: Elsevier, 2012. 643p.

MARTHA JUNIOR, G. B.; TRIVELIN, P. C. O.; CORSI, M. Absorção foliar pelo capim-tanzânia da amônia volatilizada do 15N-ureia aplicado ao solo. **Revista Brasileira de Ciência do Solo,** v.33, p. 103-108, 2009.

MASSEY, C.G.; SLATON, N.A.; NORMAN, R.J.; GBUR Jr.; E.E., DELONG, R.E.; GOLDEN, B.R. Bermudagrass forage yield and ammonia volatilization as affected by nitrogen fertilization. **Soil Science Society of America Journal**, v. 75, p. 638-648, 2011.

MATTERA, J.; ROMERO, L. A.; CUATÍN, A. I.; CORNAGLIA, P. S.; GRIMOLDI, A. A. Yield components, light interception and radiation use efficiency of lucerne (Medicago sativa L.) in response to row spacing. **European Journal of Agronomy**, v. 45, p. 87-95, 2013.

MENGEL, K.; KIRKBY, E. A. Nitrogen. In._____, **Principles of plant nutrition.** 5 ed. Kluwer Academic Publishers, 2001, p.397-434.

MILFORD, R. MINSON, D.J. Intake of tropical pasture species. In: **International Grassland Congress**, 9, 1966, São Paulo. Proceedings... São Paulo: s.ed., 1966. p.815-822.

NABINGER, C.; PONTES, L. S. Morfogênese de plantas forrageiras e estrutura do pasto. In. PEDREIRA, C. G. S.; SILVA, S. C. A Produção animal na visão dos brasileiros. **Anais...** Sociedade Brasileira de Zootecnia. p.755-771, 2001.

NEIL, C.; MELILLO, J. M.; STEUDLER, P. A.; CERRI, C. C.; de MORAES, J. F. L.; PICCOLO, M. C.; BRITO, M. Soil carbon and nitrogen stocks following forest clearing for pasture in the southwestern Brazilian amazon. **Ecological Applications**, v. 7, p. 1216-1225, 1997.

OLIVEIRA, M. A.; PEREIRA, O. G.; HUAMAN, C. A. M. Características morfogênicas e estruturais do capim-Bermuda “Tifton 85” (*Cynodon* spp.) em diferentes idades de rebrota. **Revista Brasileira de Zootecnia.** v.29, p.1939-1948, 2000.

PEREIRA, O.G.; ROVETTA, R.; RIBEIRO, K.G.; SANTOS, M.E.R.; FONSECA, D.M. e CECON, P.R. Crescimento do capim –tifton 85 sob doses de nitrogênio e alturas de corte. **Revista Brasileira de Zootecnia**, v. 41, p. 30-35, 2012.

PICCHIONI, G. A.; QUIROGA-GARZA, H. M.; Growth and nitrogen partitioning, recovery, and losses in bermudagrass receiving soluble sources of labeled $^{15}$nitrogen. **Journal of America Society of Horticulture Science**, v. 124, p. 719-725, 1999.

PREMAZZI, L.M.; MONTEIRO, F.A.; CORRENTE, J.E. Tillering of Tifton 85 bermudagrass in response to nitrogen rates and time of application after cutting. **Scientia Agricola,** v.60, p.565-571, 2003.

QUARESMA, J. P. S.; ALMEIDA, R. G.; ABREU, J. G.; OLIVEIRA, M. A.; CARVALHO, D.M. Produção e composição bromatológica do campim-tifton 85 (*Cynodon* spp.) submetido a doses de nitrogênio. **Acta Scientiarum**, v. 33, p. 145-150, 2011.

RAIJ, B. Fertilidade do solo e manejo de nutrients. **International Plant Nutrition Institute.** 2011, 420p.

REIS, R. A. Processamento e conservação de feno. In: ALVIM, M.J.; BOTREL, M. A.; PASSOS, L. P.; BRESSAN, M.; VILELA, D. (Eds.). **Anais…** Workshop sobre o potencial forrageiro do gênero *Cynodon*, Juiz de Fora, MG, 1996, p.57-68.

RIBEIRO, K. G.; PEREIRA, O. G. Produtividade de matéria seca e composição mineral do capim-tifton 85 sob diferentes doses de nitrogênio e idades de rebrotação. **Ciência agrotecnologia**, v. 35, p. 811-816, 2011.

SANGOI, L.; SCHWEITZER, C.; SILVA, P. R. F.; SCHMITT, A,; VARGAS, V. P.; CASA, R. T.; de SOUZA, C .A. Perfilhamento, área foliar e produtividade do milho sob diferentes arranjos espaciais. **Pesquisa agropecuária brasileira,** v. 46, p.609-616, 2011.

SBRISIA, A. F.; SILVA, S. C. O ecossistema de pastagens e a produção animal. In. PEDREIRA, C. G. S.; SILVA, S. C. A Produção animal na visão dos brasileiros. **Anais...** Sociedade Brasileira de Zootecnia. p.755-771, 2001.

SCURLOCK, J. M. O.; HALL, D. O. The global carbon sink: a grassland perspective. **Global Change Biology**, v. 4, p. 229-233, 1998.

SILVA, E. A.; SILVA, W. J.; BARRETO, A. C.; OLIVEIRA JUNIOR, A. B. O.; PAES, J. M. V.; RUAS, J. R. M.; QUEIROZ, D. S. Chemical composition and photosynthetically active radiation of forage grasses under irrigation. **Revista Brasileira de Zootecnia**, v. 41, p. 583-591, 2012.

SILVEIRA, M. L.; HABY, V. A.; LEONARD, A. T. Response of coastal bermudagrass yield and nutrient uptake efficiency to nitrogen sources. **Agronomy Journal**, v. 99, p. 707-714, 2007.

SILVEIRA, M. L.; VENDRAMINI, J. M. B.; SELLERS, B.; MONTEIRO, F. A.; ARTUR, A. G.; DUPAS, E. Bahiagrass response and N loss from selected N fertilizers sources. **Grass and Forage Science**, v. 70, p. 154-160, 2015.

SILVEIRA, M.L.; LIU, K.; SOLLENBERGER, L.E.; FOLLETT, R.F. e VENDRAMINI, J.M.B. Short-term effects of grazing intensity and nitrogen fertilization on soil organic carbon pools under perennial grass pastures in the southeastern USA. **Soil Biology & Biochemistry**, v. 58, p. 42-49, 2013.

SIX, J.; ELLIOT, E. T.; PAUSTIAN, K.; DORAN, J. W. Aggregation and soil organic matter accumulation in cultivated and native grassland soil. **Soil Science Society of America Journal**, v. 62, p. 1367-1377, 1998.

SOHM, G.; THOMPSON, C.; ASSEFA, Y.; SCHLEGEL, A.; HOLMAN, J. Yield and quality of irrigated bermudagrass as a function of nitrogen rate. **Agronomy Journal**, v. 106, p. 1489-1496, 2014.

STEWART, C. E.; FOLLETT, R. F.; WALLACE, J.; PRUESSNER, E. G. Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the Virginia Coastal Plain. **Soil Science Society of America Journal**, v. 76, p. 1257-1267, 2012.

STRIEDER, M. L.; SILVA, P. R. F.; RAMBO, L.; BERGAMASCHI, H. DALMAGO, G. A.; ENDRIGO, P. C.; JANDREY, D. B. Características de dossel e rendimento de milho em diferentes espaçamentos e sistemas de manejo. **Pesquisa Agropecuária Brasileira,** v.43, p.309-317, 2008.

TAIZ, L.; ZEIGER, E. **Plant physiology**. Sundervland: Sinauer Associates, 2010. 782 p.

USDA, National Resources Conservation Service. **Keys to Soil Taxonomy.** Soil Survey Center, 12 ed, 375p, 2014.

VENDRAMINI, J. M. B.; SILVEIRA, M.L.A.; DUBEUX, Jr., J. C. B.; SOLLENBERGER, L. E. Environmental impacts and nutriente recycling on pastures grazed by cattle. **Revista Brasileira de Zootecnia,** v.36, suplemento especial, p.139-149, 2007.

WERNER, J.C.; PAULINO, V.T.; CANTARELLA, H.; ANDRADE, N.O.; QUAGGIO, J.A. Forrageiras. In: RAIJ, B. van; CANTARELLA, H.; QUAGGIO, J.A.; FURLANI, A.M.C. (Ed.). **Recomendações de adubação e calagem para o Estado de São Paulo**. Instituto Agronômico, 1996. p.263-273. (IAC. Boletim Técnico, 100).