University of São Paulo
“Luiz de Queiroz” College of Agriculture

Blending polymer-sulfur coated and NBPT-treated urea to improve nitrogen use efficiency and grain yield in corn production systems

Hugo Abelardo González Villalba

Thesis presented to obtain the degree of Doctor in Science. Area: Soil and Plant Nutrition

Piracicaba
2018

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **PAULO CESAR OCHEUZE TRIVELIN**

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1. Fertilizante de eficiência aumentada 2. Acúmulo de nitrogênio 3. Recuperação do nitrogênio 4. Nitrogênio do solo 5. Sustentabilidade I. Título

To my father Lucio Hugo (*in memorian*)
My hero, my friend, my boss,
My inspiration

I DEDICATE

To my mother Blanca Sabina,
and my brothers
José, Diego and Sabrina
To my family, the most precious gift of life

I OFFER

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“***And he gave it for his opinion, that whoever could make***

two ears of corn, or two blades of grass, to grow upon

a spot of ground where only one grew before,

would deserve better of mankind,

and do more essential service to his country,

than the whole race of politicians put together”

Jonathan Swift, Gulliver’s Travels

CONTENTS

RESUMO

Misturas de ureia revestida com enxofre e polímeros e ureia tratada com NBPT para aumentar a eficiência de uso do nitrogênio em sistemas de produção de milho

A mistura de fertilizantes nitrogenados de liberação controlada e estabilizados representa uma alternativa para fornecer nitrogênio (N) em todos os estádios da cultura do milho, além de ser uma opção válida para reduzir custos em comparação ao uso exclusivo de produtos de liberação controlada. Neste sentido, conduziram-se experimentos de campo na região Sudeste do Brasil com a aplicação de um *blend* de ureia revestida com enxofre e polímeros (UREP) e ureia tratada com NBPT (U-NBPT), na proporção 70:30, aplicada na semeadura do milho, de forma incorporada. Os objetivos do trabalho foram: i) avaliar o destino do N dentro das plantas de milho proveniente dos fertilizantes misturados e determinar a eficiência de recuperação de cada um; ii) avaliar a resposta do rendimento de grãos de milho a doses de N (*blend*) em sistemas de produção contrastantes e avaliar a possibilidade de reduzir as doses de N quando aplicado o blend em comparação à ureia convencional; iii) entender e monitorar as mudanças da biomassa e o nitrogênio dentro das plantas de milho ao longo do ciclo da cultura. No primeiro estudo, o N na planta proveniente da UREP, da U-NBPT, e do solo (N-Solo) variaram ao longo do ciclo do milho. Contudo os fertilizantes nitrogenados contribuiram com menos de 50% do N total da planta em todos os estadios avaliados (V4, V12, R2 e R6). No estádio V4, a maior parte do N na planta proveniente de fertilizante (NPPF) foi fornecido pela U-NBPT, enquanto que nos estadios seguintes, a maior parte do NPPF foi fornecido pela UREP. O N-Solo foi o maior fornecedor de N para a planta, mas a contribuição diminuiu com o aumento das doses de N. Na colheita, 59% do total do N da planta foi alocado nos grãos. Do total de N da planta, 64% foi proveniente do N-Solo, 26% foi fornecido pela UREP, e 10% pela U-NBPT. A eficiência de recuperação da UREP e U-NBPT foram, respectivamente, 51 e 36%. No segundo estudo, o rendimento de grãos de milho variou entre locais, provavelmente devido às condições edafo-climáticas de cada área experimental. A aplicação do fertilizante nitrogenado influenciou o rendimento de grãos de milho, a produção de biomassa e acúmulo de N em todos os locais. O rendimento de grãos e acúmulo de N mostraram uma resposta quadrática às doses de N (*blend*). A incorporação do blend de UREP e U-NBPT na semeadura do milho mostrou-se como uma ótima estratégia para evitar perdas massivas de N e mostrou que pode atingir produtividade similar a ureia convencional com doses de N menores. O terceiro capítulo, com foco no acúmulo e particionamento da biomassa e N nas plantas de milho ao longo do ciclo, desmonstrou que a quantidade de N absorvido após o florescimento pode chegar a 50% do total de N acumulado nas plantas, pelo que adequada disponibilidade de N deve ser garantida nos estádios vegetativos finais e nos estádios reprodutivos da cultura do milho, o que pode ser conseguido com o uso de misturas de UREP e U-NBPT.

Palavras-chave: Fertilizantes de eficiência aumentada; Acúmulo de nitrogênio; Recuperação do nitrogênio; Nitrogênio do solo; Sustentabilidade

ABSTRACT

Blending polymer-sulfur coated urea and NBPT-treated urea to improve nitrogen use efficiency and grain yield in corn production systems

Blends of controlled release and stabilized nitrogen (N) fertilizer represent an alternative to provide N at all corn growth stages, and is an option to reduce costs compared to the use of solely controlled release N. In this context, field experiments were conducted in Southeast Brazil with the use of a blend of polymer-sulfur coated urea (PSCU) and NBPT-treated urea (NBPTU) at a 70:30 ratio, applied at corn planting and incorporated into the soil. The objectives of the study were: i) to quantify and measure each fertilizer-derived N fate in the plants, and determine the nitrogen recovery efficiency of each N source in the blend; ii) to evaluate corn grain yield response to N rates (blend) in contrasting cropping systems, and to assess the posibility of reducing N rate when applying a blend of two enhanced efficiency N fertilizers compared to the application of regular urea; iii) understand and monitor changes in plant biomass and N uptake during the growing season. Fertilizer N contributed with less than 50% of the total plant N uptake at all evaluated corn growth stages (V4, V12, R2, and R6). At V4 growth stage, most of the N in the plant derived from fertilizer (NPDF) was provided by NBPTU, while later in the season, most of the NPDF was provided by PSCU. At harvest, most of the plant N was allocated in the grains (59%). Of the total plant N, 64% was supplied by the native soil N pool, 26% was provided by PSCU, and 10% by NBPTU. Therefore, NBPTU provided N to corn early in the season, while PSCU played a crucial role supplying N later in the season, as plants demand for N increased. Soil N was the main N source at all GS and this fraction decreased as N rate increased. At harvest, 64% of the total plant N was derived from the soil native N pool, 26% derived from PSCU, and 10% from urea. The measured fertilizer NRE of urea was in average 36%, and the estimated NUE from PSCU was 51%. In the second study, corn grain yield varied between sites, probably due to soil and climate characteristics of each site. Corn grain yield, N uptake, and biomass production were greatly impacted by fertilizer N. Grain yield and N uptake showed a quadratic response to N rates (blend). The blend of PSCU and NBPTU, applied at corn planting and incorporated into the soil proved to be a great strategy to attain yields at N rates below those needed when using regular urea. The third chapter focused on corn biomass and N uptake and partitioning throughout the growing season, and it was demonstrated that the amount of N uptake after flowering can reach up to 50% of the total plant N, thus, N availability must be guaranteed in late vegetative corn growth stages, and especially in the reproductive stages, which can be achieved by adopting enhanced efficiency N fertilizers such as the blend of PSCU and NBPTU used in this study.

Keywords: Enhanced efficiency fertilizer; Nitrogen uptake; Nitrogen recovery; Soil nitrogen; Sustainability

1. GENERAL INTRODUCTION

Corn (*Zea Mays* L.) is a very important cereal crop worldwide and especially in Brazil. In the 2016/2017 growing season, corn occupied 17.6 million hectares in Brazil, with an average grain yield of 5.56 Mg ha^{-1} and a total grain production of 97.8 million tons (CONAB, 2018).

Nitrogen (N) is one of the most yield limiting nutrient in corn production (Raun and Johnson, 1999), and the required in greatest amount. The soil is often incapable of providing all the N crops require, making necessary the use of synthetic fertilizer N to attain optimum grain yield. In fact, as pointed out by Zhang et al. (2015), approximately half the world's people are nourished by crops grown and fertilized with synthetic N fertilizers. Mismanagement of N fertilizer and bad fertilizer management practices can have unintended adverse environmental and human health impact. Nitrogen derived from fertilizers and not taken up by plants can be lost from agricultural soils through leaching of NO_3^-, NH_3 volatilization, N_2O emission, erosion, and other loss pathways (Galloway et al., 2008; Zhang et al., 2015; Otto et al., 2016). In order to face the challenges of food security, environmental degradation and climate change, food production must grow, while agriculture's environmental footprint must shrink dramatically (Foley et al., 2011; Zhang et al., 2015), all at the same time.

Global nitrogen use efficiency (NUE) for cereal production is estimated to be between 33 – 50% (Raun and Johnson, 1999; Ladha et al., 2005). Pires et al. (2015), in a recent analysis of Brazilian cereal production systems, found that NUE is around 27%. That means 73% of the applied N is not taken up by the plants and could be damaging natural resources. Therefore, is clear that fertilizer N management needs to be improved in order to NUE.

The 4R's concept, which is a modern approach to increase nutrient use efficiency through fertilizer best management practices (FBPPs), consist on applying the right source, at the right rate, at the right time and at the right placement (IFA, 2009), and is currently being promoted and adopted worldwide as one of the best alternatives to optimize fertilizer management.

Among N sources, urea is the most used N fertilizer in Brazil (ANDA, 2015), and one of the main reasons is the lower price per unit of N, availability in most markets, high solubility, promptly available N to plants, and low soil acidification (Cantarella et al., 2008; Silva et al., 2017). When applied to the soil, urea suffers hydrolysis catalyzed by the enzyme urease, converting the R-NH_2 to NH_4^+. By consuming H^+, this reaction promotes soil pH elevation around the fertilizer granules, favoring NH_4^+ transformation to NH_3, which is a gaseous form of N highly susceptible to losses through volatilization (Trivelin et al., 2002). In a recent global review of NH_3^+ losses through volatilization from agricultural soils, Rochette et al. (2013) found that ranging from 8 to 68 % of the applied N is lost due to ammonia volatilization, being one of the main reasons for the low efficiency of N fertilizers such as urea. Therefore, the development of technology for N fertilizers (especially for urea), aiming to reduce N losses is extremely necessary.

One of the alternatives to improve NUE is the adoption of enhanced efficiency N fertilizers. Snyder (2017) define enhanced efficiency N fertilizer as N fertilizers with polymer coatings, or with the addition of urease and/or nitrification inhibitors, or those with characteristics that confer them either better agronomic response (i.e. improved grain yield), and/or lessened losses of N compared to a reference water-soluble N fertilizer (i.e. regular urea). Several studies conducted in different parts of the world and various crops have shown that generally there are positive effects of the adoption of enhanced efficiency N fertilizers on crop yield and environmental quality (Halvorson and Bartolo, 2014). Chien et al. (2009) present a review of fertilizer technology (all nutrients) aiming to improve nutrient use efficiency, while Trenkel, (2010), González-Villalba et al. (2014), Guelfi (2017), and Snyder (2017) describe current technologies for N fertilizers with a focus on enhancing regular urea performance. There are three groups of enhance efficiency N fertilizers: stabilized N fertilizers (i.e. urea treated with nitrification and urease inhibitors), slow release N fertilizers (i.e. urea condensation products), and controlled release N fertilizers (coated or encapsulated fertilizers) (Chien et al., 2009; González-Villalba et al., 2014; Guelfi, 2017).

An interesting option in the group of controlled release N fertilizers is polymer-sulfur coated urea (PSCU). This type of fertilizer consists of regular urea coated with a layer of elemental sulfur (S^0) (sulfur coated urea), coated with a layer of polymer (Trenkel, 2010), and it is intended to release N gradually, better matching the crop N requirements throughout the growing season. Polymer-sulfur coated urea has been shown to increase corn yields by around 5 to 11% (Geng et al., 2016; Zheng et al., 2016a). Halvorson and Bartolo (2014) suggest that when using this type of fertilizer under corn production, N rates could be decreased when compared to urea N rates, and yet provide grain yield benefits and economic profit. However, the main reason for the relatively small rate of adoption of this fertilizer for extensive crops is its higher price per unit of N applied (2-4 times) than regular urea (Chien et al., 2009;

Trenkel, 2010; Halvorson and Bartolo, 2014), although between 2011 and 2014 the sales of enhanced efficiency fertilizers almost doubled globally (Guelfi, 2017).

The N release pattern from PSCU depends on several factors such as soil temperature, soil moisture, density and/or thickness of the coating material, quality of the coating material and the coating process itself, among others (Trenkel, 2010). Depending on the conditions cited before, delays in N release from PSCU can occur (Rodrigues et al., 2010; Grant et al., 2012), therefore affecting early season N availability for corn (Zheng et al., 2017), causing problems in N uptake and plant growth, as reported by Grant et al. (2012). Stabilized N fertilizers such as NBPT-treated urea is also a great example of enhanced efficiency N fertilizers. It consists in regular urea treated with an enzyme urease inhibitor. The NBPT-treated urea is highly soluble, less susceptible to N losses, and meet the immediate demand of N by the crop.

Polymer-sulfur coated urea presents a gradually N release, ensuring sufficient plant available N at later crop growth stages, while the NBPT-treat urea provides the N to supply and attend early season N requirement of crops. Several studies worldwide have demonstrated the benefits of adopting of PSCU in improving crop grain yields (Halvorson and Bartolo, 2014; Geng et al., 2016; Shapiro et al., 2016; Zheng et al., 2016a). Other studies have reported great reduction in N losses (especially through ammonia volatilization) when applying NBPT-treated urea (Soares et al., 2012; Cancellier et al., 2016; Engel et al., 2017; Rajkovich et al., 2017; Silva et al., 2017). Soares et al. (2015) also demonstrated that NBPT-treated urea could reduce N_2O emissions. A great example of the value of integrating and taking advantage of the available technology is the physical mixture of PSCU and NBPT-treated urea. In theory, NBPT-treated urea provides N early in the season and PSCU later in the season as N requirement by crops increments (Payne et al., 2015; Guelfi, 2017; Guo et al., 2017; Zheng et al., 2017). This combination strategy also provides the benefit of reducing the fertilizer cost when compared to the use of solely controlled release N fertilizer (Guelfi, 2017). The use of fertilizer N blends is becoming more popular in Brazilian agriculture (González-Villalba et al., 2014; Guelfi, 2017) and worldwide (Trenkel, 2010; Snyder, 2017), being the main reason a reduction in production cost at industry level, and therefore is nowadays more accessible to farmers.

González-Villalba (2014) studied the application of several blend proportions of PSCU and regular urea for corn production, varying from 50 to 100% of the slow release source, applying the total N rate at corn planting. Compared to regular urea split applied (20% of the total N rate at planting and the remaining at V4 corn growth stage), as recommended by

Raij et al. (1996), the blends provided similar amount of plant available N (N-NH_4^+ + N-NO_3^-) at early corn growth stages and higher amount at later corn growth stages, besides providing higher yield than the regular urea treatments in the N-responsive site. Guo et al. (2017) studied blends of enhanced efficiency N fertilizers with regular urea in high yielding (>15 Mg ha^{-1}) corn production areas in China. They found that a one time application (pre-plant) of blends resulted in lower N losses, higher grain yield and economic benefit when compared to the split applied regular urea treatment. Zheng et al. (2017) reported corn grain yield increase by 7-10%, higher plant available N during the growing season, and higher economic benefits in China using a mixture of controlled release urea and regular urea, compared to the use of solely regular urea. Zheng et al. (2016b) reported that combining controlled release urea with regular urea provided higher yields, NUE, and economic profits that the treatments with solely regular urea application. Additionally, these authors mention that the mixture provided the same corn grain yield as the regular urea treatment even when the mixture supplied one-third less N, thus giving the extra benefit of possibly reducing N rate when applying this enhanced efficiency N fertilizer. In this direction, Snyder (2017) discussed that optimal mixtures or combinations of controlled release N fertilizer with regular urea could allow N rates to be reduced by 25%. However, no such studies have been conducted in Brazilian corn production conditions.

Another strategy to reduce N losses is the incorporation of N fertilizers into the soil, which is known as a great alternative to prevent N losses, especially through ammonia volatilization (Rochette et al., 2013). Nitrogen timing application also has great impact on corn grain yields and NUE (Gehl et al., 2005; Schoninger, 2014; Oliveira et al., 2018).

The present study included N rates applied as a blend of PSCU and NBPTU at a 70:30 ratio, incorporated at planting. Although not a thorough study of all 4R's concept for N management, this research included all 4 factors (i.e. N rates, N source, N placement, and N timing). In this context, field experiments were conducted in Southeast Brazil, with the application of a blend of polymer-sulfur coated urea (PSCU) and NBPT-treated urea (NBPTU) at a 70:30 ratio, applied at corn planting and incorporated into the soil in contrasting corn production systems. The objectives of the study were: i) to quantify and measure each fertilizer-derived N fate in the plants, and determine the nitrogen use efficiency of each N source in the blend; and ii) to evaluate corn grain yield response to N rates (blend) in contrasting cropping systems, and to assess the posibility of reducing N rate when applying a blend of two enhanced efficiency N fertilizers compared to the application of regular urea.

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2. N-FERTILIZER RECOVERY BY CORN FROM A BLENDED APPLICATION OF POLYMER-SULFUR COATED AND NBPT-TREATED ^{15}N-UREA

ABSTRACT

Nitrogen use efficiency (NUE) in corn (*Zea mays* L.) can be increased by using controlled-release N fertilizer (CRN). The objectives of this study were to: i) evaluate the fate of N derived from a blend of two N sources throughout the growing season; and ii) determine the fertilizer N recovery efficiency (NRE) of the two N sources. A field study was conducted during the 2015-2016 growing season. Treatments included six N rates (0, 60, 120, 180, 240 and 300 kg N ha^{-1}) band-applied at corn planting. Controlled release (polymer-sulfur coated urea - PSCU) and NBPT-treated urea (NBPTU, ^{15}N-labeled at 1.6% atoms ^{15}N) were blended at a 70:30 ratio. At harvest, most of the plant N was allocated in the grains (59%). Plant N derived from PSCU, NBPTU, and soil$_N$ varied greatly, however, the fertilizer sources contributed to less than 50% of the total plant N throughout all the growing season. At V4 corn growth stage (GS) most of the plant N derived from fertilizer was from NBPTU, while at later GS, most of the fertilizer-N in the plants was derived from PSCU. The results of this study indicate that NBPTU provided N to corn early in the season, while PSCU played a crucial role supplying N later in the season, as plants demand for N increased. Soil N was the main N source at all GS and this fraction decreased as N rate increased. At harvest, 64% of the total plant N was derived from the soil native N pool, 26% derived from PSCU, and 10% from NBPTU. The measured fertilizer NRE of NBPTU was in average 36%, and the estimated NRE from PSCU was 51%.

Keywords: Controlled release nitrogen; Nitrogen recovery efficiency; Nitrogen uptake; Maize; Urea

Abbreviations: CRN, controlled release nitrogen; Growth stage, GS; HI, harvest index; NHI, nitrogen harvest index; NRE, nitrogen recovery efficiency; PSCU, polymer-sulfur coated urea; PSCU$_N$, nitrogen in the plant derived from polymer-sulfur coated urea; Soil$_N$, nitrogen in the plant derived from the soil native N pool; NBPTU, regular urea treated with the enzyme urease inhibitor NBPT; NBPTU$_N$, nitrogen in the plant derived from NBPT-treated urea

2.1. INTRODUCTION

Cereal-grain N recovery efficiency (NRE) worldwide is estimated to be 33% in average, ranging from 30 to 50 % (Raun and Johnson 1999; Ladha et al. 2005). In a recent study, Pires et al. (2015) reported that NRE in Brazilian cereal production areas is 27%, a seriously low NRE value. The amount of N applied as fertilizer and not taken up by plants can be lost from the soil-plant system resulting in economic lost and environmental impairments (Fowler et al., 2015). Therefore, N fertilizer management by farmers are often directly linked to environmental quality (Weber and McCann, 2015).

Nitrogen fertilizer source, application time and placement are key factors that can affect N uptake efficiency in crop production (Fageria and Baligar, 2005; Amado et al., 2013). One common recommendation for N fertilization in corn is for a split application, with most of the N fertilizer applied in-season as side-dress. The split application of N fertilizer typically require about 20% of the total recommended rate to be applied at planting and the balance applied between the V4 and V6 corn growth stage (GS) (Raij et al., 1996). Surface broadcast or banded application of the N fertilizer is a common application method; however, N loss from urea volatilization can be worsen under humid and high temperature conditions with in-season urea fertilizer application (Lara Cabezas et al., 2000; Mariano et al., 2012). Urea incorporation into the soil can reduce, or even cease N losses from volatilization (Trivelin et al., 2002). Previous studies showed that incorporating urea at about 7.5 cm depth resulted in negligible N losses, compared to N volatilization losses of up to 50% when urea was surface applied (Rochette et al. 2013)

The use of controlled release N (CRN) fertilizer sources has been proposed as another alternative to improve NUE (Trenkel, 2010) in agricultural systems. The adoption of CRN fertilizer sources are intended to improve the synchronization between N supply and plant N uptake during the growing season (Chalk et al., 2015). Recent studies evaluated the impact of CRN fertilizers on N losses and corn grain yield on a tropical soil and environmental conditions (Cancellier et al. 2016). The results showed that surface application of polymer-sulfur coated urea (PSCU) at the V5 corn GS resulted in approximately 37% reduction of total N losses through volatilization compared to regular urea, whereas N uptake and grain yield were similar. Several other studies have shown increased yield and economic profit using CRN without jeopardizing the environment (Halvorson and Bartolo, 2014; Geng et al., 2016; Zheng et al., 2016).

The price of CRN is in average about 2-4 times the price of regular urea (Chalk et al., 2015; Guelfi, 2017). One approach to lower fertilizer cost is to use mixtures or blends of the controlled release source with the regular N fertilizer. For example, Guo et al. (2017) evaluated blends of CRN and regular urea in high yielding (>15 Mg ha^{-1}) irrigated corn in Northwest China and found that a single at planting application of a blend of polymer coated urea and regular urea provided similar or higher grain yield than the split applied urea treatment, resulting in higher NRE and economic return.

When applying a mixture of two N fertilizers, there are uncertainties on the real source of N to the plants. Is the N taken up by the plants coming from the controlled release source, the regular N fertilizer, or from the soil native N pool? How much does each source contribute to the total plant N? In what part of the plants does the N allocate? Answering these questions is a challenge due to the highly complex dynamic of N in the soil-plant-atmosphere system. A recent paper by Chalk et al. (2015) discusses the need or lack of scientific reports on the evaluation of NRE when applying slow or controlled release fertilizers using isotopic techniques (^{15}N-labeled fertilizers).

Nitrogen recovery efficiency in field experiments is usually calculated by either the difference or the direct method. The difference method is based on the differences in crop N uptake between fertilized and unfertilized plots (Rao et al., 1991), while the direct method is based on the application of isotopic techniques with the use of ^{15}N-labeled fertilizers to estimate the recovery of the applied N using the isotopic dilution principles (Dobermann, 2007). The direct method typically results in overall lower NUE values compared to the difference method, although both are strongly related. For example, in different research trials around the world, Ladha et al. (2005) reported an average NRE of 55% measured with de difference method compared to 44% measured with the direct method (^{15}N). Other studies showed approximately a 20% difference between estimations by the two methods (Rao et al. 1991).

Previous studies suggested that the difference method can result in NRE overestimation due to the omission of the “priming effect” or the “added N interaction” (Jenkinson et al., 1985; Rao et al., 1991; Azam et al., 1993). This consists of the increase in N mineralization rate from soil organic matter caused by fertilizer N (^{15}N) application (Jenkinson et al., 1985; Kuzyakov et al., 2000). On the other hand, NRE can be underestimated with the ^{15}N method due to possible confounding effects caused by pool N substitution, which basically consists in the immobilization of ^{15}N fertilizer by the microbial biomass and initial release of ^{14}N from the microbial biomass (Dobermann, 2007). Both methods were used in this study in order to allow us discriminate plant N derived from two blended N fertilizers (^{15}N labeled NBPT-treated urea and polymer-sulfur coated urea).

The objectives of this study were to: i) determine the fate of N derived from each N source of a blended N fertilizer applied at planting, and the allocation of the fertilizer-derived

N in the plant; and ii) to estimate the NRE from each source from a blended application including the interaction with the native soil N pool.

2.2. MATERIAL AND METHODS

2.2.1. Experimental site and treatments

A field experiment was conducted in Iracemápolis, São Paulo, Brazil (22°38'50"S, 47°30'14"W) during the 2015-2016 growing season, on a Typic Hapludox. The climate of this site correspond to Cfa (hot summer) transitioning to Cwa (dry winter and hot summer), according to the Köppen classification map for Brazil elaborated by Alvares et al. (2013). Before the establishment of the experiment, the area was cropped with cotton (*Gossypium hirsutum* L.), and prior to that the field was under citrus tree (*Citrus sinensins* L.) production for approximately 20 years.

Soil samples were collected prior to planting to characterize the soil chemical and physical properties by collecting one composite sample at 0-20 soil depth. All soil chemical characteristics determination were performed using methods outlined by Raij et al. (2001). Samples were analyzed for pH using $CaCl_2$ 0.01 mol L^{-1}, ratio of 1:2.5 for soil and solution - m/v. Organic matter (O.M.) was determined by potassium dichromate titration. Nutrients phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were extracted using ion-exchange resin and determined colorimetrically (P), by flame spectroscopy (K), and atomic absorption (Ca and Mg). To determine soil potential acidity (H+Al), the SMP buffer solution method was adopted (Shoemaker et al., 1961), and cation exchange capacity (CEC) at pH 7.0 was calculated as the sum of the exchangeable cations (K^+, Ca^{2+} and Mg^{2+}) and soil potential acidity. Base saturation was calculated by dividing the sum of exchangeable cations by CEC and multiplied by 100. Soil texture (sand, silt and clay) was determined by the hydrometer method (Bouyoucos, 1962).

Soil samples collected from the study area at the 0-20 cm depth showed the following characteristics: pH of 5.2; soil organic matter 23 g dm^{-3}; soil test P of 19 mg dm^{-3}; soil test K of 3 mmolc dm^{-3}; Ca of 35 mmolc dm^{-3}; Mg of 18 $mmol_c$ dm^{-3}; soil potential acidity (H+Al) = 34 mmolc dm^{-3}; CEC of 97 $mmol_c$ dm^{-3}; and base saturation of 65%. Soil clay, silt and sand content was 520, 153, and 327 g kg^{-1} respectively. The study site was under rainfed conditions and received a total rainfall of 719 mm from planting to harvest distributed as 87, 303, 154, 169, and 6 mm for Dec., Jan., Feb., Mar., and Apr. respectively.

2.2.2. Experimental design and treatments

The experiment was arranged in a complete randomized block design with four replications. Planting was performed on 22-Dec. 2015. Plots were 8 corn rows by 12 m long, planted at 50 cm row spacing at 70,000 plants ha^{-1} seeding rate. Nitrogen fertilizer treatments consisted of six application rates (0, 60, 120, 180, 240 and 300 kg ha^{-1} N) applied and incorporated by hand immediately after corn planting. The PSCU used in this experiment was Producote® (Patent No. EP 0574541 B1), described as regular urea coated with a layer of elemental sulfur (S) and a biodegradable insoluble in water polymer layer [(39-0-0-11), (N, P_2O_5, K_2O, S)]. The regular ^{15}N labeled urea was treated with NBPT at a 0.3 % dose (m/v). The composition of the urea was 45-0-0 (N, P_2O_5, K_2O).

Nitrogen fertilizer treatments were band-applied right after planting. Furrows were opened 10 cm from the row and approximately 5 cm deep for N fertilizer placement, and closed by hand. The corn hybrid used was DEKALB 390 VT PRO 2. Phosphorus fertilizer was applied in-furrow with the planter at 120 kg ha^{-1} P_2O_5 at planting using triple superphosphate (TSP). Potassium fertilizer was surface-broadcast applied three days before planting at 120 kg ha^{-1} K_2O using potassium chloride (KCl).

2.2.3. Isotopic ^{15}N-labeled determination

A micro-plot of 1.5 m wide by 1.5 m long (three corn rows) were installed inside each plot, and a blend of non-labeled PSCU and ^{15}N-labeled NBPT treated urea (1.6 atom% ^{15}N) were applied at the same N rate of the rest of the plot. Two plants from the micro-plots were sampled at the V4, V12, and R2 GS from the outside two rows. At harvest (R6, physiological maturity), two plants were sampled from the center row of the micro-plots. Plant samples were separated into corn plant parts depending on the GS: leaves (leaves + tassel + husk leaves), stalk, cob and grain. After separation in plant parts, the samples were dried in a forced air oven at 65ºC for 5 days, weighed and ground with a Willey mill (2 mm screen). Total N and ^{15}N abundance were determined in an isotope ratio mass spectrometer (Barrie and Prosser, 1996). Sampling dates were 14-Jan. (V4), 5-Feb (V12), 3-March (R2), and 3-May (R6, harvest) in 2016. Biomass accumulation and nitrogen uptake rate were calculated from the difference in the accumulated N uptake among sampling dates, dividing the result by the number of days in each period (Mariano et al., 2015).

Total nitrogen uptake in the control treatments (no N added) was assumed as the total N derived from the soil ($soil_N$) in our study. The value of $soil_N$ was used for other treatments with N fertilizer application, with the assumption that the N immobilization-mineralization processes and other N transformations are the same for both fertilized and non-fertilized plots (Rao et al., 1991). Due to the omission of the priming effect of the applied fertilizer N (i.e. increased N mineralization from the organic matter), the difference method used in this study to calculate the amount of N derived from PSCU may overestimate the N recovery value of this source (Stevens et al., 2005a; b). Nevertheless, this approach can be a useful tool in the estimation of N uptake by corn plants from two blended N sources, and also offer an approximate value of N uptake from the soil.

With the abundance of ^{15}N atoms in the plant, and the values of N uptake in the corn plants, nitrogen derived from fertilizer calculations were performed. The results were obtained using equations 1 to 5.

$$NBPTU_N(\%) = \left(\frac{a-b}{c-b}\right) \times 100 \text{ ------------------------ } (Eq.1)$$

$$NBPTU_N\ (kg\ ha^{-1}) = \frac{NBPTU\ (\%)}{100} \times N\ uptake \text{ ---------------- } (Eq.2)$$

$$NRE_{NBPTU}\ (\%) = \left(\frac{NBPTU_N\ (kg\ ha^{-1})}{NBPTU\ fertilizer\ (kg\ ha^{-1})}\right) \times 100\ \times 2 \text{ ---------- } (Eq.3)$$

$$PSCU_N\ (kg\ ha^{-1}) = N\ uptake - Soil_N - NBPTU_N \text{ ------------- } (Eq.4)$$

$$NRE_{PSCU}\ (\%) = \left(\frac{N\ uptake - NBPTU_N\ - Soil_N}{PSCU\ fertilizer}\right) \times\ 100 \text{ ----------- } (Eq.5)$$

Where *$NBPTU_N$*: nitrogen in the plant derived from NBPT-treated urea (% and kg ha^{-1}); *a*: abundance of ^{15}N atoms in the different plant parts (leaves, stalk, cob, and grain); *b*: natural abundance of ^{15}N atoms in the different plant parts; *c*: abundance of ^{15}N atoms in the fertilizer; *N uptake*: total N accumulated in the different plant parts (kg N ha^{-1}); *NRE*: fertilizer nitrogen recovery efficiency (%); *NBPTU fertilizer*: amount of N applied as NBPT-treated ^{15}N-urea (kg N ha^{-1}); *$Soil_N$*: nitrogen in the plant derived from the mineralization of soil organic matter (control treatment) (kg N ha^{-1}); *$PSCU_N$*: nitrogen in the plant derived from polymer-sulfur coated urea (PSCU); *PSCU fertilizer*: amount of N applied as PSCU (kg N ha^{-1}).

Equation 1 was used at the V4, V12 and R2 GS. We assumed that 50% of the N from fertilizer in the plant was taken up from the labeled fertilizer and 50% from the non-labeled fertilizer, thus we used the multiplication factor 2 on Eq. 1, as recommended by Jhonson and Kurtz (1974). However, for the sampling at the R6 corn GS the factor 2 on Eq. 1 was not included because the sampling was performed in the center row of the micro-plot, where plants had ^{15}N applied at both sides of the row.

2.2.4. Statistical analysis

All data were analyzed using the PROC GLIMMIX procedure in SAS® software (SAS Institute Inc, Cary, NC). Variables were analyzed by corn GS; the main effects of N fertilizer application rate and plant parts were included as fixed factor and blocks as random factor in the model. Corrected denominator degrees of freedom were obtained using the Kenward-Roger adjustment. To assess the significance of differences between means we used the LSMEANS and SLICE option in PROC GLIMMIX. Treatment effects were considered statistically significant at the $P \leq 0.10$ probability level.

2.3. RESULTS AND DISCUSSION

2.3.1. Corn biomass production and partitioning

Corn aboveground biomass production early in the season (V4) was not affected by N rate (Table 2.1). Low corn shoot biomass accumulation rate early in the season may contribute to the lack of N rate effect (Table 2.2). At the V4 GS, corn plants have accumulated only 1.4% of the final total biomass production. At later GS, however, the effect of N rate treatments and plant components were statistically significant for BP (Table 2.1). Average values of BP across N rates were 4.5, 8.3 and 14.7 Mg ha^{-1} for V12, R2 and R6 GS respectively (Table 2.2). Biomass production increased from V4 to R6 GS, with significantly lower BP values for the control treatment compared to the N fertilizer treatments at the V12, R2 and R6 GS (Table 2.2). We found the higher biomass accumulation rate between the V4 and V12 GS, with a rate of 196 kg ha^{-1} day^{-1} (Table 2.3). Bender et al. (2013) reported the highest corn biomass accumulation rate later in the season between the V10 and V14 GS, with 439 kg ha^{-1} day^{-1} in the USA corn belt conditions, while Schoninger (2014) found a rate of 266 kg ha^{-1} day^{-1} between the V12 and R1 GS in tropical conditions from Brazil. Results from our study as well as previous research in Brazil showed that maximum biomass accumulation rate occurs at late vegetative corn GS (Gava et al., 2010; Schoninger, 2014; Garcia et al., 2018); therefore the adequate supply of plant available N may be particularly important during this period of rapid growth.

Corn biomass partitioning showed that N rate only affected leaves, not stalk or cob biomass accumulation during the growing season (Table 2.2). At R6, N rate affected only grain BP. At the V12 and R2 GS, the plants have accumulated 31%, and 57% of the total BP, respectively (Table 2.2). After the R2 GS, corn plants typically enter a period of rapid dry matter and nutrient redistribution or remobilization to the grains (Bender et al., 2013). During this period leaves and stalk are the main suppliers (source) of resources to the grains (sink). At maturity (R6 GS), the leaf represented 31% of the total biomass, stalk 14%, cob 8% and grain 47% (Fig. 2.2). The value of 47% for the grain is slightly lower than the harvest index (HI) values reported in the literature. For example, Bender et al. (2013) reported HI values between 48 and 54% for six corn hybrids across two sites in Illinois, USA. Attia et al. (2015) reported values between 49 and 52% in a three years experiment in Nebraska, USA, and Schoninger (2014) reported average HI values of 42 and 53% for two different growing seasons in Brazil.

Table 2.1. Significance of the F values for the fixed effects of N fertilizer rate and plant parts on corn biomass production (BP), nitrogen concentration (Nc), nitrogen uptake (N uptake), nitrogen derived from the soil ($soil_N$), nitrogen derived from NBPT-treated ^{15}N-urea ($NBPTU_N$), nitrogen derived from polymer-sulfur coated urea ($PSCU_N$), ^{15}N-NBPTU nitrogen recovery efficiency (NRE_{NBPTU}), and polymer-sulfur coated urea nitrogen recovery efficiency (NRE_{PSCU}).

Fixed effect	BP	Nc	N uptake	$Soil_N$	$NBPTU_N$	$PSCU_N$	NRE_{NBPTU}	NRE_{PSCU}
	- P>F -							
				V4 Growth Stage				
N Rate (NR)†	0.357	0.204	0.237	0.671	0.777	0.711	0.003	0.959
				V12 Growth Stage				
N Rate (NR)	<0.001	<0.001	<0.001	<0.001	<0.001	0.031	0.002	0.001
Plant Part (PP)‡	<0.001	<0.001	<0.001	0.039	0.141	0.007	<0.001	<0.001
NR × PP	0.087	0.992	<0.001	0.998	1.000	0.997	0.403	0.641
				R2 Growth Stage				
N Rate (NR)	<0.001	<0.001	<0.001	0.052	<0.001	0.232	0.046	<0.001
Plant Part (PP)	<0.001	<0.001	<0.001	0.114	<0.001	0.111	<0.001	<0.001
NR × PP	0.001	0.303	0.002	0.9319	0.972	0.921	0.783	0.003
				R6 Growth Stage				
N Rate (NR)	<0.001	<0.001	<0.001	0.009	<0.001	0.380	<0.001	0.026
Plant Part (PP)	<0.001	<0.001	<0.001	0.101	0.001	0.193	<0.001	<0.001
NR × PP	<0.001	0.219	<0.001	0.941	0.999	0.970	0.011	0.607

† Five N rates (60, 120, 180, 240, and 300 kg N ha^{-1}). BP, Nc, and N uptake included the control for the analysis (six N rates total).

‡ Plant parts included leaves, stalk, cob, and grain.

Table 2.2 Corn biomass production (kg ha^{-1}), nitrogen concentration, and nitrogen uptake in corn plant parts (leaves, stalk, cob, grain, and total) at different corn growth stages as affected by nitrogen application rate using a blend of polymer-sulfur coated urea and NBPT-treated ^{15}N-urea at a 70:30 ratio.

	V4 GS†	V12 GS			R2 GS					R6 GS				
N rate	Total	Leaf	Stalk	Total	Leaf	Stalk	Cob	Grain	Total	Leaf	Stalk	Cob	Grain	Total
kg N ha^{-1}							Biomass production (Mg ha^{-1})							
0	0.15	1.9 c	0.6	2.5 c	2.5 c	1.1	0.7	0.7	4.9 e	3.3	1.6	0.9	2.1 c	8.0 e
60	0.20	3.1 ab	1.3	4.3 ab	4.1 ab	1.8	1.0	1.1	8.0 cd	3.9	1.9	1.0	5.5 b	12.3 d
120	0.19	3.3 ab	1.3	4.6 a	4.3 ab	1.8	1.0	1.1	8.2 bc	4.3	2.0	1.0	6.5 b	13.8 c
180	0.21	2.9 b	1.1	3.9 b	4.7 a	1.9	1.2	1.4	9.2 a	4.4	2.0	1.2	7.9 a	15.6 ab
240	0.19	3.4 a	1.4	4.8 a	3.7 b	1.8	0.8	1.1	7.3 d	5.1	2.3	1.3	8.1 a	16.9 a
300	0.20	3.4 a	1.3	4.8 a	4.7 a	1.9	1.1	1.2	8.9 ab	4.6	2.2	1.2	6.9 ab	14.9
P > F	0.357	<0.001	0.167	<0.001	<0.001	0.596	0.912	0.852	<0.001	0.340	0.965	0.995	<0.001	<0.001
							Nitrogen concentration (g kg^{-1})							
0	32	20 cd	12 b	18 bc	19 abc	6 b	10 b	20 c	15 b	09 c	5 c	5	13 d	9 c
60	30	19 d	9 b	17 c	18 c	6 b	11 bc	22 bc	15 b	10 c	5 bc	6	13 cd	11 b
120	33	22 cd	11 b	18 bc	18 c	6 b	13 bc	22 bc	15 b	10 c	5 bc	5	14 cd	11 b
180	35	22 bc	14 a	20 ab	19 bc	7 b	12 ab	21 bc	16 ab	11 b	6 ab	6	15 ab	12 a
240	37	24 ab	14 a	21 a	20 ab	9 a	12 ab	24 a	17 a	11 b	6 abc	5	15 abc	12 ab
300	34	25 a	15 a	22 a	21 a	9 a	13 a	22 b	17 a	13 a	7 a	5	16 a	13 a
P > F	0.200	0.002	<0.001	0.002	0.043	<0.001	0.017	0.008	0.052	<0.001	0.068	0.786	0.011	<0.001
							Nitrogen uptake (kg ha^{-1})							
0	5	39 c	7	46 d	47 c	6	6	14	74 d	29 c	8	4	28 d	69 d
60	6	60 c	12	72 c	74 b	10	10	25	120 c	40 bc	10	6	74 c	130 c
120	6	71 b	14	86 b	74 b	11	12	24	124 c	42 bc	11	5	92 c	149 b
180	7	63 b	14	78 bc	89 ab	14	14	27	144 ab	51 ab	13	6	119 a	189 a
240	7	84 a	19	103 a	75 b	17	10	27	128 bc	60 a	15	7	119 a	201 a
300	7	84 a	20	104 a	97 a	18	14	27	156 a	63 a	16	7	108 ab	193 a
P > F	0.237	<0.001	0.496	<0.001	<0.001	0.857	0.969	0.759	<0.001	0.015	0.974	0.999	<0.001	<0.001

† GS, growth stage (Abendroth et al., 2011)

Table 2.3. Whole plant biomass production (BP) rate and nitrogen uptake (NU) rate. Values estimated across N rate treatments.

Period	Range	Days after planting†	BP rate	NU rate
Growth stage	days		- - - - - - - kg ha^{-1} day^{-1}	- - - - - - -
VE – V4	16	23	12	0.4
V4 – V12	22	45	196	3.7
V12 – R2	27	72	142	1.7
R2 – R6	61	133	109	0.6

† Total days since planting

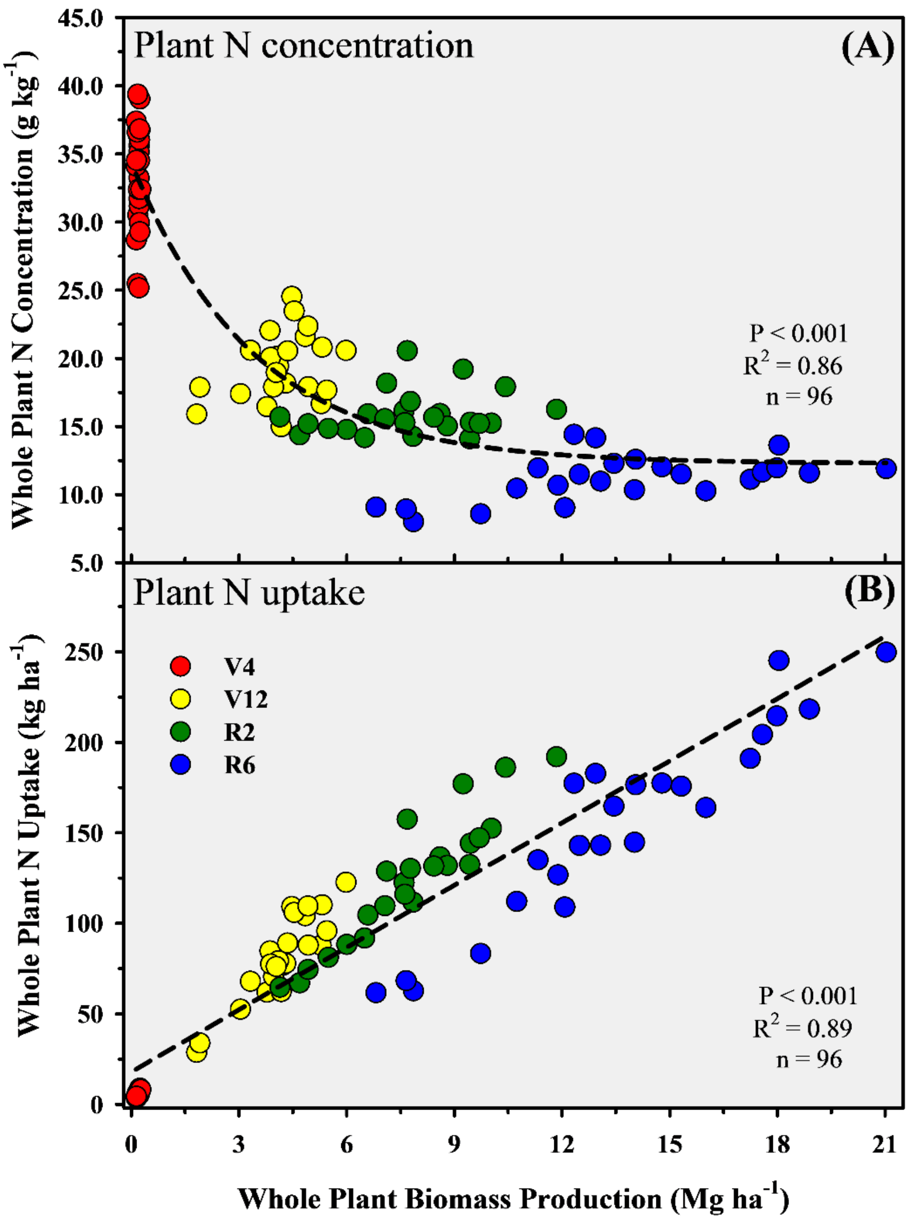

Figure 2.1. Relationship between whole plant biomass accumulation and whole plant N concentration (A); and whole plant biomass accumulation and whole plant N uptake (B). Samples were collected throughout the growing season (V4, V12, R2, and R6 growth stages).

Table 2.4. Nitrogen in the plant derived from NBPT-treated ^{15}N-urea and polymer-sulfur coated urea allocated in different corn plant parts (leaves, stalk, cob, grain, and total) at different corn growth stages as affected by nitrogen application rate using a blend of polymer-sulfur coated urea and NBPT-treated ^{15}N-urea at a 7:3 ratio.

	V4 GS†	V12 GS			R2 GS					R6 GS				
N rate	Total	Leaf	Stalk	Total	Leaf	Stalk	Cob	Grain	Total	Leaf	Stalk	Cob	Grain	Total
							-kg ha^{-1}							
kg N ha^{-1}							Nitrogen derived from NPBT-treated ^{15}N-urea							
60	1.0	5.5 d	0.8	6.4 c	3.7 c	0.4	0.4	1.0	5.5 d	2.3 b	0.5	0.2	3.0 c	7.2 d
120	1.2	9.2 cd	1.6	10.7 b	6.0 c	0.7	0.7	1.2	8.8 c	3.2 b	1.0	0.4	6.1 b	13.0 c
180	1.5	11.0 cd	2.1	13.0 b	9.5 b	1.1	1.0	1.7	13.3 b	7.1 a	1.6	0.7	12.3 a	23.0 b
240	1.3	14.2 ab	2.9	17.2 a	10.2 ab	1.8	1.1	2.5	15.8 ab	8.9 a	2.1	0.8	13.5 a	24.7 ab
300	1.6	17.0 a	3.7	20.6 a	13.0 a	1.9	1.2	2.5	18.5 a	9.2 a	2.1	0.7	11.7 a	25.4 a
P > F	0.555	<0.001	0.752	<0.001	<0.001	0.872	0.989	0.86	<0.001	<0.001	0.710	0.992	<0.001	<0.001
							Nitrogen derived from polymer-sulfur coated urea							
60	0.0	16.2	4.1	20.0 b	22.8	3.7	3.7	10.2	40.5	8.7	1.8	1.2	22.2	34.0
120	0.2	23.2	5.9	29.1 ab	22.0	4.7	5.2	9.2	41.4	9.5	2.1	0.1	40.6	52.4
180	1.0	13.7	5.5	19.2 b	32.1	6.6	6.5	11.9	57.0	15.0	3.2	1.3	40.8	60.4
240	0.7	30.5	9.5	40.0 a	17.1	9.0	2.1	10.3	38.4	22.4	4.9	1.4	22.8	51.6
300	0.2	28.2	9.0	37.3 a	37.0	9.7	6.9	10.4	64.1	24.5	6.3	1.5	34.9	67.2
P > F	0.746	0.163	0.946	0.031	0.290	0.970	0.988	0.999	0.044	0.710	0.997	1.000	0.486	0.188

† GS, growth stage (Abendroth et al., 2011)

Table 2.5. Percentage of nitrogen in the plant derived from the soil, NBPT-treated urea and polymer-sulfur coated urea allocated in different corn plant parts (leaves, stalk, cob, grain, and total) and at different corn growth stages as affected by nitrogen application rate using a blend of polymer-sulfur coated urea and NBPT-treated ^{15}N-urea at a 70:30 ratio.

	V4 GS†	V12 GS			R2 GS					R6 GS				
N rate	Total	Leaf	Stalk	Total	Leaf	Stalk	Cob	Grain	Total	Leaf	Stalk	Cob	Grain	Total
kg N ha^{-1}	%													
	Plant N derived from the soil													
60	82	64	60	63	63	61	62	75	62	83 ab	78 b	78	74 b	75 b
120	87	55	48	53	64	54	52	72	62	70 bc	77 b	89	62 b	66 b
180	68	63	49	60	55	46	47	56	59	57 cd	62 bc	70	58 b	58 b
240	73	50	39	48	64	37	70	57	59	53 cd	61 bc	72	71 b	64 b
300	77	45	36	44	50	35	47	56	53	46 d	52 c	77	62 b	57 b
P > F	0.671	0.116	0.043	0.110	0.712	0.205	0.302	0.712	0.793	0.059	0.215	0.628	0.716	0.677
	Plant N derived from NBPT-treated urea													
60	17	10 b	7 c	9 c	6 d	4 c	4 c	3 d	4 d	6 b	5 c	4 c	4 c	5 c
120	12	13 b	11 bc	12 bc	8 c	6 bc	5 c	5 cd	7 c	8 b	9 b	7 b	7 b	7 b
180	21	17 a	15 b	16 ab	11 b	9 b	8 b	6 bc	10 b	14 a	13 a	11 a	10 a	12 a
240	19	17 a	15 b	16 ab	14 a	11 a	12 a	9 a	13 a	15 a	14 a	12 a	12 a	13 a
300	22	21 a	19 a	19 a	13 ab	11 a	8 b	8 ab	12 ab	15 a	14 a	12 a	11 a	13 a
P > F	0.777	0.004	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Plant N derived from polymer-sulfur coated urea													
60	1	26	33	27	31	34	34	37	33	11	16	17	22	20
120	1	32	41	34	27	40	42	20	31	21	14	3	31	27
180	11	20	36	23	34	45	45	38	37	28	24	19	32	30
240	8	33	46	35	22	52	18	34	28	32	25	16	17	23
300	1	34	45	36	37	54	45	35	39	38	34	11	27	30
P > F	0.711	0.369	0.380	0.392	0.818	0.535	0.207	0.640	0.918	0.365	0.602	0.797	0.826	0.938

† GS, growth stage (Abendroth et al., 2011)

Table 2.6. Fertilizer nitrogen recovery efficiency from a blended application of NPBT-treated urea and polymer-sulfur coated urea, evaluated at different corn growth stages.

	NBPT-treated urea				Polymer-sulfur coated urea			
N rate	V4	V12	R2	R6	V4	V12	R2	R6
kg N ha^{-1}				%				
60	6 a	36 a	31 a	40 ab	0.1	47 a	96 a	81 a
120	3 b	30 ab	24 b	36 bc	0.3	34 ab	49 b	62 ab
180	3 bc	24 bc	24 b	42 a	0.8	15 c	45 bc	48 bc
240	2 c	24 bc	22 b	34 c	0.4	24 bc	23 d	31 c
300	2 c	23 c	20 b	28 d	0.1	18 c	31 cd	32 c
P > F	0.003	0.010	0.013	<0.001	0.959	0.008	<0.001	0.009

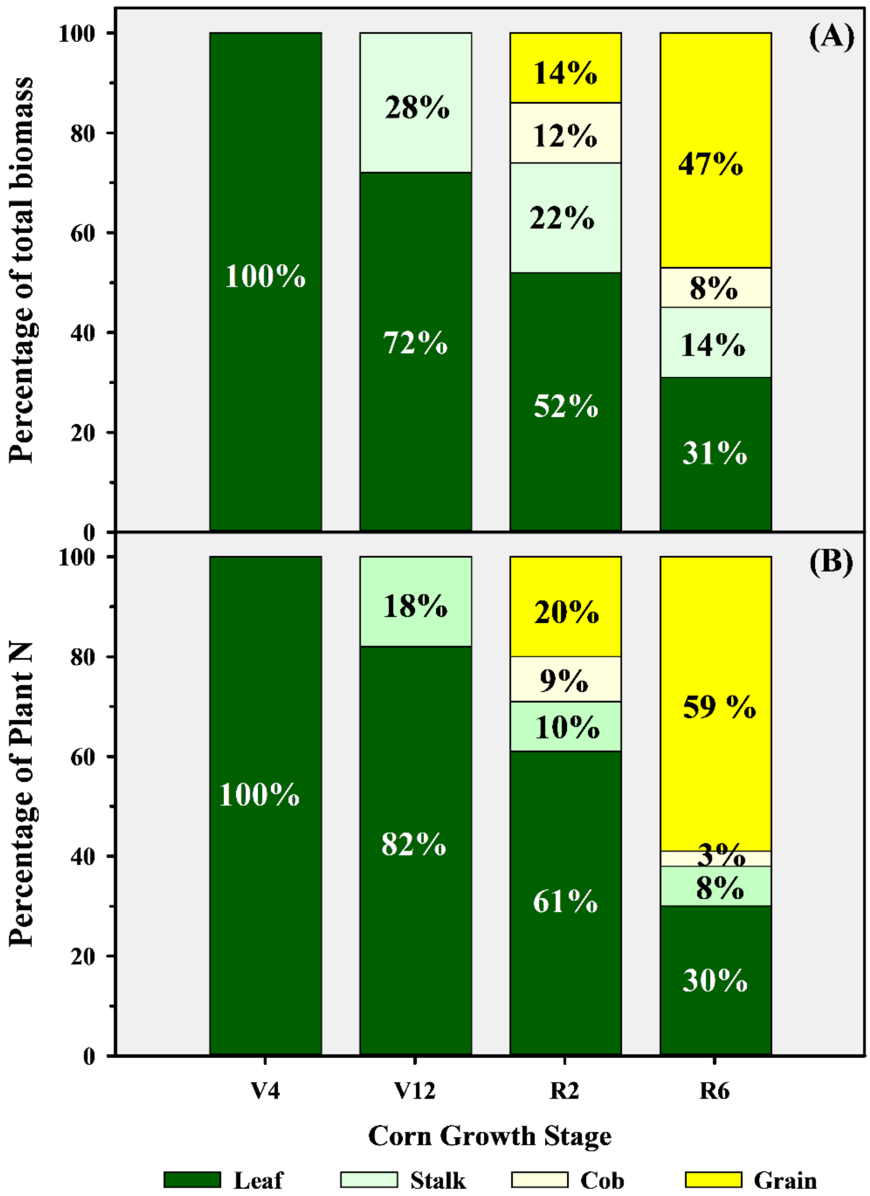

Figure 2.2. Biomass (A) and nitrogen (B) partitioning and allocation (leaf, stalk, cob, and grain) at different growth stages (V4, V12, R2 and R6). Average across six N rates.

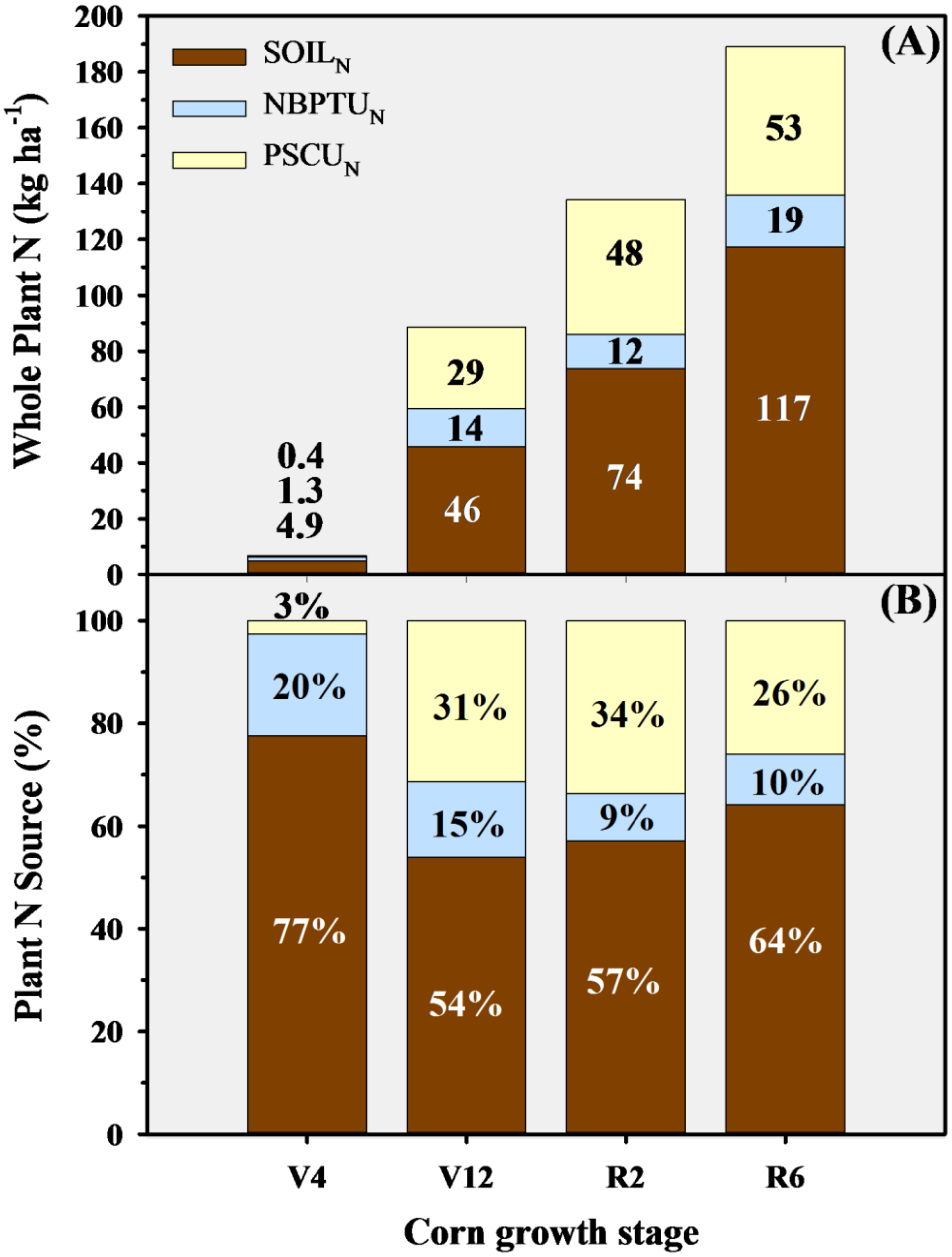

Figure 2.3. Whole plant nitrogen uptake (A); and percentage of total nitrogen in the plant (B) derived from the soil ($SOIL_N$), from NBPT-treated urea ($NBPTU_N$) and from polymer-sulfur coated urea ($PSCU_N$) at four corn growth stages (V4, V12, R2 and R6). Average across six N rates.

2.3.2. Plant nitrogen concentration, uptake, and partitioning

Plant N concentration early in the season (V4) was not affected by fertilizer N application (Table 2.2). However, fertilizer N did influence N concentration in all plant components at all sampling dates except the cob plant part at harvest (R6) (Table 2.2). Stalk and cob plant parts generally showed the lowest N concentration values compared to leaves and grains. However, N concentration values varied across different N rate treatment with no clear relation to the rate of N fertilizer applied (Table 2.2). For example, the control treatment with no N fertilizer application did not show the lowest N concentration values. On the other hand, the highest N rate treatments (240 and 300 kg N ha^{-1}) often showed the highest N concentration values, but not always. Whole plant N concentration decreased as a function of BP (Fig. 1A), showing a typical N dilution curve in plants (Gastal and Lemaire, 2002). This suggest that plant BP may have a greater influence on whole plant N concentration than N fertilizer application rate. Our results agree with previous studies showing a decrease in whole plant N concentration with the increase in corn BP (Ciampitti and Vyn, 2011).

Nitrogen application rate did not impact aboveground N uptake at the V4 GS, but it did affect N uptake at later GS (Table 2.1). Nitrogen accumulation in the different plant parts was affected by fertilizer N application except in the stalk, where no difference was observed (Table 2.2). Similar to these results, Gava et al. (2010) reported that N application rate affected plant N uptake for different plant parts except the stalk throughout the growing season, in a . Whole plant N uptake showed a linear relationship with whole plant BP (Fig. 1B), in agreement to previous studies reporting similar observations (Gastal and Lemaire, 2002; Ciampitti and Vyn, 2011).

A total of 82, 61, and 30% of the total N in the plant was allocated in the leaves at the V12, R2 and R6 GS, respectively (Fig. 2B). A total of 59% of the total N uptake was allocated in the grains at harvest (R6 GS), representing a N harvest index (NHI) within the range of 51-61 % (average 58%) reported by Bender et al. (2013), and similar to the average values of 56 and 68% found by Schoninger (2014). The cob and stalk fractions accounted for 3 and 8% of the total N uptake at harvest (R6), demonstrating a low but still important N reservoir for the N cycling process after harvest. Nitrogen uptake rate was higher between V4 and V12, with an average of 3.7 kg N ha^{-1} (Table 2.3), thus highlighting the importance of optimum supply of N during these GS.

2.3.3. Contribution of N sources to plant N uptake

The amount of plant N derived from NBPTU and PSCU is shown in Table 2.4 and Table 2.5. Nitrogen derived from NBPTU at the V4 GS was higher than N from PSCU, which at this stage resulted in little contribution to the total plant N uptake (Fig. 2.3), most likely because of the controlled release nature of this fertilizer (Trenkel, 2010). Polymer-sulfur coated urea played a bigger role in supplying N to corn later in the season. Between the V12 and R2 GS, we found a slight decrease on $NBPTU_N$ (from 13.6 at V12 to 12.4 kg N ha^{-1} at R2 for the five N rates) (Table 2.4). It is not clear why would this happen, as BP and N uptake increased in this period (Table 2.2), and the calculated plant N derived from PSCU also increased. One plausible hypothesis is that during the N redistribution process from shoot to the developing grain, which starts to intensify when corn plants reach the reproductive GS, some of the N was lost from the canopy (Schoninger et al., 2018). For example, Francis et al. (1993) reported post-anthesis losses of previously taken up ^{15}N from corn plant leaves ranging from 7 to 34 kg ha^{-1}. Another possible explanation would be the redistribution of this N, which was taken up and assimilated by the plants at early GS, and then redistributed to the roots for growth and development.

There are several studies highlighting the importance of soil N to fulfill plant N requirement. For example, Stevens et al. (2005b), in a long-term ^{15}N rate study reported that soil N accounted for 54-83% of the total plant N uptake, with decreasing soil N contribution as fertilizer N rate increased. Dourado-Neto et al. (2010) found that regardless of the ^{15}N source (organic such as cover crops, or inorganic fertilizers), the native soil N reminded the primary N source for all crops evaluated in their study [wheat (*Triticum aestivum* L spp. *aestivum*), sugarcane (*Saccharum officinarum* L.), corn, rice (*Oryza sativa* L.), sunflower (*Helianthus annuus* L.), peanuts (*Arachis hypogaea* L.) and beans (*Phaseolus aureus* Roxb.)], with an average value of 79% of the total plant N uptake at harvest for all crops. Gava et al. (2010), studying N rates impact on corn production in a tropical region of Brazil, found that the soil was responsible for 78% of the total N uptake by corn plants, while only 22% derived from the applied NBPT-treated urea fertilizer. We observed the same trend in our study (Fig. 3), corroborating that native soil N derived from N mineralization of the soil organic matter was the main N source for corn. Previous studies have demonstrated that soil N remains as the major source of N regardless of the soil and climatic conditions, with similar values for tropical and temperate regions (Stevens et al., 2005a; b; Dourado-Neto et al., 2010).

2.3.4. Nitrogen recovery efficiency

Nitrogen recovery efficiency values for the two blended N sources in this study at different GS are shown in Table 2.6. Nitrogen rates affected NBPTU and PSCU recovery values at all GS, except at the V4 GS for PSCU. In general, higher NRE was observed at lower N rates (Table 6), similar to the reported by Gava et al. (2010), who found decreasing NRE values with increasing fertilizer N rates. NBPT-treated urea resulted in higher overall NRE values compared to PSCU early in the season (at V4 corn GS). However, at later GS, we found the opposite and the PSCU showed higher NRE than NBPTU. At physiological maturity (R6), NRE was in average 36% for NBPTU and 51% for PSCU, showing a better performance of the controlled release N source, in agreement with other research (Zhao et al., 2013; Geng et al., 2016; Zheng et al., 2016; Guo et al., 2017). As stated by Shapiro et al. (2016), the use of CRN represent a risk reduction strategy by improving the synchronization between N release and crop needs, which might be especially important in areas with high susceptibility to N losses.

2.3.5. Implications for future research on ^{15}N-labeled enhanced efficiency N fertilizers

Further discussing the approach adopted and used in this study is highly important to appropriately understanding the results. While we believe it is an original approach and it provides very important and needed information about N recovery from the application of a combination of two fertilizer N sources, we are aware that an ideal experiment would use both mixed N-fertilizers (i.e. PSCU and NBPTU) ^{15}N-labeled, installing at least two micro-plots instead of one in order to have a more accurate measure of the fertilizer N recovery from PSCU and the N contribution from the NBPT-treated urea. Therefore, we would have data of N recovery from each of the blended N sources used. However, as pointed out by Chalk et al. (2015), ^{15}N-labeled slow or controlled release fertilizers for research purposes are still not commercially available, therefore they must be synthesized at small scale (i.e. laboratory conditions), with the possible outcome of presenting non identical physical and chemical characteristics of the industrial N fertilizer. This situation must be overcome first to allow us conduct field research and be able to estimate the real contribution and efficiency of CRN fertilizers, and especially when applied as a blend of N fertilizers.

Furthermore, the present study provides and represent a first step in the direction of better understanding NUE, N recovery and N dynamics when using controlled release N fertilizers by providing the (to our knowledge) first attempt to quantify the N contribution from two blended enhanced N fertilizers. A great amount of open questions remains to be answered in this subject. More laboratory and especially field research, adopting refined methods are needed. Currently, research is being conducted at the Laboratory of Stable

Isotopes, Center for Nuclear Energy in Agriculture, University of São Paulo with the objective of quantifying more accurately the improvement in N recovery when using stabilized, slow, and controlled release N fertilizers.

With the current growing public concern with the environmental consequences of agriculture and fertilizer management practices, it is imperative to develop more efficient fertilizers and fertilizer management practices. To assess the improvement on NUE and NRE with the use of enhanced efficiency N fertilizers, we need to develop methods and technology which could allow us to better quantify and determine these improvements. The use of ^{15}N enriched fertilizers have helped in the past to understand several N processes in the soil-plant-atmosphere system. Although implicates the need of research funding, it will help to increase the current knowledge and aid scientists, consultants, and farmers in the decision-making process when thinking about N fertilization. Clearly the decision will ultimately be based on the economy of the fertilization operation, but it is imperative to better understand the efficiency of these new technologies and in the different crop production systems, and for different crops.

Our findings show the agronomic benefits of blending a CRN source with a more rapidly available N source and performing a single fertilizer-N application at corn planting. Adopting this fertilizer management practice can secure N supply early in the season as well as good N supply later in the season. Results from our study showed that the timing of N release from a controlled release fertilizer (i.e. PSCU) is synchronized with periods of rapid plant N demand and growth. The approach we adopted in this study might be a useful tool to estimate the fate of fertilizer N under various cropping systems adopting slow/controlled release N fertilizers as an alternative to improve NUE and NRE.

2.4. CONCLUSIONS

A blend of PSCU and NBPT-treated urea applied at planting and incorporated into the soil was evaluated in this study as a strategy to provide N both early and late in the season, with the goal of estimating the source of the N found in the plant (i.e. $soil_N$, $NBPTU_N$ or $PSCU_N$) using the ^{15}N method integrated with the difference method. Our results indicate that the soil native pool was the main N source for the plant throughout the growing season. An average of 77, 54, 57 and 64% of the plant N was derived from the soil N pool at the V4, V12, R2 and R6 GS, respectively, with its contribution decreasing as fertilizer N application rate increased.

Evaluation of plant N at the V4 growth stage demonstrated that NBPT-treated urea provided on average 20% of the total plant N and only 3% was derived from PSCU. Later in the season, PSCU became the main fertilizer-N source for the plants. These results suggest that N release from PSCU fertilizer was gradual under field conditions and that blending this fertilizer with NBPT-treated urea can help to ensure enough N supply both early and late in the season. At harvest, an average of 64% of the total plant N uptake was derived from the soil, 26% was derived from PSCU, and 10% derived from NBPT-treated urea. The measured NRE was on average 36% for NBPT-treated urea and the estimated NRE for PSCU was 51%.

Our findings provide a unique perspective on fertilizer NRE and allocation of N in the different plant organs when a blend of two N sources is applied to the soil under field conditions. They also support the need for more research on the use of ^{15}N-labeled controlled release fertilizers to better understand the fate of N from enhacend efficiency N fertilizers in the soil-plant-atmosphere system.

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3. BLENDING POLYMER-SULFUR COATED AND NBPT-TREATED UREA TO IMPROVE GRAIN YIELD IN CONTRASTING CORN PRODUCTION SYSTEMS

ABSTRACT

Adopting enhanced efficiency nitrogen (N) fertilizers for corn production is a great management strategy for increasing N use efficiency and grain yield (GY) in different cropping systems. The objectives of this study were: i) to investigate the impact of N rates applied as a blend of polymer sulfur coated urea (PSCU) and NBPT-treated urea (NBPTU) at a 70:30 ratio at corn planting, compared to the application of regular urea, on corn grain yield in two different cropping systems; and ii) to evaluate the possiblity of reducing fertilizer N rates when applying a blend of two enhacend efficiency N fertilizer compared to the use of regular urea. Field experiments were conducted in two contrasting cropping systems: fallow-corn (FC) and sugarcane-corn (SC) successions, respectively, during the 2014/2015 and 2015/2016 growing seasons in São Paulo State, Brazil. Treatments consisted of the application of a blend of PSCU and NBPTU at 60, 120, 180, 240, and 300 kg N ha^{-1}, two treatments with the application of 180 kg N ha^{-1} of regular urea (one applied all at planting and the other split applied), and a control plot with no fertilizer N. Corn grain yield varied between sites, most likely due to climate and soil properties at each site. Nitrogen fertilizer application greatly impacted corn GY and N uptake at all sites. Yield and N uptake showed a quadratic response to N rates at all sites. The blend of PSCU and NBPTU, applied at corn planting and incorporated into the soil in order to prevent N losses, proved to be a great strategy to attain yields at N rates below those needed with regular urea.

Keywords: Enhanced efficiency fertilizer; Stabilized nitrogen fertilizer; Nitrogen use efficiency; Crop N uptake; Best fertilizer management practices

Abbreviations: PSCU, polymer-sulfur coated urea; NBPTU, regular urea treated with NBPT; NUE, nitrogen use efficiency; BP, biomass production; Nc, whole plant nitrogen concentration; NU, whole plant nitrogen uptake; GNc, grain nitrogen concentration; GNr, grain nitrogen removal; HI, harvest index; NHI, nitrogen harvest index; IUE, internal use efficiency

3.1. INTRODUCTION

To meet the growing world demand for food, feed, fiber, and fuel and do it so without endangering the environment, crop production must be doubled by 2050 (Godfray et al., 2010; Foley et al., 2011). This is known as 'sustainable intensification' (increase crop production minimizing environmental impacts), and can be addressed by closing crop ‘yield gaps’ (the difference between the potential yield and the observed yield), reducing food waste, moderating diets and reducing inefficiencies in resource use (Foley et al., 2011; Davis et al., 2016). Mueller et al. (2012) defend the case of closing yield gaps through nutrient and water management, with special emphasis on fertilizer nitrogen (N) management in corn, wheat, and rice.

Corn (*Zea mays* L.) is an important row crop in Brazilian agriculture. The national corn grain yield average in the 2016-2017 growing season was 5.6 Mg ha^{-1} (CONAB, 2018), although there are several studies reporting corn grain yield higher than 11 Mg ha^{-1} in Brazilian conditions (Schoninger, 2014; Vargas et al., 2015; Caires et al., 2016; Oliveira et al., 2018). Ray et al. (2012) classified Brazil as a region with fast yield improvement region. Therefore, even though a great yield gap exists in Brazilian corn production areas, there are also great opportunities for yield improvement, which can be attained through better management and agronomic practices (Meng et al., 2013).

Corn is a highly N dependent crop, therefore adequate fertilizer N management is important and required to obtain optimal yields without endangering the environment (Raun and Johnson, 1999; Zhang et al., 2015). Unfortunately, current low nitrogen use efficiency (NUE) values in cereal production do not meet the requirements for sustainable agriculture. Lassaletta et al. (2014) reported that only half (47%) of the total applied N in the world cropping systems is harvested as product. For Brazilian cereal production systems, however, Pires et al. (2015) reported that only 27% of the applied N is harvested. The key message to scientist, consultants, and farmers sent out by these low NUE values is that current fertilizer N management practices should be reevaluated and reassessed. Several alternative strategies to improve NUE are being currently proposed. To use the right fertilizer N sources, at the right rates, at the right placement, at the right time (IFA, 2009). All these factors need to be optimized in order to attain optimum corn grain yields, with economic profit and without causing any harms to the natural resources.

When thinking about fertilizer N sources, enhanced efficiency N fertilizers are today a reality, are available in the fertilizer market and gaining space rapidly. As pointed out by Guelfi (2017), enhanced efficiency fertilizer price depends on the technology level used for its production. Thus, their price increases as follows: regular N fertilizer < stabilized N fertilizer < blends (mixture of slow or controlled release N with regular N fertilizer) < slow release N fertilizer < controlled release N fertilizer (Guelfi, 2017). Nevertheless, enhanced efficiency fertilizer price has being constantly decreasing due to technology development, which allows the fertilizer industry to reduce costs.

The use of fertilizer N blends is becoming more popular in Brazilian agriculture (González-Villalba et al., 2014; Guelfi, 2017) and worldwide (Trenkel, 2010; Snyder, 2017), being the main reason an industrial cost production decrease, and therefore more accessible for farmers. An example of the integrated use of technology is the physical mixture of polymer-sulfur coated urea (PSCU) and NBPT-treated urea. The former is regular urea coated with a layer of elemental sulfur (S°) and a second layer of polymer as physical barriers to the release of N, while the latter is regular urea treated with an enzyme urease inhibitor known as NBPT. The NBPT-treated urea is highly soluble, less susceptible to N losses, and meet the immediate demand of N by the crop, while the PSCU provides a gradual N release, securing N availability for later growth stages (Payne et al., 2015; Guelfi, 2017; Guo et al., 2017; Snyder, 2017). Several studies around the globe have demonstrated the effectiveness of PSCU in improving grain yields (Halvorson and Bartolo, 2014; Geng et al., 2016; Shapiro et al., 2016; Zheng et al., 2016a), and the effectiveness of NBPT-treated urea to minimize N losses through ammonia volatilization (Soares et al., 2012; Cancellier et al., 2016; Engel et al., 2017; Rajkovich et al., 2017; Silva et al., 2017). Snyder (2017) discussed that optimal mixtures or combinations of controlled release N fertilizer with regular urea could allow N rates to be reduced by 25%. However, no such studies have been conducted in Brazilian corn production conditions.

González-Villalba (2014) reported a field study where blends of polymer-sulfur coated urea (PSCU) and conventional urea were studied, using blend ratios ranging from 100 to 50% of the controlled release source, applying the total N rate at planting and without further side-dress N application. These results were evaluated and compared to the commonly recommended N fertilization strategy, which consists on applying approximately 20% of the N rate at planting and the remaining of the total N rate at V4-V6 corn growth stage (Cantarella et al., 1996). The conclusion of the study was that applying a blend of regular urea and controlled release urea (polymer-sulfur coated urea) at corn planting can be considered an environmental friendly strategy that supplies N as the crop demands it, which was demonstrated with the monitoring of plant available N content (NH_4^+ + NO_3^-) throughout the cycle, finally leading to greater corn grain yield under soil and climate favorable conditions (i.e. N-responsive site).

Numerous management factors can influence corn response to fertilizer N, but discovering the right amount of N required for optimum yield is critical to maximizing net return and crop recovery of applied N (Dobermann, 2007). As pointed out by Halvorson and Bartolo (2014), being able to reduce the N rate while maintaining crop yields and economic returns would benefit the environment.

Among fertilizer N management factors that can help improving NUE, Shapiro et al., (2016) and Rosolem et al. (2017) affirm that maintaining N in the root zone is crucial for greater yield and N uptake. Incorporating fertilizer N into the soil certainly reduce significantly the amount of N losses through NH_3 volatilization, keeping more N available for plant roots to take up. However, it fails to, per se, prevent N losses via N leaching. By gradually releasing N, controlled release fertilizers represent a good strategy to prevent such losses, ensuring N retention in the root zone. Incorporating N fertilizers into the soil has been known as a great strategy to prevent N losses, especially through ammonia volatilization (Rochette et al., 2013) and nitrous oxide emission (Kessel et al., 2013). This management practice it is also known as a great avenue for improving NUE and crop yields (Trivelin et al., 2002; Vitti et al., 2007; Amado et al., 2013).

Information is needed on how the application of a blend of PSCU and NBPT-treated urea, applied at planting, incorporated into the soil, and without further side-dress N might impact corn yield in Brazilian production systems. In this context, questions remain open about how the application of this enhanced efficiency N fertilizer (N source), incorporated (N placement), and applied at planting (N timing) could impact corn grain yield. Another question is if N rate could be reduced when using these fertilizer, compared to regular urea, as has been suggested by several studies performed worldwide (Halvorson and Bartolo, 2014; Hatfield and Parkin, 2014; Shapiro et al., 2016; Zheng et al., 2016b; Guo et al., 2017).

In this context, the main hypothesis of this study was: i) the application of a blend of fertilizer N (PSCU and NBPTU at a 70:30 ratio) application incorporated into the soil at corn planting could save split N (as urea) application and result in higher grain yield.

The objectives of this study were to i) evaluate corn grain yield response to increasing N rates when applying a blend of PSCU and NBPTU (70:30 ratio) incorporated at planting, compared to the application of regular urea in two contrasting cropping systems; ii) determine if fertilizer N rate could be reduced when applying a blend of enhanced efficiency N fertilizers, compared to the application of regular urea.

3.2. MATERIAL AND METHODS

3.2.1. Site description

The study was conducted during the 2014/15 and 2015/16 growing seasons in two contrasting cropping systems: fallow-corn (FC) and sugarcane-corn (SC) successions, both under rainfed conditions. The FC experiment was located at Iracemápolis, São Paulo, Brazil (22°38’5’’S, 47°30’14’’W; 608 m altitude) on a clay textured soil (Soil Survey, 2014) during both growing seasons (Site 1 and 2, respectively). The climate of this site correspond to Cfa (hot summer) transitioning to Cwa (dry winter and hot summer), according to the Köppen classification map for Brazil elaborated by Alvares et al. (2013). The area in this cropping system was planted with sweet orange (*Citrus sinensis* L.) for approximately 20 years before being converted to a no-tillage area a few years prior to the installment of the experiment. There is no documentation of by-products application in these areas, and fertilizer application started at the time of conversion to no-tillage. At this cropping system area, corn planting was performed after desiccation of the vegetation with herbicides, with no soil disturbance. Black oat (*Avena strigosa* Schreb) was planted during the winter as a cover crop, however without good development as a consequence of the environmental conditions and limited rainfall during this period. Thus, at the time of corn planting, the amount of black oat residues left in the area was minimum.

The SC study was located at Severinia, São Paulo, Brazil (20º43’34’’S, 48º41’52’’ W; 605 m altitude) during the 2014/15 growing season (Site 3) and at Altair, São Paulo, Brazil (20º27’15’’S, 49º02’05’’W; 537 m) during the 2015/16 growing season (Site 4). The climate of these sites correspond to Aw (tropical zone with dry winter) according to Alvares et al. (2013). Sites 3 and 4 (SC) were planted with citrus for a long time before being converted to mechanized unburnt mechanized sugarcane (*Saccharum* spp.) cropping system (harvested without fire) for approximately 15 years. This cropping system represented a challenge because of the difficulty to incorporate the N fertilizer in an area with the soil covered with a high amount of sugarcane residue. A quantification of the residual biomass was performed before the experiment installment. The residual sugarcane straw the first year was 16.5 Mg ha^{-1} and the second year 18 Mg ha^{-1} (average of 5 random samples in a 1 m^2 area). However, after several “pre-trials” installation, we were able to incorporate the N fertilizer with no much inconvenience. It is also of interest that at Site 4 (Altair 2015/2016), concentrated vinasse, a byproduct of the sugarcane industry, rich in potassium content (Otto et al., 2017) application is common. At these SC sites, 10-15% of the total area used for sugarcane production is planted with corn every year, taking advantage of the sugarcane renovation operation. All grain produced in these areas is destined to beef cattle alimentation in a modern feed-lot system.

3.2.2. Soil sampling for characterization of the experimental sites

Soil samples were collected at all sites prior to corn planting to characterize the soil chemical and physical properties by collecting one composite sample for each site at 0-20, 20-40 and 40-60 cm sampling depth. Analysis of soil samples was performed using methods outlined by Raij et al. (2001). Samples were analyzed for pH using $CaCl_2$ 0.01 mol L^{-1}, ratio of 1:2.5 for soil and solution - m/v. Organic matter (O.M.) was determined by potassium dichromate titration. Nutrients phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were extracted using ion-exchange resin and determined by colorimetry (P), by flame spectroscopy (K), and atomic absorption (Ca and Mg). To determine soil potential acidity (H+Al), the SMP buffer solution method was adopted (Shoemaker et al., 1961), and cation exchange capacity (CEC) at pH 7.0 was calculated as the sum of the exchangeable cations (K^+, Ca^{2+}, and Mg^{2+}) and soil potential acidity.

Table 3.1. Selected chemical and physical soil properties, sampled at the 0-60 cm depth, in 20 cm increments at four year/sites.

Depth	pH	O.M.	P	K	Ca	Mg	H+Al	BS	CEC	V	Sand	Silt	Clay
cm		g dm^{-3}	mg dm^{-3}	- - - - - - - - - - mmol$_c$ dm^{-3} - - - - - - - - - -						%	- - - - g kg^{-1} - - - -		
				Iracemápolis 2014-2015 (Fallow-Corn, Site 1)									
0-20	5.3	25	19	3	42	18	34	63	97	65	327	153	520
20-40	4.6	17	9	1	13	8	50	22	72	31	300	149	550
40-60	4.5	15	2	1	7	4	46	12	58	21	300	114	586
				Iracemápolis 2015-2016 (Fallow-Corn, Site 2)									
0-20	5.7	23	21	2.3	28	10	20	40	60	67	347	185	469
20-40	4.8	18	10	1	13	8	50	22	72	31	300	149	550
40-60	4.5	16	2	1	7	4	46	12	58	21	300	114	586
				Severinia 2014-2015 (Sugarcane-Corn, Site 3)									
0-20	4.8	16	5	0,9	5	3	22	9	31	29	751	26	223
20-40	4.2	12	3	1,3	3	2	28	6	34	18	715	11	273
40-60	4.3	9	2	0,8	4	2	20	7	27	25	692	12	296
				Altair 2015-2016 (Sugarcane-Corn, Site 4)									
0-20	5.6	13	42	7.7	22	6	13	36	49	73	845	17	138
20-40	6.0	9	5	6.3	15	6	15	27	42	65	807	17	176
40-60	5.4	6	4	4.8	16	7	18	28	46	61	755	19	226

Base saturation was calculated by dividing the summation of exchangeable cations by the CEC and multiplied by 100. Soil texture (sand, silt and clay content) was determined by the hydrometer method (Bouyoucos, 1962). Selected soil characteristics are portrayed in Table 3.1. The two cropping systems present contrasting soil properties. In general, the soil at the FC sites show a higher clay content, O.M. content, and CEC, thus presenting higher natural fertility. High K content is observed at Site 4, which can be explained by the application of concentrated vinasse for sugarcane production.

3.2.3. Experimental design and treatments

The experiments were arranged in a complete randomized block design with four replications. Treatments consisted of N rates (0, 60, 120, 180, 240, and 300 kg N ha^{-1}) using a blend of polymer-sulfur coated urea (PSCU) and NBPT-treated regular urea (NBPTU) at a 70:30 ratio, and two treatments with 180 kg N ha^{-1} of regular urea. A control plot with no N was also included. All fertilizer N was band applied and incorporated at planting, except for one of the 180 kg N ha^{-1} of regular urea treatments (180 U-SA), where fertilizer N was split applied (20% of the total N rate applied at planting and the remaining applied when the corn plants were at the V4 growth stage), as recommended by (Cantarella et al., 1996).

Treatment details and fertilizer N application methods and timing are displayed in Table 3.2. The N sources used in the blend were NBPT-coated urea [(46-0-0), (N, P_2O_5, K_2O)], and Producote® (Patent No. EP 0574541 B1), described as regular urea coated with a layer of elemental sulfur (S°) and a biodegradable insoluble in water polymer layer [(39-0-0-11), (N-P_2O_5-K_2O-S)]. The commercial name of this fertilizer is Polyblen® and is currently being produced and commercialized by Produquimica Ind. e Com. - Compass Minerals. As Guelfi (2017) pointed out, the NBPT-treated urea is highly soluble, less susceptible to N losses, and meet the immediate demand of N by the crop, while the PSCU provides a gradual N release, ensuring N availability for later growth stages.

3.2.4. Experiment setup and crop management

Three months before the installment of the experiment, soil test was performed to characterize the area and plan for lime application, which application was completed at both cropping systems and growing seasons two months prior to corn planting, attempting to raise base saturation (BS) to 70%.

Table 3.2. Treatment details, N rate (kg N ha^{-1}), time and method of application, depth of fertilizer incorporation and distance of application from the corn row.

Treatment†	Time of application	Application method	Depth	Distance
			- - - - - - - - cm	- - - - - - - -
Control	-	-	-	-
60 Blend	Planting	Incorporated	7	10
120 Blend	Planting	Incorporated	7	10
180 Blend	Planting	Incorporated	7	10
240 Blend	Planting	Incorporated	7	10
300 Blend	Planting	Incorporated	7	10
180 U-P	Planting	Incorporated	7	10
180 U-SA	Planting + V4	Surface	-	20

† Numbers = N rate (kg N ha^{-1}). Blend = polymer-sulfur coated urea (39 % N) mixed with regular urea (45 % N) at a 70:30 ratio. U–P = regular urea applied at all planting. U–SA = regular urea split applied, 20 % of the rate at planting (incorporated) and 80 % at V4 corn growth stage (surface applied).

Phosphorus fertilizer single superphosphate (120 kg P_2O_5 ha^{-1}) was applied at planting, using an automated planter. Potassium chloride (120 kg K_2O ha^{-1}) was surface broadcast applied days before corn planting. Nitrogen fertilizer application was different at the two cropping systems. At the FC sites, N fertilizers were band applied by hand after planting. Row furrows were opened alongside the planting row using a hand hoe, at an approximately distance of 10 cm, 7 cm deep. The N fertilizers were applied uniformly to the eight rows and then the furrows were closed again with the hand hoe. At the SC sites, on the other hand, N fertilizer application was performed using a GPS driven mechanized-automated tractor plus planter. The tractor did a first pass applying P fertilizer and planting, and then a second pass applying the N fertilizer, which was placed at a 10 cm offset from the corn row and placed approximately 7 cm deep. This operation was performed several times in “pre-trials” the first year of the experiment to calibrate the N fertilizer application since the distance from the row and depth of N fertilizer placement were not a common operation for the tractor drivers/operators. Once the tractor and planter were adequately calibrated, the application and experiment setup went smoothly. To avoid areas reflecting the N rates change between plots, after plants germination a 3 m alley was cleared out between plots in the same block. The fertilizer N application rate is changed between plots by varying the number of revolutions of the auger in relation to ground speed. A calibration of the N rates was performed before the installment of each experiment and before changing the fertilizer N (i.e. PSCU or regular urea).

Planting and harvest dates were Dec 3, 2014, and May 8, 2015 (Site 1); Dec 22, 2015, and May 4, 2016 (Site 2); Dec 20, 2014, and Apr 23, 2015 (Site 3); and Nov 19, 2015, and Apr 15, 2016 (Site 4). The cultivar used at all the experimental sites was Dekalb® 390 VT PRO 2™, which includes the YieldGard VT PRO™ technology which controls *Spodoptera frugiperda*, *Helicoverpa zea*, and *Diatraea saccharallis*, and also includes the RoundupReady® technology, which provides resistance to glyphosate. Pest and disease management were performed according to appearance of any problems and following the specialist technicians recommendation.

At the FC sites, plots consisted of 8 corn rows by 12 m long, planted at 50 cm row spacing at 70,000 plants ha^{-1} population density. At the SC sites, plots consisted of 6 corn rows by 20 m long, planted at 70 cm row spacing at 64,000 plants ha^{-1} population density, following directions provided by the seed company technician for each region.

3.2.5. Sampling and analysis

At harvest (black layer, R6 corn growth stage) the aboveground part of four plants from each plot were clipped at ground level and separated into leaves, stalk, cob, and grain (depending on the growth stage the plants were at). All plant material was oven dried at 65°C for 72 h, weighed to determine the above ground biomass production (BP), and ground using a Willey mill. A subsample was then prepared for N content (Nc) analysis by micro Kjeldhal digestion (Raij et al., 2001). Total nitrogen uptake (NU) was calculated multiplying BP by N concentration of each plant part. The results reported here represent the sum of all plant parts. For grain yield (GY) determination at the FC sites (Sites 1 and 2), corn ears in the central 5 m of the two center rows of the plots were hand harvested. On the other hand, at the SC sites (Sites 3 and 4), GY was obtained through the harvest of the two central rows with an adapted combine with a precision weighing system. The first year of the experiment, to ensure there was no big differences between the hand and combine harvested GY, several small areas were hand harvested around the experimental area, weighed and subsamples taken for moisture determination, and then all the hand-harvested corn ears passed through the combine to compare the results, which were similar.

The hand-harvested ears were threshed for grain separation, and then yield was estimated by weighing the grains and expressed at 13% moisture basis. Grain N concentration

(GNc) was determined in the four plants sampled and then multiplied by the actual GY to obtain grain nitrogen removal (GNr) values. Harvest index (HI) was calculated as the ratio of grain weight (GY) to total plant weight (BP) (Sinclair, 1998). Nitrogen harvest index (NHI) was calculated as the ratio between GNU and total plant nitrogen uptake (NU) (Fageria, 2014), and internal use efficiency (IUE) was estimated as GY per unit of NU (Sadras, 2006). To understand the relationship between NU and BP, GY and NU, and GY and BP, the data of all 4 sites was pooled and plotted into graphs.

3.2.6. Statistical analysis

The response variables were analyzed using the PROC GLIMMIX procedure in SAS® software (SAS Institute Inc, Cary, NC). The main effects of the fertilizer N treatments and site were included as fixed factor and blocks as random factor in the model. Corrected denominator degrees of freedom were obtained using the Kenward-Roger adjustment. To assess the significance of differences between means we used the LSMEANS and SLICE option in PROC GLIMMIX, which provides a general mechanism for performing a partitioned analysis of the LSMEANS for a specific interaction (i.e. analysis of simple effects). An across site analysis (using site as a random factor) was also included in the analysis to allow broader generalizations of the results. Treatment effects were considered significantly different at the $P \leq 0.10$ probability level, including confidence interval estimations. For grain yield and nitrogen uptake, regression analysis (linear, quadratic, and cubic) were performed using the curves fitting option in Sigmaplot®, selecting the one with higher coefficient of determination (R^2).Then, the blend replacement value of the 180 kg ha^{-1} of regular urea (U-P and U-SA) value was calculated and plotted into the blend N rate curves. This was performed using the same approach as Mahama et al. (2016), using the intercept and the slope values of the curve, in order to determine the blend replacement value. To understand the relationship between plant nitrogen uptake and plant biomass, grain yield and plant N uptake, and grain yield and plant biomass, regression analysis (linear, quadratic, and cubic) were performed using the curves fitting option in Sigmaplot®, and selecting the one with higher coefficient of determination (R^2).

3.3. RESULTS AND DISCUSSION

3.3.1. Weather conditions

Growing season conditions varied among site-years (Table 3.3), impacting corn grain yield in different ways. There was no severe water limitation for crop development apart from April at the FC sites, where only 6 mm was observed both years, while the 5-yr average is 87 mm. At this point, corn plants were already in the reproductive stages, with possible consequences on the final yield due to water deficiency (Çakir, 2004). Comparing between sites, the first year of the experiment the FC received more rainfall than SC, while the second year the opposite was documented. Comparing each site-year with the 5-yr rainfall precipitation trend, the 2014/2015 growing season at the FC registered similar amount of rainfall, while during the 2015/2016 received below average amount of water. At the SC sites occurred the opposite to FC. In the 2014/2015 growing season the amount of rainfall was 122 mm below average, while the second growing season rainfall was 118 mm above the average 5-yr trend. The average temperature during the growing season at all site-years followed the documented 5-yr trend.

3.3.2. Corn grain yield

3.3.2.1. Among sites

The average grain yield observed in this study at all four sites are higher than Brazil's average corn grain yield, which is 5.6 Mg ha^{-1} (CONAB, 2018).

Corn GY was impacted by both site and fertilizer N application (Table 3.4). There was no interaction between the two fixed effects regarding GY. Average yield comparisons by site are portrayed in Table 3.5. Site 1 showed the highest yield (9.6 Mg ha^{-1}) while Site 3 showed the lowest corn GY (both during 2014/2015 growing season). The two cropping systems during the 2015/2016 growing season resulted in similar average GY, probably as a consequence of the higher rainfall precipitation documented at the SC site compared to the first growing season (Table 3.3). The average GY difference between the FC sites (2 Mg ha^{-1} higher in the first year) were also likely due to the higher water availability observed in the first season.

Crop yield variability between sites and years is common in agriculture. The environmental influence on crop yield is mainly dependent on soil properties and climate behavior on a specific year (i.e. biophysical attributes) (Hatfield and Walthall, 2015; Edreira et al., 2017). Climate varies greatly from year to year, which causes considerably large differences in yield potential (Cassman et al., 2002). The FC sites presented higher soil fertility (e.g. higher C.E.C., O.M. content, etc.) (Table 3.1), thus greater GY were expected in comparison to the SC sites. However, as mentioned before, the lower rainfall precipitation recorded in the second year of the experiment apparently limited the overall grain yield at Site 2. At these two sites, the amount of remaining straw from the prior crop was negligible, thus, no much N immobilization would be expected.

Grain yield at Sites 3 and 4 (SC cropping system) were 6.4 Mg ha^{-1} and 7.4 Mg ha^{-1}. The higher GY at Site 4 could be related to the higher rainfall precipitation observed and the higher soil fertility properties of Site 4 (2015/16). At these two sites, corn was planted after the desiccation of sugarcane. The dry weight of straw left in the soil surface was 16.5 Mg ha^{-1} at Site 3 and 18 Mg ha^{-1} at Site 4, which is in agreement with the numbers reported in the literature (Trivelin et al., 2013; Vieira-Megda et al., 2015; Leite et al., 2016; Mariano et al., 2016). Sugarcane straw presents a high C:N ratio of 100:1 (Fortes et al., 2012), which results in high N immobilization rates (Mariano et al., 2013), due to increased microbiological activity caused by the energy input into the soil, which is considered to be the main reason behind the low NUE values in sugarcane cropping systems.

An interesting shift in the regular urea treatments yield trend was observed at Site 3. Interestingly, the U-P treatment (regular urea applied at planting, incorporated) yielded 840 kg ha^{-1} more grain than the split applied urea treatment (U-SA). Several factors could have triggered this effect, but most likely the U-SA treatment suffered higher N losses and N immobilization, thus causing low plant available N content. However, this was not consistent across sites.

When corn is planted in a sugarcane straw covered soil, it is highly probable that high N immobilization rates will occur. Furthermore, when fertilizer N is applied on the soil surface, soil microbial community will consume all the plant available N (NO_3^- + NH_4^+), causing N deficiency in corn plants. There is also a high risk of N losses through NH_3 volatilization when fertilizer N is applied on the soil surface (Lara Cabezas et al., 2000; Trivelin et al., 2002; Lange et al., 2008; Mariano et al., 2012; Leguizamón Rojas et al., 2012; Rochette et al., 2013). It has been known that fertilizer N incorporation into the soil improves N use efficiency by preventing N losses, especially through NH_3 volatilization, and increasing crop yield (Trivelin et al., 2002; Cabezas et al., 2005; Amado et al., 2013; Rochette et al., 2013). Therefore, the GY attained at the SC (Table 3.5) are probably higher than what it would be if fertilizer N was applied on the soil surface. The results obtained in this study reinforce the importance of incorporating fertilizer N and using enhanced efficiency N fertilizer whenever it is possible, and especially in cropping systems with high susceptibility to N losses.

Table 3.3. Total monthly rainfall precipitation and average temperature for each studied site-year and respective 5-yr average (2010-2014).

Site / Year†	Dec.	Jan.	Feb.	Mar.	April	May	Total
			Monthly rainfall precipitation (mm)‡				
FC 2014/2015	255	109	357	105	6	105	937
FC 2015/2016	102	303	154	169	6	118	852
FC 5-year	194	244	202	136	87	73	936
SC 2014/2015	279	96	108	258	53	59	853
SC 2015/2016	316	371	145	143	16	102	1093
SC 5-year	234	232	172	215	66	56	975
			Monthly mean temperature (C)				
	Dec.	Jan.	Feb.	Mar.	April	May	Average
FC 2014/2015	26.5	27.7	26.5	25.0	23.6	20.6	25.0
FC 2015/2016	27.1	26.6	26.2	25.0	24.6	19.5	24.8
FC 5-year	26.0	25.7	26.3	24.8	23.2	19.9	24.3
SC 2014/2015	25.3	27.2	25.3	24.3	24.0	21.0	24.5
SC 2015/2016	25.6	25.2	25.9	25.6	25.1	21.0	24.7
SC 5-year	26.1	25.9	26.6	25.5	24.1	20,8	24.8

† FC= fallow-corn cropping system; SC= sugarcane-corn cropping system.
‡ Climate information was gathered by automated meteorology stations located at each site-year.

Table 3.4. Significance of the F values for the fixed effects of the study sites and N treatments on grain yield (GY), biomass production (BP), whole plant nitrogen concentration (Nc), whole plant nitrogen uptake (NU), grain nitrogen concentration (GNc), grain nitrogen removal (GNr), harvest index (HI), nitrogen harvest index (NHI) and internal use efficiency (IUE).

Fixed Effect	GY	BP	Nc	NU	GNc	GNr	HI	NHI	IUE
					P>F				
Site (S)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Treatment (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.120	0.148	0.134
S × T	0.270	0.635	0.037	0.781	0.012	0.141	0.557	0.511	0.915

Table 3.5. Corn grain yield (GY), biomass production (BP), whole plant nitrogen concentration (Nc), whole plant nitrogen uptake (NU), grain nitrogen concentration (GNc), grain nitrogen removal (GNr), harvest index (HI), nitrogen harvest index (NHI) and internal use efficiency (IUE) at four sites.

Site†	GY	BP	Nc	NU	GNc	GNr	HI	NHI	IUE
	Mg ha^{-1}	Mg ha^{-1}	g kg^{-1}	kg ha^{-1}	g kg^{-1}	kg ha^{-1}			
Site 1 (FC)	9.6 a‡	18.2 a	9.7 b	177 b	12.4 c	118 a	53 a	66 a	54 a
Site 2 (FC)	7.6 b	14.7 c	11.0 a	164 c	14.2 a	107 b	52 a	65 a	47 b
Site 3 (SC)	6.4 c	17.2 b	8.4 c	145 c	13.6 b	88 c	37 c	60 b	44 b
Site 4 (SC)	7.4 b	17.3 b	10.7 a	187 a	14.6 a	108 b	43 b	57 b	40 c

† FC, Fallow-Corn; SC, sugarcane-corn.

‡ Means followed by different letters in the column indicate differences (ANOVA) at $P < 0.1$ between sites.

3.3.2.2. Within sites

Fertilizer N application positively impacted GY (Table 3.4). A great yield response to fertilizer N application was documented at all sites (Fig. 3.1). Corn GY varied from 6.0 to 11.5, 5.8 to 8.7, 3.7 to 7.6, and 5.0 to 8.7 Mg ha^{-1} at Site 1, 2, 3, and 4, respectively, representing 92, 50, 105, and 74 % GY increase in N fertilized plots in relation to the control plot, with the N rate at which the highest GY was attained also varying between sites. A quadratic response of corn GY to N rate (applied as blend of PSCU and U) was documented at all sites, showing an initial rapid increase as N rates escalate, getting to a peak GY response and then slightly declining again.

Corn is a highly responsive crop to fertilizer N (Ciampitti and Vyn, 2013; DeBruin et al., 2017), as it was demonstrated with the results. However, there are also some studies performed under Brazilian conditions showing that sometimes, no response to fertilizer N is observed (Goes et al., 2012; González-Villalba, 2014; Schoninger, 2014; Garcia et al., 2018), depending mainly on soil properties and climate behavior.

3.3.2.3. Can we reduce N rate when applying a blend of polymer-sulfur coated urea and NBPT-treated urea?

Understanding that urea is the most widely used fertilizer N in Brazil and the N rate recommended to attain high corn GY (10-12 Mg ha^{-1}) is 180 kg ha^{-1} (Cantarella et al., 1996), we used urea at a 180 kg N ha^{-1} total N rate to calculate the blend (PSCU + NBPTU) replacement value of regular urea, so that we could assess the feasibility of reducing N rate when using an enhanced N fertilizer such as PSCU combined with NBPT-treated urea.

Corn grain yield at the same N rate (180 kg ha^{-1}) was higher with the application of the enhanced efficiency N fertilizer compared to the two regular urea treatments evaluated in this study (U-P and U–SA) at all sites (Fig. 3.1.). The replacement value of the blend over the U-P and U-SA regular urea treatments were of 83 and 89, 20 and 75, 104 and 66, and 79 and 110 kg N ha^{-1}, at sites 1, 2, 3, and 4, respectively. These results suggest that the application of N in the form of blend of PSCU and NBPTU (70:30 ratio) could help in reducing N rate significantly while yielding the same as U-P and U-SA.

Several studies have demonstrated and suggested that a certain GY could be attained using enhanced efficiency N fertilizers at N rates below those needed with regular urea. For example, Halvorson and Bartolo (2014) found no difference in corn GY between fertilization with regular urea and stabilized urea (Super U, which is urea treated with the enzyme urease inhibitor NBPT) in a three year N rates and sources study. On the other hand, they found higher GY (approximately 1 Mg ha^{-1} higher) when applying polymer-coated urea than when applying regular urea and NBPT-treated urea. They suggest that N rate should be reduced when using polymer coated urea in relation to regular urea or stabilized urea. Zhao et al. (2013) found that the use of different enhanced efficiency N fertilizers improved summer corn GY in average by 12%, increasing also NUE in compared to regular urea. The authors attributed the increased GY and NUE under enhanced efficiency N fertilizer to higher photosynthetic rate and lower ammonia volatilization compared to the regular urea treatment.

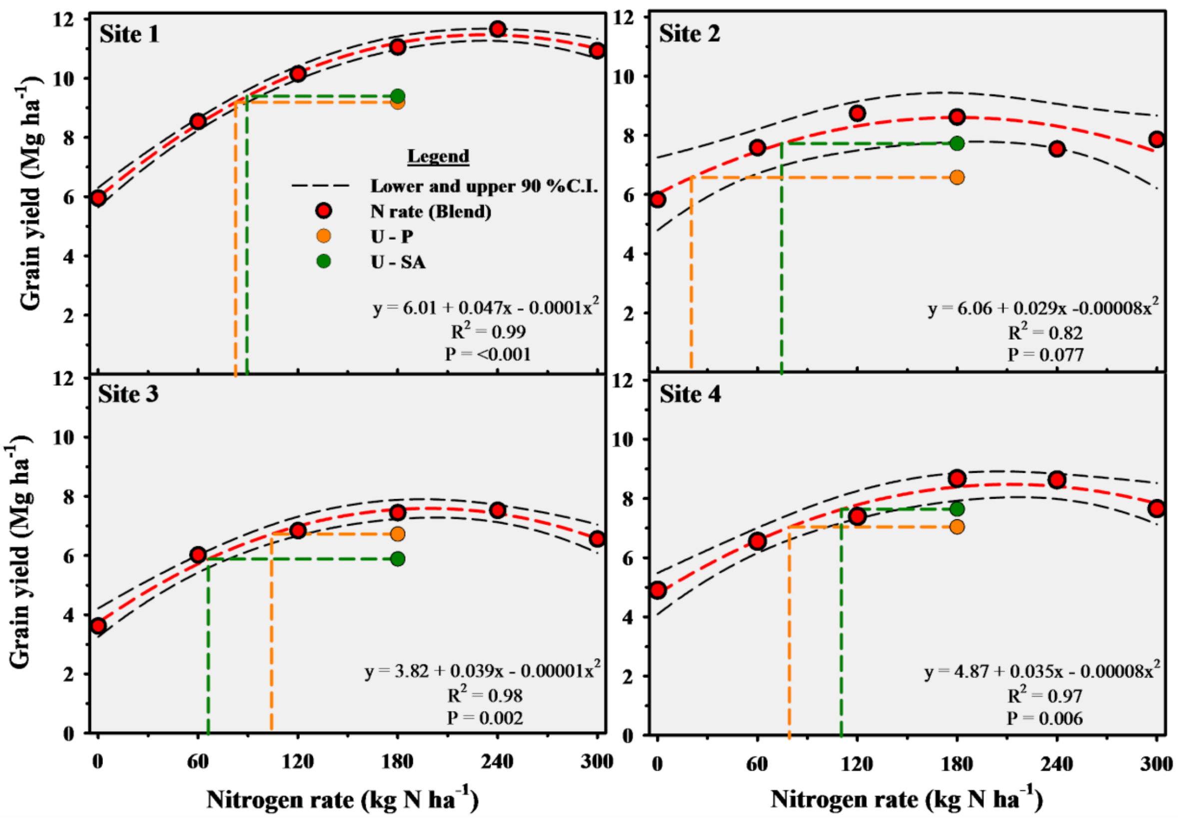

Figure 3.1. Corn grain yield response to nitrogen rates of a blend of polymer-sulfur coated urea and NBPT-treated urea at a 70:30 ratio, and blend replacement value of 180 kg N ha^{-1} as urea applied at planting (U-P) and split applied (U-SA) at four experimental sites.

Not all reports are positive for enhanced efficiency N fertilizer though. There are reports in the literature where no benefit (higher yield) was documented when applying enhanced efficiency N fertilizers compared to the use of conventional N fertilizers (i.e. regular urea). For example, Rubin et al. (2016) found that a split-urea application yielded 5% more and increased agronomic efficiency by 12% relative to single pre-plant application of enhanced-efficiency fertilizers in an irrigated area in upper USA Midwest. Grant et al. (2012) found that under growing conditions across a wide range of Canada’s ecoregions, the use of controlled release urea or split application do not appear to provide consistent improvement in crop yield and NUE as compared to standard regional timing and placement of regular urea. In the Brazilian southeast, Cancellier et al. (2016) found that the use of NBPTU reduced NH_3 volatilization by 18%, and PSCU reduced NH_3 losses by 37% when compared to regular urea. However, neither fertilizer N increased corn grain yield, N accumulation, or NUE compared to the use of regular urea. In the Brazilian south, Mota et al. (2015) found no increase in corn grain yield or NUE when treating regular urea with urease or nitrification inhibitors, related to the use of regular urea or ammonium nitrate.

The results of this study are beyond interesting. Several studies have demonstrated higher crop yields and NUE with the application of enhanced efficiency N fertilizers compared to conventional N fertilizer (Zhao et al., 2013; Halvorson and Bartolo, 2014; Geng et al., 2016; Shapiro et al., 2016; Zheng et al., 2016a). In particular, there are reports showing that PSCU, and PSCU blended with regular urea (Zheng et al., 2016b, 2017; Guo et al., 2017) provide higher grain yields and increase profits by reducing the fertilizer cost using mixtures or blends. However, there is no much information on the impacts in crop yield of mixtures of two enhanced efficiency N fertilizer in Brazilian conditions. As mentioned before, NBPT-treated urea is highly soluble, and prevent N losses, but ensures N availability to meet the early N demand by crops, and PSCU presents gradual N release, securing N provision at later growth stages (Guelfi, 2017; Silva et al., 2017; Snyder, 2017). In addition to these enhanced characteristics, the fertilizer N used in this study was incorporated into the soil, a management practice known as a great strategy to prevent N losses (Trivelin et al., 2002; Rochette et al., 2013), and as consequence, increase NUE and crop yields. Therefore, the N source used in this study and the placement (incorporated band) adopted served the purpose of minimizing N losses (through volatilization, leaching, etc.) and improve corn grain yield.

Otto et al. (2016) discusses that as long as N fertilizers are not subject to N losses through ammonia volatilization, there is no need to incorporate them. However, it is well known that N losses through NH_3 volatilization represent a great N sink and will continue to be so, and the agriculture sector will continue to struggle on improving NUE while it does not minimize N losses. The bottleneck regarding fertilizer N incorporation into the soil has always been the right equipment to perform the incorporation, especially in no-tillage systems with high amount of plants residue on the soil surface. With current modern agriculture technology and innovation (GPS guided high power tractors, high precision tractor implements, and innovations, high precision planters with in-furrow fertilizer application, etc.), however, to incorporate fertilizer N is feasible. One possible issue with this management strategy is that the planter will have to make two passes on the same field, one pass to apply P and K fertilizers together with the seeds, and another pass to apply the N fertilizers. This operation, however, may be an issue in some farms where the window for planting is small due to climatic conditions, and where planting should be done as fast as possible.

It is important to highlight, however, that the solely incorporation of N fertilizer does not ensure higher yields and/or NUE. Regular urea was incorporated in the U-P treatment, which yielded less (except at Site 3) than the U-SA treatment, where 20% of the total N rate was applied incorporated at planting and the remaining was surface applied at V4 corn growth stage. Our data suggest that to obtain higher corn grain yield the N source adopted is of great importance, and the solely fact of incorporating regular urea is not enough. It should be integrated to a higher efficiency N fertilizer.

An across site analysis was performed to allow broader generalization of the results over different cropping systems and rainfall precipitation regime. Our data suggest that the use of a blend of two enhanced efficiency N fertilizer (PSCU + NBPTU) demonstrated to be a more effective strategy compared to the use of regular urea. The same yield level obtained with the use of 180 kg N ha^{-1} of split applied regular urea (U-SA) could be attained applying only 85 kg N ha^{-1} of the blend of PSCU and NBPTU (47% N rate reduction), as portrayed in Fig. 3.2. For the U-P treatment, however, the same yield level achieved with 180 kg ha^{-1} of regular urea incorporated all at planting could be reached with 74 kg N ha^{-1} of the blend of PSCU and NBPTU.

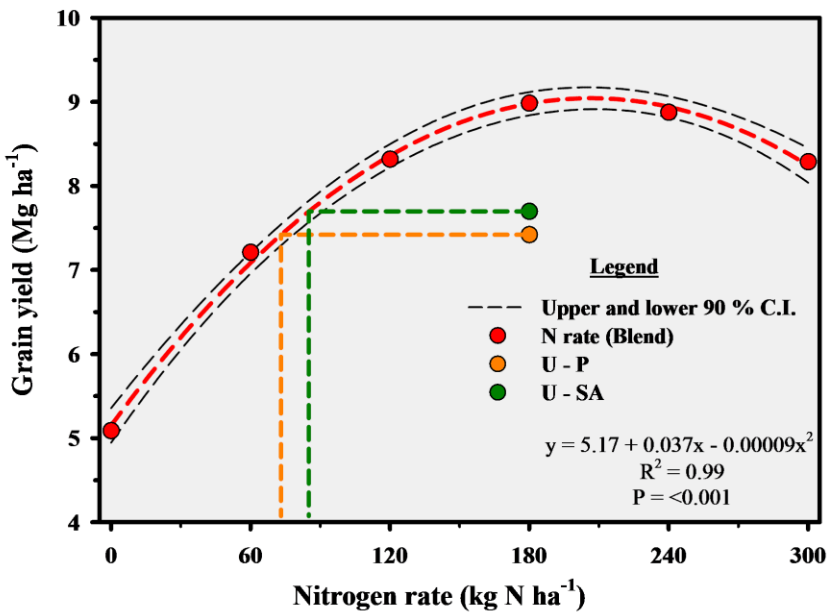

Figure 3.2. Corn grain yield as affected by nitrogen rates of a blend of polymer-sulfur coated urea and NBPT-treated urea at a 70:30 ratio, and blend replacement value of 180 kg N ha^{-1} as urea applied at planting (U-P) and split applied (U-SA) across four experimental sites.

3.3.3. Nitrogen uptake

Whole plant N uptake (NU) was affected by site and N fertilizer application (Table 3.4). Average whole plant NU varied among sites (Table 3.5), with the highest total plant NU at Site 4 with 187 kg N ha^{-1}, intermediate NU at Site 1 with 177 kg N ha^{-1}, and lower (and similar between them) NU at Sites 2 and 3 with 164 and 145 kg N ha^{-1}, respectively. To produce 1 Mg of grain, it was necessary for the corn plants to acccumulate 18, 22, 23, and 25 kg N at Sites 1, 2, 3, and 4, respectively. Sangoi et al. (2001) estimated that generally, corn plants must accumulate between 20 and 25 kg N to produce 1 Mg of grain.

Nitrogen uptake response to increasing N rates applied as a blend (PSCU + NBPTU) is portrayed in Fig. 3.3, with a quadratic response at all sites evaluated. Plant N uptake varied from 118 to 222 kg N ha^{-1} at Site 1, from 124 to 192 kg N ha^{-1} at Site 2, from 80 to 175 kg N ha^{-1} at Site 3, and from 124 to 223 kg N ha^{-1} at Site 4. These differences are directly related to yield variation, which at the same time is closely related to the rainfall precipitation regime and soil properties, which are known to affect soil N availability and whole plant NU (Tremblay et al., 2012). These NU values are lower than the observed in the USA Corn Belt by Bender et al. (2013) in a study where NU ranged from 266 to 307 kg N ha^{-1} across six hybrids grown at two sites.

Nitrogen uptake was increased at all sites (Fig. 3.3.), reflecting the increased fertilizer N supply. Regarding NU, the fertilizer (blend of PSCU and NBPTU) replacement value of regular urea as U-P and U-SA were of 59 and 99, 33 and 80, 93 and 84, and 65 and 134 kg N ha^{-1}, at sites 1, 2, 3, and 4, respectively. As it happened with GY, the amount of blend required for the corn plants to reach the same level of NU as when applying regular urea was surprisingly below the expected, most likely due to regular urea’s high susceptibility to N losses.

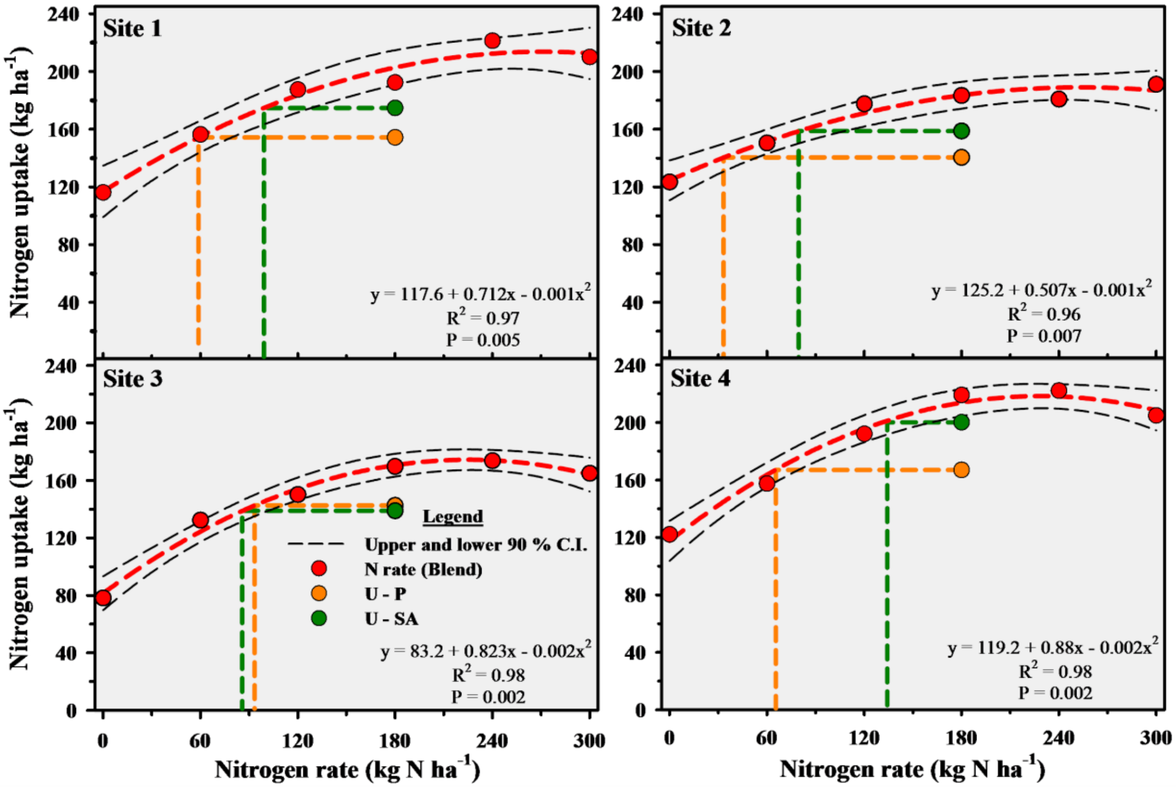

Figure 3.3. Whole corn plant nitrogen uptake as affected by nitrogen rates applied as a blend of polymer-sulfur coated urea and NBPT-treated urea at a 70:30 ratio incorporated into the soil at planting, and blend replacement value of 180 kg N ha^{-1} as regular urea incorporated into the soil at planting (U-P) and split surface applied urea (U-SA) at four sites.

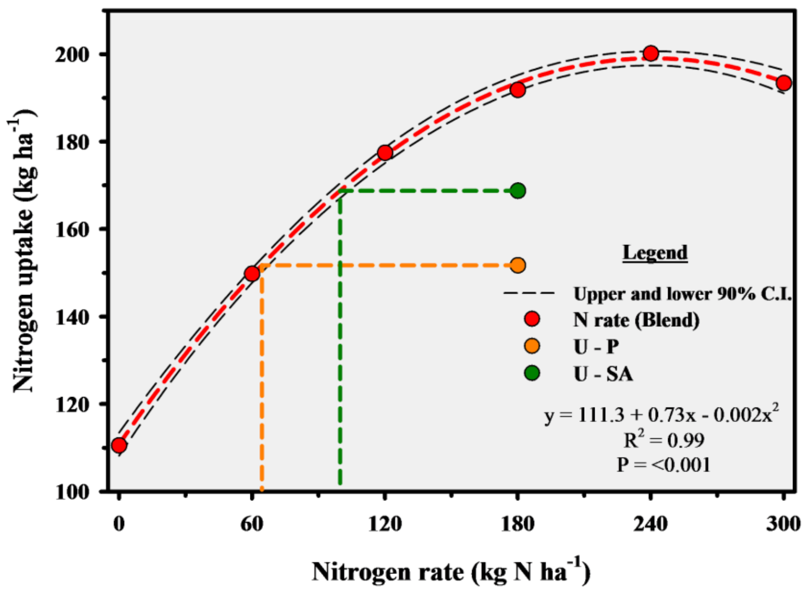

Figure 3.4. Whole corn plant nitrogen uptake as affected by nitrogen rates of a blend of polymer-sulfur coated urea and NBPT-treated urea at a 70:30 ratio incorporated into the soil at planting, and blend replacement value of 180 kg N ha^{-1} as regular urea incorporated into the soil at planting (U-P) and split surface applied urea (U-SA) across four sites.

Sadras and Lemaire (2014) mention that NU is co-regulated by plant available N and crop biomass accumulation. Furthermore, NU is highly variable within a single year, between years, sites, and crops, even when N supply (both by soil and fertilizer input) is plentiful (Gastal and Lemaire, 2002). Gastal and Lemaire (2002) reflect that when there is adequate N supply, crop NU is to a large extent determined or conditioned by crop growth rate.

The across site analysis showed that the overall blend replacement value of regular urea was 66 and 114 kg N ha^{-1} for the U-P and U-SA treatments, respectively (Fig. 3.4.), with the possibility of a significant N rate reduction when using the combined controlled release N fertilizer and the stabilized N fertilizer.

3.3.4. Biomass production and whole plant N concentration

Whole plant biomass production was affected by site and N application (Table 3.4). The average BP at all sites is portrayed in Table 3.5. Site 1 and 2 (FC) BP varied almost 3.5 Mg ha^{-1}, probably due to the higher rainfall precipitation during the first growing season. Sites 3 and 4 (SC), however, resulted in similar BP, 17.2 and 17.3 Mg ha^{-1}, respectively, regardless of the difference in rainfall precipitation between the growing seasons. These BP values are similar to those documented by Schoninger (2014) and Garcia et al. (2018), in corn N management studies developed also in the state of São Paulo, Brazil, but lower than those reported by Bender et al. (2015) and DeBruin et al. (2017), in studies realized in the high yielding areas of the USA Corn Belt.

Biomass production is an important plant component for determining grain yield in field crops (Fageria and Baligar, 2005), and as pointed out by Sadras and Lemaire (2014) the N economy of a certain crop is always a critical driver of biomass and grain production. Therefore, all these plant traits (biomass production, nitrogen uptake, and grain yield), are closely interrelated.

Biomass production greatly responded to applied N (Table 3.6). The control treatment produced less BP than all the N treatments. Regular urea treatments resulted in similar BP except at Site 4, where U-P promoted higher BP than U-SA. Compared to the treatments with blend application, U-P and U-SA resulted in lower BP than the 180, 240, and 300 kg N ha^{-1} treatments with blend application at Sites 1 and 3, while at Site 2 only U-P yielded less biomass than those N rates applied as blend.

Whole plant N concentration was impacted by both site and fertilizer N application (Table 3.4). Nitrogen concentration showed a great variation between sites (Table 3.5), with the higher values at Sites 2 and 4, with 11.0 and 10.7 g N kg^{-1}, intermediate Nc values at Site 1, with 9.7 g N kg^{-1}, and the lowest values at Site 3, with 8.4 g N kg^{-1}. Monitoring Nc is always important, since plant tissue analysis is used as a diagnostic tool to gather information about the N nutrition status of plants. It can offer information about sufficiency, deficiency, or excess of N. In fact, some authors have developed critical N curves for corn, with critical N concentration values, defined as the minimum N concentration required to achieve maximum growth (Plénet and Lemaire, 2000; Ziadi et al., 2008). Besides, nutrient (and in this case, N) uptake in plants is mainly measured by plant tissue analysis (Fageria and Baligar, 2005).

Only at Site 2 there was no impact of fertilizer N on Nc (Table 3.6). At Sites 1, 3, and 4, the response of Nc to fertilizer N was clear, with lower values observed in the control treatment and low N rate treatments. The across site analysis shows the control treatment and the 60 kg N ha^{-1} (blend application) treatment as the lowest Nc values. The U-SA promoted similar values to the 120, 180, 240, and 300 kg N ha^{-1} applied as blend.

3.3.5. Grain N concentration and removal

Grain N concentration was affected by both study site and fertilizer N application (Table 3.4). Total GNc varied among sites (Table 3.5), with the highest average values observed at Sites 2 and 4 (14.2 and 14.6 g kg^{-1}, respectively), intermediate values at Site 3 (13.6 g kg^{-1}), and the lowest at Site 1 (12.4 g kg^{-1}). As pointed out by Grant et al. (2012), GNc provides both an indication of the N sufficiency for the growing crop and an indication of grain quality in terms of protein content. Ciampitti and Vyn (2011, 2013) reported that grain N concentration has declined over time as an unintended consequence of grain yield improvement, largely because of higher tolerance to crowding intensity by modern corn hybrids (i.e. increased plant density).

Interestingly, GNc varied with fertilizer N application only at the SC sites (Sites 3 and 4) (Table 3.6). At Site 3 the control treatment (no N applied) showed the lower value (11.5 g N kg^{-1}), thus, the application of fertilizer N increased GNc. There was not much difference between N treatments. At Site 4, however, GNc response to fertilizer N did not follow a clear trend, with the control treatment presenting similar values of GNc to treatments with N application. All observed GNc values were situated in the range of the commonly observed N concentration in modern hybrids (Bender et al., 2013; Ciampitti and Vyn, 2013).

Grain N removal was also affected by site and N treatments (Table 3.4). Total GNr varied from 88 kg N ha^{-1} at Site 3 to 118 kg N ha^{-1} at Site 1, and intermediate values of 107 and 108 kg N ha^{-1} at Sites 2 and 4 (Table 3.5). These values could be considered low if compared to the average GNr of 166 kg N ha^{-1} reported by Bender et al. (2013), and the GNr values reported by González-Villalba (2014), who found average values of 146 and 160 kg N ha^{-1} removed in the grains at two experimental sites in the State of São Paulo. These low values of GNr observed in this study are most likely related to the relatively lower GY documented compared to those related in the mentioned reports, since GNc was apparently not limited (Table 3.5 and 3.6). Grain N removal was greatly influenced by fertilizer N application (Table 3.6). At all sites, the control treatment resulted in lower GNr. The regular urea treatments U-P and U-SA resulted in similar GNr except at Site 3, where U-P resulted in higher N content in the grain.

3.3.6. Harvest Index, Nitrogen Harvest Index, and Internal Use Efficiency

Plant harvest index (HI), defined by Sinclair (1998) as the ratio of grain yield to whole plant biomass production, reflects the efficiency of dry matter partitioning to the grain (Bender et al., 2013). Harvest index was impacted by site but no by fertilizer N treatments (Tables 3.4, 3.5 and 3.7). The values of HI were higher in the FC cropping system (53 and 52 at Sites 1 and 2, respectively) than at the SC cropping system, where the HI values were of 37 and 43 for Sites 3 and 4, respectively. These contrasting results are very intriguing. The HI values observed at Sites 1 and 2 are similar to the HI values of six modern corn hybrids reported by Bender et al. (2013), which varied from 48 to 54. On the other hand the values observed at Sites 3 and 4 could be considered low HI values, similar to those of older corn hybrids (Hanway, 1962; Hanway and Russell, 1969; Bender et al., 2013). However, Schoninger (2014) reported HI values similar to the observed at Sites 3 and 4, with an average HI of 42 in the first experimental year and 53 in the second year.

Sinclair (1998) explains how HI is an important trait associated with the increases in crop yield in the twentieth century. It is not clear why would HI values be that lower at the SC experimental sites. The one likely reason for the lower HI values (which suggest inefficient photosynthate partitioning between the grain and the vegetative parts of the plant) at the SC experimental areas could be that at this cropping system, corn harvest is performed as soon as the plants enter the physiological maturity stage, when corn grain presents between 35% moisture (because it is destined to silage for beef cattle consumption). This is known as high-moisture corn, and presents better nutritional properties compared to dry corn (Persichetti Júnior et al., 2014). Furthermore, harvest is performed when plants are still entirely green.

Perhaps harvest occurred before all dry matter was partitioned from the vegetative parts to the grain.

Nitrogen harvest index varied among sites, but fertilizer N treatments did not have any impact on it (Table 3.4). Fageria (2014) defines NHI as the ratio between N accumulated in the grain to N accumulated in the whole plant (grain plus straw) and represents an important agronomic tool because it helps in measuring the efficiency of the remobilization of absorbed N from vegetative plant parts to the grain. The results indicate a clear difference between the two cropping systems evaluated (Table 3.5), with higher NHI observed at the FC sites (66 and 65 for Sites 1 and 2), and lower at the SC sites (60 and 57 for Sites 3 and 4). As suggested for HI, it is also probable that the N remobilization process was still active when the plants were harvested, therefore removing less N in the grains than what would be expected. Bender et al. (2013) cite NHI values ranging from 51 to 62 in the USA across six high yielding modern corn hybrids (average of 12 Mg ha^{-1}). Schoninger (2014) on the other hand, documented and average NHI of 56 and 68 in the first and second experimental year, respectively.

Table 3.6. Corn biomass production (BP), whole plant N concentration (Nc), grain N concentration (GNc), and grain N removal (GNr) as affected by nitrogen treatments at four experimental sites and across sites.

Treatment†	Fallow-Corn		Sugarcane-Corn		Across Sites
	Site 1	Site 2	Site 3	Site 4	
			Biomass Production (Mg ha^{-1})		
Control	12.7 c‡	11.7 c	11.4 d	12.2 e	12.0 e
60 Blend	17.2 b	14.4 ab	15.5 c	16.4 cd	15.8 cd
120 Blend	19.7 a	16.1 a	18.4 ab	17.5 cb	18.0 b
180 Blend	20.4 a	16.3 a	19.7 a	20.3 a	19.2 a
240 Blend	21.1 a	16.4 a	19.2 a	20.1 a	19.2 a
300 Blend	19.9 a	15.9 a	19.9 a	18.2 b	18.5 ab
180 U-P	17.2 b	12.9 bc	16.5 c	20.3 a	15.5 cd
180 U-SA	17.2 b	14.2 abc	17.1 bc	18.4 b	16.7 c
P > F	<0.001	0.028	<0.001	<0.001	<0.001
			Whole Plant N Concentration (g kg^{-1})		
Control	9.2 c	10.5	7.0 c	10.2 bc	9.2 d
60 Blend	9.1 c	10.5	8.7 ab	9.5 c	9.5 cd
120 Blend	9.5 bc	11.0	8.0 bc	11.2 a	9.9 b
180 Blend	9.4 c	11.2	8.5 ab	10.7 ab	10.0 b
240 Blend	10.6 a	11.0	9.2 a	11.0 ab	10.5 a
300 Blend	10.6 a	11.7	8.2 ab	11.2 a	10.4 a
180 U-P	9.1 c	10.7	9.0 ab	10.7 ab	9.9 bc
180 U-SA	10.2 ab	11.2	8.2 ab	11.0 ab	10.2 ab
P > F	0.004	0.136	0.040	0.018	<0.001
			Grain N Concentration (g kg^{-1})		
Control	12.7	13.5	11.5 c	14.2 bc	13.0 d
60 Blend	12.2	13.5	13.5 b	13.7 c	13.2 d
120 Blend	12.7	14.2	13.7 b	15.5 a	14.1 abc
180 Blend	11.7	14.7	14.0 ab	15.0 ab	13.9 abc
240 Blend	13.2	15.0	14.7 a	14.2 bc	14.3 a
300 Blend	13.0	15.0	13.5 b	15.0 ab	14.1 ab
180 U-P	11.2	14.0	14.2 ab	14.5 bc	13.5 cd
180 U-SA	12.5	13.5	13.5 b	14.7 ab	13.5 bcd
P > F	0.249	0.113	<0.001	0.041	0.026
			Grain N Removal (kg ha^{-1})		
Control	73 e	79 d	42 e	70 e	66 e
60 Blend	102 d	103 bc	83 d	90 d	95 d
120 Blend	125 bc	122 ab	93 bcd	115 bc	114 b
180 Blend	127 bc	125 a	104 ab	132 a	122 ab
240 Blend	153 a	113 abc	112 a	123 ab	125 a
300 Blend	143 ab	117 ab	88 cd	115 bc	116 b
180 U-P	101 d	92 cd	98 abc	103 cd	99 cd
180 U-SA	118 cd	105 abc	79 d	113 bc	104 c
P > F	<0.001	0.018	<0.001	<0.001	<0.001

† Control, no N application; Blend, mixture of polymer-sulfur coated urea and urea (70:30 ratio); U-P, urea applied at planting; U-SA, split applied urea.
‡ Means followed by different letters in the column indicate differences (ANOVA) at P < 0.1 between treatments

Table 3.7. Harvest index, nitrogen harvest index and internal use efficiency as affected by nitrogen treatments at four sites and across site.

Treatment†	Fallow-Corn		Sugarcane-Corn		Across Sites
	Site 1	Site 2	Site 3	Site 4	
			Harvest Index		
Control	46	52	33	41	43
60 Blend	50	53	39	41	46
120 Blend	51	54	37	42	46
180 Blend	54	53	38	43	47
240 Blend	55	46	40	43	46
300 Blend	55	50	33	42	45
180 U-P	54	52	38	46	48
180 U-SA	55	54	34	42	46
P > F	0.125	0.556	0.254	0.771	0.183
			Nitrogen Harvest Index		
Control	62	64	53	56	58
60 Blend	65	68	62	57	63
120 Blend	66	68	62	59	64
180 Blend	66	68	61	60	64
240 Blend	69	63	65	55	63
300 Blend	67	62	54	56	60
180 U-P	65	66	69	61	65
180 U-SA	68	65	56	56	61
P > F	0.201	0.694	0.127	0.498	0.333
			Internal Use Efficiency		
Control	50	48	46	40	46
60 Blend	55	51	46	42	48
120 Blend	54	49	46	38	47
180 Blend	57	47	44	40	47
240 Blend	53	42	44	39	44
300 Blend	52	41	41	37	43
180 U-P	60	47	47	42	49
180 U-SA	54	49	41	38	46
P > F	0.345	0.242	0.742	0.556	0.121

† Control, no N application; Blend, mixture of polymer-sulfur coated urea and urea (70:30 ratio); U-P, urea applied at planting; U-SA, split applied urea.

Internal use efficiency was affected by site, but no by fertilizer N (Table 3.4). The average values of IUE were 54, 47, 44 and 40 for Sites 1, 2, 3, and 4 (Table 3.5), representing 54, 47, 44, and 40 kg of grains for each kg of accumulated N. Bender et al. (2013 and González-Villalba (2014) reported values of approximately 45 kg of grains for each kg of total accumulated N. These values are, however, below the indicated by Dobermann (2007) as the optimal range (55-65) for balanced nutrition at high yield levels. As stated before, IUE represents the ability of plants to transform acquired N (from the soil, from fertilizer) into economic yield (grain) (Dobermann, 2007). Furthermore, looking at the values, it appears that there was something interfering or preventing an optimal transformation of NU to GY. As a matter of fact, Dobermann (2007) explains that IUE is highly dependent on genotype, environment and crop management, and adds that low IUE suggest poor nutrient conversion due to other stresses, which could be drought stress, heat stress, pests, etc.

Crop physiological N requirements are controlled by the efficiency with which N in the plant is converted to BP and GY (Cassman et al., 2002). Cassman et al. (2002) discuss that because cereal crops such as corn are harvested for grain, the most interesting measure of physiological N efficiency is the change in GY per unite change in NU (IUE). Furthermore, the authors mention that IUE is mainly driven by two factors: i) the photosynthetic pathway (i.e. C3 or C4 plants); and ii) grain N concentration (which depends on genetics, but also affected by N supply). It results interesting that HI, NHI, and IUE (all important plant traits) were not affected by fertilizer N application. What impacted them was the experimental site instead, demonstrating the importance of the environmental factor when thinking about crop traits to be improved in corn production systems. Therefore, it becomes clear that only fertilizer N application cannot change these traits, and that they are more responsive to environmental factors.

3.3.7. Relationship between corn grain yield, biomass production, and nitrogen uptake

The relationship between NU and BP followed a linear model (Fig. 3.4 A), showing a proportional change in corn NU as BP increased. The variation in NU due to BP increment was 67%. Cassman et al. (2002) stated that crop N demand is determined by biomass yield and the physiological requirement for tissue N.

The relationship between GY and NU is portrayed in Fig. 3.4 B, showing a linear relationship. The changes in GY due to NU was 59%. As pointed out by Fageria and Baligar (2005), crop NU generally presents a positive relationship with grain yield, as portrayed in Figure 3.4. A recent review, using a big dataset, reported that the relationship between GY and NU follows a linear phase (i.e. proportional increase in GY as NU increases), and then hits a plateau, where a disproportional change in GY related to NU increment is documented, and generally occurs in high yielding corn cropping systems (Ciampitti and Vyn, 2012). The relationship between GY and NU at physiological maturity provides a valuable information of IUE.

The relationship between GY and BP fits a linear model (Fig. 3.4 C). The changes in GY due to BP increment was 50%. In general, good BP is related to high GY.

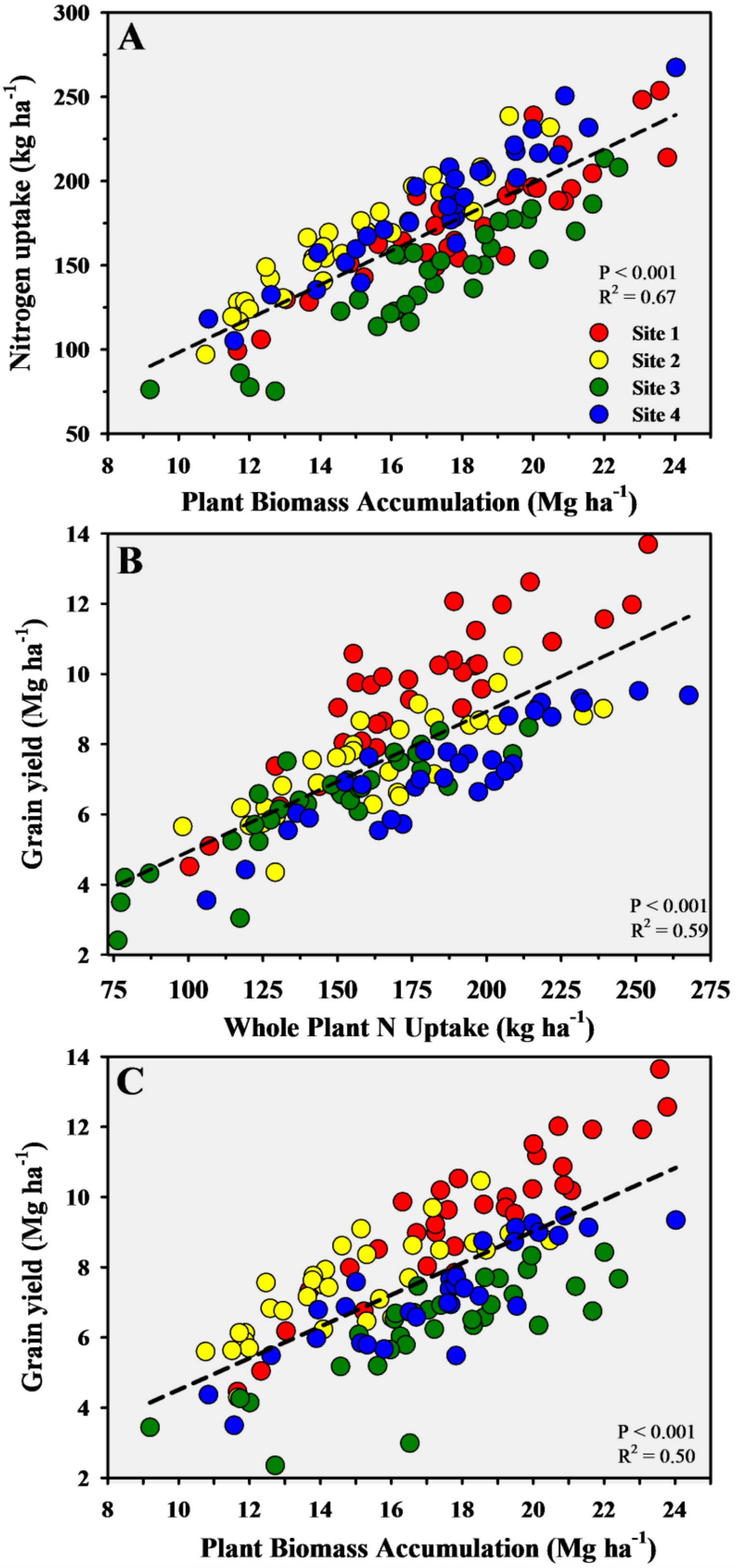

Figure 3.5. Relationship between plant nitrogen uptake and plant biomass (A), grain yield and plant nitrogen uptake (B), and grain yield and plant biomass (C) at physiological maturity at all four sites of the study.

3.3.8. Implications for future research

Future research steps should focus on conducting multiple site-year trials, comparing N rates of conventional N sources (i.e. ammonium nitrate, regular urea, ammonium sulfate) with N rates of the enhanced efficiency N fertilizers. Research on enhanced efficiency N fertilizer application timing and placement are also needed. The 4R's concept (IFA, 2009; Snyder, 2017) should be the main driver of research on fertilizer N.

Nitrogen losses (nitrate leaching, ammonia volatilization, nitrous oxide emission, etc.) need to be measured in field conditions, and if possible at the same experiment. To focus in just one of the mentioned N losses pathway does not provide enough information in order to understand N dynamics when applying enhanced efficiency N fertilizers. And of course this need to be closely related to seasonal soil and plant N status monitoring. The only way to achieve such ambitious research goals is to conduct multi-institutional, multi-disciplinary research projects, including research institutions, universities, industry, and private sector, and necessarily include farmers in such projects, in order to integrate efforts and different points of view to achieve a common goal: improve fertilizer N use efficiency while minimizing the environmental footprint of fertilizer N.

A comprehensive economic analyses of fertilizer N management should be included in order to have a better insight on the overall aspects of the fertilization practice for corn production. In this study the use of a blend of PSCU and NBPTU showed to be, at least agronomically, a preferably option compared to the application of regular urea, with a possible N rate reduction of over a 100%. However, it is not clear whether the higher corn grain yield will ultimately be reflected on higher profits. Therefore, economics of the fertilization practice (using current enhanced efficiency fertilizer N and fertilizer N incorporation costs) need to be included in order to better assist farmers in the decision-making process.

3.4. CONCLUSIONS

This study evaluated the impact of increasing N rates of a blend of polymer-sulfur coated and NBPT-treated urea applied at corn planting and incorporated into the soil, on corn grain yield in two production systems and a possible N rate reduction when adopting this N management strategy compared to the normally recommended N fertilizer application for corn production. The results obtained in this study indicate that the adoption of this N fertilizer management, applying this fertilizer N source (blend of PSCU and NBPTU), this fertilizer N placement (incorporated, 10 cm from the corn rows, 5 cm deep), and this fertilizer N timing (at planting, without further side-dress N application), proved to be a great strategy to attain corn grain yield at N rates below those needed with regular urea (over 100% N rate reduction).

This study provides a unique perspective on the use of enhanced efficiency N fertilizers as a tool to improve corn grain yield and minimize the fertilizer N environmental footprint under different production systems. Regular urea's high susceptibility to N losses is clearly exposed in the results, regardless of how it is applied (i.e. incorporated at all planting or split applied).

With the increasing pressure of nowadays society over the agriculture and especially the fertilizer sector, technologies which provide lower N losses to the environment will have to be chosen over the conventional fertilizers, although fertilizer N management decisions for corn production will ultimately be dependent (and should be accompanied with) on economic profits.

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4. CORN BIOMASS AND NITROGEN UPTAKE AND PARTITIONING AS AFFECTED BY A BLEND OF CONTROLLED RELEASE AND STABILIZED UREA FERTILIZER

ABSTRACT

Blends of controlled release nitrogen (N) fertilizers and stabilized N fertilizers are proposed as an alternative to synchronize N availability and crop N needs throughout the growing season. Field experiments were conducted in Southeast Brazil, in contrasting corn production systems, with the application of a blend of polymer-sulfur coated urea (PSCU) and NBPT-treated urea (NBPTU) at a 70:30 ratio, applied at corn planting and incorporated into the soil. The objectives of the study were: i) to determine differences in biomass and N uptake and partitioning between a 0-N treatment, a 180 kg N ha^{-1} as blend, and a 180 kg N ha^{-1} as regular urea; ii) characterize and understand N remobilization processes comparing three N fertilizer managements (i.e. 0-N, 180 kg N ha^{-1} as blend, and 180 kg N ha^{-1} as regular urea); and iii) to understand plant N dynamics throughout the growing season and across sites and its relationship with corn biomass. Corn biomass and N uptake and partitioning were affected by N fertilizer. However, no much difference was observed between the two N fertilizer managmenet strategies evaluated. Nitrogen remobilization processes are altered by N fertilization. Apparently, more N is being taken up after flowering compared to older data on N remobilization and after flowering N uptake. Plant N dynamics is complex, and the data gathered in this study will help to better understand N changes during the season. These data could be used sometime, if pooled with data from other corn production areas, to develop nitrogen nutrition indexes, which are a powerfull and intersting tool currently being used in other parts of the world.

Keywords: Crop growth rate; Uptake rate; Nitrogen remobilization; Fertilizer nitrogen; Nutrient partitioning

Abbreviations: PSCU, polymer-sulfur coated urea; NBPTU, regular urea treated with NBPT; BP, biomass production; NU, nitrogen uptake

4.1. INTRODUCTION

Modern and high yielding corn hybrids (12.0 Mg ha^{-1}) in the United States Corn Belt require in average a whole plant N uptake of 286 kg N ha^{-1}, a whole plant biomass production of 23.0 Mg ha^{-1}, and 166 kg N ha^{-1} accumulated in the grain at harvest (58% of the total plant N, harvested in the grain) (Bender et al., 2013). On the other hand, Schoninger (2014), reported an average corn grain yield of 9.6 Mg ha^{-1} in Southeast Brazil, requiring a whole plant N uptake of 192 kg N ha^{-1}, a whole plant biomass production of 19.2 Mg ha^{-1}, and 119 kg ha^{-1} accumulated in the grain at harvest (62% of N harvest index). With these high biomass production and N uptake values, it is important to understand the accumulation processes and the rates at which growth and uptake occurs. To know at what period or growth stage is the maximum growth and/or N uptake rate can help in developing better fertilizer or water management strategies in order to ensure N availability, and therefore helping the plants to growth at their maximum potential.

As stated by (Fageria and Baligar, 2005), photosynthetic products produced by green plants are divided into several plant parts (i.e. roots, shoots, and grain). A part remains in shoots and a part is translocated to roots and grain. This process is referred to as biomass (or dry weight) partitioning in plants, and the same is valid for plant N uptake.

The objectives of the study were: i) to determine differences in biomass and N uptake and partitioning between a 0-N treatment, a 180 kg N ha^{-1} as blend, and a 180 kg N ha^{-1} as regular urea; ii) characterize and understand N remobilization processes comparing three N fertilizer managements (i.e. 0-N, 180 kg N ha^{-1} as blend, and 180 kg N ha^{-1} as regular urea); and iii) to understand plant N dynamics throughout the growing season and its relationship with corn biomass.

4.2. MATERIAL AND METHODS

4.2.1. Sites description

The study was conducted during the 2014/2015 and 2015/2016 growing seasons in two contrasting cropping systems: a fallow corn succession cropping system (FC), and a sugarcane corn (SC) succession cropping system. The FC experiment was located at Iracemápolis, São Paulo, Brazil (22°38’5’’S, 47°30’14’’W; 608 m altitude) on a clay textured soil (Soil Survey, 2014) during both growing seasons (Site 1 and 2, respectively). The climate of this site correspond to Cfa (hot summer) transitioning to Cwa (dry winter and hot summer), according to the Köppen classification map for Brazil elaborated by Alvares et al. (2013). At this cropping system area, corn planting was performed after desiccation of the vegetation with herbicides, with no soil disturbance. Black oat (*Avena strigosa* Schreb) was planted during the winter as a cover crop, however without good development as a consequence of the environmental conditions and limited rainfall during this period. Thus, at the time of corn planting, the amount of black oat residues left in the area was minimum.

The SC study was located at Severinia, São Paulo, Brazil (20º43’34’’S, 48º41’52’’ W; 605 m altitude) during the 2014/15 growing season (Site 3) and at Altair, São Paulo, Brazil (20º27’15’’S, 49º02’05’’W; 537 m) during the 2015/16 growing season (Site 4). The climate of these sites correspond to Aw (tropical zone with dry winter) according to Alvares et al. (2013). A quantification of the residual biomass was performed before the experiment installment. The residual sugarcane straw the first year was 16.5 Mg ha^{-1} and the second year 18 Mg ha^{-1} (average of 5 random samples in a 1 m^2 area). At Site 4 (Altair 2015/2016), concentrated vinasse, a byproduct of the sugarcane industry, rich in potassium content (Otto et al., 2017) application is common.

For further details on the experimental sites, please refer to the section 3.2.1 in Chapter 3. Soil characterization of the experimental sites is portrayed in section 3.2.2. in Chapter 3. In general, the two cropping systems presented contrasting soil properties. In general, the soil at the FC sites show a higher clay content, O.M. content, and CEC, thus presenting higher natural fertility. High K content is observed at Site 4, which can be explained by the application of concentrated vinasse for sugarcane production.

4.2.2. Experimental design and treatments

The experiments were arranged in a complete randomized block design with four replications. The treatments of the experiments were the same as in 3.2.3. in Chapter 3: N rates (0, 60, 120, 180, 240, and 300 kg N ha^{-1}) using a blend of polymer-sulfur coated urea (PSCU) and NBPT-treated regular urea (NBPTU) at a 70:30 ratio, and two treatments with 180 kg N ha^{-1} of regular urea. A control plot with no N was also included. All fertilizer N was band applied and incorporated at planting, except for one of the 180 kg N ha^{-1} of regular urea treatments (180 U-SA), where fertilizer N was split applied (20% of the total N rate applied at planting and the remaining applied when the corn plants were at the V4 growth stage), as recommended by (Cantarella et al., 1996).

Treatment details and fertilizer N application methods and timing are displayed in Table 3.2 in Chapter 3. The N sources used in the blend were NBPT-treated urea [(46-0-0), (N, P_2O_5, K_2O)], and Producote®. For more details of the N fertilizer used, please refer to section 3.2.3 in Chapter 3. As Guelfi (2017) pointed out, the NBPT-treated urea is highly soluble, less susceptible to N losses, and meet the immediate demand of N by the crop, while the PSCU provides a gradual N release, ensuring N availability for later growth stages.

4.2.3. Experiment setup and crop management

All details on treatments application (i.e. fertilizer application), crop management details, planting and harvest dates, plant density, etc., are portrayed in section 3.2.4. in Chapter 3.

4.2.4. Sampling and analysis

Plant sampling was performed at V4, V8, V12, R1, R4, and R6. All treatments were sampled for biomass production and N concentration determination. The aboveground part of four plants per plot was clipped at ground level and separated into leaves, stalk, cob, and grain (depending on the growth stage). All plant material was oven dried at 65°C for 72 h, weighed to determine the aboveground biomass production (BP), and ground using a Willey mill. A subsample was then prepared for N content (Nc) analysis by micro Kjeldhal digestion (Raij et al., 2001). Total nitrogen uptake (NU) was then calculated multiplying BP by N concentration of each plant part. For N uptake and partitioning, as well as for biomass accumulation and partitioning, and remobilization data, only the 0-N, 180 kg ha^{-1} as blend, and 180 kg ha^{-1} as regular urea were used. To portray the N dilution curve and the relationship between N uptake and biomass production, however, all data points of the experiment are showed.

For N remobilization calculations, the percentage of N remobilized from the leaves or the stalk to the grains after flowering was calculated by the difference method (Equation 1), similar to the proposed by (Ciampitti and Vyn, 2013a; Ciampitti et al., 2013; Schoninger, 2014).

(Equation 1) $$RN\ (\%) = \left(\frac{(N_{R6} - N_{R1}}{NG_{R6}}\right).100$$

Where,

RN is the remobilized nitrogen (from the leaves and/or stalk), in percentage;

NR_6 is the accumulated N in the leaves and/or stalk at the R6 corn growth stage (i.e. physiological maturity), in kg ha^{-1};

NR_1 is the accumulated N in the leaves and/or stalk at the R1 corn growth stage (i.e. flowering), in kg ha^{-1};

NG_{R6} is the accumulated N in the grains at R6 corn growth stage (physiological maturity), in kg ha^{-1}.

The total percentage of remobilized N (TRN) was given by the sum of the remobilization from the leaves and stalk. The percentage of N in the grain at harvest derived from after flowering N uptake (NUAF) was calculated using Equation 2, in which N remobilized from the cob were considered minimal, and therefore obviated.

(Equation 2) $$\text{NUAF } (\%) = 100 - \text{TRN}$$

Where,

NUAF is the nitrogen uptake after R1 growth stage (flowering) which was accumulated in the grains, in percentage;

TRN is the total remobilized N, in percentage.

The accumulation rates and patterns (biomass and nitrogen), were calculated based in Lucchesi (1984). In this method, the rates referent to each period (between growth stages, interval between sampling dates in V4, V8, V12, R1, R4, and R6) are determined by dividing accumulated N or biomass and the time period (days) for such accumulation. From this accumulation rates, curves were fitted in Sigmaplot 12.5®, choosing the significative ones ($p \leq 0.1$), and with the higher coefficient of determination.

4.2.5. Statistical analysis

All data were analyzed using the PROC GLIMMIX procedure in SAS® software (SAS Institute Inc, Cary, NC). The main effects of the fertilizer N treatments (N fertilizer) and plant parts were included as fixed factor and blocks as random factor in the model. Growth stage was not included in the model. Corrected denominator degrees of freedom were obtained using the Kenward-Roger adjustment. Treatment effects were considered significantly different at the $P \leq 0.10$ probability level including confidence interval estimations.

4.3. RESULTS AND DISCUSSION

4.3.1. Relationship between plant N concentration and plant biomass: the N dilution curve

It is well known that N concentration in plants decreases with time (i.e. plant biomass production) (Plénet and Lemaire, 2000; Seginer et al., 2004; Ciampitti and Vyn, 2011; Ciampitti et al., 2012). Lemaire et al. (2008) discuss the reason why that happens (i.e. plant N concentration decreases monotonically as the crop grows during the vegetative growth period). The authors give two main reasons. First, there is an ontogenetic decline in leaf area per unit of biomass, and second, high remobilization of N occurs from shaded leaves at the bottom of the canopy to well illuminated leaves at the top. With this kind of information, for example, a nitrogen nutrition index (NNI) could be generated for Brazilian corn production systems. Nitrogen nutrition index is today a reliable index of the N stress level in corn plants (Lemaire et al., 2007, 2008; Ziadi et al., 2008) in several parts of the world, although it can only be used until the R1 growth stage (flowering) (Sadras and Lemaire, 2014).

The relationship between whole plant N concentration and plant biomass accumulation is portrayed in Fig. 4.1 A, where it is notorious the higher Nc early in the season, and as time passes and biomass is accumulated faster, there is a rapid decrease of Nc. This phenomenon is denominated as the N dilution curve in crops (Gastal and Lemaire, 2002; Sadras and Lemaire, 2014).

4.3.2. Relationships between nitrogen uptake and plant biomass

The relationship between crop NU and biomass production relies on the interregulation of several crop physiological processes (Gastal and Lemaire, 2002). Guo et al. (2017) found that the total corn plants N uptake after the flowering stage varied from 51 to 63% of the total N, in a high yielding corn production environment (i.e. >15 Mg ha^{-1}), demonstrating that modern corn hybrids present a higher N requirement compared to older corn hybrids, in agreement with Ciampitti and Vyn (2013), who also reported that in modern hybrids reproductive N contributes more to the total grain N uptake compared to older corn hybrids. Therefore, fertilizer N management should be focused on ensuring adequate plant available N supply during the entire growing season, even at late corn growth stages, which, as demonstrated in Chapter 2, can be attained with the application of a blend of PSCU and NBPTU.

The relationship between whole plant Nu and whole plant BP is portrayed in Figure 4.1 B. There is a strong relationship between both crop traits, and it is notorious that 96% of changes in plant N was caused by increase in plant biomass. As pointed out by Setiyono et al. (2010), understanding the relationship between N uptake requirement and grain yield (which is closely related to biomass production) is crucial to develop sound N fertilization programs and improve corn grain yield.

4.3.3. Biomass accumulation and biomass accumulation rate

Fertilizer N treatment and plant parts impacted BP and at all growth stages evaluated and at both cropping systems (FC and SC) (Table 4.1). There was also an interaction between plant parts and N treatment at the two cropping systems evaluated.

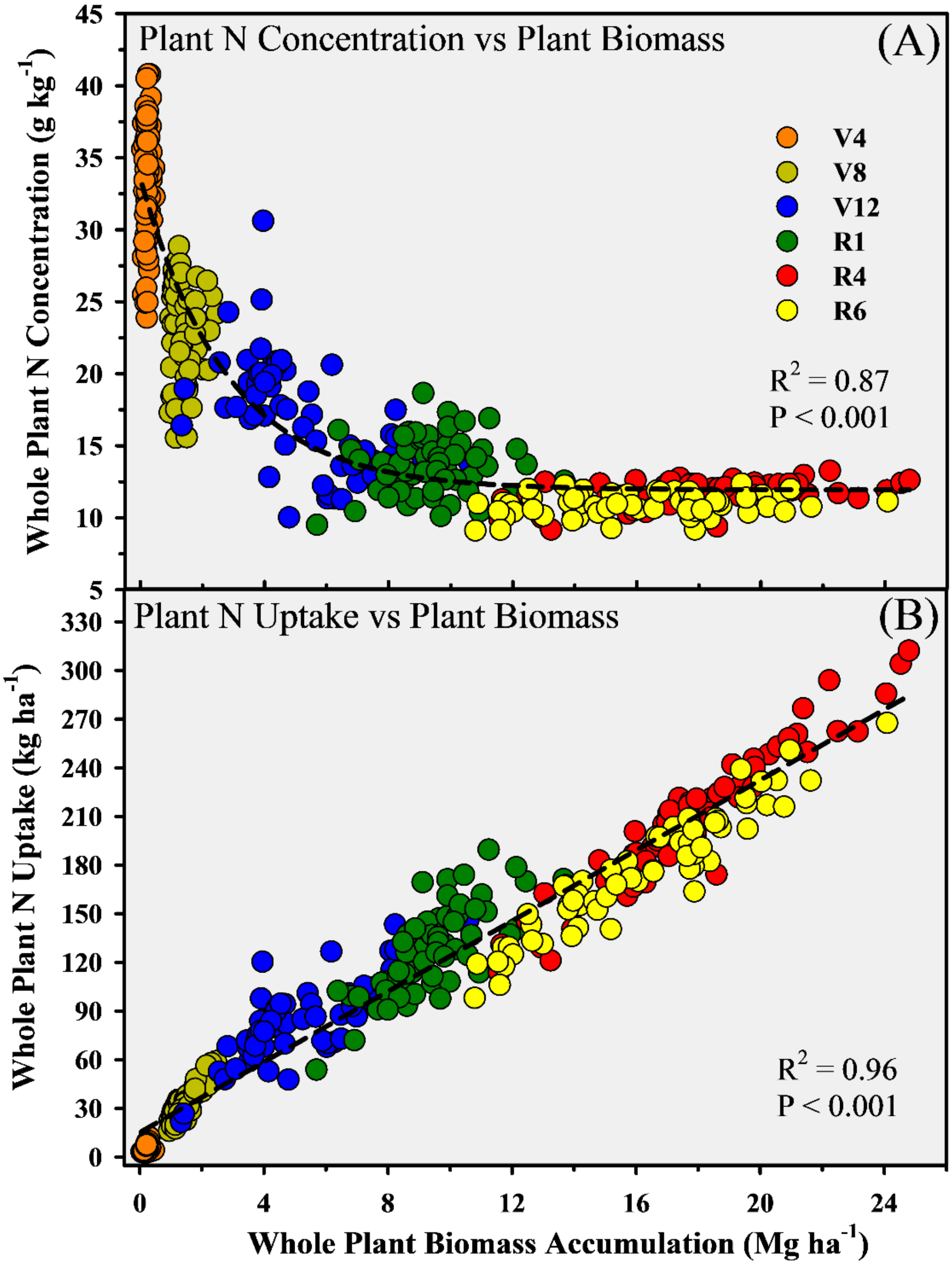

Figure 4.1. Relationship between whole plant nitrogen concentration and whole plant biomass accumulation (A), and between whole plant nitrogen uptake and biomass accumulation (B) throughout the 2015-2016 growing season (sampling dates at V4, V8, V12, R1, R4, and R6 growth stage). Two sites evaluated.

Table 4.1. Significance of the F values for the fixed effects of N fertilizer, plant part and growth stage on biomass accumulation (BP) and nitrogen uptake (NU) in two corn cropping systems (average of two years each)

Fixed effect	Fallow-Corn		Sugarcane-Corn	
	BP	NU	BP	NU
	- - - - - - - - - - -	- - - - - - - - - - - P > F	- - - - - - - - - - -	- - - - - - - - - - -
		V4 Growth stage		
N treatment (Trt)†	<0.001	0.003	<0.001	<0.001
		V8 Growth stage		
N treatment	<0.001	<0.001	<0.001	<0.001
Plant Part (PP)‡	<0.001	<0.001	<0.001	<0.001
Trt × PP	<0.001	<0.001	0.002	<0.001
		V12 Growth stage		
N treatment	<0.001	<0.001	<0.001	<0.001
Plant Part	<0.001	<0.001	<0.001	<0.001
Trt × PP	<0.001	<0.001	0.565	0.130
		R1 Growth stage		
N treatment	<0.001	<0.001	<0.001	<0.001
Plant Part	<0.001	<0.001	<0.001	<0.001
Trt × PP	0.068	<0.001	<0.001	<0.001
		R4 Growth Stage		
N treatment	<0.001	<0.001	<0.001	<0.001
Plant Part	<0.001	<0.001	<0.001	<0.001
Trt × PP	<0.001	<0.001	<0.001	<0.001
		R6 Growth Stage		
N treatment	<0.001	<0.001	<0.001	<0.001
Plant Part	<0.001	<0.001	<0.001	<0.001
Trt × PP	<0.001	<0.001	<0.001	<0.001

† Nitrogen fertilizer treatments
‡ Plant part (Leaf, Stalk, Cob, Grain)

Biomass production varied for all three evaluated treatments at the two cropping systems (FC and SC) (Figure 4.1). The control treatment accumulated less BP than the two fertilizer N treatments (180 kg ha^{-1} blend, and 180 kg ha^{-1} regular urea). The blend accumulated higher BP compared to the regular urea at the two cropping systems. Biomass accumulation pattern was different for both N treatments. At the FC, increase in BP in the blend treatment started slightly earlier than the regular urea. Around V12, however, the regular urea was accumulated biomass faster until around R2 or R3, where a decline on BP was observed in the urea treatment relative to the blend. At the SC, something similar occurred. There was a rapid BP increase by the regular urea treatment until around V14, when a shift occurred, and the blend started to cause higher and faster BP. In both cropping systems, the blend showed a slight delay to get to its maximum NU rate. While this delay at the FC was only 2 two or three days, at the SC this delay was of more than ten days. Schoninger (2014) also showed variation in BP and BP rate as a consequence of fertilizer N application (N timing).

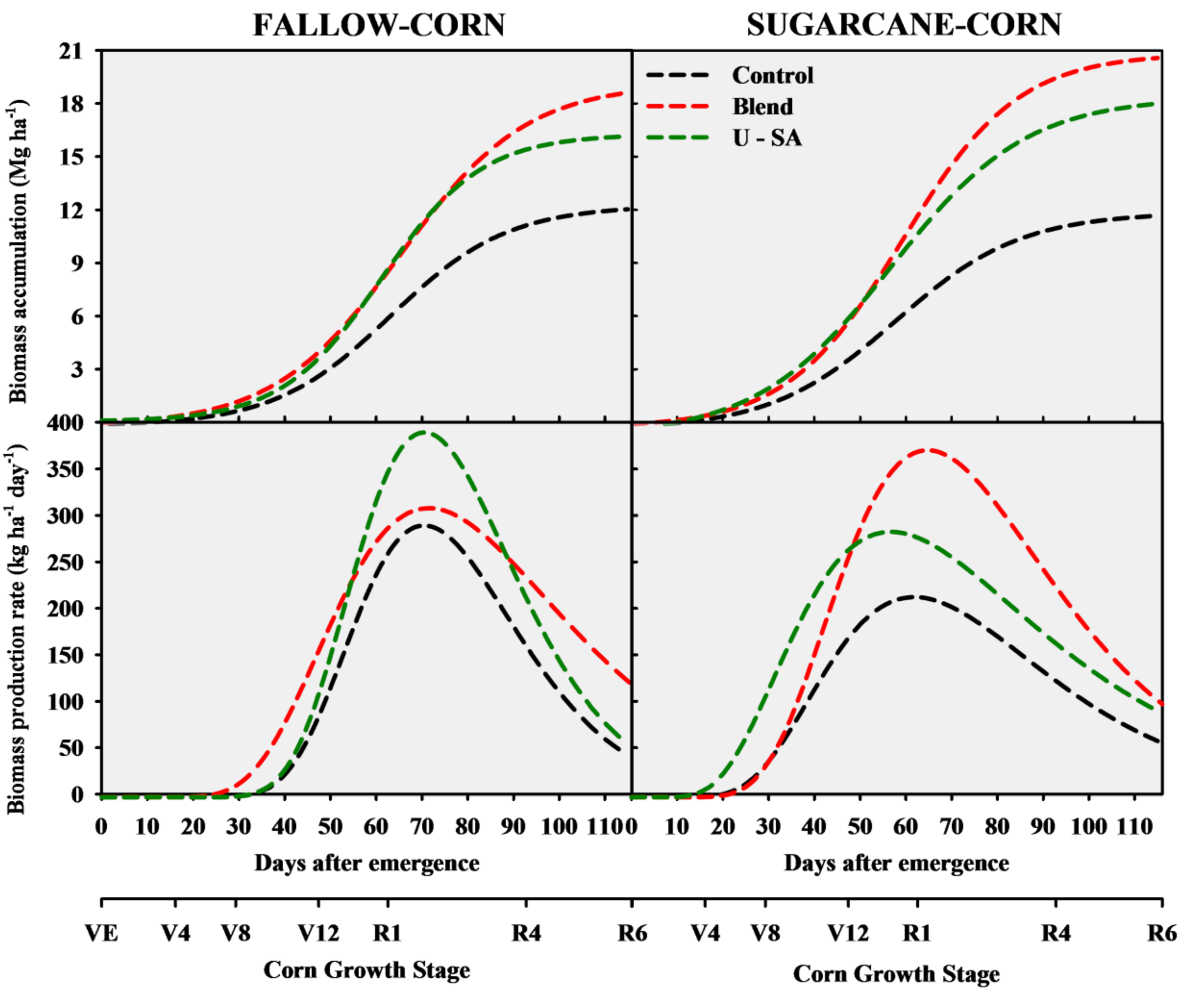

Figure 4.2. Seasonal whole plant biomass production (BP) and accumulation rate in two cropping systems (sampled at V4, V8, V12, R1, R4, and R6 growth stage). Average of two growing seasons for each cropping system.

Obviously, BP pattern reflected on BP rate. The control showed lower maximum BP rate. Regular urea promoted higher. At both sites, the blend treatment got to the end of the season with higher BP rate. The maximum BP rate for the control was around 280 kg biomass ha^{-1} day^{-1} at the FC and 200 kg biomass ha^{-1} day^{-1}, while those of N treatments showed much higher maximum BP rate.

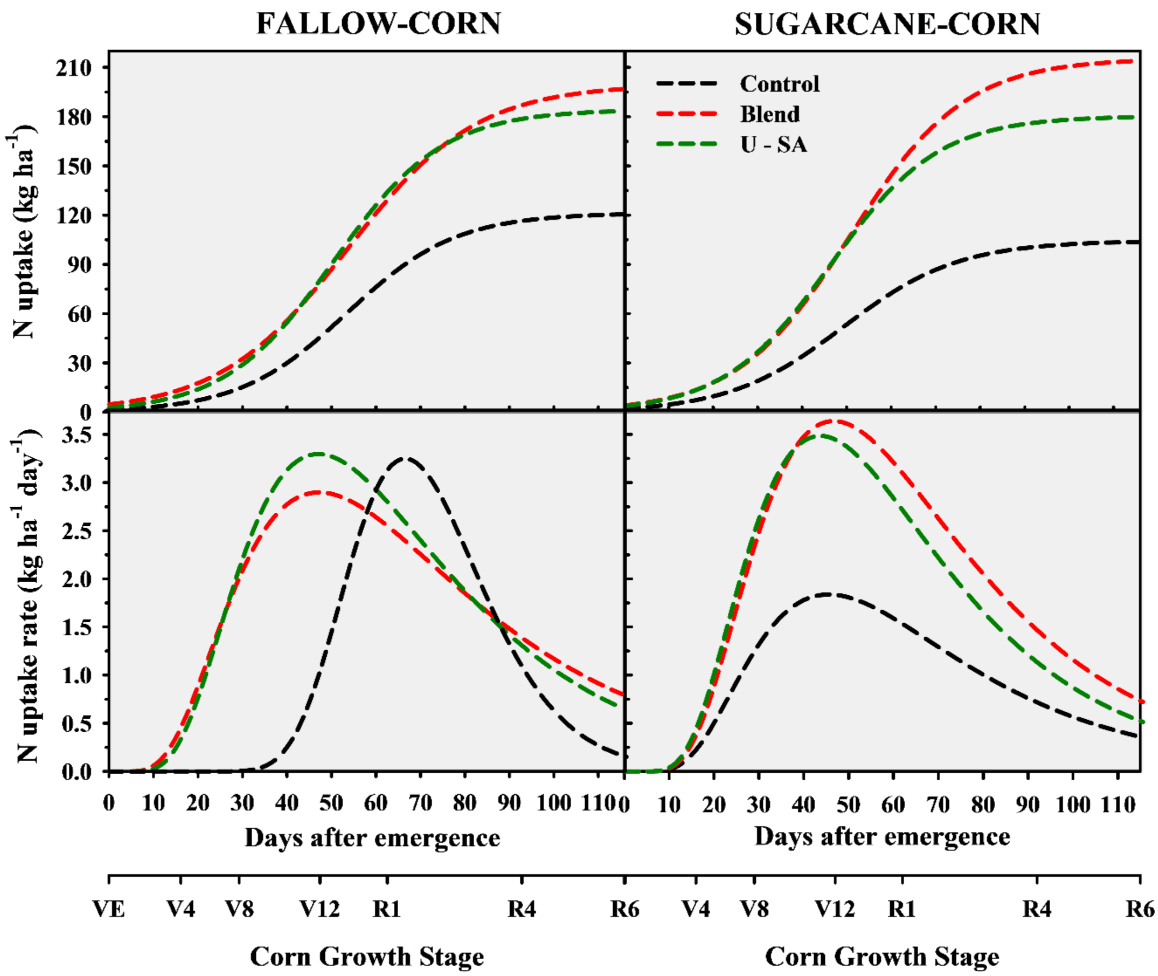

Figure 4.3. Seasonal whole plant nitrogen accumulation and uptake rate in two cropping systems (sampled at V4, V8, V12, R1, R4, and R6 growth stage). Average of two growing seasons for each cropping system.

4.2.1. Nitrogen accumulation and nitrogen accumulation rate

Nitrogen accumulation followed a similar pattern to the observed with BP (Figure 4.3). At the FC cropping systems, the amount of NU in the blend treatment was only slightly higher than the regular urea. The control, on the other hand, showed low values of NU. Regarding NU rate, a curious fact was observed at the FC. The model fitted to the NU at the control treatment resulted in a big offset of the maximum rate of NU. This was most likely due to high N deficiency during early growth stages in the control plots.

Interestingly, the maximum N uptake rate in the two fertilizer N treatments was around 45 days at the FC and around 48 at the SC. Therefore, the maximum NU rate occurred between 10 and 20 days in anticipation of the maximum BP rate observed. This observation raises the question about if it is not NU that drives BP. Could it be that the plants firs need to take up N to later increase BP (e.g. plant growth)? Although several authors defend the opposite idea (BP drives NU) (Cassman et al., 2002; Gastal and Lemaire, 2002), it represents a really good point for discussion and debate, even though highly complex physiological processes are involved. Schoninger (2014) and Gava et al. (2010) also found that the maximum NU rate occurred around 16 days before the maximum BP rate, also in Southeast Brazil. Furthermore, taking into account these values, we must ensure N availability for the plants around 40-55 days after planting, which would be probably around V9-V12, the period in which most N is taken up by corn plants (8-9 kg N ha^{-1} day^{-1}), according to Bender et al. (2013).

4.2.2. Nitrogen remobilization and N uptake after flowering

Nitrogen remobilization values and N uptake after flowering are shown in Table 4.2. Several authors have demonstrated that in modern corn hybrids, N uptake after flowering have increased significantly compared to later corn hybrids (Ciampitti and Vyn, 2011, 2013b; Bender et al., 2013; Schoninger, 2014; Chen et al., 2015; DeBruin et al., 2017). Therefore, N availability in late corn growth stages has become a major concern in corn production systems.

The values observed in this study are in agreement with those of the literature. High amount of N is taken up after corn flowering, varying between 31 and 48% of the total N at the FC cropping system, and from 52 to 79% at the SC cropping system. Thus, our results confirm that N availability late in the season is crucial for good crop development. And apparently, as time passes, and new hybrids are released, the dependence on N remobilization from vegetative parts is decreasing, even though it is still of high priority to provide a good N nutrition to corn plants in order to ensure good crop growth and development, and finally high grain yields.

Schoninger (2014) comments that the main source for N remobilization to the grains are the leaves, and that stalk has little total contribution. Therefore, any restriction on leaves N accumulation (such as diseases, weed infestation, pests) will have a direct and undesirable effect on grain N uptake.

Table 4.2. Nitrogen remobilization (kg ha^{-1} and % of total N uptake) and N uptake after flowering (kg ha^{-1} and % of total N uptake) in two corn cropping systems (average of two years each)

Treatment	Fallow-Corn				Sugarcane-Corn			
	Remobilized N		NU after flowering		Remobilized N		NU after flowering	
	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	%
Control	40 b	52	36 b	48	28 b	48	28	52
Blend	54 ab	44	38 b	56	67 a	41	52	59
U - SA	74 a	69	33 a	31	65 a	21	32	79
P > F	0.054	0.231	0.047	0.231	0.007	0.450	0.430	0.457

4.2.1. Biomass and nitrogen accumulation and partitioning

Biomass and N accumulation and partitioning are portrayed in Fig. 4.4 and 4.5, respectively. Understanding the allocation of biomass is important to understand the efficiency with which resources are used within the plant. Looking at both FC and SC biomass partitioning, it is noteworthy that at the FC cropping system, the decrease in vegetative plant parts biomass is more notable than at the SC. Maybe that happens because of the fact mentioned in Chapter 3, the high moisture grain harvest for beef cattle nutrition. Therefore, it may be that not all biomass (i.e. photosynthates) has been remobilized or translocated yet, and while remobilization is still active and happening, the crop is harvested already. The N treatments did not change much the trends observed in biomass partitioning. They did, however, affect the total biomass accumulated, as discussed before.

Similar trends were documented for N uptake and partitioning. At the FC cropping system, N remobilization was higher than at the SC. This may be due to the “earlier” harvest of the grains at this cropping system. As it happened with biomass, most of the N in the plant was harvested with the grain. However, there is a considerable amount of N left in the stalk, leaves, and cob, which depending on the management at each cropping system, will contribute with soil N and C dynamics.

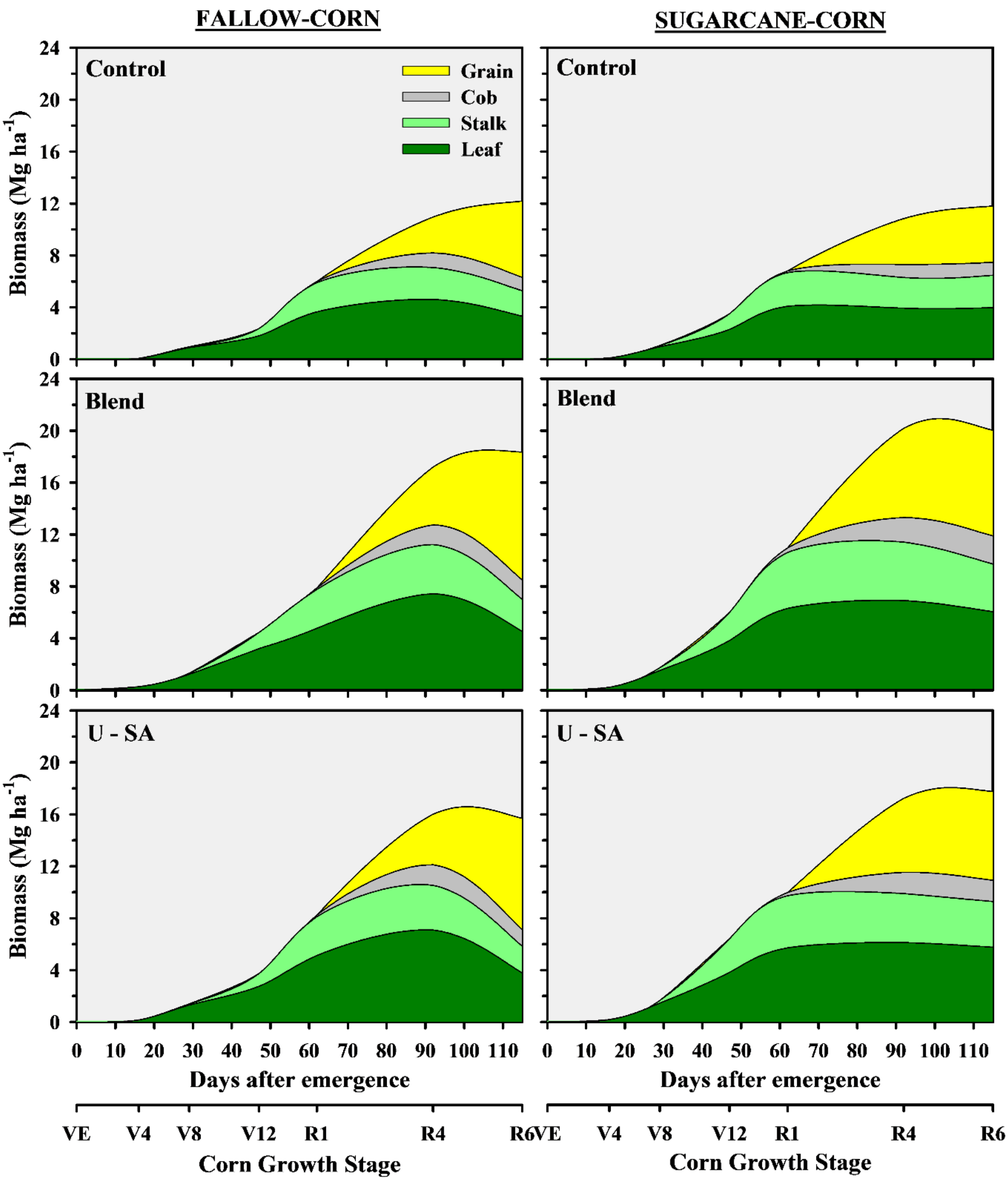

Figure 4.4. Biomass accumulation and partitioning (leaf, stalk, cob, grain) as affected by nitrogen fertilizer management in two cropping systems (sampling dates at V4, V8, V12, R1, R4, and R6 growth stage). Average of two growing seasons for each cropping system.

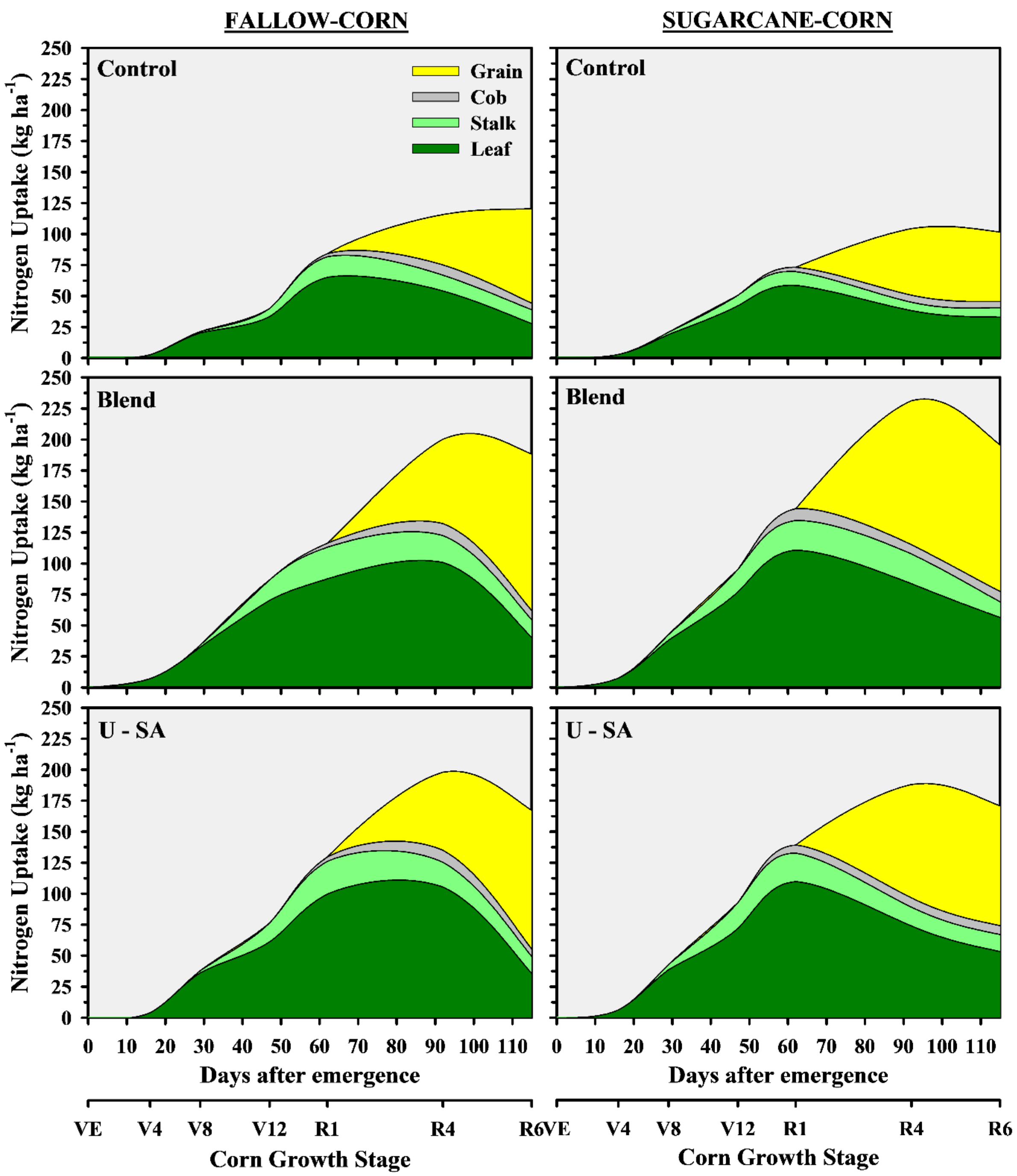

Figure 4.5. Nitrogen uptake and partitioning (leaf, stalk, cob, grain) as affected by nitrogen fertilizer management in two cropping systems systems (sampling dates at V4, V8, V12, R1, R4, and R6 growth stage). Average of two growing seasons for each cropping system.

4.3. CONCLUSIONS

Corn biomass and N uptake and partitioning were affected by N fertilizer. However, no much difference was observed between the two N fertilizer managmenet strategies evaluated. The data evidenced that N remobilization processes are altered by N fertilization.

The results of this study also demonstrate that in modern corn hybrids, more N is taken up after flowering, compared to older hybrids. Up to 50% of the total plant N could be taken up after corn flowering. Therefore, adequate N availability, either via soil organic matter mineralization or fertilizer N, must be guaranteed in late vegetative corn growth stages, and especially in the reproductive stages, which can be achieved by adopting enhanced efficiency N fertilizers such as the blend of PSCU and NBPTU used in this study. Plant N dynamics is complex, and the data gathered in this study will help to better understand N changes during the season.

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5. FINAL REMARKS

The increasing world population will continue to boost the demand for food, feed, fiber and energy (Godfray et al., 2010; Foley et al., 2011). As stated by Snyder (2017), fertilizer nitrogen (N) has been and will continue to be essential in nourishing, clothing, and providing bioenergy for the human family. At the same time, society will increase the pressure for minimizing the environmental footprint of N fertilizer use in agriculture (Zhang et al., 2015). Current values of nitrogen recovery efficiency (NRE) are low (Raun and Johnson, 1999; Ladha et al., 2005; Lassaletta et al., 2014; Pires et al., 2015; Zhang et al., 2015), and they are not sustainable in the long term. Therefore, we must increase NRE through agronomic practices in order to attain sustainable agriculture.

Nitrogen is essential for corn production and to attain optimal grain yield. Nitrogen management is very challenging due to the highly dynamic nature of this element in the soil-plant-atmosphere system (Galloway et al., 2008). However, between the proposed alternatives to improve corn (and other crops) grain yield at the same time of minimizing the environmental costs of N fertilization, the use of enhanced efficiency N fertilizers has received a lot of attention lately. Research on enhanced efficiency N fertilizers has increased dramatically in the last decade (Chalk et al., 2015; Snyder, 2017), and will very likely continue to increase. Several reports in the literature have demonstrated crop yield and NUE increase, accompanied by economic profits when using enhanced efficiency N fertilizers (Halvorson and Bartolo, 2014; Shapiro et al., 2016; Zheng et al., 2016, 2017; Guo et al., 2017). However, most of these studies have been conducted outside Brazil, under different soil and climate conditions. The few available data on enhanced efficiency fertilizer for corn production in Brazil are not replicated in years (González-Villalba, 2014; Garcia et al., 2018) or in space (Cancellier et al., 2016), therefore they provide very local information, not allowing to generalize the results and transpolate to other corn production areas. Therefore, a multi-year and site study was needed to allow broader considerations about enhanced efficiency N fertilizers performance in corn production systems.

This study consisted of the application of increasing N rates of a blend of polymer-sulfur coated urea (PSCU), a controlled release N fertilizer, and NBPT-treated urea, a stabilized urea. The N fertilizers were applied at corn planting, incorporated into the soil, aiming to minimize N losses through ammonia volatilization (Rochette et al., 2013). The study was conducted in Southeast Brazil, in two contrasting cropping systems.

The second chapter of this thesis focused on the recovery efficiency of N fertilizer from a blended application of PSCU and NBPTU (70:30 ratio) and the allocation of fertilizer derived N in corn plants. The results demonstrated most of the plant N was allocated in the grains (59%) at harvest. Fertilizer N contributed with less than 50% of the total plant N uptake at all evaluated corn growth stages. The results of this study indicated that NBPTU provided N to corn early in the season, while PSCU played a crucial role supplying N later in the season, as plants demand for N increased. Soil N (i.e. soil organic matter) was the main N source at all GS and this fraction decreased as N rate increased. At harvest, 64% of the total plant N was derived from the soil native N pool, 26% derived from PSCU, and 10% from urea. The measured fertilizer NUE of urea was in average 36%, and the estimated NUE from PSCU was 51%.

The third chapter of this thesis was directed at the effect of increasing N rates (blend) in corn grain yield and the possibility of reducing N rates using the blend compared to the application or regular urea. The results showed a great variation in corn grain yield, N uptake, and biomass production between sites and as a consequence of fertilizer N application. Grain yield and N uptake showed a quadratic response to N rates (blend). The blend of PSCU and NBPTU, applied at corn planting and incorporated into the soil in order to prevent N losses, proved to be a great strategy to attain yields at N rates below those needed (100% rate reduction) when using regular urea.

The fourth chapter deals with corn biomass and nitrogen uptake and partitioning. Valuable information was gathered in this study, which could be at some point, be used to pool other similar data of other corn production systems and develop nitrogen nutrition indexes as a diagnostic tool to monitor crop N status. Nitrogen uptake after flowering is crucial for modern corn hybrids, and this was demonstrated with our results. Therefore, ensuring N availability for corn plants after flowering will be a challenge, which can be overcome with the uses of enhanced efficiency N fertilizers that help securing N in the soil until late crop growth stages.

It is not about being an enthusiast of a certain N fertilizer type or technology. We must understand that there are too many factors affecting NRE and crop yields, therefore it is clear that one technology will not help resolve all of our problems. However, it is interesting to look at the results, reflect on them and understand that enhanced efficiency N fertilizers are a great, real, and available alternative to improve NRE and crop yields in different crop production systems. Thus, this technology represents an additional tool to be added to the "fertilizer N management toolbox". Clearly, the adoption or not of any technology or innovation will ultimately depend on each farmer at each specific situation. And of course, all decision-making in this sense will ultimately be linked to an economic analysis and assessment of the tradeoffs between crop yield gains and fertilizer costs, analysis which is missing in this thesis and prevented us from having a better idea of the real viability of this N management strategy.

Food quality (i.e. nutrient content) will be given even more importance in the future decades (Wood et al., 2018). Monitoring N concentration and N uptake in crops will play a crucial role, thus the development of N nutrition indexes with critical N concentration curves (Gastal and Lemaire, 2002; Lemaire et al., 2008; Sadras and Lemaire, 2014), as in-season diagnostic tools of the N status of different crops could also play an important role on improving N management.

Further integration of enhanced efficiency N fertilizers within the 4R framework (IFA, 2009), together with the adoption of current technologies, such as precision agriculture tools, modern farm equipment, geographic information system, in season N sensors, and all available tools to improve on farm N management should be integrated in order to pursue the objective of improving NUE.

Other simple but key management practices should not be overlooked. It is known that the inclusion of cover crops in the cropping system improves NUE (Fontes et al., 2017), in addition to the ecosystem services they provide (Frasier et al., 2016). Moreover, adequate soil correction (Caires et al., 2016), use of appropriate crop rotations, adoption of conservation tillage, correct control of insects, diseases, and weeds, all are important crop management strategies that help improving NRE (Fageria and Baligar, 2005).

Besides the strictly scientific purposes, this thesis also had practical purposes. We showed with this study that fertilizer N incorporation is possible in no-tillage systems with a smart and integrated use of technology. Even in the worst possible scenario (i.e. no-till over sugarcane residues), where an average of 16-18 Mg ha^{-1} of sugarcane straw was left on the soil surface, we were able to incorporate fertilizer N, which represents sort of a paradigm for a lot of no-till practitioners who firmly believe it is impossible.

Finalizing, we emphasize that the right approach to close up the gap existing between academia, the industry sector, extension agents, and agriculture practitioners is to perform agricultural experimentation with the necessary scientific rigor in partnership with the industry sector and farmers, such as we did in this study. If we get to integrate these views for most research activity, we will attain a long time chased goal: agricultural sustainability.

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