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Response of bermudagrass to enhanced-efficiency fertilizers, application strategies and release under tropical conditions

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Abstract

Nitrogen fertilization is an important input for crop yield; however, it can result in detrimental environmental effects due to low use efficiency of regular N sources. This study evaluated the effects of N fertilizers and application strategies (single vs. split application) on bermudagrass (*Cynodon* spp.) responses and release pattern and rate in controlled and field incubations. The bermudagrass study was arranged in a two-way factorial scheme of 6 N fertilizers, urea, Polymer Coated Urea (PCU), PCU-6 (6 months), PCU-4 (4 months), PCU-2 (2 months) and urea + urease inhibitor (U-NBPT) applied as a single (400 kg N ha^{-1} yr^{-1}) or two split applications of 200 kg N ha^{-1} (400 kg N ha^{-1} yr^{-1}). The controlled experiment was a two-way factorial of PCU-6, PCU-4, PCU-2 and 15, 45 and 90% water hold capacity (WHC), sampling period of 170 days, the field incubation used the same sources sampled up to 220 days. Enhanced-efficiency fertilizers (EEF) increased herbage accumulation (HA) by 1.3 Mg ha^{-1} compared to untreated urea, on average. Nitrogen use efficiency (NUE) was greater for EEFs (44%) than urea (36%). Results showed that increased soil moisture inferred positive responses in release pattern and a minimum of 45% WHC was necessary for optimum release. Fertilizers at field conditions resulted in an earlier release than expected, ~20 days.

Keywords: Nitrogen; Forage; Urease inhibitor; Polymer-coated urea; Use-efficiency.
Abbreviations: EEF_enhanced efficiency fertilizer; HA_herbage accumulation; NBPT_(n_butyl)_thiophosphoric triamide; NUE_nitrogen use efficiency; WHC_water hold capacity.

Introduction

Adequate N supply is essential to obtain relatively high forage production levels, particularly in intensively managed production system. However, due to the potential of causing environmental problems (Connell et al., 2011), N fertilizer management has become a topic of global importance and increased public attention. Nitrogen losses can be harmful to the environment resulting in unintended detrimental effects on water and air quality. Water quality problems are typically associated with nitrate leaching (Silveira et al., 2007), while ammonia volatilization and nitrous oxide emissions are the primary sources of air contamination (Cabrera et al., 1991). Technologies that promote better crop NUE while protecting the environment are vital to agriculture, food security, and society as a whole.

Improved fertilizer sources are expected to be advantageous, especially in tropical regions subjected to heavy rainfall events, where relatively poor crop N use is often associated with high N losses (Shoji et al., 2001; Drost et al., 2002). However, the major limitations associated with the wide spread use of improved fertilizer sources are their high cost and lack of information on crop responses. Although the technology of the coating material has evolved N release from these sources are sometimes unpredictable, especially under different field conditions (Cabrera, 1997; Halvorson and Bartolo, 2014).

Reports on crop performance responses to EEFs technology have been inconsistent. For instance, in a study conducted with a bermudagrass under a temperate climate in silt loam and loam soils fertilized with PCU receiving 336 kg N ha^{-1} per year, there was a decrease of 11% in HA and 20% in agronomic efficiency (kg kg^{-1} N) when compared to urea-ammonium nitrate and urea treated with urease inhibitor N-(n-butyl)-thiophosphoric triamide (NBPT), respectively (Silveira et al., 2007). Similar results were also observed in maize (*Zea mays* L.) (Grant et al., 2012), canola (*Brassica napus* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum spp*. L.) (Grant et al. 2012) and other warm-season forage species (Karamanos and Stevenson, 2013). However, others reported positive effects of PCU and stabilized urea compared to untreated urea on maize (Gagnon et al., 2012; Halvorson and Bartolo, 2014) and barley yield (Blackshaw et al., 2011).

Because EEFs are often claimed to be more efficient than traditional fertilizer sources, reduced application levels have been suggested as a viable approach to reduce the risks of N losses while sustaining the same level of crop production. In a study with wheat, (Zhang et al., 2010) indicated that reduced level of NBPT-treated urea resulted in similar DM yield and NUE and greater shoot N concentration than untreated urea. The latter authors suggested that greater shoot N concentrations were likely due to less ammonia (NH_3) volatilization in NBPT treatments compared to urea alone.

The inconsistent reports in the literature suggest that the positive impacts of EEFs on agronomic performance will likely vary depending on climatic, edaphic, and management conditions (Nelson et al., 2009). Further research is warranted to evaluate the economic/agronomic benefits and potential trade-offs associated with utilization of alternative fertilizer sources (Zhengping et al., 1991a; b; Farmaha and Sims, 2013), especially in tropical conditions, where uneven rainfall distribution throughout growing seasons may also affect crop response.

Therefore, the hypothesis of this study was that EEFs will promote greater bermudagrass HA, NUE and agronomic efficiency than untreated urea independently of the application strategy. The objective of the study was to evaluate the effects of different N sources and application strategies on intensively-managed bermudagrass hay production system in Brazil.

Results

Shoot N concentration and herbage accumulation as affected by N sources and application strategy

There was a fertilizer source × harvest interaction effect on shoot N concentration (Table 1). Although shoot N concentrations were lower for Urea than PCU-4, no treatment differences were observed on the last harvest. PCU-2 and U-NBPT resulted in the greatest shoot N on the first harvest, 23.1 and 21.5 g kg^{-1}, respectively, PCU-4 and PCU-6 presented higher values on the second and third harvest when compared to standard Urea. Regardless of the N source, shoot N generally decreased as the growing season progressed.

Shoo N concentration was also affected by the application strategy × harvest interaction (Table 2) in which the single application resulted in a decrease in shoot N concentrations of ~ 35% during the experimental period. Conversely, at the third harvest, split application increased shoot N concentration by 20% compared with single application. No effects of fertilizer application strategy were observed in the last harvest.

The HA was affected by the fertilizer source × harvest interaction (Table 1). The HA generally decreased as the growing season progressed. Treatments receiving urea and PCU decreased HA by ~600% while a ~400% decreased was observed for U-NBPT treatments from first to last harvest. Although U-NBPT resulted in the greatest HA in the first harvest, slow release fertilizers PCU-2, PCU-4 and PCU-6 resulted in the highest HA, average. The PCU-2, PCU-4, and PCU-6 reported a bermudagrass HA ~88% greater than other N fertilizer sources (Table 1).

Bermudagrass HA was also affected by the application strategy × harvest interaction (Table 2). When N was applied as single application, HA decreased by ~3.8 Mg ha^{-1} from first to last harvest. The split application treatments resulted in a ~2.2 Mg ha^{-1} decrease in HA, from first to last harvest. Single application increased HA in the first and second harvests, however, greater HA was associated with split N application during the third and fourth harvests.

Cumulative HA was also affected by the fertilizer × application strategy interaction (Table 3) the sources that presented the highest cumulative production compared to Urea were the PCU-2 when single applied and the U-NBPT when applied split, with an increase of 2.8 and 2.9 Mg ha^{-1}, respectively.

Forage nitrogen use efficiency and agronomic efficiency

The NUE and agronomic efficiency were not affected by fertilizer sources × application strategy interaction, therefore, single effects of fertilizer sources and application strategy (P ≤0.05) will be discussed here. NUE was affected by fertilizer source (P =0.005), it ranged from 50% to 39%, U-NBPT and PCU, respectively (Table 4).

The agronomic efficiency was affected by both N source and application strategy. U-NBPT increased agronomic efficiency by 6 kg kg^{-1} more than untreated urea, which correspond to an increase of 35%. The split application increased agronomic efficiency by ~10% compared to single application.

Nitrogen releasing pattern from fertilizers incubated in controlled environment and field studies

There was a cumulative N release effect on fertilizer source × moisture interaction (Table 5). The 90% WHC promoted the highest N releases for all sources, but for PCU-2. The 45% WHC presented similar responses as 90% for PCU-6 and PCU-2. The PCU-6 and PCU-4 did not release 80% of their N content independently on the adopted WHC (Table 5). The only source able to release 80% of its N content was PCU-2, however 50 days later than expected (Fig 1).

Fertilizer on field incubation did not present differences on cumulative N release after 220 days and by the end of the experimental period all source had released at least 80% of its total N content (Table 5). Releasing pattern of all sources showed a quadratic adjust, PCU-6, PCU-4 and PCU-2 which they reached 80% N release at 110, 130 and 35 DAI, respectively (Fig 2).

Discussion

Shoot N concentration and HA as affected by N sources and application strategy

Despite the relatively smaller HA and shoot N concentrations associated with urea treatments, urea increased HA and shoot N relative to control treatments. This means that although urea may not be the most efficient N fertilizer source, it can still sustain satisfactory levels of forage production (Silveira et al., 2007; Borges et al., 2017). Our data are in agreement with previous studies which demonstrated that bermudagrass responded positively to N fertilizations (Silveira et al., 2007; Alderman et al., 2011; Sohm et al., 2014; Borges et al., 2017). However, bermudagrass response to N fertilization can be improved by

Table 1. Bermudagrass shoot N concentration and herbage accumulation as affected by fertilizer sources × harvest interaction.

	Shoot N concentration				Herbage accumulation			
				Harvest				
Fertilizer	1	2	3	4	1	2	3	4
	------------------ g kg^{-1} ------------------				----------------- Mg ha^{-1} ------------------			
U-NBPT	21.5†abA‡	19.5bA	19.7abA	17.7aB	4.9aA	1.9aC	2.7aB	1.2bD
PCU	20.0bA	19.7abA	18.6bA	17.6aB	4.3bA	1.3bC	2.6abB	0.7bD
PCU-2	23.1aA	18.7bB	18.5bB	18.0aB	4.4bA	1.8aC	2.8aB	1.3aC
PCU-4	20.0bAB	21.9aA	18.9bB	18.9aB	2.7bA	2.1aB	2.5abAB	2.1aB
PCU-6	19.2bAB	20.2abAB	21.0aA	18.1aB	3.7bA	1.6abC	2.5abB	1.6aC
Urea	20.0bA	18.0bA	18.9bA	17.2aB	4.3bA	1.2bC	2.2bB	0.7bD
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

†Data are means across two application strategies and three replicates.

‡Means within column followed by different lower-case letter or row by different upper-case letter are different using the LSMEANS/difflsmeans procedure (*P* >0.05).

U-NBPT – urea treated with urease inhibitor N-(n-butyl)-thiophosphoric triamide; PCU – Polymer-coated Urea; PCU-2 – Polymer-Coated Urea, 2 months; PCU-4 – Polymer-Coated Urea, 4 months; PCU-6 – U-NBPT – urea treated with urease inhibitor N-(n-butyl)-thiophosphoric triamide; PCU – Polymer-coated Urea; PCU-2 – Polymer-Coated Urea, 2 months releasing time; PCU-4 – Polymer-Coated Urea, 4 months releasing time; PCU-6 – Polymer-Coated Urea, 6 months releasing time.

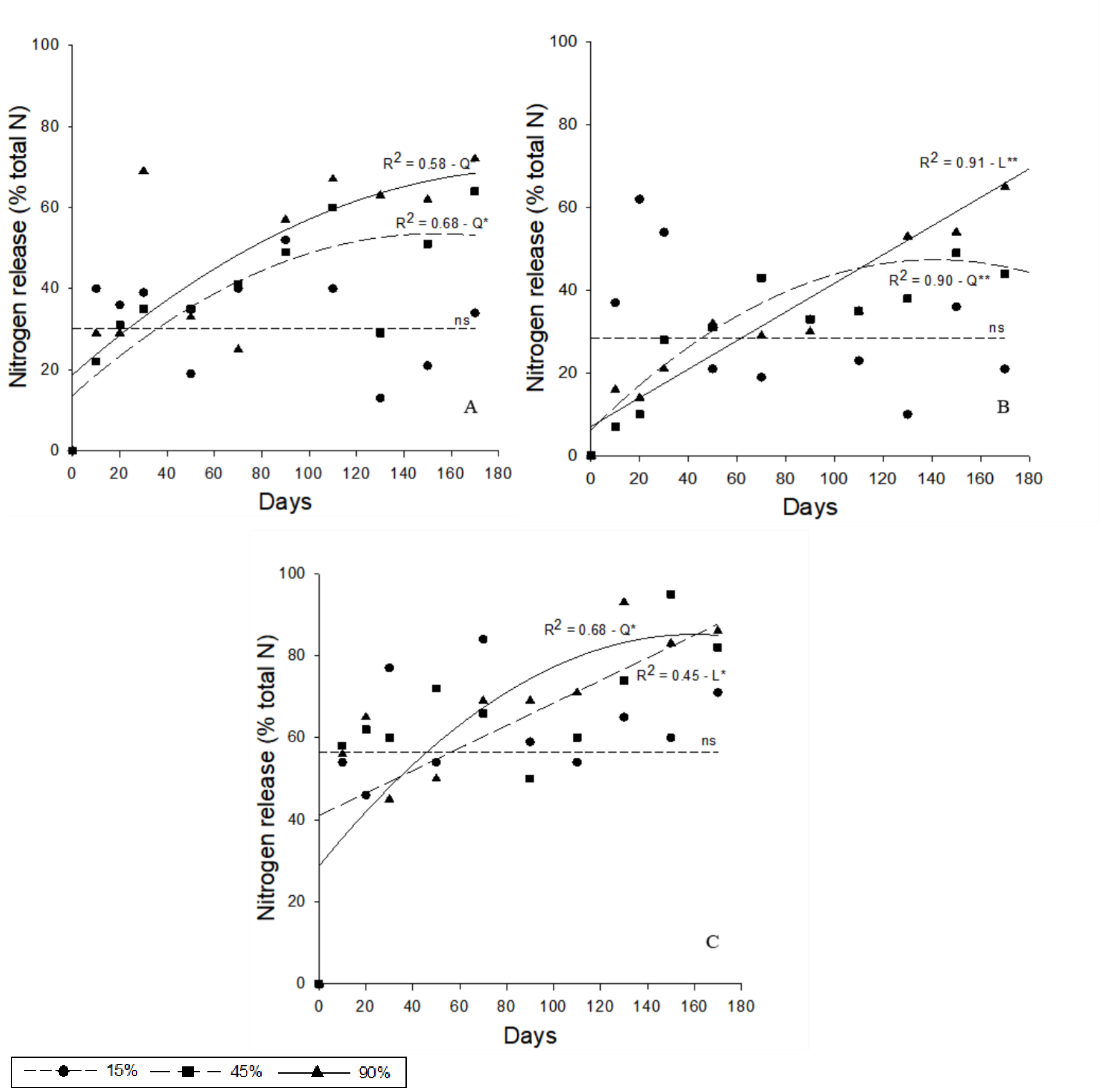

Fig 1. Nitrogen release from fertilizers over 170 days after application in an incubation study with different soil water hold capacity. Total N was analyzed by an elemental analyzer using combustion method. Each value is a mean of 2 readings. **A)** PCU-6, **B)** PCU-4, **C)** PCU-2. *, ** and ^{ns} Significant at the 0.05, 0.01 probability levels and non-significant, respectively. Q and L, quadratic and linear adjusts, respectively.

Table 2. Bermudagrass shoot N concentration and herbage accumulation as affected by application strategy × harvest interaction.

Strategy	Shoot N concentration				Herbage accumulation			
	Harvest							
	1	2	3	4	1	2	3	4
	------------------ g kg^{-1} ------------------				------------------ Mg ha^{-1} ------------------			
Single	22.7†aA‡	20.8aB	17.1bC	17.4aC	4.6aA	2.2aB	1.4bC	0.8bD
Split	18.5bB	18.5bB	21.4aA	18.4aB	3.4bA	1.0bC	3.7aA	1.7aB
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

†Data are means across five fertilizers and three replicates.

‡Means within column followed by diffeent lower-case letter or row by different upper-case letter are different using the LSMEANS/difflsmeans procedure (*P* >0.05).

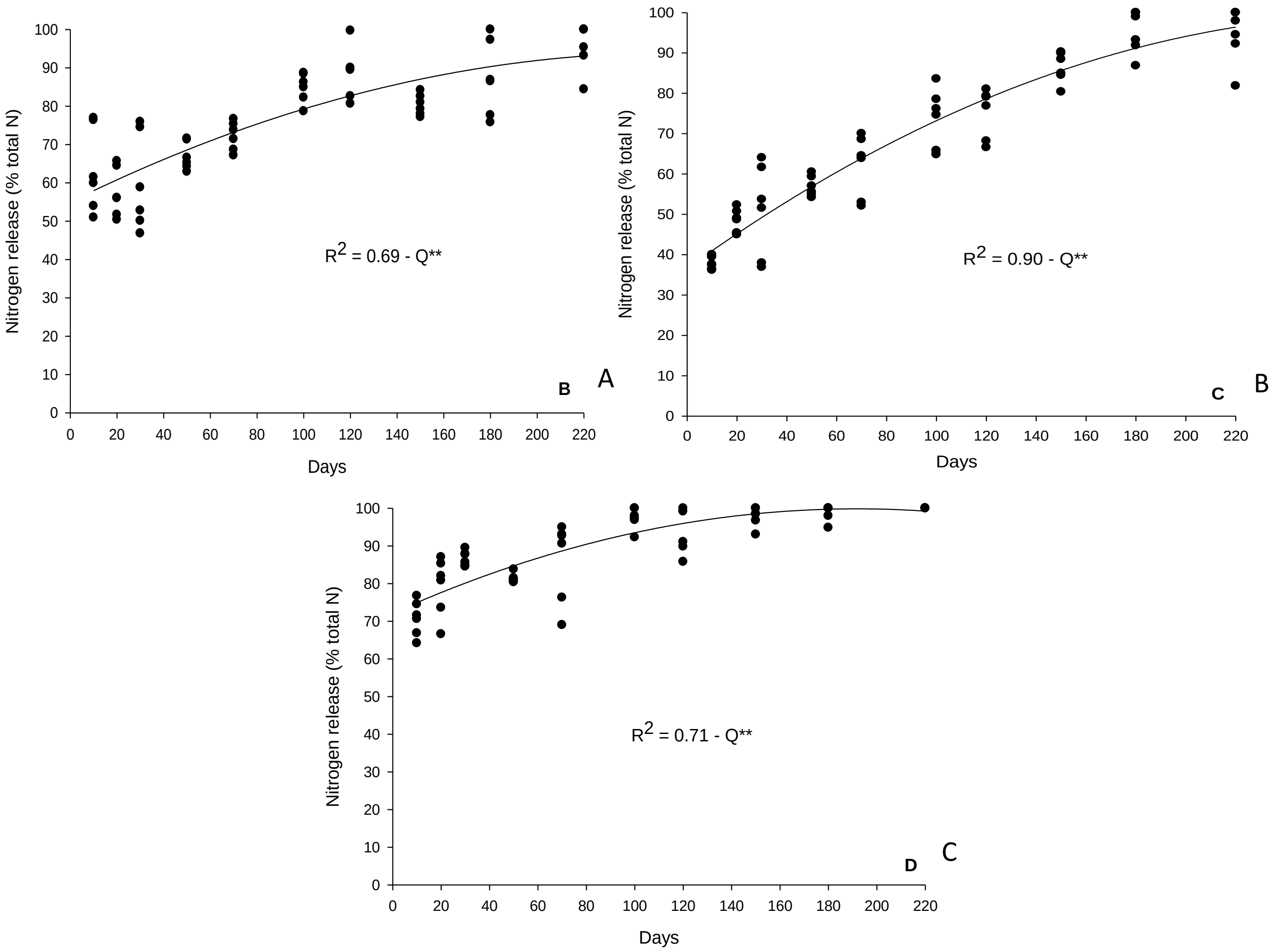

Fig 2. Nitrogen release from fertilizers over 220 days after application in a field incubation study. Total N was analyzed by an elemental analyzer using combustion method. Each value is a mean of 2 readings. **A)** PCU-6, **B)** PCU-4, **C)** PCU-2. ** Significant at the 0.01 probability level. Q quadratic adjusts.

Table 3. Bermudagrass average monthly and cumulative herbage accumulation (HA) as affected by fertilizer sources × application strategy interaction.

Fertilizer	Monthly HA		Cumulative HA	
	Single	Split	Single	Split
	------------------ Mg ha^{-1} ------------------			
U-NBPT	2.3†aB‡	3.1aA	9.1abB§	12.2aA
PCU	2.1abA	2.3bA	8.3abA	9.4bA
PCU-2	2.6abA	2.5bA	10.2aA	10.2abA
PCU-4	2.4abA	2.3bA	9.7abA	9.2bA
PCU-6	2.3abA	2.2bA	9.3abA	9.0bA
Urea	1.9bB	2.3bA	7.4bB	9.3bA
P-value	<0.001	<0.001	0.044	0.008

†Data are means across two application strategies and three replicates.

‡Means within column followed by different lower-case letter or row by different upper-case letter are different using the LSMEANS/difflsmeans procedure (*P* >0.05).

U-NBPT – urea treated with urease inhibitor N-(n-butyl)-thiophosphoric triamide; PCU – Polymer-coated Urea; PCU-2 – Polymer-Coated Urea, 2 months releasing time; PCU-4 – Polymer-Coated Urea, 4 months releasing time; PCU-6 – Polymer-Coated Urea, 6 months releasing time.

Table 4. Bermudagrass nitrogen use efficiency (NUE) and agronomic efficiency as affected by fertilizer sources and application strategy.

Treatments	NUE	Agronomic efficiency
Fertilizers (F)	%	kg kg^{-1} N
U-NBPT	50a†	23a
PCU	39bc	18ab
PCU-2	48ab	22ab
PCU-4	42abc	20ab
PCU-6	41abc	19ab
Urea	36c	17b
P-value	0.005	0.012
Strategies (S)		
Single	42	19
Split	43	21
P-value	0.690	0.031
Interaction	-------------------------------------- *P*-value	------------------------------------
(F) × (S)	0.263	0.064

†Means within column followed by different lower-case letter are different using the 'Tukey' test (P >0.05).
U-NBPT – urea treated with urease inhibitor N-(n-butyl)-thiophosphoric triamide; PCU – Polymer-coated Urea; PCU-2 – Polymer-Coated Urea, 2 months releasing time; PCU-4 – Polymer-Coated Urea, 4 months releasing time; PCU-6 – Polymer-Coated Urea, 6 months releasing time.

Table 5. Cumulative N released (% initial N) during the laboratory and field incubation studies. Values represent percentage of total N released from each N source.

Fertilizer	Laboratory incubation water hold capacity			Field incubation
	15%	45%	90%	
PCU-6	34† bB‡	64 abA	72 aA	92^{ns}
PCU-4	20 bC	45 bB	73 aA	90
PCU-2	71 aA	82 aA	86 aA	100

† Values represent the average across 3 replicates.
‡Different lowercase letters within a column are significantly different at $P \leq 0.05$; Different uppercase letters within row are significantly different at $P \leq 0.05$; ^{NS} = not significantly different (P >0.05).

selecting and properly managing different fertilizer sources. Based on Kelling and Matocha (1990), shoot N concentrations in the current study were below the critical range of 21-26 g kg^{-1} for forage production but were above the deficient plant nutritional status of <15 g kg^{-1}. The only exception was the control treatments (no N added) that exhibited shoot N below values considered deficient (data not shown).

The poor performance of urea observed in the current study contradicted previous studies that reported no differences or even detrimental effects of EEFs compared to untreated urea (Connell et al., 2011; Grant et al., 2012; Karamanos and Stevenson, 2013). Potential reduction in crop productivity was often associated to a nutritional deficiency as a result of reduced levels of N released from PCUs in early stages of plant development (Connell et al., 2011). Conversely, our results were in agreement with data reported by Blackshaw et al. (2011), Gagnon et al. (2012) and Halvorson and Bartolo (2014) which showed that EEFs can outcompete urea, but the agronomic benefits also depend on application strategy.

Although cumulative HA was not affected by the method of fertilizer application, highest monthly HA was generally associated with the single application. This response is consistent with previous studies that also concluded that bermudagrass can efficiently uptake and assimilate N (Alderman et al., 2011), even when N is applied at levels higher than 200 kg N ha^{-1}. Bermudagrass positive responses to relatively high N levels such as 400 to 670 kg N ha^{-1} yr^{-1} have been reported in previous studies under similar environmental conditions (Sohm et al., 2014).

Results indicated that split application of readily available N sources (U-NBPT and urea) resulted in higher cumulative HA while no differences in application strategies were observed for PCU sources. The highest HA associated to the split application of U-NBPT was consistent with the readily available nature of the fertilizer source. The main reason for the superior performance is that U-NBPT was designed to reduce urea hydrolysis and, consequently decrease N losses by volatilization (Rogers et al., 2015). On the other hand, PCU N fertilizers can be a viable tool to increase crop yield with a lesser number of applications in reason to their ability to provide a continuous supply of N during the crop's cycle (Shaviv, 2005). These results corroborated previous studies with corn (*Zea mays*) (Gagnon et al., 2012; Halvorson and Bartolo, 2014) and barley (*Hordeum vulgare*) (Blackshaw et al., 2011) that also reported an increase in crop production in response to PCU compared to conventional N fertilizers.

Forage nitrogen use efficiency and agronomic efficiency

NUE followed a similar pattern as cumulative HA. Nitrogen fertilizer sources that resulted in greater cumulative HA (PCU and U-NBPT) also promoted NUE. Although NUE observed in the current study were < 50% , these values were in agreement with previous ^{15}N studies in bermudagrass swards (Borges et al., 2017).

Ammonia volatilization represent an important pathway of N losses in agricultural soils worldwide especially when N-fertilizer is surface applied (Massey et al., 2011). Studies demonstrated that as much as 20% of applied N can be volatilized when urea is applied over plant residues (dead litter) (Fox et al., 1986; Hargrove 1988). Since bermudagrass pastures often contain appreciable amounts of dead plant residue materials on the soil surface, it is expected that ammonia volatilization represents a substantial loss of N. A study with sugarcane (*Saccharum* spp.) under different environmental conditions reported that U-NBPT was able to decrease N losses by as much as ~36% compared to untreated urea, mainly due to a reduction in ammonia volatilization (Cantarella et al., 2008). Therefore the use of urease inhibitors increased crop productivity and NUE (7.5% and 12.9%, respectively) (Abalos et al., 2014).

Nitrogen releasing pattern from fertilizers incubated in controlled environment and field studies

Data presented in controlled (Fig 1) and field (Fig 2) incubation studies showed that fertilizers maintained N release rate up to 150 days after incubation. This result indicates that PCU fertilizers can potentially maintain a certain level of available N in the system during the entire experimental period. However, the amount and time of N release by the sources were different, *e.i.* PCU-2 released the greatest amount of N and in the shortest period (Table 5). According to Adams et al. (2013) nutrient release rate and patter from polymer-coated fertilizers are influenced by temperature and moisture.

Regarding temperature, at the experimental area, it was above 20 ^{o}C, which, according to the same authors, should be enough for this kind of fertilizer to maintain a constant release. Another point to be highlighted is that PCU 2 was the only source that showed no difference among moisture regimes and the source that released the highest amount of N in the 15% soil moisture this can be explained by its earlier release characteristic compared to the others. On the other hand, the 90% soil moister was able to promote the same level of cumulative N released for all sources, showing how influenced by moisture the pattern and rate were affected (Table 3). This might be explained because only when soil is above 50% WHC nutrient release from fertilizer should be constant and uniform (Kochba et al., 1990).

Materials and methods

Experimental area

The experiment was conducted in an established 'Tifton 85' hay field at the Sao Paulo State University Campus of Jaboticabal, SP, Brazil (21°15'22" S, 48°15'18" W, 600 asl) on a Rhodic Haplustox clay soil. Initial soil characterization to a depth of 0-0.1 m showed the following values; pH ($CaCl_2$) 4.8; organic matter 35 g kg^{-1}; P (resin) 12 mg kg^{-1}; K, Ca, Mg, CEC of 0.5; 3.0; 1.7; 11.4 cmol$_c$ kg^{-1}, sand, silt and clay of 280, 141 and 579 g kg^{-1}, respectively. Average daily temperature and rainfall data were collected during experimental period (Borges et al., 2017).

Treatments and forage response

The field research was conducted from 26 Nov. 2012 to 26 Mar. 2013 to evaluate bermudagrass response to urea-based N sources and application strategies. The experiment was arranged as a two-way factorial in a complete randomized block design 3 three replicates. Plots were 25 m^2 (5 × 5 m) with a 2-m alley between plots. Response variables included HA and shoot N concentration.

Treatments consisted of untreated urea (46% N); four polymer-coated urea sources [Polymer-coated Urea, 46% N (PCU), according to the manufacturer this fertilizer is coated with two layers of additives – mineral and organic – and an external polymer layer; Polymer-Coated Urea, 37% N, 6 months, period of time which fertilizer releases 80% of its total N (PCU-6); Polymer-Coated Urea, 38% N, 4 months (PCU-4); Polymer-Coated Urea, 39% N, 2 months (PCU-2)]; urea treated with urease inhibitor (Urea + NBPT, 45% N (U-NBPT) and a control treatment (no N added). Fertilizers were surface broadcast either as a single application of 400 kg N ha^{-1} at the beginning of the season or two split applications of 200 kg N ha^{-1} each, one at the beginning of the season and the other after the second harvest.

The entire experimental area was fertilized with 51 kg P ha^{-1} and 52 kg S ha^{-1} as single superphosphate 15 days before first N application. Plots received 66 kg K ha^{-1} as potassium chloride with the first N application and after each harvest. Fertilizers were manually broadcasted at the surface of each plot. Bermudagrass was harvested to a 7-cm stubble height at 30 days intervals for a total of four harvesting events.

Samples were weighted fresh at the field and a sub-sample (0.5 kg) was oven dried at 65^{o}C for 72 h for HA determination. Dry samples were ground in a Wiley mill through a 1 mm sieve and analyzed for total Kjeldahl N. The NUE$_{[1]}$ and AE$_{[2]}$ were calculated by the following equations (Moll et al., 1982):

[1] $$\text{NUE (\%)} = \frac{\text{N uptake (fertilized plot)} - \text{N uptake (control plot)}}{\text{N rate applied}} \times 100$$

[2] $$\text{Agronomic efficiency (kg kg}^{-1}\text{ N)} = \frac{\text{yield fertilized plot} - \text{yield unfertilized plot}}{\text{N application rate}}$$

Releasing pattern in controlled moisture and field study

The controlled experimental design was a complete randomized design with three replicates in a factorial scheme of three N sources, PCU-6, PCU-4, PCU-2, and three soil moisture regimens 15, 45 and 90% of WHC. Approximately 66 g of air-dried soil was placed in 100 ml polyethylene flasks (density 1.3 Mg m^{-3}) and fertilizer were subsequently surface-applied at the rate of 150 kg ha^{-1} N (wt basis). Flasks contained a lid with five perforations to allow gaseous exchange (Fujinuma et al., 2009). Laboratory incubation was carried out for 170 days under constant temperature (24 ± 1 ºC) in a dark room. Flasks were weighed every other day to ensure moisture conditions. Fertilizers remaining at soil surface were collected every 10 days during the first 30 days and every 20 days to the end. At each sampling, three flaks of each source were destructively sampled, fertilizers were collected, sieved to be separated from soil and air-dried until constant weight.

The field study was arranged in complete randomized design, fertilizers were the same as in the controlled study. The equivalent of 400 kg ha^{-1} N for each source was placed in 0.1 x 0.1 m polyethylene bags, 18 mesh and heat-sealed along four edges. The bags were placed on the surface of the soil in a Tifton 85 hay field in order to simulate the surface broadcast of fertilizers. Samplings were performed from November/2012 to July/2013 at 10 – 20 – 30 – 50 – 70 – 100 – 120 – 150 – 180 – 220 days after incubation (DAI) collecting three bags in each sampling period, for each treatment.

Nitrogen release was determined by dry combustion using a LECO FP-528 Total Nitrogen Analyzer (LECO, St Joseph, MI). At each sampling event, remaining N (expressed in grams of fertilizer) was calculated multiplying the N concentration by the mass of the prills. Nitrogen release was calculated according to the following equation:

$$\%\mathrm{NR_C} = \left[1 - \left(\frac{\mathrm{N_s}}{\mathrm{N_i}}\right)\right] \times 100$$

which $\%NR_C$ is percentage of initial N released determined by combustion method, N_i and N_s are the N contents (mass of fertilizer × N concentration) determined at the beginning and at each sampling event, respectively.

Statistical analysis

Response variables, HA and shoot N concentration for each harvest, were analyzed by a linear mixed model using R 3.4.1 by the lmerTest function from package lmr4 (Bates et al., 2015). Nitrogen sources, fertilizer application strategy and harvests were considered fixed effects and replicates were random effects. Treatments were considered different when $P \leq 0.05$. Interactions not discussed here were not significant ($P > 0.05$). The mean values presented were least square means in which were compared using difflsmeans function (Lenth, 2016).

Variable that not implicated in an evaluation over time such as cumulative HA, NUE and agronomic efficiency were submitted to ANOVA, when interactions presented $P \leq 0.05$, means were compared using Tukey test. Results were analyzed as a balanced randomized block design in a double factorial scheme with fat2.rbd function from ExpDes package (Ferreira et al., 2013). Control treatments (no N added) were initially included in the statistical model; however, because all response variables increased with N addition, controls were eliminated from the mean comparison among the various N sources.

Response parameters of N release under controlled environment were analyzed using a double factorial scheme in a completely randomized design in R 3.4.1 with fat2.crd function from package ExpDes (Ferreira et al., 2013). Treatments and their interaction were considered different when P values ≤0.05. Simple regressions were calculated using SigmaPlot version 11 (Systat-Software, 2008). Data from N release field study were analyzed in a completely randomized design in R 3.4.1 with crd function from package ExpDes (Ferreira et al., 2013) when $P \leq 0.005$, means were compared using a Tukey test and simple regressions were calculate using Sigmaplot 11 (Systat-Software, 2008).

Conclusion

This study demonstrated that EEFs increased forage production, nutritive value, NUE, and agronomic efficiency compared to untreated urea. These findings support the conclusion that EEFs such as PCU and U-NBPT may be superior in supplying N to forage than untreated urea. The single application of U-NBPT resulted in greater cumulative HA than untreated urea. A minimum of 45% soil moisture was necessary to a satisfactory N release, showing that water is a factor that substantially affects the efficiency of EFFs. The technology applied to PCU-6 and PCU-4 was not accurate to precisely determine the nutrient release pattern and rate in the field study, on the other hand PCU-2 correspond as advertised.

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