Cover crop and nitrogen fertilizer influence on inbred maize performance and soil nitrogen[1, 2]

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ABSTRACT

The response of inbred maize (*Zea mays* L.) lines to nitrogen (N) fertilizer and cowpea (*Vigna unguiculata* cv. Iron Clay) cover cropping was evaluated in three consecutive croppings from 2014 to 2016 in a Guamaní (Torrifluventic Haplustepts) soil. The crop rotation sequence was maize (spring 2014), cowpea (summer 2014), maize (winter 2014-2015), cowpea (summer 2015), maize (winter 2015-2016) and cowpea (summer 2016). The N fertilizer levels were 0, 90, 135, 180 and 225 kg N/ha for 2014 and 2014-2015 seasons, and 0, 50, 100, 150, and 200 kg N/ha for the 2015-2016 season. Soils were sampled to a depth of 90 cm before and after each successive cropping. Cover crop did not affect maize yields. The effect of N fertilizer on seed yield was observed in two out of the three years with optimum seed yields of 7,034 and 4,708 kg/ha with fertilizer N of 135 and 90 kg N/ha, respectively. Residual soil N tended to increase due to N fertilizer and cover crop. A partial aboveground N budget showed that the net N balance was more positive and increased with each successive fertilizer N level, reaching values of +516 kg N/ha in fallow and +621 kg N/ha with cover crop after three consecutive croppings over a 30-month period. Part of the excess N (not taken up by the crop) was accounted for in the soil profile. A slightly greater positive N balance at higher N fertilizer rates was due to the cover crop rotation. Cover-cropping with cowpea continues to be an important practice that in the long term will result in improved N recycling due to scavenging of residual soil N after maize cropping or by N fixation. Nitrogen fertilizer rates in the range of 90 to 135 kg N/ha can result in good inbred maize yields and can be adjusted with knowledge of soil inorganic N to a depth of 30 cm, that will result in higher yields, improved N use efficiency and reduced losses to the environment.

Key words: crop response, nitrogen balance, residual soil N, nutrient use efficiency

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RESUMEN

Influencia de los cultivos de cobertura y fertilizante nitrogenado sobre líneas puras de maíz y el nitrógeno en el suelo

Se evaluó la respuesta de líneas puras de maíz (*Zea mays* L.) a niveles de fertilizante nitrogenado (N) en rotación con una cobertora de caupí (*Vigna unguiculata* cv. Iron Clay) en tres cultivos consecutivos de 2014 a 2016 en un suelo Guamaní (Torrifluventic Haplustepts). La rotación consistió en maíz (primavera 2014), cobertora (verano 2014), maíz (invierno 2014-2015), cobertora (verano 2015), maíz (invierno 2015-2016) y cobertora (verano 2016). Los niveles de fertilización con N fueron 0, 90, 135, 180 y 225 kg N/ha, para las temporadas 2014 y 2014-2015, y 0, 50, 100, 150 y 200 kg N/ha, para la temporada 2015-2016. Los suelos se muestrearon a una profundidad de 90 cm antes y después de cada cosecha. La cobertora no afectó a los rendimientos del maíz. Se observó respuesta a la fertilización con N en dos de tres años con rendimientos óptimos de semillas de 7,034 y 4,708 kg/ha con fertilizante N de 135 y 90 kg N/ha, respectivamente. El N inorgánico residual en suelo tendió a aumentar debido al fertilizante-N y la cobertora. Un presupuesto parcial del balance de N mostró que el balance neto de N era más positivo y aumentó con cada nivel de fertilización-N alcanzando valores de +516 kg N/ha en barbecho y +621 kg N/ha en la rotación con la cobertura después de tres cultivos sucesivos en un período de 30 meses. Parte del exceso de N (que no extrajo el cultivo) se encontró en el perfil del suelo. Hubo un balance de N positivo ligeramente mayor debido a la rotación con la cobertora a los niveles mayores de fertilización. La rotación del maíz con la cobertora continúa siendo una práctica importante que a largo plazo resultará en un mejor reciclaje de N debido a la utilización del N residual del suelo después de la cosecha de maíz o por la fijación de N. Niveles de fertilización entre 90 a 135 kg N/ha pueden resultar en buenos rendimientos de maíz endogámico, estos niveles se pueden ajustar si conocemos el N inorgánico del suelo a una profundidad de 30 cm, lo que resultará en un mayor rendimiento, una mayor eficiencia de uso de N y una reducción de las pérdidas para el medio ambiente.

Palabras claves: respuesta de cultivos, balance de nitrógeno, N residual en suelo, eficiencia de utilización de nutrientes

INTRODUCTION

Nitrogen (N) is the most limiting nutrient in non-legume cropping systems (Mullen, 2011; Havlin et al., 2014; Scharf, 2015). Success has been limited in the use of chemical and biological soil tests to identify nitrogen deficient soils and less progress has been made to calibrate the tests (Scharf, 2001; Morris et al., 2018). Efforts to ameliorate expected nitrogen deficiency in crops can lead to nitrogen fertilizer applications in excess of crop nutrient requirements. This can negatively impact the environment by contributing to climate change through nitrous oxide emissions, eutrophication of inland surface water and marine waters, and contamination of ground water resources (Meisinger et al., 2008). Effective nitrogen management is essential to achieve a balance between yields, nitrogen supplementation and nitrogen losses (Andraski and Bundy, 2002; Mullen, 2011). A better understanding of the relationships between N fertilizer rates and crop yields will aid in improving N management and N use efficiency (Ma et al., 2005; Fixen et al., 2015). Studies in Puerto Rico on maize (*Zea mays* L.) response to N fertilizer applications have shown mixed success regarding the optimum N fertilizer level needed for maximum crop yields (Vázquez, 1961; Capo, 1967; Fox et al., 1974; Feliciano et al., 1979; Quiles et al., 1988; Sotomayor-Ramírez et al., 2012; Rivera-Zayas et al., 2017).

The use of legume cover crops in rotation is a conservation practice that can maintain or increase soil N and extend the availability of N to the succeeding cash crop (Havlin et al., 2014; Snapp et al., 2005). An important benefit of legume cover crops is their ability to scavenge soil N and improve N availability through their N fixing capacity. Cover crops have the potential benefits of reducing soil N leaching and improving nitrogen use efficiency (Dinnes et al., 2002; Dabney et al., 2010; Blanco Canqui et al., 2012). After the legume cover crop is incorporated into the soil, there may be more N available for successive crops (Kaspar and Singer 2011; Blanco Canqui et al., 2012; Blanco-Canqui et al., 2015). Depending on the type of cover and its management, between 30 to 60 kg N is transferred to the soil and the succeeding crop (Peoples et al., 2009; Sotomayor-Ramírez et al., 2012). Legume and non-legume cover crops provide additional benefits such as increased soil organic carbon, improved soil water infiltration, protection against soil erosion, improved soil physical properties, more effective weed control, a habitat for beneficial insects, reduced populations of soil pathogens, reduced soil N and phosphorus (P) losses, and promotion of nutrient recycling (Snapp et al., 2005; Kaspar and Singer, 2011; Ritchey et al., 2015; Delgado et al., 2017). Studies have demonstrated soil benefits and seed yield increases in maize-cover crop rotation systems (Rao and Mathuva, 2000; Delgado et al., 2007; Gabriel and Quemada, 2011; Mupangwa et al., 2012; Sotomayor-Ramírez et al., 2012; Delgado et al., 2017).

The southern coast of Puerto Rico is an important winter nursery site that produces maize seed from inbreds[7]. There is a continued need to evaluate the minimum level of N fertilizer necessary to achieve satisfactory inbred maize seed yields for specific soil types and to assess soil management practices to protect soil water quality. Critical N fertilizer levels can be modified by use of cover crops in

[7]https://www.prabia.org/agricultura-moderna

rotation. The objective of this paper was to quantify the response of inbred maize lines to N fertilizer levels and cover crop rotation. A secondary objective was to assess the fate of N in the cropping systems. This information can be used to improve N fertilizer management in similar ecosystems of the island and elsewhere. The results presented are part of the efforts of UPR-AES and industry to improve on-farm N fertilizer management. It is the culmination of several years of published and unpublished on-farm experimental trials to quantify the response of inbred maize to N fertilizer and the effects of cover crops in rotation (Sotomayor-Ramírez and Barnes, 2014; Sotomayor-Ramírez, 2017).

MATERIALS AND METHODS

Site description and experimental design

The study site was located at Mycogen Seeds Corp. research farm in Guayama, Puerto Rico. The predominant soil is Guamaní (Fine-loamy over sandy or sandy-skeletal, mixed, superactive, isohyperthermic Torrifluventic Haplustepts). The soil had 2.7% organic matter, immediately available (1M KCl extractable) soil inorganic N (0 to 30 cm) in the range of 73 to 100 kg N/ha, and profile (0 to 90 cm) inorganic N in the range of 122 to 160 kg N/ha. The effects of five N fertilizer levels and a cowpea (*Vigna unguiculata* cv. 'Iron Clay') cover crop (CC) rotation on inbred maize seed yields were evaluated from spring 2014 to summer 2016.

The crop rotation sequence was maize (spring 2014), cowpea (summer 2014), maize (winter 2014-2015), cowpea (summer 2015), maize (winter 2015-2016) and cowpea (summer 2016). The experimental arrangement was a randomized complete block with four replicates for maize cropped in 2014 and thereafter modified with a cowpea cover crop as a split across each block and analyzed as strip-plot design. In Table 1 are details of inbred maize lines used, maize planting and harvest dates, maize planting density, cowpea planting, harvest and incorporation dates. The cowpea cultivar 'Iron clay' was purchased from Johnny's Selected Seeds (Waterville, ME USA)[8]. The cowpea was planted with a commercial planter (John Deere Drill 1520; Deere & Co. Moline Ill, USA) at a rate of 67.3 kg/ha for an estimated density of 554,472 plants per hectare.

[8]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

TABLE 1.—*Description of activities carried out during the experiment conducted from 2014 to 2016.*

	2014	2014-2015	2015-2016
Inbred maize line	SSH65VH	SSH65VH	SLM15VH
Maize planting	14 April 2014	10 December 2014	21 December 2015
Maize harvest	16 July 2014 (91 DAP[1])	23 March 2015 (103 DAP)	21 March 2016 (91 DAP)
Planting density (plants/ha)	28,009	26,288	24,045
Cowpea planting	8 August 2014	21 May 2015 (9 June 2015)[2]	15 June 2016
Cowpea harvest	11 October 2014	21 August 2015	18 August 2016
Cover crop soil incorporation	24 October 2014	2 November 2015	8 September 2016

[1]Days after planting
[2]A second planting took place on 9 June 2015 because of poor germination of the cover crop.

The N fertilizer levels for the maize inbreds were 0, 90, 135, 180 and 225 kg N/ha for 2014 and 2014-2015, and 0, 50, 100, 150, and 200 kg N/ha for 2015-2016. During 2014 and 2014-2015, N fertilizer was applied at planting (rate of 68 kg N/ha to all plots that received N fertilizer) and at 36 and 34 days after planting (DAP), respectively. The N fertilizer rates applied at the six-leaf stage were 22, 67, 112, and 157 kg N/ha, for each successive treatment. The N fertilizer sources were ammonium sulfate and urea at an N proportion of 3:1 in 2014 and 2014-2015 and 1:1 in 2015-2016. In 2015-2016, 30 kg N/ha was applied at planting to all plots receiving N fertilizer, and the N fertilizer rates at the six-leaf stage (38 DAP) were 20, 70, 120, and 170 kg N/ha, for each successive N fertilizer treatment. Complementary nutrients were applied at planting by banding at rates of 63, 104 and 28 kg/ha of P_2O_5, K_2O and micronutrients, as triple superphosphate, muriate of potash, and Granusol Five-Star-Mix®, respectively. Further treatment and site descriptions are detailed by Vilches-Ortega et al. (2022).

Maize stover biomass, seed yield and N extraction

Maize stover was harvested at or near seed harvest from a predetermined strip (usually 3 m). Fresh stover was weighed and a subsample was collected for moisture determination after drying in an oven at 55° C for 48 h. The dried subsample of stover was ground to pass through a 1-mm mesh and stored. Maize seed yield from a 6-m strip was harvested from the middle two rows of each plot. Seed samples were oven-dried (65° C) and adjusted to 15.5% moisture. Plant biomass was expressed on a dry-weight basis after correcting for plant tissue moisture. Seed, indicator leaves, and stover dry matter were analyzed for total N concentration by AgSource Laboratories (Lincoln, NE).

Maize-fallow subplots were maintained weed free by tillage and herbicide applications. After the maize harvest and removal of remaining cobs, the maize stover was cut and incorporated by disking. No weed or pest control was done during cowpea growth. Cowpea aboveground biomass was measured by cutting the vegetative material at the ground level in a 1 m^2 quadrant. In summer of 2014, cowpea was measured from two 1 m^2 quadrants. A portion of vegetative material was weighed and dried in an oven at 65° C for moisture determination. The material was later ground to pass through a 2-mm mesh and analyzed for total N concentration. Cowpea was terminated by glyphosate application followed by soil incorporation by disking. After incorporating the cover crop, the field was disked at approximately weekly intervals until maize planting.

Soil sampling and inorganic N analysis

In the 2014 season, soil samples (pre-plant) were taken before maize planting for the 20 main plots. Soil samples for the 2014-2015 (pre-plant) and 2015-2016 (pre-plant) seasons were taken after the cover crop had been incorporated into the soil but before each maize planting for each subplot to determine plant available N. In the 2015-2016 season (post-harvest) soil samples were taken after the last maize harvest for each subplot. Sampling was done by hand with a bucket auger. Soil samples were collected at depths of 0 to 15 cm, 15 to 30 cm, 30 to 60 cm and 60 to 90 cm per sub-plot. Soil samples were left to air dry and then sieved to pass through a 2-mm mesh. Soils were analyzed for 1M KCL extractable NH_4^+-N and NO_3-N by AgSource Laboratory (Lincoln, NE, www.agsourcelaboratories.com).

Nutrient use efficiency

Nutrient use efficiency indicators were calculated as specified by Fixen et al. (2015). The agronomic efficiency (AE) of N fertilizer applied (N_a) was calculated as

$$(Yseed,_{f} - Yseed,_{u})/N_a \quad [1]$$

where $Yseed,_{f}$ and $Yseed,_{u}$ are the maize seed yields in fertilized and unfertilized treatments, respectively. The apparent recovery efficiency (ARE) is defined as the increase in whole plant nutrient uptake per unit of nutrient applied and was calculated as

$$(U_f - U_u)/N_a \quad [2]$$

where U_f and U_u are the whole plant (seed plus stover) N content in fertilized and unfertilized treatments, respectively. The partial factor productivity (PFP) is defined as the weight of seed harvested per unit of N fertilizer applied and was calculated as

$$Yseed,_{f} / N_a \quad [3]$$

The partial nutrient balance (PNB) is defined as the amount of nutrient removed in seed (Useed-N) per unit of N fertilizer applied and was calculated as

$$Useed\text{-}N,_{f} / N_a \quad [4]$$

The ability of crop to transform nutrients acquired from all sources into seed yield was the internal utilization efficiency (IUE) and was calculated as

$$Yseed,_{f} / U_f \quad [5]$$

Partial aboveground nitrogen budget

A simplified partial aboveground N budget was constructed yearly, and cumulative values were obtained for the study period (Meisinger et al., 2008; Prasad and Hochmuth, 2013). The initial inputs were N fertilizer rate and immediately available N (inorganic soil N to a depth of 0 to 30 cm). Inputs for succeeding years included the antecedent stover N and cover crop N. Both stover and cover crop N were transient input-output reserves. These reserves will take up N and release N to the subsequent crop during mineralization and were observed to be well decomposed during the subsequent maize growth stage. The N outputs included whole-plant N and 40% cover crop N uptake. Our observations that inbred maize lines such as the ones we were using have a very shallow (<30 cm) root system (Sotomayor-Ramírez et al., 2012) led us to hypothesize that soil N beyond 30 cm would only be minimally available for the subsequent crop, thus the sum of ammonium-N and nitrate-N was termed immediately available N. Work by Sotomayor-Ramírez and Estévez (2008) has shown that cowpea can fix up to 73% of the N taken up in a similar soil, and under field conditions we assumed that said amount could reasonably be 60% of aboveground cover crop N uptake. The difference between inputs and outputs was unaccounted N, which in the case of positive values indicates an N surplus and potential N losses from denitrification, volatilization, leaching, microbial immobilization, and runoff from the soil-plant system. A negative N balance is indicative of a potential crop-N deficiency or deficit. Part of the unaccounted N could be found in the soil profile at the end of each cropping season and prior to the next.

Statistical Analysis

The data was analyzed using InfoStat (2014®) statistical software. The 2014 season plots were arranged in a randomized complete block design, with an ANOVA considering the effect of N fertilizer rate. During the 2014-2015 and 2015-2016 seasons, we used a strip-plot arrangement of a randomized complete block design, with an ANOVA where N fertilizer treatment was the main effect and cover crop the strip plot. The cowpea cover crop, maize harvest and soils data was verified for normality and homogeneity using Shapiro-Wilks and Levene tests. Significant differences among means were determined by LSD Fisher test with a $p<0.05$. The data between plant available soil N and seed yield and indicator leaf N was fitted to various linear and non-linear models such as quadratic, quadratic-plateau, exponential, square-root and linear-plateau (Cerrato and Blackmer, 1990; Mallarino and Blackmer, 1992). The model with the lowest root-mean-square error was selected. Plant available N was defined as the sum of applied N fertilizer and immediately available N to a depth of 30 cm. Indicator leaf critical levels were determined from the first derivative of each equation.

RESULTS AND DISCUSSION

Cowpea cover crop biomass and N uptake was unaffected by previous applications of N fertilizer. The cover crop dry matter (DM) biomass was 5,201; 2,595; and 2,154 kg DM/ha, for the 2014, 2014-2015 and 2015-2016 seasons, respectively. Crop N uptake as a result of recycling of N (biological N fixation plus soil N) was 126, 51 and 59 kg N/ha for the 2014, 2014-2015 and 2015-2016 seasons, respectively. Based on this information we were able to compute cowpea cover crop N extraction coefficients ranging from 19.7 to 27.1 kg N/t DM, which by knowing the cowpea yield, can be used by farmers as a legume N credit for a subsequent crop in rotation to improve N management.

Cover crop and N fertilizer x cover crop interaction did not affect maize agronomic and yield parameters for all seasons (Table 2). The lack of cover crop effect contrasts with previous positive effect of cowpea cover crop on inbred maize seed yields (Sotomayor-Ramírez et al., 2012; Espinosa-Irizarry, 2016; Rivera-Zayas et al., 2017), and other crops via improved N availability from biological N fixation or soil chemical and physical properties (Dabney et al., 2010; Blanco-Canqui, 2012).

Maize seed yield, stover and biomass. Nitrogen fertilizer rate significantly ($p<0.05$) affected seed yield in 2014 and 2014-2015 seasons. A maximum seed yield of 7,134 kg/ha was obtained at N fertilizer rates between 135 and 180 kg N/ha in 2014 (Table 3), and a maximum seed yield of 4,708 kg/ha was measured with a N fertilizer rate of 90 kg N/ha in 2014-2015 (Table 4). Stover weight was not affected by N fertilizer with means of 8,293; 3,434; and 8,059 kg/ha, in 2014, 2014-2015 and 2015-2016 seasons, respectively (Tables 3, 4 and 5). Whole-plant biomass means were 16,070 kg/ha, 8,309 kg/ha and 10,617 kg/ha in 2014, 2014-2015 and 2015-2016 seasons, respectively.

Seed yields ranging from 2,357 to 2,758 kg/ha during 2015-2016 were much lower than the previous two years and may seem low but are much more typical of seed yields reported for maize yield trials conducted on farms (Sotomayor-Ramírez et al., 2012; Sotomayor-Ramírez and Barnes, 2014). The observed optimum N fertilizer rates and seed yields in two of the three years tested are higher than reported in previous studies which evaluated inbred maize response to N fertilizer. Sotomayor-Ramírez et al. (2012) reported maximum seed yield of 1,444

TABLE 2.—*Summarized ANOVA with significant levels (p-values) of the effects of fertilizer N (N), cover crop (CC) and N x CC on various maize agronomic parameters for Guayama experiment from 2014 to 2016.*

Parameter	2014	2014-2015			2015-2016		
	N Fertilizer (N)	N Fertilizer (N)	Cover crop (CC)	N x CC[1]	N Fertilizer (N)	Cover crop (CC)	N x CC[1]
Seed yield 15.5% moisture	0.0024	0.0176	0.4409	0.9174	0.5525	0.6409	0.6874
Stover	0.7336	0.5732	0.1292	0.2557	0.0955	0.0742	0.8478
Biomass[3]	0.4694	0.1875	0.1637	0.2713	0.1526	0.2026	0.9041
Seed N concentration	0.6357	0.0091	0.7627	0.4237	0.3785	0.3765	0.4944
Stover N concentration	0.8949	0.0554	0.3582	0.5880	0.5997	0.3618	0.1457
Seed N uptake	0.0381	0.0012	0.3619	0.9079	0.2314	0.4776	0.5444
Stover N uptake	0.8241	0.3012	0.1643	0.4869	0.2054	0.0779	0.1872
Biomass N uptake[2]	0.4728	0.0148	0.1786	0.3922	0.2079	0.0695	0.2397
Harvest index	0.8276	0.5629	0.8861	0.7051	0.6242	0.8561	0.5267
Number of seeds	0.0658	0.1557	0.5512	0.8869	0.2911	0.6025	0.5381
Indicator leaf N concentration	0.2408	0.0039	0.5575	0.8970	0.5344	0.0256	0.4785
Plant density	0.0666	0.3813	0.7187	0.5159	0.0390	0.2062	0.1803
Ear density	--------[4]	0.5106	0.8126	0.5788	0.1274	0.9200	0.3411

[1]Interaction between N fertilizer (N) and cover crop (CC) rotation.
[2]Biomass N-uptake was calculated by adding stover and seed N uptake.
[3]Biomass was calculated by adding seed yield dry weight and stover dry weight.
[4]Data not measured for this time.

TABLE 3.—*Effect of N fertilizer on maize agronomic parameters for 2014 season.*

Parameter	Fertilizer treatment (2014 season) 0	90	135	180	225	Means
	----------------	----------------	kg/ha	----------------	----------------	
Seed yield[4]	6,168 a[1]	6,746 b	7,034 bc	7,234 c	6,656 b	--------[3]
Stover	7,758 ns[2]	8,490	9,608	8,255	7,356	8,293
Biomass	14,912 ns	16,150	17,700	16,570	15,018	16,070
Seed N-uptake	103.5 a	110.4 ab	119.9 bc	123.9 c	116.3 bc	--------[3]
Stover N-uptake	77.4 ns	92.0	103.3	90.9	77.9	88.3
Biomass N-uptake	180.9 ns	202.4	223.2	214.9	194.2	203.1
Harvest index	0.49 ns	0.48	0.47	0.51	0.52	0.49
Number of seeds	2.94E+07 ns	3.39E+07	3.48E+07	3.45E+07	3.28E+07	3.31E+07
Indicator leaf N concentration (%N)	2.33 ns	2.56	2.56	2.57	2.75	2.55

[1]Parameter means with different letters within N levels are significantly different at $p<0.05$.
[2]ns denotes that N application rates were not significant at $p>0.05$.
[3]Means were not calculated for parameters that had significant differences ($p<0.05$).
[4]Seed yield at a seed moisture of 15.5%.

TABLE 4.—*Effect of N fertilizer on maize agronomic parameters for 2014-2015 season.*

Parameter	Fertilizer Treatment (2014-2015)					Means
	0	90	135	180	225	
	kg/ha					
Seed yield[4]	3,693 a[1]	4,708 b	4,166 ab	4,695 b	4,663 b	--------[3]
Stover	3,336 ns[2]	3,404	3,139	3,977	3,314	3,434
Biomass	7,444 ns	8,633	7,745	9,209	8,511	8,309
Seed N uptake	50.5 a	69.0 bc	62.3 b	74.1 c	76.3 c	--------[3]
Stover N uptake	26.7 ns	28.1	27.1	37.8	35.3	31.0
Biomass N uptake	77.2 a	97.1 ab	89.4 a	111.9 b	111.6 b	--------[3]
Harvest index	0.56 ns	0.6	0.59	0.58	0.61	0.59
Number of seeds	1.01E+07 ns	1.23E+07	1.01E+07	1.06E+07	1.18E+07	1.10E+07
Indicator leaf N concentration (%N)	2.50 a	2.76 b	2.87 b	2.81 b	2.96 b	--------[3]

[1]Parameter means with different letters within N levels are significantly different at $p<0.05$.
[2]ns denotes that N application rates were not significant at $p>0.05$.
[3]Means were not calculated for parameters that had significant differences ($p<0.05$).
[4]Seed yield at a seed moisture of 15.5%.

TABLE 5.—*Effect of N fertilizer on maize agronomic parameters for 2015-2016 season.*

	Fertilizer Treatment (2015-2016)					
	0	50	100	150	200	
Parameter	kg/ha					Means
Seed yield[2]	2,357 ns[1]	2,555	2,395	2,485	2,758	2,510
Stover	7,518 ns	8,421	7,311	8,650	8,393	8,059
Biomass	9,924 ns	11,020	9,750	11,189	11,204	10,617
Seed N uptake	39.0 ns	44.0	41.8	44.3	49.6	43.7
Stover N uptake	89.9 ns	112.5	97.1	122.1	109.3	106.2
Biomass N uptake	128.9 ns	156.5	138.8	166.4	158.9	149.9
Harvest index	0.24 ns	0.23	0.24	0.23	0.25	0.24
Number of seeds	1.43E+07 ns	1.75E+07	1.56E+07	1.54E+07	1.75E+07	1.61E+07
Indicator leaf N concentration (%N)	1.68 ns	1.54	1.92	2.06	1.71	1.78

[1]ns denotes that N application rates were not significant at $p>0.05$.
[2]Seed yield at a seed moisture of 15.5%.

to 2,726 kg/ha with N fertilizer in the range of 84 to 112 kg/ha. Rivera-Zayas et al. (2017) reported a 62 kg N/ha optimal N rate with a seed yield of 2,770 kg N/ha. Espinosa-Irizarry (2016) found no significant differences in inbred seed yields with N rates of 60, 110 and 160 kg N/ha.

Seed N uptake, stover N uptake and biomass N uptake. In the 2014 and 2014-2015 seasons, N fertilizer significantly affected seed N uptake. Maximum seed N uptake of 120 kg N/ha was obtained with a N fertilizer rate of 135 kg N/ha in 2014 and 69 kg N/ha with 90 kg N/ha in 2014-2015. The mean stover N uptake and biomass N uptake was 88 and 203 kg N/ha, respectively, in 2014; stover uptake was 31 kg N/ha in 2014-2015 season; and stover and biomass uptake were 106 and 150 kg N/ha in 2015-2016 season. The 90 kg N/ha rate resulted in higher biomass N uptake in 2014-2015 season.

Harvest index, seed number and indicator leaf N. Nitrogen fertilizer did not affect (P>0.05) harvest index and number of seeds for the three seasons. Mean harvest index was 0.49, 0.59 and 0.24 for the 2014, 2014-2015, and 2015-2016 seasons, respectively. Espinosa-Irizarry (2016) reported a harvest index of 0.44 for a trial conducted in 2011. The 2015-2016 mean harvest index of 0.24 coincides with inbred maize harvest index values reported by Sotomayor-Ramírez et al. (2012) of 0.26 and 0.21, Rivera-Zayas et al. (2017) of 0.24 and Espinosa-Irizarry (2016) 2012 trial of 0.28. The mean number of seeds ($x10^6$/ha) was 33, 11, and 16, for the 2014, 2014-2015 and 2015-2016 seasons, respectively.

Indicator leaf N data best fit an exponential and a quadratic model in the 2014 and 2014-2015 seasons, respectively. Leaf N critical levels were 2.55% N and between 2.76 and 2.96% N for the 2014 and 2014-2015 seasons, respectively. In the 2015-2016 season there was no significant correlation between indicator leaf N and seed yield. Optimal indicator leaf concentration levels found for 2014 and 2014-2015 fall into reported sufficiency ranges of 2.5 to 3.5% for hybrid maize grown in southeast USA (Campbell, 2000). Rivera-Zayas et al. (2017) reported an optimal leaf N value of 2.5% at a seed yield of 2,660 kg/ha. Sotomayor-Ramírez et al. (2012) reported optimum leaf N concentration values of between 2.14 and 3.31%.

Optimum available N. The graphical relationship of the data between available soil N (fertilizer N applied plus inorganic N to a depth of 0 to 30 cm) and seed yield was best described by the linear-plateau model. Since there was so much seed yield variability among years, the individual models were fit to each year. The critical optimum available N was defined as the crop nutrient requirement (CNR) and was 143 kg N/ha for a 6,917 kg/ha seed yield and 156 kg N/ha for a 4,579 kg/

ha seed yield, for the 2014 and 2014-2015 seasons, respectively (Figure 1). The analysis was not run for the 2015-2016 season because of the non-significance of N fertilizer on seed yield and the low seed yields obtained. If soil inorganic N (ammonium-N plus nitrate-N) content to a depth of 30 cm is known prior to planting, this value can be used as

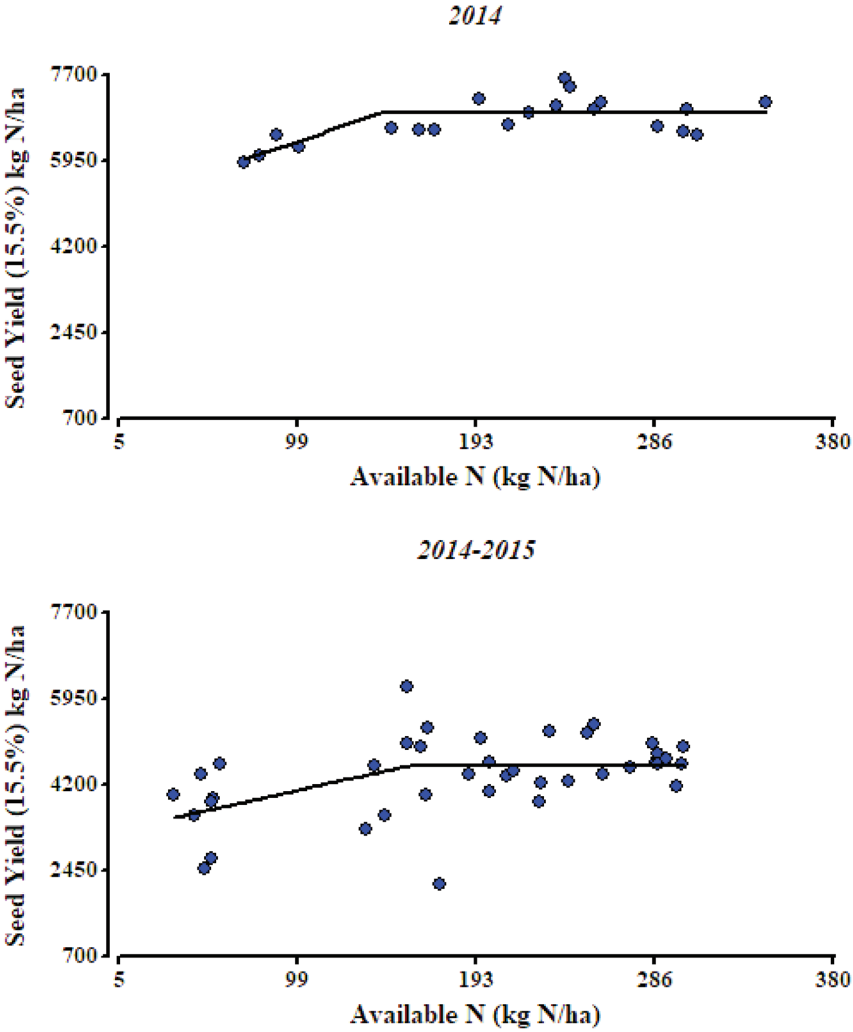

FIGURE 1. Relationship between available soil N and maize seed yield for the 2014 and 2014-2015 seasons. Available soil N was calculated as the sum of applied N fertilizer rate plus immediately available N (0-30 cm).

a credit in developing an N fertilizer recommendation for a crop and location like what we evaluated.

Soil inorganic N. Immediately available soil inorganic N (at 0 to 30 cm) 27 DAP (pre-sidedress inorganic N) for maize grown in the 2014-2015 season tended to increase with applications of N fertilizer (Table 6). At the time of sampling, the plots had received successive increments of N fertilizer the previous season and 68 kg N/ha of fertilizer N at planting. Only the 0 kg N/ha plots did not receive fertilizer. Inorganic N in soil was 26 mg N/kg at the lower levels and 41 mg N/kg at the highest N fertilizer levels. Soil inorganic N critical values of 25 mg N/kg at 0 to 30 cm have been observed (Andraski and Bundy, 2002; Morris et al., 2018). Thus, the technology transfer of soil inorganic N critical values suggests that crop response would be observed up to an N fertilizer level of 90 kg N/ha, as was determined in this study.

Soil profile inorganic N (soil N) at the beginning of the experiment ranged from 122 to 143 kg N/ha, and as expected was unaffected by N fertilizer treatments, because these had not yet been applied (Figure 2). The majority (mean of 64%) of soil N was in the top 30 cm of the soil, which on average was 85 kg N/ha (or about 21 mg N/kg). In subsequent cropping years, the proportion of soil N in the top 30 cm was fairly consistent between fallow and cover crop treatments for N fertilizer levels with means ranging from 46 to 61%. Our observation that inbred maize has a very shallow root system led us to hypothesize that soil N beyond 30 cm would not be available for the subsequent crop and thus could be considered potentially leached N.

The soil N content determined in this study suggests that inorganic N does not accumulate in the deeper soil layers, even with the higher N fertilizer treatments. At the higher N fertilizer rates applied at each successive level, inorganic N is either being taken up by the plant or is being lost to the environment, possibly by leaching. Since whole-plant N uptake was not affected by fertilizer N, increased plant N uptake may not be the most probable N-loss mechanism. Soil samples

TABLE 6.—*Soil inorganic N at 0 to 30 cm, 27 DAP (pre-sidedress inorganic N), for maize grown in 2014-2015. All plots except those with 0 nitrogen fertilizer received 68 kg N/ha (additional N was provided at 36 DAP).*

N Fertilizer Level kg/ha	Soil inorganic N mg N/kg
0	26.43 a
90	31.75 ab
135	27.90 a
180	30.00 ab
225	40.78 b

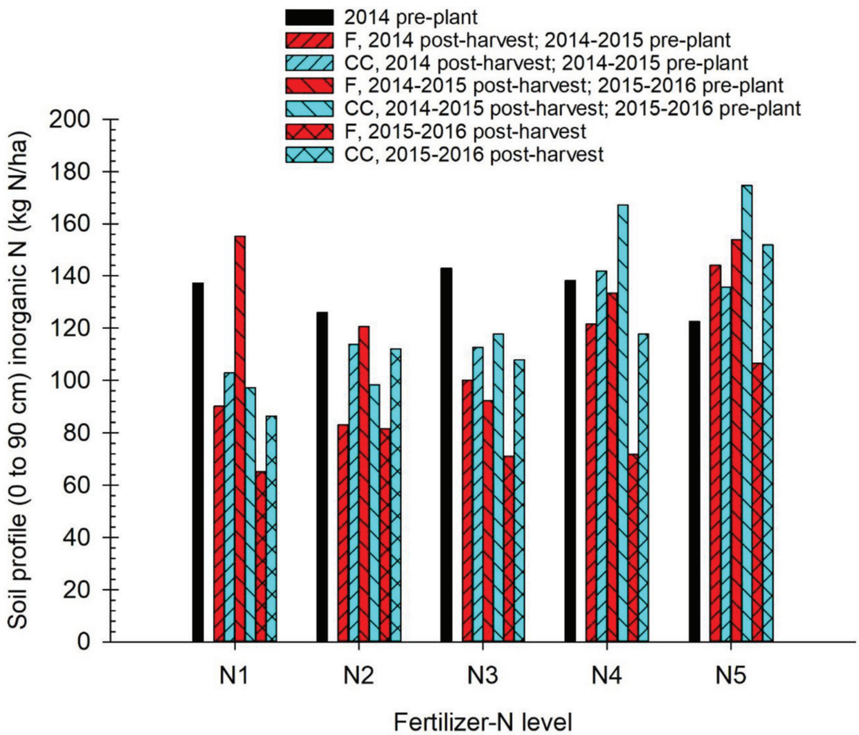

FIGURE 2. Soil profile (0 to 90 cm) inorganic N as a result of fallow (F), and cover cropping (CC) during 2014, 2014-2015, and 2015-2016 seasons.

were collected after cover crop incorporation but usually there was a 60- to 40-day period until soil samples were collected, during which about 40% of the annual rainfall fell. The percolation induced by the increased incidence of precipitation (from September to early December) in combination with the loamy surface texture and sandy texture of the subsoil may have contributed to N leaching (bottomless bucket concept).

The limited data set precludes from providing explanation regarding the temporal variation in soil profile inorganic N after each successive cropping and N fertilizer level. Soil profile inorganic N remaining after each successive harvest is the net result of crop N uptake, antecedent crop stover and cover crop mineralization, soil organic N mineralization and other losses such as denitrification, volatilization, and leaching (Rice and Havlin, 1994; Scharf, 2015), which in turn is very dynamic and was not quantified. Yet, some interesting patterns can be observed. Profile inorganic N after maize harvest and after cover crop termination, tended to increase with N fertilizer application. Soil profile inorganic N tended to be consistently higher in the cover crop treatment than in the fallow treatment at the three highest N fertilizer rates and with time. For example, with N fertilization of 180 kg N/ha during 2014 and 2014-2015 season and 150 kg N/ha in 2015-2016 season, soil profile inorganic N was 17, 24, and 64% higher than fallow for each cropping. Yet for N fertilization of 90 kg N/ha during 2014 and 2014-2015 season and 50 kg N/ha in 2015-2016 season, soil profile inorganic N was 37, 25 and 64% higher with CC than fallow for each cropping. This finding can be attributed to increased N availability as a result of scavenging of soil N by the cover crop or increased N availability due to biological fixation.

Partial aboveground N budget. After three successive maize croppings during a 30-month period, similar cumulative inorganic N inputs from applied fertilizer or soil mineralization were available to the crop in fallow and with cover crop in the range of 186 to 879 kg N/ha for the N fertilizer rates evaluated (Figure 3). The percentage of the available N that was removed in maize seed during 2014 and 2014-2015 and in whole plant in 2015-2016 was similar between fallow and cover crop for each fertilizer rate applied and decreased with increasing fertilization rates. The net N balance (inputs – outputs) was negative for the unfertilized fallow and cover crop treatments, with higher (more negative) deficits in the former. The net N balance was more positive and increased with each successive level of N fertilizer applied reaching values of 516 kg N/ha in fallow and 621 kg N/ha with cover crop. The numerical difference suggests that the annual N contribution from biological N fixation in the cover crop was about 35 kg N/ha. There was a slightly greater positive N balance due to the cover crop rotation at the higher N fertilizer rates.

Nutrient use efficiency. Optimal NUE ranges of 40 to 90 kg grain/kg nutrient, 15 to 30 kg grain/kg nutrient, 0.7 to 0.9 kg grain/kg nutrient, and 40 to 65% have been reported for PFP, AE, PNB, and RE, respectively (Fixen et al., 2015). The PFP is indicative of the relative productive level of the cropping system in comparison to the N input. Values of PFP generally decreased with increased N fertilizer rates and were highest for the 2014 season and for the lowest N fertilizer rate (Table 7). Our calculated AE values were generally lower than those that are considered optimum, possibly because of the small difference in yields between the fertilized and unfertilized treatments. Our PNB values were similar for 2014, 2014-2015 and 2015-2016 seasons at 0.89, 0.77 and 0.88 for 135 kg N/ha, 90 kg N/ha and 50 kg N/ha, respectively. Overall PNB values decreased with increased N fertilizer levels in all seasons; values greater than 1 indicate more N is being removed from the system than being applied. Most PNB values for all

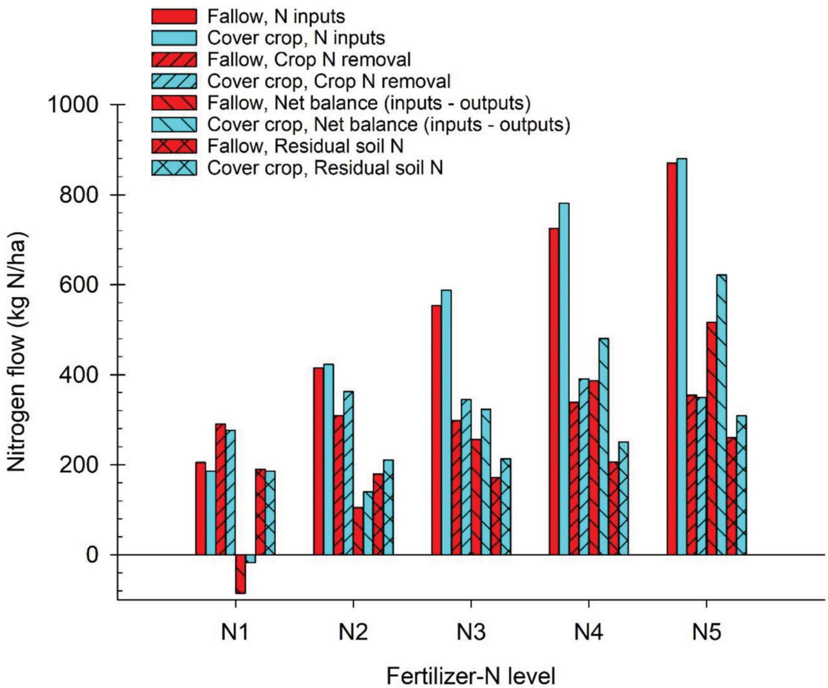

FIGURE 3. Partial aboveground nitrogen budget for fallow and cover crop rotation with increasing fertilizer rates. Each N fertilizer level represents the cumulative amount applied during three seasons where N1 is 0 kg N/ha, N2 is 230 kg N/ha, N3 is 370 kg N/ha, N4 is 510 kg N/ha, and N5 is 650 kg N/ha. The net balance represents the N inputs minus N outputs. N inputs were (i) inorganic soil N (to a depth of 0 to 30 cm), (ii) stover N uptake which is recycled back into the soil, (iii) cowpea cover crop uptake (when applicable), and (iv) added N fertilizer. N outputs were seed N uptake in the first two seasons and whole-plant biomass N in the third season. The residual soil N was the cumulative mass amount of profile N at depths of 30 to 60 cm.

seasons were below the efficiency range indicating N losses from the system.

Our IUE values were within an acceptable range only for the 2014-2015 season. The IUE can be described as the internal N requirement or the efficiency that maize plants can use N to produce grain (in this case seed). As IUE becomes larger the maize plant is able to produce more grain per unit of total N uptake, and the internal N requirement is reduced. Previous work with inbreds has shown low conversion of fertilizer N to grain N (Espinosa-Irizarry, 2016). Thus, inbreds appear to require higher N fertilizer levels to produce the same unit of grain as hybrids (Morris et al., 2018). For example, new era maize hybrids have an IUE (expressed as kg N/t) of 56; old era hybrids, 49.7 (Ciampit-

TABLE 7.—*Nutrient use efficiency (NUE) calculations for 2014-2016 Guayama experiment.*

	PFP[1]	AE[2]	PNB[3]	RE[4]	IUE[5]
N level	kg/kg	kg/kg	kg/kg	%	%
			2014		
90	74.9	6.4	1.23	24	33.33
135	52.1	6.4	0.89	31	31.51
180	40.2	6.0	0.69	19	33.67
225	29.6	2.0	0.53	6	34.27
			2014-2015		
90	52.3	11.3	0.77	22	48.48
135	30.9	3.5	0.46	9	46.61
180	26.1	5.6	0.41	19	41.96
225	20.7	4.3	0.34	15	41.79
			2015-2016		
50	51.1	4.0	0.88	55	16.33
100	24.0	0.4	0.42	10	17.25
150	16.6	0.9	0.30	25	14.93
200	13.8	0.2	0.25	15	17.36

[1]PFP = grain yield/ fertilizer application
[2]AE = (grain yield $_{\text{nutrient application}}$-grain yield $_{\text{no nutrient application}}$)/N fertilizer applied
[3]PNB = grain N-uptake/fertilizer rate applied
[4]RE = (crop N-uptake $_{\text{with nutrient applied}}$-crop N-uptake $_{\text{no nutrient applied}}$)/N fertilizer
[5]IUE = grain yield/crop N-uptake

ti and Vyn, 2012), and inbreds such as the ones we have evaluated, 31.1 (Sotomayor-Ramírez, 2017). Overall optimal PFP, AE, PNB and RE values for inbred maize in this experiment were achieved at near 90 kg N/ha for the 2014 and 2014-2015 seasons and at 50 kg N/ha for the 2015-2016 season.

CONCLUSIONS

This experiment demonstrated that cover crop rotation did not affect inbred maize yield, yet crop response to N fertilizer was observed in two of three years. With regards to the aboveground N budget, nitrogen was near balance at the cumulative N level of 230 kg N/ha (90 + 90 + 50 kg N/ha) and in deficit for the control treatment. Increasing N fertilizer resulted in N inputs that were greater than N exported in the maize seed and suggests that there is excess N and potential losses in the system. Optimal nutrient use efficiency levels were obtained at the lowest fertilizer N rate applied and appear to be lower than for maize hybrids. Our results are consistent with previous studies of N fertilizer management for inbreds and contrast with N fertilizer management for hybrids because of lower expected yields and lower internal nutrient use requirements. Although a positive effect of cover crops on crop yields was not observed in this study, the soil may benefit from a legume ground cover regarding soil biological, physical and chemical benefits.

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