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Effects of Nitrogen Fertilization and Seed Piece Applied Fungicides on Establishment, Tiller Dynamics, and Sucrose Yields in Successively Planted Sugarcane

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Abstract: Sugarcane (*Saccharum* spp.) successive planting causes 25–30% yield reduction in comparison to fallow or rice rotation planting in a three-year production cycle on Florida Histosols. Field experiments were established to manage the yield losses associated with successive planting through nitrogen fertilization and seed piece application of fungicides in plant and first ratoon crops each at two sites. Nitrogen fertilization treatments included 0 (N_0), 50 (N_{50}), and 100 (N_{100}) kg ha^{-1} applied in furrows at the time of planting, and one split application (N_{50+50}) with 50 kg ha^{-1} applied at planting and 50 kg ha^{-1} applied at 90 days after planting as side-dress. Fungicides treatments were mancozeb at 2.5 kg a.i. (active ingredient) ha^{-1}, mefenoxam at 0.57 kg a.i. ha^{-1}, and azoxystrobin at 0.30 kg a.i. ha^{-1} applied to seed cane pieces laid in the furrows at planting. Nitrogen fertilization showed increasing trends of the tiller and millable stalks production in plant and ratoon crops. N response varied with the time of ratooning. Overall, N_{50+50} produced greater tons of cane per hectare (TCH) and tons of sucrose per hectare (TSH) compared to other N treatments in plant crop and late season ratoon crop (ratooned in March). N_{100} treatment enhanced tillering and TCH in December ratooned crop. In 2016 plant crop, mefenoxam produced higher TCH than others, but no carryover effects were observed in ratoon crops. Both nitrogen fertilization and fungicides seem to be promising cultural practices to minimize yield losses in successive sugarcane planting in Histosols.

Keywords: sugarcane monoculture; yield decline management; nitrogen fertilization; fungicidal treatments; DRIS indices; Nutrient Balance Index

1. Introduction

Sugarcane (*Saccharum* spp. complex hybrid) is an important row crop in Florida with $1.3 billion of economic impact and providing 32,770 jobs [1]. Approximately 78% of the sugarcane is grown on Histosols south of Lake Okeechobee in the Everglades Agricultural Area (EAA). Sugarcane in Florida is planted successively (also called monoculture) on almost half of the total production area (47.2% in 2014), and the remainder was fallow planted after rotation with sweetcorn or rice or flooded fallow [2]. Successive planting allows more intense use of land without losing any crop year as in case of fallow planting. A study on sugarcane yield comparisons under successive, regular fallow, and late fallow planting scenarios showed approximately 25 to 30% lower yields in successive than fallow planting in a 3-year production cycle [3]. This was further confirmed in recent research that showed 20% lower cane yields in successive than regular fallow planting in two years in Histosols (plant cane

and first ratoon) [4]. Although the reasons for this yield decline in Histosols are unknown, the results from other parts of the world (especially Australia) are providing some important information on this topic. Garside et al. [5] reported that continuous sugarcane could reduce the productive capacity of the soil and induce a complex problem known as yield decline. Various factors contributing to yield decline under continuous sugarcane monoculture includes increased the incidence of plant pathogens, nematodes, weeds, deleterious rhizosphere microorganisms, excessive colonization of mycorrhizal fungi, autotoxicity, and changes in soil physical, chemical and, biological properties [6]. Yield decline under prolonged sugarcane monoculture was evidenced by poor root health associated with soil-borne root pathogens, many of which were fungi [7]. Soil application of different fungicides, such as benomyl, dithiocarbamates (mancozeb, maneb, and zineb), improved sugarcane root health and increased shoot growth on sugarcane monoculture soils in Australia [8,9]. In Louisiana soils, 16 to 27% plant crop and ratoon growth enhancements were observed with the application of oomycete specific fungicide metalaxyl [10]. In addition to *Pythium arrhenomanes*, several other species of Pythium, dematiaceous group fungi, pineapple sett rot are known to damage the sugarcane root system. Pineapple sett rot disease (caused by *Ceratocystis paradoxa*) was prevalent in successively planted sugarcane soils in the EAA and pathogen attacks on seed pieces through the cut ends, causing seed piece decay and patchy germination [11]. Seed piece application of systemic fungicides, such as propiconazole at planting, improved the stand establishment in disease-endemic fields [12].

The Histosols of the EAA began forming approximately 4400 years ago by decomposition of saw grass and other aquatic vegetation in the submerged land [13]. Due to the inherent high supply of N through mineralization far exceeding the crop requirements, nitrogen fertilization is currently not recommended for sugarcane production on EAA Histosols [14]. However, land conversion from submerged land to sugarcane production resulted in soil subsidence (at 2.5 cm yr^{-1} in the 1970s), which was primarily due to microbial oxidation [15]. Terry [16] reported mineralization of approximately 686 kg N ha^{-1} for each centimeter of Pahokee muck lost due to microbial oxidation, thus annually 900 to 1200 kg N ha^{-1} was mineralized from Histosols. In the 1990s, the subsidence rates were slowed down to 1.5 cm yr^{-1} or lower [17]. Overall, the decrease in subsidence could be due to high-water table maintenance; growers maintained higher water tables for a prolonged time to reduce phosphorus loads before releasing it to the main canals. This slows down microbial activity and oxidation of remaining organic matter [18]. The decreased subsidence may also be due to the high energy requirement for soil microbes to utilize the recalcitrant soil carbon pool [19].

In successive planting (plow-out, replant), a heterogeneous mix of sugarcane residues consisting of stubbles, roots, tops, dried leaves, unharvested stalks having wider C:N ratio (82:1) are returned to the soil [20]. Decomposer communities grow on these residues by immobilization of exogenous inorganic N to build cellular proteins and hexosamines, which results in a decrease in soil available N to the plants [21]. Further, low soil temperatures during sugarcane planting season (especially in November through January) and early growth periods (February through April) may further slowdown microbial activity and N mineralization rates in Histosols. The time lag between two sugarcane production cycles in successive planting is usually very short (less than 6 weeks); therefore, soil N supplies may not be adequate to support crop growth. Nitrogen deprivation at early stages causes a reduction in meristematic cellular activity, vegetative growth, and tillering in sugarcane. It has been estimated that a 100-ton sugarcane crop would remove 80–130 kg N [22]. In the southern zone of EAA, soil depth is reduced to less than 30 cm due to subsidence, which may not be enough to supply adequate nitrogen needed for sugarcane, unlike the deep mucks in the northern part of the EAA. Previous greenhouse pot experiments in shallow Histosols under periodically flooded conditions showed a positive sugarcane growth response to supplemental N application up to 100 kg ha^{-1} [23] and N fertilizer applications as high as 168 kg ha^{-1} in two equal splits under periodically flooded conditions in May and August [24]. However, there were no field experiments conducted on sugarcane response to nitrogen fertilization on Histosols, and, in contrast to this, adjacent sandy soils of EAA annually receive 224–246 kg N ha^{-1} [14].

Yield decline in successive planting may be due to a variety of factors. This study was guided by two hypotheses to explain yield decline in the EAA: (1) low nitrogen availability due to shallow soil depth coupled with the addition of root stubbles from previous crops may lead to temporary immobilization of mineralized nitrogen and (2) fungal pathogens that adversely affect bud germination, root health, and tillering at early growth stages. Therefore, the objectives of this research were: (i) to evaluate nitrogen fertilization and seed piece applied fungicides on sugarcane establishment, tillering, and yield attributes in plant and ratoon crops; (ii) to analyze the nitrogen fertilization effects on leaf tissue nitrogen concentration and its relationship with nutrient balance index.

2. Materials and Methods

2.1. Experimental Sites Characteristics

The first field trial was planted in November 2015 at the University of Florida, IFAS, Everglades Research and Education Center (26° 40′ 2.60″ N, 80° 37′ 53.06″ W) experimental farm, Belle Glade, Florida in IM3WE (site 1) block, and it was continued for two years (2015 plant crop and 2016 first ratoon). The second trial was planted in October 2016 in GH-10N block (site 2), and this was also conducted for two years (2016 plant crop and 2017 first ratoon). The soil type in both the sites was classified as Lauderhill series of muck or euic, hyperthermic Lithic Haplosaprists [25]. Both sites were under sugarcane cultivation for >20 years and are 800 m apart. Prior to sugarcane planting, soil samples (0 to 15 cm depth) were collected in four replicates, and each replicate represented a composite sample of 15 randomly collected soil cores (2.5 cm diameter, 15 cm depth). The soil samples were air dried at 38 °C for two days, then ground and passed through a 2 mm sieve. Soil pH was measured in 0.01 M $CaCl_2$ solution (soil to solution ratio = 1:2.5) saturated paste. Total soil carbon and total nitrogen were measured by dry combustion using Carlo Erba NA1500 CNHS elemental analyzer (Haak-Buchler Instruments, Saddlebrook, NJ, USA). Available P, K Ca, Mg were extracted with Mehlich-3 extractant, and P was determined colorimetrically [26], while K, Ca and Mg were determined by Atomic Absorption spectrophotometry (Varian SpectrAA220, Varian Inc., Palo Alto, CA, USA). Available Si content was determined with Brinkmann colorimeter (PC950 Probe Colorimeter, STH Company, River View, FL, USA) after 0.5 N Acetic acid extraction [27]. Soil chemical properties, dates for sugarcane planting, yield estimation, and machine harvest are given in Table 1. The time lag between plow-out to replant was about 4 weeks in both sites.

2.2. Nitrogen Treatments

The experimental design was split-plot design with N treatment as a whole plot and fungicide treatment as sub-plot. Combination of N rates and application timing were applied to main plots in a randomized block design. Each main plot comprised of 16 cane rows of 10 m length with 1.5 m wide row spacing. There were 4.5 m wide alleys between the two main plots and 3 m-wide alleys between two subplots. Sugarcane cultivar CP 96-1252 [28] was planted by placing pairs of whole stalks side by side in 15–20 cm deep furrows followed by chopping them into 45–60 cm pieces. The N treatments were: (i) 0 N (N_0); (ii) 50 kg N ha^{-1} applied at planting (N_{50}); (iii) 100 kg N ha^{-1} (N_{100}) at planting; (iv) 50 kg N ha^{-1} at planting, and additional 50 kg N ha^{-1} (N_{50+50}) was applied at 90 DAP (Days After Planting) as side-dress. The source of N was ammonium nitrate. In addition to different N levels, a fertilizer blend containing 115 kg K_2O, 285 kg STM-5 with 80% Sulfur and 5% Mn (Tiger Sulfur, Tiger-Sul products LLC, Atmore, AL, USA) plus micronutrients (3.4 kg Zn, 2.3 kg Cu, 1.2 kg B ha^{-1}) were applied to all the plots. This fertilizer mixture was applied in furrows as a basal dose at the time of planting. The source of P and K fertilizer used for the plant crop was triple superphosphate and muriate of potash, respectively.

Table 1. Physicochemical soil properties of experimental sites prior to sugarcane planting and calendar of events for plant cane and first ratoon crops.

	Site 1	**Site 2**
Location	26°39′53″ N 80°38′14″ W	26°65′96″ N 80°63′10″ W
Soil thickness (cm)	26	28
pH	7.4	7.6
Total carbon (g kg^{-1})	428.15	394.29
Total nitrogen (g kg^{-1})	23.07	24.23
C:N ratio	18.58	16.27
Mehlich-3 phosphorus (mg kg^{-1})	40	22
Potassium (mg kg^{-1})	108	119
Calcium (mg kg^{-1})	9438	10,106
Magnesium (mg kg^{-1})	1335	1344
Silicon (mg kg^{-1})	50	39
Crop Calendar		
Sugarcane stubble crop harvest	15 November 2015	22 October 2016
Sugarcane planting	10 December 2015	18 November 2016
Plow-out to replant duration	25 days	27 days
Plant crop sampling	10 December 2016 (12 Months)	15 November 2016 (12 Months)
Plant crop machine harvest	05 March 2017	17 December 2017
Ratoon crop sampling	19 December 2017 (9 months, 15 days)	19 November 2018 (11 months, 2 days)

Ratoon crops received the same nitrogen treatments as plant crop except for the method of N application. In ratoon crops, N was applied as a side-dress application in which fertilizers were surface applied next to the row. In addition, NPK fertilizer mix containing 0-12-43 at 454 kg ha^{-1} was surface applied using tractor mounted mechanical spreader (Tarter fertilizer spreader and seeder, Dunnville, KY, USA), after initiation of ratooning (within 30 days after machine harvest). For ratoon crops, ammoniated polyphosphates fertilizer having 2.54% ammoniacal nitrogen was used as a source of P fertilizer. The P, K, and micronutrient fertilization rates used in this experiment were according to pre-plant soil test recommendations for sugarcane grown on Histosols (McCray et al., 2016). Standard agronomic practices, such as post-planting inter-row cultivation, weed management, sub-surface irrigation, was followed throughout the cropping cycles at both sites.

2.3. Fungicide Treatments

Fungicides were applied to the subplots (10 m × 6 m (4 rows)) at the time of planting, prior to sugarcane row closure. The treatments were: (i) Untreated, (ii) Mancozeb 2.5 kg a.i ha^{-1} (Manzate Max®, United Phosphorus Inc., King of Prussia, PA, USA), iii) Mefenoxam, 0.57 kg a.i ha^{-1} (Ridomil Gold® 2E, Syngenta Corporation, Greensboro, NC, USA), and (iv) Azoxystrobin, 0.3 kg a.i ha^{-1} (Quadris® 2.08SC, Syngenta Corporation, Greensboro, NC, USA). Further description of commercial formulations of these fungicides can be found in Vuyyuru et al. [29]. Fungicides were topically applied as a 15 cm concentrated band over seed cane pieces in the furrows prior to covering with soil. The fungicides were applied by using backpack sprayer (Solo backpack sprayer 15 L tank capacity, model:425-101, Root-Lowell manufacturing Co., Lowell, MI, USA) in 150 L ha^{-1} water with flat pan nozzle at 138 kPa pressure. Additionally, Phorate (Thimet® 20-G, AMVAC, Los Angeles, CA, USA) was applied at 21.9 kg ha^{-1} in furrows to prevent wireworm (*Melanotus communis*) damage to the planted cane.

2.4. Tissue Analyses

In sugarcane nutrient diagnosis, the leaf-blade lamina of youngest fully matured leaf, specifically third leaf from the top (known as Top Visible Dewlap or TVD leaf), was used to determine the extent of crop-nutrient supplies, which are adequate for optimum crop growth. Leaf samples were collected during the sugarcane grand growth period (April to July), and in each sampling occasion, 20 randomly selected TVD leaves were collected between 9:00–10:00 a.m. Leaf midveins were removed, and then leaf blades were rinsed in deionized water to remove any adherent soil particles. Leaf blades were dried at 60 °C in a sample dryer room for 7 days. The dried tissues were ground in Willey plant tissue grinding mill (Thomas Scientific, Swedesboro, NJ, USA) fitted with 1 mm sieve and were used for nutrient concentration determination. Total Kjeldahl nitrogen (TKN) was measured by digesting 0.10 g of the ground tissue with 3.5 mL concentrated H_2SO_4 and 1.0 g Kjeldahl digestion mixture (10 g K_2SO_4 + 0.30 g $CuSO_4$) for 3.5 h at 160 °C to 380 °C temperature in a block digester. TKN content was determined by following the semi-automated colorimetric analytical procedure in the digested samples by method 351.2 [30]. Tissue P, K, Ca, Mg, Cu, Mn, Fe, and Zn were determined after ashing 0.4 g of the ground sample at 500 °C for 12 h [31]. The ash was dissolved in 20 mL of 6.0 M HCl, and concentration of elements in the extract was determined by inductively coupled plasma emission spectrometer (Agilent 5110 ICP-OES, Santa Clara, CA, USA). The tissue nutrient concentrations were compared using critical nutrient level (CNL) approach and Diagnosis and Recommendation Integrated System (DRIS) indices (Table S1).

Sugarcane tissue critical and optimum nutrient ranges were compared with the published values [32,33], and DRIS application was developed for Florida sugarcane [34]. DRIS uses means and standard deviations of the nutrient ratios to calculate the relative indices of individual nutrients that range from negative to positive, and their sums are always equal to zero. Higher negative index for a given nutrient indicates insufficient nutrient uptake, while near zero or positive values indicate adequate nutrient uptake by the plants. DRIS indices were calculated by inputting the nutrient concentrations into excel spreadsheet calculator, and the sum of absolute DRIS indices was presented as a nutrient balance index (NBI). Conducting analysis of variance on DRIS indices of individual nutrients would not be appropriate as negative indices have equal weights as of positive indices. Hence, we used NBI parameter as an indicator of relative nutrient balance as influenced by nitrogen application.

2.5. Tiller Dynamics

Sugarcane exhibits various degrees of bud dormancy, and shoot emergence takes 4 to 6 weeks after planting under Florida weather conditions. After crop establishment, a 9 m^2 area (2 rows × 1.5 m row spacing × 3 m length) was flagged in the middle two rows of each subplot. Sequential tiller counts were made in the flagged area to quantify the dynamics of tillering, i.e., starting from first primary shoot emergence, secondary, or higher order shoots (also known as tillers) and subsequent shedding of excess tillers to final stabilization of millable canes. The first count was taken between 6 to 8 weeks after planting to estimate the number of primary shoots emerged from the planted setts. No attempt was made to differentiate the primary, secondary, or higher order tiller types, but the emphasis was made to readily observable differences in tiller counts at different times of data collection. In 2015 plant crop, tillers were counted at 54, 74, 96, and 119 DAP (Days After Planting). To differentiate N side-dress effects on tillering, we considered two distinct periods relative to the side-dress application, i.e., pre-side-dress and post-side-dress tiller count changes.

2.6. Yield Measurements

The number of millable stalks ha^{-1} in each subplot was estimated by counting the number of mature stalks in the two middle rows in early August. For estimating the average stalk weight, 10 randomly selected mature stalks per plot were hand harvested from the middle two rows. Tops were cut, and the leaves were stripped off the stalks before weighing. The average mature stalk weight was

calculated by dividing the total bundle weight with number of stalks in that bundle. Sugarcane yields, TCH (tons of cane per hectare), were calculated by multiplying the average stalk weight with number of stalks per hectare. The harvested stalks were crushed in a three-roller mill for juice extraction. Juice percent Brix (total soluble solids) was determined with an automatic temperature compensated (20 °C) refractometer (Bausch & Lomb, Rochester, NY, USA). About 200 mL juice was clarified by adding 5 g of Octapol Plus^{TM} (Baddley Chemicals Inc, Baton Rouge, LA, USA) and filtered through Whatman Grade 4V fluted filter paper. The filtrate was used for the determination of polarity (Juice sucrose concentration) with Saccharimeter (Rudolph Research Instruments, Flanders, NJ, USA). In 2017 and 2018 harvests, Cane Presentation System, CPS (Bruker Optik instruments, Ettlingen GmbH, Germany) was used to measure brix, pol, fiber, and moisture in each sample. In CPS, the sampled stalks were shredded in the disintegrator (Disintegrator DM 540, IRBI machinery and equipment, Aracatuba, Brazil) and then analyzed with FT-NIR spectrum (Fourier Transform Near Infra-Red spectrum). The Brix, Pol, and fiber were used to calculate juice sucrose concentration as commercially recoverable sucrose (KST, kg sucrose t^{-1} cane) [35]. Sucrose yield, TSH (tons of sucrose per hectare), was calculated as the product of TCH and KST. Dry biomass production (t ha^{-1}) was calculated based on the fresh weight of stalks (without tops and leaves), and percent moisture data were obtained from the FT-NIR spectrum.

2.7. Statistical Analyses

Statistical analyses were performed using SAS for Windows version 9.4 (SAS Institute, Cary, NC, USA 2018). Mixed model procedures for split-plot designs were used to analyze the combined data set of plant and first ratoon crops. In the case of significant year effect, the data were analyzed separately for plant crop and ratoon crops. Error degrees of freedom was determined by Satterthwaite option. Blocks and their interaction with the whole plot (nitrogen) were considered as random effects in separate analyses for each year. Year, block, and their interaction effects were considered as random in the analysis of combined data for two years. Means were compared using Tukey-Kramer grouping whenever ANOVA showed significant differences ($p \leq 0.05$ or $p \leq 0.1$). Measurements taken over time to see the treatment differences on the nutrient concentration of tissues were analyzed using repeated measures mixed procedure with restricted maximum likelihood method in which plot was considered as subject and date or month as time factor or repeated variable [36]. Unstructured model (type = un) was used to describe the repeated measures covariance in all the analyses.

3. Results

3.1. Weather Conditions

In multi-year trials, weather plays an important role in crop performance, and we experienced significant changes in amount and distribution of rainfall in different experimental years, which is common in southern Florida. According to the Koppen climate classification, the EAA lies in a transition zone of humid subtropical dry (Cfa) and tropical wet (Aw) climatic zones [37]. Typically, November–April months are dry winters that are characteristic to humid subtropical climate, which coincides to sugarcane maturity/harvesting, re-planting, and early growth periods. When the crop is in the grand growth period (May–September months), climate transitions to a tropical wet (Aw) climate with >70% annual precipitation received during these five months. Daily precipitation, minimum and maximum temperatures during the experimental years was collected from the Everglades Research and Education Center, Belle Glade, weather station [38] located within the radius 500 m distance from experimental sites. In 2015–2016, the minimum air temperature was ranged between 1.8 to 24.7 °C (mean 17.7 °C), and maximum temperature was ranged between 13.7 to 37.1 °C (mean 29.7 °C), while total precipitation received was 1115 mm (Figure 1A). In 2017, the minimum air temperature was ranged between 1.4 to 24.7 °C (mean 17.5 °C), and maximum temperature was ranged between 12.6 to 34.8 °C (mean 28.5. °C), while total precipitation received was 1604 mm (Figure 1B). Until sampling period for yield estimates in 2018, the minimum air temperature was ranged between 1.5 to 25.2 °C

(mean 18.42 °C), and maximum temperature was ranged between 13.1 to 33.9 °C (mean 28.9 °C), while total precipitation received was 1006.3 mm (Figure 1C). Precipitation patterns during sugarcane planting to the early growth period varied in experimental years.

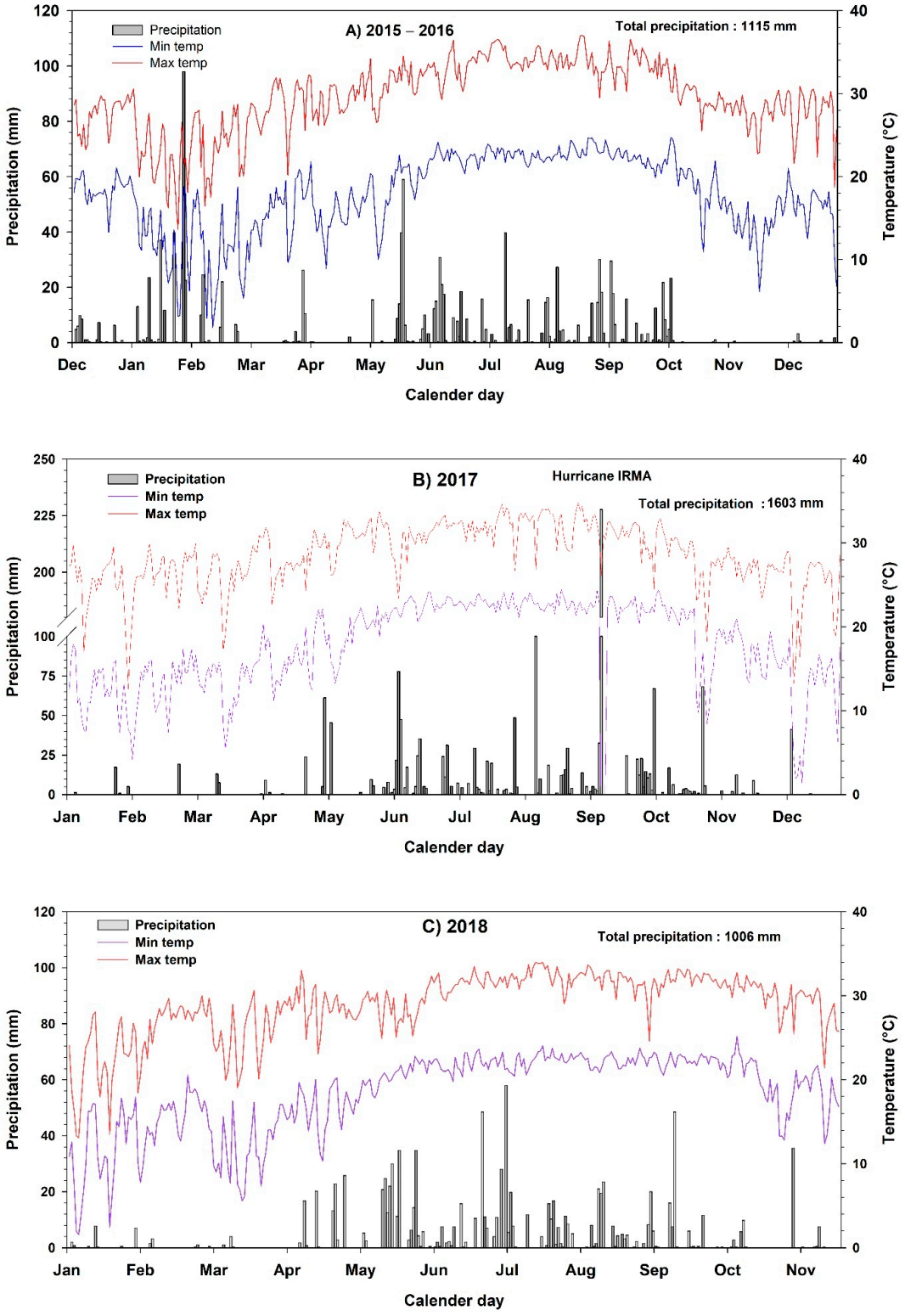

Figure 1. Daily precipitation and air temperatures (minimum and maximum) during the experimental years (**A**) 2015–16, (**B**) 2017, and (**C**) 2018.

3.2. Sugarcane Establishment and Tiller Dynamics

Tillering response to nitrogen fertilization and fungicides in plant crop varied with experimental years (2015 and 2016). The tiller count in 2015 was lower than in 2016. This may have been due to prolonged flooding after heavy precipitation in early weeks after planting in 2015. Sequential tiller counts data indicated maximum tillering at 147 DAP in 2016 plant crop (Figure 2B) and at 105 DAH (Days After Harvest)in both the ratoon crops (Figure 2C,D).

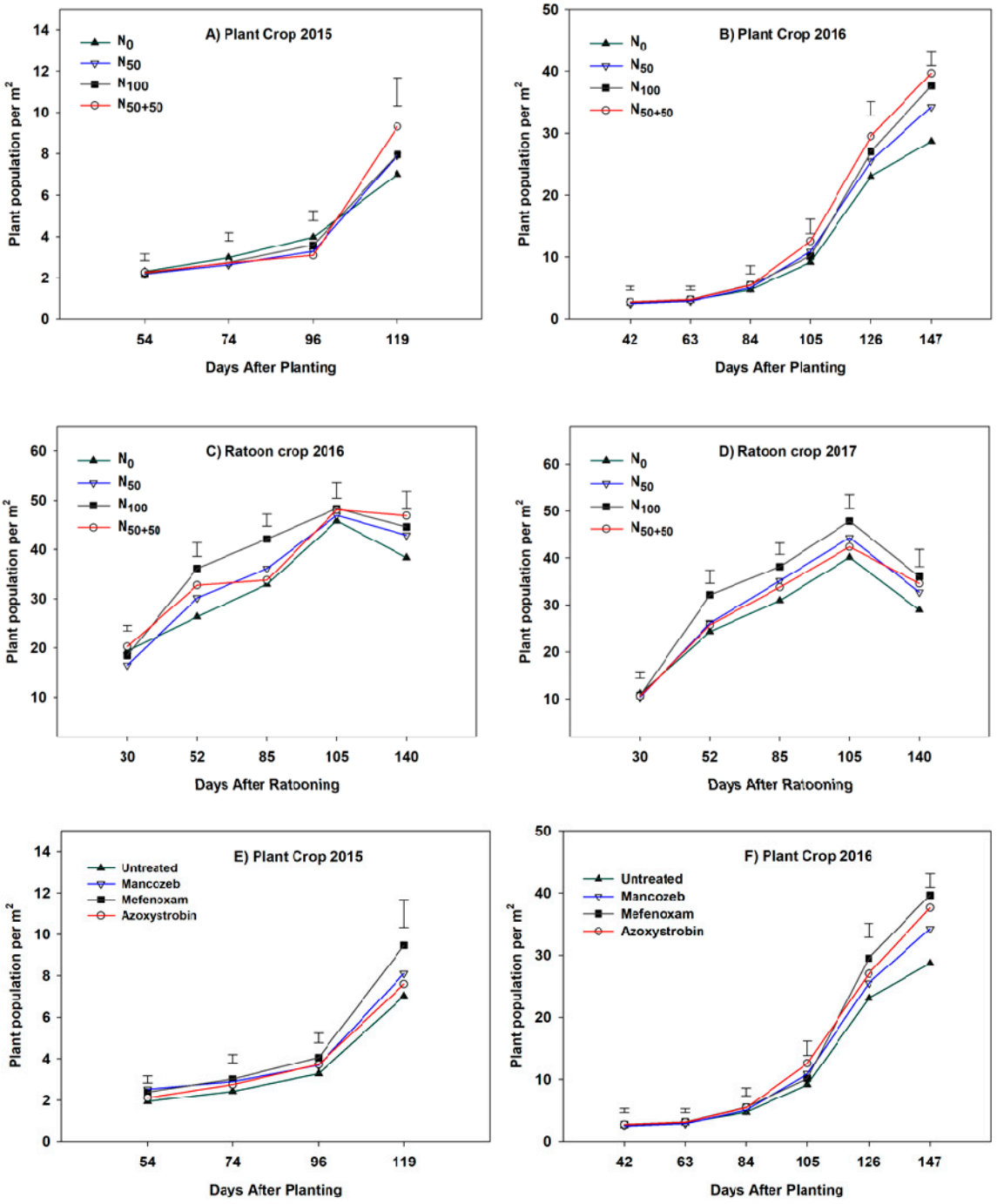

Figure 2. Sugarcane establishment and tiller dynamics as influenced by nitrogen fertilization and fungicides (**A**) plant crop 2015, (**B**) plant crop 2016, (**C**) ratoon crop 2016, (**D**) ratoon crop 2017; Fungicides in (**E**) plant crop 2015 and (**F**) plant crop 2016; Error bars represent standard error of the mean.

In general, the initial establishment of plant crop was not affected by nitrogen fertilization, but secondary or higher order tillering was influenced by N fertilization. In the plant crop, tiller production was similar in all treatments at an early stage in both years (Figure 2A,B), but in counts taken after 100 DAP, all N applied plots showed higher tiller production than N_0. Split application of N (N_{50+50}) produced higher tillers than other treatments. The ratoon crop of 2016 started under favorable temperature and moisture conditions (early-March machine harvest) than the ratoon crop of 2017 (mid-December machine harvest); hence, more tillering was observed in ratoon crop of 2016 than 2017. In the ratoon crop of 2016, after side-dress N application at 90 DAH (N_{50+50}), increased trends of tiller production were observed (Figure 2C), whereas, in the ratoon crop of 2017, N_{100} produced more tillers than other treatments at all sampling dates (Figure 2D).

In comparison to N treatments, fungicidal treatments did not show any effect on early season plant population (Figure 2E,F). Trends of increased tiller production were evident at 3rd or 4th sequential counts where mefenoxam showed greater tiller production than in the untreated. However, this fungicidal effect in plant cane was not carried to ratoon crops, and there was no significant treatment difference (data not shown).

3.3. Tissue Nutrient Concentrations

3.3.1. Plant Crop

In plant crop of 2016, repeated measures analyses of nine nutrients' (N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu) concentrations and nutrient balance index in tissues did not show significant effects on nitrogen fertilization (Table 2). Fungicides showed significant effects on Mg and nitrogen x fungicides interaction effects on P, Ca, Mg, and Zn concentrations (Figure 3). Seasonal fluctuations in N concentrations in leaf blades were evident with significant three-way interactions of nitrogen x fungicide x month effects (p = 0.0317). Sampling month effects were significant on N, P, K, Ca, Mg, and Mn nutrients and are presented in Table 3. In all sampling instances, the nitrogen concentration in plant crop was above the critical value (18 g kg^{-1} tissue) and within optimum range (20–26 g kg^{-1} tissue) for the leaf blades [32,39]. N concentrations were relatively high in April (24.12 g kg^{-1}) compared to May (23.03 g kg^{-1}). Overall, Ca (4.90–6.17 g kg^{-1}) and P (2.36–2.56 g kg^{-1}) content of leaf blades were high, K (10.91–12.12 g kg^{-1}) and Mg (1.68–1.83 g kg^{-1}) were within optimum ranges, and Cu (3.52–3.72 mg kg^{-1}) content was low (Table 3) when compared to published nutrient levels in TVD leaves (Table S1). Absolute DRIS value ranges within 10 does not indicate nutrient limitation for optimum yields. Relatively higher Ca concentrations led to positive DRIS values for Ca, while relatively lower K and Mg concentrations led to negative DRIS values for these nutrients (Table S2).

Table 2. Repeated measures analyses ANOVA p-values for the effects of nitrogen, fungicides, sampling months, and their interactions on sugarcane leaf tissue nutrient composition of 2016 plant crop.

Fixed Effects	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	NBI
					Plant crop 2016					
Nitrogen (N)	0.4879	0.5836	0.7305	0.8379	0.9707	0.8486	0.9693	0.8971	0.8132	0.6094
Fungicide (F)	0.7359	0.7596	0.8570	0.0835	0.0447	0.8567	0.8738	0.2139	0.5661	0.0610
N × F	0.9057	0.0051	0.3963	0.0078	0.0238	0.8255	0.2769	0.0237	0.0876	0.7388
Month (M)	0.0044	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1670	0.2856	<0.0001	0.1939
N × M	0.3480	0.8649	0.6978	0.3737	0.4212	0.5646	0.9556	0.9985	0.1405	0.4705
N × F × M	0.0317	0.6764	0.4719	0.2116	0.6076	0.8580	0.9958	0.3790	0.5748	0.8639

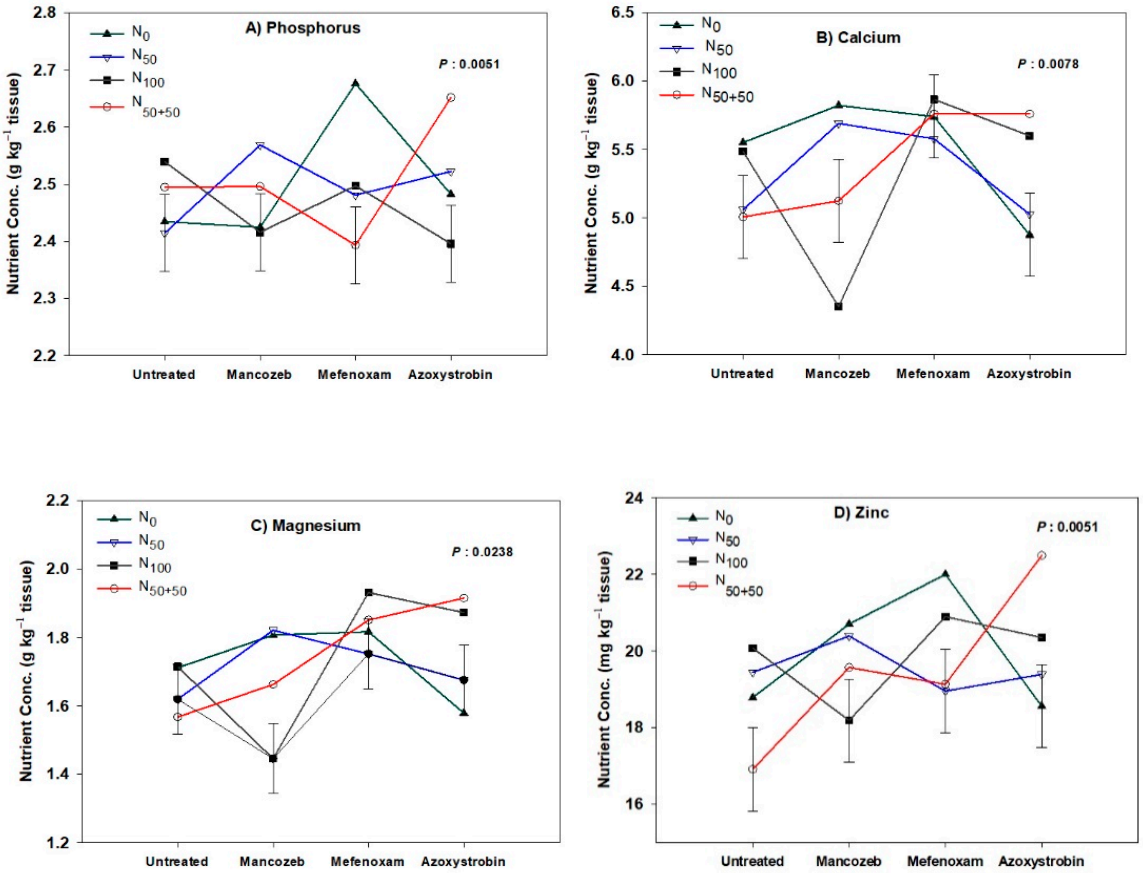

Figure 3. Interaction effects of Nitrogen × Fungicide on sugarcane leaf tissue concentrations of (**A**) Phosphorus, (**B**) Calcium, (**C**) Magnesium, and (**D**) Zinc nutrients in plant crop 2016. Error bars represent standard error of the mean.

Table 3. Least Square means for seasonal sugarcane leaf tissue nutrient concentrations of macro and micro-nutrients and nutrient balance index (NBI) in 2016 plant crop.

Month	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	NBI
			g kg^{-1}					mg kg^{-1}		
April 2017	24.12 a	2.56 a	10.91 b	5.09 a	1.68 b	77.79 a	3.73 a	20.82 a	15.09 b	99.05 a
May 2017	23.03 b	2.53 a	11.05 b	6.17 a	1.68 b	80.93 a	3.73 a	19.38 a	25.62 a	91.72 a
June 2017	23.31 ab	2.36 b	12.12 a	4.90 a	1.83 a	69.95 b	3.52 a	19.75 a	26.46 a	89.14 a

Values followed by different letters in a column are significantly different; Tukey's HSD (honestly significant difference) test at $p \leq 0.05$.

3.3.2. Ratoon Crops

Nitrogen fertilization effects on the N composition of leaf blades varied from year to year with significant differences in ratoon crop of 2016 while no differences in ratoon crop of 2017 (Table 4). In 2016 ratoon crop, N fertilization tend to show higher leaf N concentration than N_0, with no significant difference among N treatments (Table 5). Other nutrients, such as Ca, were significant in both the years, while Mg and Zn were significant only in ratoon crop of 2017. Calcium content of leaf blades was ranged within optimum ranges. There was no specific pattern in N fertilization effects (Table 5) or seasonal changes for Ca, Mg, and Zn nutrients (Table 6) in both the ratoon crops. As in plant crop, relatively higher Ca concentrations in ratoon crops also reduced K and Mg concentration in leaf blades. There was a decreasing trend of NBI values with N fertilization and lower values in both the years with N_{50+50} treatment compared to N_0. DRIS analyses indicated positive balances with N and Ca and

negative balances for K, Zn, Cu in both the ratoon crops except for Fe. For Fe, negative DRIS values were observed in the ratoon crop of 2016 but not in ratoon crop of 2017 (Table S3).

Table 4. Repeated measures analyses ANOVA *p*-values for the effects of nitrogen, fungicides, sampling months, and their interactions on sugarcane leaf tissue nutrient composition of first ratoon crops.

Fixed Effects	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	NBI
Ratoon crop 2016										
Nitrogen (N)	0.0394	0.5356	0.4445	0.0376	0.7050	0.2111	0.6541	0.9006	0.5941	0.0347
Month (M)	0.0064	<0.0001	<0.0001	<0.0001	0.0016	<0.0001	<0.0001	0.3149	0.1753	0.6225
N × M	0.4427	0.5323	0.2029	0.6689	0.3739	0.3296	0.4168	0.1403	0.6337	0.5053
Ratoon crop 2017										
Nitrogen (N)	0.8993	0.4838	0.7590	0.0352	0.0223	0.7859	0.2115	0.0237	0.6762	0.0418
Month (M)	0.3007	<0.0001	0.0024	<0.0001	0.1872	0.5827	0.0098	0.0360	0.0002	0.1001
N × M	0.0543	0.0984	0.4709	0.8468	0.7066	0.5313	0.0689	0.0595	0.3876	0.1688

NBI = Nutrient Balance Index.

Table 5. Least Square means for nitrogen main effects on sugarcane leaf tissue nutrient concentration and nutrient balance index (NBI) of ratoon crops.

Nitrogen	N g kg^{-1}	Ca g kg^{-1}	NBI	Ca g kg^{-1}	Mg g kg^{-1}	Zn mg kg^{-1}	NBI
	Ratoon crop 2016			Ratoon crop 2017			
N_0	23.94 b	4.31 a	207.12 a	4.03 ab	1.14 ab	10.40 ab	152.09 a
N_{50}	25.50 a	3.78 b	173.15 ab	3.72 b	1.04 b	9.94 b	112.06 ab
N_{100}	24.77 ab	4.05 ab	124.84 b	4.32 a	1.27 a	11.09 ab	95.32 ab
N_{50+50}	25.04 ab	4.27 ab	134.56 b	4.26 ab	1.23 ab	12.05 a	82.43 b

Values followed by different letters in a column are significantly different; Tukey's HSD (honestly significant difference) test at $p \leq 0.05$.

Table 6. Least Square means for seasonal leaf tissue nutrient concentrations of macro and micro-nutrients and nutrient balance index (NBI) in sugarcane ratoon crops.

Month	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	NBI
	g kg^{-1}					mg kg^{-1}				
2016 Ratoon crop										
May 2017	23.80 b	1.83 b	8.55 b	4.38 a	1.21 a	44.37 b	2.08 b	11.93 a	11.93 a	168.80 a
June 2017	26.09 a	1.63 c	7.50 c	4.57 a	1.24 a	44.90 b	2.48 a	12.51 a	12.51 a	163.36 a
July 2017	24.50 ab	2.01 a	11.82 a	3.35 b	1.10 b	53.37 a	2.07 b	13.25 a	13.25 a	147.59 a
2017 Ratoon crop										
April 2018	20.53 a	2.55 a	9.94 a	4.49 a	1.22 a	60.43 a	1.79 a	11.96 a	16.45 b	165.36 a
May 2018	20.13 a	2.08 b	8.33 b	3.93 b	1.20 a	63.83 a	1.48 ab	9.99 b	21.51 a	155.13 a
June 2018	20.32 a	2.30 b	7.59 b	3.82 b	1.08 a	59.04 a	1.45 b	10.67 ab	19.43 a	158.91 a

Values followed by different letters in a column are significantly different; Tukey's HSD (honestly significant difference) test at $p \leq 0.05$. NBI = Nutrient Balance Index.

3.4. Yield Attributes and Sucrose Yields

In a combined dataset of two years' plant cane and ratoon crops, ANOVA *p*-values for stalks m^{-2}, TCH, and TSH yields showed significant year effects with N fertilization and fungicides (except in TSH) but no significant interaction between N and fungicides (Table 7). Significant year effects on TCH indicated that cane yields varied across environments. Significant year effects in KST were probably due to deterioration of cane quality in 2016 plant cane due to hurricane Irma and difference in crop age at the time of harvest. The interaction effects of year and crops (Y × C) and three-way interaction effects with nitrogen, years, and crops (N × Y × C) indicated the effect of N fertilization on stalks m^{-2},

TCH, and TSH yields that varied in different years. Hence, data were analyzed separately for each crop year to detect nitrogen and fungicidal effects on yield attributes and sucrose yields.

Table 7. Fixed effects on ANOVA *p*-values of nitrogen, fungicides, year, crop, and their interaction effects on sugarcane yield attributes and yields with a combined dataset of plant and ratoon crops.

Effect	d.f	Stalks m^{-2}	Stalk Weight	TCH	KST	TSH
Nitrogen (N)	3	0.0051	0.2259	0.0004	0.6237	0.0188
Fungicides (F)	3	0.0920	0.7040	0.0175	0.8881	0.1592
N × F	9	0.6689	0.2902	0.3668	0.9508	0.3730
Year (Y)	1	0.0837	0.0004	<0.0001	<0.0001	0.1338
N × Y	3	0.1783	0.8602	0.1738	0.4977	0.8351
F × Y	3	0.7233	0.0603	0.2570	0.0635	0.4632
N × F × Y	9	0.8990	0.9718	0.9172	0.6495	0.9363
Crop (C)	1	0.0031	<0.0001	<0.0001	<0.0001	<0.0001
N × C	3	0.5961	0.8299	0.2608	0.7421	0.4319
F × C	3	0.0853	0.1167	0.1291	0.6843	0.0863
N × F × C	9	0.5674	0.2653	0.4970	0.5402	0.8165
Y × C	1	<0.0001	<0.0001	<0.0001	0.0007	<0.0001
N × Y × C	3	0.0047	0.2033	0.0003	0.7163	0.0258
N × F × Y × C	3	0.3343	0.4237	0.5952	0.3643	0.9329

d.f = degrees of freedom, TCH = tons of cane per hectare, KST = kilograms of sucrose per ton, TSH = tons of sucrose per hectare.

3.4.1. Plant Crops

Successive plant crop N responses on yield attributes and sucrose yields were observed in 2016, but not in the 2015 year (Table 8). In 2016 plant crop, millable stalks, stalk weights, TCH, TSH, dry biomass yields were significantly increased with N fertilization, generally followed in the order $N_0 < N_{50} \leq N_{100} < N_{50+50}$. Sugarcane juice quality (KST) was unaffected by N fertilization. Significant effects of seed piece applied fungicides on yield attributes, especially, millable stalks m^{-2}, stalk weights, were observed in 2015 plant crop. Fungicides also showed higher trends in 2016 TSH and dry biomass, and on TCH in both the years. Among fungicides, mefenoxam showed significantly higher stalks m^{-2} and TCH yields than azoxystrobin or mancozeb fungicides in 2015 plant crop. Significant N × F interaction effects on final millable stalks suggested that mefenoxam, in combination with N fertilization, achieved greater millable stalks than other fungicides (Figure 4). In 2016, dry biomass yields were significantly increased with N fertilization. Fungicides did not show significant effects on KST in both the years, and a significant increase in TSH yields in 2016 plant crop was observed. As sugarcane yields are reported on a fresh weight basis, the cane was harvested at different stages of maturity. It is imperative to know whether there were significant differences in dry biomass yields due to N fertilization or Fungicides. The dry biomass yields of 2016 plant crop showed significant effects due to N fertilization and followed in the order: $N_0 < N_{50} \leq N_{100} < N_{50+50}$. While seed piece applied fungicides showed significant effects on dry biomass yields and followed in the order: untreated < mancozeb ≤ azoxystrobin < mefenoxam.

Table 8. Least Square means of sugarcane yield attributes and yields in nitrogen and fungicide treatments in 2015 and 2016 plant crops.

Main Effects	Plant Crop 2015					Plant Crop 2016					
	Stalks m^{-2}	Stalk Weight (kg)	TCH (t ha^{-1})	KST (kg t^{-1})	TSH (t ha^{-1})	Stalks m^{-2}	Stalk Weight (kg)	TCH (t ha^{-1})	KST (kg t^{-1})	TSH (t ha^{-1})	Dry Biomass (t ha^{-1})
Nitrogen (N)											
N_0	10.97 a	1.15 a	126.17 a	131.11 a	16.53 a	11.54 b	1.14 b	132.31 c	109.01 a	14.41 c	37.23 c
N_{50}	10.92 a	1.16 a	127.71 a	131.86 a	16.83 a	11.88 ab	1.19 ab	141.94 b	106.41 a	15.11 bc	40.13 b
N_{100}	10.95 a	1.14 a	124.94 a	127.51 a	15.94 a	12.04 ab	1.20 a	145.09 b	106.45 a	15.44 b	40.32 b
N_{50+50}	11.07 a	1.14 a	126.53 a	130.94 a	16.57 a	12.38 a	1.22 a	150.94 a	108.34 a	16.28 a	42.74 a
Fungicide (F)											
Untreated	10.63 c	1.18 a	125.65 ab	128.29 a	16.11 a	11.70 a	1.17 a	137.06 b	107.48 a	14.73 b	38.55 c
Mancozeb	11.02 b	1.09 b	120.50 b	132.24 a	15.93 a	11.92 a	1.20 a	143.04 b	106.04 a	15.15 ab	40.03 b
Mefenoxam	11.45 a	1.15 ab	131.88 a	131.20 a	17.34 a	12.30 a	1.19 a	146.69 a	107.51 a	15.77 a	41.52 a
Azoxystrobin	10.80 bc	1.17 a	127.32 ab	129.67 a	16.50 a	11.92 a	1.20 a	142.89 b	109.19 a	15.61 a	40.42 ab
p-Values											
N	0.6247	0.8279	0.8937	0.6192	0.6395	0.0151	0.0346	<0.0001	0.4924	<0.001	<0.0001
F	<0.0001	0.0191	0.0240	0.6995	0.1991	0.1268	0.6549	<0.0001	0.5184	0.0075	<0.0001
N × F	0.0449	0.1785	0.0728	0.9456	0.5309	0.4596	0.1365	0.2377	0.1856	0.0525	0.4473

TCH = tons of cane per hectare, KST = kilograms of sucrose per ton, TSH = tons of sucrose per hectare. Values followed by different letters in a column are significantly different; Tukey's HSD (honestly significant difference) test at $p \leq 0.05$).

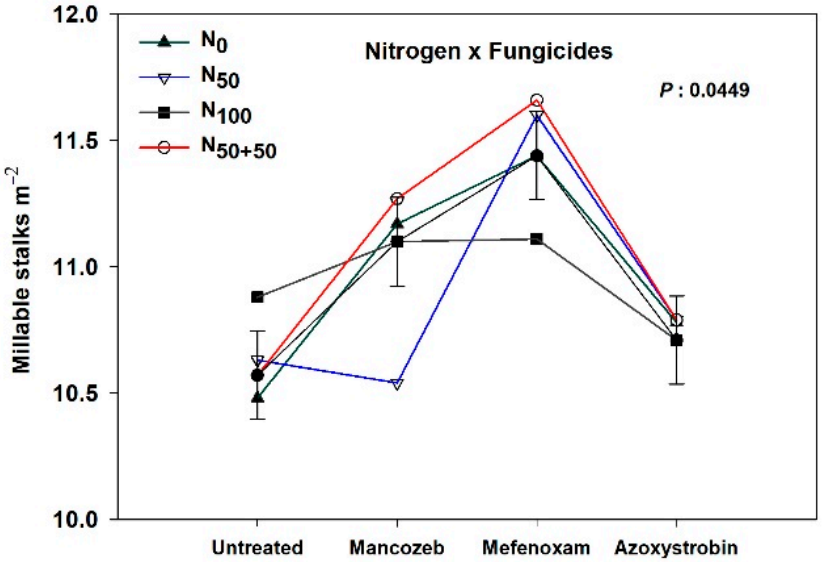

Figure 4. Interaction effects of nitrogen × fungicide on sugarcane millable stalks in 2015 plant crop. Error bars represent standard error of the mean.

3.4.2. Ratoon Crops

In both the ratoon crops, nitrogen fertilization showed significant effects on yield attributes primarily on number of stalks m^{-2} but not on stalk weight and KST content of stalks (Table 9). Sugarcane yields (TCH) showed increasing trends with N application and higher response at 100 kg N ha^{-1} than unfertilized. Ratoon crop response to N_{100} vs. N_{50+50} treatments varied between years. In 2016 ratoon crop, N_{50+50} showed significantly higher cane and sucrose yields than N_{100}, while the opposite was true in 2017 ratoon crop yields. No carryover effects of seed piece applied fungicides from plant crop to ratoon crops were observed in both the ratoon crops.

3.4.3. Average Yields

The average yields of all plant and ratoon crops (Figure S1) combined data analyses showed that N fertilization significantly increased TCH over unfertilized (Figure S1A). Significant enhancement in TSH yields was observed in N split application (Figure S1C). In fungicide treatments, only mefenoxam improved TCH compared to untreated (Figure S1B), and there were no significant fungicidal effects on TSH yields (Figure S1D).

Table 9. Least Square means of sugarcane yield attributes and yields in nitrogen and fungicide treatments in 2016 and 2017 ratoon crops.

Main Effects	Ratoon Crop 2016						Ratoon Crop 2017					
	Stalks m^{-2}	**Stalk Weight (kg)**	**TCH (t ha^{-1})**	**KST (kg t^{-1})**	**TSH (t ha^{-1})**	**Dry Biomass (t ha^{-1})**	**Stalks m^{-2}**	**Stalk Weight (kg)**	**TCH (t ha^{-1})**	**KST (kg t^{-1})**	**TSH (t ha^{-1})**	**Dry Biomass (t ha^{-1})**
Nitrogen (N)												
N_0	11.05 b	0.844 a	93.36 b	128.47 a	11.94 b	29.66 b	10.90 b	1.33 a	145.51 b	94.84 a	13.79 a	43.50 b
N_{50}	10.99 b	0.863 a	94.88 b	126.57 a	12.01 b	30.02 b	10.82 b	1.36 a	147.28 ab	92.50 a	13.63 a	44.09 ab
N_{100}	10.99 b	0.890 a	96.79 b	131.09 a	12.65 b	31.01 ab	11.37 a	1.34 a	152.67 a	92.42 a	14.10 a	45.80 a
N_{50+50}	12.15 a	0.878 a	106.37 a	127.57 a	13.66 a	34.14 a	10.92 b	1.33 a	145.37 b	94.90 a	13.78 a	43.95 ab
Fungicide (F)												
Untreated	11.12 a	0.863 a	95.58 a	127.97 a	12.33 a	30.25 a	11.01 a	1.30 b	144.18 a	95.33 a	13.73 a	43.49 a
Mancozeb	11.23 a	0.875 a	98.40 a	125.76 a	12.38 a	31.26 a	10.93 a	1.37 a	149.75 a	94.19 a	14.10 a	44.98 a
Mefenoxam	11.10 a	0.880 a	97.11 a	130.69 a	12.73 a	31.25 a	11.05 a	1.33 ab	148.93 a	89.07 a	13.27 a	44.21 a
Azoxystrobin	11.70 a	0.856 a	100.32 a	129.62 a	11.91 a	32.07 a	10.91 a	1.35 ab	147.98 a	96.06 a	14.20 a	44.66 a
p-values												
N	0.0739	0.6790	0.0990	0.6725	0.0872	0.0448	0.0002	0.6247	0.0275	0.7448	0.8228	0.0497
F	0.6216	0.9275	0.8540	0.5774	0.7916	0.7642	0.2407	0.0522	0.1741	0.1111	0.2665	0.3361
N × F	0.8382	0.8897	0.7969	0.9598	0.8239	0.7044	0.5071	0.3012	0.6188	0.5392	0.2858	0.6047

TCH = tons of cane per hectare, KST = kilograms of sucrose per ton, TSH = tons of sucrose per hectare. Values followed by different letters in a column are significantly different; Tukey's HSD (honestly significant difference) test at $p \leq 0.05$.

4. Discussion

The Histosols of EAA contains 2 to 4% total nitrogen [40], and growing sugarcane without external nitrogen inputs on these soils is unique to the Florida sugarcane industry. Nitrogenous fertilizers are reported to induce succulence, and plants become more susceptible to frost injury once air temperature falls below 0 °C in Dec–Feb months [14]. However, the frequency of frost events is very low in South Florida, and no frost event has been reported since 2010. The current University of Florida, IFAS nutrient recommendations do not include N fertilization for growing sugarcane on Histosols. Based on soil test results for Mehich-3 extractable phosphorus, the annual P banded applications recommended for sugarcane ranges from 0 to 36.7 kg ha^{-1} [41]. However, due to non-availability of single superphosphate, fertilizer blending plants were substituting with monoammonium phosphate (NPK content 11-52-0), at this recommended maximum rate of P fertilization, monoammonium phosphate can supply up to 17.8 kg N ha^{-1}. As the soils are getting shallower due to soil C oxidation, there may not be adequate N availability in shallow Histosols (<50 cm soil depth). Furthermore, less gap between two crop cycles in successive sugarcane planting results in the accumulation of higher plant residue (leafy residue and stubbles) than fallow planting. This situation is likely to increase C:N ratio and reduce N availability to plants in successive than fallow planting. Also, there may be harmful fungal pathogens incidence in successive planting on stand establishment. Therefore, in the present study, we used N_{50}, N_{100} fertilization treatments at planting and split application as N_{50+50} (50 kg at planting + 50 kg as side-dress at 90 DAP) to observe N effects on sugarcane growth and yield. We used seed piece fungicidal applications for better crop establishment. Overall, sugarcane growth and yield in successive planting showed a positive response to N application in three out of four experimental years.

4.1. Nitrogen Effects on Sugarcane Tiller Dynamics and Crop Yields

A positive tillering response to applied nitrogen in both plant (except 2015 plant crop) and ratoon crops indicated that mineralized nitrogen supply might not be adequate for the crop to produce maximum tillers (Figure 2). The previous study in EAA showed less nitrogen in the top soil profile in sugarcane fields than in prairie lands (also called virgin lands that are not cropped to sugarcane, but drained Histosols) possibly due to crop export [42]. To achieve better yields, vegetable crops (sweetcorn, lettuce, and celery) grown on Histosols are recommended with 27 to 58 kg N ha^{-1} during spring season [43–45]. Tillering response to N rate and split application was varied in different crop years. There was no significant N effect on tillering in 2015 which might be due to high precipitation (>20 mm) during early growth period that might have caused N leaching from the root zone (Figure 1A). In 2016 plant crop, split application of N (N_{50+50}) produced more tillers than other treatments, especially after the side-dress application at 90 DAP. This suggested that 100 kg N ha^{-1} might not be required at the time of planting, and split application might result in better tillering.

In ratoon crops, N_{100} showed higher tiller counts than other N treatments during most of the dates. The split N application enhanced tiller production after side-dress application at 90 DAH in 2016 ratoon but not in 2017 ratoon crop. Compared to plant crops, profuse and synchronous tillering is a characteristic feature of ratoon crops and, therefore, competition for nutrients starts earlier than plant crop [46], and it may be the reason for better tillering with N_{100} than other N treatments. Response to split application (N_{50+50}) was dependent on ratooning time and precipitation events. The 2016 ratoon crop started in March 2017 (Table 1), and N side-dress application (N_{50+50}) was completed in June 2017 when the field was moist, that enhanced late season tillering. The 2017 ratoon crop was started in December 2017, and during early tillering, crop experienced dry conditions in January through April 2018. Consequently, N side-dress effects on tillering were lower than N_{100} treatment. Therefore, the time of ratooning is important to decide the rate and timing of N application.

Maximum tillering response and final millable stalk counts showed similar trends in all crop harvests. It indicates that better early season tiller production with N application could be translated to a higher number of millable stalks than unfertilized. However, stalk weights were not influenced by

N application except in 2016 plant crop where N_{100} and N_{50+50} treatments produced heavier stalks than unfertilized. Yield improvement with N applications was primarily due to increased tillering and conversion to millable stalks in these experiments.

High N rates have been previously reported to reduce sucrose yields [47]. In the present study, we did not find significant effects on KST measurements, and an increase in sucrose yields (TSH) was due to higher cane tonnage with N applications. The leaf N concentration was always above optimum ranges (N > 2.0%) in the present study. This explains why the KST was similar across treatments in plant and ratoon crops. Sugarcane yields (TCH and TSH) followed similar trends as millable stalks (except no significant effects in 2017 ratoon crop TSH yields). Overall, the split application of N improved TCH and TSH yields of plant and ratoon crops harvested in 2016. However, N_{100} produced higher TCH in 2017 ratoon crop. As discussed previously, in tiller production, split application (N_{50+50}) seems to be a better option in late season ratooning and N_{100} for early to mid-season ratooning. However, N response was limited by high precipitation events in these shallow Histosols. As an alternative to breaking the monoculture, different N rates and split applications were tested on sugarcane yield improvements in plow-out replant system in Australia. In general, significant N fertilization effects on sugarcane yields were either due to enhanced tillering followed by tiller retention to produce final millable stalks or increase in individual stalk weights, as previously reported elsewhere [48–50].

4.2. Fungicidal Effects on Sugarcane Tiller Dynamics and Crop Yields

Sugarcane monoculture yield decline research in Australia reported large-scale yield improvements by introducing longer breaks (more than 2 years), fumigation treatments, and heavy doses of fungicides (e.g., mancozeb at 1200 kg ha^{-1}) [51,52]. In this study, we used seed piece fungicidal applications to improve stand establishment in plant crop. Although fungicide application improved initial crop establishment with higher late season tillering than untreated in both plant crops (Figure 2E,F), significantly higher numbers ($p \leq 0.05$) were achieved with mefenoxam in the 2015 plant crop (Table 8). The reasons for the lower responses with mancozeb and azoxystrobin may be limited by mode of action, persistence in the environment, or rate of fungicide used. For instance, mancozeb, being a contact fungicide, ensuring that it protects the cut ends of seed cane may not be possible using an in-furrow seed piece application method. An alternate method, such as dipping the sets in a fungicidal solution, may be a better choice [12]. This may be the reason that mancozeb caused a significant improvement in sugarcane root and shoot growth in a greenhouse study [29] where the soil was drenched with a fungicide solution. In high organic matter soils, azoxystrobin was reported to be degraded faster by soil microbial communities [53], and greater response with mefenoxam may be due to its more systemic and persistent nature. The positive response with mefenoxam agrees with Hoy and Schneider [10] findings on metalaxyl using the compound at lower application rates to mitigate sugarcane stubble decline.

4.3. Effects on Tissue Nutrient Concentrations

Leaf analyses showed no significant differences in the leaf N concentrations across N fertilization treatments and were above the established critical value (N = 1.8%) in plant crops. No significant effect of N fertilization on tissue N concentration might be due to the dilution effect in the leaf tissues since the tillering and dry biomass production were increased with N treatments. Ratoon crop of 2016 showed higher leaf N% with N applications but not in ratoon crop of 2017; N uptake might be dependent on environmental conditions. Regardless of nitrogen fertilization, the mean N values reported in these trials were within the optimum ranges. Tissue analyses of plant crop indicated no significant differences between treatments on NBI calculated from DRIS indices. In these trials, a relatively higher concentration of Ca in leaf blades might have created antagonism among three cations and induced imbalance in K and Mg concentrations. Increased negative DRIS indices for K with N fertilization could be related to antagonism with Ca or maybe high-affinity K^+ transport system inhibited by NH_4^+ in plants [54]. Another explanation for lower K and Mg concentrations was attributed to a decrease in

the permeability of cells with high Ca uptake [55]. Relatively high NBI values show a larger extent of nutrient imbalance and need for fertilization [34]. While in 2016 ratoon crop, N fertilization indicated luxury consumption and a decrease in overall NBI values with N fertilization. Better micronutrient uptake in both the ratoon crops was possibly due to acidifying nature of ammonium nitrate. We did not measure soil pH after ammonium nitrate applications, and a decrease in soil pH due to shallow soil acidification with ammonium nitrate application and consequently enhanced uptake of Mn, Zn, Cu, and Fe nutrients were reported in bromegrass [56].

Significant nitrogen and fungicidal complex interaction effects in plant crops for P, Ca, Mg, and Zn uptake in leaf blades (Figure 4) could be attributed to spatial variations for available calcium within the experimental plots (10,106 ± 806 mg kg^{-1} soil). During the process of land preparation and post-planting inter-row cultivations to improve aeration and weed control, the calcium carbonate particles from underlying limestone were mixed up with the surface soil. Consequently, soil pH in shallow Histosols are within the ranges of 6.5 to 8.5, and sulfur amendments are recommended up to 500 kg ha^{-1} to improve micronutrient availability to plant crops [57]. Besides Ca-induced lower K and Mg cations uptake, higher negative indices for Fe, Cu, Mn, Zn indicated the need for ameliorating soil pH and micronutrient application for ratoon crops to improve yields. In the present study, sugarcane yield enhancements with N fertilization could not be explained either by critical nutrient limits or DRIS approaches as they indicated within optimum ranges across treatments in both plant and ratoon crops.

4.4. Economic Analyses

In the present study, we used ammonium nitrate as a source of nitrogen as Florida sugarcane growers prefer to use ammonium nitrate over urea as a source of N on mineral soils for sugarcane production [50]. Ammonium nitrate (34% N) supplies N in both ammonium and nitrate forms equally. Unlike other cereal grain crops, such as sorghum and maize, which absorb both N forms equally, sugarcane strongly prefers ammonium over nitrate [58]. Overall, N fertilization has shown to increase TSH yields over unfertilized by 0.39, 0.26, and 0.90 t ha^{-1}, respectively (Figure S1C), and economic benefits of N fertilization were \$162, \$0.17, and \$381 ha^{-1}, respectively, for N_{50}, N_{100}, and N_{50+50} treatments. In this economic analysis, additional N fertilization costs of ammonium nitrate at \$560 t^{-1} [59], application costs \$16.25 ha^{-1} [60], and cost of unrefined sugar \$625.23 t^{-1} [61] are used in calculations.

5. Conclusions

Our results portrayed that sugarcane yields in successive plantings might be improved through nitrogen fertilization and seed piece application of fungicides. Nitrogen fertilization has shown to enhance tillering and the number of millable stalks in three out of four crop harvests with increasing N rates (N_0 to N_{100}). Nitrogen split application (N_{50+50}) enhanced tillering, millable stalks, and TCH yields in plant crop and late ratooning (March). While N_{100} improved yields in mid-season (December) ratooning. Among the fungicides, mefenoxam improved TCH and TSH yields in the plant crop, but no carryover effects to ratoon crops were observed. On average, incremental yield increases with nitrogen fertilization ranged between 1.87 to 6.42%. It appears that nitrogen fertilization and fungicide soil application are promising practices to ameliorate the impact of sugarcane monoculture on sugarcane yields in Histosols.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/7/387/s1. Table S1. Critical and optimum ranges for Sugarcane leaf nutrient concentrations for TVD leaf (without midveins) during June–July months. Table S2. Main effects of nitrogen and fungicides on DRIS indices at different months in plant crop. Table S3. Main effects of nitrogen on DRIS indices at different months in ratoon crops. Figure S1. Overall effects of nitrogen fertilization and seed piece application of fungicides on Tons of cane per hectare (TCH) and Tons of sucrose per hectare (TSH) yields.

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Abbreviations

EAA, Everglades Agricultural Area; TCH, Tons of cane per hectare; TSH, Tons of sucrose per hectare; KST, Kilograms of sucrose per ton of cane; DRIS, Diagnosis and recommendation integration system; NBI, Nutrient Balance Index.

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