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Long-Term Effects of Nitrogen Sources on Yields, Nitrogen Use Efficiencies, and Soil

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Abstract: Corn is both the most important and nitrogen (N)-fertilized crop, but N losses from agricultural systems impact the environment. There is a lack of long-term data comparing effects of tilled systems receiving organic N fertilizer (TONF; e.g., manure) versus inorganic N fertilizer (TINF; e.g., urea) on N balance and losses. A long-term study assessing effects of TONF and TINF and comparing them to no till (NT) and strip till (ST) systems receiving inorganic nitrogen fertilizer (INF) was conducted with an irrigated continuous corn (CC) system with minimal erosion in Fort Collins, Colorado, USA. An N balance found a system nitrogen use efficiency (NUE_{Sys}) for TONF (manure) of 86.3%, which was higher than the NUE_{Sys} of TINF (enhanced efficiency fertilizer, EEF) of 60.2%. TONF (manure) contributed to higher HGY (2 of 11 yr.), higher C sequestration ($P<0.05$), and higher soil organic N content ($P<0.05$) than TINF. Soil phosphorus (P) and nitrate were higher with TONF than TINF ($P<0.05$). Both TONF and TINF had higher HGY and HGY N content (HGYN) than NT and ST ($P<0.05$). From 2012 to 2023, the HGY of the TONF did not decrease, while the HGY of TINF decreased with time ($P<0.05$). TONF is a best management practice to increase yields and N and C sequestration while reducing N losses compared to TINF and achieving higher yields than NT and ST. A combination of manure (30% of N input) with EFF (70% of N input) contributed to a synergistic effect that increased agronomic productivity.

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1. Introduction

One of the most urgent issues confronting humanity in the 21st century is the need to increase agricultural production to feed the continuously growing global population while adapting agriculture to a changing climate using soil and water conservation practices that contribute to sustainable systems and minimize losses of agrochemicals to the environment [1]. Corn (Zea mays L.) is the most nitrogen (N)-fertilized crop in the United States and there is a need to continue increasing N use efficiencies (NUE) to reduce N losses from corn systems [2]. Although to date hundreds of millions of dollars have been invested in countless studies on N management, the national surplus of the N not used by crops was recently estimated to be 41% (27 to 55%), indicating there remains a need to increase NUE to reduce losses of this surplus N to the environment [3].

Only a few long-term (8+ years) studies have been conducted that monitor NUE of corn while conducting an N mass balance, and findings have been mixed. A 30-yr. study on continuous corn (CC) under tillage and irrigation monitored soil N in the top 30 cm of soil and found that more N is being harvested with the corn compartments than was applied as N inputs [4]. In contrast, a 22-yr. CC study under tillage and irrigation that monitored soil N in the top 120 cm found significant N losses to the environment, with up to 60% of the applied inorganic N fertilizer (INF), 41% of the applied swine manure N, 30% of the applied INF when applied with P and potassium (K) fertilizers, and 35 to 43% N lost from combinations of manure and INF [5]. An 8-yr. no-till dryland CC study monitoring soil N in the 0-20 cm soil profile found N losses ranging from 19 to 31% [6]. A 13-yr. study of an irrigated no-till CC system that monitored soil N in the top 120 cm found that N losses from the system ranged from 19 to 57% for INF rates ranging from 67 to 246 kg N fertilizer ha^{-1} y^{-1}, respectively [7]. Few studies have conducted a long-term N balance accounting for soil N changes, especially down to 120 cm.

We only found one long-term N balance for corn systems where manure inputs had been monitored [5]. However, we did find a few long-term manure studies that had monitored effects of manure inputs on yields. Manure-N-based inputs have been reported to increase yields compared to inorganic N fertilizer (10 yr. study; composed and uncomposed beef feedlot manure) [8]; (25 yr. study; mixing swine manure, plant residues, and soil) [9]; (23 yr. study; pig manure) [10]; (28 yr. study; dairy cattle manure) [11]. A long-term 100 yr. non-irrigated manure study showed that manure can provide more stabilized yields than INF systems [12]. Other long-term studies have found that the manure-treated plots had yields similar to INF (10 yr. study with cattle manure and swine effluent [13]; 10 yr. study with broiler litter [14]). Other researchers that have found similar yields with the manure-treated plots have found that in the years with the poorest yields, the non-irrigated manure plots produced higher yields than the plots receiving INF (8 yr. non-irrigated study) [15].

Numerous benefits have been reported from using manure inputs such as broiler litter and swine and cattle manures. Long-term studies ranging from 10 to 70 yr. in duration have found that compared to INF, manure applications increase soil properties that are beneficial to soil productivity, such as soil cation exchange capacity (CEC), organic matter (OM), and total nitrogen (TN) [5,9,10,14,16,17]. However, some long-term studies ranging from 10 to 100 yr. with broil litter and cattle manure have found that manure applications can increase the potential for increased losses of P and N from the system [8,12,14]. It has been established that systems receiving large applications of manure changed from serving as sinks for P to sources of P to the environment [18–20].

Conservation practices such as NT and ST have been recommended to improve sustainability of agricultural systems [1]. There is a lack of long-term studies that have conducted an N balance assessing N uptake and dynamics of tillage management practices. There is also a need to compare the long-term yields of intensively cultivated systems (e.g., TONF) that are receiving incorporated manure N to those of TINF, NT, and ST systems. The review of literature conducted in metadata analyses by Pittelkow et al. [34] and Ogle [35] showed that the harvested grain yields (HGY) of corn systems under NT are lower than those of corn under intensive cultivation [34]. Delgado et al. [26] compared the long-term yields of conventional tillage (CT), NT, and ST systems that received INF and found that after five years of higher yields with CT, the NT yields started to increase and were similar to or greater than CT and ST. However, since Pittelkow et al. [34] and Ogle et al. [7] found that over time NT yields continue to decline, there is a need to compare the effects of longer time periods of NT with CT and ST to see if these improved NT yields are maintained or decline over a longer period of implementation. Additionally, there is a need to compare the corn yields of NT, ST, and CT systems with INF to the corn yields of systems receiving manure (ONF) that could potentially contribute to improved soil properties such as increased carbon sequestration [5,9,10,14,16,17] and have more stable yields with time [12].

Our review of the scientific literature found only one long-term manure N balance study that measured changes in N soil pools down to 120 cm, N uptake and N removal in the aboveground compartment, and compared it to an INF system in an irrigated and tilled corn system [5]. However, we did not find any long-term manure study that conducted an N balance and that compared the HGY and HGYN of TONF and TINF to long-term ST and NT studies, making the present work a unique study and a new contribution. The objective was to assess the long-term N balance from 2012 to 2017 as well as the NUE of the system (NUE_{Sys}) and N losses from the system (NL_{sys}) while accounting for N inputs, N outputs, and changes in soil N of an irrigated, tilled CC system during this period. Additionally, we compared the yields of the TONF and TINF to those of the NT and ST from 2012 to 2023 and conducted a lot of additional assessment on effects of TONF and TINF. We hypothesized that a) there will not be C and N sequestration with the TINF, b) the NL_{sys} with TINF

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will be higher than TONF, and c) TONF will contribute to C and N sequestration via the manure inputs. There is a need to conduct these additional long-term studies.

2. Materials and Methods

2.1. Site Information

This study was conducted at the Halvorson long-term research plots at Colorado State University's Agricultural Research, Development and Education Center (ARDEC) near Fort Collins, Colorado (40◦39′6′N, 104◦59′57′W, 1535 m above sea level) on a Fort Collins clay loam soil (fine-loamy, mixed, mesic Aridic Haplustalfs) with a 1 to 2% slope. The site has a semiarid temperate climate, and had average minimum, mean, and maximum air temperatures; growing degree days (GDD); and precipitation of 10.5 °C, 18.7 °C, 27 °C, 1341 °C, and 199 mm, respectively, during the planting to physiological maturity (R6, black layer) stages (Tables S1, S2) of the 2012 to 2023 growing seasons. The Halvorson plots area is 162.5 m wide by 162.5 m long with different crop rotations established in 2000 in an area that was previously in cultivated corn for seven years.

2.2. Manure and Inorganic N Fertilizer Application

The primary rotation used for this study was the manure rotation, which was 21.9 m wide by 162.5 m long, and divided into four randomized blocks with the treatments established in 2012. Prior to the start of the manure studies, the area was in cultivated continuous corn from 2000 to 2008, and in strip till continuous corn from 2009 to 2011. All the plots were tilled and the tilled treatments were: i) an average application of 186 kg N ha^{-1} of dry dairy manure (TDM; Table S3; an application rate that represented 45% of the total manure N applied released during the first growing season **[21–23]**); ii) an average application of 185 kg N ha^{-1} of DM with AgrotainPlus (TDMAP), which contains a urease inhibitor containing the active ingredient N-(n-butyl)-thiophosphoric triamide (NBPT) and the nitrification inhibitor dicyandiamide (DCD; Table S3); iii); 179 kg N ha^{-1} of urea fertilizer (TUF); iv) 179 kg N ha^{-1} of the enhanced efficiency fertilizer SUPERU (TSU), which is a urea fertilizer that contains DCD and NBPT (Table S3); and v) a control of zero nitrogen (TC0N) fertilizer input (0 kg N ha^{-1}; Table S3).

For mechanical reasons, the plot size varied slightly, with each TDM, TDMAP, TUF, and TSU plot being 4.6 m wide and 21.3 m long with six corn rows with 76-cm spacing. The TDM and TDMAP plots were longer than the TUF and TSU plots, which were 4.6 m wide and 15.2 m long. The TC0N plots were 9.1 m wide with 12 rows, and 6.1 m long. However, the area sampled for yield data was consistent for a total sample area of 11.6 m2 from each experimental unit. The TDMAP treatment was changed in 2017 to 30% of the rate of TDM (an average application of 55 kg N ha^{-1}) plus 70% of the rate of TSU (an average application of 117 kg N ha^{-1}) for a total of 172 kg N ha^{-1}. In other words, for the 2017 to 2023 period, the TDMAP treatment was changed to a TDMSU treatment. Additionally, the 179 kg N ha^{-1} TUF treatment was changed in 2021 to 179 kg N ha^{-1} TSU, so the TSU area doubled to cover an area 9.1 m wide by 15.2 m long. However, we continued collecting soil and plant samples at the original TSU area that had been receiving 179 kg TSU N ha^{-1} since 2012. Each year post corn emergence, TSU and TUF were surface broadcasted in the years 2012 to 2014 and 2018. They were surface banded in the years 2015 to 2017 and 2019 to 2020, and TSU was surface banded from 2021 to 2023. Immediately after fertilizer application, the plots were irrigated to incorporate the surface-applied fertilizer.

The dairy manure was obtained from the same commercial dairy operation since 2012 and it was composted for about 30 d from mid-February to mid-March. The composted solid manure's water, total N% and total C% content was determined the week before application to be approximately 179 kg of available N rate ha^{-1}. To get the final manure N input, a tractor loader tared using a weighbridge for the tractor and empty loader was used. After the manure was moved from the compost pile, it was returned to the weighbridge and adjusted until the manure content in the loader was one fourth of the intended full-plot application. A few hundred grams of manure were subsampled from the bucket into a small, steel can and sealed with a lid and weighed. The composted solid manure's water,

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total N% and total C% content was determined again to calculate the final applied manure N rate (Table S3).

The loader delivered the bucket of manure to its assigned one quarter of the plot area where it was distributed as uniformly as possible across the soil surface by hand using pitch forks. This process was repeated four times for each TDM, TDMAP, and TDMSU plot. For the TDMAP plots only, immediately after manure application AP was dissolved in water and applied to the TDMAP plots (2012 to 2015) with a field sprayer before manure incorporation (see Table S3 for manure, AP, UF, and SU rates). The manure was applied each year about one month before planting, and on the same day of manure application to the treatments a rotor-tiller was used to incorporate the manure to a depth of 15 cm, simulating moldboard plow tillage (Table S3). The TUF, TSU, and TC0N plots were also rototilled at the same time so all plots had a similar tillage effect.

2.3. General Management, Plant and Soil Sample Collection, and Chemical Analysis

Corn was planted close to the end of April or first week of May. Plant samples were collected at R5.5 for silage (dent) and at the R6 (physiological maturity) stage, which traditionally occurred in September. At R5.5 and R6, samples were divided into stalks, leaves, cobs, and grain. After the drydown process, which usually occurred in October, harvested yields were collected by manually removing the ears from all plants in two adjacent rows, each 7.6 m long, for a total sample area of 11.6 m2 from each experimental unit. All harvested ears were air-dried and mechanically threshed; the grain was then subsampled and oven-dried. (All oven-dried samples were left in a 60° C oven for at least 48 h.). After the collection of hand samples to assess harvested grain, all plots were harvested with a small plot harvester. Then a silage system was simulated by shredding and windrowing the corn stover and bailing it out, leaving just about 5-cm tall stalk stubs.

Soil samples down to 180 cm were collected in spring 2012 before manure application and fall 2012 after harvesting the corn plots with a small plot harvester. Soil samples down to 150 cm were collected in fall from 2013 to 2017 at similar times. A Valley® linear-move sprinkler was used to irrigate the corn as needed and irrigation samples were collected each time to determine background NO_3-N and NH_4-N content in water. Aboveground plant samples were analyzed for carbon and nitrogen content. Soil samples were analyzed for total carbon, total nitrogen, inorganic carbon, extractable soil phosphorous (P), and inorganic nitrogen (NO_3-N and NH_4-N). Additional details of plant, soil, and irrigation sampling and analysis, as well as management information about irrigation, N inputs/applications, pesticide applications, and plant sampling operation are shown in Tables S4 and S5, and Figure S1. Each of the soil N pools was expressed on the basis of an equivalent mass as described by Ellert et al. (2001)[24] and others that have used the equivalent soil mass procedure to conduct soil N balance as done by Delgado et al. (2023) [7].

2.4. Weather

Weather data was collected at the ARDEC research station from 2012 to 2023 (COlorado AGricultural Meteorological nETwork [CoAgMet] station #: FTC03). In the few instances where temperature or precipitation data was missing, this was supplemented by the CoAgMET station FCL01, which is located about 9 miles away from FTC03 on CSU's main campus. The data from CoAgmet station #FTCO3 and FCL01 was used to calculate the GDD. Additional information about weather is also shown in Tables S1 and S2.

2.5. N Budgets, NUE, Statistical Analysis, and Comparison to No-Till and Strip Till Treatments

An N budget similar to that conducted by Delgado et al. (2023) [7] was done for the manure plots (TDM, TDMAP, TUF, TSU, and TC0N) from 2012 to 2017 [7]. For the N budget, all inputs of N with the fertilizer and/or manure were accounted for, and the changes in total and soil organic N (SON) were determined. Estimated atmospheric wet and dry deposition were also accounted for, as well as the N input with background irrigation N, following the Delgado et al. (2023) [7] approach (Table S4). For a detailed description of how the N budget analysis was conducted, see Table S4 in

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the **Supplementary Materials** and Delgado et al. (2023) [7]. The NUE_{Sys} and NL_{sys} were calculated similarly to how it was done in Delgado et al. (2023; Table S4) [7]. The NUE analyses were calculated using the traditional method of subtracting the N uptake using the control plots.

To test for differences due to treatments within the manure rotation, we used an ANOVA [41] for nitrate N and ammonium N and other soil parameters (2012 to 2017), yields (2012 to 2023), and N uptake (2012 to 2023). Additionally, we conducted soil regression analysis [41] for the N budget studies from February 1, 2012 (day 0) to November 13, 2017 (day 2,112). Other regression analyses were conducted from August 30, 2013 (day 576) to September 12, 2023 (day 4,241) for silage; from September 24, 2013 (day 601) to September 27, 2023 (day 4256) for biomass (R6); and from October 22, 2013 (day 629) to October 17, 2023 (day 4276) for harvest.

We conducted a t-test analysis [41] by year to determine if from 2012 to 2023 the HGY and HGYN of TDM vs. NT; TDM vs. ST; TDMAP vs. NT; TDMAP vs. ST; TDMSU vs. NT; TDMSU vs. ST; TSU vs. NT; TSU vs. ST; TUF vs. NT; TUF vs. ST; and NT vs. ST were different. The differences between the tilled (TC0N), NT (NTC0N) and ST (STC0N) controls were also tested. Detailed information about the NT and ST rotations have been reported in previous publications **[7,25–27]** but no long-term comparison of the manure rotation to other rotations of the Halvorson plots has been conducted. The randomized block design of NT and completely randomized design of ST received five different N rates ranging from 0 to 240 kg N ha^{-1}, and were located just 50 meters to the east (ST rotation) and 50 m to the west (NT rotation) of the manure rotation study (TDM, TDMAP, TSU, TUF, and TC0N). The NT and ST N rates that were used for the pair t-test analysis above were the 202 (NT202 and ST202) and 0 kg N ha^{-1} (NTC0N and STC0N) rates. Both NT from 2012 to 2023 and ST from 2012 to 2019 received the same amount of irrigation as the manure study, which was applied the same day with the same linear sprinkler system, except in 2014, when the manure study received 384 mm vs. the NT and ST that received 472 mm. Both NT and ST received 25.4, 31.2, and 31.2 mm of additional irrigation during August and September 2014 compared to the manure study. Additionally, the ST and NT rotations were planted with the same hybrids and harvested on about the same days as the manure rotation plots and were managed similarly for pest control and weed management (Table S4 and S5).

No plant biomass outliers were removed to conduct the 2012 to 2017 N balance and to assess the NUE_{Sys}, nor to calculate the N losses from the system (NL_{Sys}) [7]. To assess the N balance, N losses, and NUE_{Sys}, the total N removed from each individual experimental unit was accounted for, independent of hail and/or other factors. Plant biomass outliers were only removed to assess the changes in corn dry biomass production and N content trends from 2012 to 2023. 2012 (bird damage), 2016 (severe bird damage), 2018 (hail damage), 2019 (bird damage) and 2022 (severe hail damage) were not used to assess changes in corn biomass production because yields were significantly reduced or, in the case of bird damage, plants around the sampled plants were damaged. Since the experimental units were of sufficient size to enable us to sample corn plants that were not affected by bird damage, in cases where there was significant corn crop damage in a sample row or for a given year, another plant within the experimental units would be sampled at random from the adjacent row instead. We believe that this strategy allows us to assess the effects of treatments on yields independently of bird damage to yields for a given year; however, in order to be conservative in the case of using linear regression to assess changes in production with time, we removed the years when we observed bird damage, and also the years were there was hail damage and entire plots were impacted by hail (2018, 2022).

For the statistical analysis of soil response variables, we identified outliers using an R Core Team language regression analysis [41] for each response variable versus the number of days since the start of the study. The regression analysis was done by treatment level and soil depth segment. For each fit, studentized residuals were calculated using the function rstudent(), and observations having a studentized residual with absolute value greater than or equal to 3 were marked as outliers and removed from the dataset. In 2014, a large level of soil NH_4–N was detected, which was identified as substantial noise in the NH_4–N analysis, with the control (non-fertilized) soils having an impossible value of 1,740 kg ha^{-1} of available NH_4-N (0 to 180 cm depth). Since the yields of the control plots in 2014 were low, as in other years, and we did not know the source of this noise, the 2014 fall soil samples were not used for any analyses. For full transparency, all datasets, including versions with and without outliers removed, have been provided with this paper as supplementary material so readers could see both the entire data collection, as well as the datasets that were used for this study.

3. Results

3.1. Changes in Soil N and C Pools, N Balance, and N and C Sequestration

The changes in inorganic N expressed in total equivalent soil mass (IN_{em}-N) from 2012 to 2017 were −77, 50, and 107 kg IN_{em}-N ha^{-1} for the TC0N, TDM, and TSU, respectively. These changes in IN_{em}-N reflect the sum of the changes in NO_3-N expressed in equivalent soil mass (NO_3-N_{em}) and NH_4-N expressed in equivalent soil mass (NH_4-N_{em}). The changes in soil organic N (SON) expressed in equivalent soil mass (SON_{em}-N) were 0, 708, and −597 kg SON_{em}-N ha^{-1} for TC0N, TDM, and TSU, respectively. Thus, the total changes in total soil N expressed in equivalent soil mass (TNem-N) were −77, 758, and −490 kg N ha^{-1} for TC0N, TDM, and TSU, respectively. These changes in TN_{em}-N suggest that N from the SON_{em}-N pool of the TSU treatment was being lost.

This loss of N from the TN_{em}-N of TSU agrees with a previous N balance study conducted for a no-till CC system at this site that found N is being lost from the TN_{em}-N and SON_{em}-N pools [7]. The loss of 98 kg N $ha^{-1}yr^{-1}$ from the TSU treatment in this five-yr. N balance study is four times higher than the loss observed in the NT study (24 kg N $ha^{-1}yr^{-1}$) that was conducted with a 13-yr. N balance at this site [7]. The greater N losses from the TN_{em}-N and SON_{em}-N pools observed with the TSU treatment than with the NT studies agree with findings from various studies that have reported greater C losses from tilled systems than no-till systems [28, 29, 30 and 31]. Although we did not detect N being lost from the SON_{em}-N pool with the TC0N using the Delgado et al. (2023) [7] peer-reviewed method that used the $P<0.18$ level. We suggest that five years was not enough time to detect these changes in SON_{em}-N loss from the TC0N at $P<0.18$. The negative N loss detected in TN_{em}-N with TC0N (−77 kg TN_{em}-N ha^{-1}) suggests that the control plots are also losing N from the SON_{em}-N pool at this site. The NH_4-N_{em} of TC0N increased across the soil profile while the NO_3-N_{em} decreased across the soil profile, an additional indication that N was being lost from the SON_{em}-N with TC0N and/or that a significant amount was removed with the harvested grain and bailed out of the TC0N. In contrast to these findings of losses of SON_{em} with no-till and tilled systems fertilized with inorganic N, the TDM is sequestering N in the soil at a rate of 126 kg N ha^{-1} yr^{-1}.

While the 2012 to 2017 losses of TSU SON_{em}-N were occurring, TSU was also losing −1190 kg ha^{-1} of soil organic C expressed in equivalent soil mass (SOC_{em}-C; −198 kg C $ha^{-1}yr^{-1}$). This finding agrees with previous research conducted at this site that found soil C losses with NT [32,33]. We were not able to detect the loss of SOC_{em}-C from the TC0N at $P<0.18$. We suggest that five years was not enough time to detect these changes in SOC_{em}-C loss from the TC0N at $P<0.18$. In contrast, we found that the manure-fertilized plots are sequestering C in the soil at a rate of 2,830 kg C ha^{-1} y^{-1}, for a total of 17,000 kg C ha^{-1} sequestered from spring 2012 to fall 2017. The TDM plots received 200,000 kg dry manure ha^{-1}, which contained 33,000 kg C ha^{-1}. Although we don't know how much C was sequestered from the manure versus how much was sequestered from crop residue (e.g., roots), if we divide the C sequestration by the total C applied with the manure, we estimate that about 31% of the applied C with manure was sequestered in the soil. If we also account for the fact that applying inorganic N and tilling the system (TSU) generates a loss of 1190 kg C ha^{-1}, then TDM sequestered 43% of the applied C with manure compared to the tilled system receiving inorganic fertilizer. This is important for farmers to know if they are interested in seeking potential compensation for sequestering C in their agricultural system.

The total organic or inorganic N fertilizer inputs were 2097, 1070, and 0 kg N ha^{-1} with TDM, TSU, and TC0N, respectively. We applied an additional 121 kg N ha^{-1} with the irrigation water to all the plots. Using the same method as Delgado et al. (2023) [7], we found that the estimation of atmospheric N inputs at the site was 31.2 kg N ha^{-1} for 2012 to 2017

The amount of N removed with the harvested grain was 373, 809, and 855 kg N ha^{-1} for the TC0N, TDM, and TSU treatments, respectively; with bailing and removal of crop residue after

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harvesting, the amount of N removed was 193, 436, and 373 kg N ha^{-1} for the TC0N, TDM, and TSU treatments, respectively. An N balance accounting for changes in the soil N pools in the 0 to 120 cm soil profile found that the system nitrogen use efficiency (NUE_{Sys}) for TDM is 86.3%, which is higher than the NUE_{Sys} for TSU (60.2%). The TDM loss of 13.7% of the N inputs to the environment is lower than the 39.8% observed with TSU. For total N mass loss, the N balance found that the N loss from TSU from 2012 to 2017 of 488 kg N ha^{-1} was higher than that of TDM, which was 309 kg N ha^{-1}.

Although the manure system (TDM) was sequestering C and N, it still had an average loss of 300 kg N ha^{-1}, for an average 50 kg N ha^{-1}yr^{-1}, exceeding the average loss of 40 kg N ha^{-1} yr^{-1} under no tillage with the 202 kg N ha^{-1} rate reported by Delgado et al. (2023) [7] at this site. These findings show that tilled systems (TSU) are losing significant C and N at a higher rate than the no-till system at this site, and that although on a percentage basis the manure system is sequestering C and N, with significant increases in total N balance, the amount of N lost from the system is still significantly higher than the amount of N lost with a no-till system. The rate of SOC_{em}-C and SON_{em}-N sequestration for the 0 to 7.6 cm and 7.6 to 15 cm depths was higher with TDM than the rate of SOC_{em}-C and SON_{em}-N losses with TSU (P<0.05, Table S25).

3.2. Effects of Organic and Inorganic N Inputs on Soil Parameters: 2012 to 2017

For the 2012 to 2017 period, we did not detect any differences in inorganic C expressed in total equivalent soil mass (ICem-C) at any of the depths from 0 to 120 cm (P<0.05, Table S6). In contrast, extractable total equivalent soil mass P (Pem-P) started to increase quickly after the first year of manure application, and Pem-P was significantly higher in the TDM, TDMAP, and TDMSU treatments than the other treatments (P<0.05, Table S6). This signal of higher extractable Pem-P was also observed for the 7.6 to 15 cm and 15 to 30 cm depths with just one application of manure. Higher Pem-P content was constantly observed with the manure treatments in 2013, 2014, and 2016 (P<0.05, Table S6). For example, in 2016 the average extractable Pem-P for the manure treatments was 103, 103, and 27 kg P ha^{-1} for the 0 to 7.6, 7.6 to 15, and 15 to 30 cm depths, respectively (P<0.05, Table S6), which was higher than the average Pem-P for TSU and TC0N of 13, 9, and 5 kg P ha^{-1} for the 0 to 7.6, 7.6 to 15, and 15 to 30 cm depths, respectively (P<0.05, Table S6).

Similarly to Pem-P, we detected significant increases in SOC_{em}-C at the 0 to 7.6 cm soil depth with the TDM and TDMAP treatments in 2012, which were higher than TSU, TUF, and TC0N in 2012, and through 2013 to 2017 (P<0.05, Table S6). By 2015, the average SOC_{em}-C of TDM and TDMAP was 16,800, 17,200, and 29,900 kg C ha^{-1} at the 0 to 7.6, 7.6 to 15, and 15 to 30 cm depths, respectively, which exceeded the average SOC_{em}-C of the TUF, TSU, and T0N of 11,400, 11,900 and 21,300 kg C ha^{-1} at the 0 to 7.6, 7.6 to 15, and 15 to 30 cm depths, respectively (P<0.05, Table S6). Similarly to the observed immediate increases in extractable Pem-P and SOC_{em}-C, there was also an immediate increase in SON_{em}-N in 2012, that continued during 2013, 2015, 2016, and 2017 (P<0.05, Table S6). The average SON_{em}-N values for TDM and TDMAP were 1,850 and 1,910 kg N ha^{-1} for the 0 to 7.6 and 7.6 to 15 cm soil depths, respectively, which exceeded the average SON_{em}-N values of 1,380 and 1,440 for TSU, TUF, and TC0N, respectively (P<0.05, Table S6).

The total C expressed in equivalent soil mass (TCem-C) with the TDM was higher than the TCem-C for TC0N, TSU, and TUF in the top 30 cm in 2014, 2015, and 2017 (P<0.05, Table S6). The average TN_{em}-N with TDM and TDMAP was higher from 2012 to 2017 than with TC0N, TSU, and TUF. In 2017, the average TN_{em}-N values for TDM and TDMAP of 1,876 and 1,940 kg N ha^{-1} at the 0 to 7.6 and 7.6 to 15 cm depths, respectively, were higher than those of the TC0N, TSU, and TUF treatments, which averaged 1,410 and 1,470 kg N ha^{-1} for the 0 to 7.6 and 7.6 to 15 cm depths, respectively (P<0.05, Table S6).

3.3. Effects of Organic and Inorganic N Inputs on Soil Parameters: 2012 to 2022

Similarly to the changes that we observed in EPem-P, we detected changes in IN_{em}-N in 2013, 2018, 2019, and 2022, when lower IN_{em}-N was detected for TC0N at various depths from 0 to 120 cm (P<0.05, Table S6). We also detected differences in IN_{em}-N between the organic and inorganic N inputs at the lower depths of 30 to 61 cm, and occasionally at even greater depths, in 2013, 2018, and 2019

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(P<0.05, Table S6). This suggests that inorganic NO_3 was being moved to lower depths and perhaps leaching out of the system, in agreement with findings by Delgado et al. (2023) [7]. The differences in NO_3-N_{em} were observed constantly from 2013 to 2019 and from 2021 to 2022 in fall soil sampling events, with TC0N constantly having the lower NO_3-N_{em} content. This supports the conclusion that organic and inorganic N fertilizer inputs increased NO_3-N_{em} levels above background levels and that NO_3-N_{em} is one of the pathways for movement of IN_{em}-N through the soil profile and out of the system. The data shows constantly higher NO_3-N_{em} levels above control levels at the 30 to 61 cm depths, and even up to the 150 to 180 cm soil depths (P<0.05, Table S6). The INF sources consistently had higher concentrations at the lower depths, and we only detected higher concentrations for the manure treatments at the 15 to 30 cm depth. These results suggest that among all the organic and inorganic N inputs, the INF sources were more mobile, contributing to the movement of NO_3-N_{em} to greater soil depths, and thus higher NO_3-N_{em} than the non-fertilized TC0N and manure plots (TDM and TDMAP) at this site (P<0.05, Table S6).

We did not detect practically any differences in NH_4-N_{em} among soil organic and inorganic and control treatments from 0 to 120 cm during spring 2012 to fall 2022 sampling (P<0.05, Table S6). Only on one occasion in fall 2021 at a depth of 13 to 30 cm did we identify a difference in NH_4-N_{em} between the control and SU treatments (P<0.05, Table S6). Since the N balance showed that: a) the NH_4-N_{em} was increasing for TDM, TSU, and TC0N (P<0.05, Table S9); b) SON_{em}-N was increasing for the TDM and decreasing for TC0N and TSU (P<0.05, Table S9); c) there were practically no differences in NH_4-N_{em} among treatments from 2012 to 2017; and d) there were differences among treatments in NO_3-N_{em} from 2012 to 2017 (P<0.05, Table S6); the mineralized organic matter from manure and soil NH_4-N_{em} was quickly changed to NO_3-N_{em} and/or being taken up by the corn treatment. The Delgado et al. (2024) [25] climate change study at the site also found that temperatures are increasing and suggested that the mineralization rates are increasing at this site. Delgado et al. (2023) [7] also found that NH_4-N_{em} was increasing for the no-till plots at this site; however, our data suggest that due to quick transformation to NO_3-N_{em}, there is no significant difference due to treatments in NH_4-N_{em} (P<0.05, Table S6).

3.4. Effects of Organic and Inorganic N Inputs on Yields and N Uptake (2012-2023)

Silage (R5.5): We found that all organic and inorganic N inputs increased silage yields beyond TC0N during 2012, from 2015 to 2018, and in 2022 (P<0.05, Table S10). In 2013, 2014, 2019, and 2022, the two manure treatments had higher silage yields than TC0N (P<0.05, Table S10). In 2021, only the TDMSU silage yields were higher than those of TC0N (P<0.05, Table S10). In 2019 and 2021, the silage production of TSU was higher than that of TC0N (P<0.05, Table S10). In 2020 and 2023, the organic and inorganic N inputs did not increase the silage yields above TC0N (P<0.05, Table S10). In summary, for the vast majority of years from 2012 to 2023, both organic and inorganic N inputs increased the silage yields over those of TC0N (P<0.05, Table S10). Among organic and inorganic comparisons in 2021 and 2022, the TDMSU had higher silage production than TSU, suggesting a positive effect of adding manure with EEF vs. adding the EEF alone (P<0.05, Table S10).

Physiological maturity (R6): We found that all organic and inorganic N inputs increase total aboveground biomass production above those of TC0N every year from 2012 to 2023 (P<0.05, Table S10). Comparison of organic and inorganic N inputs revealed that in 2016 the R6 aboveground biomass production with TUF and TSU were higher than with the TDM and TDMAP; however, this was the year that we did not apply manure, showing that the manure application from 2012 to 2015 did not have sufficient recycling of N to maximize aboveground production (P<0.05, Table S10). When organic and inorganic N inputs were applied only in 2020, 2021, and 2022, total biomass production at R6 with TDMSU was higher than TSU, supporting the hypothesis that there was a positive interaction effect of adding manure with EEF vs. EEF alone (P<0.05, Table S10).

Harvested grain: We found that all organic and inorganic N inputs increased harvested grain yields above those of TC0N every year from 2012 to 2023 (P<0.05, Table S10). Comparison of organic and inorganic N inputs found that in 2016 the TUF and TSU harvested grain yields were higher than those of the manure treatments; however, this was the year that we did not apply manure (P<0.05,

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Table S10), showing that the manure application from 2012 to 2015 did not have sufficient recycling of N to maximize harvested grain production (P<0.05, Table S10). Comparison of organic and inorganic N inputs when N inputs were applied, found that in 2014 harvested yields of TDM were higher than those of TUF (P<0.05, Table S10) and that in 2023 the harvested yields of TDMSU and TDM were higher than TSU, suggesting a positive effect of adding manure with EEF compared to EEF alone (P<0.05, Table S10).

N uptake at R5.5: We found that all organic and inorganic N inputs increased total N uptake aboveground silage (R5) production above that of TC0N every year from 2012 to 2022, except in 2016 and 2020 (P<0.05, Table S10). A comparison of organic and inorganic N inputs found that only in 2016 was the N uptake from inorganic N sources (TSU and TU) with silage production higher than that of TC0N, but the organic manure treatments were not higher than TC0N (P<0.05, Table S10). This shows that the N cycling from the applied manure from 2012 to 2015 did not cycle enough N to maximize N uptake with the manure treatments to increase N uptake beyond that of the control plots, but it was high enough to have N uptake with the manure treatments in the year that the manure was not applied, so it was not different from the plots with inorganic N inputs (P<0.05, Table S10). There were no differences in N uptake in 2020 among treatments (P<0.05, Table S10). In 2023, only TDMSU had significantly higher N uptake than TC0N, but it was not different from TDM and TSU (P<0.05, Table S10). However, the TDM and TSU treatments did not have higher N uptake than TC0N (P<0.05, Table S10).

N uptake at R6.0: We found that all organic and inorganic N inputs increased total N uptake aboveground biomass production at R6 beyond TC0N every year from 2012 to 2023 (P<0.05, Table S10). Comparison of organic and inorganic N inputs found that in 2016 the N uptake of aboveground biomass at R6 from TSU and TUF were higher than that of TDM, and TSU was higher than TDMAP. This shows that the N cycling from the applied manure from 2012 to 2015 did not cycle enough N to maximize N uptake with the manure treatments at R6 (P<0.05, Table S10). Since the N uptake from TC0N was 91 kg N ha^{-1} and the N uptake average the year that manure was not applied for TDM and TDMAP was 167 kg N ha^{-1}, we estimate that the applied manure from 2012 to 2015 was cycling 76 kg N ha^{-1} that was taken up by the aboveground biomass at R6 (167 kg N ha^{-1} − 91 kg N ha^{-1} = 76 kg N ha^{-1}), showing the importance of accounting for N sources applied in previous years (P<0.05, Table S10). Additionally, among organic and inorganic comparisons, when N inputs were applied only in 2021, we detected a difference among N inputs, with TDMSU resulting in higher N uptake than the TDM and TSU treatments, supporting the hypothesis that manure applications with EEF had a positive effect of recovering N compared to EEF alone (P<0.05, Table S10).

N uptake at harvest of grain: We found that all organic and inorganic N inputs increased total N uptake content by the harvested grain above TC0N every year from 2012 to 2022 (P<0.05, Table S10). Comparison of organic and inorganic N inputs found that in 2016 when manure was not applied, the harvested grain N uptake from TSU and TUF were higher than with TDM and TDMAP (P<0.05, Table S10). This shows that the N cycling from the applied manure from 2012 to 2015 did not cycle enough N to maximize N uptake by the harvested grain (P<0.05, Table S10). Since the harvested grain N uptake from TC0N was 61 kg N ha^{-1} and the N uptake average from the two manure treatments was 87 kg N ha^{-1}, the N cycling from the applied manure from 2012 to 2015 absorbed by the grain was estimated at 26 kg N ha^{-1} (87 kg N ha^{-1} − 61 kg N ha^{-1} = 26 kg N ha^{-1}). Additionally, among organic and inorganic comparisons, when N inputs were applied only in 2014 and 2023, the HGYN of TDM and TDMAP was higher than that of TSU and TUF (P<0.05, Table S10). In 2023, TDM and TDMSU resulted in higher harvested grain N uptake than TSU (P<0.05, Table S10).

NUEs: The NUE of the total aboveground biomass at R6 was not significantly different among treatments in 8 of the 12 years in the 2012 to 2023 period (P<0.05, Table S10); only in 2018 (TUF > TDM), 2019 (TDM > TUF), 2020 (TDMSU > TUF) and 2021 (TDMSU > TSU and TDM) (P<0.05, Table S10). Since we only had TDMSU since 2017, in two of the seven years TDMSU had higher NUE than the other treatments (TDM, TSU, and/or TUF; P<0.05, Table S10). These results suggest that the application of manure with EEF may contribute to increased NUE of manure systems beyond SU alone or manure alone. Similarly, in eight of the 11 years there were no significant differences in silage

NUE among treatments (P<0.05, Table S10). Due to the onset of the covid pandemic, silage samples were not collected in 2020; silage NUEs were only significant in 2012 (TUF > TDMA), 2015 (TSU and TUF > TDM), and 2022 (TDMSU > TDM and TSU). This suggests that when we detected the differences in three of the 12 years, the advantages in NUE were for the inorganic N fertilizer treatments or a combination of inorganic fertilizer and manure treatments, rather than the organic (manure) treatments (P<0.05, Table S10). We suggest that the continued N cycling from manure contributes to increased availability of N and increased NUE at the later harvesting stages of R6 and harvested grain, with relative advantages to TDM and/or TDMSU compared to just INF, which can potentially be vulnerable to losses early in the growing season via various loss mechanisms, while the manure can function as a slow-release fertilizer and continue to cycle N from manure from the R5.5 to R6 stages and then move the N that was taken up to the harvested grain.

The NUE of the harvested grain was more dynamic with only five of the 12 years (less than half) not being significant (P<0.05, Table S10). We found differences in 2012 (TSU > TDMA and TDM; TUF>TDMA), 2013 (TDMA > TUF); 2014 (TDM and TDMA > TUF and TSU); 2017 (TDMSU > TUF, TDM); 2018 (TSU > TDM); 2021 (TDMSU>TDM) and 2023 (TDMSU, TDM > TSU). In the first year (2012), the NUE was higher with TSU and TUF than with TDM. Out of the other six years that we saw differences in NUE, in four years the NUE was higher with one or both of the manure treatments than at least one of the inorganic N treatments. In three of the seven years that we had the TDMSU treatment, TDMSU had higher NUE for the harvested grain. This supports the conclusion that after a year of manure applications, the cycling of the N from previous application starts contributing to higher N uptake and higher NUE compared to other treatments, since NUE is estimated assuming that just 45% of the applied N with the manure in a given year will be available for uptake. It also supports the conclusion that combined applications of manure and EEF contribute to increased NUE compared to other treatments (P<0.05, Table S10).

3.5. Harvested Grain Yields and Harvested Grain Yield N Content from 2012 to 2023: TDM and

TSU vs. NT and ST

We compared the HGY and HGYN of TDM and TSU to those of NT202 and ST202 by year. From 2012 to 2023, the HGY of TDM were higher than the HGY of NT202 in 2014, 2021, 2022, and 2023 (P<0.05, Table S11). The NT202 treatment resulted in higher HGY than those of TDM only in 2016, when there was no manure applied to the TDM (P<0.05, Table S11). Thus, in four of the 11 years that manure was applied, the average HGY of TDM were higher than those of NT202 (P<0.05, Table S11). In the 11 years that manure was applied, the average HGY of TDM (11,000 kg ha^{-1}) were higher than the average HGY with NT202 (9,820 kg ha^{-1}; Table S11). The comparison between TDM and ST202 was done only from 2012 to 2019 when the ST study was stopped. The HGY of TDM were higher than HGY of ST202 only in 2014 (P<0.05, Table S12). The ST202 treatment had higher HGY than TDM only in 2016, when there was no manure applied to the TDM (P<0.05, Table S12). The HGY of TDM was higher than those of ST202 in only one of the 7 years (P<0.05, Table S12). In the 7 years that manure was applied, the average HGY of TDM (10,800 kg ha^{-1}) were higher than the average HGY of ST202 (10,100 kg ha^{-1}; Table S12).

During the 2012 to 2020 period, the HGY of TUF were higher than those of NT202 in 2017 and 2019, suggesting a small advantage of TUF over the NT202 plots (P<0.05, Table S13). In the 9 years that urea was applied and tillage was implemented, the average HGY of TUF (10,700 kg ha^{-1}) were higher than those of NT202 (Table S13; 9,830 kg ha^{-1}). These studies suggest that compared to NT202, tilling and TUF contribute to higher HGY (P<0.05, Table S13). The HGY were not higher with the NT202 than TUF in any of the 9 years of this period (P<0.05, Table S13).

For the 2012 to 2019 period, the HGY of TUF were higher than those of ST202 in 2017 and 2019 (P<0.05, Table S17). In two of 8 years, TUF had higher average HGY than ST202, suggesting a small advantage of the TUF over the ST202 plots. In the 8 years that urea was applied and tillage was implemented, the average HGY of TUF (10,800 kg ha^{-1}) were higher than those of ST202 (Table S17; 10,200 kg ha^{-1}). These studies suggest that compared to ST202, tilling and UF contributes to higher

HGY. The HGY were not higher with the NT202 than the TUF in any of the 8 years of this period ($P<0.05$, Table S17).

During the 2012 to 2016 period, the HGY of TDMAP were higher than with NT202 in 2013 and 2014 ($P<0.05$, Table S14), suggesting a small advantage of TDMAP over the NT202 plots. These studies suggest that compared to NT202, the combination of tillage and manure with AgrotainPlus contributes to higher HGY. The NT202 treatment had higher HGY than TDMAP only in 2016, the year that the manure was not applied to the TDMAP plots ($P<0.05$, Table S14). In the four years that manure was applied with AgrotainPlus, the average HGY of TDMAP (11,300 kg ha^{-1}) were higher than those of NT202 (10,200 kg ha^{-1}; Table S14).

For the 2012 to 2016 period, the HGY of TDMAP were higher than those of ST202 in 2014 ($P<0.05$, Table S18), suggesting a small advantage of TDMAP over the ST202 plots. These studies suggest that compared to ST202, tilling and manure application with AgrotainPlus contributes to higher HGY. The ST202 had higher HGY than the TDMAP only in 2016, the year that the manure was not applied to the TDMAP plots. In the four years that manure was applied with AgrotainPlus, the average HGY of TDMAP (11,300 kg ha^{-1}) were higher than those of ST202 (10,400 kg ha^{-1}; Table S18).

For the 2012 to 2023 period, the HGY of TSU were higher than those of NT202 in 2013, 2017, 2020, and 2023 ($P<0.05$, Table S15). In four of 12 years, TSU had higher average HGY than NT, suggesting a small advantage with TSU over the NT202 plots. In the 12 years that SU was applied and tillage was implemented, the average HGY of TSU (10,800 kg ha^{-1}) were higher than those of NT202 (9,820 kg ha^{-1}; Table S15).

For the 2012 to 2019 period, the HGY of TSU were higher than those of ST202 in 2017, 2018, and 2019 ($P<0.05$, Table S16); however, in 2016 the HGY of ST202 were higher than those of TSU ($P<0.05$, Table S16). In three of the eight years, TSU had higher average HGY than ST202. In the 8 years that SU was applied and tillage was implemented, the average HGY of TSU (11,000 kg ha^{-1}) were higher than those of ST202 (10,200 kg ha^{-1}; Table S16). These studies suggest that compared to no till and strip tillage, tilling and adding manure and/or SU contributes to higher HGY.

For the 2017 to 2023 period, the HGY of TDMSU were higher than those of NT202 in four of the seven years (2017, 2020, 2021, and 2023; $P<0.05$, Table S23), suggesting an advantage of TDMSU over the NT202 plots. These studies suggest that compared to NT202, tilling and manure application with SU contributes to higher HGY. The NT202 did not have higher HGY than the TDMSU in any of the seven years. For the 2017 to 2019 period, the HGY of TDMSU were higher than those of ST202 in two of the three years (2017 and 2019; $P<0.05$, Table S24), suggesting an advantage of TDMSU over the ST202 plots. These studies suggest that compared to ST202, tilling and manure application with SU contributes to higher HGY. The ST202 did not have higher HGY than TDMSU in any of the seven years.

These results are agreement with studies at this site that found that cultivation contributed to higher yields than no till from 2001 to 2007 [26,27]. These plots, which had been under no tillage since 2000 and were found to have lower yields than cultivated plots support the conclusions of other studies that have found that HGY of NT start to decrease compared to cultivated systems [34–40]. This result shows that after almost two decades of NT, the average HGY are lower compared to cultivated systems ($P<0.05$, Tables S11, S13, S14, and S15). Additionally, on average cultivated systems had higher HGY than strip till systems ($P<0.05$, Tables S12, S16, S17, and S18). Our assessment comparing NT and ST found that there were no differences between long-term no till and long-term strip till from 2012 to 2019, which aligns with findings from Delgado et al. [7] ($P<0.05$; Table 19).

3.6. Harvested Grain N Content from 2012 to 2023: TDM and TSU vs. NT and ST

The TDM achieved greater HGYN than the NT202 treatment in 2014 and 2021 and was also higher than ST202 in 2014 and 2017 ($P<0.05$, Tables S11 and S12). The ST202 treatment had greater HGYN than TDM in the year that there was not a manure application ($P<0.05$, Table S12). The data suggest that average HGYN for manure is higher with TDM than the NT202 and ST202 treatments ($P<0.05$, Tables S11 and S12).

For the 2012 to 2020 period, the HGYN of TUF was higher than the HGYN of NT202 in 2013, 2015, and 2017 ($P<0.05$, Table S13), suggesting an advantage of the TUF over the NT202 plots. The HGYN was not higher with NT202 than TUF in any of the 9 years ($P<0.05$, Table S13). For the 2012 to 2019 period, the HGYN of TUF was higher than the HGYN of ST202 in 2017, 2018, and 2019 ($P<0.05$, Table S17), suggesting an advantage of the TUF over the ST202 plots. These studies suggest that compared to ST202, tillage and UF application contributes to higher HGYN. The HGYN was not higher with NT202 than TUF in any of the 8 years.

For the 2012 to 2016 period, the HGYN of TDMAP was higher than that of NT202 in 2013 and 2014 ($P<0.05$, Table S4), suggesting an advantage of TDMAT over NT202, and that tillage and manure contributes to higher HGYN. The HGYN was not higher with NT202 than TDMAP in any of the 4 years, including 2016 when manure was not applied. For the 2012 to 2016 period, the HGYN of TDMAP was higher than that of ST202 in 2014 ($P<0.05$, Table S18), suggesting an advantage of TDMAP over the ST202. The ST202 treatment had higher HGYN than TDMAP only in 2016, the year that the manure was not applied to the TDMAT plots ($P<0.05$, Table S18).

For the 2017 to 2022 period, the HGYN of TDMSU were higher than those of NT202 in four of the six years (2017, 2019 to 2021; $P<0.05$, Table S23), suggesting an advantage of TDMSU over the NT202 plots. These studies suggest that compared to NT202, tilling and manure application with SU contributes to higher HGYN. The NT202 did not have higher HGYN than TDMASU in any of the six years. For the 2017 to 2019 period, the HGYN of TDMSU were higher than those of ST202 in all years ($P<0.05$, Table S24), suggesting an advantage of TDMSU over the ST202 plots. These studies suggest that compared to ST202, tilling and manure application with SU contributes to higher HGYN. The ST202 did not have higher HGYN than TDMASU in any of the seven years.

3.7. Comparison of Controls: Zero N Fertilizer with Tillage (TC0N), No Till (NT0N), and Strip

Till (ST0N) from 2012 to 2023

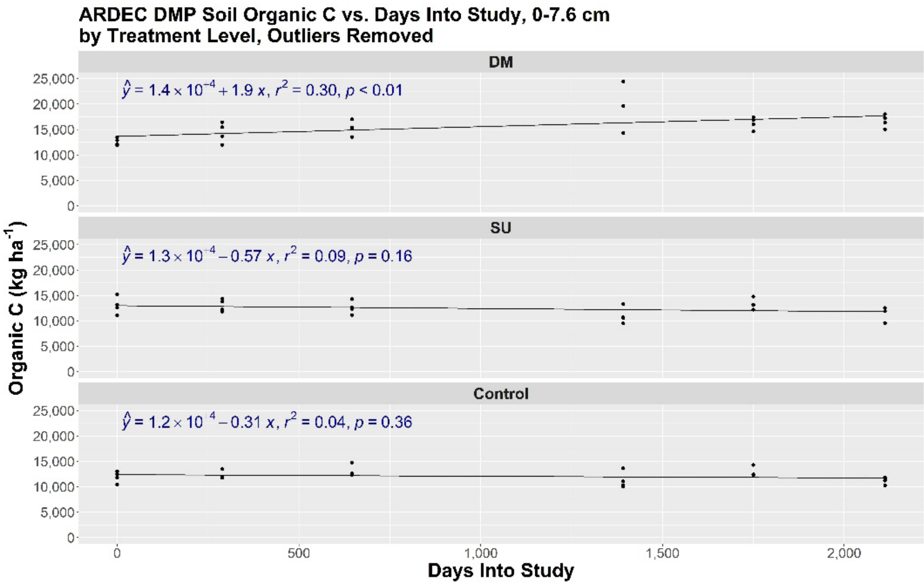
The HGY were higher in eight out of 12 years with TC0N than with NTC0N ($P<0.05$, Table S20), and higher with TC0N than STC0N in five of the eight years ($P<0.05$, Table S21). The HGYN were higher with TC0N than NTC0N in four out of 12 years ($P<0.05$, Table S20) and higher with TC0N than STC0N in five of the eight years ($P<0.05$, Table S21). There were no differences in HGY and HGYN between NTC0N and STC0N ($P<0.05$, Table S22). These results are in agreement with previous studies conducted at this site showing greater N cycling from background N sources such as SON_{em}-N in tillage management than with no till and strip till management [7,27].

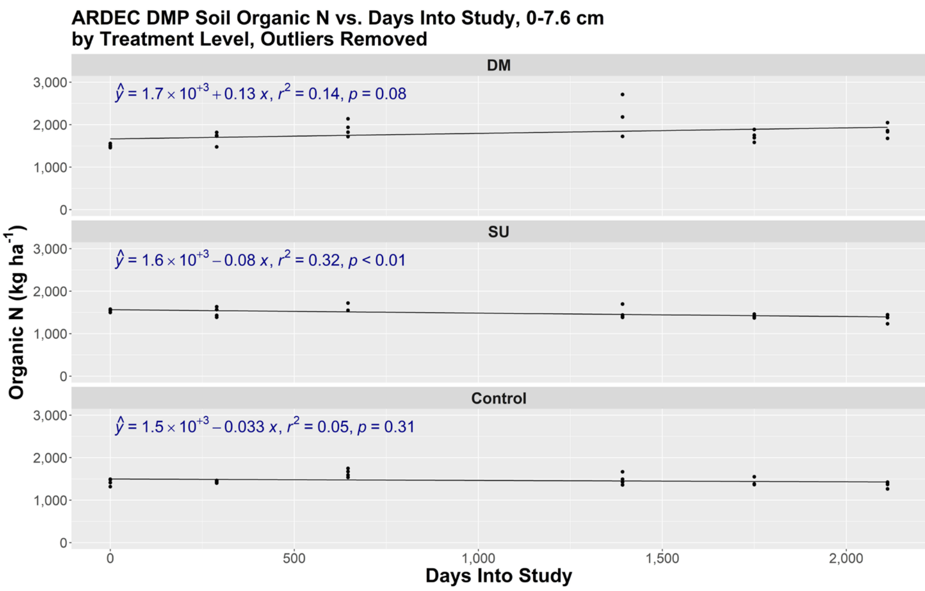
3.8. Summary of Agronomic Production System Results from 2012 to 2023

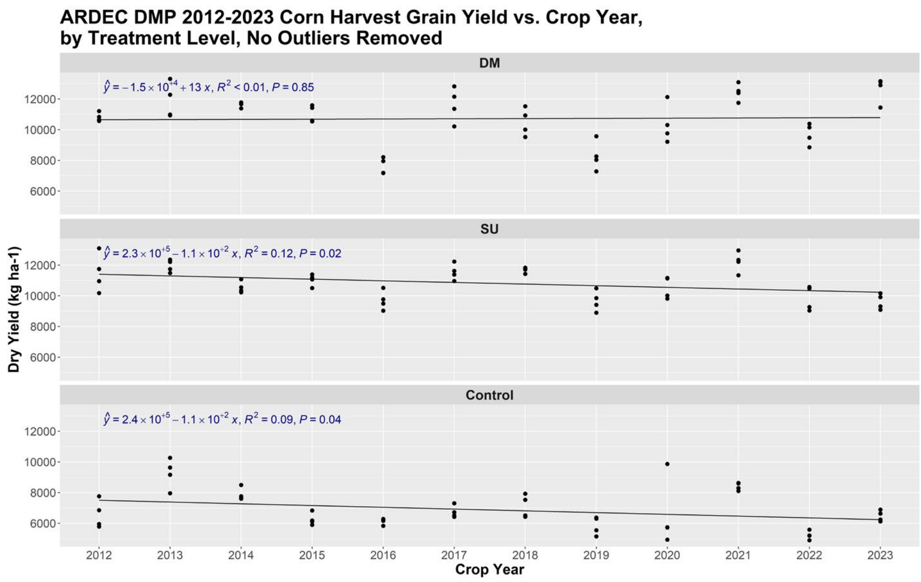
Analysis of the effects of organic and inorganic N inputs on aboveground biomass production at silage (R5), physiological maturity (R6), and harvest of grain shows that during the first 8 years of the 2012 to 2023 period (excluding 2016, when no manure was applied), the HGY of TDM was higher than those of TUF in only one year (2014). However, from 2020 to 2023, there were significantly higher yields with the manure treatments than the inorganic N fertilizer treatments. Significant agronomic yield increases were found in 2020 for aboveground biomass at R6 (DMSU > SU); in 2021 for biomass at R6 (DMSU > SU); in 2022 for biomass at R6 (DMSU >SU) and silage yields (DMSU and DM > SU); and in 2023 for HGY (DMSU and DM > SU). These results suggest that there is a synergistic effect when DM is applied with SU, contributing to higher aboveground biomass production and harvested yields than DM or SU alone.

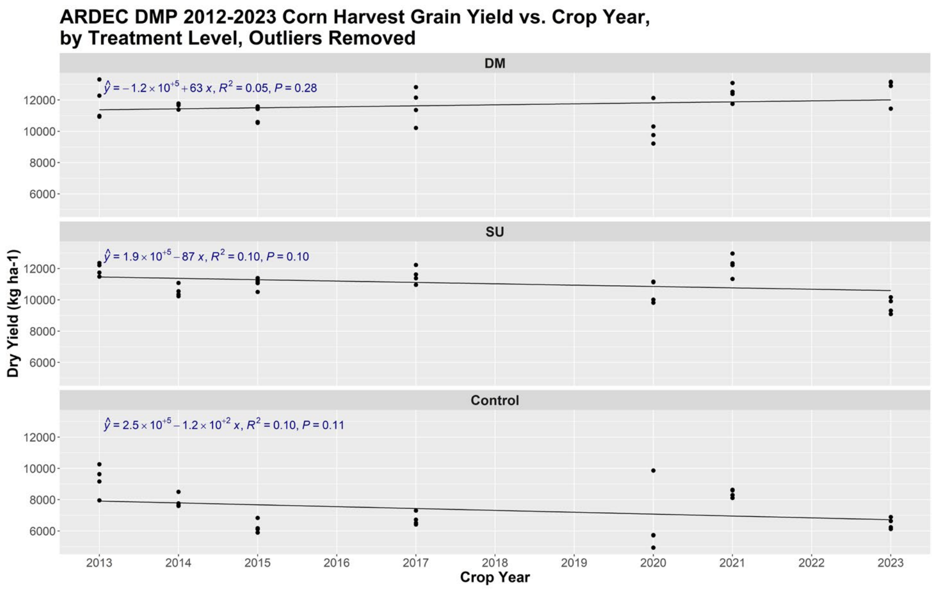
Tilled systems receiving manure applications at this site with minimal erosion and irrigated with an aboveground sprinkler had higher agronomic productivity for CC than tilled systems that received INF applications. When long-term comparisons of the manure systems with tillage were compared to long-term NT and ST systems that were receiving INF, tilled systems with manure applications had the advantage in agronomic productivity. Similarly, when the long-term inorganic systems that received INF as an input were compared to the long-term NT and ST systems, tilled systems receiving INF had an advantage over NT and ST systems receiving INF.

As far as agronomic sustainability, the tilled systems receiving manure applications had increased soil organic C and N content. While the tilled systems with manure applications were sequestering C and N in the soil organic matter pool, the tilled systems with INF were losing organic C and organic N (Figs. 1, 2). The N cycled from manure applications from current and previous years is significant and contributed to higher N uptake by aboveground biomass (R5.5 and R6) and harvested grain. These desirable increases in N cycling are contributing to higher NUE for the manure systems than the INF systems with a tillage system. The mobility of N in the NO_3-N pool was higher with the INF systems than with the manure systems, and more changes were detected at lower depths with the INF systems. These desirable changes in soil health properties such as C sequestration, greater cycling of N, higher system N use efficiencies, and lower N losses are contributing to increased yields and greater yield stability (with no decrease in yields with time) compared to tilled systems receiving INF, which are seeing decreasing yields with time (Figs. 3, 4). Independently of these effects, the inorganic systems under tillage have higher agronomic productivity as far as HGY than NT and ST systems. Although application of manure was identified as a best agronomic management practice to increase yields and sustain productivity with time, the best combination appears to be the enhanced efficiency fertilizer (70% of N input) with manure organic N (30% of N input), a combination which appears to have a synergistic effect that increases agronomic productivity.









4. Conclusions

This unique study is the second study that we found in the world literature that conducted an N balance when manure is applied that accounted for changes in soil organic N pools. However, the present work is the only study that conducted an N balance where yields of manure systems under cultivation were compared to yields of no-till and strip till systems. This study proved our hypothesis that TONF (manure) systems will contribute to higher C and N sequestration compared to TINF (inorganic N fertilizer). It also proved our hypothesis that the N losses from a TONF system with minimal erosion will be lower than those from a TINF that has more mobile N, showing the importance of conducting long-term studies.

These long-term studies show that applying manure (N) to a system that is under minimal soil erosion contributes to significant C sequestration and stable HGY with time compared to tilled systems receiving INF. The tilled systems receiving manure applications also had higher HGY than NT and ST systems fertilized with INF; all these systems had the same management practices (irrigation, weed control, and pest management) and similar N rates. The increases in organic C and N in soil organic pools increase the N cycling and uptake by the corn crop, which increases the NUE of these TONF systems compared to those receiving TINF applications. The N losses from the manure system were lower those from INF systems, which are receiving N in a form that is more mobile and dynamic. Among other effects, mobile N fertilizer input contributes to greater N losses by moving NO_3-N to lower depths to levels above the background concentration of the non-fertilized plots, showing that NO_3-N leaching is a potential mechanism for N losses from this tilled system receiving INF.

A best agronomic management practice is the application of manure (30% of N input) in combination with inorganic N fertilizer (70% of N input) using an EEF as a source of N. This appears to cause a synergistic effect that is contributing to higher HGY and increased agronomic productivity and C and N sequestration at this site. Although manure applications appear to be a best management practice that increases agronomic productivity, the combination of manure (30% of the N rate) and EEF (70% of the N rate) appears to offer even more agronomic benefits. We found that at this long-term research site that although neither tillage nor NT contributed to C and N sequestration, when we add manure to this site that has minimal erosion, we can improve soil health by increasing C and N sequestration, N cycling, and agronomic productivity. Long-term research demonstrates the importance of monitoring agricultural systems and best management practices to determine changes in agronomic productivity over the long term.

Supplementary Materials: Supporting information (databases and metadata) can be downloaded at website of this paper posted on Preprints.org.

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