**Long-term effects of nitrogen sources on biomass and grain yields, and N uptake, losses and use efficiencies in irrigated corn grown for silage and grain production**

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**Supplementary Information**

**In this file:**

* Supplementary tables (Tables S1-S??)
* Supplementary figures (Figures S1 – S??)

**Table S1.** Summary of weather variables§ for different time periods of the manure (DM) study covering the planting to physiological maturity [R6]) growing seasons. Weather data for the period 2012-2018 was downloaded on July 20, 2022 and weather data for the period 2019-2023 was downloaded on July 26, 2024 via CoAgMet from ARDEC station FTC03 (<http://www.coagmet.colostate.edu/station/ftc03_main.html>) and CSU station FCL01 (<http://www.coagmet.colostate.edu/station/fcl01_main.html>).

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| **Time Period** | **Average Mean**  **Daily Max**  **Temperature (°C)** | **Average Mean**  **Daily Minimum Temperature (°C)** | **Average Mean Daily Mean Temperature (°C)** | **Mean Annual GDD (°C)** | **Mean Annual Precipitation (mm)** |
| 2012 - 2017  2012 - 2023  2013 - 2023 | 26.7  27.0  29.8 | 10.4  10.5  10.4 | 18.6  18.7  18.6 | 1333  1341  1337 | 217  199  207 |

§Relative to the manure (DM) study plots and the treatments in this study, due to the large number of R6 field samples to collect during the above years, NT200, NT C0N, ST200 and ST C0N R6 samples were collected a couple of days earlier or later than DT treatments during these years.

**Table S2.** Summary of weather variables by growing season of each year for the manure (DM) study that include organic and inorganic treatments from planting to physiological maturity [R6]). Weather data from 2012-2023 was downloaded on July 20, 2022, and data from 2019-2023 was downloaded on July 26, 2024 via CoAgMet from ARDEC station FTC03 (<http://www.coagmet.colostate.edu/station/ftc03_main.html>) and CSU station FCL01 (<http://www.coagmet.colostate.edu/station/fcl01_main.html>).

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| --- | --- | --- | --- | --- | --- |
| **Year** | **Mean Daily Max Temperature (°C)** | **Mean Daily Minimum Temperature (°C)** | **Mean Daily Mean Temperature (°C)** | **GDD (°C)** | **Precipitation (mm)** |
| 2012  2013  2014  2015  2016  2017  2018  2019  2020  2021  2022  2023 | 28.9  28.0  25.3  26.0  26.2  26.1  27.6  25.8  28.2  28.1  28.0  25.8 | 10.9  11.5  9.3  10.4  9.7  10.5  10.8  9.6  10.4  11.2  11.0  10.3 | 19.9  19.7  17.3  18.2  18.0  18.3  19.2  17.7  19.3  19.7  19.5  18.1 | 1392  1354  1276  1268  1358  1351  1351  1362  1403  1302  1369  1308 | 114  217  233  254  108  378  163  207  109  99  149  360 |

**Table S3.** Nitrogen (N) fertilizer treatment details and application rates by year.

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| --- | --- | --- | --- | --- | --- |
| Treatment level | Year(s) | Total N applied (kg N ha-1) ¥ | Total C applied (kg N ha-1) ¥ | Dry manure applied (t ha-1) ¥ | Fertilizer placement |
| TDM\*  (assumed 45% manure N mineralization for each year applied) | 2012  2013  2014  2015  2016  2017  2018  2019  2020  2021  2022  2023  **average** | 461 [207]  344 [155]  406 [183]  418 [188]  0 [0]  469 [211]  590 [266]  191 [86]  364 [164]  512 [230]  351 [158]  431 [194]  **412 [186]** | 7540  4810  5310  4380  0  6600  7390  2930  5180  7070  5470  5610  **5663** | 52.8  34.1  36.3  36.1  0  49.7  78.9  21.5  32.1  32.1  28.8  21.4  **38.5** | Manually broadcast with shovels before rototilling to 15 cm depth (all years) |
| TDMAP  (assumed 45% manure N mineralization for each year applied) | 2012  2013  2014  2015  2016  **average** | 497 [224]  347 [156]  401 [180]  398 [179]  0 [0]  **411 [185]** | 7810  4860  5270  4140  0  **5520** | 53.0  33.2  37.0  36.3  0  **38.9** | Manually broadcast with shovels before rototilling to 15 cm depth (all years) |
| TDMSU  (assumed 45% manure N mineralization for each year applied) | 2017  2018  2019  2020  2021  2022  2023  **average** | 145 [65] (DM) + 100 (SU)  180 [81] (DM) + 116 (SU)  57 [26] (DM) + 137 (SU)  111 [50] (DM) + 120 (SU)  156 [70] (DM) + 110 (SU)  91 [41] (DM) + 116 (SU)  116 [52] (DM) + 120 (SU)  **122 [55+117]** | 2050  2250  888  1490  2100  1510  1510  **1685** | 17.0  24.6  6.59  9.64  9.94  7.19  5.95  **11.6** | Manually broadcast with shovels before rototilling to 15 cm depth (all years) |
| TUF  (Urea 46% N) | 2012 - 2014  2015 - 2017  2018  2019 – 2020  **average** | 179  179  179  179  **179** |  |  | Surface broadcast by hand  Surface band in corn row  Surface broadcast by hand  Surface band in corn row |
| TSU  (Super U 46% N) | 2012 - 2014  2015 - 2017  2018  2019 – 2023  **Average** | 179  179  179  179  **179** |  |  | Surface broadcast by hand  Surface band in corn row  Surface broadcast by hand  Surface band in corn row |

**¶**In addition to N, P in the form of triple superphosphate (0 – 45 – 0) was applied to all non-manure experimental units as follows: 56 kg P ha-1 on 03/18/2013; and 56 kg P ha-1 on 03/13/2018. P in the form of triple superphosphate (0 – 46 – 0) was applied to all non-manure experimental units as follows: 56 kg P ha-1 on 04/15/2015.

\*Dairy manure N value for each year is the mean over four replicates of the corresponding treatment level.

¥Values in brackets represent 45% of the corresponding total N amount provided by manure, which was the assumed rate of N mineralization during the application year.

**Table S4.** Descriptions of the weed and pest control; nitrogen management; tillage management; plant, soil, and irrigation sampling and analysis; calibration of instruments for plant N analysis; the linear-plus-plateau model analysis; and nitrogen use efficiency (NUE) equations used.

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| --- | --- |
| **Practice** | **Description** |
| Weed control[7,25] | In general, all of the continuous corn experimental units were managed the same as far as weed control, regardless of tillage treatment or row spacing. On average, a pre-plant or pre-crop-emergence broad-spectrum herbicide like glyphosate (Roundup®) or glufosinate-ammonium (Liberty®) were applied to control emerged broadleaf and grass weeds. This herbicide was usually tank mixed with a broad-spectrum, residual herbicide (Zidua®) to suppress un-emerged weeds, followed by a post-emergence herbicide mix (Roundup® or Liberty and Status®) to control or suppress any escaped weeds just prior to crop canopy closure. Problem weeds such as Canada thistle were spot sprayed by hand as needed using clopyralid (Stinger®). Over the 20+ years of maintaining these experimental units, the chemicals have varied based on the EPA-approved products available at that time, but the intended weed management strategy has remained consistent. The same weed control used for the manure study plots was used for the NT and ST plots. |
| Pest control[7,25] | Spider mites have been a significant issue with the corn crop, affecting some experimental units in 2012 (6 experimental units with the NT study). To rescue the crop, the field was aerial-sprayed with one of two pesticides (Onager® and Warrior®) or both, depending on timing (one targets the adult stage of the mites and the other targets the juvenile stage). Bird damage is significant at ARDEC. Sunflower experimental units in the area have attracted huge flocks of blackbirds that also damaged the corn. Several deterrent strategies have been employed including predator decoys, balloons with eyes, kites, flashing lights, banging sounds, and once a propane cannon. One practice employed was to switch to a hybrid of corn with a tighter husk to try to deter the birds. 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. The same pest control used for the manure study plots was used for the NT and ST plots. |
| Nitrogen management[7,25] | For fertilizer applications we used Granular SUPERU® (46-0-0) was applied post-emergence from 2012 to 2013 for NT and ST. From 2014 to 2023, granular urea (46-0-0) was also applied post-emergence using a Barber drop spreader with surface side bands and 30” spacing. Granular triple super phosphate (0-46-0) was applied to the surface in 10’ bands at pre-planting in 2004, 2005, 2009, 2010, 2013, 2015, 2018, 2021, and 2023. For the manure study the UF plots received urea fertilizer from 2012 to 2023 and the SU received SUU from 2012 to 2017. The DM and inorganic urea fertilizer plots also received UF from 2018 to 2023 [7,25]. |
| Tillage management[7,25] | The crop tillage management for these systems was as follows. From 2013 to 2023 we used a 15’ wide roll chopper, mounted on the front of 165 hp tractor, on corn stalks at pre-planting for both the NT, ST and manure plots. For the ST plots, from 2012 to 2019 during pre-planting we strip tilled to a soil depth of 9 inches with a 15’ Orthman 1tRIPr strip tiller® pulled with a 140 or 165 hp tractor. For the manure study, from 2012 to 2023 a silage system was simulated by shredding and windrowing the corn stover and bailing it out, leaving just about 5 cm tall stalk stubs. After the DM application to the manure treatments a rotor-tiller was used to incorporate the manure to a depth of 15 cm simulating moldboard plow tillage. All plots, UF, SU and C0N plots were also rototilled to a depth of 15 cm. |
| Plant, Soil, and Irrigation Sampling and Analysis[7,25] | To assess the effects of manure and N sources on yields and total biomass, plant samples were collected during three distinct field operations each year. At silage (R5.5) and physiological maturity (R6), 15 randomly selected plants were cut by hand at ground level and removed from the field to assess silage (R5.5) and biomass production of stalks and leaves (R6). After silage and the R6 biomass samples were collected, ears were manually removed from plants, oven dried, and then shelled, while the stalks and leaves were together shredded, subsampled, and oven dried. After shelling, the cobs were also retained and oven dried. The third sampling was performed after dry down at harvest, during which ears were manually removed 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. The manually 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.) Plant samples (2012 to 2023) were coarsely ground to pass through a 2-mm screen and were then finely ground to pass a 1-mm screen and finally analyzed for total N using an Elementar Vario Macro C-N analyzer (Elementar Americas) from 2012 to 2023.  The crop residue biomass was determined by summing the dry weight of the stalks, leaves, and cobs at R6. A small plot harvester was used to remove the harvested grain alone. 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. The total N removed from the plots was estimated as the sum of the N removed with the harvested grain plus the N that was bailed out. The N bailed out was estimated as the sum of the N content of stalks, leaves, and cobs at R6. Irrigation samples were monitored and with most irrigation events, water samples were collected and analyzed for N (NO3-N and NH4-N) analysis. Water NO3-N and NH4-N content were determined with the Lachat QuickChem flow injection analyzer and total application of N during the study period from 2012 to 2023 was estimated at 266.6kg NO3-N ha-1 with an average of 15.7kg NO3-N ha-1 yr-1. The average quantities of NO3-N applied per year from 2012 to 2023 are reported in Table S5. All manure, NT and ST plots received the same amount of irrigation and NO3-N per hectare per year. |
| Calibration of instruments for plant N analysis | Delgado et al. published and released the datasets about the use of plant standards and soil standards that were run continuously from 2005 to 2018, and the long-term analysis of plant standards showed that the long-term Elementar analysis was accurate and precise during these long-term studies and repeatedly provided accurate and precise analysis of the plant samples that were run continuously during this period (Fig. S2). |
| N Budget (Soil and plant sampling and N analysis) | A silage sampling was performed at R5.5 and a biomass sampling was performed at R6. For the silage sampling, 10 continuous plants were cut by hand just above ground level and for biomass sampling 15 randomly selected plants were cut by hand just above ground level. A final, third sampling was conducted at harvest after standing plants had field-dried, in which ears were manually removed 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. After the R6 biomass samples were collected, ears were manually removed from plants, oven dried, and then shelled, while the stalks and leaves were together shredded, subsampled, and oven dried. After shelling, the cobs were also retained and oven dried. The harvest 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.) Plant samples (2012 to 2023) were ground to pass through a 2-mm screen and were analyzed for total N using an Elementar Vario Macro C-N analyzer. Total aboveground biomass (R6) N was determined by summing the N content of cobs, stalks plus leaves, and grain. Total N removed from the experimental units was determined from the N content of the dried down corn at harvest. We simulated a silage system by shredding and windrowing the corn stover and bailing it out, leaving just about 5 cm tall stalk stubs. The leaves stalks and cobs N at R6 were also summed to calculate the N removed from the plots that were bailed out. The N harvested with grain was also accounted for as part of the N that was removed with a plant compartment.  Soil samples were collected each fall after harvest, using a tractor-mounted Giddings hydraulic sampler. Two cores were collected at a single sampling location within each experimental unit. The first core (approximately 5 cm wide), which went to a depth of 30 cm, was divided into depth segments of 0 to 7.6, 7.6 to 15, and 15 to 30 cm. A second, narrower core (approximately 2.7 cm wide) was collected at the same location as the first, and was divided into depth segments of 30 to 61, 61 to 91, 91 to 122, 122 to 152, and 152 to 183 cm. From 2014 to 2017, the greatest depth sampled was 152 cm. After collection, the soil samples were left open to air dry in weighing boats with fans blowing over them at room temperature for several weeks. Once dried, samples were passed through a 2-mm sieve and picked clean of crop residue and root matter larger than 2 mm in diameter before grinding in a flail grinder. Some of the organic matter that is longer than 2 mm in length but oriented correctly and thin enough can make it past the sieve and required further picking to remove, which entailed removing essentially all the visible root that passed through the sieve.  Sieved and picked soil samples (2012 to 2018) were ground to pass a 150-μm screen and analyzed for total N using an Elementar Vario Macro C-N analyzer. Air-dried soil nitrate nitrogen (NO3-N) and ammonium nitrogen (NH4-N) were extracted with 1 M potassium chloride (KCl) with a soil:solution ratio of 1:5, and the content was determined using a Lachat QuickChem® flow injection analyzer. Soil samples were analyzed for extractable soil phosphorus (P) using a sodium bicarbonate (NaHCO3) method (Olsen et al. 1954) and extractant solutions were ana­lyzed using a Lachat QuickChem® flow injection analyzer.  The mass of each N pool from the fall of 2012 to the fall of 2018, by soil depth and treatment level, was calculated using the respective soil bulk density and N pool concentration measured with the Elementar instrument, and the NO3-N and NH~~4~~-N measured with the Lachat instrument, unless calculated by a difference. Each of these soil N pools was expressed on the basis of an equivalent mass as described by Ellert et al. (2001) and others that have used the equivalent soil mass procedure to conduct soil C balances. N pools residing in soil depth sub-segments expressed in equivalent soil mass that mirror the depth segments sampled in the field were considered for this analysis. Thus, the 0 to 7.6 cm sub-segment is the same as the top soil-depth segment expressed in equivalent soil mass; the 7.6 to 15 cm sub-segment is the difference between the 0 to 15 cm and 0 to 7.6 cm soil depth segments expressed in equivalent mass, and so on. Inorganic N expressed in total equivalent soil mass (INem-N) was calculated based on the sum of measured NO3-N expressed in equivalent soil mass (NO3-Nem) and NH4-N expressed in equivalent soil mass (NH4-Nem). Organic N expressed in equivalent soil mass (SONem-N) was calculated based on the difference between measured total soil mass for N (TNem-N) and INem-N.  Soil bulk density was measured in each experimental unit at 0 to 7.6 cm, 7.6 to 15.2 cm, and 15.2 to 30.5 cm in the fall of each study year. From 2012 to 2013, for depths below 30.5 cm, bulk density values that were measured in the alleys between experimental units in the fall of 2001 were used, in 30.5-cm increments to a depth of 183 cm, and then averaged by depth. From 2014 to 2023, for depths below 30.5 cm, bulk density values that were measured in the fall of 2014 in each experimental unit were used, in 30.5-cm increments to a depth of 152.5 cm. Since the deep bulk densities in 2001 were collected in the alleys and in 2014 in each experimental unit, testing for differences in bulk densities was not done [7,25].  Irrigation samples were monitored and with most irrigation events, water samples were collected and analyzed for N (NO3-N and NH4-N) analysis. Water NO3-N and NH4-N content were determined with the Lachat QuickChem flow injection analyzer (Table S5) and total application of N during the study period was 243 kg NO3-N ha-1 (Table S5) with an average of 19 kg NO3-N ha-1 yr-1. In a few years NH4-N was detected but dismissed as noise and not reported since the median ppm of 0.05 NH4-N was close to detection limits. |
| NUE equations | The harvested grain NUE (NUEHG) (Eq. 1) was calculated by subtracting the N content (kg N ha-1) in the harvested grain of the control (non-fertilized) experimental units (HGNCCtl) from the N content (kg N ha-1) in the harvested grain of the N-fertilized experimental units (HGNCEU), and dividing the difference by the N fertilizer added (available) to the fertilized experimental unit (kg N ha-1):  NUEHG (%) = HGNCEU – HGNCCtl  ----------------------------------------------- x 100 (Eq. 1)  N fertilizer applied  The NUE of the total aboveground biomass N content at R5.5 (NUEBR5.5) (Eq. 2) was calculated by subtracting the N content (kg N ha-1) in the total aboveground biomass at R5.5 of the control (non-fertilized) experimental units (BR5.5NCCtl) from the N content (kg N ha-1) in the total aboveground biomass at R5.5 of the N-fertilized experimental units (BR5.5NCEU), and dividing the difference by the N fertilizer (kg N ha-1) added to the fertilized experimental unit:  NUEBR5.5 (%) = BR5.5NCEU – BR5.5NCCtl  -------------------------------------------------- x 100 (Eq. 2)  N fertilizer applied  The NUE of the total aboveground biomass N content at R6 (NUEBR6) (Eq. 3) was calculated by subtracting the N content (kg N ha-1) in the total aboveground biomass at R6 of the control (non-fertilized) experimental units (BR6NCCtl) from the N content (kg N ha-1) in the total aboveground biomass at R6 of the N-fertilized experimental units (BR6NCEU), and dividing the difference by the N fertilizer (kg N ha-1) added to the fertilized experimental unit:  NUEBR6 (%) = BR6NCEU – BR6NCCtl  -------------------------------------------------- x 100 (Eq. 3)  N fertilizer applied  The harvested grain NUE accounting for changes in the SONem-N and INem-N (NUEHGSeq) (Eq. 4) was calculated first by subtracting HGNCCtl from the HGNCEU. Then, since increases in INem-N for all the treatments were measured, and this net increase (in kg N ha-1) in the soil was not lost to the environment, this INem-N was accounted for as recovered N and was added to the difference of HGNCCtl and HGNCEU. This sum was then divided by the sum of the amount of N fertilizer added to the fertilized experimental unit and the kg of N per hectare lost through mineralization of the SONem-N in the fertilized experimental unit. Since negative N sequestration for all the treatments was measured, and this loss (kg N ha-1) of the SOM was practically a net input (kg N ha-1) increasing the net N (kg N ha-1) available to the corn system, this net loss through mineralization of the SONem-N was accounted for as an N input:  NUEHGSeq (%) = (HGNCEU – HGNCCtl (kg N ha-1)) + net increase INem-N  ------------------------------------------------------------ x 100 (Eq. 4)  (N fertilizer applied + net loss SONem-N)  The NUE of the total aboveground biomass N content at R5.5 accounting for changes in the SONem-N and INem-N (NUEBR5.5Seq) (Eq. 5) was calculated by first subtracting the N content (kg N ha-1) in the total aboveground biomass at R5.5 of the control (non-fertilized experimental units) (BR5.5NCCtl) from the N content (kg N ha-1) in the total aboveground biomass at R5.5 of the N-fertilized experimental unit (BR5.5NCEU) and adding this difference to the increases in INem-N. This sum was then divided by the sum of the amount of fertilizer (kg N ha-1) added to the fertilized experimental unit and net N loss (kg N ha-1) through mineralization of the SONem-N in the fertilized experimental unit:  NUEBR5.5Seq (%) = (BR5.5NCEU – BR5.5NCCtl) + net increase INem-N  -------------------------------------------------------- x 100 (Eq. 5)  (N fertilizer applied + net loss SONem-N)  The NUE of the total aboveground biomass N content at R6 accounting for changes in the SONem-N and INem-N (NUEBR6Seq) (Eq. 6) was calculated by first subtracting the N content (kg N ha-1) in the total aboveground biomass at R6 of the control (non-fertilized experimental units) (BR6NCCtl) from the N content (kg N ha-1) in the total aboveground biomass at R6 of the N-fertilized experimental unit (BR6NCEU) and adding this difference to the increases in INem-N. This sum was then divided by the sum of the amount of fertilizer (kg N ha-1) added to the fertilized experimental unit and net N loss (kg N ha-1) through mineralization of the SONem-N in the fertilized experimental unit:  NUEBR6Seq (%) = (BR6NCEU – BR6NCCtl) + net increase INem-N  -------------------------------------------------------- x 100 (Eq. 6)  (N fertilizer applied + net loss SONem-N)  The system NUE (NUESys) (Eq. 7) accounting for N inputs from N fertilizer application (NFA), N added with irrigation water (IrN) (19 kg N ha-1 y-1), N added with wet atmospheric deposition (Nwatd) (5.2 kg N ha-1 y-1 ; National Atmospheric Deposition Program (NADP; <https://nadp.slh.wisc.edu/networks/national-trends-network/>), and the N changes in SONem-N, and accounting for the output of N with the harvested grain plus the increases in INem-N, was calculated as follows:  NUESys (%) = HG (kg N ha-1) + net increase INem-N  ------------------------------------------------------ x 100 (Eq. 7)  NFA + IrN + Nwatd + net loss SONem-N  The N loss from the system (NLsys) to the environment from fall 2005 to fall 2018 was calculated with an N balance equation (Eq. 8) that finds the difference between the N inputs to the corn system and the sum of the removed N in harvested corn grain and the increases in INem-N. Accounting for all the N inputs was not done, since dry N deposition has been reported for this region to range from 6 to around 24 kg N ha-1 y-1. Additionally, any potential deposition from birds that may have visited the experimental units has also not been accounted for. It is unknown if there was any atmospheric N fixation from free-living N-fixing bacteria in the experimental units. All of these unaccounted-for N inputs, if they occurred, will potentially contribute to increases in the unknown N losses from the system. The N balance was conducted by assessing changes in SONem-N from the 0 to 120 cm depth and changes in INem-N from 0 to 183 cm.  NLsys (kg N ha-1) = (NFA +IrN +Nwatd +net loss SONem-N) – (HG +net increase INem-N) (Eq. 8) |

**Table S5.** Summary of irrigation water applied by calendar year (2012-2023). Although most of the irrigation was applied between planting and physiological maturity (R6), on occasion irrigation events may have occurred before planting or after R6.

|  |  |  |
| --- | --- | --- |
| Year | Irrigation Water Applied (mm) | kg NO3-N ha-1 |
| 2012  2013  2014  2015  2016  2017  2018  2019  2020  2021  2022  2023 | 506  438  384  475  488  375  527  394  476  387  472  305 | 21.1  33.3  16.5  19.8  14.8  10.9  17.9  14.8\*  14.4\*  9.9\*  14.3\*  9.1¥ |

\*For irrigation events in this year with no associated nitrate analysis, water was assumed to contain nitrate N at a concentration equal to the mean concentration of those events in the same year for which nitrate N concentration was measured.

¥As no nitrate analysis was performed for any irrigation events in 2023, we assumed that nitrate N in that year was delivered in a concentration equal to the mean concentration of nitrate N reported in 2022

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| --- | --- |
| ANFA  ARDEC  BR6NCEU  BR6NCCtl  CoAgMet  CSU  CC  HGNCEU  HGNCCtl  HP  INem-N  KCl  MAON  NADP  NH3-N  N2O-N  NOx-N  NO3-Nem  NH4-Nem  NT  NO3-N  NH4-N  NUE  NUEHG  NUEBR6  NUEHGSeq  NUEBR6Seq  NUESys  N  NLsys  NFA  POM-N  POMem-N  R1  R2  R3  R4  R5  R6  SONem-N  SON  TNem-N  V1 to V8 VT | annual N fertilizer application  Agricultural Research, Development and Education Center  total aboveground biomass N content at R6 of the N-fertilized experimental units  total aboveground biomass N content at R6 of the control (non-fertilized)  experimental units  COlorado AGricultural Meteorological nETwork  Colorado State University  continuous corn  N content in the harvested grain of the experimental unit  N content in the harvested grain of the control (non-fertilized) experimental unit  horsepower  inorganic N expressed in total equivalent soil mass  potassium chloride  mineral-associated organic nitrogen  National Atmospheric Deposition Program  ammonia  nitrous oxide  NOx is shorthand for nitric oxide (NO) and nitrogen dioxide (NO2)  NO3-N expressed in equivalent soil mass  NH4-N expressed in equivalent soil mass  no-till (NT)  nitrate  ammonium  nitrogen use efficiency  nitrogen use efficiency of harvested grain  NUE of the total aboveground biomass N content at R6  NUE of harvested grain accounting for changes in the SONem-N and INem-N  NUE of the total aboveground biomass N content at R6 accounting for changes  in the SONem-N and INem-N  system NUE (NUESys)  nitrogen  N loss from the system  nitrogen fertilizer application  particulate soil organic matter nitrogen  particulate organic matter N expressed in equivalent soil mass  pollination (silking)  blister  milk  dough  dent  physiological maturity (black layer)  soil organic N expressed in equivalent soil mass  soil organic N  total N expressed in equivalent soil mass  stages of growth  tasseling |

**Table S22.** List of abbreviations used in this work.

Graphical user interface, application

Description automatically generated

**Figure S1.** Standards run as samples in the Elementar C and N analyzer during this long-term study from 2005 to 2018, where the same sample was run on multiple occasions during the same year and for a large number of years. Soil, corn leaf, and corn stalk standards were run. Note that since on occasion, more than one run was done on the same day, the timescale of the regression equations in the graph of Figure S1 are in seconds (y = % N; x = seconds). However, the timescale of the graph’s x-axis is shown in years (y = % N; x = year).