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# Sustainable manure management in intensified corn production systems for maintaining crop quality and yield, managing soil phosphorus, and increasing soil health Gabriella Burkett

A thesis submitted to the University Honors Program in partial fulfillment of the requirements for the Honors Certificate with Thesis

Approved by

Dr. Amir Sadeghpour

Southern Illinois University, Carbondale

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#### Acknowledgement and biographical note of author

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1

#### **Biographical Note**

Gabriella Burkett was a triple major in Plant Biology (specializing in Ecology); Crop, Soil, and Environmental Management (specializing in Soil Science); and Criminology and Criminal Justice, with minors in Forensic Sciences and Agricultural Education. She began research in Plant Biology her freshman and sophomore years under Dr. Sedonia Sipes, assisting with pollination field work and database collection and studying *Bombus pensylvanicus*, a declining Midwestern bumblebee. Through SUPERB, Ms. Burkett continued analysis of *B*. *pensylvanicus*' foraging habits and presented her research inter-collegiately and at SIU.

She began researching under Dr. Amir Sadeghpour her sophomore year, where she continued researching throughout her undergraduate years at SIUC, studying sustainable cropping practices. She was awarded a REACH grant for this research and has won several awards while presenting this research: a 1<sup>st</sup> place poster at. ASA-CSSA-SSSA 2022 international meeting, 2<sup>nd</sup> place oral at ASA-CSSA-SSSA 2023 international meeting, top undergraduate poster at the CARP 2024 research symposium at SIUC, and more.

Outside of her research, Ms. Burkett was active in community service through the Honors program and University Excellence Scholarship program and is a SASES Golden Opportunity Scholar. She took part in leading STEM workshops such as building and leading a soil-based mystery for 200 middle and high schoolers for SIU Saluki Sleuths. Additionally, she served as the SIU Soil Judging Team President, Horticulture Association and Saluki Science Dawgs Vice President, and Ag Council Rep for Horticulture Association; as well as being a part of Zeta-Theta-Omega, Collegiate Farm Bureau and the ALE&FFA 2024 PAS soil specialist team (took 1<sup>st</sup>individual and 1<sup>st</sup> team in state, and 2<sup>nd</sup> individual and 1<sup>st</sup> team in nationals).

She intends to begin a Ph.D program in Agricultural Sciences at SIUC in Fall 2024.

# Sustainable manure management in intensified corn production systems for maintaining crop quality and yield, managing soil phosphorus, and increasing soil health

# Abstract

Dairy farmers often surface apply phosphorus (P)-based liquid manure to corn (Zea mays L.) for silage, supplementing with N fertilizers for optimum corn nitrogen (N), optimizing crop production while decreasing P loss to the environment. However, injecting manure may further conserve losses and reduce synthetic N fertilizer need. An experiment was conducted on a dairy farm in Breese, IL from May 2019 to April 2022 with two main treatments including (i) surface application of manure at P-based rate supplemented with 123 kg ha<sup>-1</sup> synthetic N and (ii) manure injection at P-based rate supplemented with 17 kg ha<sup>-1</sup> of synthetic N fertilizer. Both treatments delivered 201 kg N ha<sup>-1</sup> to meet corn N need. Our results indicated that yield and quality of silage corn and cereal rve were similar in both treatments. This suggests that injection can limit manure ammonium-N fraction losses and decrease the need for supplemental N fertilizer by 106 kg ha<sup>-1</sup>, which translates into up to \$150 ha<sup>-1</sup>, while not affecting the quality and quantity of vields. Moreover, the effect of manure injection on soil test P (STP) was similar to that of surface application and did not increase STP over a three-year period. Elevated STP in high Psupplying soils can be an environmental concern, but our results show that neither treatment increased STP. Future research should focus on quantifying N loss through denitrification and leaching when manure is injected versus surface applied to provide a more holistic overview of the soil and environmental impact of each system.

**Keywords:** Corn silage, manure injection, manure surface application, phosphorus management, winter rye forage

1

# **1. Introduction**

Corn (*Zea mays*) is a major cash crop in the U.S. Midwest, and corn grown for silage in particular is an important component of dairy rationing. Harvesting corn for silage removes a substantial amount of nutrients such as N, P, and potassium (K) from the soil [1] that needs to be replenished to sustain crop production. Dairy farmers often apply liquid dairy manure to meet the N requirement of a corn crop (N-based management) [2–4] and to enhance soil quality [5,6]. However, the relatively high P:N ratio in manure, when compared to the nutrient needs of a corn crop, can lead to a gradual increase of soil test phosphorus (STP) concentrations over time [7,8] Elevated STP levels can result in greater phosphorus loss into surface and groundwater [9,10].

Shifting from an N-based approach to a P-based approach in the management of manure for corn has been proposed as a strategy to regulate STP levels [8,11]. However, such a shift necessitates reducing the manure application rate, thus lower N for the corn crop. In this case, the application of supplemental N via synthetic fertilizer has been proposed in soils that have high STP [12–14].

In no-till systems, incorporation of manure is not practiced, and thus, surface application of manure often results in loss of ammonium-N (NH<sub>4</sub>-N) fraction through ammonia volatilization [13,15,16]. Manure injection is an effective approach that not only increases the N utilization of manure through reduction in NH<sub>4</sub>-N loss but also addresses odor concerns linked to surface application [12]. In cases where the corn's N requirements are not fully met through manure injection at the P-based rate, a modest N fertilization can be applied to ensure that an adequate amount of N is supplied to the corn crop [17]. Several studies have shown that P-based manure management, even with incorporation, might result in a corn yield penalty [4,5], and that adding fertilizer could eliminate that influence [14].

Cover crops are becoming increasingly popular among farmers for many benefits, including their use as a forage crop in corn for silage systems. In soil with high STP, reducing the P build-up by adding winter cereals into rotation with corn for silage is becoming common [18–20]. Winter cereals can uptake some P from the soil during the spring [21,22] and reduce STP levels while potentially using residual N after corn for silage harvest and providing homegrown feed for dairy operations [19,20,23,24]. Literature is scant on evaluating P-based manure application methods (injection versus surface application) effects on both corn for silage and the following winter rye in rotation. Therefore, the primary objective of our research was to assess the consequences of switching from a surface application of P-based liquid dairy manure supplemented with sidedress N fertilizer at a rate of 123 kg ha<sup>-1</sup> to an injection of manure at the P-based rate supplemented with 17 kg ha<sup>-1</sup> of sidedress N fertilizer on corn and winter rye performance in rotation. The higher N fertilizer application in surface-applied manure treatment aims to compensate for the loss of NH<sub>4</sub>-N due to surface application. Our hypothesis was that a transition from P-based rate surface application to injection could produce similar corn yield at lower fertilizer N requirement and therefore, benefit the growers by saving N fertilizer and also by benefiting the environment through reduction in P runoff and odor concerns associated with the surface management practices.

#### 2. Materials & Methods

#### 2.1 Experimental site and weather conditions

In 2019, a field experiment was initiated in Breese, IL (36°69'51" N, 89°53'61" W), with an elevation of 139 m above sea level. The soil was classified as poorly drained silt loam (Fine, smectitic, mesic Udollic Endoaqualfs) with a slope of 0-2%. The soil contained 245 g kg<sup>-1</sup> sand, 520 g kg<sup>-1</sup> silt, and 235 g kg<sup>-1</sup> clay; with organic C content of 17.5 g kg<sup>-1</sup> in the top 20 cm and pH (1 water: 1 soil) of ~7.9. Initial levels of nitrate-N (NO<sub>3</sub>-N), Bray-1 STP (Bray and Kurtz, 1945), and Mehlich-3 STK (Mehlich, 1984) were 2.9, 77.6, and 206.9 mg kg<sup>-1</sup>, respectively, within the top 20 cm of soil. According to the IL Agronomy Handbook

(http://extension.cropsciences.illinois.edu/handbook/pdfs/chapter08.pdf), STP and STK concentrations were classified as very high and high, respectively. This experiment was conducted on a dairy farm; therefore, there was a prior manure application to the study area which was producing corn for silage before the start of this experiment.

Cumulative corn growing season (May – Sept.) precipitation was higher in 2020 (649.1 mm) followed by 2019 (536.4 mm) and 2021 (472.2 mm). During the winter rye growing seasons (Oct-Apr), cumulative precipitation was higher in 2020 (717 mm) than in the two other years. Cumulative monthly precipitation during the corn phase is shown in Table 1. Cumulative precipitation was higher in May 2019 than in other years (Table 1). Precipitation in June was comparable among all three years, but 2020 had several instances of excessive rain during July and August (231.3 and 191.2 mm, respectively) which influenced the N availability in the field and thus crop production. September, when the harvest for corn occurred was slightly wetter in 2021 (69.8 mm) than the two other years (20.0 mm for 2019 and 15.4 mm for 2020). Precipitation during the winter rye phase (Oct. -April) was often variable over the years (Table 1). While Oct. was similar among all years, precipitation in 2019 (91.4 mm) and 2020 (130.0 mm) was much higher than that of 2021 (21.0 mm). Similarly, Jan. was much wetter in 2019 and 2020 than the 2021. In contrast, 2021 received much higher precipitation in Feb. (124.2 mm) than the other years and both 2021 and 2022 had higher precipitation than 2020 in March.

Average corn growing season temperature was 22.9, 22.6, and 22.2 °C for 2019, 2020,

and 2021, respectively. The average air temperature for winter rye was 6.7, 6.4, and 6.8 °C

during 2020, 2021, and 2022, respectively. Air temperature was comparable among the three

study years for corn (2019, 2020, and 2021) and rye (2020, 2021, and 2022). The air temperature

was slightly higher in May 2019 (19.5 °C) than in 2020 (17.7 °C) and 2021 (17.0 °C) (Table 1).

Nov. 2019 was also much cooler than those of 2020 and 2021. Some of these weather differences

could explain differences in corn or winter rye yields.

#### Table 1.

Cumulative precipitation and mean air temperature during corn and winter rye growing seasons at each study year.

Month/Voor	Precipitati	on (mm)		Air Temperature Average (C)				
	2019/20	2020/21	2021/22	2019/20	2020/21	2021/22		
May	161.0	108.9	72.3	19.5	17.7	17.0		
June	100.3	102.3	99.8	22.7	24.9	24.4		
July	103.3	231.3	168.4	25.6	26.4	24.2		
August	151.8	191.2	61.9	23.7	23.8	24.4		
September	20.0	15.4	69.8	23.2	20.0	21.1		
October	114.3	128.5	130.5	12.9	12.8	16.1		
November	91.4	130.0	21.0	4.5	9.9	6.3		
December	50.5	30.7	80.0	3.0	2.4	6.8		
January	183.8	103.6	36.5	2.1	0.5	-2.6		
February	59.9	43.6	124.2	2.6	-3.54	0.2		
March	97.0	122.9	164.5	9.6	9.8	8.3		
April	120.1	78.4	97.7	12.2	12.9	12.5		

#### 2.2 Experimental design and treatments

The experiment was conducted employing a randomized complete block design replicated four times. The two main treatments of this study were (i) surface application of liquid dairy manure at a P-based rate (120.5 kL ha<sup>-1</sup>) with 123 kg ha<sup>-1</sup> (to match 201 kg N ha<sup>-1</sup>) requirement for corn and (ii) injection manure at P-based rate (120.5 kL ha<sup>-1</sup>) plus 17 kg ha<sup>-1</sup> of

N fertilizer to match 201 kg N ha<sup>-1</sup> requirement for corn. Differences in N fertilizer rate in each management were due to the assumption of NH<sub>4</sub>-N loss through volatilization of ammonia in the surface application of manure, as explained below.

The experiment considered a silage yield potential of 16.4 Mg DM ha<sup>-1</sup> based on the growers yield data history (past five years). The estimation for corn P removal was approximately 85 kg  $P_2O_5$  ha<sup>-1</sup>, assuming an average of 2.3 g of phosphorus per kg of dry matter [5]. The total N requirement of 201 kg ha<sup>-1</sup> was based on growers' typical application that matches the Maximum Return to N decision support tool (MRTN) as described by [25]. The added inorganic N rate for sidedressing was set to meet the expected N demand of the corn crop. We assumed liquid dairy manure had 35% organic-N availability in the year of application, followed by 12% and 5% in years 2 and 3, respectively [5,26]. For the surface P-based liquid dairy manure, no inorganic N fraction (NH<sub>4</sub>-N) was considered available because the manure was surface applied and never incorporated after application. The guidelines assume a 100% NH<sub>4</sub>-N loss through volatilization of ammonia. In contrast, for the injected, P-based manure, a conservation rate of 100% for inorganic N (NH<sub>4</sub>-N) was assumed, given the direct placement of manure in the soil [26]. The rate of application was determined based on the composition of manure sourced from the farm supplying the resource in 2019 (Table 2).

weight basis).						
Year	Total N	Ammonia N	Organic N	$P_2O_5$	K <sub>2</sub> O	Total solids
			g k	g-1		
			Dairy liqui	d manure		
2019	2.0	0.9	1.1	0.3	1.7	69
2020	1.6	0.8	0.9	0.3	1.6	28
2021	1.7	0.6	1.1	0.3	1.7	22

Table 2.

Characteristics of liquid dairy manure during 2019, 2020, and 2021 (nutrient measurements on a dry-weight basis).

#### 2.3 Liquid manure sampling and analysis

Liquid dairy manure was collected each year from agitated manure storage lagoons of the dairy farm. Subsamples were collected before land application, kept cool at the time of collection, and frozen before being sent to the laboratory for analysis. Manure samples were analyzed at Ward Laboratory in Kearney, NE. Total solids, total N, organic and inorganic N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O were measured on the manure samples as described [27]. Briefly, total C and N were quantified using a LECO FP-2000 CN analyzer (LECO Corporation, St. Joseph, MI) through dry combustion. Inorganic N levels were assessed colorimetrically with a Lachat Quikchem 8500 (Hach Company, Loveland, Colorado), while organic N was estimated as the difference between total and inorganic N concentrations. The concentrations of total P (P<sub>2</sub>O<sub>5</sub>) and K (K<sub>2</sub>O) were determined using Inductively Coupled Plasma (ICP) spectrometry with an iCAP 6500 Duo ICP instrument from Thermo Fisher Scientific Inc. (Waltham, MA).

#### 2.4 Cultural management practices

The corn was planted with a John Deere 7000 Max Emerge planter at 76 cm row spacing. A Pioneer standard corn silage was planted on May 10, May 21, and May 12 in 2019, 2020, and 2021, respectively at a population of 79,010 seeds ha<sup>-1</sup>. Weeds, insects, and pests were controlled according to Illinois Agronomy Handbook (https://extension.illinois.edu/global/agronomyhandbook). Corn for silage was harvested on Sept. 20, 2019, August 28, 2020, and Sept. 15, 2021. Winter rye (cv. Wintergrazer) was planted using a small grain drill after silage harvest on Oct. 12, 2019, Oct. 6, 2020, and Oct. 10, 2021. The row spacing of WR was 19 cm, and the seeding rate was 100 kg ha<sup>-1</sup> in each year of the study.

#### **2.5 Measurements**

#### 2.5.1 Soil Pre sidedress Nitrate Test (PSNT)

Each year, soil samples were collected at the V6 stage of corn (six cores per plot, 0–30 cm depth) to test for PSNT. Samples were kept cool while in the field, air dried for several days on arrival at the laboratory and crushed to pass 2 mm before analysis for soil NO<sub>3</sub>-N following standard soil preparation procedures reported in Brookside laboratory (New Bremen, OH). Soil NO<sub>3</sub>-N was analyzed using 1 N KCl cadmium reduction with a Flow Injection Analyzer (FIAlab Instruments Inc., Bellevue, WA).

#### 2.5.2 Corn yield, Corn Stalk N Test (CSNT), and silage quality

Corn was machine harvested from the middle of each plot (3 m by 9.1 m area) after removing the edge effects. After weighing the harvested area, a subsample was collected, weighed again, and then placed in an air-forced oven until it reached constant weight to measure dry matter yield for silage corn. Biomass sub-samples were then ground until they could pass through a 1 mm sieve, facilitating silage quality analysis. Ward laboratories performed the forage quality analysis according to their analysis guideline (<u>https://www.wardlab.com/resources/ward-</u> <u>guide/</u>). At harvesting time, we collected CSNT samples from the corn stalks of six healthy plants followed by a procedure explained in [8].

#### 2.5.3 Winter rye morphology, above- and below-ground biomass, and quality

Before harvesting the aboveground biomass of winter rye, the height of nine plants, extending from the ground to the top of the canopy, was measured using a yardstick. Also, a chlorophyll meter (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Aurora, IL)

which functions as a spectral instrument by quantifying the disparity in light transmittance between a red (at 650 nm) and an infrared (at 940 nm) wavelength as it passes through the leaf was used. This process yields a three-digit SPAD (Soil-Plant Analysis Development) value, as introduced by [28]. These SPAD readings were obtained by measuring the flag leaf of 9 randomly selected plants from each plot. During each site-year assessment, a GreenSeeker Handheld Crop Sensor HCS 100 (Trimble Ltd., Sunnyvale, CA) was used to assess canopy reflectance and Normalized Difference Vegetation Index (NDVI) along the two central rows covering the entire plot length. This specific sensor was a low-cost option without any connection or data logging capabilities. This device utilizes an active light source optical sensor emitting at 660 nm (red) and 780 nm (near infrared) with an approximate full-width halfmaximum of 25 nm. It continuously displays the NDVI of the scanned area at intervals of around 0.5 seconds as long as the trigger is held down. Upon releasing the trigger, it displays the average of measurements taken over the last 60 seconds, as described by White et al. [29].

To calculate the leaf area index (LAI), an AccuPAR ceptometer (LP–80; manufactured by METER Group in Pullman, USA) was used. This device utilized measurements of photosynthetically active radiation (PAR) both above and below the canopy. All measurements were conducted between 1100 and 1400 hours, ensuring that the sun's angle was close to zenith, and under clear sky conditions as explained before [30]. The AccuPAR ceptometer calculates fractional beam radiation and solar zenith angle based on the global position and time of day, which can be adjusted by the operator from the setup menu. It also employs the typical leaf angle distribution (LAD, $\chi$ ) parameter, denoted as  $\chi$ , to determine the LAI through a radiative transfer model, as described by [31].

To determine the extinction coefficient K for the canopy, we used the equation reported by Campbell [32]:

$$K = \sqrt{(x^2 + \tan \ \ \mathbb{I}\theta^2 \ \mathbb{I})/(x + 1.744 \ \mathbb{I}(x + 1.182) \ \mathbb{I}^{(-0.733)})}$$

In this equation, x represents the leaf angle distribution parameter, and  $\theta$  denotes the solar zenith angle.

Winter rye's aboveground biomass was collected using grass shears (GS model 700; Black and Decker Inc., Towson, MD) during late April. Precisely, the winter rye harvest occurred on April 23, 2020, April 15, 2021, and April 19, 2022. The harvesting area was 0.675  $m^2$ , achieved by employing three frames measuring 2.25  $m^2$  each, also positioned at the center of the plots to minimize edge effects. Subsequently, all aboveground biomass samples underwent a 72-hour oven-drying process at 48°C to determine their dry matter (DM) yield. Belowground (root) samples were collected using a shovel from a  $0.45 \text{ m}^2$  area to 30 cm depth. Depth of sampling was selected based on the findings of [33] who showed that almost 90% of the root biomass of winter rye was at the top 37.5 cm. Root samples were pressure washed and then oven-dried at 48°C for at least a 72-hour period to determine their dry matter (DM) yield. Aboveground biomass sub-samples were then ground until they could pass through a 1 mm sieve, facilitating forage quality analysis as per the methodology outlined by researchers [34]. Forage quality indices evaluated in this study included CP, ADF, NDF, NDFD, ash, and lignin which were measured using near-infrared reflectance spectroscopy (NIRS). Samples from both sites were analyzed for quality in a single lab. Total digestible nutrients, digestible dry matter (DDM), in vitro dry matter digestibility (IVTDMD), dry matter intake (DMI), relative feed value (RFV), RFQ, and net energy for lactation (NE<sub>L</sub>), net energy gain (NE<sub>G</sub>) along with maintenance  $(NE_M)$  were estimated based on the formula [35,36]:

10

 $TDN = (-1.291 \times ADF) + 101.35,$ 

DMI = 120% NDF dry matter basis,

 $DDM = 88.9 - (0.779 \times \%ADF; dry matter basis),$ 

 $RFQ = (DMI, \% \text{ of body weight for dairy cattle}) \times (TDN, \% \text{ of DM})/1.23$ 

 $NE_L = [1.044 - (0.0119 \times \%ADF)] \times 2.205.$ 

DE (Mcal kg<sup>-1</sup>) =  $0.04409 \times \%$  TDN

ME (Mcal kg<sup>-1</sup>) =  $0.82 \times DE$ 

NE<sub>M</sub> (Mcal kg<sup>-1</sup>) =  $1.37ME - 0.138ME^2 + 0.0105ME^3 - 1.12$ 

 $NE_G$  (Mcal kg<sup>-1</sup>) = 1.42ME – 0.174ME<sup>2</sup> + 0.0122ME<sup>3</sup> – 1.65Mineral concentration in plant tissue, including P, K, total C, and ash, was also measured with NIRS. Total N was calculated by dividing CP concentration by 6.25.

For assessing C, N, and C:N ratio, above- and belowground biomass samples were ground to pass a 1-mm sieve and then pulverized using a bowl mill (Spex Sample Prep LCC, Metuchen, NJ). Carbon and N content of above- and below-ground biomass were determined by dry combustion (950 °C) using a Thermo Scientific, Flash 2000 C, and N analyzer (CE Elantech, Inc., Lakewood, NJ). This is well explained in Weidhuner et al. [37].

#### 2.6 Statistical analysis

Data for soil PSNT, corn yield, CSNT, silage quality, winter rye morphology (plant height, SPAD values, NDVI, and LAI) along with winter rye above- and below-ground biomass and their quality were first evaluated for the normality of the residuals using PROC Univariate and Shapiro-Wilk test in SAS (SAS version 9.4, SAS Institute, Cary, NC, USA). All data were analyzed using a repeated measure approach in PROC Mixed. To evaluate the differences

between the two manure application methods, data were analyzed with year, application method (surface application versus injection), and their interaction as fixed effects, and block and year nested within the block (indicating plots) as a random effect similar to the approach by Weidhuner et al. [37]. In addition, autoregressive covariance structure was specified for the plots being repeatedly measured over the years to account for the artifact of significance from years at one sampling point, and convergence criteria were met. Where treatment results were found significant ( $P \le 0.05$ ), least square means and standard error were withdrawn using the LSMEANS option in SAS PROC Mixed adjusted for Tukey.

Principal component analysis (PCA) biplot was analyzed on correlations and two principal components were chosen based on eigenvalues and scree plot outputs. The PCA was ran for corn and winter rye separately. For corn, corn silage yield, CSNT, PSNT, along with N, P, K removals, and quality data were included. Similarly, for winter rye, above- and belowground biomass, their C, N, and C:N ratio, along with N, P, K removals, Ca, Mg, Ca:Mg, and forage quality parameters were included.

# **3. Results**

#### 3.1 Corn silage yield, PSNT, and CSNT

Corn silage yield was affected by year but not treatment (manure application method) or the interaction of year by treatment (Supplemental Table 1). This suggests that the manure application method does not affect the following corn yield. Corn silage yield (averaged over years) was 15.3 Mg ha<sup>-1</sup> for surface application and 14.6 Mg ha<sup>-1</sup> for manure injection (Fig. 1A).

Soil PSNT data showed the effect of year and treatment-by-year interaction was significant but not by treatment alone (Supplemental Table 1). Although the PSNT was similar between surface application and injection within each study year, surface application had higher PSNT in 2021 (23.6 mg kg<sup>-1</sup>) than those of injection in 2019 (17 mg kg<sup>-1</sup>). In general, only surface application of manure in 2021 had higher PSNT values than the threshold of 25 mg kg<sup>-</sup> <sup>1</sup> (indicating no need for sidedress N fertilization). This suggest that whether manure was injected or surface applied, there was a need for N fertilization at sidedress timing to optimize corn



Fig. 1. Effect of year and manure application method on corn yield (a), corn stalk nitrate test (CSNT) (b), and pre-sidedress soil nitrate test (PSNT) (c) across years. The bars indicated standard error. INJ: inject manure, SP: spread manure. Means with the same letter for each year are not significantly different (Tukey=0.05).

production (Fig. 1B). Corn stalk nitrate test data were affected by year but not treatment or the interaction of year by treatment (Supplemental Table 1), reflecting weather conditions and high

yields. These results indicate that a producer could apply manure by either injection or surface

application and as long as the N requirement is met, corn yields would remain unaffected.

#### 3.2 Corn N, P, and K concentrations, removals, and balances

Corn N and P concentrations were not affected by year, treatment, or their interactions

(Supplemental Table 1; Table 3).

#### Table 3.

Effect of year and manure application method on Corn N, P, and K applied, removal and balance across the years.

Year	Treatment	N applied (kg ha <sup>-1</sup> )	N (g kg <sup>-1</sup> )		N Removal (kg ha	<b>a</b> <sup>-1</sup> )	N balance (kg ha <sup>-1</sup>	)
2010	INJ		120.4 ±17.56 a*		186.44 ± 34.1 ab	1.	$15.25 \pm 34.09$ bc	1.
2019	SP		124.4 ± 2.28 a	а	$216.87 \pm 8.0$ a	D	$-15.16 \pm 7.98$ bc	b
2020	INJ	201 70	113.6 ± 1.95 a		$93.22\pm16.5~b$		108.47 ± 16.55 a	
2020	SP	201.70	124.25 ± 8.0 a	а	$136.87 \pm 11.9 \text{ ab}$	с	$64.83 \pm 11.94$ ab	а
2021	INJ		131.6 ± 2.72 a		$246.19 \pm 16.6$ a		-44.49 ± 16.56 c	
2021	SP		141.2 ± 2.46 a	а	$248.76 \pm 8.0$ a	а	$-47.05 \pm 7.99 \text{ c}$	с
		P applied (kg ha <sup>-1</sup> )	P (g kg <sup>-1</sup> )		P Removal (kg ha	<b>1</b> <sup>-1</sup> )	P balance (kg ha <sup>-1</sup>	)
2010	INJ		$2.60 \pm 0.3$ a		39.88 ± 5.1 a		$-1.08 \pm 5.08$ b	
2019	SP		$2.32\pm0.1~a$	а	$40.59 \pm 2.3$ a	b	$-1.79 \pm 2.32$ b	D
	INJ	20.00	$2.65\pm0.1~a$		$21.49 \pm 3.3$ a		21.67 ± 3.27 a	
2020	SP	38.80	$2.80\pm0.1\ a$	а	$30.79\pm1.5~a$	с	12.38 ± 1.46 a	а
2021	INJ		$2.70\pm0.1$ a		$50.37 \pm 2.9$ a		$-9.10 \pm 2.94$ b	1
2021	SP		$2.80\pm0.1\ a$	а	49.47 ± 3.1 a	а	$-8.19 \pm 3.06$ b	b
		K applied (kg ha <sup>-1</sup> )	K (g kg <sup>-1</sup> )		K Removal (kg ha	<b>a</b> <sup>-1</sup> )	K balance (kg ha <sup>-1</sup>	l)
	INJ		9.67 ± 0.8 a		147.65 ± 12.2 a		$185.03 \pm 12.28$ a	
2019	SP		$10.1 \pm 0.6$ a	b	176.38 ± 12.9 a	b	$156.29 \pm 12.92$ a	а
	INJ	222 (9	$9.55 \pm 0.6 \text{ a}$		$78.11 \pm 14.02$ a		$175.84 \pm 14.02$ a	
2020	SP	332.68	$9.53 \pm 0.9$ a	b	$104.35 \pm 9.04$ a	с	$149.59 \pm 9.04$ a	а
2021	INJ		$13.00 \pm 0.5$ a		$243.05 \pm 17.8$ a		$-31.83 \pm 17.80$ b	1.
2021	SP		$12.85 \pm 0.5 \text{ a}$	а	$226.62 \pm 12.3$ a	a	$-15.41 \pm 12.37$ b	D

\*Means  $\pm$  standard error. INJ: inject manure, SP: spread manure. Means with the same letter are not significantly different (Tukey=0.05).

Corn N concentrations ranged from 9.53 (SP in 2020) to 13.0 g kg<sup>-1</sup> (INJ in 2021), while P concentrations ranged from 2.32 (SP in 2019) to 2.80 g kg<sup>-1</sup> (INJ in 2020 and SP in 2021). Potassium concentrations were only influenced by year (Supplemental Table 1; Table 3), higher in 2021 (12.92 g kg<sup>-1</sup>) than in 2019 (9.88 g kg<sup>-1</sup>) and 2020 (9.54 g kg<sup>-1</sup>). These N, P, and K levels

suggest that manure application method doesn't affect concentration of nutrients within the corn plants. Corn N removal was affected by year only. Corn N removal was higher in 2019 (201.6 kg ha<sup>-1</sup>) and 2021 (247.5 kg ha<sup>-1</sup>) than in 2020 (115.0 kg ha<sup>-1</sup>), reflecting weather conditions that resulted in different yields in each year (Supplemental Table 1; Table 3). Nitrogen removal was similar between INJ and SP in all years(Supplemental Table 1; Table 3). Similarly, corn P and K removals were only influenced by year, and both INJ and SP had similar P and K removal within each year (Supplemental Table 1; Table 3). Nitrogen, P, and K removals correlate with corn yields, suggesting application method does not affect corn yields.

# **3.3 Corn silage quality**

Corn crude protein was not affected by year, treatment, or the interaction of the two (Supplemental Table 2; Table 4). Corn crude protein ranged from 71 g kg<sup>-1</sup> (INJ in 2020) to 88.2 g kg<sup>-1</sup> (INJ and SP in 2021). Corn ADF, NDF, NEM, NEG, NEL, TDN, and RFV were all only influenced by year and not by treatment or the interaction of year by treatment (Supplemental Table 2; Table 4). This indicates that growers can manage manure by injecting or surface applying as long as N is supplemented to match the corn N needs.

# 3.4 Rye morphology, forage, and root biomass

Data for SPAD was influenced by year, but not treatment or year-by-treatment interactions (Supplemental Table 3; Fig. 2A). SPAD values were higher in 2022 than in 2021 and 2020, reflecting higher N in the plant in 2022.

# Table 4.

Effect of year and manure application method corn crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), net energy for maintenance ( $NE_M$ ), net energy for gain ( $NE_G$ ), net energy for lactation ( $NE_L$ ), total digestible nutrients (TDN), and relative feed value (RFV) in different years.

Year	Treatment	CP (g kg <sup>-1</sup> )		ADF (g kg <sup>-1</sup> )		NDF (g kg <sup>-1</sup> )		NE <sub>M</sub> (Mcal kgDM <sup>-1</sup> )	
2010	INJ	*75.25 ± 11.0 a		238.50 ± 15 a	1	$434.00 \pm 16$ a	1	$1.44 \pm 0.1$ a	
2019	SP	$77.75 \pm 1.4 \text{ a}$	а	$231.75\pm11~a$	b	$413.50\pm20~a$	b	$1.48\pm0.1~a$	а
2020	INJ	$71.00\pm1.2~a$		$221.75 \pm 10$ a	1	$385.25\pm18~a$	1	$1.56 \pm 0.1 \ a$	
2020	SP	$77.66 \pm 5.0$ a	а	$231.50\pm28~a$	b	398.00 ± 39 a	b	$1.55\pm0.3~a$	а
2021	INJ	$82.25 \pm 1.7$ a		$316.50 \pm 15$ a		$498.00\pm9~a$		$0.96\pm0.1~a$	1-
2021	SP	$88.25 \pm 1.5$ a	а	$292.00\pm21~\text{a}$	а	457.00 ± 12 a	a	$1.10 \pm 0.1$ a	D
		NE <sub>G</sub> (Mcal kgDI	<b>M</b> <sup>-1</sup> )	NE <sub>L</sub> (Mcal kgD	M <sup>-1</sup> )	TDN (g kg <sup>-1</sup> )		RFV	
2010	INJ	$0.91 \pm 0.09 \ a$	1	$1.42 \pm 0.14$ a		$711.50 \pm 10.9$ a		151.5 ± 7.8 a	
2019	SP	$0.94\pm0.07~a$	b	$1.46\pm0.10\;a$	а	$716.25 \pm 8.0 \text{ a}$	а	$160.5 \pm 9.2$ a	а
	INJ	$1.57 \pm 0.09$ a		$1.00\pm0.06~a$		$723.50 \pm 7.6 \text{ a}$		$174.5\pm9.8~a$	
2020	SP	$1.56\pm0.24~a$	а	$1.00\pm0.17~a$	b	$716.25 \pm 19.8$ a	а	$172.0 \pm 22.1$ a	а
0001	INJ	$0.58\pm0.04~a$		$0.92\pm0.07~a$	1	$657.00 \pm 10.9$ a	1	$120.0 \pm 3.2 \text{ a}$	
2021	SP	$0.68 \pm 0.08$ a	с	$1.06 \pm 0.12$ a	b	$674.00 \pm 15.2$ a	b	$135.5 \pm 6.8$ a	b

\*Means  $\pm$  standard error. INJ: inject manure, SP: spread manure. Means with the same letter are not significantly different (Tukey=0.05).



**Fig. 2.** Effect of year and manure application method on rye SPAD (a), NDVI (b), and Plant height (c) in different years. The bars indicated as standard error. INJ: inject manure, SP: spread manure. Year comparison, means with the same letter are not significantly different (Tukey=0.05).

Unlike SPAD, NDVI was not influenced by year, treatment, or year-by-treatment interactions (Supplemental Table 3; Fig. 2B). Plant height was related to winter rye shoot biomass ( $R^2 = 0.67$ ; RMSE = 0.53; P value = 0.0001) and was higher in 2021 (92.6 cm) than in 2020 (69.16 cm) and especially 2022 (48.52 cm) (Supplemental Table 3; Fig. 2C). Rye forage yield (aboveground biomass) was similar between INJ and SP in all years (Supplemental Table 3). This suggests that when supplemented with N fertilizer, the manure application method had a limited effect on the succeeding winter rye production in the rotation. Rye forage yield ranged from 1.93 Mg ha<sup>-1</sup> in 2022 to 3.18 Mg ha<sup>-1</sup> in 2021, mainly reflecting harvesting time (Supplemental Table 3; Fig. 3A).



**Fig. 3.** Effect of year and manure application method on rye shoot biomass (a), root biomass (b), shoot N (c), root N (d), shoot C:N (e) and root C:N (f) in different years. The bars indicated as standard error. INJ: inject manure, SP: spread manure. Year comparison means with the same letter are not significantly different (Tukey=0.05).

Rye root biomass was very similar to those of the aboveground biomass in which in a year that forage yield was higher (2021), root biomass was high too resulting in a root:shoot ratio

0.2, 0.17, and 0.19 for the year 2020, 2021, and 2022, respectively (Supplemental Table 3; Fig. 3B). Shoot N was similar between the two manure application methods (Supplemental Table 3; Fig. 3C). Shoot N had a negative relation to plant height ( $R^2 = 0.60$ ; RMSE = 2.71; P value = 0.0001) indicating earlier harvest in 2022 growing season resulted in smaller plants that had higher N concentrations as compared to those in 2021 and 2020. Unlike shoot N, root N was influenced by treatment differences in which INJ had higher root N than SP treatment (Supplemental Table 3; Fig. 3D). Shoot and root C:N ratios were similar between INJ and SP treatments and were only affected by year-to-year variation (Supplemental Table3; Fig. 3E-F). The shoot C:N ratio was 1.5 to 2-fold lower than that of the root C:N ratio (Fig. 3E-F).

#### 3.5 Rye N, P, and K concentrations and removals

Winter rye N and P concentrations were only influenced by year and were similar between the two application methods (Supplemental Table 4; Table 5).

#### Table 5.

Year	Treatment	N (g kg <sup>-1</sup> )		P (g kg <sup>-1</sup> )		K (g kg <sup>-1</sup> )	
2020	INJ	$148.4 \pm 6.80 \ b^*$	1.	$2.07\pm0.14~a$	1	$16.12 \pm 0.62$ a	
2020	SP	$156.8 \pm 8.02 \text{ b}$	D	$2.37\pm0.2~0a$	D	$16.90 \pm 0.98$ a	а
2021	INJ	$138.0\pm3.60~b$	1.	$2.47\pm0.02~a$	- h	$15.27 \pm 0.37$ a	
2021	SP	$140.0 \pm 11.6 \text{ b}$	b	$2.55\pm0.05~a$	ab	$15.62 \pm 0.34$ a	а
2022	INJ	$208.8 \pm 4.81$ a	0	$2.52\pm0.04~a$	0	$16.07 \pm 0.86$ a	0
2022	SP	$216.8 \pm 5.55$ a	а	$2.52\pm0.04~a$	a	$15.60 \pm 0.24$ a	а
		N uptake (kg ha <sup>-1</sup>	1)	P uptake (kg h	a <sup>-1</sup> )	K uptake (kg ha	-1)
2020	INJ	<b>N uptake (kg ha</b> <sup>-1</sup> $32.45 \pm 4.90$ a	<sup>1</sup> )	<b>P uptake (kg h</b> $4.17 \pm 0.54$ a	a <sup>-1</sup> )	<b>K uptake (kg ha</b> 33.16 ± 5.34 a	<sup>-1</sup> )
2020	INJ SP	<b>N uptake (kg ha</b> <sup>-1</sup> 32.45 ± 4.90 a 33.57 ± 6.19 a	<sup>1</sup> ) a	<b>P uptake (kg h</b> $4.17 \pm 0.54$ a $5.32 \pm 1.32$ a	<b>a</b> -1) b	<b>K uptake (kg ha</b> 33.16 ± 5.34 a 38.24 ± 8.61 a	<sup>-1</sup> ) b
2020	INJ SP INJ	N uptake (kg ha <sup>-1</sup> 32.45 ± 4.90 a 33.57 ± 6.19 a 45.84 ± 5.12 a	<sup>1</sup> ) a	P uptake (kg h 4.17 ± 0.54 a 5.32 ± 1.32 a 7.53 ± 0.95 a	<b>a</b> -1) b	K uptake (kg ha $33.16 \pm 5.34$ a $38.24 \pm 8.61$ a $46.29 \pm 5.29$ a	b
2020 2021	INJ SP INJ SP	N uptake (kg ha <sup>-1</sup> 32.45 ± 4.90 a 33.57 ± 6.19 a 45.84 ± 5.12 a 41.16 ± 9.18 a	<sup>1</sup> ) a a	P uptake (kg h $4.17 \pm 0.54$ a $5.32 \pm 1.32$ a $7.53 \pm 0.95$ a $8.50 \pm 1.70$ a	<b>a</b> <sup>-1</sup> ) b a	K uptake (kg ha $33.16 \pm 5.34$ a $38.24 \pm 8.61$ a $46.29 \pm 5.29$ a $51.75 \pm 9.94$ a	b
2020	INJ SP INJ SP INJ	N uptake (kg ha <sup>-1</sup> 32.45 $\pm$ 4.90 a 33.57 $\pm$ 6.19 a 45.84 $\pm$ 5.12 a 41.16 $\pm$ 9.18 a 42.28 $\pm$ 5.52 a	<sup>1</sup> ) a a	P uptake (kg h $4.17 \pm 0.54$ a $5.32 \pm 1.32$ a $7.53 \pm 0.95$ a $8.50 \pm 1.70$ a $4.90 \pm 0.76$ a	<b>a</b> <sup>-1</sup> ) b a	K uptake (kg ha $33.16 \pm 5.34$ a $38.24 \pm 8.61$ a $46.29 \pm 5.29$ a $51.75 \pm 9.94$ a $31.49 \pm 5.55$ a	b b

\*Means  $\pm$  standard error. INJ: inject manure, SP: spread manure. Means with the same letter are not significantly different (Tukey=0.05)

This indicates that the manure application method during the corn years does not affect the concentration of N and P in winter rye. Winter rye N concentration was much higher in 2022 (212.8 g kg<sup>-1</sup>) than in 2021 (139 g kg<sup>-1</sup>), and 2020 (152.6 g kg<sup>-1</sup>), in line with lower plant height and biomass yield in 2022, reflecting earlier harvest time in that year. Phosphorus concentration in winter rye was higher in 2022 (2.5 g kg<sup>-1</sup>) than in 2020 (2.2 g kg<sup>-1</sup>) but not in 2021 (2.5 g kg<sup>-1</sup>). Potassium concentration was not influenced by any of the year, treatment, and year-by-treatment interactions. Nitrogen removal was not influenced by year, treatment, or year-by-treatment interactions. Nitrogen removal was mainly influenced by shoot yield ( $R^2 = 0.53$ ; RMSE = 8.80; P value = 0.0001) than N concentration ( $R^2 = 0.07$ ; RMSE = 12.41; P value = 0.19). Unlike N, P, and K removals were influenced by year but not treatment or year-by-treatment interaction (Table 5). Phosphorus and K removals were higher in 2021 (8.05 g kg<sup>-1</sup> for P and 49.02 g kg<sup>-1</sup> for K) than in 2020 (4.74 g kg<sup>-1</sup> for P and 35.7 g kg<sup>-1</sup> for K) and 2022 (4.93 g kg<sup>-1</sup> for P and 31.05 g kg<sup>-1</sup> for K).

#### **3.6 Rye forage quality**

All forage quality parameters (CP, ADF, NDF, NDFD, NE<sub>M</sub>, NE<sub>G</sub>, NE<sub>L</sub>, lignin, IVTDMD, fat, TDN, and RFQ) for winter rye influenced by year but not treatment or year-by-treatment interaction (Supplemental Table 5; Table 6). Generally, forage quality parameters like CP, TDN, and RFQ were higher when winter rye was harvested earlier in 2022, reflecting the important role of the growth stage in getting high-quality winter rye. Both TDN and RFQ were the highest in 2022 (773.5 g kg<sup>-1</sup> for TDN and 209.1 g kg<sup>-1</sup> for RFQ) however, TDN was similar between 2020 and 20201, while RFQ was the lowest in 2020 (124.3 g kg<sup>-1</sup>) (Table 6). These

parameters, generally, have a negative relation with ADF, NDF, and lignin and a positive

relation with NDFD and energy parameters.

**Table 6.** Effect of year and manure application method rye crud protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), net energy for maintenance ( $NE_M$ ), net energy for gain ( $NE_G$ ), net energy for lactation ( $NE_L$ ), Lignin, in vitro dry matter digestibility (IVTDMD), Fat, Ash, total digestible nutrients (TDN), and relative forage quality (RFQ).

Year	Treatment	CP (g kg <sup>-1</sup> )		ADF (g kg <sup>-1</sup> )	ADF (g kg <sup>-1</sup> )		NDFD (g kg		
2020	INJ	$92.75 \pm 4.25 \ b^*$	h	344.0 ± 19.28 a		646.25 ± 21.28 a		$577.42 \pm 20.63$ a	h
2020	SP	$98.00\pm5.01\ b$	D	$334.5 \pm 20.51$ a	а	$648.00 \pm 23.55$ a	a	$575.72 \pm 22.83$ a	D
2021	INJ	$86.25 \pm 2.25 \text{ b}$	h	$329.0\pm4.00~a$		631.25 ± 7.85 a		$591.96 \pm 7.61$ a	h
2021	SP	$87.50\pm7.27~b$	D	$321.0 \pm 13.72$ a	а	$618.25 \pm 22.07$ a	a	604.56 ± 21.39 a	D
2022	INJ	130.5 ± 3.01 a	0	$223.5 \pm 7.28$ a	h	$462.00 \pm 10.70$ a	h	$760.00 \pm 7.07$ a	0
2022	SP	135.5 ± 3.47 a	a	218.5 ± 4.17 a	U	454.25 ± 9.31 a	U	782.5 ± 15.47 a	a
		NE <sub>M</sub> (Mcal kg <sup>-1</sup>	)	NE <sub>G</sub> (Mcal kg <sup>-1</sup>	)	NE <sub>L</sub> (Mcal kg <sup>-1</sup> )		Lignin (g kg <sup>-1</sup> )	
2020	INJ	$1.41 \pm 0.07$ a	L	$0.83 \pm 0.06 \text{ a}$	L	$1.43 \pm 0.05$ a	1.	39.17 ± 5.28 a	
2020	SP	$1.45 \pm 0.07$ a	D	$0.86 \pm 0.06$ a	D	$1.45 \pm 0.05$ a	D	35.15 ± 6.19 a	а
2021	INJ	$1.47 \pm 0.01 \text{ a}$	1.	$0.88 \pm 0.01$ a	1.	$1.47 \pm 0.01$ a	1.	$28.95 \pm 0.38$ a	1.
2021	SP	$1.50 \pm 0.05$ a	b	$0.91 \pm 0.04$ a	b	$1.49 \pm 0.03$ a	b	$26.20 \pm 1.46$ a	b
2022	INJ	$1.85 \pm 0.02$ a		$1.21 \pm 0.02$ a		$1.76 \pm 0.02$ a		$18.05 \pm 1.02$ a	
2022	SP	$1.86\pm0.01~a$	а	$1.23\pm0.01~a$	а	$1.78\pm0.01~a$	а	15.55 ± 2.12 a	с
		IVTDMD (g kg <sup>-1</sup>	<sup>1</sup> )	Fat (g kg <sup>-1</sup> )		TDN (g kg <sup>-1</sup> )		RFO	
	INJ	733.75 ± 22.62 a	/	42.40 ± 7.93 a		633.50 ± 22.00 a		118.75 ± 12.76 a	
2020	SP	743.75 ± 23.59 a	с	36.37 ± 8.50 a	b	644.00 ± 23.26 a	b	$130.00 \pm 13.57$ a	c
2021	INJ	767.00 ± 4.69 a		$26.57 \pm 2.06$ a		650.25 ± 4.75 a		$142.00 \pm 1.68$ a	
2021	SP	775.50 ± 12.35 a	b	$26.27 \pm 5.00$ a	b	659.25 ± 15.64 a	b	149.50 ± 7.39 a	b
2022	INJ	873.25 ± 3.96 a	0	36.82 ± 3.95 a	0	770.75 ± 8.39 a	0	$206.75 \pm 4.42$ a	c
2022	SP	$877.50 \pm 5.13$ a	а	$39.02 \pm 2.32$ a	а	776.25 ± 4.64 a	a	211.50 ± 3.79 a	a

\*Means  $\pm$  standard error. INJ: inject manure, SP: spread manure. Means with the same letter are not significantly different (Tukey=0.05).

# 3.7 Total forage yield, N, P, and K balances

Total forage yield (corn DM yield plus winter rye DM yield) was only influenced by year

and was similar between the two manure application methods (Supplemental Table 6; Fig. 4A).

Total forage yield was higher in 2020 (18.48 Mg ha<sup>-1</sup>) and 2022 (20.14 Mg ha<sup>-1</sup>) than 2021

(12.35 Mg ha<sup>-1</sup>) mainly due to low yields in corn in 2021 (Fig. 4B).



**Fig. 4.** Effect of year and manure application method on total yield (sum of corn and rye DM yield) in different years. The bars indicated as standard error. INJ: inject manure, SP: spread manure. Year comparison means with the same letter are not significantly different (Tukey=0.05).

# Table 7.

Effect of year and manure application method on total N, P, and K removal and balance (Corn plus Rye) in different years.

Year	Treatment	N removal (kg ha <sup>-1</sup> )		P removal (kg h	a <sup>-1</sup> )	K removal (kg ha <sup>-1</sup> )	
2019-	INJ	218.9 ± 37.69 abc*	h	$44.05 \pm 5.35$ abc	h	180.81 ± 13.84 bc	h
2020	SP	$250.44 \pm 9.08 \text{ ab}$	U	$45.92 \pm 1.50$ ab	U	214.63 ± 10.97 ab	U
2020-	INJ	$139.06 \pm 18.42$ c	0	$29.03 \pm 3.12$ c	0	$124.41 \pm 14.20 \text{ c}$	0
2021	SP	$171.22 \pm 17.44$ bc	C	38.31 ± 2.79 bc	C	$150.75 \pm 9.58 \ bc$	C
2021-	INJ	$288.48 \pm 20.88$ a	я	55.27 ± 3.13 a	я	274.54 ± 18.31 a	я
2022	SP	$293.08 \pm 14.12 \text{ a}$	u	$54.43 \pm 3.29$ a	u	$257.25 \pm 14.05 \; a$	u
			1		1		1
		N balance (kg ha	<sup>-1</sup> )	P balance (kg h	a <sup>-1</sup> )	K balance (kg ha	<sup>-1</sup> )
2019-	INJ	-17.19 ± 37.69 ab	h	$-5.25 \pm 5.35$ bc	h	151.87 ± 13.84 a	9
2020	SP	$-48.74 \pm 9.08$ bc	U	-7.11 ± 1.5 bc	U	$118.05 \pm 10.97$ a	a
2020-	INJ	62.63 ± 18.42 a		14.13 ± 3.12 a	0	$129.54 \pm 14.20$ a	h
2021	SP	30.48 ± 17.44 abc	a	$4.86 \pm 2.79 \text{ ab}$	a	$103.20 \pm 9.58$ a	U
2021-	INJ	$-86.78 \pm 20.88$ c	0	-14 ± 3.13 c	0	-63.32 ± 18.31 b	0
2022	SP	-91.37 + 14.12 c	С	-13.16 + 3.29 c	С	-46.03 + 14.05 b	C

\*Means  $\pm$  standard error. INJ: inject manure, SP: spread manure. Means with the same letter are not significantly different (Tukey=0.05).

Total N removal, P and K removals were all only affected by year and were similar between INJ and SP indicating total forage yield drives the N, P, and K removals. Nitrogen balances were negative in two years (2019-2020 and 2021-2022) except for 2020-2021, which still had a positive N balance even by integrating winter rye into corn for the silage system. Total P and K removals were influenced by year and year by treatment interactions (Supplemental Table 6; Table 7).

#### 3.8 Soil phosphorus levels

Bray-1 STP concentrations were 78.5 mg kg<sup>-1</sup> for INJ and 76.7 mg kg<sup>-1</sup> for SP in spring 2019. After three years of P-based rate manure management, in spring 2022, STP levels remained unchanged. INJ had an STP level of 79.0 mg kg<sup>-1</sup> while SP had an STP level of 78.5 mg kg<sup>-1</sup> (Fig. 5), suggesting that P-based manure management in combination with winter rye as the double crop can maintain STP over a three-yr period regardless of application method.



**Fig. 5.** Effect of year and manure application method Bray-1 extractable P in different years. The bars indicated as standard error. INJ: inject manure, SP: spread manure. Year comparison means with the same letter are not significantly different (Tukey=0.05).

#### 3.9 Principal component analysis (PCA)

For corn, the first two ordination axes were responsible for 70.6% of the variability in the factors that affected corn silage yield (Fig 6A). The principal component 1 (PC1), had higher correlation with corn yield and N availability (CSNT, PSNT, CP), and nutrient removals and less with silage quality parameters including ADF, NDF, and K, P, and Ca concentrations.



The principal component 2 (PC2) represented silage quality parameters better. There was a strong and positive correlation between RFQ, TDN, and NEG which were negatively correlated with corn yield suggesting in this study, higher yields were associated with lower quality. For winter rye, the first two ordination axes were responsible for 73.5% of the variability in the factors that affected winter rye forage yield (Fig. 6B). The principal component 1 (PC1) which explained 53.3% of the variation, had higher correlation with forage quality of winter rye (RFQ) which was related to fat, winter rye aboveground N concentration, (similar to CP), and SPAD reading. The principal component 2 (PC2), which explained 20.2% of the variation, represented winter rye above- and belowground biomass and their association with plant height, and nutrient removals and to a lesser extend factors of low forage quality such as ADF, lignin, lignin:N ratio, and C:N ratio.

# 4. Discussion

The first objective of this study was to compare the effects of INJ versus SP manure application methods on silage corn yield and quality as well as nutrient uptake and balances. Corn silage yield was similar between INJ and SP treatments. This similar yield was achieved while the INJ treatment received 106 kg ha<sup>-1</sup> less synthetic N fertilizer compared to SP treatment. This reflects the importance of injecting manure to save the ammonium-N from being lost and thus, increasing the value of manure [12,17,38]. From the environmental perspective, the reduction of ammonia volatilization is essential for mitigating air pollution and its associated impacts. In the current study, manure was applied plus supplemental N to meet corn crop N requirement. If manure was applied at P-based (P removal) rates without supplemented N fertilizer, then we expected to observe differences between the two treatments with INJ producing higher yields than the SP treatment [1,8]. Similar to our results, Maguire et al. [14] showed that when manure at a P-based rate is supplemented with N fertilizer, all treatments, including N- and P-based rates, had similar corn yields across the various sites and years. This was also observed in a recent study by Sadeghpour et al. [4] in which when supplemental N was added to P-based manure or compost plots, similar corn yields to those of N-based manure or UAN fertilizer were recorded. The PCA indicated that corn silage yield was associated with the CSNT [correlation coefficient (r) = 0.59], PSNT (r = 0.34), and CP (r = 0.30) indicating N availability explains the corn silage yield variation (Supplemental Table 7; Fig. 6A). In our study, lack of CSNT, PSNT, and CP differences between the two manure application methods was mainly related to similar N input in INJ and SP treatments [1,3]. Differences in N availability in this study were mainly driven by year-to-year differences, particularly because of weather differences [1,8,12, 41, 42, 43 44]. Corn N, P, and K removals were mainly driven by

corn silage yield in which the correlations for N, P, and K removals were 0.86, 0.84, and 0.87, respectively, with the PCA showing no differences between the two manure application methods (Supplemental Table 7; Fig. 6A). Nitrogen, P, and K balances were also similar between the two application methods and followed yield patterns [4,12,45, 46, 47]. Corn silage quality was also similar across treatments and similar to other studies [5,12,48, 49, 52]; while application rates can affect CP [4,20]; when corn N requirements are met, differences in CP [50,51] are seldom observed. Also, literature often agrees that manure and fertilizer management practices do not influence ADF, NDF, NE<sub>M</sub>, NE<sub>G</sub>, NEL, TDN, and RFV [1,12]. Previous literature had shown that manure application can increase corn silage quality parameters such as RFV [53,54], however such changes in silage quality in Midwest US are not reported. The results of the PCA indicated that higher corn silage yields were negatively correlated with TDN (r = -0.55) and RFV (r=-0.61) which are indicators of high silage quality (Supplemental Table 7; Fig. 6A). This could be explained by higher fiber concentrations (ADF and NDF) in corn at higher yields.

Our second objective was to compare the effects of INJ versus SP manure application methods on the following winter rye above- and belowground biomass, nutrient uptake, and balances, as well as its quality. Winter rye aboveground biomass was similar between INJ and SP methods indicating similar residual N after harvesting corn for silage and thus, similar winter rye growth. Winter rye aboveground and belowground biomass was significantly correlated with NDVI (r = 0.53; average for both above- and belowground) and plant height (r = 0.81; average for both above- and belowground) (Supplemental Table 8, Fig. 6B). There was a strong positive correlation between winter rye above- and belowground biomass (r = 0.96) suggesting a potential for developing prediction models to estimate belowground biomass from aboveground data for winter rye (Supplemental Table 8; Fig. 6B). The ratio of above- to belowground biomass

(root:shoot ratio) across treatments and years were similar. The root:shoot ratios in this study are similar to reported values by others [55, 56]. These results show implications for improving performance of soil erosion models, including the revised universal soil loss equation (RUSLE) and the water erosion prediction project (WEPP), which would further our goals of building management plans to reduce resource losses [55].

Similar to corn for silage, N, P, and K removals were similar between the two treatments and were highly correlated with the winter rye aboveground biomass (0.73 for N, 0.97 for P, and 0.97 for K) (Supplemental Table 8; Fig. 6B). These data also indicate that estimating crop uptake through aboveground biomass of winter rye could be accurate and urging for developing models for nutrient uptake estimations. Quality of winter rye (RFQ) was negatively correlated with its aboveground biomass (r = -0.40) and several factors that impact RFQ including ADF, NDF, and lignin concentration. The RFQ for winter rye was positively associated with N concentration in the plant (r= 0.88) and fat concentration (r = 0.93) (Supplemental Table 8; Fig. 6B). These associations could be explained by differences among years and in general, in years that winter rye was harvested earlier (2021), it produced higher quality forage but yielded less.

Our third and most important objective was to evaluate whether STP can be maintained by adding manure with INJ or SP method to corn in rotation with winter rye. We observed that INJ had a cumulative P balance of -5.1, while SP had a cumulative P balance of -15.4 kg ha<sup>-1</sup>. Based on the Illinois Agronomy Handbook, this should have resulted in a 1.2 and 3.5 unit STP reduction in INJ and SP by spring 2022. However, we observed 0.5 and 1.8 unit increase in STP, which could be due to high initial STP levels and/or buildup of STP on surface soil compared to injection bands (Fig. 5). Our results are different from those of Sadeghpour et al. [4,5], who reported when P balance was negative, a reduction in STP was observed and when it was

26

positive, STP was linearly increased with P balance increase. We found that STP levels did not significantly change, despite negative P balances in both manure applications (Fig. 5). We believe this is due to these soils being of high P-supplying power and perhaps organic P release. Our findings are significant in that this study was using both a reduced manure rate (P-based rate) and an integrated winter crop to take up excess nutrients yet yielded only maintenance of STP in this system. Our results emphasize the importance of P-based rate manure management regardless of method of application as a practice that, if coupled with the intensification of the corn system with winter rye, could sustainably manage STP while providing homegrown forage for dairy farmers.

#### **5.** Conclusions

Based on our findings, at high P soils, corn growers can shift from surface application of liquid manure to subsurface injection at the P-based rates and supplement the limited N with a much smaller amount of N fertilizer to ensure optimum crop production. In this study, switching from surface application of manure at a P-based rate to injection resulted in saving 106 kg N ha<sup>-1</sup>. This was equivalent to \$150 ha<sup>-1,</sup> indicating that injection comes with a great reduction in fertilizer cost. This saving is high enough to cover the additional cost of manure injecting compared to surface application. Similar corn silage yield, quality, winter rye yield, and quality in rotation, along with similar changes in STP, all indicated that implementing manure injection can help growers reduce the use of N fertilizer, minimize ammonia volatilization, and reduce odor issues associated with surface application of manure. We concluded that by P-based manure management, both surface application and injection of manure in rotation with winter rye can

sustain STP sustainably. Still, the injection could reduce the potential for increasing the value of manure and reducing N fertilizer use.

#### 6. Future Research

The work in this honors thesis was a portion of a larger study which studied 10 treatments. 1) control: 180 lb/acre sidedress N (UAN) (150 lbs N/acre + 30 lbs N/acre as starter); 2) Injected P-based manure at 12,900 gal/acre with 15 lbs N/acre N fertilizer + rye (INJPCC); 3) Injected P-based manure at 12,900 gal/acre; 5) Surface N fertilizer (INJPNOCC); 4) Injected P-based manure at 12,900 gal/acre; 5) Surface applied P-based manure at 12,900 gal/acre with 110 lbs N/acre N fertilizer + rye; 6) Surface applied P-based manure at 12,900 gal/acre with 110 lbs N/acre N fertilizer; 7) Injected N-based manure at 14,000 gal/acre + rye; 8) Injected N-based manure at 14,000 gal/acre; 9) Injected N-based manure at 16,500 gal/acre + rye (INJNCC); 10) Injected N-based manure at 16,500 gal/acre (INJNNOCC). (Fig 7)



# Road Application to Corn (May)

Fig. 7. Map of full project and treatments.

The preliminary results are included below. The whole scope of this study was to evaluate the impact of organic fertilizer (manure application) as compared to inorganic (UAN control) on soil

health, crop yield and quality and carbon; evaluate the addition of a cover crop (rye double crop) on soil health, carbon, crop yield and quality, and economics; the impact of manure rates and incorporation of

cover crops on STP and crop yield and





quality; and the impact of manure application on crop yield and quality and on STP. From the work completed in this honors thesis, the application type doesn't affect yield and quality. Economic analyses and full nutrient cycling should be done to evaluate any additional impacts; however, these results show, and injected manure can be more economical and environmental as compared to surface application. How do rates affect the system? We hypothesized that like manure application, rate would not affect corn or rye yield (Fig 8&9) or quality (Fig 10). For double crops, only rye roots would be contributing to carbon accumulation.



Fig. 9. Preliminary results for effect of manure rate management on rye yield and accumulation.



**Figure 10.** Preliminary results of the effect of manure and fertilizer management on corn N concentration (A) and corn stalk nitrate test (CSNT; mg kg<sup>-1</sup>) over three years (B). Data are averaged over three years. We hypothesized that the P-based manure management with rye would account for the best STP management and yield similar results as a N-based manure management in regard to soil health, yield, and carbon accumulation (Fig 11). While N-based manure would add additional carbon, we hypothesized this effect would not be significant and there could be environmental impacts from the increased STP in N-based management (Fig 12).



**Figure 11.** Preliminary results of the effect of manure and fertilizer management on particulate (A) and light (B) soil organic matter fractions.







When considering a manure management system, the addition of rye increases organic matter (Fig 11), as well as increasing the potential to build carbon and improve aggregate stability (data not shown). The rate of manure does not significantly affect the increase in organic matter nor accumulation of rye dry matter, and so, it seems at these rates, P-based vs. N-based, do not affect the potential benefits on soil health. However, STP in N-based manure application is significantly higher than in P-based manure application, and so, could potentially increase the negative environmental impacts. Thus, a P-based, injected manure application, with the incorporation of a cover crops such as WCR seems to be a way to achieve soil health benefits; remove nutrient losses through volatilization, runoff, and leaching; help maintain STP; and maintain corn and rye yield and quality. Our future work will be to analyze the soil health parameters in the study under injected manure and analyze carbon cycling and contribution. Finally, additional research would be to analyze greenhouse gas emissions through manure and cover crop management (N<sub>2</sub>O, CO<sub>2</sub>, and CH<sub>4</sub>), determine nutrient loss pathways, study different systems and cover crops to increase carbon input, and continue building more sustainable practices which provide ecosystem services while remaining economical to local producers and suppling food and resources to an increasing population.

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