MANAGING THE NITROGEN TRADE-OFFS OF WINTER RYE AS A COVER CROP

By

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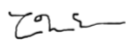
SILVA, ERIN M			*12/5/2022 6:52:24 PM*
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Abstract

Winter rye (*Secal cereale* L.) is a commonly used cover crop in Wisconsin due to its effectiveness in reducing soil erosion, scavenging nitrogen, and improving soil health. However, the potential trade-offs include limited nitrogen (N) availability from manure for the following crop. Developing an understanding of the impact of rye residue on plant available N will lead to an increase in widespread cover crop adoption and conservation benefits to dairy-based systems. This study aims to determine the single year effect of rye seeding rate on rye biomass and optimum nitrogen rate of the subsequent corn (*Zea mays* L.). Rye cover crop was planted in fall at five seeding rates (0, 34, 67, 101, 135 kg ha^{-1}) following corn silage harvest and liquid dairy manure application. Corn was planted following chemical termination of rye and fertilized with eight nitrogen rates (0-360 kg-N ha^{-1}). Rye shoot biomass accumulation ranged from 1.2-2.2 Mg dry matter (DM) ha^{-1} and root biomass 0-8-1.9 Mg DM ha^{-1} with a carbon to nitrogen ratio ranging from 10-13 in the shoot biomass and 22-29 in the root. Following rye, soil nitrogen decreased from fall to spring due to N uptake into the rye biomass, but in-season reductions in soil N through microbial immobilization during residue decomposition did not occur. In all but one case, maximum corn yield following rye was greater or equal to max yield following no cover (12.3-13.5 Mg ha^{-1}) when nitrogen fertilizer was applied. Optimum nitrogen fertilizer rates were inconsistent from year to year, with an additional 50-100 kg-N ha^{-1} fertilizer needed to reach optimum yield when corn was following rye compared to no cover in year 1, but no additional N fertilizer needed in year 2. Knowing how to accurately adjust nitrogen fertilization after a cover crop is critical to ensure optimum corn yield while still gaining the conservation benefits of winter rye.

Introduction

Corn silage is integral to dairy production systems and is grown annually on 10% (36,000 ha) of Wisconsin cropland (USDA NASS, 2022). However, corn silage production also poses environmental concern due to high nitrogen fertilizer requirements and lack of residue on the field post-harvest. The fall application of manure following silage harvest is a common practice in dairy systems, leaving temporal distance between nutrient application and plant uptake the following spring. The timing of this manure application often occurs out of necessity as storage space for manure is limited, and corn silage harvest provides a field available for application. Manure can provide soil health and agronomic benefits (Rayne & Aula, 2020), but manure application in this way poses a threat to the environment through runoff and nitrate leaching (Krueger et al., 2012). Nitrate lost from agricultural lands in the Midwest can enter the Mississppi River Basin and contribute to hypoxia in the Gulf of Mexico or contaminate groundwater locally (Kladivko et al., 2014; Krueger et al., 2013).

Cover crops are a common conservation practice that can address this issue by protecting the soil from erosion and preventing nutrient loss (Fiorini et al., 2020). While many species can make a suitable cover crop, winter rye is a good fit for northern climates due to its ability to establish quickly in the fall and survive the winter (Kaspar & Singer, 2015; Malone et al., 2014; Strock et al., 2004). Winter rye is effective at scavenging soil nitrogen and can ultimately lead to decreases in nitrate leaching and a more sustainable production system (Ketterings et al., 2015; Thelen & Leep, 2002; Tonitto et al., 2006). When used in a no-till system, cover crops can also increase soil health by increasing soil organic carbon (Sharma et al., 2018). In Wisconsin

specifically, seven years of cover crop use in a corn silage rotation was previously found to lead to a soil organic carbon increase of 13% (West et al., 2020).

Despite the benefits of winter rye as a cover crop, adoption is limited due to potential risk of yield decline to the following corn crop (Singer et al., 2007). Declines in corn grain yield have been observed following a rye cover crop, but this effect is inconsistent and dependent on many management factors. Decline in corn yield following grass cover crops can sometimes be due to allelopathic effects of rye (Dhima et al., 2006) or delayed planting of the cash crop due to cover crop termination timing in spring. The nitrogen effect due to N uptake in rye biomass or microbial immobilization during decomposition is a main driver of yield declines due to soil N pools failing to meet corn N requirements. In Iowa, there was a 6% decrease in corn yield following rye cover crop at the economic optimum nitrogen rate (Martinez-Feria et al., 2016; Pantoja et al., 2015). However, results from other work suggest that decreased cash crop yield after rye does not always occur, especially after long-term cover crop use (Thapa et al., 2022). The risk of yield decline is higher in the first years of rye adoption, as benefits to nutrient cycling and improved soil quality may not occur until years after adoption of the practice. The variability within these results demonstrate the need for research to better understand how rye can be managed to avoid these yield declines. Even though benefits of cover crops may not be accrued until several years after adoption, these nuances occurring in the initial years of adoption must be understood in order to avoid any negative effects. Addressing ways to overcome these hurdles will help to increase adoption of the practice on the landscape and alleviate environmental concerns in corn production systems.

Proper management of rye biomass accumulated in the spring is one way to avoid potential corn yield decline following a cover crop. Excessive rye biomass growth depletes soil N pools through both nitrogen uptake into the biomass and nitrogen immobilization by soil microorganisms as rye residue decomposes during the growing season. Large biomass accrual leads to greater carbon to nitrogen C to N ratios (C:N) of below- and above-ground rye residue at the time of termination (Pantoja et al., 2016). Rye biomass accumulation can be managed by adjusting planting or spring termination timing, but these field activities are weather dependent and not always feasible for producers. Adjusting cover crop seeding rate gives producers the opportunity to influence rye biomass without any additional management steps. However, little research has been conducted specifically looking at the seeding rate of single species cover crops, and no research has investigated rye seeding rate in a corn rotation. Thus, it is important to evaluate the short-term nitrogen dynamics associated with rye growth and decomposition to better understand the causes and effects of these processes and support long term adoption of the practice.

Understanding above- and below-ground residue decomposition is key to gain insight into nutrient cycling in a corn production system. This decomposition can be difficult to predict and measure due to the variation in environmental factors that heavily influence residue breakdown (Varco et al., 1993). A multi-state study in the U.S. shows that residue decomposition is positively correlated with cumulative rainfall, number of rainy days, and mean daily air relative humidity (Thapa et al., 2022). Previous work hypothesizes that in terms of residue quality, C:N and lignin content are the drivers of rye decomposition rates (Ibewiro et al., 2000; Lupwayi et al., 2004). However, other work suggests C:N has greater influence on initial biomass decomposition

than lignin, with lower C:N leading to faster residue decomposition and nitrogen release (Lawson et al., 2013; Lindsey et al., 2013, Ruffo & Bollero, 2003; Sievers & Cook, 2018). The rate and timing of nitrogen release through rye residue decomposition is not yet fully understood, and more work must be done to predict and synchronize N release from cover crops to periods of high N demand of corn.

While biomass amount and residue composition are important factors in rye decomposition, soil characteristics such as biological activity also play a role in nutrient return. Having a growing cover crop protecting the soil instead of a period of fallow could provide legacy effects on soil biota that increase the decomposition potential after rye termination (Barel et al., 2019). A global meta-analysis of 60 studies focused on cover crops found a significant increase in microbial abundance in cover crop systems compared to those without (Kim et al., 2020). While variation occurs within the management practices of the systems, this analysis revealed that cover crops increased microbial activity by 22%. Soil enzyme activity is one biological measure which can give insight to soil functions and metabolic activity (Stott et al., 2010). Enzymes mediate and catalyze nutrient cycling processes within a soil and can be responsive to early changes in soil management (Dick et al., 2015). Beta glucosidase (BG) is an enzyme that acts as a proxy for microbially facilitated carbon cycling and is active during the initial stages of residue decomposition. Activity of BG can serve as an indicator for the soil's ability to breakdown plant residues. The enzyme urease converts urea to ammonia and can provide insight to N cycling in the soil. Previous work conducted in Indiana found that rye cover crop stimulates BG activity and suppresses urease activity and ammonification (Nevins et al., 2020). When measured throughout

the growing season, urease and BG can give insight to soil biological functioning and nitrogen cycling as rye residue decomposes.

The relationship between rye seeding rate and corn optimum N rate is not well documented in the North Central Region, especially in dairy production systems. The objectives of this study were i) to determine how the seeding rate of winter rye affects the quantity and quality of rye biomass, ii) to determine the effect of rye biomass on soil nitrogen pools, and iii) to determine the effect of rye cover crop biomass on subsequent corn yield and optimum nitrogen rate. We hypothesize that winter rye biomass will increase incrementally as seeding rate increases, but will have a lower nitrogen content at high seeding rates. We expect that when large amounts of rye cover crop biomass are accumulated (>2,200 kg ha^{-1}) soil nitrogen pools will be depleted and optimum corn nitrogen fertilizer rate will increase. To further answer questions regarding the influence of rye on soil biology, a secondary objective was added to the study in the second year to determine if rye cover crop and/or nitrogen fertilization influences microbial activity and residue decomposition. We hypothesize that enzyme activity and residue decomposition will be greater following rye compared to fallow, and the addition of nitrogen fertilizer will further increase these soil biological processes.

Materials and Methods

Field description and design

The two year field study was conducted at University of Wisconsin Arlington Research Station (43°18'9.47"N, 89° 20'43.32"W) from 2020-2022. Each year of the study was conducted at a different field site located within 5 km of one another. The first field was used from 2020-2021 (year 1) and the second used from 2021-2022 (year 2). Field soils were a Plano silt loam (fine-silty, mixed, superactive, Mesic Typic Argiuodoll). Both fields are non-tillage with a field history of alfalfa grown for three years prior to one year of corn silage. The study site has a mean annual temperature of 7.55 ºC and a mean annual precipitation of 93.7 cm (National Climate Data Center). Accumulated growing degree days were similar in both years (Figure 1).

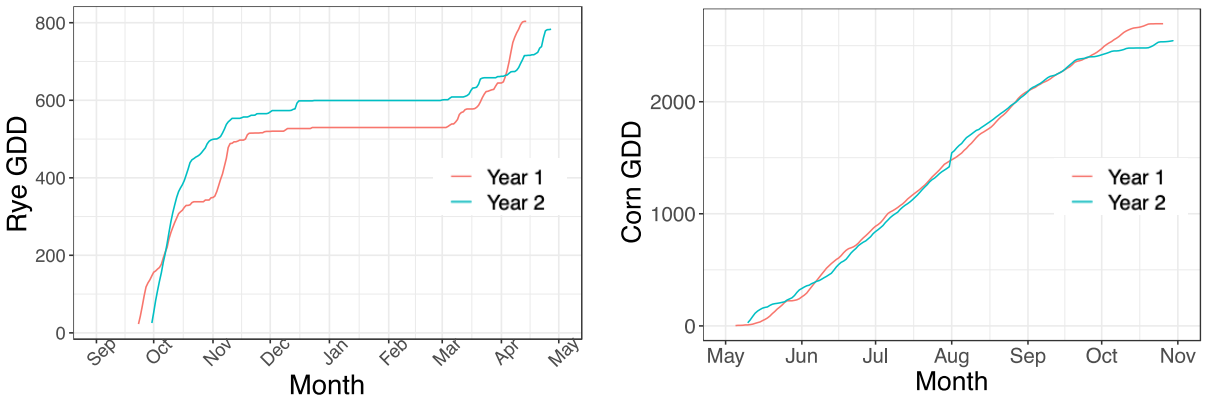

Figure 1 Accumulated growing degree days (GDDs) for both site years from rye planting to termination and corn planting to harvest. GDDs were calculated with temperature data from MSU Enviroweather using $GDD = \frac{T_{max} + T_{min}}{2} - T_{base}$ where T_{base}=4 ºC for rye and 10 ºC for corn.

The experimental design was randomized complete block split-plot replicated five times. Whole block treatments were rye seeding rates of 0, 34, 67, 101, 135 kg ha^{-1} (4.6 m x 97.5 m, six corn rows wide). Split plot treatments were nitrogen fertilizer application rates of 0, 45, 90, 135, 180, 225, 270, 360 kg-N ha^{-1} (4.6 m x 12.2 m).

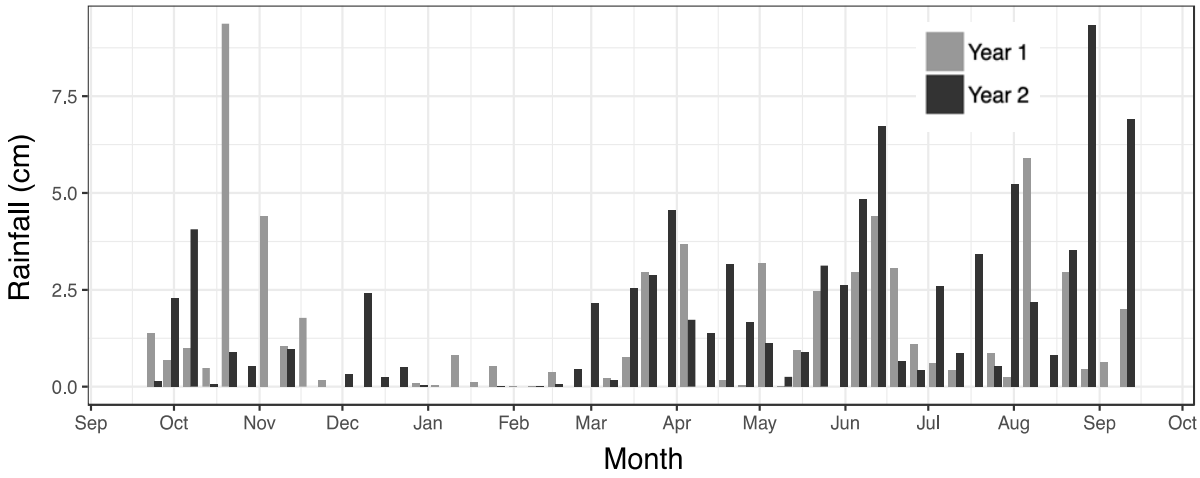

Figure 2 Weekly accumulated precipitation (cm) for Arlington, WI year 1 (2020-2021) and year 2 (2021-2022) from time of manure application through the corn growing season. Rainfall data was gathered through MSU Enviroweather.

Routine soil analysis was conducted in the fall prior to manure application to indicate any inherent field differences (Table 1). The analysis for years 1 and 2 indicated SOM of 3.6% and 3.4%, pH (1:1 water) of 7.06 and 7.0, total soil P of 75.8 and 130 ppm, and total K of 120.4 and 118 ppm, respectively. Separated liquid dairy manure was surface applied at a rate of 93,490 L ha^{-1} in the fall after corn silage harvest but before planting of rye (Table 1). The manure analysis for year 1 and 2 indicated 1.3% and 1.8% dry matter with total nutrient application of 36.48 and 36.40 kg ha^{-1} phosphorus, 146.94 and 155.23 kg ha^{-1} potassium, 6.61 and 9.74 kg ha^{-1} sulfur, and total N applied of 102.70 and 138.9 kg ha^{-1} with an estimated nitrogen credit of 22 and 37.18 kg ha^{-1}, respectively.

Winter rye was drill seeded in 19 cm rows at seeding rates of 0, 34, 67, 101, and 135 kg ha^{-1} with a 4.57 m grain drill in early fall after corn silage harvest (Table 1). Rye was chemically terminated in spring with Glyphosate and 2, 4-D. Corn was planted in May each year in 76 cm rows with starter fertilizer application of 20 kg-N ha^{-1} at time of planting. Pre-emergent herbicides (Sharpen, Medal II EC) were applied after corn planting. Corn was fertilized at growth

stage V3 with Urea coated with a urease inhibitor (Agrotain®) surface applied at rates of 0, 45, 90, 135, 180, 225, 270, 360 kg-N ha^{-1}.

Table 1 Field activity timeline for year 1 (2020, 2021) and year 2 (2021, 2022).

Field Activity	Year 1	Year 2
	2020	*2021*
Corn silage harvest	Sep 16	Sep 20
Routine soil sample	Sep 17	Sep 22
Manure application	Sep 18	Sep 24
Rye planted	Sep 23	Sep 30
Rye shoot biomass sample	Oct 30	Nov 1
Fall soil sample	Nov 5	Nov 5
	2021	*2022*
Rye root biomass sample	---	Apr 20
Spring soil sample	Apr 19	Apr 26
Rye shoot biomass sample	Apr 14	Apr 27
Rye terminated	Apr 18	Apr 28
Corn planted	May 5	May 10
Nitrogen fertilizer applied	Jun 1	Jun 1
In-season soil sample	July 10	July 1
Corn grain harvest	Oct 26	Oct 31

Soil and biomass sampling and analysis

Soil was sampled by collecting cores from a random distribution per plot using a 2.5 cm diameter probe. Cores were homogenized during sampling to create a composite bulk sample for each plot. Routine soil was collected prior to any field activity for the study as a composite of 10 soil cores per block at a depth of 0-15 cm. Samples were sent to UW Soil and Forage Analysis Lab for routine pH, Bray-1 P, Bray-1 K, and SOM-loss on ignition. Additional soil sampling occurred at three times throughout the growing season: in fall before the first heavy frost, spring at time of rye termination, and in-season during peak of corn growing season (Table 1). Fall and spring samples were composites of 10 sub-samples per treatment at depths of 0-30 cm and 30-

60 cm. In-season samples were collected as composites of 5 sub-samples per plot at 4 different depths, 0-30 cm, 30-60 cm, 0-15 cm and 15-30 cm. Samples were only collected in only plots with fertilizer application of 0 and 180 kg-N ha^{-1} for a total of 200 samples. All samples were force air dried at for at least 7 d and ground to pass through a 1 mm sieve. All samples at depths of 0-30 cm and 30-60 cm were sent to the UW Soil and Forage Analysis Lab for nitrate and ammonium analysis.

In-season samples at depths of 0-15 and 15-30 cm were analyzed for potentially mineralizable nitrogen (PMN) in-house at UW-Madison using the Drinkwater protocol for anaerobic PMN (Dick et al., 2015). A 7-d anaerobic biological incubation was conducted and PMN was calculated from the difference of quantity of ammonium in the non-incubated samples and the incubated samples. For the incubated samples (2 replicates), 10 mL of deionized water was added to 5 mg of dried and ground soil, and then incubated for 7 d at 40 °C. After incubation, ammonium was immediately extracted by adding 40 mL of 2.5 M KCl to the sample, shaking for 1 h, centrifuged, and filtered. The non-incubated samples were analyzed in the same way, except the water and 2.5 M KCl were added to the sample just prior to shaking without any incubation. The filtered supernatant was stored in sealed vials at -18 °C and was sent frozen to UW Soil and Forage Analysis Lab for quantification of ammonium.

Rye shoot biomass was sampled in fall before the first hard frost and in spring four days before termination. Biomass from two 0.25 m^2 quadrats (3 rye rows) from each plot were clipped at ground level, bagged, and force air dried at 65 ° C for at least 7 d. Dried biomass was weighed, ground, and milled to a fine powder. Between 4-5 mg of milled biomass was packed

into a 6 x 3 mm tin capsule for total carbon and nitrogen analysis using Flash EA 1112CN Automatic Elemental Analyzer.

Root biomass was sampled in spring of year 2 only at the time of rye termination. A truck mounted hydraulic Giddings soil probe was used to collect 10 cm diameter cores to a depth of 60 cm. Two cores were collected from each seeding rate treatment, one core centered in the rye row and one directly between rows. Cores were stored in plastic liners (Giddings #ZC-246), capped, and stored at 4 ºC until processing. At processing, each core was split into 0-5 cm, 5-20 cm, 20-35 cm, 35-50 cm fractions. Each section was placed in a tote with enough water to cover the soil and a drop of Liquinox^{TM} dish detergent to assist in dispersing soil aggregates. The soil was gently broken up by hand and left to sit in the solution for at least 15 minutes. The solution was then poured through a 2 mm and 0.5 mm nested sieve to capture all roots. Sieves were rinsed with deionized water, and any material that did not pass through the sieves was combined into a plastic container with deionized water. Roots were then separated from other soil organic matter using tweezers. Separated roots were placed on a 0.5 mm sieve and gently rinsed with DI water to remove any soap or soil residue, and placed on a Petri dish to air dry for 72 hrs. This process was repeated on each depth section of each core. Samples were then weighed and milled to a fine powder for C and N analysis as for shoot biomass.

Enzyme activity and rye decomposition measurement

Soil for enzyme activity analysis was collected in year 2 from only 4 plots within the study: seeding rates of 0 and 67 kg ha^{-1} with fertilizer rates of 0 and 180 kg ha^{-1}. Soil was collected at the time of termination and 2, 4, 6, 10, and 14 weeks after termination. Before fertilizer was

applied at week 6, soil samples were collected as a composite sample of 10 cores to a depth of 10 cm from the seeding rate treatments only. After fertilization, samples were collected as a composite of 5 cores from each rye seeding rate and fertilizer combination to a depth of 10 cm. Samples were transferred to the laboratory in coolers and fresh sieved through a 2 mm sieve. For each sample, 2 g of soil was weighed into a tin dish and dried at 105 ºC for 3 hrs to determine moisture content. Soil was stored at 4 ºC and analyzed at field moisture within 48 hrs of sampling.

Potential soil β-glucosidase activity (nmol para-nitrophenol released g dry soil^{-1} hr^{-1}) was quantified using a high-throughput fluorometric method (Bell et al., 2013). Briefly, 1 g dry soil equivalent was combined with 100 mL water and mixed using a stir plate for 15 mins to create a soil slurry. The enzyme assay was set up in 96-well microplates where 9 rows were filled with 200 µg soil slurry and the remaining rows filled with 200 µg Nanopure water (Thermo Scientific, Barnstead Nanopure). Three rows of each sample slurry were filled with an additional 50 µg of water, 3 rows with 50 µg substrate (4-methylumbelliferone), and 3 with the standard (4-MUB-b-D-glucoside). A subset of control wells received the same treatments. The assay plate was incubated in the dark at 25 ºC for 4 hours. Enzyme activities were corrected using a quench control. Fluorescence was measured using a microplate fluorometer at 365 nm excitation and 450 nm emission filters.

Potential soil urease activity (µg NH_4-N g dry soil^{-1} 2 hr^{-1}) was quantified according to Kandeler and Gerber (1988). Duplicates were performed for each sample, as well a control. Briefly, 5 g dry soil equivalent was added to 2.5 mL urea (0.08M) and incubated at 37 °C for 2 h. After incubation, 50 mL of 2 M potassium chloride was added to the samples and placed on a

rotary shaker for 30 mins. Control samples did not incubate, and urea and potassium chloride were added immediately before shaking. Soil suspensions were filtered following shaking and the NH_4^+-N released during incubation was determined by combining a 1 mL subsample of the supernatant and 9 mL of Nanopure water. A standard curve was prepared by mixing 0, 0.5, 1, 2, 4, 6, 8, 10 mL of 200 uM ammonium-N solution with the appropriate amount of 2 M potassium chloride to reach a final volume of 10 mL. Ammonium was analyzed by loading 20 µg sample extracts or standards into 96-well plate. Color began to develop after adding 100 µL of salicylate cocktail and 100 µL of hypochlorite. After 45 minutes at room temperature, absorbance was read at 650 nm on a microplate spectrophotometer.

Rye biomass decomposition was measured in the second year of the study only. Five days after rye termination (at time of chlorosis), two 0.36 m^2 quadrats of rye were clipped at ground level from each treatment of 67 kg ha^{-1} seeding rate and force-air dried 65 ° C for three days. All rye was combined to create one composite biomass sample. Litter bags were created by sewing together two 20x20 cm panels of plastic mesh material with 1 mm openings, leaving a 10 cm gap on one side for adding the rye. Exactly 10 g of air-dried biomass from the composite sample was placed into each litter bag, and the 10 cm opening was closed using excess wire from the plastic identification tag. Litter bags were installed in the same subset of plots in the study as for the enzyme analyses: seeding rates of 0 and 67 kg ha^{-1} with fertilizer rates of 0 and 180 kg ha^{-1}. Bags installed in the 67 kg ha^{-1} rye plots were placed where the rye shoot biomass was clipped. Six litter bags were placed in each plot and fastened to the soil surface using lawn staples. Any debris was removed from the soil to ensure maximum soil to bag contact. Litter bags were removed at 0, 2, 4, 6, 10, and 14 weeks after bag installment. Litter bags were removed from the

field during any field activity throughout the season but immediately replaced. After collection, soil was carefully removed from the exterior of the bag and remaining rye was dried and weighed.

Statistical analysis

All statistical analysis was conducted with RStudio version 2021.9.0.351 using R statistical software version 4.1.1 (R Core Team, 2020). Analysis of variance (ANOVA) was used to compare the treatment effect of rye seeding rate on soil nitrate and rye biomass. In the model, rye seeding rate and block were both fixed effects. Additional analysis was conducted in R with tukeyHSD() or emmeans() to determine differences among groups if deemed significant by ANOVA. After the split plot fertilizer treatment was applied, the effect of both rye seeding rate and nitrogen fertilizer rate on PMN and soil nitrate were determined using agricolae and lmer packages in R (Bates et al., 2015; Mendiburu, 2020). In the mixed model, block, block*seeding rate, block*nitrogen fertilizer rate, block*seeding rate* nitrogen fertilizer rate were all random effects in a split plot design. Rye decomposition and enzyme activity at fertilizer and seeding rate were analysed across sampling time using repeated measure analysis with compound symmetry correlation structure. Assumptions for normality and equal variance were tested using QQ-plots and plotting residuals from un-transformed data. Box-Cox transformation procedure was conducted to determine transformation if assumptions were not met. Log, reciprocal, and square root transformations were conducted when necessary, and values were back-transformed before being visualized or reported.

Corn yield in response to nitrogen fertilizer was modeled with quadratic-plateau response curve (Eq 1) using easynls package in R (Arnhold, 2014).

Quadratic plateau was fitted using Equation 1.

$$y_{ij} = \begin{cases} a_i + b_i x_{ij} + c_i x_{ij}^2 \ , & if\ x_{ij} < x_{m,i} \\ y_{m,i} & ,otherwise \end{cases} \quad [1]$$

where $x_{m,i} = \frac{-b_i}{2c_i}$ is the optimal n rate and $y_{m,i} = a_i + b_i x_{m,i} + c_i x_{m,i}^2$ is the maximum yield response to N rate for the i-th treatment. A bootstrapping technique with the FertBoot package (Ma & Francis, 2020) was used to determine statistical differences in treatment fertilizer response curve models, maximum yield, and optimum nitrogen fertilizer between rye seeding rate treatments. Residuals of the fitted quadratic plateau model were resampled with replacement 1000 times to produce a population estimate data set of a, b, and c coefficients, and of optimum N rates and maximum y values. Differences in model parameters between seeding rates were determined using ANOVA and TukeyHSD.

Economic optimal nitrogen rate was determined using Equation 2.

$$Economic\ optimal\ N\ rate = \left(\frac{P_N}{P_C}\right)\left(\frac{1}{2c}\right) - \left(\frac{b}{2c}\right) \quad [2]$$

where P_N is the price per unit nitrogen, P_C is the price per unit corn, and a, b, c are parameter estimates from the quadratic plateau model determined by bootstrapping residuals (Reed & Karsten, 2022).

Results

Rye biomass growth and decomposition

Aboveground rye biomass increased as seeding rate increased (Table 2). The rye seeding rate of 34 kg ha^{-1} had the least growth at 1180 kg ha^{-1} in year 1. More rye biomass was accumulated in year 2 across all seeding rates (difference of 260-610 kg ha^{-1}). The seeding rate of

135 kg ha^{-1} accumulated more biomass than all other treatments in year 2. The lowest seeding rate had greater nitrogen content in aboveground biomass compared to other seeding rates. Carbon to nitrogen ratio was low across all treatments (10-13) but was lowest at the 34 kg ha^{-1} seeding rate. Nitrogen yield increased as seeding rate increased, but this effect was only observed in year 2 when more rye biomass was accumulated.

Rye root biomass was sampled in year 2 only. Root biomass was lowest at the seeding rate of 34 kg ha^{-1} with 800 kg ha^{-1} accumulated biomass (Table 2). Seeding rates of 67 and 135 kg ha^{-1} accumulated more root biomass (1700-1890 kg ha^{-1}) than all other seeding rates. Total rye biomass was greatest (4040 kg ha^{-1}) at the highest seeding rate. Nitrogen content in root biomass was less than aboveground but followed the same trend of decreasing as seeding rate increased. Root biomass had a higher C:N than shoot biomass, but differences among treatments were not statistically significant. Total nitrogen yield (root and shoot nitrogen uptake) was smallest at the lowest rye seeding rate compared to all other treatments.

Rye biomass lost at least 70% of its mass in litterbag studies for all treatments by 98 days after rye termination (Figure 3). Less rye residue remained in plots where rye was grown compared to plots without rye at 14-42 days after termination. Rye residue decomposed faster in plots where a rye cover crop was present compared to the no cover plots ($p=0.00169$). The interaction of fertilizer, seeding rate, and time was statistically significant ($p<0.001$).

Table 2 Summary of cover crop biomass quantity and quality across rye seeding rate treatments in year 1 and 2. Root biomass was measured in year 2 only. ANOVA results as affected by rye seeding rate treatment are reported for each year. Letters within columns indicate significant differences in values between treatments within a year (α = 0.05).

		Biomass				N content		C:N		N yield		
	Seeding rate	Shoot	Root	Total	Root: Shoot	Shoot	Root	Shoot	Root	Shoot	Root	Total
	kg ha^{-1}	Mg ha^{-1}				%				kg ha^{-1}		
Year 1	34	1.18b	-	-	-	3.91a	-	10.2b	-	46.1	-	-
	67	1.45a	-	-	-	3.34b	-	12.2a	-	48.7	-	-
	101	1.46a	-	-	-	3.32b	-	12.3a	-	48.2	-	-
	135	1.54a	-	-	-	3.16b	-	13.1a	-	49.1	-	-
		p-value										
	Block	0.004	-	-	-	0.005	-	0.004	-	0.001	-	-
	Seeding rate	<0.001	-	-	-	<0.001	-	<0.001	-	0.761	-	-
Year 2	34	1.52b	0.801c	2.31c	0.536	3.75a	1.66	11.2b	25.3	56.9b	12.9b	69.7b
	67	1.71b	1.70a	3.41b	0.994	3.46b	1.68	12.2a	21.9	59.3b	28.3a	87.6a
	101	1.79b	1.26b	3.05b	0.705	3.57ab	1.40	12.2a	28.6	64.0ab	17.9b	81.9a
	135	2.15a	1.89a	4.04a	0.889	3.41b	1.45	12.5a	27.7	73.1a	27.3a	100a
		p-value										
	Block	0.634	0.011	0.004	0.165	0.114	0.575	0.007	0.685	0.812	0.170	0.030
	Seeding Rate	<0.001	<0.001	<0.001	0.575	0.045	0.165	0.006	0.258	0.012	<0.001	<0.001

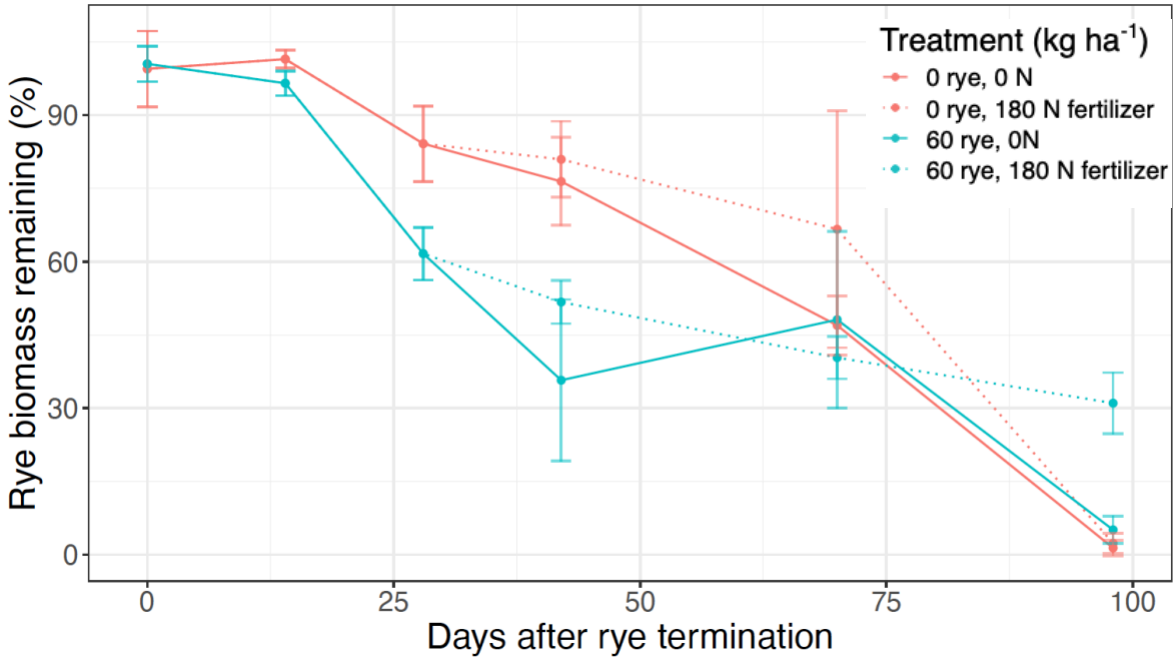

Figure 3 Rye biomass percent (%) remaining in litter bags up to 98 days after rye termination. Litter bag decomposition was measured in year 2 only (2022). Bags were placed in rye seeding rate treatments of 0 and 67 kg ha^{-1} at nitrogen rates of 0 and 180 kg ha^{-1}. Nitrogen fertilizer was applied 34 days after rye termination, between the 3rd and 4th sampling times. Error bars represent two standard deviations from the mean.

Soil nitrogen response

Trends in total soil nitrogen were only found in nitrate, so nitrate was the only form of inorganic N reported. Fall soil nitrate decreased at the 0-30 cm depth as seeding rate increased, but this difference was not statistically significant (Table 3). In spring of year 1, all rye treatments had less soil nitrate than without rye at both sampling depths (difference of >6 mg kg^{-1}). In spring of year 2, soil nitrate was lower in all rye treatments compared to without rye at both depths.

Table 3 Soil nitrate (NO_3-N) in fall and spring sampling periods in the field at depths of 0-30 cm and 30-60 cm with ANOVA results as affected by seeding rate treatment. Within each column, means followed by the same letter are not significantly different (α = 0.05).

	Soil nitrate (NO_3-N)							
	Year 1				Year 2			
	Fall		Spring		Fall		Spring	
	cm							
Seeding rate	0-30	30-60	0-30	30-60	0-30	30-60	0-30	30-60
kg ha^{-1}	mg kg^{-1}							
0	11.0	6.52	7.98a	7.90a	27.6	14.7	3.68a	12.9a
34	11.7	5.44	1.26b	1.50b	23.7	14.7	2.43b	3.82b
67	9.44	5.48	1.02b	0.88bc	20.9	11.7	2.72ab	2.70b
101	8.40	4.68	1.00b	0.73c	18.7	11.1	2.82ab	3.24b
135	8.18	6.08	1.12b	0.80bc	17.4	12.3	2.06b	1.86b
	p-value							
Source of variation								
Block	0.201	0.099	0.259	0.036	0.110	0.337	0.068	0.161
Seeding rate	0.196	0.641	<0.001	<0.001	0.072	0.278	0.010	<0.001

In season soil nitrate in year 1 was lower when rye was present (difference of >6 mg kg^{-1}) compared to the treatment without rye at both depths (Table 4). The same trend was observed in year two, but only at the 30-60 cm depth. Nitrogen fertilizer application increased soil nitrate at both depths.

Table 4 In-season average nitrate (NO_3-N) values with ANOVA results as affected by rye seeding rate treatment, nitrogen fertilization rate, and block. Within each column for significant treatment factors, means followed by the same letter are not significantly different (α = 0.05).

	Soil nitrate (NO_3-N)			
	Year 1		Year 2	
	----------cm----------			
Seeding rate	0-30	30-60	0-30	30-60
kg ha^{-1}	----------mg kg^{-1} ----------			
0	24.8a	15.0a	18.0	15.6a
34	17.9b	7.46b	14.4	8.26b
67	20.7ab	8.23b	18.3	9.26b
101	17.7b	9.92b	19.2	10.6b
135	15.7b	9.19b	20.7	9.18b
Nitrogen rate				
0	8.22b	6.22b	4.46b	5.37b
179	30.5a	13.7a	31.2a	15.8a
	p-value			
Source of variation				
Block	0.112	0.112	0.118	0.328
Seeding rate (SR)	0.026	0.020	0.652	<0.001
Nitrogen rate (N)	<0.001	<0.001	<0.001	<0.001
SR x N	0.449	0.223	0.732	0.750

Potentially mineralizable nitrogen (PMN) was also sampled in-season at depths of 0-15 cm and 15-30 cm. PMN was lower at the depth of 15-30 cm, but there was no significant effect of seeding rate or fertilizer treatments (Table 5). A significant interaction effect occurred in both year (p=0.002 and p=0.032, respectively) between seeding rate and fertilizer application at the 15-30 cm depth (Figure 3).

Table 5 In-season potentially mineralizable nitrogen values with ANOVA results as affected by rye seeding rate treatment, nitrogen fertilization rate, and block. Within each column for significant treatment factors, means followed by the same letter are not significantly different (α = 0.05).

	Potentially mineralizable nitrogen			
	Year 1		Year 2	
	----------cm----------			
Seeding rate	**0-15**	**15-30**	**0-15**	**15-30**
kg ha^{-1}	----------mg kg^{-1}----------			
0	59.6	21.0	67.7	19.7
34	67.0	21.2	66.9	19.9
67	58.2	21.7	70.8	15.5
101	62.3	22.2	62.4	16.3
135	60.8	27.5	67.0	17.5
Nitrogen rate				
0	63.8	22.7	65.7	18.7
179	59.3	22.8	68.2	16.8
	p-value			
Source of variation				
Block	0.164	0.261	0.168	0.883
Seeding rate (SR)	0.723	0.476	0.650	0.674
Nitrogen rate (N)	0.235	0.939	0.332	0.164
SR x N	0.173	0.002	0.654	0.032

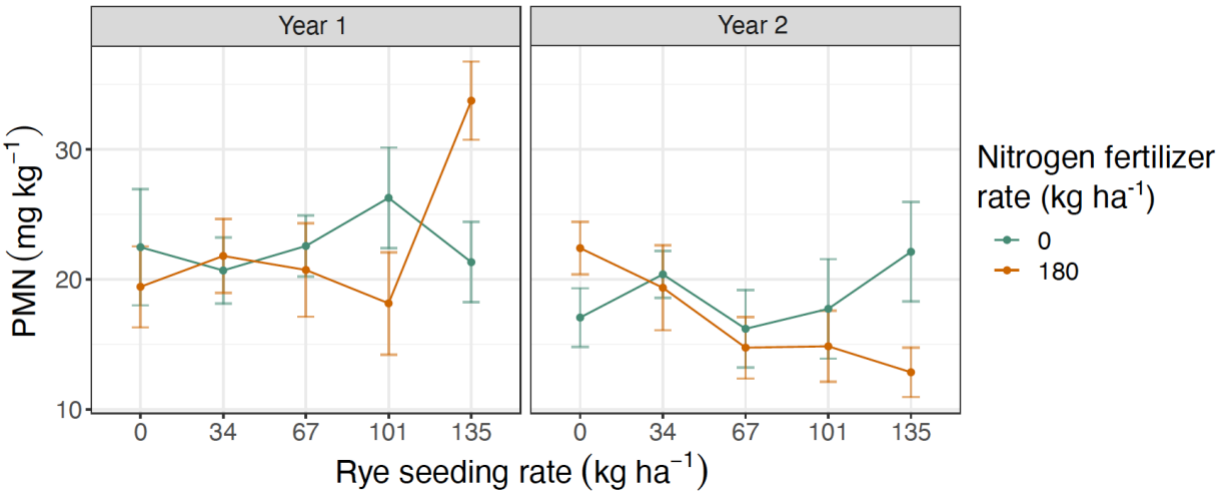

Figure 3 Year 1 and 2 potentially mineralizable nitrogen interaction plot for the 15-30 cm depth with nitrogen fertilizer rates of 0 and 180 kg ha^{-1}. Error bars represent two standard deviations from the mean.

Enzymes beta glucosidase and urease were measured in year 2 only as another in-season assessment of rye decomposition and nitrogen return. Potential BG activity was affected by sampling time only (Table 8). Activity was greatest at 70 days after termination compared to other sampling periods (Figure 4). Potential soil urease activity was greatest the first sampling period (26.26 μgNH_4-N g dry $soil^{-1}$ hr^{-1}) for the rye treatment and decreased as the growing season continued (17.77 μgNH_4-N g dry $soil^{-1}$ hr^{-1}) (Figure 5). There were significant interaction effects between sampling time and rye treatment on potential soil urease activity (Table 8). In the treatments without rye, potential urease activity was greatest at the time of rye termination (25.29 μgNH_4-N g dry $soil^{-1}$ hr^{-1}) and decreased as the season went on. At time of fertilizer application, treatments without rye had lower potential urease activity than the rye treatment.

Table 8 Analysis of variance summary table for response variables soil Beta glucosidase activity and urease activity.

	Beta glucosidase	Urease
Block	0.052	0.869
Sampling time	<0.001	<0.001
Rye	0.693	0.008
Fertilizer	0.764	0.855
Sampling time*rye	0.990	0.063
Winter rye*fertilizer	0.249	0.673
Sampling time*fertilizer	0.825	0.773
Sampling time*fertilizer*rye	0.504	0.449

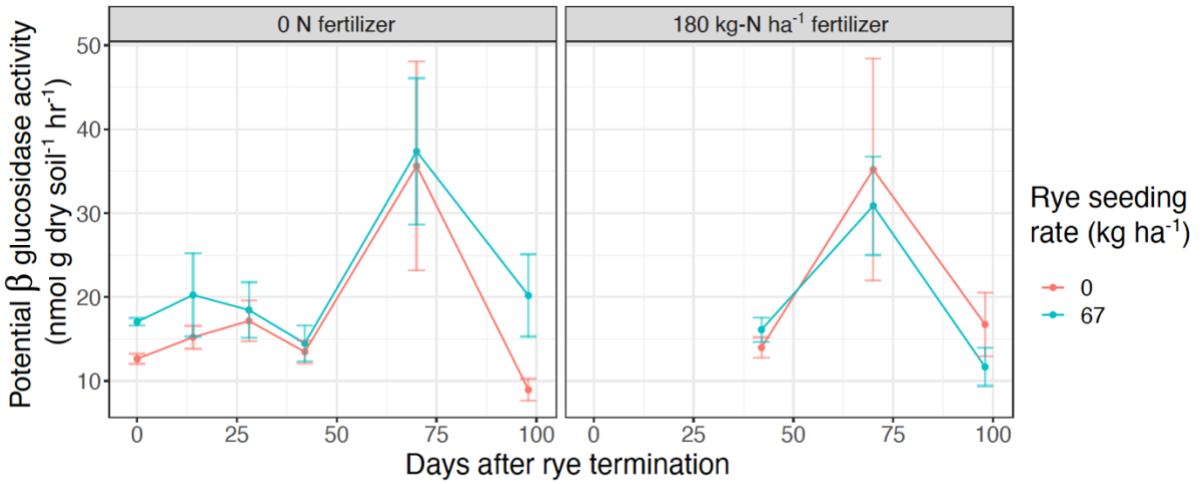

Figure 4 Beta glucosidase enzyme activity at sampling times over the growing season. Enzyme activity was measured in rye seeding rate treatments 0 and 67 kg ha^{-1} at fertilizer rates of 0 and 180 kg-N ha^{-1}. Fertilizer was applied to corn 34 days after termination between the 2nd and 3rd sampling times. Error bars represent two standard deviations from the mean.

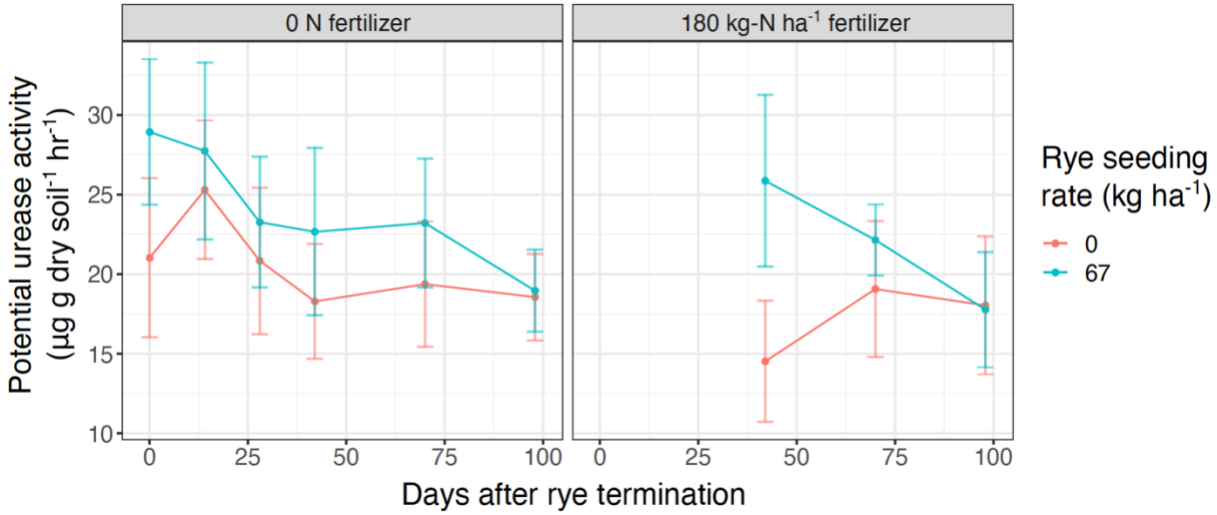

Figure 5 Urease enzyme activity at sampling times over the growing season. Enzyme activity was measured in treatments with and without rye (0 and 67 kg ha^{-1} rate) at with and without nitrogen fertilizer (0 and 180 kg-N ha^{-1} rate). Fertilizer was applied to corn between sampling time T2 and T3. Error bars represent two standard deviations from the mean.

Corn yield effects

Bootstrapped residuals of the quadratic plateau model were used to calculate 95% confidence intervals of the agronomic optimum nitrogen fertilizer rate and maximum yield for corn grain following each cover crop treatment (Figure 6). Maximum nitrogen yield varied significantly between treatments (Table 6). When nitrogen fertilizer was not applied, corn yields following rye compared to no rye were 10-19% lower in year 1 and 8-11% lower in year 2. In year 1, rye seeding rate treatment of 135 kg ha^{-1} had a lower maximum yield than all other treatments, but this difference was small (0.1-0.2 Mg ha^{-1}). Seeding rates of 34 and 67 kg ha^{-1} had the greatest maximum yield of 13.5 Mg ha^{-1}. More nitrogen was needed (15-30 kg ha^{-1}) to reach maximum yield as seeding rate of rye increased when compared to the no rye treatment (Figure 7). However, this difference was not observed at the 67 kg ha^{-1} seeding rate treatment which required the least amount of nitrogen fertilizer (93.0 kg ha^{-1} N) to reach max corn yield compared to corn yield following other rye seeding rates (Table 6).

While the bootstrapping technique allowed for determination of statistical difference between yield parameters, the differences here were not reflected in the economically optimum nitrogen rate (EONR). Using parameters based on the bootstrapped results for the quadratic plateau model, the economic optimum nitrogen rate for corn following no cover is 27 kg-N ha^{-1} (Table 7). Following rye, corn required 50-100 kg-N ha^{-1} more nitrogen fertilizer to reach EONR compared to no cover. The EONR for corn following rye at any seeding rate were 38-44 kg ha^{-1} lower than the agronomic optimum, except for the seeding rate of 67 kg ha^{-1}, which had a difference of only 12 kg ha^{-1}.

In year 2, corn yield following rye was the same or 1.5% greater at the optimum nitrogen rate compared to corn following no cover, except for the seeding rate of 101 kg ha^{-1} which was 5.5% lower (Table 6). The no cover treatment required the most N fertilizer (168 kg-N ha^{-1}) to reach the maximum yield, while max yield was reached with 16-55% less N fertilizer following the rye cover crop (Figure 8). The rye seeding rate of 34 kg ha^{-1} required the least amount of fertilizer to reach maximum corn yield compared to all other treatments (Figure 9). The EONR for no rye was 74 kg ha^{-1}, which was greater than the EONR for rye seeding rates of 34 and 101 kg ha^{-1}, and less than EONR for seeding rates of 67 and 135 kg ha^{-1} (Table 7).

Table 6. Year 1 (2021) and year 2 (2022) optimum nitrogen fertilization rate and corn yield determined by bootstrapping residuals from the quadratic plateau model. Values with the same letter within a column are not significantly different (α = 0.05).

		Parameter estimate									
	Seeding rate	a		b		c		Optimum N		Maximum yield	
Year 1	kg ha^{-1}	Estimate	sd	Estimate	sd	Estimate	sd	kg ha^{-1}	sd	Mg ha^{-1}	sd
	0	12.9a	0.272	0.00800e	0.00742	-0.0000441a	0.0000601	148c	76.0	13.4b	0.129
	34	11.7b	0.226	0.0232d	0.00843	-0.0000854b	0.0000669	163b	46.4	13.5a	0.121
	67	10.9d	0.261	0.0587a	0.0156	-0.000352e	0.000174	93.0d	22.9	13.5a	0.116
	101	11.3c	0.290	0.0263c	0.00900	-0.0000890c	0.0000605	174a	48.1	13.4b	0.152
	135	10.7e	0.247	0.0296b	0.00740	-0.0000914d	0.0000444	178a	38.4	13.3c	0.141
Year 2											
	0	11.4a	0.542	0.0192d	0.0150	-0.0000919a	0.000125	168a	79.7	12.7b	0.257
	34	10.5b	0.493	0.0617a	0.0256	-0.000421e	0.000282	96.3d	47.5	13.0a	0.214
	67	10.4c	0.451	0.0485b	0.0209	-0.000283d	0.000218	113c	46.6	12.7b	0.206
	101	10.2d	0.452	0.0432c	0.0204	-0.000256c	0.000208	116bc	55.6	12.3c	0.212
	135	10.2d	0.291	0.0441c	0.0142	-0.000210b	0.000133	123b	35.7	12.7b	0.149

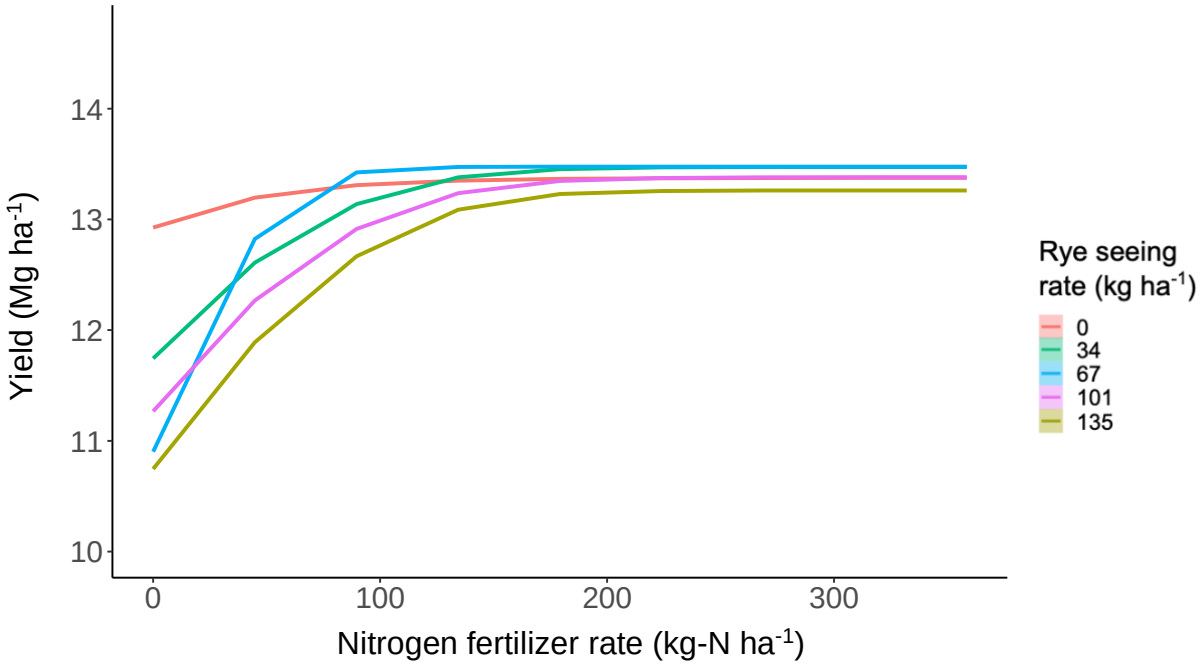

Figure 6 Year 1 (2021) corn yield fertilizer response quadratic plateau models determined by bootstrapping residuals for rye seeding rates.

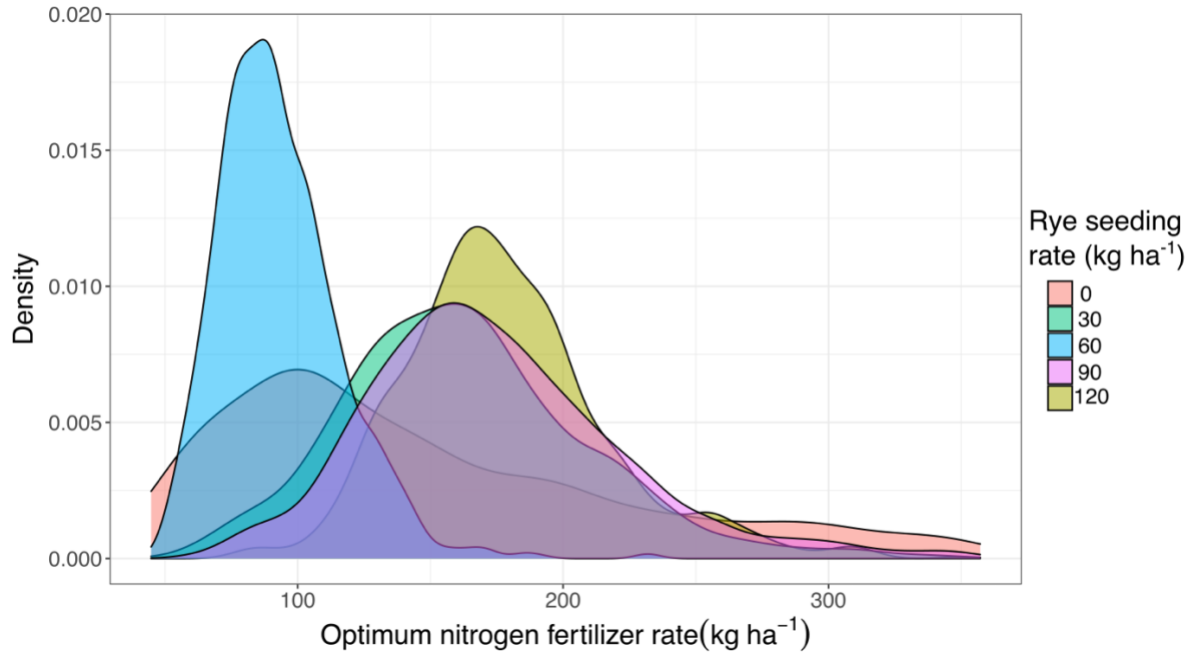

Figure 7 Density plot of optimum nitrogen fertilizer rate for year 1 (2021) of all rye seeding rate treatments. The density plots are constructed with results from bootstrapping residuals where the data was resampled 1000 times.

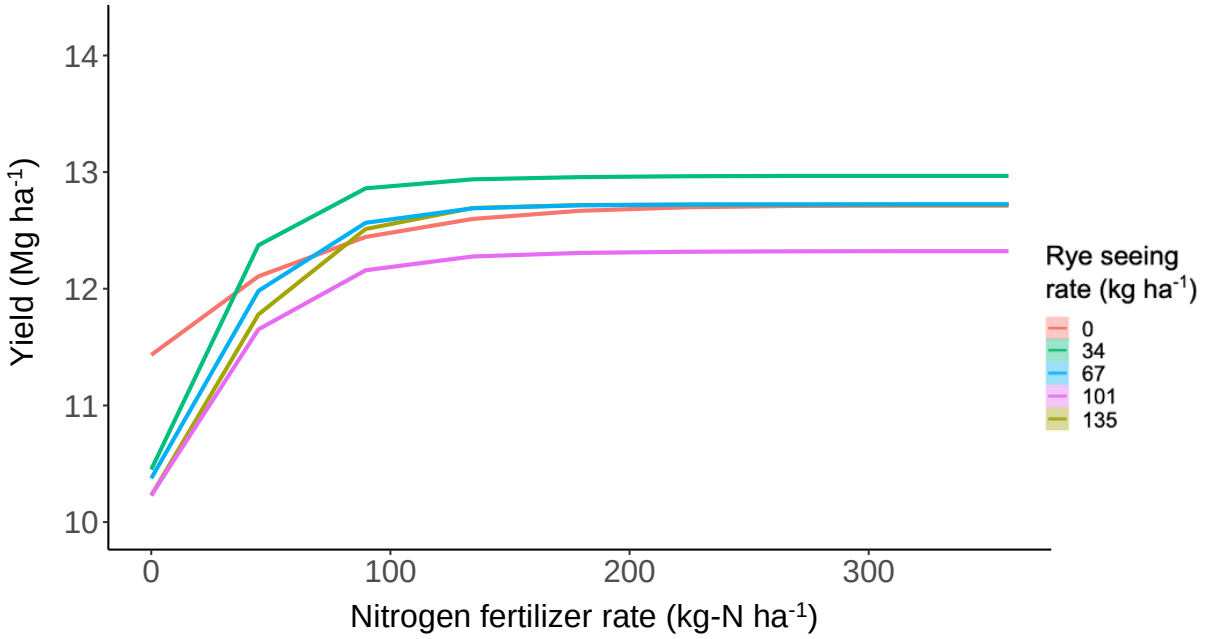

Figure 8 Year 2 (2022) corn yield fertilizer response quadratic plateau models determined by bootstrapping residuals for rye seeding rates.

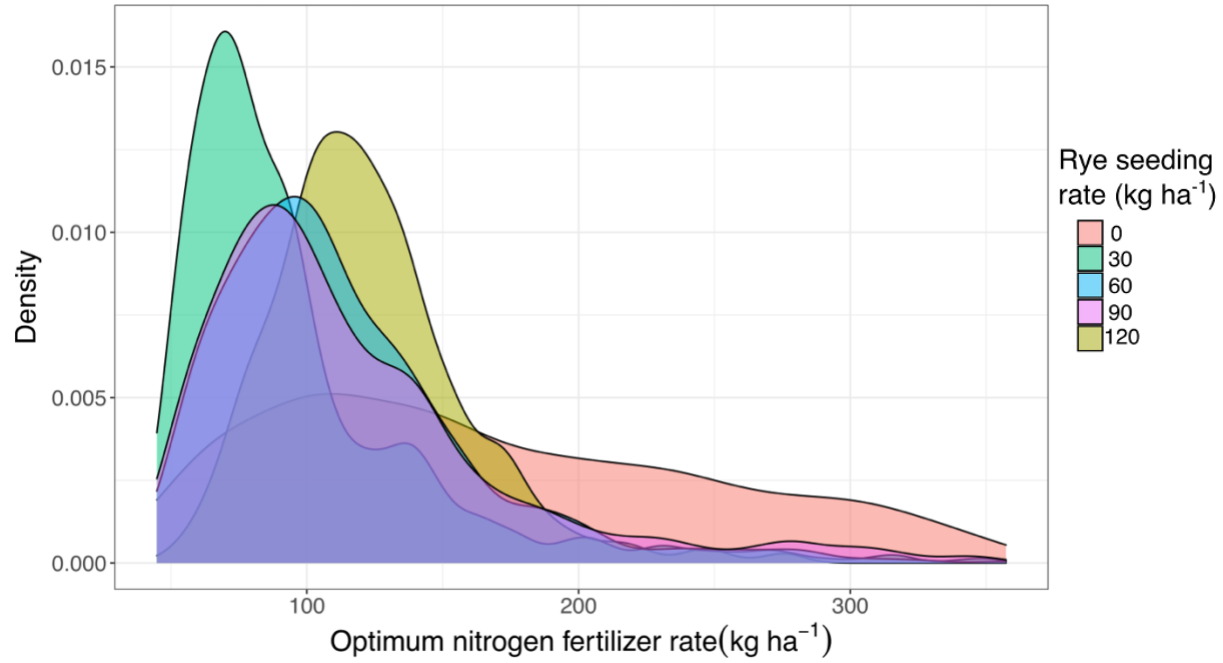

Figure 9 Density plot of optimum nitrogen fertilizer rate for year 2 (2022) of all rye seeding rate treatments. The density plots are constructed with results from bootstrapping residuals where the data was resampled 1000 times.

Table 7 Economic optimum corn grain yield and nitrogen rate based on parameter estimates from quadratic plateau model of original data for years 1 and 2. Economic optimum values calculated using a nitrogen fertilizer to corn price ratio of 0.0056.

	Seeding rate	Economic optimum N rate	Corn yield at 0 N fertilizer	Corn yield at optimum N rate	Corn yield at plateau N rate
	kg ha^{-1}	kg ha^{-1}	---------- Mg ha^{-1} ----------		
Year 1	0	0	12.9a	13.1	13.4b
	34	103	11.7b	13.2	13.5a
	67	75.4	10.9d	13.3	13.5a
	101	116	11.3c	13.2	13.4b
	135	131	10.7e	13.0	13.3c
Year 2	0	74.0	11.4a	12.3	12.7b
	34	66.6	10.5b	12.7	13.0a
	67	75.8	10.4c	12.5	12.7b
	101	73.4	10.2d	12.0	12.3c
	135	91.7	10.2d	12.5	12.7d

Discussion

Rye Biomass

While total rye biomass was greater at the max seeding rate (135 kg ha^{-1}) compared to the lowest seeding rate (34 kg ha^{-1}), intermediate rates did not consistently differ in biomass accumulation. Reed & Karsten (2022) did not find a difference in biomass between seeding rates of 34 kg ha^{-1}, 67 kg ha^{-1} and 134 kg ha^{-1} but did find that a later spring termination doubled biomass. Boyd et al. (2009) also failed to find a difference in biomass at time of termination across seeding rate, even at greater seeding rates of 90, 180, and 270 kg ha^{-1}. The amount of rye shoot biomass accumulated in our study (1.2-2.2 Mg-DM ha^{-1}) was consistent with other work in the Midwest with a mid-fall planting window (Pantoja et al., 2016). This other work has demonstrated seasonal variation in rye biomass growth, but our work was the only study to find an effect of rye seeding rate on aboveground biomass accumulation. Root biomass is less commonly measured than aboveground biomass due to the laborious task of root excavation and cleaning. However, the measurement of root biomass nearly doubled total rye biomass in our study, with a root to shoot ratio ranging from 0.54-0.99. This ratio is slightly less than other work which measured rye cover crop root:shoot from 0.75-1.94 (Martinez-Feria et al., 2016) and 0.68-1.05 (Griffin et al., 2000). The large amount of root biomass accumulated by rye as a cover crop demonstrates the need to include root biomass in estimates of total nutrient uptake.

As the amount of total rye biomass increased, biomass C:N also increased while nitrogen content decreased. Other work also demonstrates this inverse relationship between biomass and C:N (Pantoja et al., 2016; Sievers & Cook, 2018; Vaughn et al., 2022). Nitrogen content in the root biomass was lower with C:N from 22-29. Roth et al. (2022) found that during

decomposition, <11% of rye root N and >84% of shoot N was recovered in the soil in organic or inorganic form after 120 days, and that higher C:N was a driver of this difference. However, in order to measure decomposition, the root biomass in Roth et al. (2022) was completely removed from the soil and rinsed before being reincorporated into the soil. This process completely disrupted the natural soil conditions influencing decomposition, thus results may not be representative of in situ root decomposition. Dornbush et al. (2002) used an intact core root decomposition method and found that root biomass decomposes more rapidly than aboveground biomass due to greater contact with soil microorganisms and activity of the rhizosphere. Sievers & Cook (2018) estimated low nitrogen returns from rye root biomass with 80% root biomass decomposed throughout the growing season. However, only 20% of biomass N was released to the soil during decomposition, indicating a majority of N from the root biomass does not become available to corn during the growing season.

In our study, C:N remained relatively low across all seeding rates. A study across four sites in Iowa with rye as a cover crop reported C:N from 12-17 with 0.7-1.13 Mg ha^{-1} biomass accumulation (Pantoja et al., 2016). Martinez-Feria et al. (2016) reported C:N ranging from 11.3-27.1 in shoot biomass and 23.2-33.4 in root biomass. Two separate studies out of Indiana reported rye shoot C:N of 16 (Nevins et al., 2020; Roth et al., 2022). Our C:N values for rye biomass were lower than other work in the Midwest, but aligned with the C:N of 12.8 reported by West et. al., (2020) in Wisconsin, which also utilized fall manure N inputs. The low C:N in our study is due to early termination of rye while still in the vegetative state, as well as a nitrogen rich soil due to fall manure application prior to rye planting. Generally, residues with C:N <25-40 tend to favor net mineralization upon decomposition (Vigil & Kissel, 1991). However, it is not

likely that grass cover crops supply N to the following crop (Martinez-Feria et al., 2016; Roth et al., 2022). In our study, the rye residue had very low C:N and we would expect net mineralization to occur as rye residue decomposes.

Soil response

At all seeding rates, winter rye reduced soil nitrate from fall to spring. Where no cover crop was grown, soil nitrate decreased from fall to spring in year 2, indicating this nitrogen leached from the field due to rainfall in fall and spring (Figure 2). This effect was not observed in year 1 – i.e., the nitrate did not greatly decrease from fall to spring. However, the fall soil sampling occurred in late fall before the first hard freeze, so it is possible that nitrate leached from the field in the six week period between manure application and soil sampling time, especially in the two weeks between manure application and rye planting in year 1. We observed that most soil nitrate remained from fall to spring in year 1, but this could be an underestimation due to late fall sampling time which did not capture a true value for baseline fall nitrate before any uptake or leaching. However, soil nitrate content increased from spring to in-season soil test time points following rye. The change in soil nitrate from spring to in-season is larger when rye was grown compared to no cover, indicating net mineralization of the biomass during decomposition at the in-season sampling time point in the first 60 cm of the soil profile.

While changes in soil nitrate suggest rye biomass is mineralizing, potentially mineralizable nitrogen analysis (PMN) did not differ across seeding rate treatments or fertilizer rates. Other work has found a 20-80% increase in soil PMN when following a cover crop compared to no cover (Moore et al., 2014). Based on litter bag decomposition in this study, only about 50% of

aboveground rye residue was decomposed at time of in-season soil sampling, 9-10 weeks after rye termination. This sample timing is long after the most rapid period of decomposition occurring between 14-28 days after termination, which aligns with patterns in other work (Jahanzad et al., 2016; Lacey et al., 2020; Sievers & Cook, 2018; Singh et al., 2020). A future strategy to capture any variation in PMN across treatments would be to have multiple sampling times throughout the period or rye decomposition, especially during the period of rapid decomposition 2-4 weeks after termination. This would be a better approach to capture any changes occurring in in the potentially mineralizable nitrogen pools throughout the growing season.

Soil urease was the only measure of soil biological activity influenced by the presence of the cover crop. Soil urease activity was greater following a rye cover crop compared to no cover but was not influenced by nitrogen fertilizer. Activity was greatest at the time of rye termination and early stages of decomposition and decreased over time. Beta glucosidase (BG) did not increase following a rye cover crop or nitrogen fertilizer. Nevins et al. (2020) found an increase in both urease and BG following a rye cover crop. Additional work found with the presence of additional carbon and nitrogen inputs in the system, enzyme activity is greater than no cover (Allison et al., 2007; Nevins et al., 2020). West et al. (2020) observed greater BG activity when rye was used as a cover crop in continuous corn silage after seven years of use. However, these changes were not exhibited in our study potentially due to the already nitrogen rich environment due to three years of alfalfa in the rotation prior to corn silage and fall manure application. Both fields in this study are also under long term no-till management and have

relatively high organic matter, so the addition of cover crop residue inputs from one growing season did not influence these measurements.

Corn yield response

In year 1, more nitrogen was needed to reach economic optimum yield when corn was following rye compared to no cover. Previous work has found similar results, with a direct relationship between rye biomass accumulation and corn yield penalty (Krueger et al., 2011; Pantoja et al., 2015). However, in the first year of our study, yields were able to recover with the addition of N fertilizer, except at the 135 kg ha^{-1} seeding rate of rye. In contrast to year 1, no yield penalty was observed when corn followed rye with nitrogen fertilizer application in year 2. More aboveground rye biomass was accumulated (24%) in the second year of this study, so an even larger subsequent corn yield decline might have been expected. Martinez-Feria et al. (2016) conducted a meta-analysis alongside a field experiment which indicated both experimental and modeling results do not fully support the hypothesized relationship between corn yield and rye biomass production, demonstrating the complexity that exists in the system. They suggest that, depending on the growing season, other factors such as water and nitrogen stresses at corn flowering and grain filling periods may have a stronger influence than cover crop induced changes in soil water and nitrogen at corn planting. Nevertheless, in agreeance with the second year of our study, other studies did not observe a yield effect of corn following rye (Duiker & Curran, 2005; Kuo & Jellum, 2002; McSwiney et al., 2010; Snapp & Surapur, 2018). A review by Miguez and Bollero (2005) considered corn yield following winter cover crops in the

USA and Canada and found corn following a grass cover crop yielded the same as corn following no cover.

Year to year yield effects were inconsistent, but corn yield was not impacted by rye cover crop at low seeding rates. This year to year inconsistency is also reported in Kaspar & Bakker (2015), where they observed a change in corn yield when following rye from 0 to -0.69 Mg ha^{-1} compared to no cover across four growing seasons. In contrast to the work of Martinez-Feria et al. (2016), Kaspar & Bakker (2015) found a significant relationship (R^2= 0.39) between cover crop biomass accumulation and change in corn yield, with increased rye biomass leading to lower yields. Despite numerous studies assessing this relationship, the literature lacks consensus on the effects of winter rye on corn yields. This lack of consensus is reflected in the difference in results from the two site-years of this study, highlighting the intricoes in the system and lack of predictability.

Differences of EONR from year to year are likely due to changes in manure-N availability. In the no rye control in year 2, about 26 mg kg^{-1} NO_3-N was lost from fall to spring. This loss can be attributed to leaching during large rain events in the early spring (Figure 2). This loss was not observed in the first year of the study. Thus, the greater differences in EONR in year 1 when comparing rye treatments to no rye are likely best attributed to manure nitrogen carrying over from fall to spring where no rye was grown. With limited manure-N lost from fall to spring, corn had sufficient plant available soil N to reach optimum yield with only 27 kg ha^{-1} provided. The relatively non-responsive nature of this curve lead to greater differences in value when comparing EONR following rye. In year 2, nitrogen was lost to the environment from fall to spring, and no manure-N carried over. This likely gave rye treatments an advantage in terms of

nitrogen retention, so EONR was not impacted or had a slight positive effect. Any differences in EONR across seeding rates were likely attributed to nitrogen uptake into the rye biomass rather than immobilization by soil microorganisms during rye decomposition. With the amount of rye biomass accumulated and low C:N in this biomass, nitrogen was not taken up from the soil by microorganisms while rye residues were decomposing. Rather, nitrogen effects were due to depleted soil nitrogen pools through rye plant uptake in fall and spring. The nitrogen tied-up in this biomass was not yet returned to the soil at the time when corn nitrogen demand was greatest. Even though this nitrogen is temporarily unavailable to the subsequent cash crop, the nutrient is kept in the field and will eventually be returned as inorganic N.

Conclusion

Cover crops have clear potential to improve the water quality and soil conservation in dairy cropping systems in the Midwestern Corn Belt. When corn is grown following a winter rye cover crop, a short-term lack in soil nitrogen lead to yield decline at low nitrogen rates, but these yields recovered upon additional nitrogen fertilizer application, and maximum grain yield was not impacted. The extent of this effect is inconsistent from year to year, and economic optimum nitrogen rate following rye compared to no cover is dependent on soil N carryover from fall to spring. Given there was no evidence of microbial immobilization during rye decomposition, any negative corn yield effect was likely caused by nitrogen uptake into the rye biomass. Given our results, there appears to be little benefit to seeding rye at rates above 67 kg ha^{-1}. Grain yield following seeding rates of 34 and 67 kg ha^{-1} were able to reach optimum yield with less nitrogen fertilizer inputs compared to rates of 101 and 135 kg ha^{-1}. Even though we saw a nitrogen effect occurring with corn following rye, all seeding rates of rye scavenged nitrogen from the field and

provided water quality benefits through nitrogen uptake. Thus, there is a clear trade-off in terms of nitrogen cycling with rye cover crop use with manure, as water quality benefits are obtained at the cost of agronomic benefit of the applied manure. One way the trade-off can be managed is by keeping rye seeding rates low, especially at initial years of rye adoption.

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