

Nitrogen use with integrating cereal rye grain production and clover cover crop into a soybean-corn system

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

To my mother.

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ABSTRACT

Winter cereal rye (*Secale cereale* L.) has been a common cover crop choice due to seed cost, winter hardiness, rapid growth, and excellent potential to reduce NO₃ losses to the environment. It could also be an alternative grain crop to include in a corn (*Zea mays* L.) – soybean [*Glycine max* (L.) Merr] rotation, although detailed information on management practices for cultivation in Iowa is lacking. In addition, insertion into the cropping system of a different cover crop, like the legume red clover (*Trifolium pratense* L.), would be possible due to inter-seeding with the cereal rye small grain crop. Such a cover crop would also provide an alternative source of N for a subsequent cereal crop such as corn and positively impact NO₃ leaching due to extended growth after rye grain harvest. The objectives of this study were to determine N response and optimal fertilizer N application rate in cereal rye production, the impact of a red clover cover crop on rye growth and yield and on a subsequent corn crop optimal N fertilization requirement and yield, and the influence of the cropping system on soil profile NO₃. Two cereal rye varieties were no-till planted following soybean harvest in the fall 2017 and 2018 at two locations each year, Ames and Kanawha, Iowa. Six total fertilizer N rates (0, 28, 56, 84, 112 and 140 kg N ha⁻¹) were fall-spring split applied. The study was split with and without late winter inter-seeded medium red clover. Rye crop canopy sensing was collected multiple times during the growing season, and plant height, number of seed heads, leaf and grain N concentration, and grain yield were measured. In addition, soil profile NO₃ was determined after rye harvest. The red clover was allowed to grow until termination the next spring prior to no-till corn planting. Six N rates (0, 56, 112, 168, 224 and 280 kg N ha⁻¹) were planting-sidedress split applied to the corn. Corn canopy sensing was collected at mid-vegetative growth and ear leaf sensed at silking, and grain yield determined. Soil profile NO₃ samples were collected multiple

times during the season. The inter-seeded red clover had no effect on rye N response or yield. Both rye varieties responded to N rate with increased plant canopy sensing indexes, leaf greenness, head count, plant height and grain yield, but with growth and yield differences between varieties. The yield at the agronomic optimum N rate (AONR) was higher for ND Dylan at 3120 kg ha⁻¹ versus 2119 kg ha⁻¹ for Elbon. However, the mean (AONR) was similar for both rye varieties at 98 kg N ha⁻¹. Overall, the rye yields were relatively low. Post-rye harvest soil profile NO₃-N was low with all N rates. Corn canopy sensing was greater with the presence of clover when N application was deficient, showing evidence of N contribution from the terminated red clover. The clover supplied corn with an estimated mean 57 kg N ha⁻¹; therefore reduced the optimal N rate needed to maximize grain yield. Soil profile NO₃-N was minimally increased with the clover at termination and late spring, however, following corn harvest was elevated with the higher N rates indicating care is needed to account for N supplied from the red clover cover crop. Further research is needed to assess management practices that promote better rye performance. Even so, this integrated grain crop-cover crop production system has potential for an alternative rye crop within the traditional corn-soybean rotation, and to help reduce system-wide profile NO₃ loss.

CHAPTER 1. INTRODUCTION

The need for extended cropping systems

The corn-soybean rotation is the predominant cropping system in the U.S. Midwest, covering about 75% of the cropland surface (Hatfield et al., 2007). That rotation has lowered overall crop diversity and led to growth in pest pressure such as weeds, insects, and diseases (Oliveira et al., 2019). An important environmental concern related to annual crop intensification is extensive fertilization and resultant impacts on water and air quality, including from N fertilizer use.

Nitrogen fertilizer has a fundamental importance in global agriculture, particularly for staple food crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), corn, and grain sorghum (*Sorghum bicolor* L. Moench) (Preza-Fontes, 2017). According to Tilman et al. (2001), the doubling of worldwide food production in the period from 1965 to 2000 was accompanied by a 7-fold growth in global N mineral fertilization. Corn productivity in the USA increased approximately 50% in the last 40 years (FAOSTAT, 2021), a result of successful breeding programs and improvements in irrigation and nutrient management (Hilker, 2021). Nitrogen use on corn also increased. In 2018, 11 644 MT of N were used for crop fertilization in the USA, compared to 8835 MT in the early 1980s (FAOSTAT, 2021). The increased use of N fertilizer, however, has led to increased risk for environmental impacts – for instance increasing NO₃-N loads in surface waters and atmosphere nitrous oxide from cropland (Byrnes, 1990; Gaudin et al., 2013; Westra, 2015).

Iowa is one of the states with the highest occurrence of NO₃ contamination to drinking water in the USA (Temkin, 2019). Nitrogen from soil and applied fertilizer, in the form of NO₃, moves downward into the subsoil by leaching and reaches shallow groundwater resources

(Allison, 1966), which are used for drinking water and connect to surface water bodies such as streams, lakes, coastal waters and rivers, including the Mississippi River. High NO_3 loading in the Mississippi River causes an abnormal proliferation of harmful aquatic plants known as algal blooms (Conley et al., 2009), which will consume most of the available water O_2 , forming zones called hypoxic (i.e., less than 2.0 mg l^{-1} of dissolved O_2) (Ribaud et al., 2005).

Aiming to improve water quality, the Iowa Nutrient Reduction Strategy (INRS) set an action plan to assess and reduce nutrient loads to Iowa waters and the Mississippi River. The goal is a 41% reduction of annual $\text{NO}_3\text{-N}$ load from nonpoint sources, such as agricultural lands (IDALS, 2013). Although this is a continental scale problem, the key to addressing it involves the management of thousands of individual farm fields across the Midwest (Tomer et al., 2015). Kling (2013) estimated that 8.5 million ha of cropland in Iowa could be targeted in the strategy. In addition to water quality improvement, the nutrient reduction practices also provide complementary benefits, such as soil health, greenhouse gas emission reduction, and wildlife habitat (Hoque and Kling, 2016).

Multiple practices are necessary to meet reduction goals. The INRS science assessment identified several N management practices that could reduce $\text{NO}_3\text{-N}$ loss. Three in-field practices that have a major impact are cover crops, N application rate, and extended crop rotations (Thompson et al., 2014). Of the three, extended rotation has the largest potential effect (42%), followed by rye cover crops (31%) and N rate to corn the lowest (10%). If implementing these practices is cost effective and user friendly, there is great potential for widespread adoption. Farmers will most likely adopt the easiest transition regarding in-field practices and most economically profitable without cost share incentives. Regarding goals for implementation of these practices, use of adequate integrated agronomic practices has the potential to improve

sustainability of agricultural production, increase soil health and productivity, and increase crop grain yield while reducing negative effects on the environment and providing economic profitability to farmers. The combination of these methods can be the key to the sustainable use of N in agriculture (Godfray, 2013).

Extended rotations and cover crops

Increasing plant diversity in agricultural production has the potential to reduce N application and loss of NO_3 , among other environmental benefits, compared to the conventional short-term corn-soybean rotation (Thompson et al., 2014). In general, cash crops produce the greatest amount of residue, but improved environmental management programs frequently include a winter cover crop to enhance soil coverage and nutrient management (Ruffo and Bollero, 2003). Cover crop species selection depend on specific goals. Legume forages are mainly used as an alternative N source for the next cereal crop, and cereal grasses are used with the main purpose of reducing NO_3 leaching. However, a legume forage cover crop could also take up NO_3 and reduce leaching. An intercropping combination of legume and grass cover crops could concurrently provide both benefits (Ranells and Waggoner, 1996), and has shown to provide high cover crop production (Decker et al., 1994). Other benefits from the use of cover crops include soil erosion control and improved soil organic matter and water-holding capacity (Hartwig and Ammon, 2002; Roth et al., 2018; Seman-Varner et al., 2017).

Adding a cereal small grain crop, inter-seeded with a forage legume such as red clover, to the corn-soybean rotation has been a viable practice to extend rotations in the US Midwest (Blaser et al., 2012). In that alternative cropping system, the cover crop provides active plants and soil coverage in fall and winter and can provide potential N supply to an annual crop like corn. The presence of the small grain increases warm season weed suppression, and crop roots

take up soil and fertilizer NO_3 in the early spring thus reducing leaching loss. Further, extending the time between soybean crops may break disease cycles and be a non-host for soybean cyst nematode (*Heterodera glycine*) (Posner et al., 1995; Stute and Shelley, 2009).

Karlen et al. (2006) assessed extended crop rotations in long-term experiments in Iowa and Wisconsin. They observed that longer rotations, with at least 3 years of forage crops, positively affected soil quality indicators (bulk density, organic C, microbial biomass, and penetration resistance). In addition, corn, soybean, and wheat (with red clover) in a rotation can produce as much as 90% of the conventionally managed individual crop yields (Posner et al., 2008). An alternative cereal small grain to include in the rotations could be cereal rye.

Cereal rye originated in western Asia, the same area as common wheat, barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.). According to Bushuk (2001), as most rye is grown as a fall-seeded annual crop, it is generally called “winter rye.” Due to its winter tolerance, rye has been grown successfully in areas considered too severe for winter wheat or barley. In crop rotations, rye is often used for livestock pasture and harvested forage, and grains are used for livestock feed, feedstock in alcohol distilling, and as flour in baked products (Bushuk, 2001; Kornecki and Balkcom, 2020). From 2018 to 2019, the annual national rye production in the USA ranged from 214180 to 269810 MT, an annual yield growth of 9.8%. (FAOSTAT, 2021).

Because of its superior winter hardiness, cereal rye has been suggested as the most reliable cover crop choice in the U.S. Midwest (Snapp et al., 2005). It is widely used for the purpose of accumulating inorganic soil N in the period between annual crops (Clark, 2007). In Iowa, Kaspar et al. (2007) reported that the use of rye as a cover crop can reduce $\text{NO}_3\text{-N}$ leaching up to 61%, with the INRS nutrient reduction strategy average at 31%. Other studies report further benefits to the soil system such as soil coverage that helps control wind and water

erosion, inorganic N recycling, and soil structure and water holding capacity improvement (Burket et al., 1997; Kessavalou and Waters, 1999; Stivers-Young, 1998; Weinert et al., 2002).

Besides the current use as a successful cover crop in Iowa, cereal rye can be an additional cash grain crop easily incorporated into existing soybean-corn production systems. The USDA-NASS 2020 crop values summary (USDA-NASS, 2021) had rye at \$59 million in value. Fall seeded rye for grain production can act as a cover crop following soybean or corn, providing soil profile NO₃ reduction and improving soil health. Harvested rye grain after spring growth and maturation will provide an economic return to growers. The harvested grain can then be used either as seed for a cover crop after corn or soybean in the following season or sold as grain to the livestock or food industry.

Clover can also be used as a cover crop. Red clover can successfully thrive within a small grain intercrop as it tolerates more shading than most other legumes (Wheaton, 1993). Better results are usually achieved when clover is seeded early in the spring season, and then growth continues after cereal grain harvest. Seeding a legume forage after cereal grain harvest can compromise establishment due to potential dry conditions and a short growing period (Stute and Shelley, 2009). That seeding timing also does not provide for continuous cover crop growth after small grain harvest. Frost-seeding clover in a small grain crop in late winter or early spring is a low cost and simple establishment method (Gettle et al., 1996). The freeze-thaw cycles from low overnight and warm daytime temperatures along with early spring precipitation cause soil movement and improve seed-soil contact, eliminating the need for drilling the clover.

Red clover can be left to grow or mowed after small grain harvest, so plants continue active growth in order to maximize N fixation, with mowing to help with weed growth control as needed (Stute and Shelley, 2009). The clover can also be harvest in the late summer for livestock

forage. Spring termination of clover can be preferred as it provides greater N supply compared to fall termination (Ketterings et al., 2011). Termination can be easier in the fall, with initial degradation in the fall and a longer period for plant N release. However, fall termination removes active plants from the land during the late fall and early spring – thus eliminating the opportunity for active growth and NO_3 uptake, and increasing the chance for greater NO_3 in the soil profile that would be subject to early spring leaching.

Environmental and management factors affect organic decomposition dynamics, making it tricky to correctly predict the amount of N supplied by a legume forage such as clover, and when the legume N will be available for a following cereal crop like corn (Crews and Peoples, 2005). More detailed research on the impact of red clover as a cover crop on the corn N fertilization requirement is needed. Assessing the N contribution of a cover crop is essential when determining corn N fertilizer rates in order to optimize profitability and mitigate environmental N issues related to potential carryover soil NO_3 (Andraski and Bundy, 2002).

Cereal rye and corn nitrogen fertilization requirements

There is only older guidance on N fertilization recommendations within Iowa for small grain cereals such as wheat and oats (Voss and Killorn, 1988), and none for cereal rye. Those recommendations were yield goal based. Small grains suggested N fertilizer rate in the U.S. Midwest varies, 0 to 146 kg N ha⁻¹ for wheat production (Gibson and Paulsen, 1999; Lindsey et al., 2017), 34 to 78 kg N ha⁻¹ for oat (Gailans et al., 2020), and 40 to 80 kg N ha⁻¹ for barley (Sainju et al., 2013; Vyn et al., 1999), depending on soil N status, texture, and organic matter level, prior crop, and yield goal. According to Nafziger (2009) in Illinois, rye N rate should be 22 to 34 kg N ha⁻¹ less than for wheat due to lower yield potential and for small grains N rate

reduced 20 to 25% below the optimal rate to reduce small grain competition to inter-seeded legume forage seedlings such as clover or alfalfa (*Medicago sativa* L.).

There has been little or no research to investigate the N fertilization rate requirement for cereal rye seed production in Iowa or the north-central region, or cereal rye with inter-seeded red clover. Laboski and Peters (2012) base N rate recommendations for rye production in Wisconsin on soil organic matter level, range across 45 to 67 kg N ha⁻¹, although the publication states limited research data. Fertilization guidelines in Minnesota base recommendations on expected yield, suggesting the N rate for rye following soybean at 22 to 134 kg N ha⁻¹ for soils with medium-high organic matter level (Kaiser, 2018).

Recent cereal rye variety trials in Iowa have applied various N rates, ranging from 34 to 114 kg N ha⁻¹ (Pecinovsky et al., 2020). Consequently, yield performance could be less than optimal and inconsistent due to sub-optimal N, with yields ranging from 26 to 121 kg ha⁻¹. (Pecinovsky et al., 2020; USDA-NASS, 2021).

Development of efficient N management programs in corn, a crop with high N requirement, is extremely important to mitigate the negative impacts of N losses to the environment. The use of red clover in this system is important not only for the potential N contributions, but also because of its ability to mineralize N slower building a more stable organic N reserve (Westra, 2015). Gentry et al. (2013) reported a mean red clover N contribution of 39 kg N ha⁻¹, when clover was frost seeded into winter wheat preceding first-year corn. An estimate across field crop systems using inter-seeded red clover in the USA and Canada found average N-credit values of 97 kg N ha⁻¹ (Gaudin et al., 2013).

Although many studies have evaluated cereal rye cover crop effects on a following corn crop (Basche and DeLonge, 2019; Pantoja et al., 2015; Patel et al., 2019), little research has

assessed integrating winter cereal rye as a grain crop in a soybean-corn rotation. New research on rye management will produce a better understanding of how rye responds to red cover inter-seeding and N fertilization rate response. This knowledge can further be used to develop management recommendations to optimize profit for farmers. Therefore, region- and site-specific research need to assess variety behavior, evaluate how and if an inter-seeded cover crop affects the respective cropping system, and adjust N fertilizer rates applied to cereal rye and a following corn crop. This information will be of major importance to help producers better manage and realize the benefits of including winter cereal rye as a grain crop in cropping systems.

Research project objectives

The overall goal of this research was to study the soybean-cereal rye seed production–red clover cover crop–corn grain cropping sequence. The specific objectives were to determine cereal rye response to N fertilization and optimal N rate requirement, the effect of inter-seeded red clover cover crop on the rye production and yield, impact of the red clover cover crop on corn optimal N fertilization requirement and yield, and the influence of the cropping system on soil $\text{NO}_3\text{-N}$.

CHAPTER 2. MATERIAL AND METHODS

Site description

The study was conducted within a three-year project established in the fall of 2017 at two Iowa State University Research and Demonstration Farms. The overall project consisted of a no-till, three-year cropping system of soybean - cereal rye grain crop/inter-seeded red clover – corn. The field experiments were conducted consecutively for the cereal rye and corn phases of the rotation. The cereal rye phase took place during the 2018 and 2019 growing seasons, with a new rye experimental area each year; the corn phase took place during the 2019 and 2020 growing seasons. One site was the Agricultural Engineering and Agronomy Research Farm, located approximately 10 km west of Ames, Iowa (42° 01' N lat; 93° 46' W long) with an annual mean precipitation of 881 mm and temperature of 9.1 °C (30-yr average). The other site was the Northern Research and Demonstration Farm near Kanawha, Iowa (42° 55' N lat; 93° 47' W long) with an annual mean precipitation of 787 mm and temperature of 8.2 °C. Both sites have a hot summer continental Dfa climate (Köppen Climate Classification System). The soil series at both sites were an intergrade across Clarion loam (Fine-loamy, mixed, superactive, mesic Typic Hapludolls) and Nicollet clay loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls), with 1-3% slopes.

Soil samples were collected prior to study initiation for routine soil testing, depth 0- to 15-cm. Samples were dried at 27 °C and ground to pass a 2-mm sieve. Soil test P and K were determined by Mehlich-3 extraction, with P determined colorimetrically (Frank et al., 2011) and K by atomic absorption spectrometry (Warncke and Brown, 2011). Soil pH was determined in a 1:1 soil to water suspension (Watson and Brown, 2011) and organic carbon by dry combustion with a LECO CHN-2000 analyzer (LECO Corporation, St. Joseph, MI, USA) (Combs and

Nathan, 2011). Routine soil test results are presented in Table 1. The results of the soil analysis indicated K level was Optimum to High both years (Mallarino et al., 2013). Soil test P was also above the Optimum level at both sites each year. At Ames the 2018 cereal rye site tested higher in organic matter content than the 2019 cereal rye site; however, at Kanawha soil organic matter content was similar for the 2018 and 2019 sites. In the fall 2017 before rye planting, at the Ames site triple-superphosphate was applied at 15 kg P ha⁻¹ and at the Kanawha site limestone was applied at 900 kg ha⁻¹ effective calcium carbonate equivalent. At each site and year in the fall before rye planting and in the spring at corn planting, gypsum (CaSO₄) was applied at 17 kg S ha⁻¹ to avoid potential S deficiency.

Cereal Rye Phase

The rye phase was conducted for two production years, fall 2017-2019. The experimental design at both sites in the rye phase was a split-split-plot randomized complete block with four replications. The main plot was with or without inter-seeded medium red clover, the split-plot cereal rye variety, and the split-split-plot fertilizer N rate. The main plots were 6 x 90 m, split-plots 3 x 90 m and the split-split-plots 3 x 15 m. The rye varieties were planted with 14 rows, row spacing 0.19 m.

Two cereal rye varieties were used, ‘ND Dylan’ and ‘Elbon’. The rye varieties were chosen as contrasting varieties. ND Dylan is a recent North Dakota 2016 release (“Northern variety”), Elbon an older Oklahoma 1956 release (“Southern variety”) with a lower winter hardiness than ND Dylan. Also, Elbon is commonly used as a rye cover crop in Iowa. Each fall the rye was planted no-till after soybean harvest, 19 Oct. 2017 for both sites and 24 Sept. 2018 at Ames and 23 Oct. 2018 at Kanawha. The planting dates were later than desired due to late soybean harvest and wet soil conditions that delayed planting. The target rye planting rate was

approximately 2.7 million pure live seeds (PLS) ha⁻¹, planted with a John Deere 750 no-till drill (Deere & Co., Moline, IL, USA). The drill planting rates were calibrated before planting.

Planting depth was 2.5 to 4 cm.

Urea fertilizer was applied at six total N rates (0, 28, 56, 84, 112 and 140 kg N ha⁻¹). At planting, 28 kg N ha⁻¹ was hand-applied to all plots except the zero N rate. In the spring, the remainder of the N rates were hand-applied shortly after spring plant green-up (for Ames and Kanawha, respectively, 5 Apr. and 24 Apr. 2018 and 28 Mar. and 1 Apr. 2019). Urea at each application was treated with Agrotain Advanced 1.0 urease inhibitor (NBPT) at the 2.1 L Mg⁻¹ product label rate (Koch Agronomic Services, Wichita, KS, USA).

The medium red clover, ‘Ruby’ brand, was “frost seeded” by hand application to the soil at 17 kg PLS ha⁻¹, respectively, for Ames and Kanawha on 12 and 13 Mar. 2018 and 27 and 28 Mar. 2019. The red clover seed was inoculated with *Sinorhizobium Meliloti* and *Rhizobium leguminosarum biovar trifolii* (Exceed, Visjon Biologics, Wichita Falls, TX, USA). No pest management products were applied to the rye during the growing season. When needed, hand weeding was done in the rye to reduce weed pressure.

Weather information (Figs. 1 and 2) was collected from recording stations located at each research farm (Arritt and Herzmann, 2019). Precipitation greater than normal preceding rye planting occurred in the late summer and early fall at each site and year. This resulted in wet soils and delayed soybean harvest and subsequently later than desired rye planting. Cool to below normal temperatures in the later part of Oct. and in Nov. contributed to slow rye emergence and a low amount of growth each fall, which would also impact fall tillering potential. Despite the low fall rye growth, and normal winter cold period, rye plants survived each year. Precipitation well above normal in May and June each year may have contributed to

observed degraded and dead rye leaf tissue (likely including disease incidence). Temperatures were near normal in the spring through summer, but summer temperatures high enough to potentially accelerate rye maturity. Accelerated rye maturity, along with high rainfall, likely compromised rye grain yield potential. The adequate spring and early summer precipitation allowed for successful red clover establishment.

Rye plant measurements

To minimize border effects, rye measurements and plant samples were collected from plot interior rows. A RapidScan CS-45 (Holland Scientific Inc., Lincoln, NE, USA) active canopy sensor was used to monitor in-season rye plant growth and N status. The sensor was hand carried at a constant 1.3 m s^{-1} speed and 0.6 m above the rye canopy, positioned perpendicular to the crop row (Barker and Sawyer, 2010). Mean values were recorded by the sensor (from 95 to 110 sensor readings per plot), collected from an approximate 12 m length of each plot. Sensing was conducted three times during the growing season: at jointing (Feekes' growth stage 6-7), full flag leaf emergence (Feekes' 9-10), and full head emergence (Feekes' 10.5). This sensor has been used as a tool for N management in field crops (Bronson et al., 2020). Mean near-infra-red (NIR, 880 nm), visible-red (VIS-R, 670 nm), and visible-red edge (VIS-RE, 730 nm) reflectance data were determined for each plot and used to calculate NDVI and NDRE [(NIR-VIS)/(NIR+VIS)] indexes for estimating plant canopy N status and growth response for each treatment.

Minolta SPAD-502 meter (Konica Minolta, Tokyo, Japan) readings were collected from the rye flag leaf or first leaf below the flag leaf at full flag leaf emergence. Measurements were collected midway between the stem and leaf tip. Each plot reading comprised an average from 20 randomly selected plants.

At full flag leaf emergence, the top two rye leaves (flag leaf or first leaf below the flag leaf) were collected from 50 random plants in each plot to determine leaf N concentration. Leaf samples were dried at 60 °C in a forced-air oven. The dried samples were ground to pass a 1-mm screen using a UDY Cyclone Sample Mill, model 3010-014 (UDY Corporation, Fort Collins, CO, USA). Leaf N concentration was determined by micro-Kjeldahl TKN digestion (Bremner, 1996) with colorimetric determination by flow-injection analysis (Egan, 2002).

Rye plant height and number of seed heads were measured at full head emergence (Feekes' 10.5). The height from the soil surface to the top of the seed head was measured on 10 random plants per plot. The number of heads were counted from three 0.9-m row segments in each plot.

Each entire rye split-split plot was harvested with a John Deere 9450 combine (Deere & Company, Moline, IL USA) modified and equipped for research plot grain harvesting in July 2018 and 2019. Rye grain yields were adjusted to 14 g kg⁻¹ moisture. Grain samples were collected at harvest for N analysis. The samples were dried in a forced-air oven at 60 °C, ground with a Baldor Mill (Baldor Electric Co., Ft. Smith, AR, USA), and N analyzed as described for leaf N analysis. Following grain harvest, the rye straw was baled and removed from the entire study area.

Soil profile sampling

Soil profile samples for NO₃-N determination were collected after rye harvest from the 0, 84, and 140 kg N ha⁻¹ rates, separately for with and without clover, but across rye varieties. Composite samples from 6 random cores were collected at 0-0.3 and 0.3-0.6 m depths using a soil probe with a 0.02-m dia. The samples were dried in a forced-air oven at 27 °C and ground to pass a 2-mm sieve. Soil was extracted with 2 M KCl (Gelderman and Beegle, 2011) and NO₃-N

determined with a Timberline TL-2800 dual channel analyzer (Timberline Instruments, Boulder, CO, USA) after reduction to ammonia (Carlson et al., 1990). Reported soil NO₃-N concentrations are on a dry weight basis.

Statistical analysis

Rye plant measurements were analyzed using a split-split-plot randomized complete block design across sites and years using PROC GLIMMIX in SAS 9.4. Site, year, replicates, and their interactions were considered random, with treatments and interactions considered fixed effects. Significant differences between treatment means were determined using LSMEANS with the LINES option providing t-grouping differences for mean comparisons and were considered significantly different at $P \leq .10$. Plant measurement responses to fertilizer N rate were fit with linear-plateau (LP) regression models using PROC NLIN. Analysis of covariance, PROC GLM, was also used to compare the linear regression component rate response between rye varieties. Also, single degree of freedom estimates determined with PROC GLIMMIX for N rate response and rate interactions with variety. With the LP response, the AONR is at the regression yield response join point, as is the economic optimum N rate (EONR) when the slope of the line is greater than the price ratio.

Corn Phase

The corn phase was conducted for two production years, 2019 and 2020. The experimental design at both sites in the corn phase was a split-plot randomized complete block with four replications. Main plots consisted of the red clover from the previous year seeding or no clover, and six N rates followed the rye phase N rate sub-plot sequence across rye variety.

Each main plot measured 6 x 90 m and each subplot 6 x 15 m. Corn was no-till planted with 8 rows per plot, 0.76 m row spacing.

The six fertilizer N rates (0, 56, 112, 168, 224 and 280 kg N ha⁻¹) were applied as SUPERU, urea treated with Agrotain and DCD (Koch Agronomic Services, Wichita, KS, USA). At planting, 56 kg N ha⁻¹ was broadcast-applied to all plots except the zero N rate. At the V3-V4 corn growth stage (Abendroth et al., 2011), the remainder of the N rates were broadcast-applied on 10 June 2019 and 4 June 2020 at Ames and on 11 June 2019 and 8 June 2020 at Kanawha.

The red clover had been “frost seeded” the previous spring in the cereal rye grain crop phase. The clover was left to grow within the rye crop and following rye harvest. The clover, as a cover crop, was not mowed or harvested throughout the summer or fall and left untouched until termination before the next year corn planting. There was no volunteer rye in the clover main plots. The no-clover main plots were mowed for weed control and had some volunteer rye growth. Prior to termination of the clover and volunteer rye, aboveground biomass samples were collected using a 0.25 m² area PVC square randomly placed in three spots from each of the corn 0, 168 and 280 kg N ha⁻¹ plots to determine dry matter and N content. The samples contained all aboveground biomass, including rye straw, clover material from the prior-year growth and new spring regrowth, or volunteer rye. The samples were oven-dried at 60 °C, weighed, ground to pass a 2-mm screen with a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and analyzed for N concentration by micro-Kjeldahl TKN digestion (Bremner, 1996) with colorimetric determination by flow-injection analysis (Egan, 2002).

The clover and any volunteer rye were terminated on 24 April 2019 at both sites with glyphosate [N-(phosphonomethyl) glycine] and 2,4-D amine (2,4-dichlorophenoxyacetic acid). In 2020, clover and any volunteer rye were terminated on 21 April and 24 April at Ames and

Kanawha, respectively, with application of glyphosate and flumetsulam plus clopyralid potassium salt (Hornet®, Dow Agrosiences, Midland, Michigan, USA). The rates varied between sites and years, as needed.

Corn was no-till planted at 86,000 seeds ha⁻¹ both years at each site, intended 1-2 weeks after clover and volunteer rye termination. At Ames, Pioneer P0825AMXT (108-d relative maturity), pre-treated with Poncho +Votivo (BASF, Florham Park, NJ, USA) was planted on 16 May 2019 and 29 April 2020. At Kanawha, Dekalb DKC54-38SS Smartstax (104-d relative maturity), pre-treated with Acceleron (Bayer CropScience, Research Triangle Park, NC, USA) was planted on 16 May 2019 and 1 May 2020. In 2019 rainfall and wet soil conditions caused delayed planting. The planters at each site were equipped with row cleaners, furrow openers, and closing attachments appropriate for no-till planting into a cover crop.

Weed control at Ames included pre-emerge application of thienencarbazone-methyl plus isoxaflutole (Corvus, Bayer Cropscience) and glyphosate in 2019 and dimethenamid-P (Outlook, BASF) and glyphosate in 2020. Post-emerge herbicide application was topramezone (Impact, AMVAC Chemical Corporation) both years. Weed control at Kanawha included pre-emerge application of Dimethenamid-P in 2019 and dimethenamid-P and glyphosate in 2020. Post-emerge herbicide application was clopyralid (Stinger, Dow AgroSciences), mesotrione (Callisto, Syngenta) and glyphosate in 2019 and glyphosate and tembotrione (Laudis, Bayer CropScience) in 2020.

The summer through early fall precipitation each year (after rye grain harvest) at both sites allowed for excellent clover growth and low weed competition in the clover cover crop, especially in 2019 (Figs. 1 and 2). Air temperatures were near normal through the 2019 corn growing season, but above normal in the summer 2020. Spring through early summer

precipitation at each site was above normal in 2019, but below normal in the summer 2020. The difference in summer precipitation and temperature in 2020 compared to 2019 resulted in slightly lower yield in 2020, however, corn yield was still high in 2020.

Corn plant measurements

Canopy sensing measurements were collected at the V8-V9 growth stage using a RapidScan CS-45 active canopy sensor (Holland Scientific Inc., Lincoln, NE, USA). The sensor was hand carried at a constant 1.3 m s⁻¹ speed and 0.6 m directly above the crop row (Bean et al., 2018). Reflectance values recorded by the sensor were collected from an approximate 12-m length of center rows in each plot. Mean reflectance values were recorded by the sensor (from approximately 90 to 110 sensor readings per plot). This sensor has been used as a tool for N management in field crops (Bronson et al., 2020). Mean near-infra-red (NIR, 880 nm), visible-red (VIS-R, 670 nm), and visible-red edge (VIS-RE, 730 nm) reflectance data were determined for each plot and used to calculate NDVI and NDRE [(NIR-VIS)/(NIR+VIS)] indexes for estimating plant canopy N status and growth response for each treatment. Minolta SPAD-502 chlorophyll meter (Konica Minolta, Tokyo, Japan) readings were collected from the ear leaf at the R1 growth stage. Measurements were collected midway between the stem and leaf tip and midway between the leaf edge and midrib (Peterson et al., 1993). Each plot mean comprised an average from 20 randomly selected plants from the two center rows. For the canopy sensing indexes and chlorophyll meter readings, relative values were calculated by dividing the average value for each N rate by the corresponding average value from the highest N rate (Hawkins et al., 2007).

Corn plant population was evaluated after the second N application (V4-V6 growth stages) by counting a 6-m length in the two center rows in each plot. Corn grain yield was

determined by harvesting the middle 4 rows of each sub-plot with a plot combine and reported at 155 g kg⁻¹ moisture. Across years and sites, corn was harvested between 22 September and 28 October.

Soil profile sampling

Soil samples (0-0.6-m depth in 0.3-m increments) were collected from the 0, 168 and 280 kg N ha⁻¹ rates to determine soil profile NO₃-N, separately for with and without clover in the Spring (at time of biomass sampling), Late Spring (early -to-mid-June, when corn height was approximately 15- to 30-cm from soil surface to center of corn whorl), and Fall (post-corn harvest). The samples were a composite of six random cores collected using a 0.02-m dia soil probe. Soil samples were dried in a forced air oven at 27 °C and ground to pass a 2-mm sieve. Soil was extracted with 2 M KCl (Gelderman and Beegle, 2011) and NO₃-N determined with a Timberline TL-2800 dual channel analyzer (Timberline Instruments, Boulder, CO, USA) after reduction to ammonia (Carlson et al., 1990). Reported soil NO₃-N concentrations are on a dry weight basis.

Statistical analysis

Corn plant measurements were analyzed using a split-plot randomized complete block design, main plot with and without clover cover crop and N rate sub-plot, across sites and years using PROC GLIMMIX in SAS 9.4. Site, year, replicates, and their interactions were considered random, with treatments and interactions considered fixed effects. LSMEANS with the LINES option was used to assess the difference between treatment means. Main effects and all interactions were considered significantly different at $P \leq .10$. Plant measurement responses to fertilizer N rate were fit with a quadratic-plateau regression model using PROC NLIN. The N

supply to corn from the clover cover crop was estimated as the difference in the AONR from the regression model fits for with and without the clover cover crop. The EONR was determined from each regression model at a 5.6 N fertilizer to corn grain price ratio (\$0.88 kg⁻¹ N and \$0.157 kg⁻¹ grain) (Cerrato and Blackmer, 1990). The N rate difference from the EONR (dEONR) was calculated for each applied N rate as the N rate minus the EONR. To determine the relationship between dEONR and relative sensing index values, the relative values were regressed against the corresponding dEONR (Hawkins et al., 2007) with PROC NLIN used to fit corresponding quadratic-plateau regression models.

Soil NO₃-N concentrations for each phase were analyzed separately by each sample depth for with and without clover cover crop and the three N rates, across sites and years using PROC GLIMMIX, as well as for the profile NO₃-N amount within the 0-0.6 m depth. Site, year, replicates, and their interactions were considered random, with clover, N rate, and interactions considered fixed effects. Main effects and interaction LSMEANS were considered statistically different at $P \leq .10$ using the LINES option.

CHAPTER 3. RESULTS AND DISCUSSION

Cereal Rye Phase

As cereal rye is not a traditional cash grain crop grown in Iowa, information was lacking on response to N fertilization. Therefore, one main goal of the project was to study rye plant and yield response to applied N rate and determine the optimal N application rate. Therefore, several plant measures were studied to confirm response and productivity. A second main goal was to determine effect of inter-seeded red clover on rye N response and productivity, and the subsequent impact on N response in a corn crop following rye and clover. This section focuses on the rye phase of the project.

Plant measurements

The number of rye seed heads at maturity (indication of rye plant density and tillering) was different for the two varieties and was influenced by N rate (Table 2). Inter-seeded clover had no effect on head count and there was no interaction between variety, N rate, and clover (Table 2). The number of seed heads was greater for ND Dylan than Elbon (Table 3), approximately 590,000 more seed heads ha^{-1} for ND Dylan. The variety difference was also visually observed in the fall plant establishment and spring growth each year. The PLS planting rate was planned to be the same for both rye varieties, so differences noted and measured are assumed to be due to seed quality or adaptation differences to the late planting and winter cold period. Nitrogen response was evident in both varieties, with a linear response to 122 and 128 kg N ha^{-1} for ND Dylan and Elbon, respectively (Tables 2, 3, and 4 and Fig. 3). Similar N rate effect was reported by Graham et al. (1983), with rye number of heads reaching a maximum at 105 kg N ha^{-1} . The head count response to N rate is evidence that tillering increased with N application

and would be an important component for grain yield. However, N application did not remove the head count difference between varieties.

Rye plant height was different for the two rye varieties, although the difference was small (Tables 2 and 3), and height was influenced by N rate (Table 2). Inter-seeded clover had no effect on rye plant height and there was no interaction between variety, N rate, and clover. Wiersma et al., 2005 reported in another small grain, hard red spring wheat (*Triticum durum* Desf.), that height was not affected by inter-seeded clover. Plant height N response was evident in both rye varieties, with a linear response to 70 and 83 kg N ha⁻¹ for ND Dylan and Elbon, respectively (Table 4 and Fig. 3). However, the N rate effect on plant height was small at approximately 10 cm. Graham et al. (1983) also reported an increase to a maximum at 70 kg N ha⁻¹ in rye plant height from applied N. Overall, cereal rye varieties can be quite tall, and that was the case in this study for ND Dylan and Elbon (mean height 140 cm). Due to the general tall nature of the rye, and the relatively small N rate effect on height, there was some but small observed increase in plant lodging with N application (noted at grain harvest). Visual lodging scores were attempted in the study, but were found to be difficult to score due to random wind induced lodging across the study areas and as rye plants tended to lodge with no N applied or across the N rates. Therefore, lodging scores are not presented, just the mention that N application appeared to increase lodging and that the highest N rates did not result in extreme lodging (flattened plants) as can occur with too much N applied to small grains.

Seed count (seeds g⁻¹) was determined on harvested grain, however, the counts were determined after drying grain for N analysis. This may have affected results as there was no difference in the seed counts for variety or N application (data not shown).

Leaf and grain nitrogen

Rye leaf and grain N concentrations were different for the two rye varieties, although the difference was small for grain N, and both influenced by N rate (Tables 2 and 3). For leaf and grain N, Elbon had greater concentrations than ND Dylan. There was an interaction between variety and N rate for leaf N. Interestingly rye leaf N concentration was lower when inter-seeded with clover, but the difference was small (Table 3). That could be an indication of clover competing for soil N, but that would be minimal competition as the clover growth was small at the leaf sampling stage. The season-long clover presence did not affect grain N concentration. For leaf and grain N there was no interaction between clover, variety, and N rate. Nitrogen response was evident in both varieties, with a linear response to the highest applied N rate for grain N concentration, and nearly the highest rate for leaf N (Table 4 and Fig. 4). The increase in grain N concentration from zero to the highest N rate was small, and larger for the leaf N concentration (Table 4 and Fig. 4). This indicates a greater plant tissue N response to N rate than in grain. Despite the variety x rate linear interaction for leaf N concentration, the maximum response rate was similar at 117 and 137, respectively, for Elbon and ND Dylan. This indicates Elbon achieved a greater leaf N concentration than ND Dylan at a lower applied N rate. This may be a reflection of the plant density difference, where Elbon had a lower seed head count and thus less plant stand and biomass at time of leaf sampling (and as observed visually).

Cereal rye canopy sensing

The rye plants were sensed at three growth stages with an active canopy sensor and leaves once with a SPAD meter. Two canopy sensing indexes were determined, NDRE (red-edge based) and NDVI (red-based). The NDRE is a newer index as it employs the red-edge band. The NDVI is a well-established index that employs the red-band, and has been documented many

times to express relative vegetative biomass and plant N stress. It is assumed NDRE will be similar, although less studied in crops for N stress measurement. The absolute index values are different between NDRE and NDVI, and thus cannot be used directly for index comparisons of N stress or N response – that is each needs to be evaluated separately unless a relative approach would be employed and determined to be effective (potential rye variety effects, as noted in this research, would also need to be studied).

For all of the sensing methods and timings throughout the season, there were no measured effects due to the presence of inter-seeded clover (Tables 5 and 6). This is likely due to the small amount of clover biomass present as it established and grew under the rye canopy. Only after rye grain harvest, and with adequate moisture, will the clover grow rapidly.

For the first and second canopy sensing timings, and SPAD leaf measurement, N rate, variety, and their interactions influenced sensing results (Table 5) – an indication of N response and concurrent leaf/plant N status – and differences between varieties. In the third canopy sensing, no interaction between variety and N rate was found.

Overall, NDRE and NDVI index values were greater for ND Dylan than Elbon at all three sensing times (Table 6), reflecting the lower Elbon variety plant stand visually observed early in the spring and measured later in the seed head counts. That is, lower stand after the winter period and less tillering with Elbon than ND Dylan. The lower index values for Elbon would also indicate that ND Dylan responded more (more plant material or tillering) to N fertilizer application in the early part of the season. The difference in canopy index values between varieties were also consistent across N rates (Figs. 5 and 6).

Both rye varieties responded positively to N rate, with a linear increase in sensing index value to a maximum response (Table 4 and Figs. 5 and 6). Despite differences in index values

between varieties (first two sensing timings), the maximum response N rate was similar. Also, the maximum response rate increased with the sensing timing, that is, increased as the season progressed – ranging from about 90 to 120 kg N ha⁻¹ as sensing timing progressed. The sensing response was similar for both indexes, indicating each index produced a similar measure of plant canopy and N response. The lack of variety difference at the third sensing (full head emergence) could be due to a full canopy and seed head presence causing saturation of the wavelengths or seed head interference.

Opposite of the canopy index values, SPAD readings collected at full flag leaf emergence (second sensing timing), were significantly greater for Elbon than ND Dylan (Table 6). This is the same variety effect as found for the leaf N concentration and thus a possible reflection of the plant density difference between varieties (less with Elbon), or simply a varietal difference. Leaf SPAD values were less responsive to N rate than canopy sensing indexes (Table 4 and Figs. 5, 6, and 7). Despite a variety x N rate interaction (the difference in SPAD values narrowed with increasing N rate), the maximum N rate response was the same at 118 kg N ha⁻¹.

Rye grain yield

Rye grain yield was different for the two rye varieties, increased by fertilizer N addition, and with an interaction between variety and N rate (Table 2). The ND Dylan variety on average produced approximately 900 kg ha⁻¹ more than Elbon (Table 3). There was no effect of the interseeded clover on grain yield for either rye variety. The positive effect of N rate found for the various plant measures – canopy sensing, leaf SPAD, leaf N, and head count translated into increased grain yield.

The yield response to N rate was linear, reaching a plateau for each variety (Tables 2 and 4). However, the rate of yield increase was greater for ND Dylan than Elbon. The yield for ND

Dylan without N applied was higher than Elbon, and with the greater linear response for ND Dylan, ND Dylan also had higher yield than Elbon at the AONR, 3120 versus 2119 kg ha⁻¹, respectively (Table 4 and Fig. 8). Despite the variety difference in yield level, the AONR was similar for both varieties, 95 and 100 kg N ha⁻¹, respectively, for ND Dylan and Elbon. As long as the N price to grain price ratio remains less than the linear slope of each variety (Table 4), the economic optimum N rate will be at the AONR and application of N will be profitable for cereal rye grain production. This research clearly shows that improved rye grain production occurs with N application when rye follows a soybean crop in Iowa environmental conditions – for Elbon a 41.8% yield increase and for ND Dylan a 44.3% yield increase. And that a profitable application rate occurs at 95 to 100 kg N ha⁻¹. Study of more rye varieties, and at more sites (environments), would be helpful to confirm these results and help to further inform needed application rate across Iowa.

Post-harvest soil nitrate

Post-rye harvest soil profile NO₃-N (0.6 m depth) was sampled across rye varieties. The concentration for each soil depth and the profile amount of NO₃-N was influenced by N rate, but no effect of the inter-seeded clover and no interaction between clover and N rate (Table 7). An impact of inter-seeded clover would be expected to be low as the clover growth is small during the rye production period until harvest time. The concentration and amount of NO₃-N increased with increasing N rate, however, the profile increase was small at only 2 and 5 kg N ha⁻¹ more than the zero N rate for the 84 and 140 kg N ha⁻¹ applied N rates, respectively (Table 8). The AONR for both varieties was at 95 to 100 kg N ha⁻¹, which is close to the 84 kg N ha⁻¹ rate sampled for profile NO₃. This indicates that at the optimal N rate, there would be minimal effect

on residual NO_3 . Even with an N rate 40 kg N ha^{-1} higher than the AONR, the effect on profile residual $\text{NO}_3\text{-N}$ was small.

These results reflect the rapid uptake of N during small grain growth, and during the spring period when the fraction of total loss of NO_3 from the soil to drainage water is highest. Hence, a small grain crop like cereal rye grown for seed production would have a positive effect on NO_3 loss compared to annual crops like corn and soybean. In addition, although the inter-seeded clover had no effect on post-rye harvest profile $\text{NO}_3\text{-N}$, continued clover growth (not mowed) observed after rye harvest each year would indicate continued uptake of NO_3 from the soil profile and thus a positive influence on moderating NO_3 in the soil from mineralization or residual from N applied to the rye crop. There was some volunteer rye and weed growth following harvest in the non-clover plots (managed by mowing), which would also act as a cover crop taking up residual or mineralized N. However, although not measured in the summer or fall, the amount of volunteer rye/weed growth was substantially less than the clover growth.

Corn Phase

Following the project's main objectives, this section comprises results obtained from the corn phase of the project. Our primary goal for this phase was to document the effects of including a cereal rye small grain crop in the rotation with the subsequent impact of a red clover cover crop on corn N response, N fertilization requirement, and grain yield.

Aboveground cover crop biomass

The aboveground biomass dry matter at time of the red clover control in the spring was greater with the clover cover crop than without (Tables 9 and 10). The same result occurred for total N uptake. The N fertilizer rate applied to the prior rye crop ($0, 84, \text{ and } 140 \text{ kg N ha}^{-1}$) did

not affect dry matter biomass or total N, and there was no interaction between the clover cover crop and the prior year N rate. The lack of N rate effect on biomass due to the N applied to the prior-year rye could be expected as the post-rye harvest profile $\text{NO}_3\text{-N}$ was low with little difference between N rates.

The aboveground vegetation sampled at time of clover control contained all material present; which included straw from the prior rye crop, volunteer rye, and clover. In the clover cover crop, the predominant vegetative material was dead clover material from the prior year growth and new clover spring growth, and any remaining rye straw (the rye straw was baled the prior summer). In the non-clover cover crop, the predominant vegetative material was volunteer rye, remaining rye straw, and dead weed material from the prior year growth (no weed growth in the spring).

At clover termination, the mean aboveground vegetative dry matter was $2047 \text{ kg DM ha}^{-1}$ greater (2.4 times more) with the clover cover crop than without the clover (Table 10). Although not separated, a main component of the clover biomass was dead material from the prior year clover growth. In conjunction with the greater biomass, there was 34 kg N ha^{-1} more (2.1 times more) total aboveground N with the clover cover crop than without the clover (Table 10). The below ground clover root system was not sampled, so there was potential for more clover N present than just in the aboveground material; especially as the spring sampling occurred as the clover was just beginning regrowth. Also, the presence of rye straw, with low N concentration, in the sampled material would dilute the N contribution of clover alone. Therefore, the material sampled at time of clover control would be representative of above ground material present in the clover following a baled but not mowed prior-year rye crop that was harvested for grain.

Those effects could make it difficult to directly relate aboveground biomass and N to corn response to clover N supply amount.

In both years of study, abundant precipitation in the summer after rye grain harvest provided adequate soil moisture and therefore contributed to good clover growth and some volunteer rye establishment. The soil moisture also allowed for weed growth in the non-clover, but not in the clover cover crop. Clover cover crop (green manure crop) aboveground biomass has varied considerably in various studies. Liebman et al. (2011) compared grain oat residue (inter-seeded with red clover, and no clover with some volunteer oat growth after grain harvest) for aboveground biomass and N content. In that study with termination in the spring, oat-clover produced 5863 kg DM ha⁻¹ across years and sites, compared to 3843 kg DM ha⁻¹ for oat alone, with 197 and 45 kg total N ha⁻¹, respectively. Those amounts are more than measured in our study, which could be due to timing of sampling/control in the spring. A meta-analysis study assessing cereal rye cover crop aboveground biomass in the U.S. Midwest reported mean dry matter production of 1032 kg ha⁻¹ across years (Martinez-Feria et al., 2016). That mean dry matter with the cereal rye was similar to the no clover/volunteer rye in our study, but less than the clover cover crop. Liebman et al. (2018) found that across various legume cover crops planted in the fall the mean biomass was 6225 kg dry matter ha⁻¹ and 176 kg N ha⁻¹. Again, more than in our study with clover, but that could also be due to length of growth period in the spring. Cover crop biomass accumulation is influenced by environment, cultivar, date of termination, and other factors (Appelgate et al., 2017). Therefore, cover crop biomass measured at the time of spring termination is not by itself a good predictor for cover crop effects on N supply and corn grain yield response (Martinez-Feria et al., 2016).

Corn canopy sensing

The corn canopy was sensed at the V8-V9 growth stage with an active canopy sensor and ear leaf at the R1 stage with a SPAD meter. Two canopy sensing indexes were determined, NDRE (red-edge based) and NDVI (red-based). The NDRE is a relatively new index as it employs the red-edge band. The NDVI is a well-established canopy index and has been document to express vegetative biomass and N stress. The NDRE index is less studied in corn for N stress measurement.

Canopy sensing NDRE and NDVI indexes were influenced by N rate and the interaction of clover and N rate (Table 11). The clover alone did not affect canopy NDVI or NDRE index values, indicating no overall change in corn early plant canopy with the clover cover crop. There was a clover x N rate quadratic interaction for canopy NDRE, a quadratic NDVI response to N rate, and a linear interaction for NDVI. These interactions indicate different corn growth response to N rate with and without the clover cover crop.

Table 12 gives the NDRE and NDVI regression models, with graphs provided in Fig. 9. The overall increase in canopy sensing values was small across N rates. The NDVI detected only a small difference in corn canopy response to the clover, that is, did not readily detect an N supply from the clover legume cover crop. However, the NDRE index did have an increase when no N was applied and at the lowest fertilizer N rate. That indicates an early season corn N response to N from the clover cover crop. As is typical with early - to mid-season canopy sensing, the overall absolute index value response to N rate was low, and canopy index values reached a plateau with adequate N. Canopy sensing provides an indication of corn response to N supply and when a maximum response (plateau join point) is achieved. For the without clover, the maximum response N rate (AONR) was at 152 and 126 kg N ha⁻¹ with NDRE and NDVI, respectively, but was only 95 and 87 kg N ha⁻¹ with the clover. That response difference with

clover indicates a rate difference of 39-57 kg N ha⁻¹ being supplied from the clover (Table 12). As the NDRE was more sensitive to N response (and the larger interaction with clover), the N rate difference estimation with and without the clover was more reliable with the NDRE than NDVI.

The mid-vegetative corn canopy response to N rate was similar to other studies in the U.S. Midwest (Sripada et al., 2008; Kitchen et al., 2010; Roberts et al., 2010). The measured small index responses indicate low N stress when N application was deficient, as was visually observed in zero N plots at the time of sensing; especially with clover. As N rate increases, the amount of plant available N increases, improving canopy biomass and therefore increasing sensing values. Furthermore, the red-edge waveband was better at detecting N deficiency, meaning it would be more sensitive to N stress than the red band. It has been documented that the NDVI underestimates optimum N rate and is less sensitive to variation in EONR values when compared to NDRE (Bean et al., 2018). However, as expected with canopy closure and adequate N, both NDVI and NDRE indexes remained constant, indicating that more than adequate N does not translate into greater corn canopy development or change N stress indication.

Relative sensing index values are often more useful than index values when other field factors (corn hybrid, weather, soil conditions, plant stand, other nutrient deficiency) cannot be controlled (Barker and Sawyer, 2010). That is, differences in canopy structure and plant color can be reduced with relative values, with more emphasis on N response. One method to use relative index values for estimating N response is to determine the relationship between a relative index value and the N rate difference from the EONR (dEONR); that is, regress the relative index values (rNDRE and rNDVI) vs. dEONR. Those quadratic-plateau regression parameters are provided in Table 13, and graphically presented in Fig. 10. The relative index values with

and without the clover follow the same regression response, but with an dEONR offset with the clover cover crop. This result is important as it indicates that dEONR and relative indexes can be used for evaluating a legume cover crop effect on corn N response at the mid-vegetative period. That is, the canopy sensing N stress was not indirectly affected by with or without the prior clover.

As other research has found, the rNDRE and rNDVI values are lowest with the greatest N deficiency, increase as the N rate approaches the dEONR, and then plateau at adequate to more than adequate N (Barker and Sawyer, 2010) (Fig. 10). The rNDRE had a greater spread in relative values than the rNDVI when N was deficient, as found for the absolute index values, which indicates greater sensitivity with the red-edge band (NDRE). Both the rNDRE and rNDVI reached a plateau at 1.0, and with a similar join point at -59 and -62 kg N ha⁻¹, respectively. Prior research has shown that additional N application is indicated when the relative index value is less than zero dEONR (Barker and Sawyer, 2010). In this study, for both indexes, the value at zero dEONR was the same as the value at the joint point, as the latter was less than zero. Often the relative index values near 0.95 are used for indication of N application need. In this study the plateau was at 1.0 and a rate <0 dEONR for both indexes, possibly indicating the V8-V9 canopy sensing was underestimating season long N stress.

Like the earlier season canopy sensing, SPAD meter ear leaf sensing at the R1 growth stage indicated an N rate response and a quadratic interaction with the clover cover crop (Table 11). However, the leaf SPAD values showed a larger difference between with and without clover when N was deficient than found with the canopy sensing (Table 12 and Fig. 11), and with a much larger range in relative values (rSPAD) (Table 13 and Fig. 12). This larger difference compared to canopy sensing was probably due to the SPAD readings being taken later in the

season (R1 stage), meaning there was more time for N mineralization from the clover terminated in spring, increasing N supply for the following corn plants, and therefore increasing the SPAD difference between with and without clover cover crop. However, the AONR difference between with and without clover was only 23 kg N ha⁻¹, a much lower N supply amount from the clover than estimated with canopy sensing.

The rSPAD values plateaued below 1.0 (at 0.98), which is more like sensing values in other research (Hawkins et al., 2007) and lower than found with the canopy sensing results. Also, the dEONR join point was close to zero dEONR (-12 kg N ha⁻¹) which is a better relation to optimal N. As expected, with N rates greater than the zero dEONR, rSPAD values did not increase with increasing applied N or N supplied from the clover, the same as other research that has shown SPAD meter sensing is not useful to detect more than needed plant available N (Hawkins et al., 2007). And as expected, rSPAD values decreased with increasing deficit N and were an indicator of deficit N amount. The rSPAD values followed the same regression response, with an offset of dEONR due to N being supplied by the clover. That result indicates that SPAD readings can be useful for determining corn N response with or without a legume cover crop, that is no interference due to corn growing after the clover, and useful for detecting potential clover N supply to corn.

Corn grain yield

Weather conditions during both growing seasons were good for corn production, with some in-season moisture stress in 2020 (Figs. 1 and 2). Corn plant population was the same with and without the clover cover crop (data not shown). Grain yield responded to N fertilizer rate and to the clover cover crop (Tables 11 and 12). There was an interaction between clover and N rate, with a clover x N rate quadratic response (Table 11). Mean grain yield across all fertilizer N rates

was 8.5% higher with clover (10850 kg ha^{-1}) than with no clover (10000 kg ha^{-1}). Rutan and Steinke (2019) observed an average 13% higher corn grain yield following cover crops compared to a no cover crop control (with no mineral N fertilization). According to a study conducted by Norris et al. (2020), regardless of N rate, corn yield following a legume cover crop was greater than following rye. Decker et al. (1994) also observed higher corn yields following legume cover crops compared to a winter wheat cover crop, cover crop mixtures, or fallow. This is probably because clover fixed/accumulated more N than other cover crop species, increasing corn grain yield.

As there was an interaction of clover and N rate for grain yield, and like canopy sensing, the corn yield response to the clover without N applied and at the low N rates was different than at adequate N (Fig. 13). The yield without applied fertilizer N, and at low N rates, was higher with the clover than without, indicating N supply from the clover and therefore higher yield due to additional N supply to the corn. This was also noted in the canopy and leaf N sensing measurements. The higher corn yield at no to low N rates would therefore affect an overall mean yield comparison (across all N rates) for with and without the clover cover crop – that is, a higher yield following the clover cover crop. However, at the optimal fertilizer N rate (AONR) to more than the optimal rate, the yield with clover was 4% lower than without clover (Table 12 and Fig. 13), but not different at the highest fertilizer rate applied. It is not known why that yield difference at optimal N occurred. Such yield difference at optimal N has been noted in Iowa with a cereal rye cover crop (Pantoja et al., 2015; Patel et al., 2019). Others have found that at low water stress conditions, cover crops may not adversely affect corn yield (Reese et al., 2014). Pantoja et al. (2015) found no optimal N rate difference with or without a cereal rye cover crop.

That indicates the N response in our study where there was some volunteer rye would be the same as if no volunteer rye were present.

Like canopy (NDRE) and leaf (SPAD) sensing, lower grain yield occurred without clover when fertilizer N application was deficient, indicating corn N responsiveness to the clover cover crop. There was clearly an N supply from the prior clover growth. At zero N, grain yield was 2610 kg ha⁻¹ greater following clover than without clover. The AONR with no clover (228 kg N ha⁻¹) was greater than the AONR with clover (171 kg N ha⁻¹), indicating approximately 57 kg N ha⁻¹ supplied from the clover cover crop. The N amount supplied from the clover is similar to that found in prior research (Gaudin et al., 2013; Gentry et al., 2013; Westra, 2015) and within ranges provided in state nutrient management guidelines for red clover N credits (Fernandez et al., 2009; Gibson et al., 2006; Kaiser et al., 2020; Laboski and Peters, 2012). The NDRE canopy sensing indicated a 57 kg N ha⁻¹ lower AONR with the clover, the NDVI canopy sensing a 39 kg N ha⁻¹ lower AONR, and the SPAD leaf sensing a 23 kg N ha⁻¹ lower AONR. While there was variation in the various estimates, all of the plant measures indicated N supply from the clover. Interestingly, the NDRE canopy sensing, while an early season measure and with a low difference in sensing values between with and without the clover, had the same difference in AONR between with and without clover (at 57 kg N ha⁻¹) as for the yield response. Having the same indication of clover N supply to the corn for the yield and NDRE is an indication that the red-edge NDRE index could be a good indicator of N stress and potential optimal N fertilization. That is also indicated in the dEONR response where there was a greater spread in rNDRE values than with rNDVI.

Soil profile nitrate

Soil profile $\text{NO}_3\text{-N}$ varied throughout the corn growing season, and generally was influenced by the clover cover crop and N rate (depending on sample timing, either the rate applied to the prior-year rye crop or the N applied to the corn) (Tables 14 and 15). There was only occasional interaction between clover and N rate. The soil sampled at the time of clover control (Spring) would reflect $\text{NO}_3\text{-N}$ due to the clover, volunteer rye, and N applied to the prior-year rye crop. The soil sampled later in the spring (Late Spring) would reflect $\text{NO}_3\text{-N}$ due to the clover and split-N applied at time of corn planting (56 kg N ha^{-1} to all rates except the no-N control). The soil sampled post-corn harvest (Fall) would reflect the combination of the total fertilizer N applied to the corn and N supplied by the clover.

At the time of clover control, soil $\text{NO}_3\text{-N}$ concentration and profile total was influenced by N rate applied to the prior-year rye, but the difference was small and interestingly was lowest for the highest fertilizer N rate (140 kg N ha^{-1} rate) (Table 15). Overall, the amount of profile $\text{NO}_3\text{-N}$ was low at the time of clover control. The $\text{NO}_3\text{-N}$ concentration and profile total was increased with the clover cover crop, with a mean 5 kg ha^{-1} higher profile total $\text{NO}_3\text{-N}$ compared to without clover. While not a large difference, the clover increased profile $\text{NO}_3\text{-N}$. Since there was some volunteer rye in the non-clover, that volunteer rye would act as a cover crop and hence could have some effect on reducing the amount of profile $\text{NO}_3\text{-N}$ at time of control (Pantoja et al., 2015). The rye is also not a legume, and therefore would not supply N before control as could occur with the clover. It is possible that dead aboveground clover tissue (clover was not mowed or harvested the prior summer/fall and above ground tissue had been left to die the prior fall) had released N into the soil in the fall and early spring period.

At the Late Spring sampling time (early-mid June), the effect of the split-N applied at time of corn planting was evident, where the $\text{NO}_3\text{-N}$ concentration at each depth and profile total was greater than the zero N control where no N was applied (Table 15). This sampling time occurred before the second N fertilizer application and at this point the amount of soil $\text{NO}_3\text{-N}$ would be comprised of any residual rye crop N fertilization from the previous year, the first split-N fertilizer applied to the corn, (same for all N rates except the no-N control), and the effect of the clover and volunteer rye. Hence, the concentrations and total $\text{NO}_3\text{-N}$ for the 168 and 280 kg N ha^{-1} rates were not different (11.8 mg kg^{-1} , and 67 to 70 kg $\text{NO}_3\text{-N ha}^{-1}$, respectively), but differed from the zero N rate (4.7 mg kg^{-1} and 30 kg $\text{NO}_3\text{-N ha}^{-1}$, respectively). The overall higher $\text{NO}_3\text{-N}$ values at the Late Spring sampling time compared to the Spring sampling at clover control were expected as time after clover control, adequate moisture, adequate temperature, and killed clover would allow soil and clover N mineralization to occur. The increase in profile $\text{NO}_3\text{-N}$ from the Spring to Late Spring was 18 kg N ha^{-1} . The soil $\text{NO}_3\text{-N}$ concentration and profile total was greater with the clover cover crop than without. However, the concentration in the 0-0.3m depth (4.7 mg $\text{NO}_3\text{-N kg}^{-1}$) was well below the late spring soil nitrate test (LSNT) 25 mg kg^{-1} $\text{NO}_3\text{-N}$ critical concentration (Sawyer and Mallarino, 2017) with or without the clover, indicating expected response to additional N application. The lack of difference in LSNT concentration (zero N rate) between with and without the clover also indicated a low supply of N from the clover cover crop at that late spring timing. The mean profile total increase with clover was only 9 kg N ha^{-1} , which could be a reflection of N being supplied from both the killed clover and volunteer rye, and a reflection of the short time period for N to be released from decaying clover above- and below-ground tissues. This effect (low clover N supply effect on sensing index values) was found in the corn canopy sensing at the V9

corn stage. Time for more N mineralization from the clover was indicated by the R1 stage SPAD sensing values.

In the post-harvest sampling (Fall), the soil $\text{NO}_3\text{-N}$ concentration and profile total were increased by the corn N rate and the clover cover crop. In the first soil depth samples, and in the profile total, there was an interaction between N rate and clover. The effect was a greater concentration and greater profile total with the clover than without as N rate increased. Those results indicate the N being supplied from the clover and the lower optimal N application rate required for corn following the clover. Therefore, the combination of high N rate and clover N supply resulted in greater soil profile $\text{NO}_3\text{-N}$ when the fertilizer N rate was more than optimal; leaving increased $\text{NO}_3\text{-N}$ in the profile. To avoid increased end of season residual profile $\text{NO}_3\text{-N}$, the corn fertilization rate must take into account N being supplied by a clover cover crop. Use of a cover crop after the corn would help to lessen residual profile $\text{NO}_3\text{-N}$.

Research has broadly reported that the replacement of bare fallow periods with cover crops increase retention of soil inorganic N, a source of NO_3 to water systems and an obligatory substrate for gaseous production of nitrous oxide (McCracken et al., 1994; Behnke et al., 2020). In our study, the with and without clover cover crop had different amounts of profile $\text{NO}_3\text{-N}$ in the Spring sampling. However, the amount was not widely different because non-clover plots were not absent a cover crop as volunteer cereal rye was present, potentially sequestering N in the growing biomass since seed was left after rye grain harvest the previous summer. Furthermore, the efficiency of cover crops in reducing soil NO_3 is directly related to the amount of biomass produced, which is mainly affected by cover crop uniformity, precipitation, and temperature conditions in the fall and early spring (Kaspar et al., 2012; Kladvko et al., 2014; Meisinger and Ricigliano, 2017). With the clover inter-seeded with the rye grain crop, the clover

has an advantage as a cover crop as it has an extended period for establishment from March through July (seeding to rye grain harvest) and non-competitive growth after the rye harvest from July through the fall.

Agronomic practices that reduce excess soil NO_3 are globally important as NO_3 is the essential substrate for nitrous oxide production (Behnke et al., 2020) and that NO_3 leaching contaminates surface water systems, resulting in eutrophication and drinking water issues (Kladivko et al., 2014; Tonitto et al., 2006). A meta-analysis study conducted by Tonitto et al. (2006) compared conventional (inorganic fertilizer with a winter-bare fallow) with several diversified (legume and non-legume cover crops) systems. They found that post-harvest soil $\text{NO}_3\text{-N}$ was similar in both fertilizer-based and green manure systems. However, $\text{NO}_3\text{-N}$ leaching was reduced by 40% in legume cover crop systems (unfertilized) and 70% in non-legume cover crop systems (fertilized), compared to the conventional (fertilized with no cover crops). In our study, soil profile $\text{NO}_3\text{-N}$ was increased with the clover cover crop, with or without fertilizer N addition, compared to no clover at all three soil sample timings. The clover did add to post-harvest profile $\text{NO}_3\text{-N}$, especially in conjunction with the high rate of fertilizer N. Therefore, it is important to estimate clover cover crop N supply and account for that in corn N fertilization.

CHAPTER 4. CONCLUSIONS

Cereal rye consistently responded to increasing N rate with increased plant canopy, leaf greenness, tillering, and therefore grain yield. A variety difference was observed, first visually during the growing season and later in the data analysis. ND Dylan established better with greater plant stand and early season plant canopy development. Nitrogen response, the yield increase to applied N, was also greater with ND Dylan than Elbon. The optimal N fertilization rate for both varieties, however, was similar at a mean 98 kg N ha^{-1} , but the maximum yield was 1000 kg ha^{-1} greater for ND Dylan. ND Dylan is a relatively new “northern” released variety (North Dakota), and Elbon an older “southern” released variety (Oklahoma). The ND Dylan appeared to be better adapted to Iowa growing conditions, especially fall through winter. Inter-seeded medium red clover did not interfere with rye production and positively impacted the following corn crop, providing approximately a mean 57 kg N ha^{-1} equivalent N supply, reduced optimal N rate, and with little to no impact on grain yield. Minimal N fertilization effect on soil profile $\text{NO}_3\text{-N}$ was observed following rye harvest. Since rye is a low N rate requirement crop, and with major N uptake in the early spring, profile $\text{NO}_3\text{-N}$ values near the AONR fertilization rate indicate minimal potential for carryover N after rye grain harvest. However, greater levels of soil profile $\text{NO}_3\text{-N}$ were found in the corn year with the clover cover crop, especially following harvest in conjunction with a high N fertilizer rate. Therefore, corn N fertilization following a clover cover crop must take into account the N contribution from the clover N supply.

The findings of this study support the initial idea to integrate cereal rye grain production with a clover cover crop into soybean-corn rotations. Moreover, the project fits in the strategy of integrating different landscape management practices for crop production, soil resources, and water quality. Further research is needed to assess management practices that promote high

cereal rye yields and improved standability. Fall planted rye and frost-seeded red clover would provide a green soil cover with living roots year-round, helping with NO_3 loss reduction between annual crops. Clover as a legume can also increase plant-available N for corn, reducing N fertilization rates. Although not studied in this project, using the harvested rye seed as a cover crop after corn would provide additional green cover and continuous plant growth both above and below ground. Therefore, this integrated grain crop-cover crop production system has potential to positively affect NO_3 loss, provide soil improvement, and provide an alternative cropping sequence for producers.

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Table 1. Routine soil tests for the 0- to 15-cm soil depth collected prior to rye planting.

Year	Site	pH	STP ^a	STK ^a	SOM ^b
			-----mg kg ⁻¹ -----		g kg ⁻¹
2018	Ames	6.7	21 (H)	212 (H)	44
	Kanawha	5.3	30 (H)	229 (H)	38
2019	Ames	6.1	22 (H)	161 (O)	30
	Kanawha	6.0	33 (VH)	236 (H)	38

^a Mehlich-3 soil test P (STP) and soil test K (STK). Soil test interpretation category for O, optimum; H, high; VH, very high (Mallarino et al., 2013).

^b SOM, soil organic matter.

Table 2. Statistical significance levels for the effect of clover presence, rye variety, fertilizer N rate, and their interactions on number of seed heads, plant height, leaf N concentration, grain N concentration, and grain yield.

Source of variation	Head count	Height	Leaf N	Grain N	Yield
	-----P > F-----				
Clover (CL)	.182	.490	.096	.147	.450
Variety (V)	<.001^a	.001	<.001	<.001	<.001
N rate (NR)	<.001	<.001	<.001	<.001	<.001
NR _L	<.001	<.001	<.001	<.001	<.001
NR _Q	.314	<.001	.288	.005	<.001
CL x V	.472	.994	.462	.935	.171
CL x NR	.913	.895	.290	.311	.810
V x NR	.358	.662	.011	.954	.008
V x NR _L	.591	.634	<.001	.930	<.001
V x NR _Q	.898	.355	.114	.351	.434
CL x V x NR	.981	.694	.976	.531	.969

^a Bold values indicate significance ($P \leq .10$).

Table 3. Main effects of clover presence, rye variety, and fertilizer N rate on number of seed heads, plant height, leaf N concentration, grain N concentration, and grain yield.

Treatment	Head count	Height	Leaf N	Grain N	Yield
	heads ha ⁻¹ x 10000	cm	g kg ⁻¹	g kg ⁻¹	kg ha ⁻¹
Clover					
With	319a ^a	137a	37.5b	23.8a	2291a
Without	302a	138a	38.1a	24.1a	2350a
Variety					
Elbon	281b	134b	40.7a	26.9a	1877b
ND Dylan	340a	140a	34.9b	21.1b	2764a
N rate (kg N ha ⁻¹) ^b					
0	261	130	33.5	23.7	1838
28	284	134	34.4	23.6	2043
56	301	138	37.1	23.5	2283
84	324	140	39.2	23.9	2520
112	341	141	40.8	24.4	2596
140	351	139	42.0	24.7	2643

^a Means followed by different letters within a column treatment set indicate significant difference ($P \leq .10$).

^b Nitrogen rate response statistics provided in Table 2.

Table 4. Linear-plateau regression parameters describing rye responses to fertilizer N rate by variety.

Measurement	Rye variety	Regression parameters				R ²	P ≥ F
		a	b	Joint point ^a kg N ha ⁻¹	Plateau		
First ^b NDRE	Elbon	0.164	0.00029	86	0.189	0.98	.003
	ND Dylan	0.211	0.00080	94	0.286	0.99	.003
Second NDRE	Elbon	0.202	0.00061	99	0.262	0.99	.008
	ND Dylan	0.230	0.00079	122	0.326	0.99	.009
Third NDRE	Elbon	0.213	0.00057	119	0.281	0.99	.006
	ND Dylan	0.220	0.00053	136	0.292	0.99	.006
First NDVI	Elbon	0.348	0.00085	78	0.415	0.98	.026
	ND Dylan	0.490	0.0018	91	0.657	0.99	.002
Second NDVI	Elbon	0.484	0.0016	93	0.627	0.99	.009
	ND Dylan	0.584	0.0019	95	0.766	0.99	.008
Third NDVI	Elbon	0.543	0.0013	101	0.670	0.99	.007
	ND Dylan	0.590	0.0009	124	0.705	0.99	.003
SPAD	Elbon	38.2	0.025	119	41.2	0.90	.030
	ND Dylan	34.0	0.037	118	38.3	0.99	.002
Headcount (n ha ⁻¹ x 10000)	Elbon	236	0.669	128	321	0.97	.006
	ND Dylan	288	0.763	122	381	0.95	.009
Height (cm)	Elbon	128	0.112	83	138	0.94	.014
	ND Dylan	132	0.154	70	143	0.99	.009
Leaf N (mg kg ⁻¹)	Elbon	36.5	0.063	117	43.9	0.97	.005
	ND Dylan	29.7	0.076	137	40.1	0.99	.005
Grain N (mg kg ⁻¹)	Elbon	26.3	0.008	140	27.4	0.83	.011
	ND Dylan	20.5	0.008	140	21.6	0.64	.058
Yield (kg ha ⁻¹)	Elbon	1494	6.249	100	2119	0.99	<.001
	ND Dylan	2162	10.094	95	3120	0.99	<.001

^a Nitrogen rate, which is the agronomic optimum rate, at which the linear equation joins the plateau value.

^b Rye canopy sensing stages are First, jointing; Second, full flag leaf emergence; Third, full head emergence; and SPAD at full flag leaf emergence.

Table 5. Statistical significance levels for the effect of clover presence, rye variety, fertilizer N rate, and their interactions on three different canopy sensing timings for NDRE and NDVI, and leaf SPAD reading.

Source of variation	First ^a		Second		Third		SPAD
	NDRE	NDVI	NDRE	NDVI	NDRE	NDVI	
	-----P > F-----						
Clover (CL)	.371	.362	.960	.795	.852	.173	.829
Variety (V)	<.001^b	<.001	<.001	<.001	.186	.003	<.001
N rate (NR)	<.001	<.001	<.001	<.001	<.001	<.001	<.001
NR _L	<.001	<.001	<.001	<.001	<.001	<.001	<.001
NR _Q	<.001	<.001	<.001	<.001	.360	.007	.029
CL x V	.241	.355	.343	.480	.634	.170	.787
CL x NR	.925	.979	.998	.999	.424	.026	.531
V x NR	<.001	<.001	<.001	.092	.730	.354	.075
V x NR _L	<.001	<.001	<.001	.005	.846	.112	.005
V x NR _Q	.100	.223	.575	.992	.152	.238	.811
CL x V x NR	.858	.934	.904	.835	.990	.995	.957

^a Rye canopy sensing stages are First, jointing; Second, full flag leaf emergence; Third, full head emergence; and SPAD at full flag leaf emergence.

^b Bold values indicate significance ($P \leq .10$).

Table 6. Main effects of clover presence, rye variety, and fertilizer N rate on three different canopy sensing timings for NDRE and NDVI, and leaf SPAD reading.

Treatment	First ^a		Second		Third		SPAD
	NDRE	NDVI	NDRE	NDVI	NDRE	NDVI	
Clover							
With	0.222a ^b	0.502a	0.261a	0.639a	0.254a	0.644a	38.2a
Without	0.217a	0.488a	0.261a	0.635a	0.254a	0.630a	38.1a
Variety							
Elbon	0.181b	0.393b	0.239b	0.575b	0.251a	0.621b	39.9a
ND Dylan	0.258a	0.597a	0.283a	0.698a	0.257a	0.653a	36.4b
N rate (kg N ha ⁻¹) ^c							
0	0.188	0.419	0.217	0.536	0.220	0.569	36.1
28	0.201	0.455	0.231	0.576	0.229	0.592	36.6
56	0.220	0.498	0.257	0.637	0.246	0.629	38.3
84	0.233	0.528	0.277	0.677	0.264	0.663	38.6
112	0.237	0.536	0.289	0.696	0.279	0.679	39.4
140	0.238	0.536	0.294	0.697	0.287	0.689	39.7

^a Rye canopy sensing stages are First, jointing; Second, full flag leaf emergence; Third, full head emergence; and SPAD at full flag leaf emergence.

^b Means followed by different letters within a column treatment set indicate significant difference ($P \leq .10$).

^c Nitrogen rate response statistics provided in Table 5.

Table 7. Statistical significance levels for the effect of clover presence, rye variety, fertilizer N rate, and their interactions on post-harvest soil profile NO₃-N.

Source of variation	0-0.3 m depth	0.3-0.6 m depth	0-0.6 m avg
-----P > F-----			
Clover (CL)	.299	.261	.230
N rate (NR)	.002^a	<.001	<.001
CL x NR	.821	.493	.632

^a Bold values indicate significance ($P \leq .10$).

Table 8. Effect of clover presence and fertilizer N rate on post-rye harvest soil profile NO₃-N.

Source of variation	0-0.3 m depth	0.3-0.6 m depth	0-0.6 m avg
-----mg kg ⁻¹ -----			
kg N ha ⁻¹			
Clover			
With	3.2a ^a	1.4a	21a
Without	3.3a	1.5a	22a
N Rate			
0	2.9c	1.2c	19c
84	3.2b	1.4b	21b
140	3.6a	1.7a	24a

^a Means followed by different letters within a column treatment set indicate significant difference ($P \leq .10$).

Table 9. Statistical significance levels for the clover cover crop, fertilizer N rate, and the interaction on aboveground biomass and total N at time of control in the spring before corn planting.

Source of variation	Dry Matter	Total N
	-----P > F-----	
Clover (CL)	<.001 ^a	<.001
N rate (NR)	.311	.588
CL x NR	.534	.942

^a Bold values indicate significance ($P \leq .10$).

Table 10. Main effect of the clover cover crop and fertilizer N rate on aboveground biomass and total N at time of control in the spring before corn planting.

Treatment	Dry Matter	Total N
	kg ha ⁻¹	kg N ha ⁻¹
Clover		
With	3527a ^a	66a
Without	1480b	32b
N Rate (kg N ha ⁻¹) ^b		
0	2266a	47a
168	2557a	49a
280	2686a	52a

^a Means followed by different letters within a column treatment set indicate significant difference ($P \leq .10$).

^b Nitrogen fertilizer rate to be applied to corn. Nitrogen rates applied to the prior year rye grain crop were 0, 84, and 140 kg N ha⁻¹.

Table 11. Statistical significance levels for the clover cover crop, fertilizer N rate, and their interactions on corn canopy sensing, leaf SPAD, and grain yield.

Source of variation	NDRE	NDVI	SPAD	Yield
	-----P > F-----			
Clover (CL)	.104	.203	<.001	<.001
N rate (NR)	<.001 ^a	<.001	<.001	<.001
NR _L	<.001	<.001	<.001	<.001
NR _Q	<.001	<.001	<.001	<.001
CL x NR	.019	.290	<.001	<.001
CL x NR _L	.002	.048	<.001	<.001
CL x NR _Q	.053	.163	<.001	.067

^a Bold values indicate significance ($P \leq .10$).

Table 12. Quadratic-plateau regression parameters describing corn response to fertilizer N rate with and without the clover cover crop.

Measured	Clover	Regression parameters					R ²	P ≥ F
		a	b	c	Joint point ^a	Plateau		
					kg N ha ⁻¹			
NDRE	With	0.372	0.0006	-0.0000032	95	0.401	0.97	.006
	Without	0.337	0.0009	-0.0000030	152	0.406	0.99	.001
NDVI	With	0.801	0.0006	-0.0000032	87	0.826	0.89	.036
	Without	0.791	0.0007	-0.0000027	126	0.835	0.96	.008
SPAD	With	42.1	0.194	-0.00064	151	56.8	0.98	.002
	Without	31.5	0.286	-0.00082	174	56.5	0.99	.001
Yield (kg ha ⁻¹)	With	6739	65.09	-0.1899	171	12317	0.98	.003
	Without	3866	78.84	-0.1733	228	12834	0.99	<.001

^a Nitrogen rate, which is the agronomic optimum rate, at which the quadratic equation joins the plateau value.

Table 13. Quadratic-plateau regression parameters describing relative corn canopy sensing index (rNDRE and rNDVI) and relative SPAD (rSPAD) response to N rate difference from the EONR (dEONR), across with and without the clover cover crop.

Measured	Regression parameters					R ²	P ≥ F
	a	b	c	Joint point ^a kg N ha ⁻¹	Plateau		
rNDRE	0.97	-0.000872	-0.0000074	-59	1.00	0.98	<.001
rNDVI	0.99	-0.000296	-0.0000024	-62	1.00	0.90	<.001
rSPAD	0.98	-0.000255	-0.000011	-12	0.98	0.98	<.001

^a Nitrogen rate, at which the quadratic equation joins the relative value plateau.

Table 14. Statistical significance levels for the clover cover crop, fertilizer N rate, and the interaction on soil profile NO₃-N concentration by soil depth and profile total by sampling time in the corn crop.

Source of variation	0-0.3 m	0.3-0.6 m	0-0.6 m
-----P > F-----			
Spring ^a			
Clover (CL)	<.001 ^b	.003	<.001
N rate (NR)	.033	.551	.039
CL x NR	.713	.371	.368
Late Spring			
Clover (CL)	.020	.331	.016
N rate (NR)	<.001	<.001	<.001
CL x NR	.047	.690	.143
Fall			
Clover (CL)	<.001	.039	.002
N rate (NR)	<.001	<.001	<.001
CL x NR	.042	.264	.025

^a Soil profile sampling at termination of the clover cover crop, Spring; June, Late Spring; and post-harvest, Fall.

^b Bold values indicate significance ($P \leq .10$).

Table 15. Effect of clover cover crop and fertilizer N rate on soil profile NO₃-N by soil depth and profile total by sampling time in the corn crop.

N Rate ^a		Soil depth (m)								
		0-0.3			0.3-0.6			0-0.6		
		Clover								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
kg N ha ⁻¹		-----mg NO ₃ -N kg ⁻¹ -----						-----kg NO ₃ -N ha ⁻¹ -----		
<u>Spring</u> ^b										
0	2.1 ^c	1.0	1.6AB ^d	1.5	0.9	1.2A	16	9	12AB	
168	2.4	1.6	2.0A	1.3	1.0	1.1A	16	12	14A	
280	1.7	1.0	1.3B	1.2	1.0	1.1A	13	9	11B	
Mean	2.1A ^d	1.2B		1.3A	1.0B		15A	10B		
<u>Late Spring</u> ^b										
0	5.3d	4.1d	4.7B	2.2	1.6	1.9B	33	26	30B	
168	11.9b	11.7b	11.8A	3.5	3.1	3.3A	69	66	67A	
280	13.8a	9.8c	11.8A	3.7	3.8	3.7A	79	61	70A	
Mean	10.3A	8.5B		3.1A	2.8A		60A	51B		
<u>Fall</u> ^b										
0	2.4d	1.6d	2.0C	0.7	0.6	0.6C	14d	10d	12C	
168	4.9bc	3.3cd	4.1B	2.0	1.0	1.5B	31c	19d	25B	
280	11.2a	6.4b	8.8A	5.2	3.5	4.3A	74a	44b	59A	
Mean	6.2A	3.8B		2.6A	1.7B		39A	24B		

^a Nitrogen fertilizer rate applied to corn.^b Soil profile sampling at termination of the clover cover crop, Spring; June, Late Spring; and post-harvest, Fall.^c Means followed by different lower case letters within a treatment set indicate significant interaction effect ($P \leq .10$).^d Main effect means followed by different upper case letters within a treatment set indicated significant difference ($P \leq .10$).

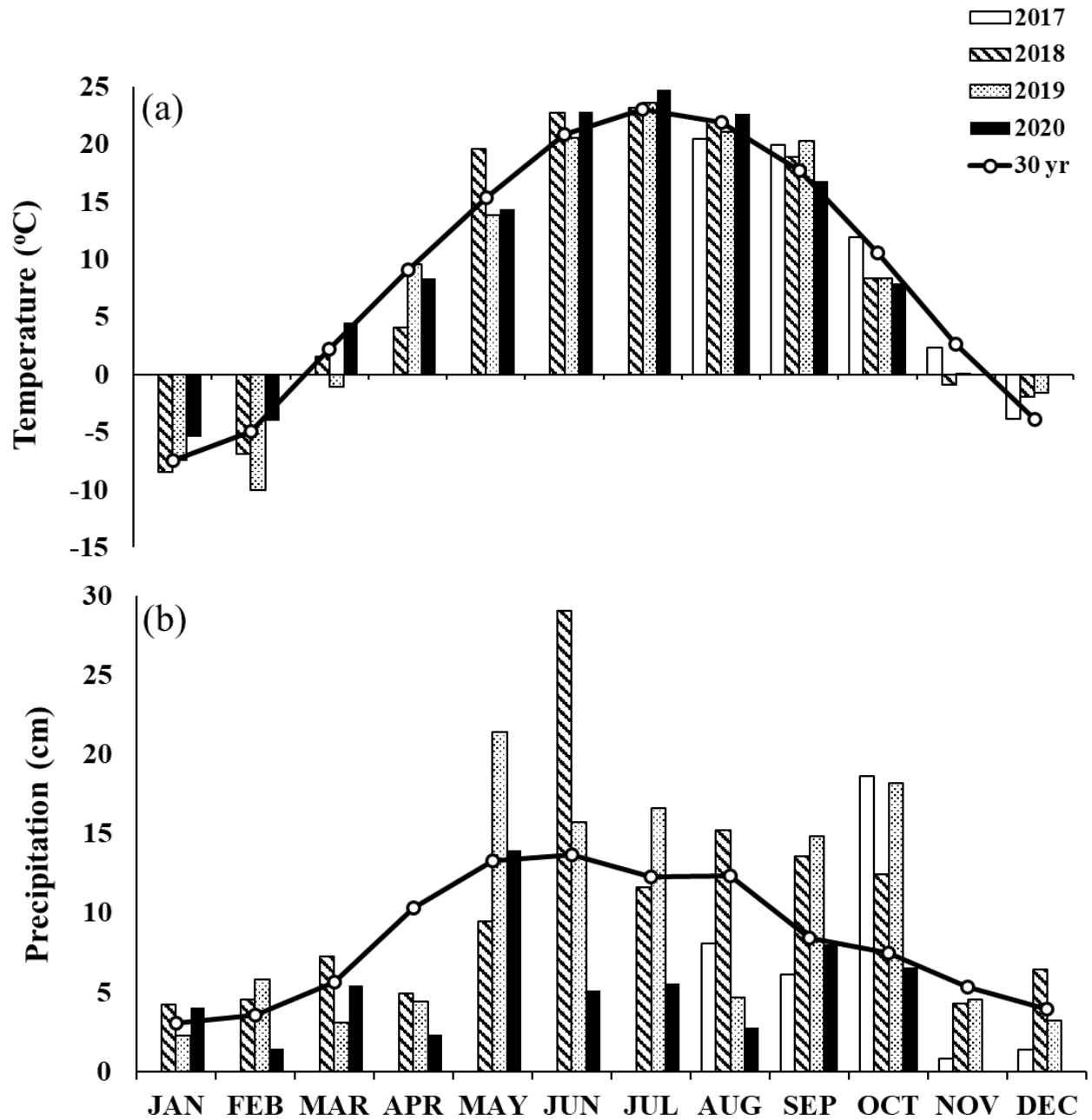


Fig. 1. Monthly mean air temperature (a) and total monthly precipitation (b) and the 30-yr mean at the Ames site.

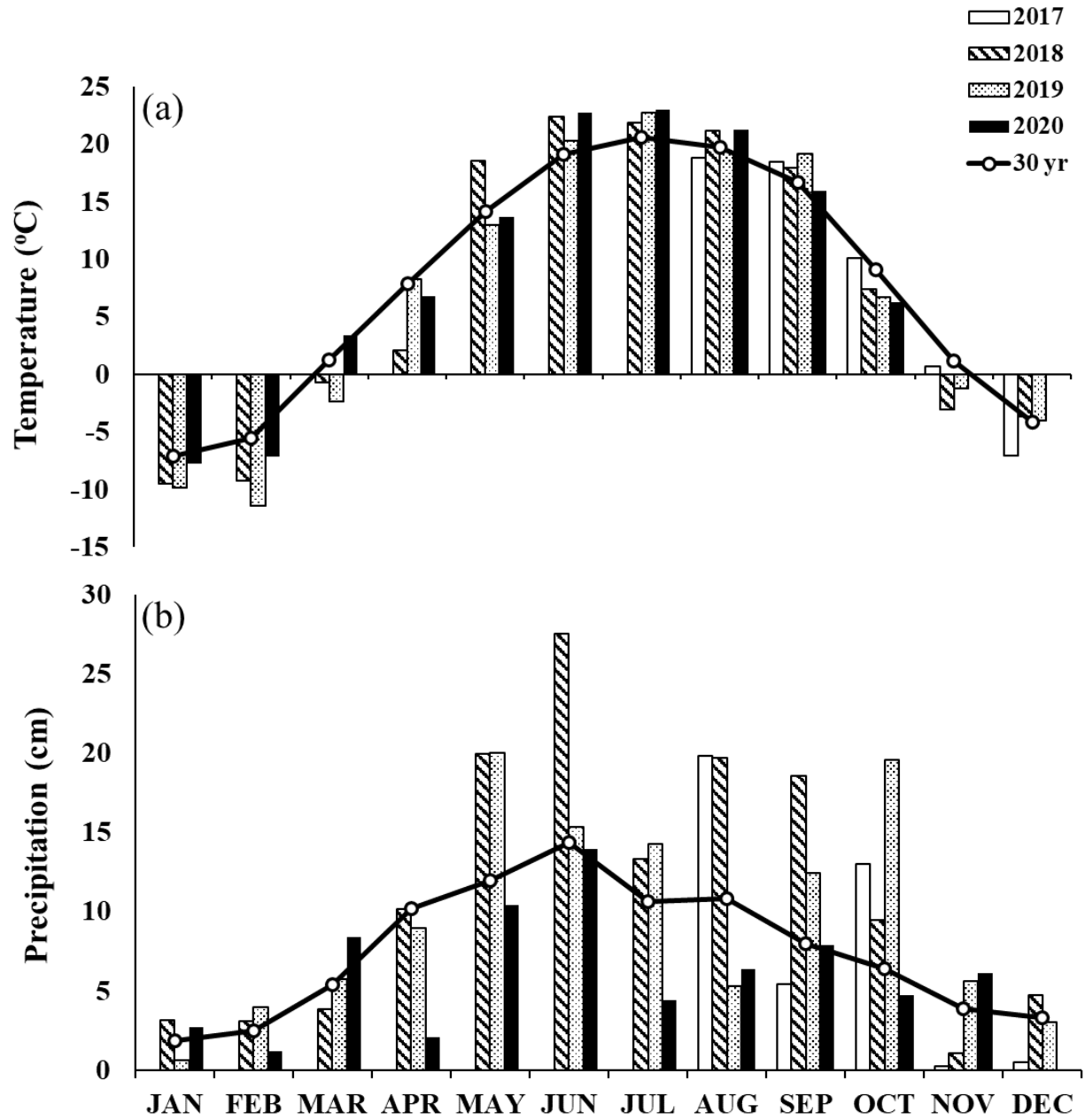


Fig. 2. Monthly mean air temperature (a) and total monthly precipitation (b) and the 30-yr mean at the Kanawha site.

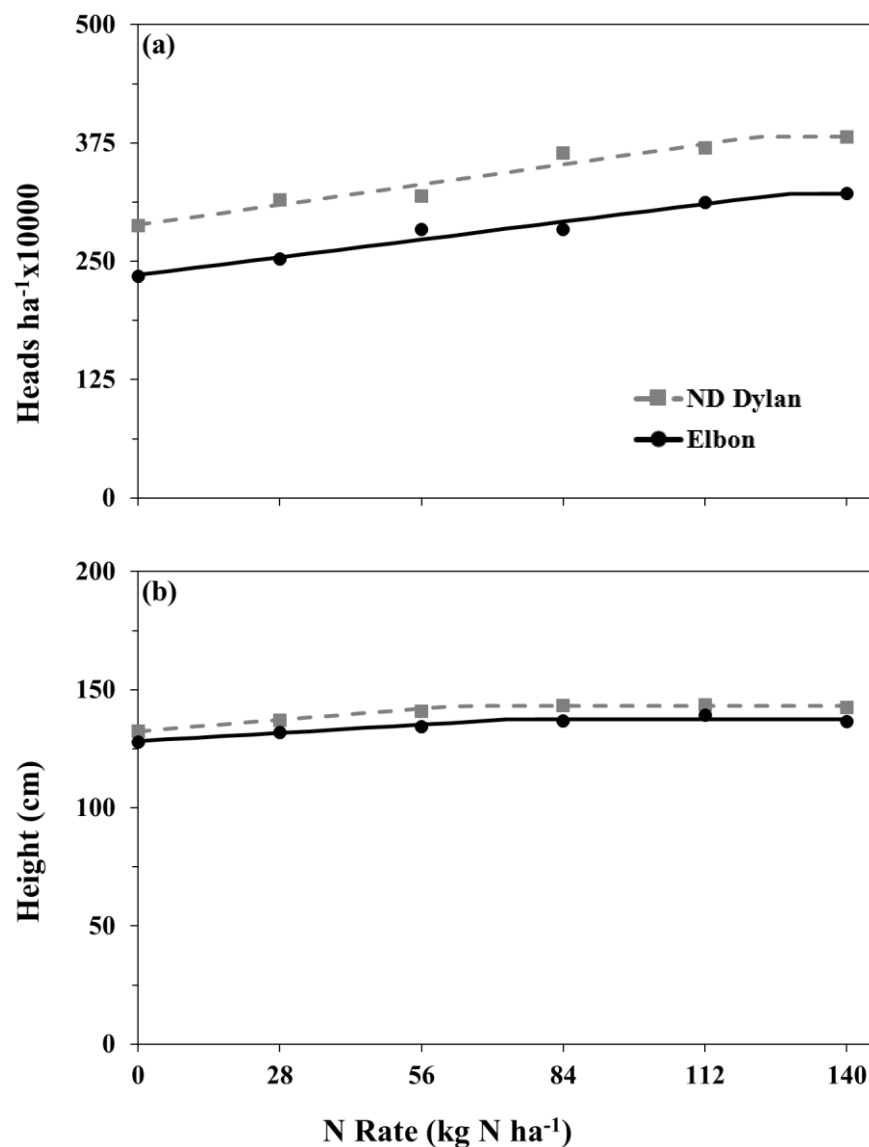


Fig. 3. Rye seed head count (a) and plant height to top of seed head (b) response to N rate at full head emergence. Nitrogen rate and variety \times N rate response significance in Table 2, with regression parameters presented in Table 4.

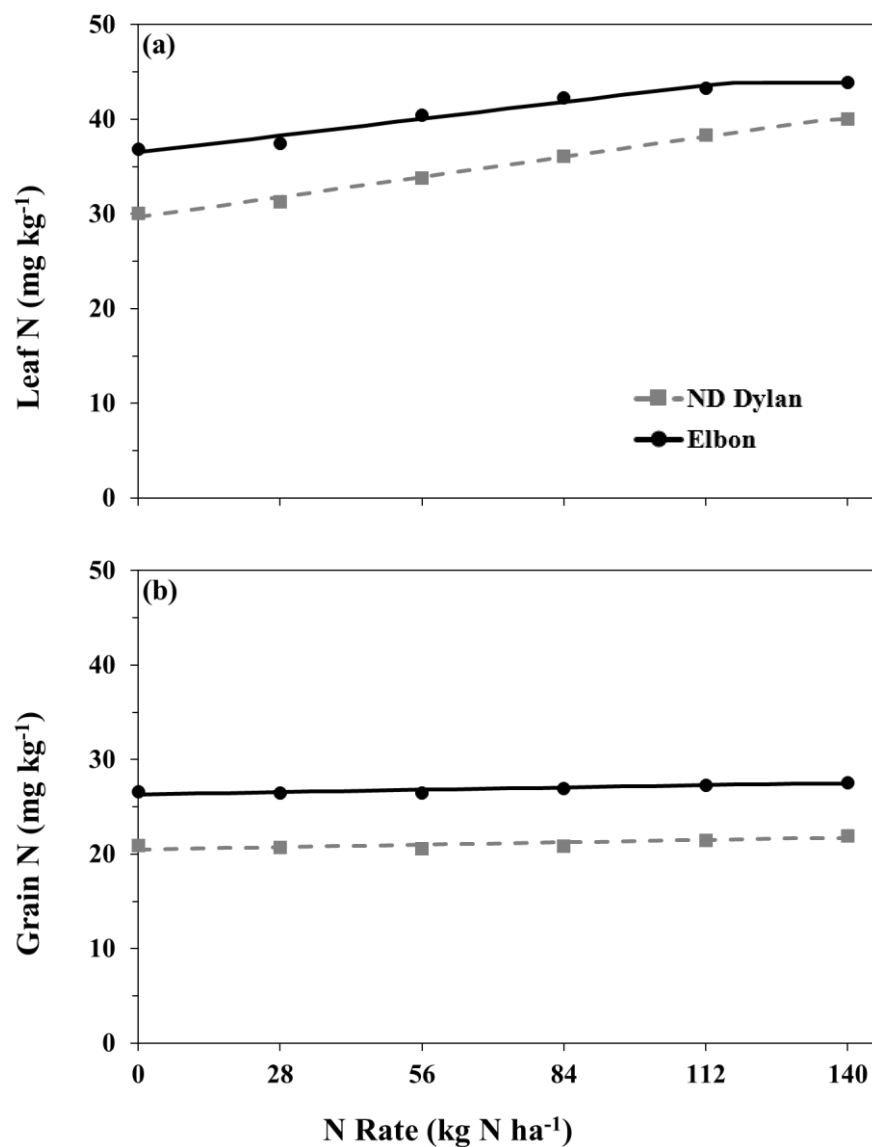


Fig. 4. Rye leaf (a) and grain (b) N concentration response to N rate. Nitrogen rate and variety x N rate response significance in Table 2, with regression parameters presented in Table 4.

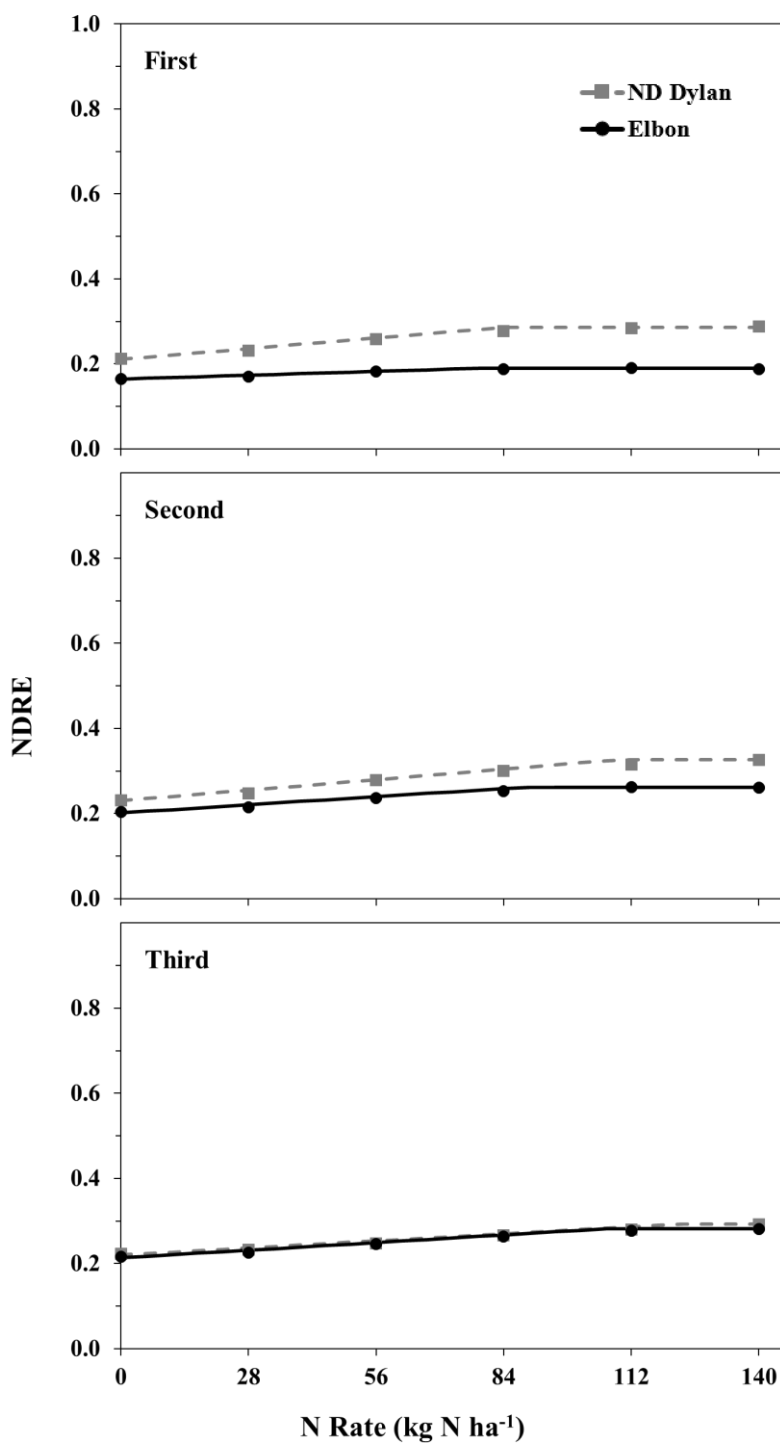


Fig. 5. The NDRE index response to N rate at three rye crop stages (First, jointing; Second, full flag leaf emergence; Third, full head emergence). Nitrogen rate and variety x N rate response significance in Table 5, with regression parameters presented in Table 4.

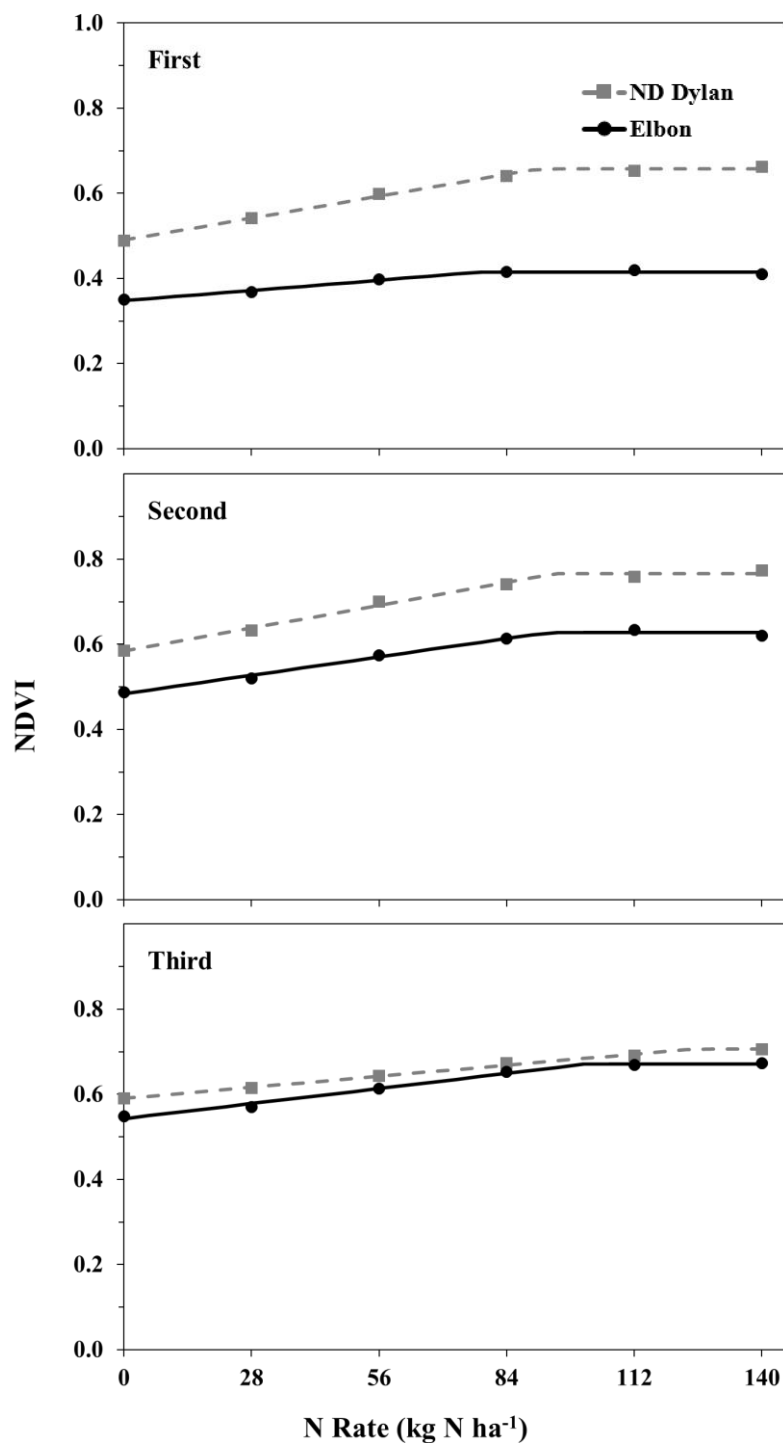


Fig. 6. The NDVI index response to N rate at three rye crop stages (First, jointing; Second, full flag leaf emergence; Third, full head emergence). Nitrogen rate and variety x N rate response significance in Table 5, with regression parameters presented in Table 4.

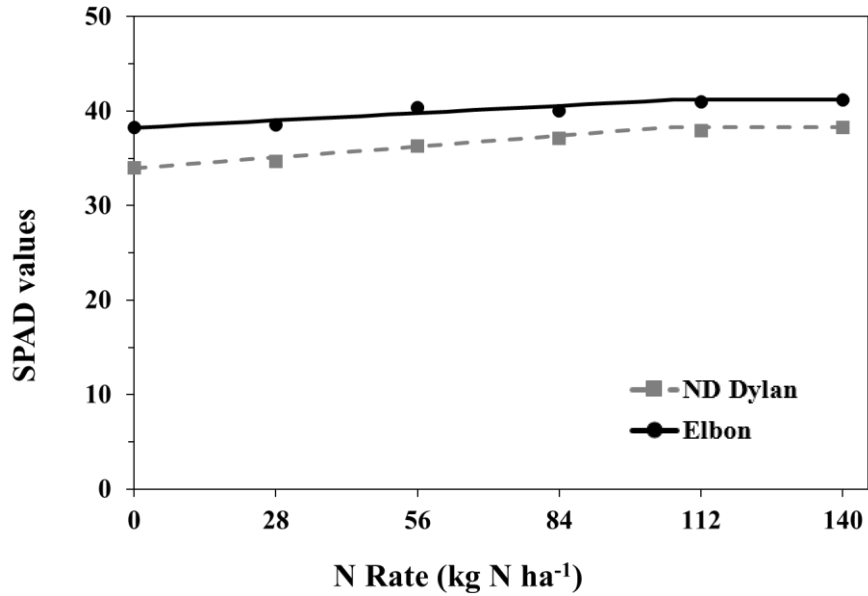


Fig. 7. Rye leaf Minolta SPAD reading response to N rate (flag leaf or leaf below flag leaf at full flag leaf emergence). Nitrogen rate and variety x N rate response significance in Table 5, with regression parameters presented in Table 4.

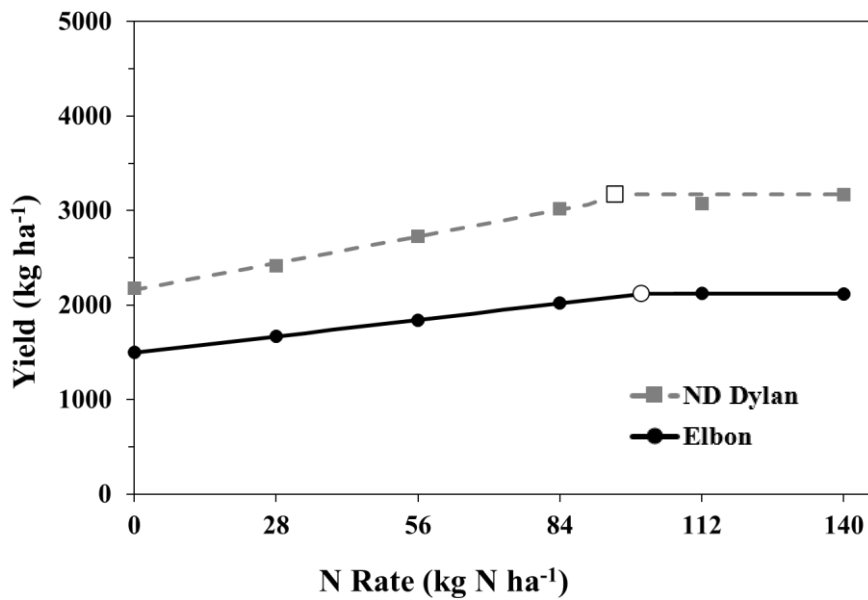


Fig. 8. Rye grain yield response to N rate. Nitrogen rate and variety x N rate response significance in Table 2, with regression parameters presented in Table 4. Open symbols show the agronomic optimum N rate for ND Dylan and Elbon.

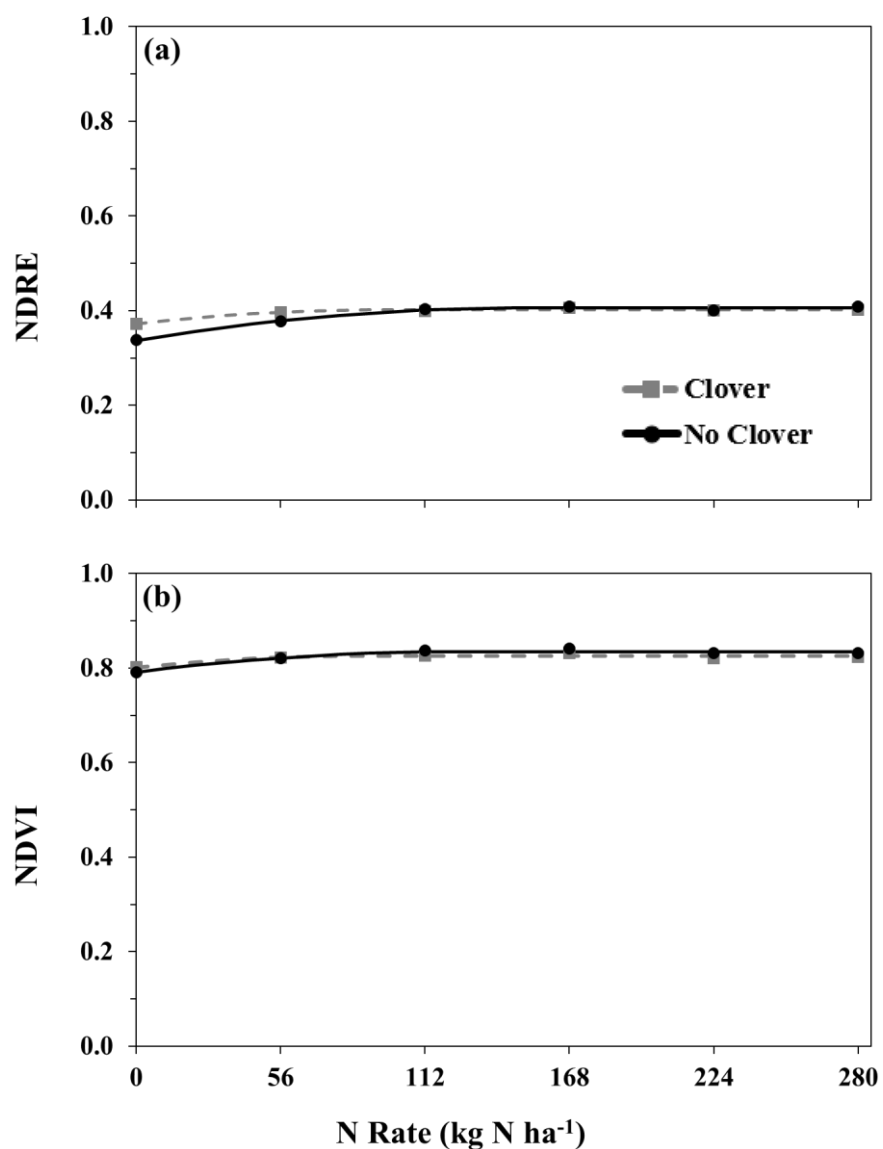


Fig. 9. The NDRE (a) and NDVI (b) corn canopy sensing index response to N rate by with and without the clover cover crop. Nitrogen rate and clover x N rate response significance provided in Table 11, with regression parameters provided in Table 12.

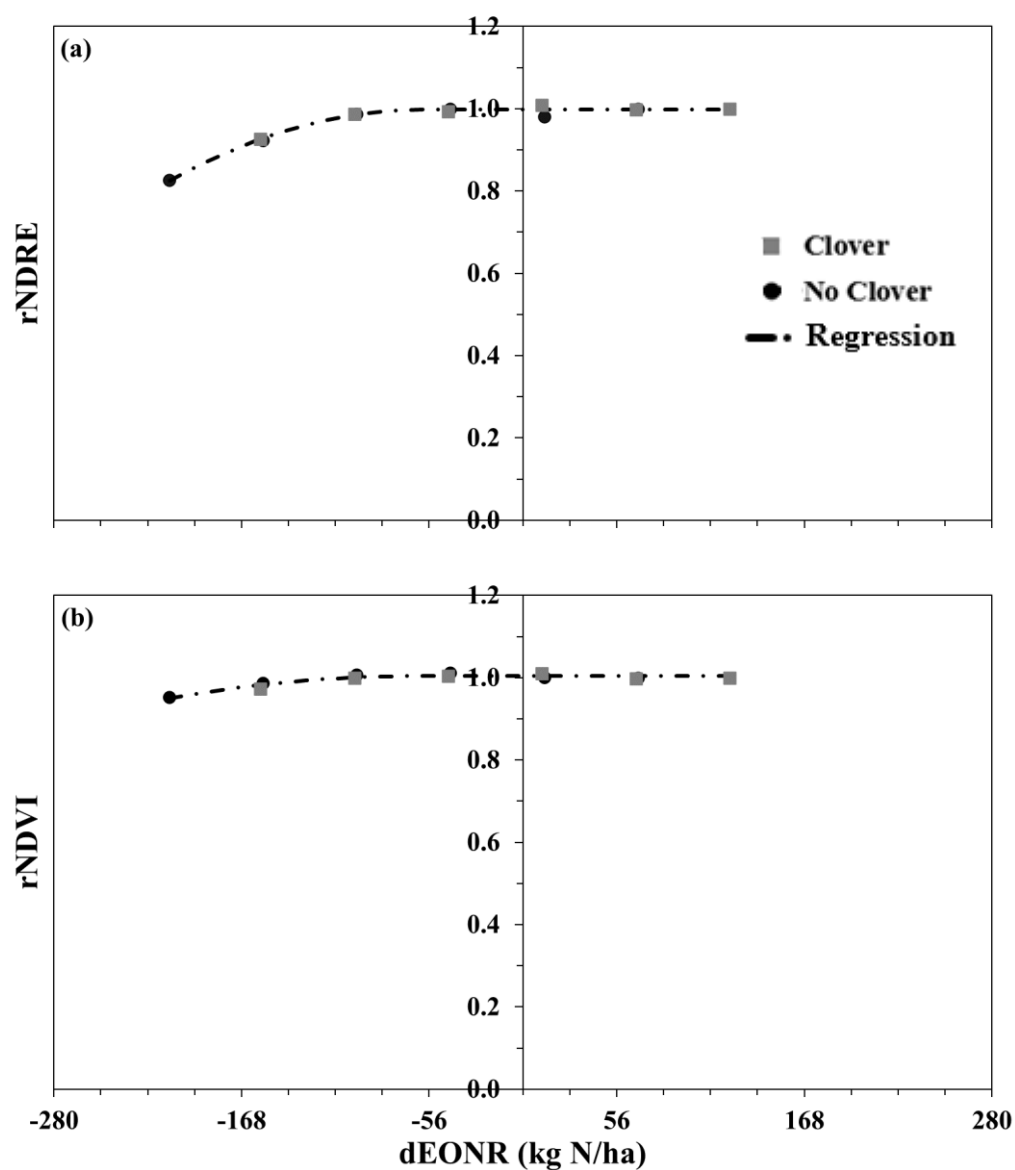


Fig. 10. Relative corn canopy sensing index values [rNDRE (a) and rNDVI (b)] as related to the N rate difference from the economic optimum N rate (dEONR) for with and without the clover cover crop. Regression parameters provided in Table 13.

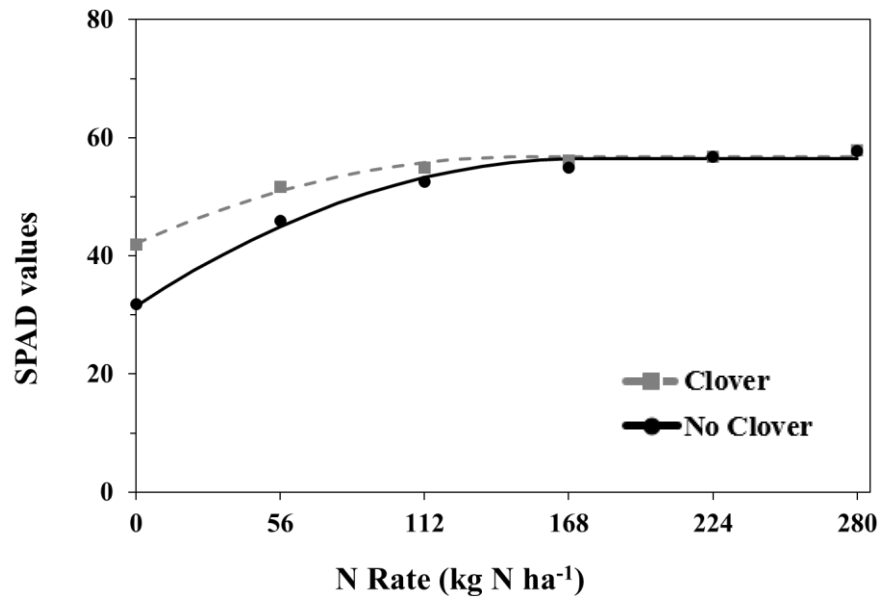


Fig. 11. Corn ear leaf SPAD response to N rate by with and without the clover cover crop. Nitrogen rate and clover x N rate response significance provided in Table 11, with regression parameters provided in Table 12.

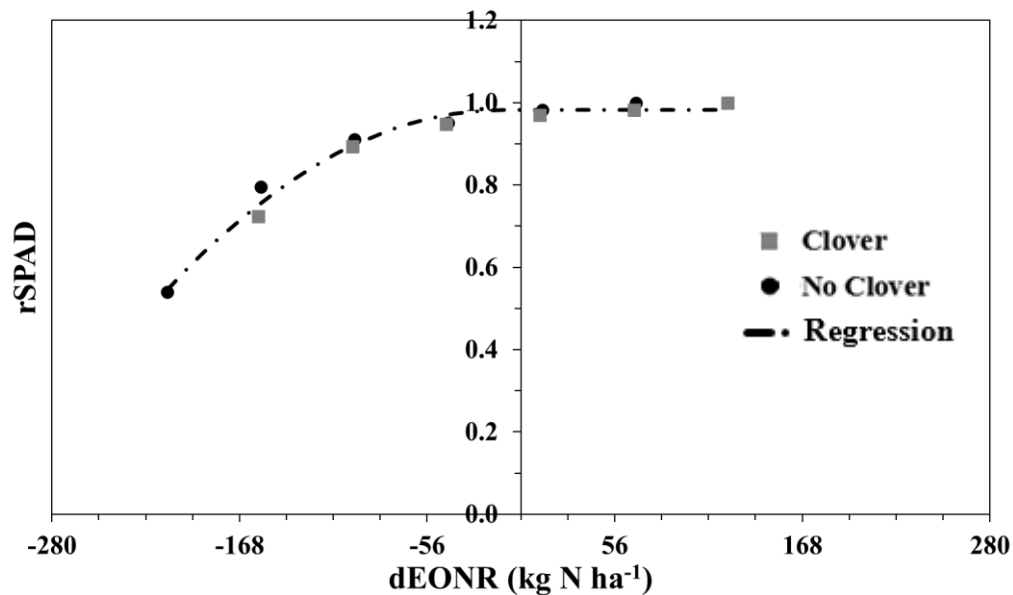


Fig. 12. Corn ear leaf relative chlorophyll meter (rSPAD) values as related to the N rate difference from the economic optimum N rate (dEONR) for with and without the clover cover crop. Regression parameters provided in Table 13.

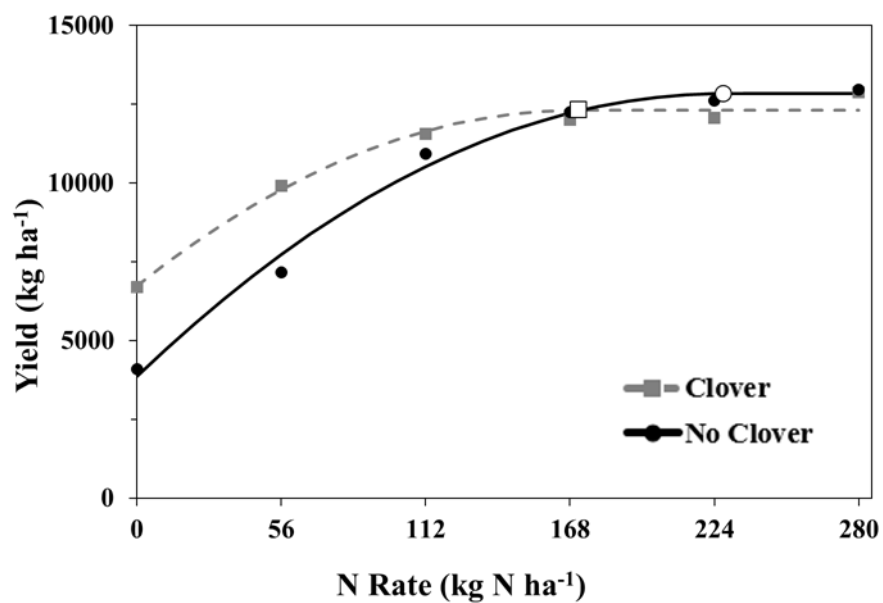


Fig. 13. Corn grain yield response to N rate by with and without the clover cover crop. Nitrogen rate and clover x N rate response significance provided in Table 11, with regression parameters provided in Table 12. Open symbols show the agronomic optimum N rate (AONR) for with and without the clover cover crop.