Winter rye cover crop biomass, nutrient uptake, and quality in response to fall and spring N fertilization

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Abstract: Growers in Illinois often apply manure or add starter fertilizers that contain nitrogen (N) in the fall to save time for other farming operations in the spring. Fall manured fields sometimes receive a starter N in spring as part of the operation. We hypothesized that winter rye (Secale cereale) (wr) cover crop could capture the applied N which in turn changes wr dry matter (dm) biomass, nutrient uptake, and c accumulation capacity, and wr quality (ash, lignin, and c concentrations along with c:n, and lignin:n ratios). Two separate trials with randomized complete block design at two separate locations in Southern Illinois were conducted in 2020 and 2021. Trial one had two rates of fall-applied N (0 vs. 56 kg N ha⁻¹), and trial two had initial fall manure to all plots plus four rates of N fertilizer in spring (0, 23, 47, and 71 kg ha⁻¹). Results for trial one indicated that fall N fertilization had no effect on wr biomass, nutrient uptake, and quality parameters due to loss of applied N in the fall. Spring N application did not affect wr dm biomass but linearly increased N, phosphorus (P), and potassium (K) concentrations, their uptake, c concentration, and decreased c:n and lignin:n ratios. We concluded that spring N fertilization could facilitate N decomposition of wr by decreasing its c:n and lignin:n ratio and help increasing the wr nutrient uptake capacity, but fall N application should be avoided to prevent N loss to the environment and decrease the cost of operation.

Subjects: Agriculture and Food; Soil Conservation Technology; Agronomy

Keywords: carbon; cover crop; fertilizer; nitrogen; nutrient composition; winter cereal rye

1. Introduction
Corn (Zea mays L.) and soybean (Glycine max L.) rotation is the main crop rotation in the Midwest, USA (Lacey et al., 2020). In Illinois, prior to corn, growers often apply manure or add starter nitrogen (N) fertilizer in the fall to save time for other farming operations in the spring. Fall manured fields sometimes, depending on their operation, receive a starter N in spring to allow time for other operations later in spring due to time limitations. Research has shown that N is often lost mainly through leaching in the fall or spring when cover crops are not utilized (Farsad et al., 2012). Illinois is one of the 12 states with a nutrient loss reduction strategy aiming to reduce 15% of N and 25% of P loss by 2025. According to Illinois nutrient loss reduction strategy, an effective management to minimize nitrate-N loss through leaching is to plant a winter cereal cover crop (WCCC) in rotation with corn and soybean (Adeyemi et al., 2020). Among WCCCs, winter rye (Secale cereale) (WR) is the most widely used in the Midwest, USA.
(Lacey et al., 2020). Apart from N loss reduction potential, winter rye provides several ecosystem benefits, including soil erosion protection (Rorick & Kladivko, 2017), weed suppression (Mirsy et al., 2011; Kumar et al., 2021), nutrient uptake and cycling (Jahanzad et al., 2016; Singh et al., 2020), soil temperature regulation (Reed & Karsten, 2022) and soil C addition that could potentially sequester C in the soil and improve soil health (Blanco-Canqui et al., 2015; Liptzin et al., 2022). Many of the abovementioned benefits increase when WR cover crop biomass is maximized, which often occurs when WR develops into the heading stage (Finney et al., 2016). This is less desirable for growers that plant WR prior to corn due to possible N immobilization (high C:N or lignin:N ratio) and difficulty in planting into the WR residue (Sadeghpour et al., 2021). When WR is present, applied N in spring or fall could potentially increase WR biomass while decreasing its C:N ratio (Balkcom et al., 2018; Reiter et al., 2008). Response of WR to N is site-specific and depends on soil type, soil moisture, temperature, and previous manure or crop history (Lyons et al., 2019). For example, Lyons et al. (2019) found that at least one-third of the studied sites did not respond to spring N fertilization. This indicates the importance of evaluating whether WR captures N applied in the fall or spring. We hypothesized that winter rye (Secale cereale) (WR) cover crop can capture the applied N, gain greater biomass, and potentially return more carbon (C) to the soil and sequester C, which is desirable for mitigating climate change. We also hypothesized that spring N fertilization could potentially increase WR biomass yield while decreasing C:N ratio as a result of an increase in plant N concentration that drives C:N ratio (Weidhuner et al., 2019). Therefore, our objectives were to evaluate whether fall or spring N fertilization influences WR (i) biomass accumulation, (ii) nutrient concentration and uptake, (iii) quality parameters (ash, lignin, and C concentrations along with C:N, and lignin:N ratios), and (iv) C concentration and accumulation.

2. Materials and methods

2.1. Experimental site

Two separate field experiments were conducted in Southern Illinois, USA. Trial one was located at Belleville Research Center (BRC) in Belleville, Illinois (38.52° N, 89.84° W). Trial two was conducted on a grower’s farm near Breese, Illinois (36.69 N, 89.53 W). Both trials were conducted in 2019 (2019–2020) and 2020 (2020–2021) growing seasons. The soil at the BRC site was a mixture of Bethalto silt loam (fine-silty, mixed, superactive, mesic Udolic Endoaqualf) and Winfield silt loam (fine-silty, mixed, superactive, mesic Oxyaquic Hapludalfs) in 2020 and Pierron (Fine, smectitic, mesic Typic Albauqualfs) silt loam in 2021 (Soil Survey Staff, 1999). The on-farm site in Breese, Illinois, was classified as poorly drained silt loam (Fine, smectitic, mesic Udolic Endoaqualfs) with a slope of 0–2%. Before trial initiation, composite soil samples (24 cores per trial) were taken by a soil probe from 0 to 15 cm soil depth at each site to ensure no nutrient limitation. In trial one (the BRC site), in 2020, soil pH (1 soil: 1 water) was 6.3, organic matter (loss on ignition at 360 °C) was 18.0 g kg⁻¹, Bray-1 P (Bray & Kurtz, 1945) was 22.0 mg kg⁻¹, and Mehlich-3 K (Mehlich, 1984) was 157.0 mg kg⁻¹. In 2021, soil pH was 6.4, organic matter was 23.0 g kg⁻¹, Bray-1 P was 26.0 mg kg⁻¹, and Mehlich-3 K was 148.0 mg kg⁻¹. In trial two (the Breese site), in 2020, soil pH (1 soil: 1 water) was 7.8, organic matter (loss on ignition at 360 °C) was 31.0 g kg⁻¹, Bray-1 P was 63.6 mg kg⁻¹, and Mehlich-3 K was 172.3 mg kg⁻¹. In 2021, soil pH was 7.6, organic matter was 28.1 g kg⁻¹, Bray-1 P was 58.7 mg kg⁻¹, and Mehlich-3 K was 148.2 mg kg⁻¹. Average monthly air temperatures and monthly cumulative precipitation recorded at each site are shown in Table 1.

2.2. Experimental design and details

2.2.1. BRC site (Trial 1)

The trial at the BRC site was arranged in a randomized complete block design with four replicates. Plots were 3.3 m wide and approximately 13 m long. In this trial, no fall manure was applied, and the site did not have a known manure history. Treatments were as follows: (i) a no-N control and (ii) application of 56 kg N ha⁻¹ in the fall. The N fertilizer source was urea which was surface applied
Table 1. Mean air temperature (°C) and cumulative monthly precipitation from Belleville weather station used for both trials in 2019–2020 and 2020–2021 growing seasons

| Month   | Mean air temperature (°C) | Cumulative monthly precipitation (mm) |
|---------|---------------------------|--------------------------------------|
|         | 2019–2020 | 2020–2021 | 30-year average | 2019–2020 | 2020–2021 | 30-year average |
| September | 23        | 23        | 21            | 20        | 16        | 83            |
| October  | 13        | 13        | 15            | 114       | 129       | 87            |
| November | 4         | 4         | 9             | 91        | 130       | 99            |
| December | 3         | 3         | 1             | 50        | 31        | 74            |
| January  | 2         | 1         | 2             | 183       | 104       | 58            |
| February | 3         | –4        | 3             | 60        | 44        | 57            |
| March    | 10        | 10        | 8             | 97        | 123       | 81            |
| April    | 12        | 13        | 14            | 120       | 78        | 98            |
| May      | 17        | 17        | 19            | 108       | 72        | 124           |

at the planting date. At the BRC site, WR was planted following soybean in both years. The planting dates were October 16 and Oct 23 in 2019 and 2020, respectively. The seeding rate for WR was 100 kg ha$^{-1}$ in each year of the study.

2.2.2. Breese site (Trial 2)
The experiment was arranged in a randomized complete block design with five replicates at the Breese site. Treatments were four N rates (0, 23, 47, and 71 kg N ha$^{-1}$) applied in spring at WR Feeks 3–4 (Large, 1954) or green-up (in early March). Similar to the BRC site, we surface-applied urea. Plots were 3.3 m wide and approximately 10 m long. We applied (via injection) liquid dairy manure to WR in the fall (typical dairy farmer practice) at 37.4 kiloliters ha$^{-1}$ before WR sowing. Based on manure composition (Table 2), N applied with the liquid manure was approximately 33 and 37 kg ha$^{-1}$ in 2020 and 2021, respectively, assuming 35% of organic N was available to WR (Sadeghpour et al., 2017). Consulting with farmer advisors, Illinois uses a similar N crediting system to New York (NY), and therefore, we based our estimated N credits according to the NY N decay series of 35% organic N. At the Breese site, the previous crop was corn for silage each year. The planting dates at the Breese site were Oct 8 and Oct 2 in 2019 and 2020, respectively. The row spacing of WR was 19 cm in all sites and years. The seeding rate for WR was 100 kg ha$^{-1}$ in each year of the study.

2.3. Measurements
Winter rye aboveground biomass was sampled with grass shears (GS model 700; Black and Decker Inc., Towson, MD) in all sites and years. The sampling dates for the BRC site were April 10 and April 15 in 2020 and 2021, respectively. Sampling dates for the Breese site were April 9 and April 11 for 2020 and 2021, respectively. The harvesting area for both trials and in both years was 0.675 m$^2$

Table 2. Characteristics of liquid dairy manure applied in each year of the study (nutrient measurements on a dry weight basis)

| Year | Total N | Ammonia N | Organic N | $P_2O_5$ | $K_2O$ | Total solids |
|------|---------|-----------|-----------|----------|--------|--------------|
|      | g kg$^{-1}$ |           |           |          |        |              |
| Dairy liquid manure |
| 2020 | 1.6     | 0.7       | 0.9       | 0.8      | 1.9    | 28.4         |
| 2021 | 1.6     | 1.0       | 0.6       | 0.9      | 2.1    | 19.2         |
(three frames of 0.225 m² from the center of plots to avoid edge effects). Biomass samples were oven-dried for 72 h at 48°C to determine DM yield. Biomass sub-samples were ground to pass through a 1 mm sieve for nutrient and cover crop quality analysis (Weidhuner et al., 2019). Nitrogen, P, K, ash, and lignin were measured using near-infrared reflectance spectroscopy (NIRS) at Ward Laboratories (Kearney, NE, USA). Carbon was measured using the combustion method as explained in Weidhuner et al. (2019). Nitrogen, P, K uptake, and C accumulation were calculated by multiplying WR DM biomass yield with the percent concentration of each element.

2.4. Statistical analysis
Prior to the analysis, data were evaluated for normality of the residuals using the Shapiro–Wilk test reported from Proc Univariate (SAS Institute, 2015). Data were then analyzed using Proc Mixed in SAS. For both trials, year and N were fixed effects, and the block was considered as a random effect. If the N effect or the interaction of year × N was significant for a response variable, data for that response variable were regressed with N treatments using linear and non-linear models, including quadratic, two, and three parameter exponential regression in JMP software (JMP Pro 14; SAS Institute, 2015). The best trend (model) was used based on lower P values, lower root mean square error (RMSE) values and higher R-squared (R²) values. If linear was a good fit (R² > 0.95 and low RMSE), we used linear and did not overfit the data. When significant at p ≤ 0.05, Fisher’s least significant difference (LSD) test was used for mean separation.

3. Results and discussions

3.1. BRC site—fall N effect on winter rye biomass, nutrient concentration, and uptake
Fall N addition did not affect WR DM biomass accumulation (Table 3). The biomass of WR was higher in 2020 than in 2021, reflecting on the earlier planting date for that year despite warmer soil temperature in 2021 than in 2020 (Table 1), emphasizing the importance of timely planting of WR (Hashemi et al., 2013). Fall N did not increase N concentration in WR either, suggesting that N applied in fall was possibly lost to the environment. Fall N fertilization had no effect on either WR P and K concentrations or N, P, and K uptake, indicating that fall N was most likely lost into the environment. Year-to-year differences existed for most of the variables, mainly reflected in the earlier planting date in 2020 and later termination date in 2021 (data not shown).

3.2. BRC site—fall N effect on winter rye quality and carbon accumulation
Fall N did not affect ash, lignin, and C concentrations (Table 3). Winter rye lignin:N and C:N ratio and C accumulation in WR were not influenced by spring N addition (Table 3). These results suggest that N which was applied in the fall was not used by WR. If WR had utilized the fall N, we would have expected at least three of the quality parameters to be impacted: (i) ash concentration was expected to increase by the addition of N (Coblenz et al., 2014), and (ii) lignin:N and C:N ratios were expected to decrease. Ash, lignin, lignin:N and C:N ratio as well as C accumulation differ from year to year resulting from the later termination date in 2021 (data not shown). Greater ash content resulted from higher nutrient concentration in 2021 than in 2020 in WR. Greater lignin, lignin:N, and C:N reflected the maturity of WR in 2021 than in 2020, confirming that if growers are using WR for weed suppression or are interested in the slower decomposition of WR or WR as a source of C that decomposes slower, a delayed termination is an effective approach (Mirsly et al., 2011; Roth & Waite, 2021).

3.3. Breese—spring N effect on winter rye biomass, nutrient concentration, and uptake
Spring N fertilizer did not affect WR biomass yield but linearly (P < 0.01) increased WR N concentration, indicating that a combination of field manure history, fall manure addition, soil organic matter (>30 g kg⁻¹) and N mineralization had provided N required for WR (Table 4). For each kg of spring N fertilizer, the N concentration of WR increased by 0.05 g kg⁻¹. This is in line with the results of Lyons et al. (2019), who reported a linear increase in N concentration of WCCC with N rates beyond the plant requirements for optimum growth. Nitrogen fertilization also increased WR N uptake. Nitrogen uptake of WR increased by 0.31 kg ha⁻¹ for each kg of N ha⁻¹ fertilizer application (Table 4). Similar to N, P and K concentrations and uptake were
| N rate | DMY | N | P | K | NU<sup>b</sup> | PU | KU | Ash | Lignin | C | L:N | C:N | CA |
|--------|-----|---|---|---|-----|----|----|-----|--------|---|-----|-----|----|
| kg ha<sup>-1</sup> | Mg ha<sup>-1</sup> | —- g kg<sup>-1</sup>—- | —- kg ha<sup>-1</sup>—- | —- g kg<sup>-1</sup>—- | Mg ha<sup>-1</sup> |
| 0 | 2.89 | 20.3 | 2.9 | 18.6 | 60.6 | 8.4 | 53.0 | 40.7 | 24.1 | 452.1 | 1.4 | 27.4 | 1.31 |
| 56 | 2.96 | 20.9 | 2.3 | 19.0 | 63.6 | 8.9 | 55.8 | 40.6 | 24.7 | 455.5 | 1.4 | 27.1 | 1.35 |
| LS<sup>a</sup> | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

<sup>a</sup>LS: level of significance; NS: not significant (P > 0.05).

<sup>b</sup>NU, PU, KU, L:N, and CA are N uptake, P uptake, K uptake, lignin:N, and C accumulation.
Table 4. Average effect of spring N application rate in 2020 and 2021 on WR DM yield (DMY), nitrogen (N), phosphorus (P), and potassium (K) concentrations and uptake along with ash, lignin, lignin:N, carbon (C), C:N, and C accumulation.

| N rate | DMY | N  | P  | K  | NU\textsuperscript{b} | PU  | KU  | Ash | Lignin | C  | L:N | CN | CA  |
|--------|-----|----|----|----|---------------|-----|-----|-----|--------|----|-----|----|-----|
| kg ha\textsuperscript{-1} | Mg ha\textsuperscript{-1} | g kg\textsuperscript{-1} | kg ha\textsuperscript{-1} | g kg\textsuperscript{-1} |             | Mg ha\textsuperscript{-1} |
| 0      | 3.17 | 13.9 | 2.30 | 12.7 | 44.1 | 7.5 | 42.0 | 23.1 | 32.4 | 460.2 | 2.34 | 33.2 | 1.46 |
| 23     | 3.55 | 15.0 | 2.40 | 13.6 | 52.2 | 8.6 | 50.3 | 28.9 | 32.8 | 462.2 | 2.17 | 30.9 | 1.68 |
| 47     | 3.74 | 16.8 | 2.45 | 13.9 | 60.9 | 9.3 | 53.2 | 29.8 | 32.7 | 465.6 | 2.00 | 27.9 | 1.74 |
| 71     | 3.99 | 17.5 | 2.55 | 15.3 | 66.0 | 10.1 | 60.9 | 34.9 | 33.8 | 464.8 | 1.96 | 27.0 | 1.85 |
| LS\textsuperscript{c} | NS | ** | ** | ** | ** | ** | ** | NS | ** | ** | NS | ** | NS |
| Trend\textsuperscript{c} | NA | L  | L  | L  | L  | L  | L  | NA | Q  | Q  | Exp | NA |   |
| RMSE   | 0.33 | 0.02 | 0.32 | 1.29 | 0.2 | 1.69 | 1.49 | NA | 1.19 | 0.02 | 0.59 | NA |   |

\textsuperscript{a}LS: level of significance; NS: not significant (P > 0.05); **: significant at P < 0.01.

\textsuperscript{b}NU, PU, KU, L:N, and CA are N uptake, P uptake, K uptake, lignin:N, and C accumulation.

\textsuperscript{c}L: linear; Q: quadratic; Exp: exponential; NA: not available.
higher when spring N was applied (Table 4). Each kg of N fertilizer increased P and K concentration by 0.003 and 0.035 mg kg⁻¹, respectively, resulting in P and K uptake of 10.1 and 60.9 kg ha⁻¹ at 71 kg N ha⁻¹, which were 26% and 31% higher than the no-N fertilizer control (Table 4). Similar to our results, Obour et al. (2019) reported an increase in WCCCs tissue P and K concentrations with N fertilizer application and related the increase in concentration to the overall increased capacity for growth in WCCCs. An increase in P uptake is desirable, especially in high P soils typical of dairy farms with repeated manure application (Sadeghpour et al., 2017), and could improve the sustainability of manure management in corn silage systems (West et al., 2020). If cover crops such as WR can uptake P, it could be used as a mechanism to reduce soil test P concentration by harvesting WR when rotated with corn and soybean. This should allow for a more sustainable P management system that limits P loss through erosion (sediment P) while capturing P and decreasing dissolved reactive P in corn-soybean cropping systems. This indicates that WR could play a role in minimizing water quality issues associated with the loss of P in the Midwest, USA.

3.4. Breese site—spring N effect on winter rye quality and carbon accumulation
Unlike fall N, spring N fertilization influenced ash, C, lignin:N and C:N ratio but not C accumulation of WR (Table 4). Lignin concentration was not affected by N fertilization, but due to an increase in N concentration, lignin:N was decreased quadratically by N addition from 2.34 (0 kg N ha⁻¹) to 1.96 (71 kg N ha⁻¹). Increased ash content by N fertilization was related to higher N, P, and K concentrations in N treatments (Read et al., 2021; Sadeghpour et al., 2022). Ash content increased by 0.15 g kg⁻¹ for every kg of N fertilizer (Table 4). Interestingly, N fertilization also increased C concentration in WR as C was 2% higher in the 47 kg N ha⁻¹ treatment than in the no-N control. The significant effect of N on C concentration was possibly due to small block-to-block variability. Carbon:N was decreased exponentially by N fertilization, indicating that spring N fertilization could be an effective strategy for reducing lignin:N and C:N ratio (desirable for crop production). Spring N fertilization should be avoided if the goal is to reduce decomposition and maintain residue. With 0, 23, 47, and 71 kg N ha⁻¹, C:N was 33.2, 30.9, 27.9, and 27.0, respectively (Table 4). This result indicated that a 47 kg N ha⁻¹ addition to WR could minimize N immobilization and potentially accelerate N release from the WR residue. Winter cereal rye C accumulation was unaffected by N fertilization, indicating that C accumulation is more yield-driven, and an increase in C concentration does not necessarily translate into increased C accumulation. These results indicate that spring N fertilization could improve WR nutrient uptake and decrease C:N and lignin:N ratios but not C accumulation in Southern Illinois. Similar to the trial at BRC, there were year-to-year variations among most of the response variables, mainly due to warmer temperatures in 2021 than 2020.

4. Conclusions
We conclude that fall N fertilizer application does not impact WR biomass, its nutrient uptake, or its quality parameters, including lignin, C, lignin:N and C:N ratio, and should be avoided to minimize N losses and farming costs, especially if WR is not planted early. Spring N fertilization in a field that had received fall manure could provide several benefits, including N, P, and K uptake (at 71 kg N ha⁻¹) and reduction in lignin:N and C:N ratios (at 47 kg N ha⁻¹) desirable for N availability to the following cash crop. Trials should be conducted to evaluate the interactive effects of fall and spring N fertilization with WR planting and termination date to assess the C accumulation benefits of WR in Southern Illinois.

Acknowledgements
The two trials were funded by Illinois Farm Bureau and Illinois Nutrient Research and Education Council. We thank Lauren Lurkins and Austin Omer for their kind support. We thank Randy Lange, Yuan Luo, Cliff Schutte, and Meyer’s family for their assistance in conducting the trials.

Funding
This work was supported by the Illinois Farm Bureau; Illinois Nutrient Research and Education Council;
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