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Rye cover crop had a nitrogen credit one year after termination in a frigid environment

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Abstract: Our prior study showed that a dormant seeded rye (*Secale cereale* L.) cover crop reduced carbon dioxide equivalence (CO_{2c}) in a frigid submesic climate. However, this research did not discuss the impact of the cover crop on temporal changes in soil inorganic nitrogen (N), soil organic carbon (SOC), microbial biomass, and community structure, which is the purpose of the present study. In this replicated no-tillage study, winter cereal rye was drilled in October of 2018 and 2019 and terminated using glyphosate (*N*-[phosphonomethyl] glycine) on June 11, 2019, and June 8, 2020, at the V4 growth stage in corn (*Zea mays* L.). Rye biomass collected at termination was air dried and placed into fiberglass litter bags at a rate of 3,750 kg ha^{-1}. Bags were placed on the soil surface at the original collection zone. Four replicate bags from each plot were collected after 87, 248, and 365 days. Following removal, the material remaining in the removed bags was analyzed for amount and chemical composition. The decomposing cover crop biomass increased the microbial biomass and the amount of soil inorganic N and SOC in the surface 15 cm at 365 days (one year after litter was applied). These results suggest that soil nutrient benefits from fall-planted rye cover crop may not occur until the year following termination. However, the N credit benefits may vary depending on the type of cover crop, such as legume or brassica; therefore, this should also be considered in future studies. Overall, these findings provide practical implications for cover crop management in frigid submesic climates, suggesting that growers' adoption of cover crops can improve soil health, enhance crop yields, and reduce reliance on chemical fertilizers, thereby contributing to more sustainable and economically viable farming systems.

Key words: cover crops—nitrogen credit—soil organic carbon

Planting cover crops can be a climate smart practice that increases carbon (C) sequestration while reducing greenhouse gas (GHG) emissions (Joshi et al. 2021, 2023, 2024; Reicks et al. 2021). What is less understood is how rapidly the C and nitrogen (N) contained in cover crop plant biomass is returned to the soil through decomposition. The purpose of this study is to investigate this question.

Prior studies have shown that cover crops during the termination year have a mixed impact on the release of inorganic N from decomposing cover crop biomass to the growing cash crop. For example, under two contrasting environments, neither Momesso et al. (2022) in Brazil nor Pantoja et al. (2016) in Iowa (United States) showed a cover crop N credit in the year of termination from nonlegume cover crop. Further, in Iowa, the rye (*Secale cereale* L.) cover crop reduced the corn (*Zea mays* L.) yield by 5% (Sawyer et al. 2017). This yield reduction could be attributed to the lack of synchronization between microbial decomposition and plant N uptake (Crandall et al. 2005; Greub and Roberts 2023; Kettering et al. 2015). These findings highlight the importance of integrating the role of soil microbial populations in N cycling and their direct influence on the nutrient use efficiency of cash crops. The dynamics of soil microorganisms in cover cropping systems are crucial, as different cover crops can have a significant impact on microbial biomass and community structure via varying root exudates and residue quality (Muhammad et al. 2021). This microbial activity influences nutrient cycling, especially the availability of N, by facilitating decomposition and mineralization processes. For example, nonlegume cover crops increased microbial biomass C (MBC) more than legume cover crops due to higher biomass yield and C content, which can ultimately improve soil structure and nutrient availability for succeeding crops (Nevins et al. 2018; Sainju et al. 2007; Thapa et al. 2021). Additionally, it is crucial to understand how the timing and rate of microbial processes vary across different seasons and decomposition durations, especially in cold environments. These variations are key to aligning nutrient availability with the demands of cash crops, which can significantly affect yield outcomes (Nevins et al. 2020).

Taken all together, these studies lead to the question, what happened to the N contained in the cover crop biomass? Limited research has been conducted on soil N and C cycling, as well as the influence of microbial communities, from cover crops over multiple growing seasons. Multiple-season research is needed because time is required to convert inorganic N to organic N and vice versa. For example, Clay et al. (1990) showed that N immobilized in microbial biomass following application of urea can be subsequently mineralized and taken up by a growing crop in the current and future growing seasons. For the mineralized N to reduce the crop's N requirement, the mineralization and plant N uptake kinetics need to be synchronized (Clay et al. 1990, 2012, 2015). When they are not synchronized, the mineralized N can be lost through leaching or denitrification. Because these processes are not instantaneous, additional research is needed to determine temporal changes causing the mineralization kinetics. The objectives of this study were to discuss the impact of

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the cover crop on temporal changes in soil inorganic N, soil organic C (SOC), microbial biomass, and community structure.

Materials and Methods

Study Site, Experimental Design, and Treatments. This study was a component of a large project that investigated the impact of cover crops on the C dynamics within rotations that include corn. Previous publications resulting from this experiment reported on the impact of cover crop termination timing on the cover crop and corn growth (Moriles-Miller 2023; Moriles-Miller et al. 2021, 2024), nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions (Reicks et al. 2021), cover crop impacts on C sequestration (Joshi et al. 2023), and the ability of machine learning techniques to predict daily N_2O and CO_2 emissions from a decomposing rye cover crop (Joshi et al. 2024).

The study was conducted at South Dakota State University Aurora Research farm located at 44°18'20.57" N and 96°40'14.04" W in two cropping years, 2019 to 2020 and 2020 to 2021. Köppen climatic subtype of the study site was DFb (humid continental climate). Daily rainfall, air and soil temperatures, soil moisture, and snowfall were obtained from the South Dakota Mesonet that maintains a weather station approximately 6.5 km from the research farm (SD Mesonet 2022). More details about the soil type and chemical and physical properties of the soil are provided in Joshi et al. (2024) and Reicks et al. (2021). The cropping rotation of the experiment was rye-corn–rye-corn. In this study, a chisel plow disk cultivation system was switched to a no-tillage system in 2018 when soybeans (*Glycine max* [L.] Merr.) were planted. Nitrogen fertilizer was not applied to the plots used in this study.

The experiment used a split plot design with two treatments: cover crop and no-cover crop. Each treatment was replicated four times. The dimensions of each plot were 9.1 m long and 3 m wide. Within the cover crop treatment, cereal rye was dormant drilled at a recommended seeding rate of 56 kg ha^{-1} using a five-double row (20 cm between rows) cover crop drill at a depth of 2.5 cm following main crop harvest on October 16, 2018, and October 23, 2019 (Reicks et al. 2021; Joshi et al. 2024). The seeded rye remained dormant throughout the winter and started to germinate and grow in the following spring. Aboveground rye biomass was clipped within a 0.5 × 0.5 m quadrant at four random locations in each plot on June 24 of 2019 and 2020. Additional information on the cover crop harvesting is available in Moriles-Miller et al. (2023). The cover crop biomass was dried at 60°C, weighted, and used in the incubation experiments described below. The activities and operations related to this experiment are summarized in table 1.

Rye Residue Decomposition. Fifteen grams of dried cover crop biomass that was harvested from the plots on June 24 of 2019 and 2020 were placed into 48, 1.5 mm mesh fiberglass litter bags that had dimensions of 20 × 20 cm. The cover crop application rate was equivalent to 3,750 kg ha^{-1}. The biomass used in 2019 had a C/N ratio of 31, and the biomass used in 2020 had a C/N ratio of 25.

At the rye termination date, corn was at the V4 growth stage and rye was at the boot stage. The remaining cover crop in the plots was treated with glyphosate (N-[phosphonomethyl] glycine; Roundup Power Max) at the rate of 2.34 L ha^{-1}. A nonionic surfactant was added at 0.25% of the spray solution. The filled 48 litter bags were placed on the soil surface of four cover crop treatment plots on July 27, 2019 (Year 1), which corresponded to corn's V8 growth stage. There were 12 bags in each plot, of which 4 were collected on October 20, 2019 (87 days of incubation), 4 were collected on March 30, 2020 (248 days of incubation), and 4 were collected on July 26, 2020 (365 days of incubation). As a result, 16 litter bags were collected after 87, 248, and 365 days of incubation. This experiment was repeated in the second year with a total of 48 litter bags installed on July 27, 2020 (Year 2), and 12 litter bags were collected on October 20, 2020, March 30, 2021, and July 6, 2021. Each year the litter bags were placed in the same area within a plot. The aboveground rye residue decomposition rates were quantified using the approach described by Joshi et al. (2020). Corn was planted on May 16, 2019, and May 14, 2020. When corn was planted, between 248 and 365 days of incubation, the litter bags in the plots were temporarily removed to prevent damage by the planters' tires. Following planting, they were returned immediately to the identical location.

After collection, the residue in the litter bag was rinsed with water to remove soil, dried, and weighed (Joshi et al. 2020). The percentage residue loss was determined following equation 1:

$$\text{Litter loss (\%)} = \left(\frac{\gamma_0 - \gamma_t}{\gamma_0}\right) \times 100\%, \quad (1)$$

where, γ_0 was the initial amount of biomass and γ_t was the amount of biomass remaining after each decomposition time (t). Rye

Table 1
Summary of activities and dates of operations performed during the two-year experiment.

Field activities and operations	Year 1 (2018 to 2020)	Year 2 (2019 to 2021)
Rye cover crop dormant seeded	Oct. 16, 2018	Oct. 23, 2019
Corn planting	May 16, 2019	May 14, 2020
Rye cover crop termination and biomass sampling	June 24, 2019	June 24, 2020
Soil sampling and litter bag installation, i.e., 0 days of incubation	July 27, 2019	July 27, 2021
Corn harvest	Sept. 26, 2019	Oct. 8, 2020
Soil sampling and litter bag collection after 87 days of incubation	Oct. 21, 2019	Oct. 21, 2021
Soil sampling and litter bag collection after 248 days of incubation	Mar. 30, 2020	Mar. 30, 2022
Soil sampling and litter bag collection after 365 days of incubation	July 26, 2020	July 26, 2022

Note: At the time of cover crop termination, the corn was at the V4 growth stage, and by the time of litter bag installation, it had reached the V8 growth stage.

residue first order decomposition rates were determined with equation 2:

$$\text{Ln}\ (\gamma_t) = \ln\ (\gamma_0) - kt, \quad (2)$$

where ln was the natural logarithm, γ_t was the amount of biomass remaining at time t, γ_0 was the biomass at time 0, and k was the first order rate constant (Clay et al. 2017). The remaining rye residue was analyzed for total C and total N using ISO-MS technology (Clay et al. 2015; Joshi et al. 2020).

Soil Sampling. Each year when litter bags were installed (July 27, 2019 and 2020), soil samples (0 to 15 cm depth) were collected from an area adjacent to the litter bags. After each litter bag removal, soil samples (0 to 15 cm) were collected from the zone directly below each bag. These samples were split for inorganic N, SOC, and microbial analysis. Subsamples for microbial analysis were immediately placed on dry ice in the field and frozen at −80°C until analysis (Veum et al. 2019). For comparison, soil samples were also collected from the no-cover crop treatment. Subsamples for inorganic N and SOC analysis were dried at 40°C followed by grinding for chemical analysis. Inorganic N was extracted with 1M KCl, and nitrate (NO_3^-) and ammonium (NH_4^+) concentrations determined calorimetrically (Clay et al. 2015). SOC was determined by dry combustion (Clay et al. 2015).

Soil Microbial Biomass and Community Structure. Microbial community size and structure were determined using Phospholipid Fatty Acid (PLFA) analyses as described by Buyer and Sasser (2012), Fiedler et al. (2021), and Joshi et al. (2024). For PLFA analysis the internal standards were 19:0 phosphatidylcholine and 19:0 trinonadecanoin glyceride. A Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Japan) with a flame ionization detector was used to analyze the extracts. The gas chromatograph was calibrated using a standard provided by MIDI Sherlock (No. 1208, MIDI, Inc., Newark, Delaware), and using the MIDI Sherlock Software the extracted fatty acids were classified into distinct microbial communities (MIDI, Inc., Newark, Delaware). The Sherlock PLFA Analysis Software assigns fatty acids to distinct functional groups associated with each community type to determine the number and kind of microbial population (Veum et al. 2019). Terminally branched chain fatty acids were used to identify gram-positive bacteria, while monounsaturated and hydroxy substituted fatty acids were used to identify gram-negative bacteria. Methyl branched chain fatty acids were used to identify actinomycetes (Zhang et al. 2016). To determine total microbial biomass, saturated straight chain fatty acids, C16:0 and C18:0, which are produced by prokaryotes and eukaryotes organisms, were added with all other fatty acids (Quideau et al. 2016). Similarly for fungi biomass determination, various biomarkers used in these analyses include fatty acids such as 18:2 w6c, 18:2 w9c, 18:1 w9c, 18:3 w6c, and 18:3 w3c, which are indicative of fungal cell membranes.

Statistical Analysis. The experimental design was a split plot design having cover crop treatment as the main factor and different litter bag incubation times as subplots. To assess the effects of different decomposition durations on response variables such as total N, SOC, and microbial biomass composition (total microbial, bacterial, and fungal biomass) within each year, Repeated Measures ANOVA was performed by comparing each variable at various sampling times within the same year for both the cover cropped and no-cover cropped treatments. This approach acknowledged the repeated measures design of our data by considering the "same plot effect" to account for the repeated measures taken from the same plots over different time points. To conduct this analysis, we used the "ezANOVA" function from the "ez" package in R (R Core Team 2019). Following the ANOVA, we explored the differences among various days of decomposition, conducting a post hoc analysis using the "emmeans" package in R to determine significant differences among the levels of different days of decomposition. The response variables between the two treatments (cover crops versus no cover crop) were also compared for each specific day of decomposition using two-way ANOVA. This comparison was performed using the "agricolae" package in R. Each of these analyses helps to isolate the effects of cover cropping and decomposition duration on soil health indicators at specific time points. Moreover, the mineralization rates of the cover crop were determined using equation 2 (Wagner and Wolf 1999). The a and k values were estimated using a linear least square function included in the R software (R Core Team 2019). Soils were compared under litter bags and with no surface litter within and across sampling dates. Unless otherwise stated, significant differences were $p \leq 0.05$.

Results and Discussion

Weather and Climatic Condition. The seasonal precipitation in Year 1 (July 27, 2019, to July 26, 2020) was 699 mm, which was higher than Year 2 (July 27, 2020, to July 26, 2021) precipitation, which was 359 mm (table 2). The accumulated snowfall between 2019 and 2020 was 3,600 mm, and the accumulated snowfall between 2020 and 2021 was 1,165 mm. The average air temperature in Year 1 was 9.1°C with a maximum and minimum temperature of 34°C and −30°C, respectively. The average temperature in Year 2 was 10.0°C, with a maximum and minimum temperature of 36°C and −33°C, respectively.

Rye Biomass Production. The rye aboveground biomass was 1,120 (±105) kg ha^{-1} in 2019 and 702 (±133) kg ha^{-1} in 2020 when terminated at corn's V4 growth stage. The higher biomass yields in 2019 compared to 2020 were attributed to higher rainfall and cooler temperatures in 2019 than in 2020 (table 2). However, because these biomass yields were relatively low, the experiment used cover crop biomass application rates that were similar to Greub and Roberts (2023), Joshi et al. (2023), Pantoja et al. (2016), and Ruis et al. (2018). These higher rates were selected to allow tracking of cover crop induced changes in SOC, inorganic N, and microbial biomass.

Cover Crop Biomass Loss and Nitrogen Release during Decomposition. During the first 87 days in Year 1 and Year 2, 13.5% and 19.2% of the residues were lost, respectively. From 87 to 248 days of incubation, which represents the winter season, an additional 9.9% and 7.22% were lost in Year 1 and Year 2, respectively. This loss occurred even though the soil was snow covered and frozen (table 2). From 248 to 365 days of incubation, which represents spring through mid-summer, an additional 22.9% and 18.5% of the biomass was lost in Year 1 and Year 2, respectively. Over the entire 365 days, the cumulative weight loss for Year 1 was 56.7%, whereas cumulative loss during Year 2 was 61.8% (table 3). Though climatic factors such as rainfall and temperature were more favorable for decomposition during Year 1 than Year 2, the greater amount of decomposition and loss of residue in Year 2 could be attributed to the two-year cumulative impact of cover crop on the soil N content (Joshi et al. 2023).

During the first 248 days of both years, N was largely conserved in the cover crop biomass, resulting in a gradual decrease in

Table 2
The average air temperature, average soil temperature (0 to 5 cm), average soil moisture (0 to 5 cm), and total rainfall and snowfall during Year 1 (July 27, 2019, to July 26, 2020) and Year 2 (July 27, 2020, to July 26, 2021) (South Dakota Mesonet 2022).

Time interval	Activities	Total rainfall (mm)	Average air temp. (°C)	Average soil temp. (°C)	Average soil moist. (cm^3 cm^{-3})	Total snowfall (mm)
Year 1 (2019 to 2020)						
Oct. 16, 2018, to June 24, 2019	Rye planting to termination	500	2	6.0	0.25	1,560
July 27, 2019, to Oct. 21, 2019	Litter bags incubation for 0 to 87 days	339	16	17.0	0.32	
Oct. 22, 2019, to March 30, 2020	Litter bags incubation for 88 to 248 days	85	–4	0.7	0.24	2,040
April 1, 2020, to July 26, 2020	Litter bags incubation for 249 to 365 days	275	16	17.0	0.38	
Year 2 (2020 to 2021)						
Oct. 23, 2019, to June 24, 2020	Rye planting to termination	374	4	8.0	0.24	803
July 27, 2020, to Oct. 21, 2020	Litter bags incubation for 0 to 87 days	86	16	18.0	0.23	
Oct. 22, 2020, to March 30, 2021	Litter bags incubation for 88 to 248 days	107	–3	0.8	0.22	362
April 1, 2021, to July 26, 2021	Litter bags incubation for 249 to 365 days	165	16	17.0	0.29	

Table 3
Change in chemical composition and amount of the rye residue loss at various stages of decomposition. Numbers in parentheses represent standard error (n = 16).

Days of decomposition	Date	Cover crop biomass remaining (kg ha^{-1})	C (kg ha^{-1})	N (kg ha^{-1})	C:N
Year 1 (2019 to 2020)					
0 days	July 27, 2019	3,750 (9.7)	1,594 (20.3)	51 (3.8)	31 (5.4)
87 days	Oct. 21, 2019	3,241 (35.4)	1,203 (33.1)	48 (3.6)	25 (9.3)
248 days	March 30, 2020	2,920 (28.4)	908 (40.3)	42 (2.9)	22 (13.8)
365 days	July 26, 2020	1,622 (41.4)	422 (15.4)	22 (1.6)	19 (9.7)
Year 2 (2020 to 2021)					
0 days	July 27, 2020	3,750 (21.5)	1,447 (30.0)	58 (4.9)	25 (6.2)
87 days	Oct. 21, 2020	3,030 (34.5)	1,057 (44.0)	47 (2.1)	23 (20.7)
248 days	March 30, 2021	2,811 (40.3)	920 (34.7)	41 (1.1)	22 (30.8)
365 days	July 26, 2021	1,431 (37.3)	451 (32.0)	23 (3.7)	20 (8.7)

Notes: C = carbon. N = nitrogen. C:N = C/N ratio.

the C/N ratio (table 3). At 365 days, 73.5% and 68.8% of the initial organic C contained in the rye had been released during Years 1 and 2, respectively. Associated with the loss of C was 56.8% and 60.3% loss of the total N contained in the decomposing rye biomass in Years 1 and 2, respectively. During Years 1 and 2, the total amount of N released from the biomass at 365 days was 29 and 35 kg N ha^{-1}, respectively, with the biomass N starting at an average of about 55 kg ha^{-1}. Most of this release occurred between 85 and 365 days, which was between harvest and corn's V6 growth stage in the following year. The amount of N released from the rye biomass was greater than the 10.6 kg N ha^{-1} reported by Sievers and Cook (2018). Our study's higher N release may be attributed to the short duration (120 days) of the Siever and Cook (2018) experiment. The loss of C from 60% to 70% of the surface biomass suggests that the biomass-C was emitted as CO_2 to the atmosphere (Joshi et al. 2024) or transported into the soil below the residue bags in the form of SOC (Joshi et al. 2020, 2023). The biomass loss rates for C and N presented in table 4 were comparable to the SOC mineralization rates reported by Clay et al. (2010), Joshi et al. (2021), and Otte et al. (2019). For example, biomass loss rates were similar to Otte et al. (2019), where the decomposition rate constant ranged from 1 to 3 g biomass kg^{-1} d^{-1}. Additionally, the decomposition rates varied by year. Specifically, the biomass decomposition rate (k) was lower in the first year (2.3 g kg^{-1} d^{-1}) compared to the second year (2.6 g kg^{-1} d^{-1}), as shown in table 4. This variation between years was attributed to cooler temperatures in Year 1, as noted in table 1. Moreover, the rate at which C was lost from the biomass was more rapid in the first year compared to the second year. However, the rate at which N was released from the biomass was similar across both years.

Change in Total Soil Inorganic Nitrogen. When the experiments were initiated on July 27 (day 0) in 2019 and 2020, the amount of inorganic N (NO_3-N + NH_4-N) contained in the surface 15 cm was similar in the cover crop and no-cover crop treatments (table 5). As the experiment progressed from 0 to 87 and 248 days, treatment differences in inorganic N were not observed. These findings are different from Gentry et al. (2013), where the red clover (*Trifolum pratense* L.), an N-fixing legume, produced a N credit in the first year of introduction, and similar to Seman-Varner et al. (2017), where rye did not produce a credit in the first year.

However, from 248 to 365 days the cover crop litter increased the amount of inor-

Table 4
The first order decay constant (*k*) and half-life for the rye cover crop on the soil surface.

	Model parameter estimates			
Response variable	***k* (g biomass kg^{-1} d^{-1})**	**Adj. R^2**	***P* > *F***	**Half-life (d)**
Year 1 (2019 to 2020)				
Biomass	2.3	0.96	0.001	495
Carbon	3.7	0.99	0.004	257
Nitrogen	2.4	0.91	0.001	495
Year 2 (2020 to 2021)				
Biomass	2.6	0.97	0.020	533
Carbon	3.1	0.98	0.001	330
Nitrogen	2.5	0.99	0.020	462

Notes: *k* = relative decomposition rate coefficient. Adj. R^2 = adjusted correlation coefficient.

ganic N in the soil. During this period, newly planted corn transitions from a reliance on seed N to an increased reliance on soil derived inorganic N. During this transition, the amount of N in corn seedling can increase from 10 to 15 kg N ha^{-1} (at V3) to 60 to 90 kg N ha^{-1} (at V10) (Bender et al. 2013). These findings suggest that a cover crop N credit may not be observed following termination but in the following year when corn is taking up N from the soil. In addition, early season mineralization from the previous year's decomposing cover crop biomass may reduce the need for starter N fertilizer.

Cover Crop Impact on Soil Organic Carbon. In Year 1, the cover crop litter did not increase SOC in the surface 15 cm at any sampling date. These results were expected because the surface residue was not incorporated into the soil. However, slightly different results were observed for Year 2, where rye increased SOC at 365 days (table 5). These results were attributed to the partial breakdown of the rye biomass from the previous year and/or the transport of organic C to the soil below the bags. These results indicate that not all the C lost from the litter bags is released to the atmosphere as CO_2. Once in the soil, the SOC can be further decomposed, incorporated into a soil C pool or biological biomass.

Cover Crop Impact on Microbial Biomass. In Years 1 and 2, the cover crop increased microbial biomass at 87 and 365 days (table 6). The increase in total microbial biomass in the cover crop treatment is significant, especially at day 365, where microbial biomass in cover crop treatment was more than twice that in the no-cover treatment in both years. This finding aligns with the meta-analysis conducted by Kim et al. (2020) and Boweles et al. (2017), who found that the introduction of diverse cover crop mixes increased microbial biomass due to improved soil organic matter and nutrient availability. Associated with these increases was a rise in bacterial C. Several other studies have also documented the similar impact of incorporating high-C cover crops like rye on the microbial community. For example, the findings from Kallenbach et al. (2015) demonstrated that cover crops lead to an increase in bacterial biomass. This suggests that cover crops can enhance microbial activity by increasing the breakdown of organic matter, thus promoting a more dynamic and robust soil ecosystem. Fungal C was also increased by the cover crop at 365 days. This change is important because bacteria generally mineralize SOC more rapidly than fungi (de Graaff et al. 2010; de Vries et al. 2012; Wardle et al. 2004). In addition, fungi produce hyphae that improves soil structure and the plant's ability to obtain water and nutrients from the soil (Allen 2007).

Summary and Conclusions

This study quantifies the temporal changes in cover crop decomposition, cover crop chemical composition, SOC, inorganic N, and microbial biomass for the year following termination. Between the V8 (0 days) and

Table 5
The impact of the rye cover crop in Year 1 (2019 to 2020) and Year 2 (2020 to 2021) on the amount of inorganic nitrogen (NO_3 + NH_4) and soil organic carbon (SOC) contained in the surface 15 cm after different decomposition days. Different letters following numbers within a column indicate significant differences among various levels of "days of decomposition" at $p = 0.05$. The *p*-value comparing cover crop and no-cover crop treatment within each "days of decomposition" is provided in the last row for each year.

Year	Days of decomposition (d)	Cover crop (kg N ha^{-1})	No-cover crop (kg N ha^{-1})	*p*-value	Cover crop (Mg C ha^{-1})	No-cover crop (Mg C ha^{-1})	*p*-value
Year 1	0	25.89a	28.38a	0.37	39.36a	39.18a	0.45
Year 1	87	23.21a	26.85a	0.18	39.7a	38.36a	0.38
Year 1	248	23.99a	23.85a	0.13	42.25a	41.24a	0.26
Year 1	365	30.50a	22.84a	0.01	44.07a	41.83a	0.20
p-value		0.32	0.47		0.25	0.29	
Year 2	0	26.45a	24.63a	0.21	43.42a	40.81a	0.16
Year 2	87	28.63a	25.79a	0.19	45.33a	41.29a	0.18
Year 2	248	29.51a	24.96a	0.11	46.34a	41.4a	0.12
Year 2	365	37.44b	22.89a	0.02	47.34a	42.81a	0.04
p-value		0.02	0.81		0.73	0.78	
Averages							
Year 1		25.9	25.48	0.81	41.35	40.15	0.36
Year 2		30.51	24.57	0.03	45.61	41.58	0.04

Table 6
The impact of the rye cover crop on the total microbial, bacterial, and fungal population in the surface 15 cm after different decomposition days. Different letters following numbers within a column indicate significant differences among various levels of "days of decomposition" at $p = 0.05$. The *p*-value comparing cover crop and no-cover crop treatment within each "days of decomposition" is provided in the last row of each year.

		Total microbial biomass			Total bacteria			Total fungus		
Year	Days of decomposition (d)	Cover crop (µg C g^{-1} soil)	No-cover crop (µg C g^{-1} soil)	*p*-value	Cover crop (µg C g^{-1} soil)	No-cover crop (µg C g^{-1} soil)	*p*-value	Cover crop (µg C g^{-1} soil)	No-cover crop (µg C g^{-1} soil)	*p*-value
Year 1	0	6.5b	4.7a	0.06	2.9b	2.3a	0.13	1.3a	0.5a	0.01
Year 1	87	2.5b	1.4b	0.03	1.2b	0.8b	0.002	0.4a	0.1b	0.07
Year 1	248	2.3b	2.2b	0.91	1.4b	1.3b	0.6	0.3a	0.3b	0.57
Year 1	365	9.8a	3.1a	0.03	4.2a	1.6b	0.04	2.1a	0.3b	0.01
p-value		0.008	0.0006		0.01	0.0006		0.11	0.004	
Year 2	0	4.6b	3.2a	0.7	2.1b	1.4a	0.01	0.4a	0.3a	0.03
Year 2	87	2.7b	1.7b	0.03	1.3b	1.0a	0	0.4a	0.2a	0.11
Year 2	248	2.5b	2.2c	0.25	1.4b	1.3a	0.11	0.3a	0.3a	0.92
Year 2	365	7.8a	2.1c	0.02	4.5a	1.6a	0.01	1.6a	0.3a	0.01
p-value		0.002	<0.001		0.02	0.09		0.1	0.3	
Averages										
Year 1		5.3	2.9	0.003	2.47	1.53	0.01	1.01	0.35	0.03
Year 2		4.4	2.3	0.001	2.29	1.35	0.01	0.29	0.27	0.7

maturity (87 days) growth stages, the cover crop did not influence inorganic N; however, an increase in microbial biomass was observed. Different results were observed at 248 days where the cover crop did not influence inorganic N, microbial biomass, or organic C. However, from 248 to 365 days, which includes the period between the V3 and the V8 growth stages in the following year, the decomposing cover crop biomass increased inorganic N and microbial biomass. These results suggest that the benefits from the cover crop may not be realized in the year in which the cover crop is terminated but in the following year. Like soil inorganic N, at the beginning of the experiment, the cover crop and no-cover crop treatments had similar amounts of SOC. In Year 1, which is first year of cover crop, no effects on SOC were observed after 87, 248, or 365 days of decomposition (2019 to 2020). However, in Year 2 the cover crop increased SOC at 365 days. These differences are attributed to the cover crop increasing the amount of C stored in the soil. Associated with the increase in SOC was a shift in the microbial population toward fungi.

This study highlights the significant yet delayed impact of cover crops on soil health, particularly in nutrient cycling and changes in microbial community dynamics across different decomposition periods and seasons in frigid environmental conditions. The increase in microbial biomass, especially fungi, alongside higher SOC levels by the second year, suggests that the full ecological advantages of cover crops, like improved soil structure and nutrient cycling, require multiple seasons to fully develop. This delayed effect implies that evaluations of cover crop benefits immediately after termination might not fully capture their long-term contributions to soil health. Additionally, the variation in effects on microbial biomass and inorganic N across different stages of crop growth and decomposition highlights the complex interactions between cover crop residue dynamics and soil microbial functions. The notable increase in microbial activity, particularly from 248 to 365 days, indicates a better synchronization of nutrient availability with the nutritional needs of crops over time. This improved synchronization may enhance the N credit benefits of cover crops, potentially reducing the dependence on synthetic fertilizers in subsequent growing seasons. It is crucial to determine the optimal timing and conditions for cover crops to maximize their contribution to N cycling and to refine their application in agricultural systems.

These insights not only deepen our understanding of the benefits of cover crops within agroecosystems but also emphasize the importance of strategic long-term planning and management in cover crop programs. By tailoring cover crop management to align with the natural cycles of microbial activity and nutrient dynamics, farmers can maximize both the ecological and economic returns of their farming practices. Such strategic approaches, informed by studies like this one, can pave the way for more sustainable agricultural practices that enhance both productivity and environmental stewardship.

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