

## In Field Measurements of Nitrogen Mineralization Following Fall Applications of N and the Termination of Winter Cover Crops

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**ABSTRACT:** Little is known about the timing and quantity of nitrogen (N) mineralization from cover crop residue following cover crop termination. Therefore, the objective of this study was to examine the impact of cover crop species on the return of fall applied N to the soil in the spring following chemical and winter terminations. Fall N was applied (200 kg N ha<sup>-1</sup>) into a living stand of cereal rye, tillage radish, and control (no cover crop). After chemical termination in the spring, soil samples were collected weekly and were analyzed for inorganic N (NO<sub>3</sub>-N and NH<sub>4</sub>-N) to investigate mineralization over time. Cereal rye soil inorganic N concentrations were similar to that of the control in both the spring of 2012 and 2013. Fall N application into tillage radish, cereal rye, and control plots resulted in an average 91, 57, and 66% of the fall N application rate as inorganic N in the spring at the 0–20 cm depth, respectively. The inclusion of cover crops into conventional cropping systems stabilized N at the soil surface and has the potential to improve the efficiency of fall applied N.

**KEYWORDS:** cover crops, fall applied nitrogen, mineralization, plant available N

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### Introduction

Nitrogen (N) is an essential element for crop growth and production in row-cropping systems. Many studies have compared fall and spring N applications and found that spring application of N often resulted in greater yield.<sup>1–3</sup> For example in Minnesota, researchers found that spring applications (40% preplant, 60% sidedress) increased corn N recovery by 13% and yield by 14 kg ha<sup>-1</sup> relative to fall applied N without a nitrification inhibitor.<sup>4</sup> Despite the majority of the literature demonstrating agronomic advantages of spring applied N, other considerations such as avoiding adverse spring weather and N fertilizer prices often outweigh the benefit of spring application when farmers make N management decisions. Thus, across the Midwest, the literature estimates that fall applied N among farmers ranges from 25%–75% depending on soil texture and climate.<sup>5–9</sup>

Nitrate leaching from both fall and spring N applications is a leading cause of N loading to surface water and the development of the Gulf of Mexico Hypoxic Zone.<sup>10,11</sup> The timing of N application can have a significant impact on N leaching and loss from agriculture fields. For example, a study conducted over a period of seven years investigated the impact of N application timing on NO<sub>3</sub>-N leaching via tile water.<sup>5</sup> The researchers observed that over the seven year study, fall application of N resulted in an average of 14% higher N leaching than spring application. However, in a single year of the study (1999), which had 47% greater precipitation than the 30 year average for the region, nitrate leaching accounted for 48% of all the nitrate lost from fall applied N. The vulnerability of fall applied N was attributed to nitrification and leaching that occurred before the plant had a chance to absorb the N fertility from the soil. Therefore there is need to identify



adaptive management practices that will improve the efficacy of fall applied N.

Cover crops are one example of an adaptive management practice that has the potential to improve the efficiency of fall applied N. Commonly, cover crops are used in spring N application systems to scavenge N between cash crop growing seasons. Nitrogen taken up by cover crops is prevented from leaching and denitrifying and is stored within the structure of the plant to be released to the soil for the following cash crop. Cover crops have been shown to reduce nitrate leaching when N is applied in the spring in a variety of regions: Maryland,<sup>12</sup> Georgia,<sup>13</sup> and Iowa.<sup>14</sup> However, there is a lack of research that investigates the use of cover crops to improve the efficiency of fall applied N and that demonstrates the impact of cover crop species on the mineralization of soil N in the spring. Furthermore, in multiple studies, spring nitrogen immobilization in fields that contained cover crop residue has been linked to less available N for cash crops, as a result of N not being released from the cover crop residue.<sup>13,15–17</sup> Therefore, the objective of this study is to determine the quantity and timing of N mineralization from cover crop residue following winter and chemical termination in the spring. Winter termination is the process by which cover crops die because of exposure to winter frost and chemical termination is the process of terminating cover crops with chemical herbicides.

## Materials and Methods

The experimental site was located at the Illinois State University Teaching and Agriculture Research Farm in Lexington, IL, USA. The dominant soils within the site were Drummer and El Paso silty clay loams. Both soils were poorly drained, contained a 0%–2% slope, and required tile drainage. A background analysis of the nutrient content and chemical properties of the soil is present in Table 1. The cropping

**Table 1.** Background soil nutrient status and chemical properties of the experimental site.

ELEMENT	SOIL TEST
	mg kg <sup>-1</sup>
P	150.5
K	221.9
Ca	4285.2
Mg	636.8
<b>Inorganic N</b>	
NO <sub>3</sub> -N	18.5
NH <sub>4</sub> -N	7.7
<b>Soil chemical properties</b>	
pH	6.3
CEC (cmol <sub>c</sub> kg <sup>-1</sup> )	31.8
TOC (%)	2.4

**Table 2.** An activity schedule for the cultural practices within plots for both the 2012 and 2013 growing seasons.

FIELD ACTIVITY	MAIN CROP YEAR		
	2011	2012	2013
Spring cover crop sampling	–	Mar. 17	Apr. 07
Spring soil sampling 1	–	Mar. 18	Mar. 30
Cover crop termination	–	Mar. 21	Apr. 09
Spring soil sampling 2	–	Mar. 28	Apr. 05
Spring soil sampling 3	–	Apr. 03	Apr. 15
Spring tillage	–	Apr. 03	May 09
Spring soil sampling 4	–	Apr. 11	Apr. 29
Spring soil sampling 5	–	Apr. 18	May 15
Corn planting date	–	Apr. 23	May 15
Spring soil sampling 6	–	Apr. 25	May 19
Harvest sampling	Sep. 05	Aug. 24	–
Cover crop planting date	Sep. 08	Sep. 13	–
Fall cover crop sampling	Nov. 15	Nov. 27	–
Fall N fertilizer date	Nov. 15	Nov. 19	–

history of the site was continuous corn (*Zea mays L.*) for the last five years to support silage production. During the current two year (2011–2013) experiment, the continuous corn silage rotation was maintained (Table 2). The experimental site consisted of nine plots (2023 m<sup>2</sup>, half-acre) arranged in a complete randomized block design with three replications and three treatments: control (no cover crop), tillage radish (*Raphanus sativus L.*), and cereal rye (*Secale cereale L.*).

In 2011, 50 kg ha<sup>-1</sup> of N as (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was applied in October to provide adequate cover crop nutrition and to maximize cover crop growth. In November 2011, anhydrous ammonium (150 kg ha<sup>-1</sup>) was knifed into the living stand of cover crops. In November of 2012, anhydrous ammonium (200 kg ha<sup>-1</sup>) was knifed into cover crops and no starter fertilizer was applied in 2012, because of elevated available N concentrations in the soil following low N absorption during the 2012 drought stricken growing season. In both years tillage radish plants winter terminated by early January and cereal rye plants were chemically terminated in March with an application of glyphosate. Spring tillage was used to incorporate cover crop residue into the soil prior to corn planting to simulate the dominant cultural practice of the region.

**Plant sampling.** Aboveground shoot biomass was collected from random sampling locations at least 1 m from the edge of the plot. Tillage radish was sampled in November of each year before it was winter killed and cereal rye was sampled in the spring before it was chemically terminated. No biomass was collected from control plots at either the fall or spring plant samplings dates. Dry weights of three 0.25 m<sup>2</sup> quadrants were measured in each plot and used for nitrogen uptake determination and analysis. Corn plants were sampled at harvest each year and total tonnage was measured from the



middle six rows of each plot. Corn subsamples were collected from each plot, oven dried at 55°C, ground and then analyzed for N concentration. Ground samples were analyzed for %N using a combustion analyzer (FP523 N Analyzer, LECO St. Joseph, MI). Total N was used to establish N uptake for corn and cover crop plant samples. Nitrogen uptake was calculated by multiplying %N by total biomass (kg ha<sup>-1</sup>).

**Soil sampling.** In order to estimate the mineralization of N from cover crop residue, soil samples were collected on a weekly and biweekly weather dependent schedule in the spring before corn planting and were analyzed for soil NO<sub>3</sub>-N and NH<sub>4</sub>-N. Soil NH<sub>4</sub>-N was determined on two spring dates within each year, April 3 and April 18 of 2012 and April 5 and May 15 of 2013. These two dates in each year coincide with one week before cover crop termination and corn planting date. Three intact soil cores were collected from random locations in each plot to a depth of 0–20 cm and combined to create a composite sample. All soil samples were dried for 24 hours at 105°C, ground, and sieved through a 1 mm sieve before analysis. To determine NO<sub>3</sub>-N and NH<sub>4</sub>-N, a 5 g subsample from each depth was shaken with 50 ml of 0.01M CaCl<sub>2</sub> solution, filtered with Whatman 42 filter paper,<sup>18</sup> and analyzed using the Ion Chromatography (Dionex ICS 1100, Thermo Fisher) and Ion Selective Diffusion (Timberline TL-2800, Timberline Instruments), respectively.

**Statistical analysis.** The statistical analysis was conducted using two-way ANOVA as calculated by the Proc Mixed command in SAS.<sup>19</sup> Comparisons of soil inorganic N concentrations between treatments were analyzed and a *P* value of ≤0.10 was considered significant. Plant samples were analyzed using one-way ANOVA as calculated by Proc Mixed command in SAS and a *P* value of ≤0.05 was considered significant. For all comparisons, Tukey multiple means comparisons were used to compare treatments to each other and the control.

## Results and Discussion

### Weather, soil temperature, and soil moisture data.

Ambient air temperature and precipitation influences soil temperature and moisture, two vital variables that impact the soil microbial activity and the rate of mineralization. Moreover, both air temperature and precipitation are critical factors that dictate the vigor and length of winter cover crop growth. The average air temperature from January 2012–April 2012 was 6.13°C compared to 1.04°C during the same period in 2013. For instance, the months of February, March, and April of 2012 were 3.50, 12.50, and 2.61°C warmer relative to the same months in 2013, respectively (Table 3). Like air temperature, monthly precipitation was different between January–April of 2012 compared to 2013. For example, the months of January and April of 2012 had 53.59 and 56.13 mm less precipitation than the same months in 2013. Furthermore in 2012, there was 160.02 mm less total precipitation than in 2013 (Table 4).

**Table 3.** Average air temperature data and 30 year average for the region by month. Bold values represent air temperature during the cover crop growing season.

MONTH	2011	2012	2013	30 YEAR AVERAGE
<b>Air temperature (°C)</b>				
January	-6.78	<b>-0.78</b>	<b>-2.50</b>	-4.39
February	-3.22	<b>0.78</b>	<b>-2.72</b>	-1.50
March	3.28	<b>12.61</b>	<b>0.11</b>	4.67
April	9.72	<b>11.89</b>	<b>9.28</b>	11.06
May	15.28	<b>19.61</b>	<b>17.61</b>	16.83
June	21.50	21.78	21.78	21.67
July	26.39	27.61	22.72	23.78
August	<b>22.89</b>	<b>22.72</b>	22.39	22.72
September	<b>17.28</b>	<b>18.11</b>	20.39	18.83
October	<b>13.00</b>	<b>11.00</b>	–	12.00
November	<b>6.89</b>	<b>4.22</b>	–	4.83
December	<b>1.61</b>	<b>2.22</b>	–	-2.44

In the spring of 2012 and 2013, soil temperature and moisture (gravimetric water content) were measured on each soil sampling date to better understand soil mineralization and nitrification. In both years, there were no detectable differences in soil temperature and moisture among treatments. However, within treatments, soil temperature and moisture were different between years. The average soil moisture in the spring of 2012 (late March–late April) was 25.3% and the average soil temperature was 11.4°C. For the same time period in the spring of 2013, the average soil

**Table 4.** Monthly precipitation for the experimental site and the 30 year average monthly precipitation. Bold values represent air temperature during the cover crop growing season.

MONTH	2011	2012	2013	30 YEAR AVERAGE
<b>Precipitation (mm)</b>				
January	61.21	<b>36.07</b>	<b>89.66</b>	51.99
February	74.17	<b>33.27</b>	<b>58.67</b>	49.36
March	75.95	<b>21.08</b>	<b>45.97</b>	69.77
April	139.45	<b>70.36</b>	<b>126.49</b>	93.90
May	120.65	<b>54.10</b>	<b>171.20</b>	113.37
June	124.46	42.93	66.29	100.92
July	107.95	37.08	32.00	104.06
August	<b>58.42</b>	<b>114.81</b>	74.93	100.92
September	<b>73.41</b>	<b>148.08</b>	30.48	79.59
October	<b>60.71</b>	<b>125.98</b>	–	81.36
November	<b>78.99</b>	<b>25.40</b>	–	83.82
December	<b>92.71</b>	<b>51.31</b>	–	71.29



**Table 5.** Cover crop biomass and N uptake means for both the 2012 and 2013 growing seasons.

YEAR	BIOMASS (kg ha <sup>-1</sup> )		NITROGEN UPTAKE (kg N ha <sup>-1</sup> )	
	2011–2012	2012–2013	2011–2012	2012–2013
Tillage radish	6561.9 Aa	3707.5 Ac	226.8 Bd	131.9 Bd
Cereal rye	3906.5 Ba	5585.5 Bc	188.1 Bd	249.9 Bd

**Note:** Capital letters indicate significant differences between treatments within years and lowercase letters indicate significant differences between years within treatment. (Alpha level of 0.05).

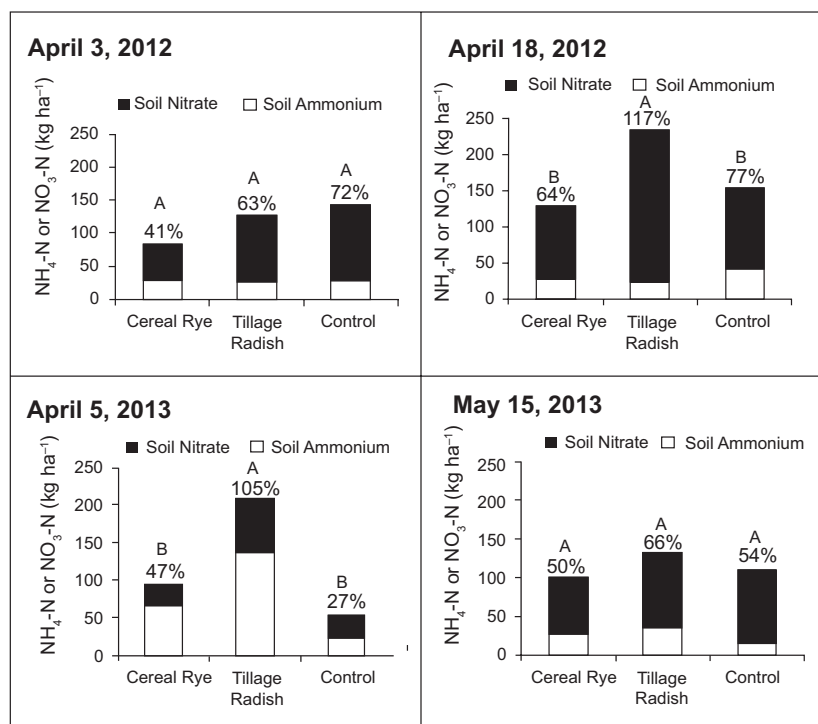
moisture was 30% and average soil temperature was 7.6°C. Soil temperature and moisture data in 2013 were measured into mid-May because of delayed corn planting. In May 2013, average soil temperature was 15.7°C and average soil moisture was 26.6%.

**Cover crop shoot dry matter and nitrogen uptake.** In the 2011/2012, cover crop growing season tillage radish accumulated 6,561.9 kg ha<sup>-1</sup> dry matter and absorbed 226.8 kg N ha<sup>-1</sup> of total N uptake. In the 2012/2013 cover crop growing season, tillage radish had significantly less biomass (3,707.5 kg ha<sup>-1</sup>,  $P \leq 0.001$ ) and N uptake (131.9 kg N ha<sup>-1</sup>,  $P \leq 0.001$ ). Cereal rye accumulated 3,906.5 kg ha<sup>-1</sup> dry matter in the 2011/2012 growing season and absorbed 188.1 kg N ha<sup>-1</sup> dry matter (Table 5). In the 2012/2013

season, cereal rye resulted in significantly greater biomass (5585.5 kg ha<sup>-1</sup>,  $P = 0.044$ ) and N uptake (249.9 kg N ha<sup>-1</sup>,  $P \leq 0.001$ ).

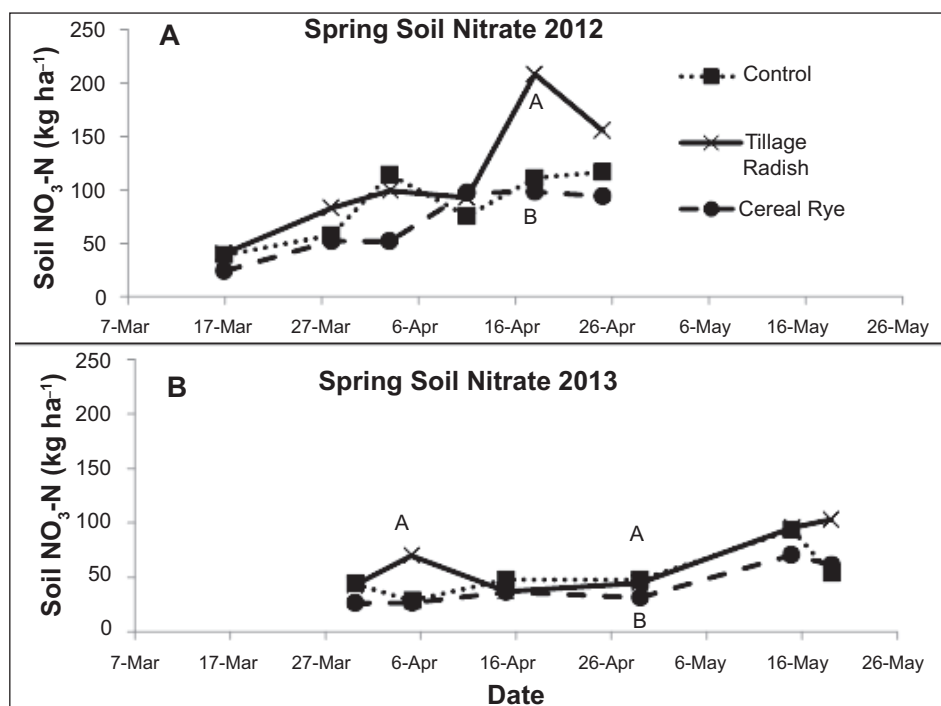
Both tillage radish and cereal rye had significantly different biomass and N uptake values between the first and second cover crop growing season. It is likely that the variability in weather impacted the growth of both cover crops. Colder air temperatures in late September, October, and November 2012 relative to 2011 likely contributed to less tillage radish biomass and N uptake. Cereal rye was less affected by colder air temperatures and grew vigorously in the fall and spring of each year. However, despite the differences in weather, tillage radish and cereal rye demonstrated the ability to absorb nearly the full rate of N applied in the fall. In fact, when averaged over two years, tillage radish and cereal rye N uptake were 179.4 and 219.0 kg ha<sup>-1</sup>, respectively (90 and 110% of N that was fall applied). This observation is vital because sequestered N is less vulnerable to loss since it is assimilated into the cover crop biomass and not susceptible to leaching or denitrification.

**Spring soil mineralization and nitrification.** The spring of 2012 was relatively warm and dry compared to the spring of 2013. As a result of warm ambient air temperatures, soil temperatures in 2012 were 3.8°C warmer than in the spring of 2013. Thus, in the spring of 2012, soil NH<sub>4</sub>-N was not significantly different between treatments on either sampling date



**Figure 1.** Total inorganic soil N at the dates of cereal rye termination and corn planting in 2012 and 2013. The percentage on top of each bar represents the amount of inorganic N as a percentage of fall applied N (200 kg N ha<sup>-1</sup>). The percentage was calculated by dividing the concentration of inorganic kg N ha<sup>-1</sup> found in the 0–20 cm depth of soil in the spring by the kg N ha<sup>-1</sup> applied in the fall as anhydrous ammonia. Capital letters that are different indicate significant difference among treatments at alpha level 0.10.





**Figure 2.** Soil nitrate from the 0–20 cm depths for both the springs 2012 (A) and 2013 (B). Capital letters indicate significant differences ( $P \leq 0.10$ ) between treatments within sampling date.

(Fig. 1). However, over the same time period, soil  $\text{NO}_3\text{-N}$  concentration significantly increased between the first and last soil sampling dates (41 days after cereal rye termination) by 152.1, 71.361, and 56.3  $\text{kg ha}^{-1}$  for tillage radish, cereal rye, and the no cover crop control, respectively (Fig. 2). Furthermore, tillage radish significantly ( $P < 0.08$ ) increased soil  $\text{NO}_3\text{-N}$  by at least 97.1  $\text{kg ha}^{-1}$  relative to all other treatments, 28 days after cereal rye termination.

In contrast to 2012, in the spring of 2013, fall application of N into tillage radish resulted in significantly more soil  $\text{NH}_4\text{-N}$  (137.7  $\text{kg ha}^{-1}$ ) compared to both the control (24.7  $\text{kg ha}^{-1}$ ,  $P = 0.02$ ) and cereal rye (66.7  $\text{kg ha}^{-1}$ ,  $P = 0.06$ ) (Fig. 1). At the same sampling date, fall application into tillage radish resulted in significantly greater  $\text{NO}_3\text{-N}$  relative to the control plots (41.2  $\text{kg ha}^{-1}$ ,  $P = 0.06$ ) and cereal rye plots (43.3  $\text{kg ha}^{-1}$ ,  $P = 0.05$ ) (Fig. 2). Additionally, 20 days after cereal rye termination, the control resulted in significantly more soil  $\text{NO}_3\text{-N}$  compared to cereal rye ( $P = 0.10$ , 16.3  $\text{kg ha}^{-1}$ ).

In the spring of 2012, soil temperatures above 10°C resulted in increased mineralization of tillage radish residue followed by rapid nitrification of mineralized  $\text{NH}_4\text{-N}$ . Rapid nitrification contributed to consistently greater soil nitrate in tillage radish plots compared to control and cereal rye plots (Fig. 1). In contrast to 2012, soil temperatures below 10°C in April of 2013 reduced the rate of soil nitrification. As a result, a greater concentration of mineralized  $\text{NH}_4\text{-N}$  was observed in the soil. However, from April to May of 2013, soil temperatures increased above 15°C and the plots received 173 mm of

precipitation. Thus, soil  $\text{NH}_4\text{-N}$  in tillage radish plots decreased by 101.4  $\text{kg ha}^{-1}$  potentially because of nitrification and N loss by leaching or denitrification. Similarly, in Maryland, researchers found that early mineralized N from forage radish resulted in increased spring nitrate leaching on sandy soils.<sup>12</sup> Another cover crop mineralization study in the Netherlands observed soil inorganic N from May through July and concluded that the increases in inorganic N were a result of mineralization of natural organic matter, not from the mineralization of forage radish.<sup>20</sup> However, our data indicate that in two weather extremes mineralization of tillage radish residue directly increased soil inorganic N immediately before planting cash crops.

Consistently lower soil nitrate concentrations in cereal rye plots compared to tillage radish and control plots are potentially the result of three factors. First, cereal rye has a much later termination date relative to tillage radish and there was less time for cereal rye residue to mineralize each spring. Second, a higher C/N ratio of cereal rye biomass compared to tillage radish potentially slowed the rate of cereal rye mineralization. Third, cereal rye residue with a C/N ratio above 30/1 can lead to microbial immobilization soil inorganic N.<sup>12</sup> Visual observations illustrated that by corn planting little mineralization of cereal rye biomass had occurred in the spring of 2013. In agreement with our results, Adeli et al<sup>15</sup> concluded that the lack of N mineralization from winter rye biomass reduced available N in the soil. This is a potential benefit to farmers because N sequestered in cereal rye biomass is much less vulnerable to loss than soil N or early mineralized N from tillage radish residue.



**Table 6.** Corn silage and N uptake for the 2012 and 2013 growing seasons.

YEAR	CORN SILAGE (Mg ha <sup>-1</sup> )		NITROGEN UPTAKE (kg N ha <sup>-1</sup> )	
	2012	2013	2012	2013
Tillage radish	14.9a	51.6b	99.2a	273.1b
Cereal rye	16.9a	52.3b	128.4a	266.4b
Control	20.4a	50b	140.6a	263.8b

**Note:** Lowercase letters indicate significant differences between years within treatment. (Alpha level of 0.05).

**Silage yields.** Corn silage data was collected from the middle six rows of each plot at harvest each year and the moisture was adjusted to 55%. Across both years, fall applied nitrogen into a standing cover crop did not significantly impact corn yield or corn N uptake compared to the control (Table 6). Drought conditions in the summer of 2012 resulted in extremely low average corn silage (18.8 Mg ha<sup>-1</sup>) and N uptake (127.3 kg N ha<sup>-1</sup>) across all treatments (Table 6). In contrast, the normal growing conditions of 2013 resulted in nearly three times greater silage production (51.3 Mg ha<sup>-1</sup>) and two times more N uptake (267.9 kg N ha<sup>-1</sup>) across treatments.

In both years, tillage radish increased the amount of inorganic N available at planting compared to the control and cereal rye plots. Despite this benefit, there were no significant differences in silage yield in either year. It is likely that stress from drought conditions in the summer of 2012 limited our ability to detect any impact cover crops might have had on corn silage and N uptake. In 2013, excess residual N was present in the soil after harvest because of low N uptake by corn. Furthermore, the full rate of fall N was applied resulting in an abundance of N available to the corn plant and any yield response from improved N management in the second year with cover crops was not detectable.

## Conclusions

The mineralization and nitrification of fall applied N from cereal rye residue was slower compared to tillage radish. Less time for cereal rye to mineralize in the spring and a greater C/N ratio could have resulted in a slower release of fall applied N to the subsequent cash crop. As a result, N absorbed by cereal rye remained as organic N to be slowly released after planting of the cash crop. In contrast, rapid mineralization of tillage radish residue resulted in a two year average of 91% of the equivalent rate of fall applied N as inorganic soil N in spring at the 0–20 cm depth compared to 66% for the control and 57% for cereal rye plots.

Currently, the cover crop industry recommends terminating cereal rye three to four weeks before the expected cash crop planting date in order to benefit from the mineralization of N from cereal rye residue. However, the data

from this study suggests that this termination date does not allow enough time for the mineralization of absorbed N in cereal rye to occur, despite weather extremes. Therefore, there is a need to further examine the influence of cereal rye and other commonly adopted cover crop species, such as annual ryegrass (*Lolium multiflorum*), hairy vetch (*Vicia villosa*), and oats (*Avena sativa*) on soil mineralization during the cash crop growing season. This would allow farmers to better synchronize mineralization and N release from cover crop residue with the growth stages of the subsequent cash crop.

As expected, with several months to decompose tillage radish, plots mineralization of absorbed N was greater relative to cereal rye. This early release of N could be a concern because of the potential for N loss by leaching or denitrification. One potential solution to this concern is planting a mixture of winter kill and over wintering cover crops species. Planting a mixture of tillage radish and winter hardy cover crops that grow vigorously in the spring could reduce the susceptibility of early mineralized N loss from tillage radish. Despite variable weather across both experimental years, cereal rye and tillage radish demonstrated the potential to absorb the full rate of fall applied N and stabilized the N within the 0–20 cm depth which has the potential to improve the effectiveness of fall applied N.

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## Author Contributions

Conceived and designed the experiments: SDA, CGL. Analyzed the data: SDA, CGL. Wrote the first draft of the manuscript: SDA, CGL. Contributed to the writing of the manuscript: SDA, CGL. Agree with manuscript results and conclusions: SDA, CGL. Jointly developed the structure and arguments for the paper: SDA, CGL. Made critical revisions and approved final version: SDA, CGL. All authors reviewed and approved of the final manuscript.

## DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.



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