A DIRECT APPROACH TO MEASURE COVER CROP NITROGEN UPTAKE FROM DAIRY MANURE VIA ^{15}N ENRICHMENT

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ABSTRACT

Fall manure applications are a standard practice across Wisconsin, primarily due to manure storage constraints and unpredictable spring field conditions. Unfortunately, manure derived nitrogen (N) is at risk for runoff and leaching into groundwater without an appropriate mechanism for N retention. Fall-planted grass cover crops can serve as N scavengers, reducing losses of manure N to the environment, especially post-silage harvest, however potential tradeoffs between sufficient N uptake and spring termination must be considered between winter-killed and overwintering varieties. This study aims to directly quantify manure N in fall-planted cover crops utilizing ^{15}N labeled dairy manure to directly determine differences in N uptake between winter-killed and overwintering varieties, while also tracking manure N in the soil profile. Spring Barley, spring oats, winter wheat, and winter rye were drill-seeded in the fall at 60 lb ac^{-1}, in addition to a no cover crop treatment, following semi-solid manure application and incorporation at approximately 220 lb total N ac^{-1} at the Arlington Agricultural Research Station (Arlington, WI). Main plots received unlabeled manure (control) and microplots within the main plots received ^{15}N labeled manure. Cover crop root and shoot samples and soil samples were taken in December and April to measure Total N/^{15}N of plant and soil. Presented results will directly quantify the amount of manure N in cover crop biomass with a stable isotope informed approach of ^{15}N manure labeling. These results will illuminate our knowledge regarding the fate of fall applied manure N when cover crops are utilized in the North Central US.

INTRODUCTION

In Wisconsin, cover crops are a promising management strategy for addressing environmental and producer concerns pertinent to manure applications. Nitrate is the greatest contaminant concern for groundwater in the state of Wisconsin, and nitrate pollution continues to increase statewide (WDNR, 2022). Runoff from applied nutrients, including manure, accounts for nearly 90% of groundwater nitrate (WDNR, 2022), which also equates to an economic loss for producers. Nearly 63% of Wisconsin acres grown for corn received dairy manure in 2010, and almost 56% of it was fall applied, according to a study done by Mitchell et al. (2021). Storage constraints and unpredictable spring weather make fall manure applications ideal, however, no growing crop is typically in place by manure application time. Non-leguminous, fall-planted cover crops can potentially utilize a significant amount of manure-derived nitrogen (N), but increased producer confidence for practice adoption is still needed in the state. This study aims to better describe manure N availability in-field under cover crop utilization, and the success of different grass cover crop varieties as N scavengers. The objectives of this study were to i) compare root and shoot biomass between winterkilled and overwintering cover crop varieties in fall and spring prior to termination, ii) measure manure-N uptake across cover crop varieties, iii) determine residual manure-N in soil following cover crop termination and when no cover crops were planted.

MATERIALS AND METHODS

Experimental design and field setup: This two-year study is being conducted from Fall 2023- Spring 2025 at the University of Wisconsin Arlington Agricultural Research Station (Arlington, WI) (43º18'9.47"N, 89 º 20'43.32"W). The soil is Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll, US Department of Agriculture [USDA] Soil Taxonomy) with 6% sand, 72% silt, and 22% clay. 20- 9×12ft field plots were established in a pseudo-replicated block design with 3×3ft microplots installed within each field plot that received labeled ^{15}N manure. The remaining outside area of the main received unlabeled manure (control). Four cover crop varieties, two overwintering (winter wheat and winter rye) and two winter-killed (barley and oats) were planted in the four block reps of the experiment. Additionally, a no cover crop treatment was established that only received manure. Cover crops were drill-seeded in early October 2023 at a seeding rate of 60 lb acre^{-1}. Prior to planting, both unlabeled and labeled manure were spread and incorporated into the soil at an approximate rate of (220 lb total N acre^{-1} or ~60 lb available N acre^{-1}). Refer to table 1 for a timeline of research activities related to this project.

Timeline	Summer 2023/2024	Fall 2023/2024	Spring 2024/2025
Plans/ measurements	Installation of microplots, labeled/unlabeled manure production.	Manure application, plant cover crops. Baseline soil samples for TN/^{15}N. TN/^{15}N of plant biomass in late fall.	Cover crop termination, yield data, TN/^{15}N of plant biomass and soil. End Field Experiment

Table 1. Project timeline outlining years 1 and 2 of field and lab setup/sampling.

Production of unlabeled and ^{15}N labeled manure: The protocol described has been approved by UW-Madison Institutional Animal Care and Use Committee (IACUC) prior to the start of the experiment. Two cannulated mid-lactation dairy cows from the UW-Madison Dairy Cattle Center were utilized to produce both labeled and unlabeled manure (feces + urine). Over a 12-day period, cows were bedded on mattresses in a tie-stall barn. No additional wood or hay bedding was used to better control the C:N of manure between collection periods.

Cow feeding regimes were normal daily herd total mixed rations (TMR), but urea N was supplemented by direct addition into the rumen of the cannulated cows. Pharmaceutical grade unlabeled urea was fed to both cows on days 1-8 to produce enough unlabeled (control) manure in a timely manner. On days 9-11, reagent grade (>99% purity) ^{15}N urea @ 98at% was mixed with unlabeled urea to achieve ^{15}N urea @ 10at%, which amounted to ~5g of reagent grade ^{15}N urea in the total 50g fed daily. ^{15}N urea was only fed to one cow due to the lower amount of manure needed for microplots and expense of ^{15}N urea. To allow for animal adaptation to a nonprotein nitrogen source, unlabeled urea was supplemented through the cannula in increasing increments of 10g per day, up to 50g of urea per day by day 5 (table 2). All urea was dissolved in 100mL of distilled water to evenly apply over rumen contents. By day 12, both cows were returned to their daily TMR with no urea supplementation.

Day	Feed amount/type	Fecal Collection (Feces + Urine)
1	10g unlabeled urea	None
2	20g unlabeled urea	Unlabeled manure collection
3	30g unlabeled urea	Unlabeled manure collection
4	40g unlabeled urea	Unlabeled manure collection
5	50g unlabeled urea	Unlabeled manure collection
6	50g unlabeled urea	Unlabeled manure collection
7	50g unlabeled urea	Unlabeled manure collection
8	50g unlabeled urea	Unlabeled manure collection
9	50g labeled urea (10at% ^{15}N) (1 cow)	^{15}N labeled manure collection
10	50g labeled urea (10at% ^{15}N) (1 cow)	^{15}N labeled manure collection
11	50g labeled urea (10at% ^{15}N) (1 cow)	^{15}N labeled manure collection
12	Normal TMR	^{15}N labeled manure collection

Table 2. Daily unlabeled and labeled urea feeding and manure collection schedule.

Manure (feces + urine) was collected twice daily in pans below the gutter grates that allow cows to defecate normally. Manure was stored in 5-gallon buckets at 4°C for < 2 weeks to minimize chemical decomposition of manure beyond a single day to accommodate the logistics of field application. The manure was sub-sampled after being mixed with a paint mixer prior to storage and air-dried at 40°C or frozen at -20°C to conduct isotopic and routine analysis of the manure. Prior to field application, both unlabeled and labeled manure was mixed and subsampled again for isotopic and routine manure analysis. For labeled manure, digested ^{15}N urea takes between 32-96 hours to evenly label between urine and feces (urine and endogenous fecal N pools), so manure collected on days 11 and 12 (days 3 and 4 of ^{15}N feeding) were prioritized for microplot application. A minimum ^{15}N at% of 10% over natural abundance can be used for short-term (1-2 year) crop N cycling studies (Powell et al., 2004).

Soil and Plant Sampling: Soil sampling occurred prior to planting and manure application for baseline soil ^{15}N/TN, in late fall after spring barley and oat winterkill, and in the spring following winter wheat and rye termination. Within each plot, five cores were taken using a hand probe and divided between 0-20cm and 20-40cm, then homogenized by depth and plot, yielding two homogenized soil samples per plot (control ^{15}N). Microplot sampling followed the same protocol except 2 cores were taken (sample ^{15}N). Soils were air-dried, passed through a 2mm sieve, and prepared for ^{15}N/total N analysis. Whole plant biomass sampling occurred for all four cover crops within the field plots (control ^{15}N) and microplots (sample ^{15}N) in late fall once the winter-killed varieties began to winterkill, and immediately prior to chemical termination of overwintering varieties. 5 individual plants from the main and microplots were dug up to encompass the root mass, then roots were soaked in water to remove all soil prior to separating from shoot and drying. Plant samples were oven dried @ 60°C, weighed, and ground for ^{15}N/total N analysis. Winter wheat and rye aboveground biomass were sampled from a 3ft^2 quadrat (three rye rows).

TN/^{15}N analysis: Soil and plant material were prepared for IRMS analysis according to protocols set forth by the Freedman Lab Core at UW-Madison.

RESULTS AND DISCUSSION

Dairy manure enrichment: ^{15}N dairy manure produced in August 2023 was successfully labeled >10% of natural abundance (0.37at%)(fig. 1). Within 72 hours after the initial ^{15}N urea feeding, isotopic enrichment increased by almost 50%, peaking at 0.57at% at 4am on day four of collection. These results indicate that the approach used for ^{15}N labeling of dairy manure was successful, and we would expect that the isotopic signal is strong enough to trace ^{15}N manure into both the cover crops and soil profile.

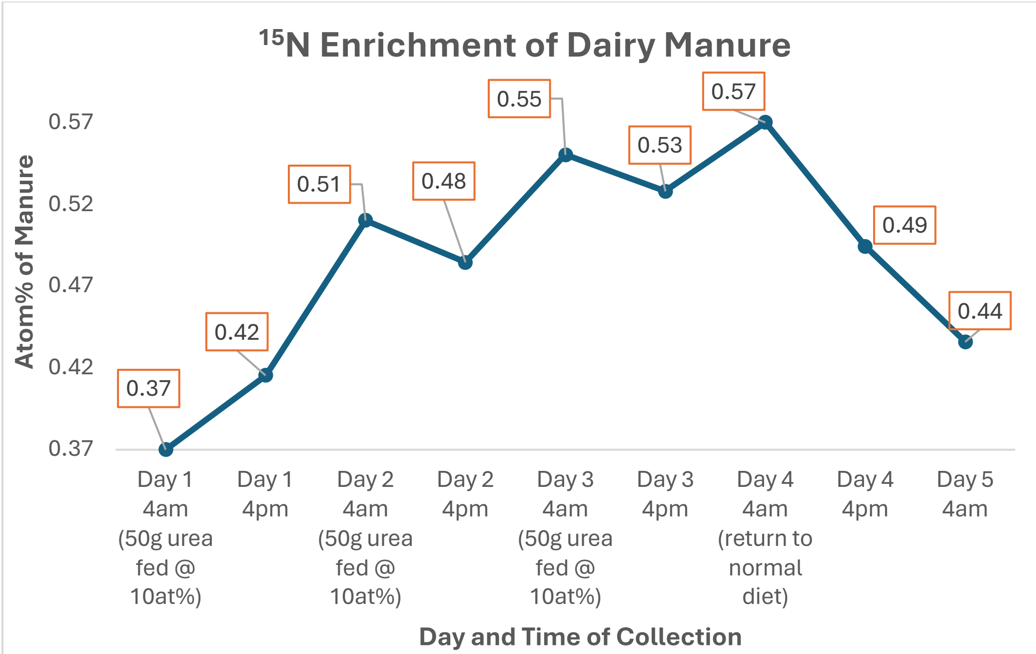

Fig. 1. Change in 15N at% of collected manure across three days of feeding with two manure collections daily.

Cover Crop Biomass: In Fall 2023, cover crop growth was limited by a late September seeding date and early snowfall. All four cover crop varieties successfully established in the plots (fig. 2), however spring barley and oats unlikely obtained enough growth to retain an environmentally relevant amount of manure N. At Fall plant sampling, shoot biomass made up >60% of the total biomass on average in winter rye, winter wheat, and barley (fig. 3). By spring sampling, shoot biomass still accounted for >50% of total biomass in winter rye and winter wheat, however root biomass accounted for nearly 40% of the total biomass (fig. 4). Calculated yield for winter rye and winter wheat were approximately 2000lb acre^{-1} and 1500lb acre^{-1}, respectively.

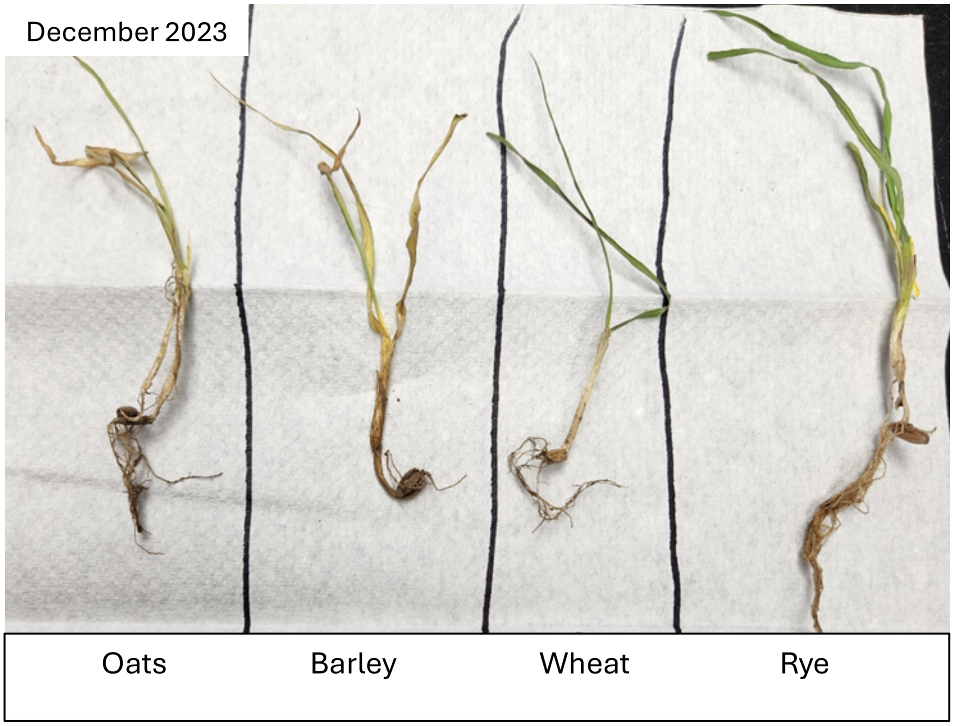

Fig. 2. Spring oats, spring, barley, winter wheat and winter rye sampled in December 2023

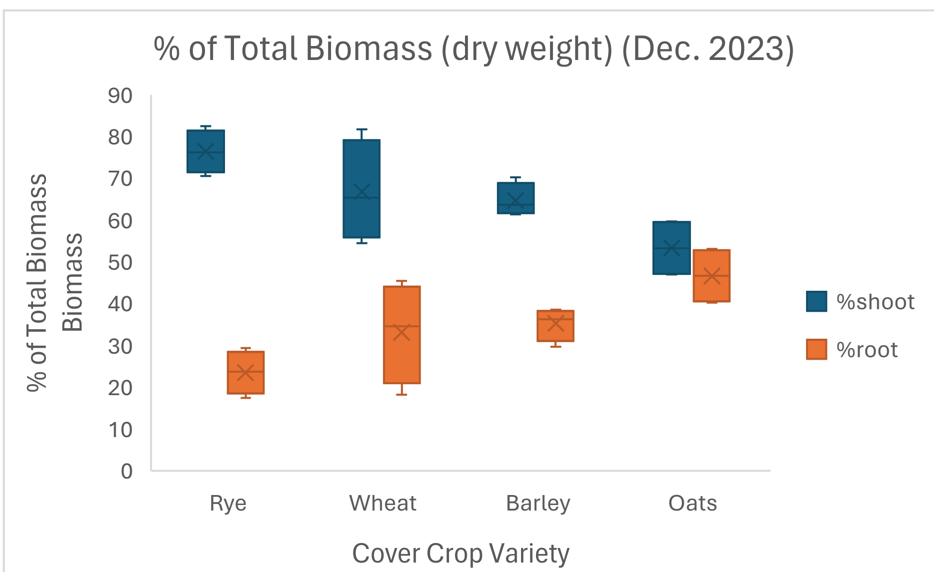

Fig. 3. % of total biomass attributed to root or shoot between four cover crop varieties samples in December 2023 following winterkill of spring barley and oats.

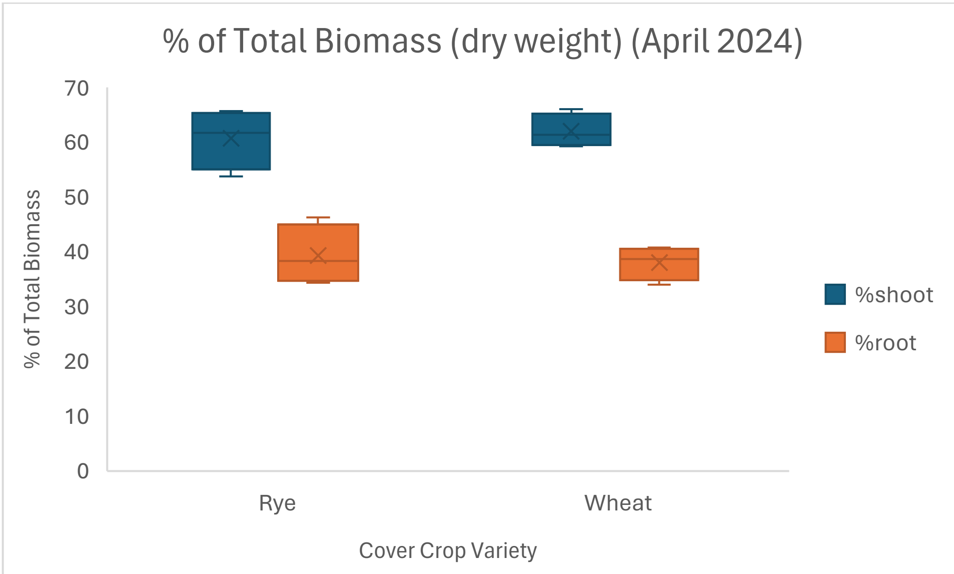

Fig. 4. % of total biomass attributed to root or shoot between four cover crop varieties samples in April 2024 following winterkill of spring barley and oats.

Manure N uptake by cover crops: Cover crop samples are still being analyzed via IRMS for ^{15}N/TN but will be presented at the conference. We expect that all four cover crop varieties sampled in late fall to have a similar amount of manure N uptake given their growth. From Spring sampling, we expect that winter rye will retain more manure N than winter wheat, but winter wheat still served as an effective N scavenger. We expect to see that spring-sampled winter rye and winter wheat will have a significant amount of their total N stored in their root biomass.

CONCLUSION

^{15}N labeled urea fed to dairy cattle successfully produced dairy manure with an isotopic label between 10-50% above natural abundance, likely sufficient for 1–2 year N cycling studies in-field. In study year 1 (Fall 2023- Spring 2024), winterkilled varieties were likely not planted early enough to obtain sufficient biomass to serve as effective N scavengers, however all varieties did establish. Winter rye obtained the greatest biomass prior to spring termination, and we expect it also retained the greatest amount of manure N.

REFERENCES

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