

LABILE CARBON APPLICATION AND VINE REMOVAL
TO REDUCE NITRATE LEACHING IN SANDY POTATO SYSTEMS

by

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ABSTRACT

Groundwater nitrate (NO_3^-) contamination is a growing concern in potato (*Solanum tuberosum* L.) production systems in sandy soils due to high nitrogen (N)-demand crops and coarse texture soil. We tested whether adding labile carbon (C) to soil in the form of glycerol, a biodiesel production byproduct, could decrease NO_3^- leaching by stimulating microbial activity and increasing N immobilization. Glycerol was broadcast-applied at 0, 1122, and 2244 L ha⁻¹ following potato vine kill at Hancock Research Station, Hancock, WI and Langlade Research Station near Antigo, WI. A similar study was done at a commercial farm in the Wisconsin Central Sands, where glycerol was applied at potato hilling. We used a combination of field sampling, resin lysimeters, and laboratory experiments to assess the effects of glycerol on NO_3^- leaching, soil NO_3^- content, and N and C cycling. At Hancock and Antigo, soil NO_3^- decreased by 33.4 and 101 kg N ha⁻¹ at the high glycerol rate 22.3 and 54.3 kg N ha⁻¹ at the low rate, respectively, compared to the control, but this effect did not last after six weeks at Hancock. Six weeks after glycerol application at Hancock, soil NO_3^- decreased by 3.4 and 16.5 kg N ha⁻¹ but was not statistically significant. Glycerol did not have a detectable effect on estimated NO_3^- leaching at Hancock or the commercial farm and there was high variation in NO_3^- leaching values among resin lysimeters. During all lab incubations soil NO_3^- was depleted up to 96% regardless of glycerol treatment or sampling timepoint, possibly driven by N immobilization in particulate organic matter. When glycerol was applied to soil in the lab, cumulative 28-d C-mineralization increased by 63% (high glycerol rate) and 29% (low glycerol rate) compared to the control. Although glycerol initially reduced soil NO_3^- , the larger question is when the immobilized N might reappear. In a separate experiment at Hancock Research Station, we measured the N content of potato vines at the onset of senescence to quantify how much NO_3^- leaching could be prevented with their removal. Previous work found that removing potato vines from the field can

potentially reduce pathogen pressure, but we are unaware of any research on the impact of vine removal on NO_3^- leaching in potato systems. In addition to vine removal, we also tested the potential of intercropping with winter rye and banded fertilizer at a reduced rate as strategies to reduce NO_3^- leaching. Data from resin lysimeters shows that when winter rye was present, estimated NO_3^- leaching was $35.7 \text{ kg N ha}^{-1}$ less than when no intercrop was present and NO_3^- leaching tended to be higher under broadcast fertilizer ($42 \pm 6.7 \text{ kg N ha}^{-1}$) than fertilizer banded at a reduced rate ($31.9 \pm 5.6 \text{ kg N ha}^{-1}$). The N stock and N percentage of potato vines were not affected by intercropping but were increased by broadcast fertilizer by 11.1% (N percentage) and 22.9% (N stock). The vines had a high average N percentage ($1.6 \pm 0.03\%$) and had an average N stock of $38.1 \pm 2.0 \text{ kg N ha}^{-1}$, meaning if those vines were removed from the field after desiccation, that amount of leaching could be prevented. Intercropping with rye, banded fertilizer, and vine removal have the potential to at least temporarily reduce NO_3^- leaching in potato systems, thereby decreasing NO_3^- inputs to groundwater.

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Chapter 1: Glycerol application to reduce nitrate leaching

1. Abstract

Groundwater nitrate (NO_3^-) contamination is a growing concern in potato (*Solanum tuberosum* L.) production systems in sandy soils due to high nitrogen (N)-demand crops and coarse textured soil. We tested whether adding labile carbon (C) to soil in the form of glycerol, a biodiesel production byproduct, could decrease NO_3^- leaching by stimulating microbial activity and increasing N immobilization. Glycerol was broadcast-applied at 0, 1122, and 2244 L ha⁻¹ following potato vine kill at Hancock Research Station, Hancock, WI and Langlade Research Station near Antigo, WI. A similar study was done at a commercial farm in the Wisconsin Central Sands, where glycerol was applied at potato hilling. We used a combination of field sampling, resin lysimeters, and laboratory experiments to assess the effects of glycerol on NO_3^- leaching, soil NO_3^- content, and N and C cycling. At Hancock and Antigo, soil NO_3^- decreased by 62% and 65% at the high glycerol rate 41% and 31% at the low rate, respectively, compared to the control, but this effect did not last after six weeks at Hancock. In terms of N stock, glycerol at the high and low rate respectively decreased soil NO_3^- by 33.4 and 22.3 kg N ha⁻¹ at Hancock, and by 101 and 54.3 kg N ha⁻¹ at Antigo, compared to the control. Six weeks after glycerol application at Hancock, soil NO_3^- tended to decrease by 3.4 and 16.5 kg N ha⁻¹ compared to the control, but was not statistically significant. Glycerol did not have a detectable effect on NO_3^- leaching at Hancock or the commercial farm, as estimated using resin lysimeters, and there was high variation in NO_3^- leaching values among lysimeters. During all lab incubations soil NO_3^- was depleted up to 96% regardless of glycerol treatment or sampling timepoint, possibly driven by N immobilization in particulate organic matter. When glycerol was applied to soil in the lab, cumulative 28-d C-mineralization increased by 63% (high glycerol rate) and 29% (low glycerol

rate) compared to the control. Although glycerol initially reduced soil NO_3^- , the larger question is when the immobilized N might reappear. Nevertheless, labile C application potentially holds promise as a method to temporarily immobilize N, prior to later uptake by cover crops or cash crops.

2. Introduction

Groundwater nitrate (NO_3^-) contamination is a long-standing and ongoing issue globally and across North America, particularly in agricultural land (Power & Schepers, 1989; Singh et al., 2022). A major contributor is agricultural systems with high inorganic nitrogen (N) fertilization and long periods without living cover in annual cropping systems (Nouri et al., 2022). Not all the N applied to agricultural fields is taken up by plants; some is left in the soil-water solution as NO_3^- , which is highly soluble and easily leached through soil into groundwater. Groundwater is the primary household water source in many rural areas, and NO_3^- consumption has been linked to various negative health impacts including cancer and fetal development issues (Wolfe and Patz, 2002; Mathewson et al., 2020). Potato (*Solanum tuberosum* L.) production systems in the Central Sands Region of Wisconsin are particularly vulnerable to NO_3^- leaching due to their high N fertilizer demand and coarse soil texture (Kraft and Stites, 2003). Additionally, in this region groundwater recharges to surface water, transporting NO_3^- and contributing to water quality issues further from the source of contamination (Bundy and Andraski, 2005). The typical cropping system of this region includes a three- or four-year rotation of potato, field corn or sweet corn (*Zea mays* L), soybean (*Glycine max* L.), and/or snap beans (*Phaseolus vulgaris*) or other processing crops (Heineman and Kucharik, 2022). University of Wisconsin Extension recommends 162 to 280 kg N ha⁻¹ fertilizer depending on potato yields (Laboksi and Peters, 2012), but growers in the region often apply higher rates. Even following University Extension

fertilizer recommendations, estimated $\text{NO}_3\text{-N}$ leaching in the Central Sands averaged 119 and 203 kg ha^{-1} for sweet corn and potato, respectively (Stites & Kraft, 2001). One study in the Central Sands found 133 kg ha^{-1} NO_3^- leached during the potato phase of the rotation and 150 kg ha^{-1} leached during the sweet corn phase, with 60% of leaching occurring after the growing season during the potato phase of the rotation (Masarik, 2023). Due to the concern of groundwater NO_3^- contamination, many studies have tested potential strategies for reducing NO_3^- leaching in potato cropping systems on sandy soils, including nutrient and irrigation management, nitrification inhibitors, increasing water-holding capacity, and cover crops (Shrestha et al., 2010).

Another potential strategy to reduce NO_3^- leaching is applying carbon (C)-rich material to the soil to stimulate microbial activity and increase N immobilization, making NO_3^- less vulnerable to leaching. Applying a material with a high C:N can provide C-limited microorganisms with C, stimulating growth and promoting immobilization of inorganic N (NO_3^- and ammonium (NH_4^+)) (Schimel and Weintraub, 2003). There are many high-C amendments that have been effective in reducing NO_3^- leaching, including wheat straw residue and biochar, which reduced NO_3^- leaching by 35% and 27%, respectively, compared to the control treatment in one study (Demiraj et al., 2018). However, the mechanism for biochar reducing leaching is not the same as for other high-C amendments; it can adsorb NO_3^- and increase water holding capacity, among other mechanisms (Borchard et al., 2019). Another study that tested the effects of three C-rich organic amendments (barley straw, effluent solids, woodchip bedding) on reducing NO_3^- leaching from cow urine found that straw and effluent solids could reduce NO_3^- leaching by up to 25% compared to the control treatment (Malcolm et al., 2019). A soil column experiment in Florida found sugarcane bagasse was effective at reducing N, phosphorus (P), and

potassium (K) leaching (Xu et al., 2022). Some of these amendments may have some drawbacks; for example, materials that are not easily decomposed, such as woodchips, would not necessarily be effective at promoting rapid N immobilization (Malcolm et al., 2019). Moreover, materials that slowly decompose and immobilize N during the crop growing season could compete for N with crops. While biochar can reduce NO_3^- leaching (Demiraj et al., 2018), it has also been found to increase soil salinity, pH, and toxic organic contaminants in some cases (Brtnicky et al., 2021). For an organic amendment to be practical, it needs to be readily available and not impact N availability to the subsequent crop or negatively influence soil properties.

In our study, we tested the potential of glycerol derived from soybeans to be used as a source of labile C to reduce NO_3^- leaching. Glycerol is a byproduct of biodiesel production, so it might be readily available in regions with biodiesel refineries, and it is easily mineralized by microbes (De et al., 2022). Several previous studies examined effects of glycerol on N dynamics and NO_3^- leaching. Potter et al. (2023) applied crude glycerol to a field corn and soybean production field in Iowa and found that glycerol decreased soil NO_3^- to 45 cm depth and increased microbial biomass C. In the same study, glycerol did not adversely impact soil properties such as electrical conductivity or pH (Potter et al., 2023). A lab incubation study of agricultural soils amended with crude glycerol found that almost 100% of NO_3^- was depleted by the end of the incubation period when amended with glycerol, while control soils mineralized N (De et al., 2022). Another field study in Canada where glycerol and urea were applied together prior to seeding canola reported a decrease in crop yield and N uptake in the first year followed by increases in the second year, potentially attributable to remineralization of N previously immobilized by glycerol (Alotaibi and Schoenau, 2016). Redmile-Gordon et al. (2014) reported

that inorganic N leaching measured in mesocosm-lysimeters was 99% lower when treated with glycerol.

To test the effectiveness of glycerol application on NO_3^- leaching reduction, we conducted field studies in three potato production locations in Wisconsin. At two sites, glycerol was applied shortly after potato vine desiccation, and at the third, it was applied at the beginning of the growing season at the request of the grower. A major portion of NO_3^- leaching tends to occur during the fall and winter where remaining soil NO_3^- can be easily leached by precipitation or snowmelt, especially when there is no living cover to take up inorganic N (Shrestha et al., 2010), so applying glycerol before this period could optimize NO_3^- leaching-reduction. Many growers use a winter rye (*Secale cereale* L.) cover crop in this region, so application of a labile C source immediately after termination of the main crop could in principle immobilize some NO_3^- prior to establishment of the cover crop. To examine glycerol's effects on N dynamics, we combined field soil sampling, incubation of sampled soils, resin lysimeters, and additional lab measurements to understand the mechanisms by which glycerol may have altered N cycling. We expected soil NO_3^- to decrease following glycerol application due to increased N immobilization from mineralization of glycerol, and for NO_3^- leaching to be lower in glycerol-treated plots. During lab incubations we expected N and C to be mineralized, and that glycerol would suppress N mineralization and increase C mineralization.

3. Methods

3.1 Site description and plot design

The main experiment took place at Hancock Agricultural Research Station, Hancock, WI (44.1186 °N, -89.5358 °W) and a commercial farm near the station. The experiment was also

replicated at the Langlade Research Station near Antigo, WI (45.14838 °N, -89.1331 °W) following a slightly modified design. The soil at Hancock is classified as Plainfield sand, at the commercial farm the soils were mapped as Sparta loamy sand, Billett sandy loam, and Richford loamy sand, and at the Antigo site the soil was an Antigo silt loam. Total carbon (C) and total nitrogen (N) at Hancock measured 4.18 g kg⁻¹ and 0.48 g kg⁻¹, respectively (C:N ratio of 8.7). At the commercial farm, hereafter referred to as the farm, total C and total N measured 7.10 g kg⁻¹ and 0.67 g kg⁻¹ (C:N ratio of 10.6). Based on a 30-year average, the mean monthly temperature at Hancock and the farm ranges from -4 °C to 28 °C, with the lowest temperature in January and the highest in July. The mean annual precipitation was 870 mm, with 70% occurring during April through September (“Wisconsin State Climatology Office,” 2021). The 30-year averages at Antigo were -11 °C to 20 °C (highest in July and lowest in January) and 745 mm of precipitation with 70% occurring from April to September (“Wisconsin State Climatology Office” 2021). Precipitation data was recorded at the Hancock Wisconet Station (“Wisconet”). All sites were planted with potato at the time of the experiment but have been in rotation with corn, soybean, and other crops over recent decades. A winter rye (*Secale cereale* L.) cover crop was typically grown after the cash crop on these fields, as it was during our experiments. The fields were managed according to conventional regional agronomic practices.

At Hancock, we used a split-plot design with fertilizer rate as the main-plot treatment and glycerol rate as the sub-plot treatment. The N fertilizer was applied at 112 or 336 kg ha⁻¹ to each of eight main plots (3.6 x 7.6 m), and glycerol was applied at 0, 1122, or 2244 L ha⁻¹ (concentrated glycerol volume) to each of three subplots (2.4 x 3.6 m) within each main plot. Due to the high viscosity of glycerol, we mixed it with water (1:1 ratio) prior to application, and the same was done at the commercial farm and Antigo. Glycerol rates were within the range of

those used in a previous study in Iowa where decreased NO_3^- was observed (Potter et al., 2023). Glycerol was applied with a backpack sprayer on Sept 13, 2024, four days after herbicide was applied to terminate potato vines. For each treatment combination (N fertilizer and glycerol) there were four replicate subplots. The experiment at Antigo followed the same plot design as Hancock but there was only one N fertilizer rate (202 kg N ha^{-1}). At the farm, glycerol was broadcast applied with a sprayer immediately prior to potato hilling in May 2024, at the same rates as used at Hancock. Seven plots, each with one treatment combination of glycerol and fertilizer, were treated with either the grower standard rate of N fertilizer, a reduced rate (67 kg ha^{-1} lower), or no fertilizer. Due to the design of the experiment at the commercial farm, where each plot received a different treatment combination and there was no replication within plots, we cannot use traditional statistical methods. Plots were 23 m wide and approximately 175 m long with 7.6 m between them.

3.2 Soil sampling and lysimeters

We used a stainless-steel core with 2 cm diameter to sample soil at depths of 0–30 cm and 30–60 cm at Hancock. Within each plot, we took one sample from the top of the hill, the shoulder, and the furrow, and composited them by depth. At Hancock, we collected soil samples three weeks before glycerol application, then four and six weeks after glycerol application. At the farm, five samples per plot were collected from 0–30 and 30–60 cm and composited by depth. Soils were sampled on April 29, 2024, one week before applying glycerol on May 29, 2024, then again two weeks after. Additional soil samples were collected approximately monthly until October.

At Hancock and the farm, we installed ion exchange resin lysimeters to measure nitrate leaching. The resin captures NO_3^- from the leachate that flows through the lysimeter that can subsequently be extracted using salts (Susfalk and Johnson, 2002). Lysimeters were constructed

from 5 cm diameter polyvinylchloride tubing with 25 g of ion exchange resin contained between mesh, with sand packed above and below the mesh (Hall et al., 2023). Lysimeters were installed at 60 cm depth by augering a hole (20 cm diameter) and carving a pocket in the side of the hole to place the lysimeter below undisturbed soil, repacking sand in the pocket, and then refilling the hole. We installed three lysimeters in each subplot, at hill, shoulder, and furrow locations, respectively, for a total of 72 lysimeters. Lysimeters at Hancock were installed on August 20 and 21, 2024, and retrieved March 21, 2025. At the farm 42 lysimeters, 6 per treatment, were installed on May 10, 2024, and 14 were retrieved on Nov 11, 2024, and the rest were retrieved on March 28, 2025. Their locations were recorded using a high precision GPS (RTK facet, Sparkfun Electronics, Niwot CO) to enable retrieval.

3.3 Sample pre-processing and lab incubation

Soils were refrigerated at 4 °C until they could be air-dried in the lab and were then sieved to remove coarse fragments and plant material > 2 mm. A subset of 12 samples (six from each site) collected before treatment was pulverized with mortar and pestle and prepared for C and N elemental analysis by combustion. To prepare for incubation, 40 g of dry soil was weighed into 50 mL plastic beakers with holes in the bottom to allow water infiltration (Haney and Haney, 2010). Glass microfiber filters were placed in the bottom of the beaker to keep the soil from falling out. The plastic beakers were placed in 1 L glass jars where 25 mL of ultrapure water was added outside the beaker and taken up by the soil by capillary action. Immediately after water was added, the jars were sealed with gas-tight gaskets and stainless-steel lids with butyl septa and flushed with CO₂-free air for 10 minutes to remove CO₂, then placed in a dark room at approximately 23 °C (Leeford et al., 2023). After 24 hours, CO₂ concentration and its C stable isotope ratio ($\delta^{13}\text{C}$) were measured by manual injection into a Campbell Scientific trace gas

analyzer (TGA200A, Logan, UT) following the method of Hall et al. (2017). We measured C stable isotopes because they can provide information about the source of the C that is being mineralized, where different isotope ratios indicate different organic matter sources (Potter et al., 2023). A set of CO₂ standards was measured along with the samples to create a standard curve. After the initial 24 hours, CO₂ and its $\delta^{13}\text{C}$ were measured once a week for four weeks, and jars were flushed with CO₂-free air following every measurement. The maximum CO₂ concentration measured in a given jar was < 5,000 ppm, indicating a negligible decrease in headspace oxygen (O₂) due to microbial respiration. Cumulative production of CO₂ over the incubation was calculated as the sum of each measurement.

3.4 Soil and lysimeter extraction and nutrient analysis

After the 28-day incubation, 7 g of moist soil was subsampled from the incubation beaker and added to a centrifuge tube with 35 mL of 2 M potassium chloride (KCl) and shaken for one hour on an orbital shaker. The samples were centrifuged for 10 minutes at 3600 g, decanted into a clean bottle and acidified with 0.2 mL 6 M hydrochloric acid (HCl) for storage at 23 °C. Subsamples of the original (un-incubated) soils were similarly extracted.

Resin beads from lysimeters were transferred to glass jars, where 125 mL of 2 M KCl was added, and beads were shaken on an orbital shaker for 2 hours. The extract was then filtered through Whatman #1 filters and acidified with 6 M HCl. We chose a subset of nine samples to extract for a second time to determine extraction efficiency. We added 125 mL KCl to the jars containing resin and remaining KCl from the first extraction, then shook and filtered them following the same procedure as the first extraction. On average, the second extractions yielded 32% of the initial NO₃⁻ concentration and 64% of the initial NH₄⁺ concentration, so the first extraction of all lysimeters was adjusted by this amount.

$$\text{Adjustment factor} = \frac{(\text{NO}_3^- \text{ released in second extraction})}{(\text{initial NO}_3^-)}$$

Soil NH_4^+ and lysimeter NO_3^- and NH_4^+ were measured using colorimetric methods implemented in a microplate reader (BioTek Epoch, Agilent, Santa Clara, CA). For soil NO_3^- analysis, we used a scanning UV-Vis spectrophotometer (UV-3100PC, VWR, Randor, PA) to measure NO_3^- from the second derivative of absorbance at 237 nm (Crumpton et al., 1992). A subset of NO_3^- samples from soil 2 M KCl extractions was measured with both methods and a linear regression of spectrophotometer NO_3^- against microplate NO_3^- showed the methods yielded very similar results ($R^2 = 0.97$).

3.5 Lab glycerol incubation and sorption experiment

To test the effect of glycerol on C and N dynamics in a controlled lab setting, we set up a jar incubation in the same way as described previously and pipetted 1.4 and 2.8 μL of glycerol into the soil cup in a 1:1 glycerol and water mixture, equivalent to 0, 1122, and 2244 L ha^{-1} , assuming that the applied glycerol was mixed with 60 cm soil. Over a 4-week period, we measured CO_2 as described above and measured N_2O concentrations on the same gas samples by placing a LiCOR 7820 analyzer (Lincoln, NE) with a low-flow kit in-line with the TGA200A. The measured N_2O concentrations were calibrated using peak heights of separate standards, analogous to the approach used for CO_2 (Hall et al., 2017).

Retention of glycerol by soil could influence its effectiveness at reducing NO_3^- leaching, so we conducted a sorption experiment. Four soils with contrasting texture and organic matter were selected for this experiment: Plainfield sand (Hancock) and Sparta loamy sand (commercial farm), and Clarion loam and Webster silty clay loam collected from an Iowa field described by (Loper et al., 2024). We included the Clarion and Webster soils to see if glycerol sorption would

differ among soil textures, and these soils are the same classification as the soil present at the glycerol application experiment by (Potter et al., 2023) who found glycerol to 45 cm depth several weeks after field application. From each soil, three samples were selected, and 4 g of dry, sieved soil was weighed into a 50 mL centrifuge tube, and 40 g of a mixture of water and glycerol equivalent to the rates applied in the field was added. We included 10 tubes containing only the glycerol-water mixture, and four containing only water to measure baseline dissolved organic carbon (DOC). The samples were shaken on an orbital shaker for 1 hour, then centrifuged at 3000 g for 15 minutes. Samples were syringe-filtered through 0.45 μm Teflon filters and acidified with 6 M HCl and stored at 10 °C in glass bottles until analysis. Total organic carbon was measured on a TOC analyzer (TOC-L/TN, Shimadzu, Kyoto, Japan). To determine how much glycerol stuck to the soil we calculated what the DOC would be if all of the glycerol that was added was measured in the extract, which we refer to as “predicted DOC”. The predicted DOC was the DOC of soil without glycerol + the DOC of the glycerol/water mixture.

3.6 Denitrification potential

To determine if denitrification could explain the loss of inorganic N we observed during the lab incubations, we conducted a denitrification enzyme assay. Three soils with two depths were selected from both study sites (12 samples total) for the assay following the procedure described by (Groffman et al., 1999). A media containing NO_3^- as KNO_3 , dextrose, and chloramphenicol was added to 25 g of dry soil in a glass jar with a septum. The jars were first flushed with dinitrogen (N_2) gas for 3 minutes to remove O_2 , then purified acetylene was added to fill 10% of the headspace. Samples were incubated for 90 minutes on a rotary shaker at 125 rpm. Gas samples were collected at 30 and 90 minutes and analyzed for N_2O concentrations using a gas chromatograph with electron capture detector (Shimadzu 2014A, Columbia, MD,

USA). In our analysis we used the rate of N₂O captured between 30 minutes and 90 minutes to calculate potential denitrification following the calculations of (Groffman et al., 1999).

3.7 Statistical analysis

All statistical analysis was done in R Studio version 4.5.1. To analyze the effects of glycerol across treatment combinations of N fertilizer rate and depth (when applicable), we used type III analysis of variance (ANOVA) from the package “car” (Fox et al., 2026). When effects were significant ($\alpha = 0.05$) in the ANOVA, we assessed the estimated marginal means from the package “emmeans” (Lenth et al., 2026). When necessary, a Grubb’s test for single outliers from the package “outliers” (Komsta, 2022) was used to test if the highest value was a statistical outlier. When applicable, a T-test or paired T-test was used to compare the means of two groups ($\alpha = 0.05$).

4. Results

4.1 Change in soil nitrate in the field after glycerol addition

4.1.1 Hancock and Antigo

Glycerol addition decreased soil nitrate measured four weeks after glycerol application, but no differences were noted at six weeks, as indicated by a three-way interaction between sampling period, glycerol rate, and depth ($p < 0.01$; Figure 1). To simplify interpretation, we then fit separate linear models for each sampling period. Even prior to glycerol application, soils from the low glycerol rate treatment had higher NO₃⁻ concentrations in the high N fertilizer treatment than the others (glycerol rate x N fertilizer rate interaction, $p = 0.04$). However, by four weeks after glycerol application, there was a more consistent trend in NO₃⁻ among treatments, with highest values in the no-glycerol controls, and lower values in the glycerol treatments across all

N fertilizer rates and depths ($p < 0.001$). Soil NO_3^- levels were 6.9 and 4.6 mg kg^{-1} less than the control when amended with the higher and lower glycerol rate, respectively ($p < 0.0001$). The same trend occurred at Antigo four weeks after glycerol application, where there was 67% less soil NO_3^- in the high glycerol treatment ($p < 0.001$) and 36% less under the low glycerol treatment ($p = 0.06$) compared to the control. However, 6-weeks post glycerol treatment at Hancock, soil NO_3^- did not differ by glycerol rate. Soil NH_4^+ was very low relative to NO_3^- and did not differ between treatments so we will subsequently focus on soil NO_3^- , although NH_4^+ was used to calculate net N mineralization.

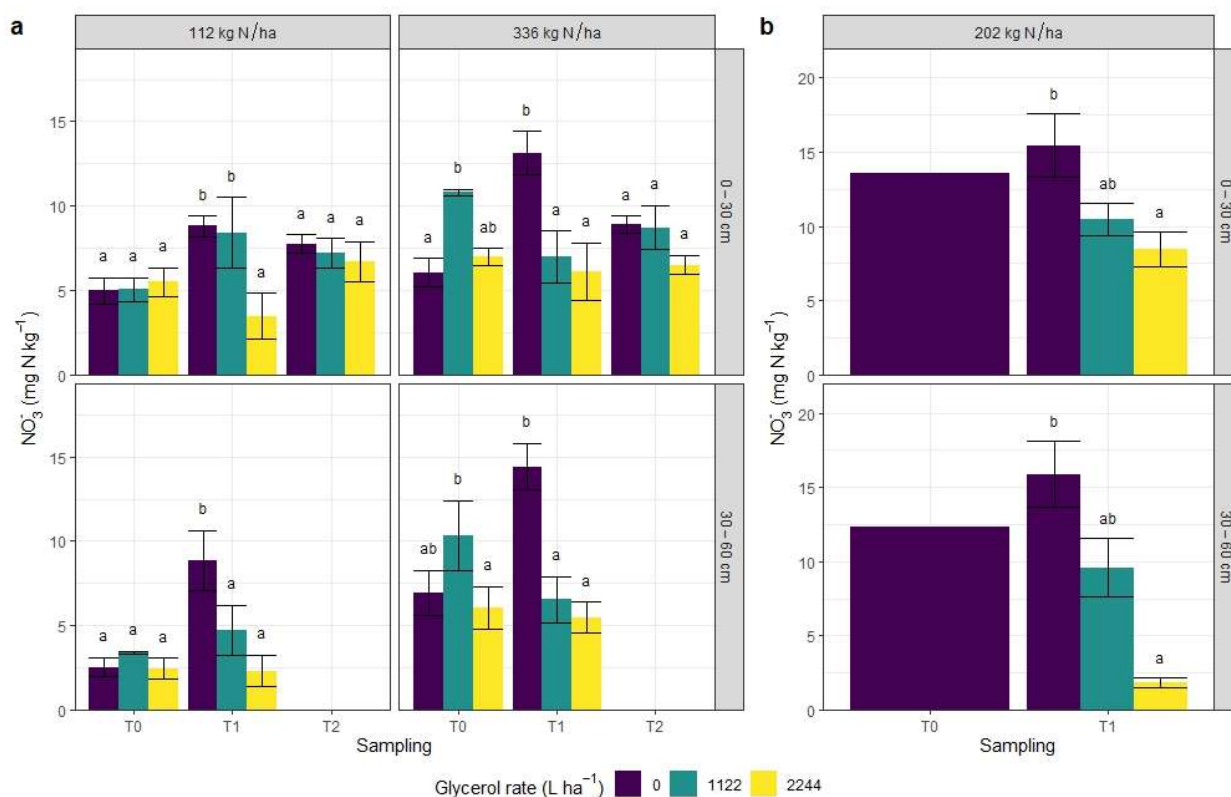


Figure 1. Soil NO_3^- concentration (mg N kg^{-1} soil) at Hancock Research Station (a) and Antigo (b). N fertilizer rates are shown in columns, depth is by rows, and glycerol rates are indicated by bar colors. The error bars are standard errors ($n = 4$). Different lowercase letters indicate

significant differences among means for glycerol rate for each depth x N rate x sampling combination (Sidak-adjusted comparisons, $p < 0.05$). Sampling period is on the x-axis, where T0 is 3 weeks pre-treatment, T1 is 4 weeks post-treatment, and T2 is 6 weeks post-treatment. Subsoil samples weren't collected during the T2 sampling period at Hancock due to a broken sampler. At Antigo only two samples were taken pre-treatment, so error bars are not presented.

4.1.2 Farm

Addition of glycerol at the farm tended to decrease soil NO_3^- two weeks after application (T1) at the high glycerol rate and the lower rate under standard fertilizer treatment (Figure 2). However, soil NO_3^- had no consistent trend among glycerol treatments in subsequent sampling periods. There was a brief increase in soil NO_3^- after N fertilizer addition, indicated by the T1 samples, but soil NO_3^- levels subsequently returned to baseline levels and did not vary consistently among N fertilizer rates or sampling period (Figure 2).

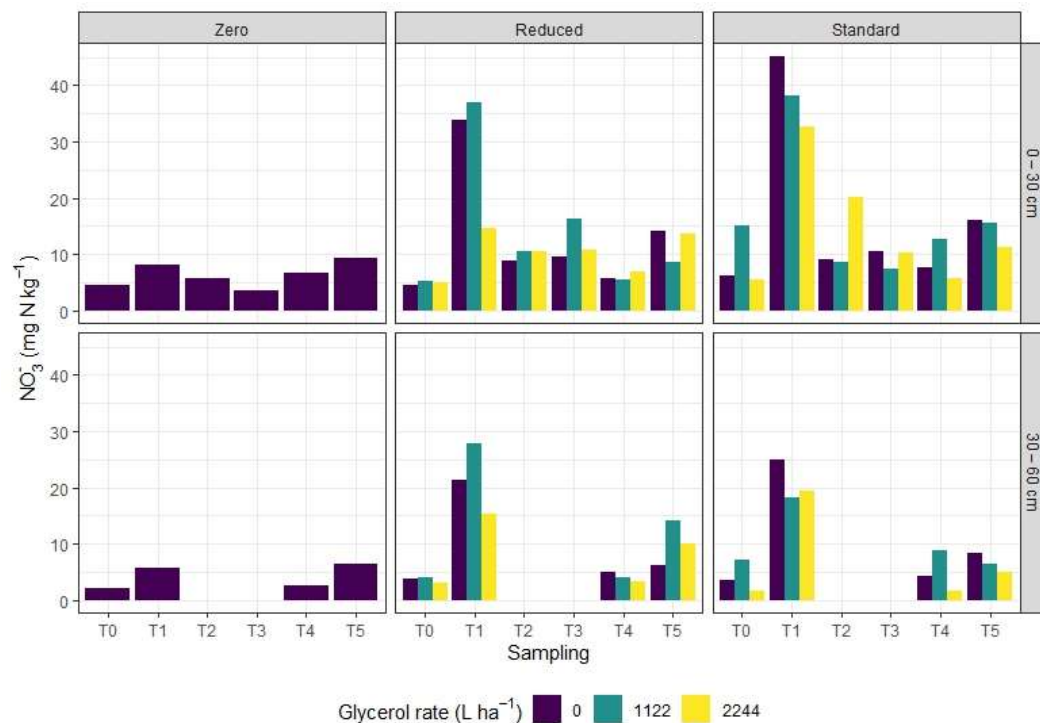


Figure 2. Soil NO_3^- concentration (mg N kg^{-1} soil) at the commercial farm. Fertilizer rates are in columns, depth is in rows, and glycerol rates are indicated by the bars. Sampling period is on the x-axis. T0, 4 weeks before glycerol application; T1, 2 weeks after; T2, 4 weeks after; T3, 5 weeks after; T4, 3 months after; T5, 4 months after. Subsoil samples were not taken during the T2 and T3 sampling periods.

4.2 Change in soil nitrate during lab incubation

4.2.1 Hancock and Antigo soil incubations

During the incubation of Hancock soil samples taken before and four weeks after glycerol application in the field, almost all inorganic N was depleted, decreasing by an average of 84% ($p < 0.0001$; Figure 3). There was a significant effect of glycerol rate ($p < 0.0001$) and N rate ($p < 0.001$) on net N mineralization. Compared to the control, soils that were amended with glycerol had 36 and 56% less N loss ($p < 0.01$ and $p < 0.001$) at the lower and higher glycerol addition

rates, respectively (Figure 3). This is because almost all inorganic N was depleted across treatments, and the pre-incubation NO_3^- concentrations were lower when treated with glycerol, especially under the higher N rate (Figure 4), and the soils that had higher initial NO_3^- (control soils) had more negative net N mineralization rates. Final NO_3^- concentrations didn't differ among any of the treatments, because almost all of the inorganic N was immobilized (Figure 4). The incubation data of Antigo soils followed the same trends as the Hancock soils; almost all inorganic N was depleted (Figure 3).

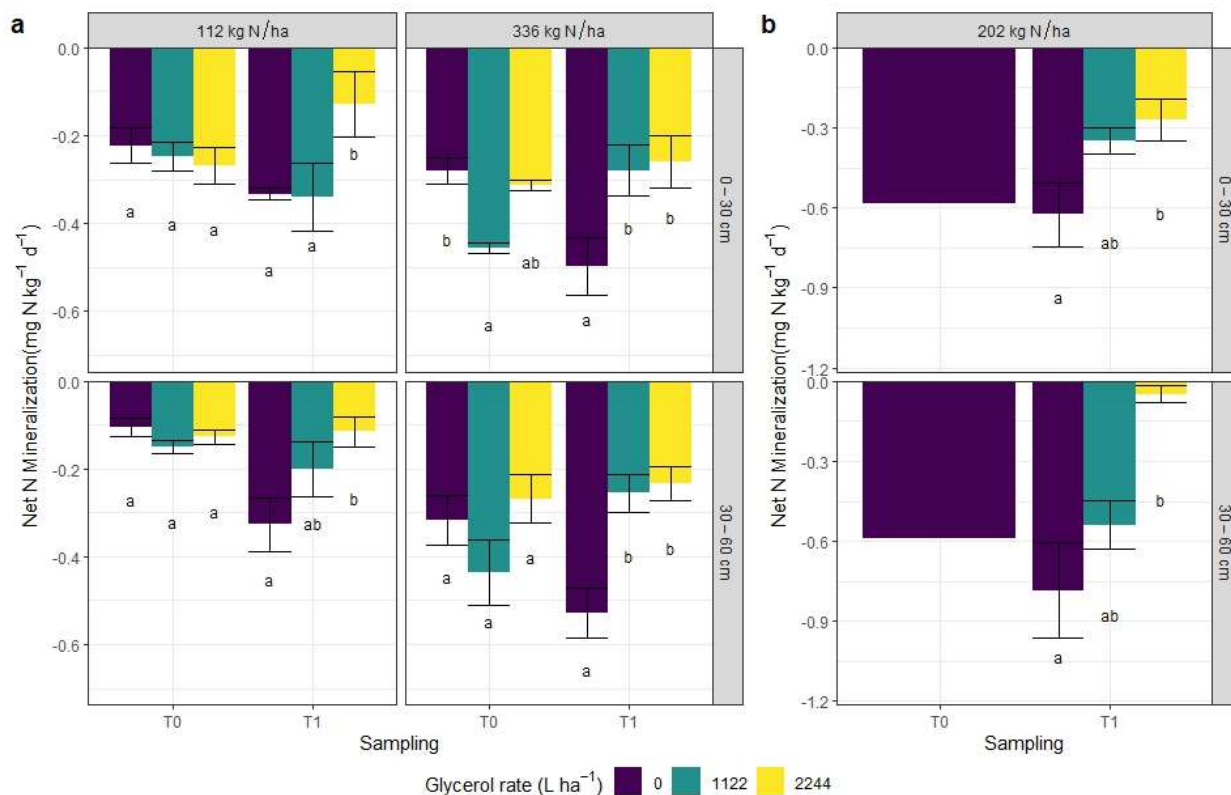


Figure 3. Net N mineralization ($\text{mg kg}^{-1} \text{d}^{-1}$) at Hancock (a) and Antigo (b). N fertilizer rate is in columns, depth is in rows, and glycerol rate is indicated by bar colors. Error bars are SE ($n=4$). Different lowercase letters indicate significant differences among means for glycerol rate for

each depth x N rate x sampling combination (Sidak-adjusted comparisons, $p < 0.05$). The x-axis is the sampling period (before vs four weeks after glycerol application; T0 and T1, respectively).

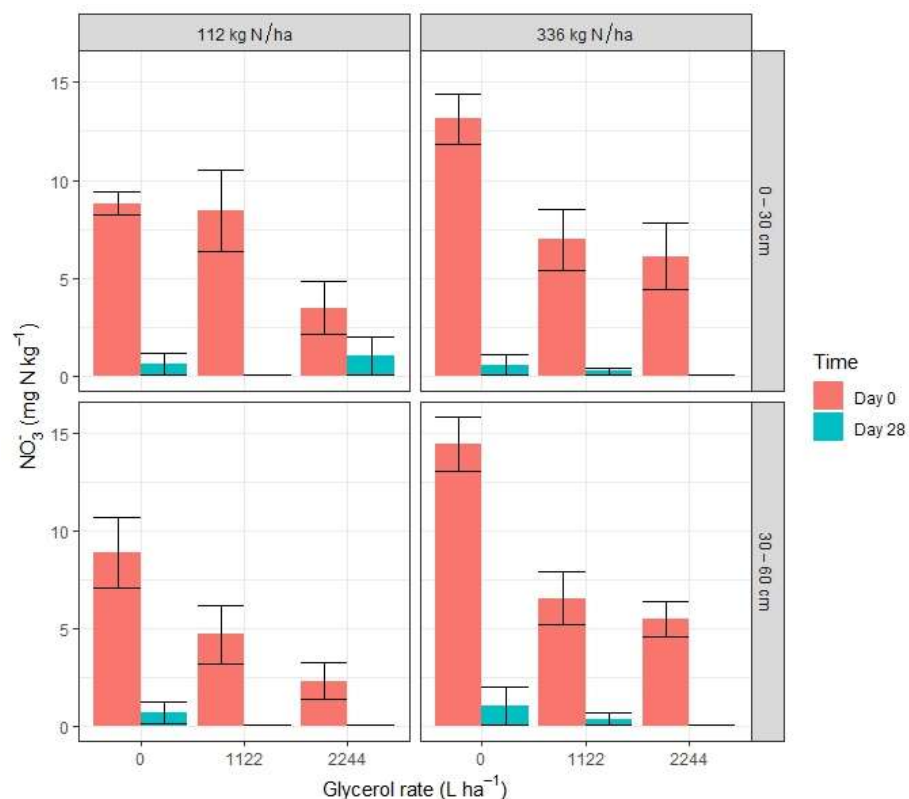


Figure 4. Soil NO₃⁻ concentrations at Hancock before (Day 0) and after (Day 28) being incubated for 28 days. Samples were from four weeks after glycerol application (T1 in figures above). N fertilizer rate is in columns and depth is in rows. The x-axis is glycerol rate and before/after incubation is indicated by the bar color.

4.2.2 Farm soil incubations

Similar to the findings from Hancock and Antigo, across all treatments and sampling periods, inorganic N decreased by 81% ($p < 0.0001$) on average during incubation of farm soils. All treatments resulted in net negative N mineralization (Figure 5), except for 2244 L ha⁻¹ glycerol, standard N, 0-30 cm, four weeks after glycerol application, which had net positive

mineralization. This sample was an outlier and was removed before further analysis ($G = 5.5$, $p < 0.0001$). Two weeks after glycerol application, the higher glycerol rate tended to have less negative N mineralization than the lower glycerol rate and the control, due to the lower initial NO_3^- of the soils treated with glycerol (Figure S1).

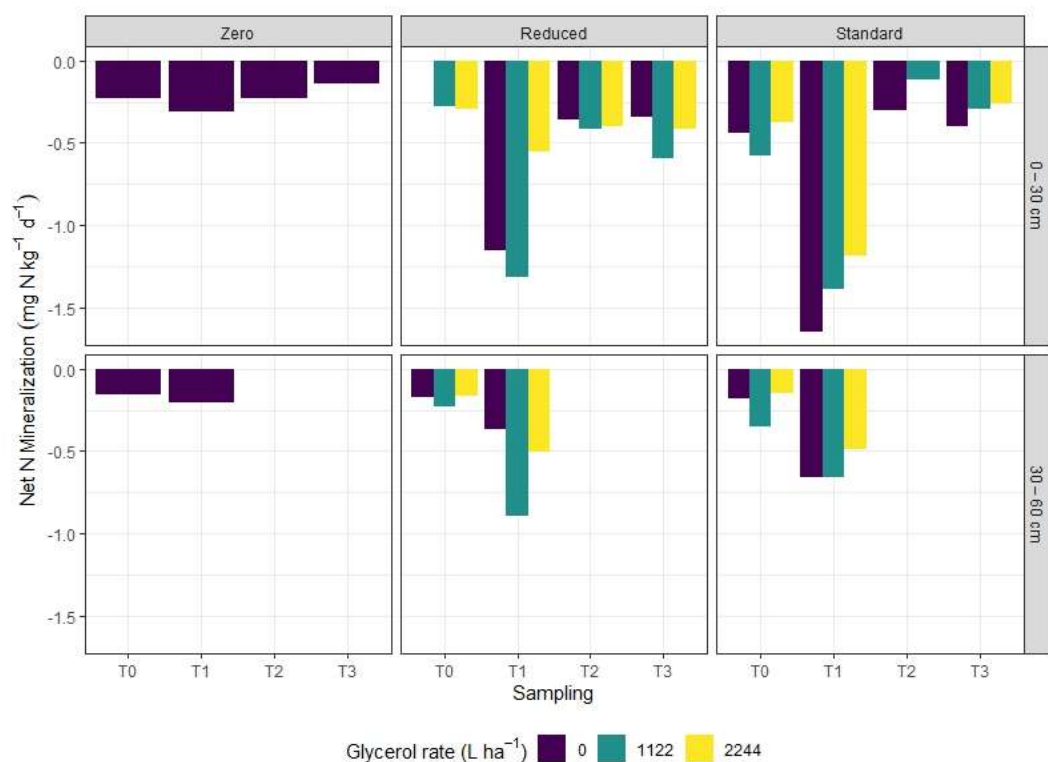


Figure 5. Net N mineralization ($\text{mg kg}^{-1} \text{d}^{-1}$) at the farm. N fertilizer rate is in columns, depth is in rows, and glycerol rate is indicated by bar colors. The x-axis is sampling time. T0, 4 weeks before glycerol application; T1, 2 weeks after; T2, 4 weeks after; T3, 5 weeks after; T4, 3 months after; T5, 4 months after. Only T0 through T3 samples were incubated.

4.3 Carbon mineralization and $\delta^{13}\text{C}$ during lab incubation

4.3.1 Hancock soil incubations

Glycerol addition did not affect C mineralization in soils collected from the field at Hancock (Figure 6a) or Antigo (Figure S2), but it increased $\delta^{13}\text{C}$ of respired CO_2 measured after 24 h at Hancock (Figure 6b). The average $\delta^{13}\text{C}$ of CO_2 respired in the first 24 h for the control (0 L ha^{-1} glycerol) was $-25.7 \pm 0.6\text{‰}$, averaged over depth and N rate. The higher glycerol rate increased the $\delta^{13}\text{C}$ by 2.2‰ ($p < 0.0001$) and the lower glycerol rate increased it by 1.3‰ ($p < 0.01$). The effect of glycerol was less prominent by the end of the incubation period, but $\delta^{13}\text{C}$ still increased under the high and low rates by 1.4‰ ($p < 0.0001$) and 0.8‰ ($p < 0.01$), respectively, relative to the control.

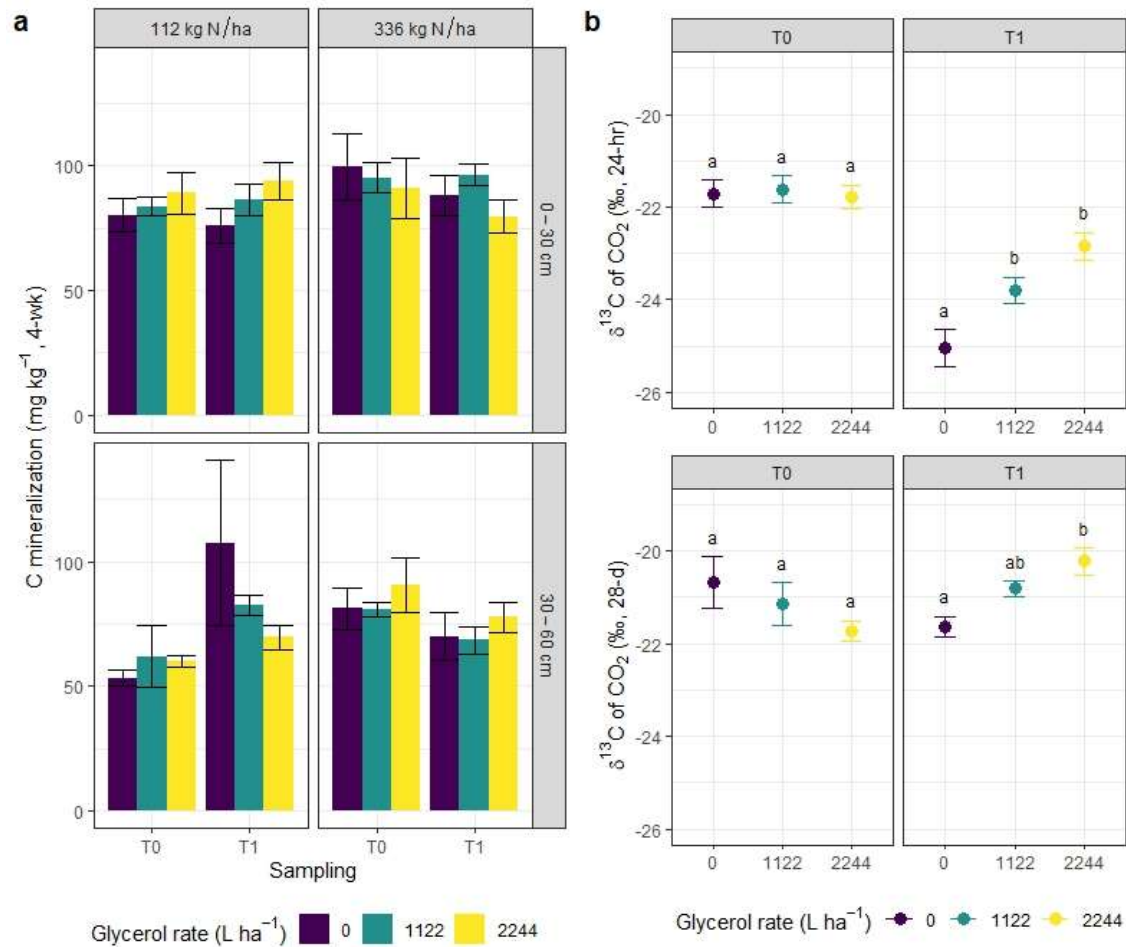


Figure 6. Cumulative C mineralization (a) during four-week incubation of soils collected from Hancock. N fertilizer rate is in columns, depth is in rows, and glycerol rate is indicated by bar colors. Error bars are SE (n=4). Sampling period is on the x-axis, where T0 is 3 weeks pre-treatment, T1 is 4 weeks post-treatment. Initial (24-h) and final (28-d) $\delta^{13}\text{C}$ of cumulative respired CO_2 (b) from incubated soils from Hancock. Columns indicate soils collected before and four weeks after glycerol application. The glycerol rate is indicated by the color of the points, which are averages and bars are standard error (n = 16). Different lowercase letters indicate significant differences among means for glycerol rate for each N rate x sampling combination (Sidak-adjusted comparisons, $p < 0.05$).

4.3.2 Farm soil incubations

There were no systematic trends in C mineralization among glycerol treatments in soils collected from the farm after glycerol application (Figure 7). The average initial 24-hr C mineralization was $4.8 \pm 0.2 \text{ mg kg}^{-1}$ and final 28-day C mineralization was $78.0 \pm 3.2 \text{ mg kg}^{-1}$. Initial $\delta^{13}\text{C}$ of respired CO_2 generally did not differ between glycerol rates, but by the end of the incubation, $\delta^{13}\text{C}$ tended to be higher when amended with the higher glycerol rate ($-20.2 \pm 1.2 \text{ ‰}$) compared to no glycerol ($-22.1 \pm 0.3 \text{ ‰}$).

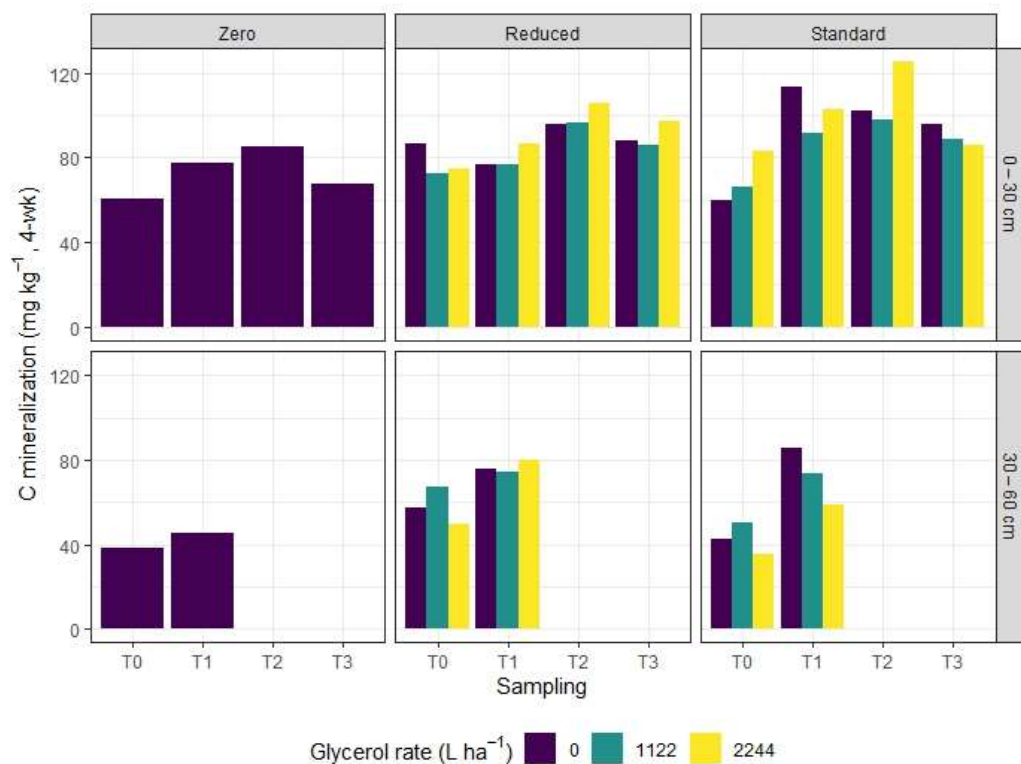


Figure 7. Cumulative C mineralization during four-week incubations of soils collected from the farm. Sampling period is on the x-axis. T0, 4 weeks before glycerol application; T1; 2 weeks after; T2, 4 weeks after; T3, 5 weeks after; T4, 3 months after; T5, 4 months after. N fertilizer rate is in columns, depth is in rows, and glycerol rate is indicated by bar colors.

4.4 Lab glycerol incubation

4.4.1 Carbon mineralization and stable isotope ratio

The addition of glycerol to soil in the lab increased cumulative 28-day C mineralization by 29% and 63% at the lower and higher glycerol rates ($p = 0.03$ and $p > 0.001$; Figure 8a). C mineralization also varied by depth and N rate ($p < 0.01$ and $p = 0.02$).

The $\delta^{13}\text{C}$ of respired CO_2 during the lab glycerol incubation differed among glycerol addition treatments (Figure 8c). The average $\delta^{13}\text{C}$ of CO_2 after 24 hours of incubation was 1.8‰ lower ($p = 0.01$) when amended with the higher glycerol rate compared to the no-glycerol control. The average $\delta^{13}\text{C}$ at the end of the experiment was 1.2‰ higher ($p < 0.01$) under the higher glycerol rate compared to the control, and the lower glycerol rate was intermediate.

4.4.2 N_2O production

Cumulative 28-day N_2O -N production was not affected by glycerol rate (Figure 8b), but N rate and depth were significant predictors ($p < 0.001$ and $p < 0.001$), with higher N_2O -N production under the high N rate in topsoil. The average cumulative N_2O -N production after the first 24 hours of the lab incubation was $0.31 \pm 0.06 \text{ mg kg}^{-1}$ and it measured $0.45 \pm 0.05 \text{ mg kg}^{-1}$ by the end of the incubation; 68% of the total N_2O production occurred within the first 24 hours.

4.4.3 Soil nitrate change during lab incubation

Across all glycerol treatments, soil NO_3^- was depleted by 96% ($p < 0.0001$) by the end of the incubation period (Figure 8d). The average final soil NO_3^- was $0.28 \pm 0.05 \text{ mg N kg}^{-1}$ and the average initial NO_3^- was $7.13 \pm 0.55 \text{ mg N kg}^{-1}$. Glycerol did not significantly affect soil NO_3^- at the end of the incubation ($p = 0.5$), but NO_3^- tended to be lower when amended with glycerol

(Figure 8d). The average final soil NO_3^- for no-glycerol soils was 0.39 mg kg^{-1} and was 0.23 and 0.22 mg kg^{-1} under the low and high glycerol rates, respectively.

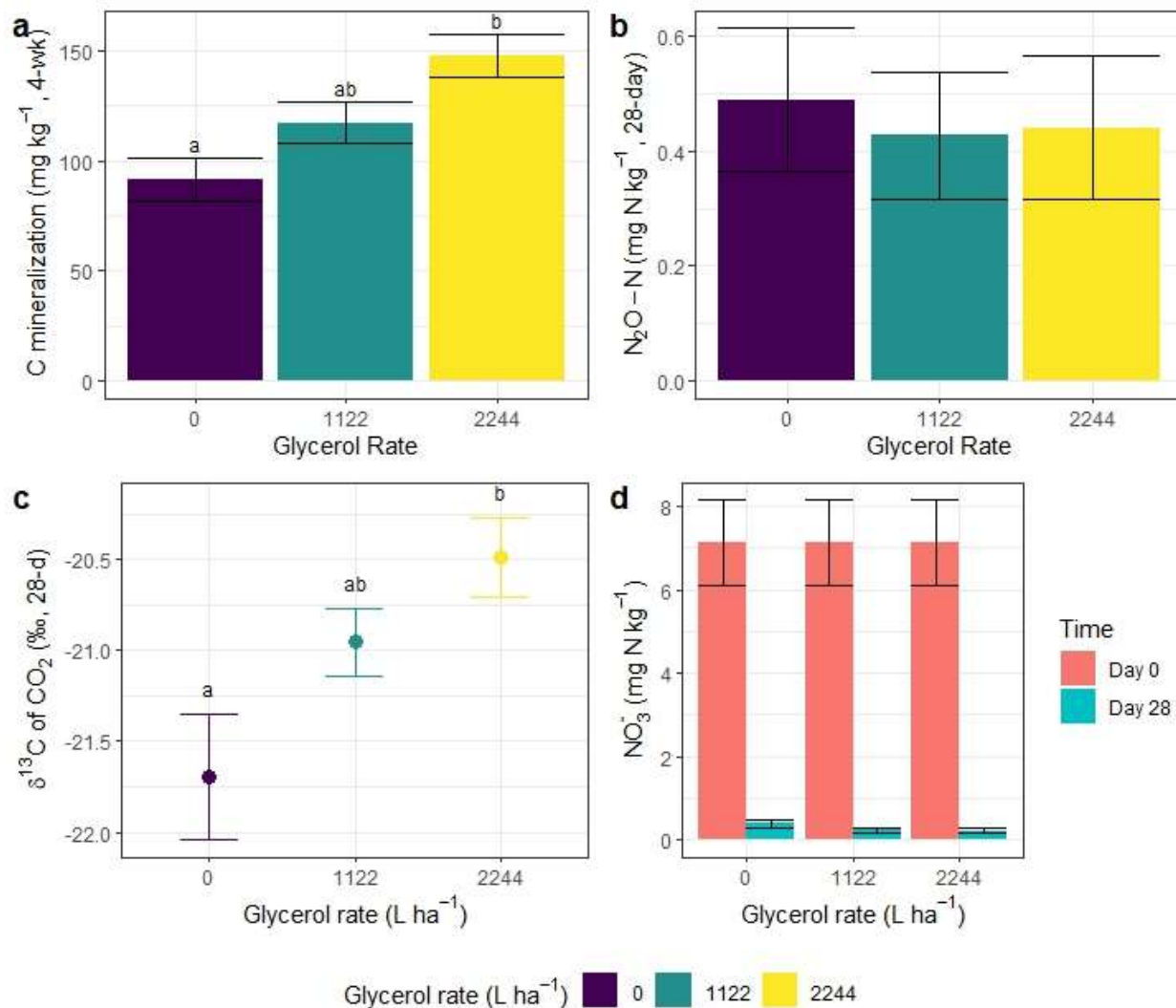


Figure 8. Plots of C mineralization (a), N_2O production (b), $\delta^{13}\text{C}$ of respired CO_2 (c), and soil NO_3^- (d) from an incubation of six soil samples from Hancock that were amended with glycerol in the lab. For C-min (mg C kg^{-1} soil), $\text{N}_2\text{O}-\text{N}$ (mg N kg^{-1}), and $\delta^{13}\text{C}$ (‰), values are the cumulative at the end of the 28-day incubation. Glycerol rate is indicated by bar and point colors in the C mineralization, N_2O , and $\delta^{13}\text{C}$ plots. Soil NO_3^- concentrations before (Day 0) and after (Day 28) being incubated for 28 days are shown in the bottom right panel. Different lowercase

letters indicate significant differences among means for glycerol rate for each N rate x sampling combination (Sidak-adjusted comparisons, $p < 0.05$). Samples were taken before glycerol application in the field, but glycerol was added to them before the incubation. The x-axis is glycerol rate and before/after incubation are indicated by the bar color.

4.5 Sorption experiment

There was no difference between predicted and measured DOC after glycerol addition for three of the four soils (Figure 9). Only the Clarion soil, which had the second highest average DOC ($413.3 \pm 11.3 \text{ mg C kg}^{-1}$, mean and SE), had a difference between the measured and predicted DOC, which were 9.7 mg C kg^{-1} ($p = 0.04$) and $28.5 \text{ mg C kg}^{-1}$ ($p = 0.03$) higher than predicted under the low and high glycerol treatments, respectively. The two soils with the lowest average DOC were the farm ($86.8 \text{ mg C kg}^{-1}$) and Hancock ($49.7 \text{ mg C kg}^{-1}$). For both sites, there was no difference between the predicted and measured DOC at either glycerol rate. The Webster soil had the highest average DOC ($1137.8 \pm 65.2 \text{ mg C kg}^{-1}$, mean and SE), but the measured DOC did not differ significantly from the predicted DOC.

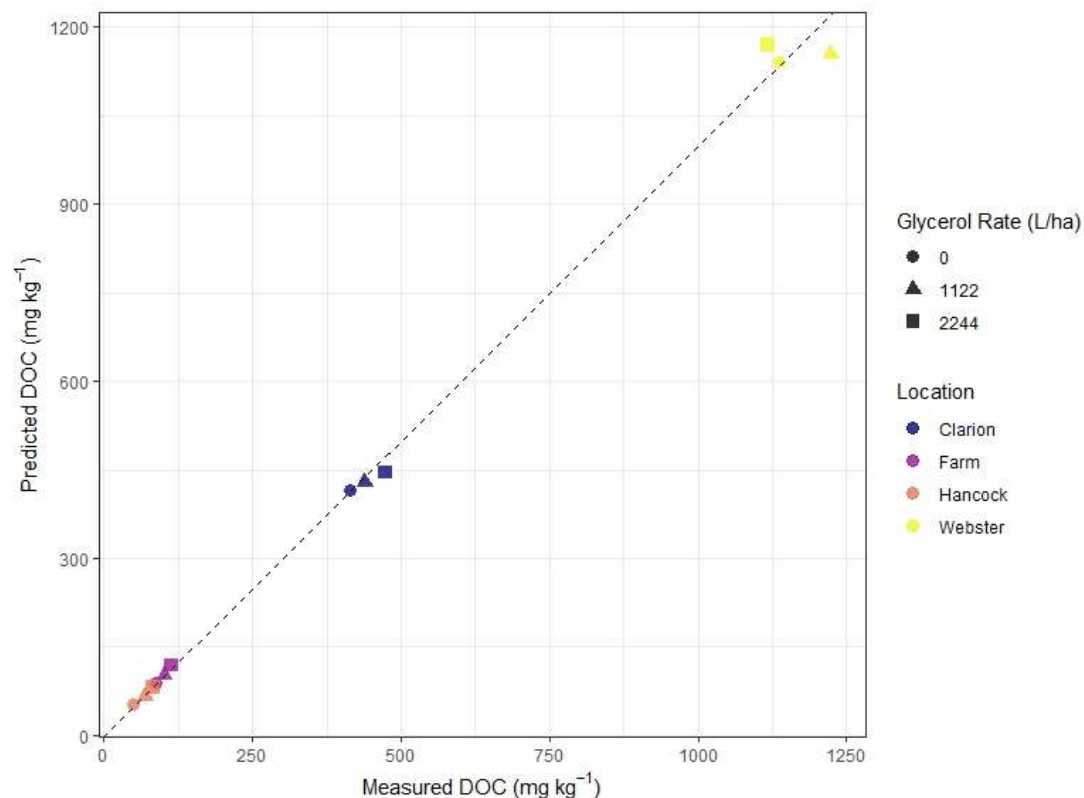


Figure 9. Plot of measured DOC and predicted DOC mg C kg^{-1} . Predicted DOC is the DOC of soil only + DOC of glycerol in water. Location of soil sample is indicated by color and glycerol rate is by shape of the point. The dashed line is the 1:1 line of measured = predicted.

4.6 Denitrification experiment

The average potential denitrification rates for Hancock and the farm were 6.5 ± 1.6 and 7.7 ± 1.1 $\text{ng N g soil}^{-1} \text{ hr}^{-1}$ (mean and SE), respectively. The denitrification rate in the subsoil at Hancock was $5.8 \text{ ng g}^{-1} \text{ hr}^{-1}$ greater than in the topsoil ($p = 0.04$). At the farm, denitrification rate was not different between sampling depths.

4.7 Nitrate leaching captured by lysimeters

4.7.1 Hancock

Overall, fall/winter NO_3^- leaching measured by the resin lysimeters varied spatially and was generally very low ($3 \pm 1 \text{ kg N ha}^{-1}$, mean and SE), but the range of values was very high, from 0.07 to 107.5 kg ha^{-1} . A Grubb's test indicated that the highest value (107.5 kg ha^{-1}) was an outlier ($G = 7.98$, $p < 0.001$) so it was removed before further analysis. The full model containing N rate, glycerol rate, and their interactions revealed no significant difference among the treatments. Under the high N fertilizer rate, both glycerol rates tended to have lower average nitrate leaching values compared to the control (Figure 15). However, under the low fertilizer rate NO_3^- leaching values tended to be higher when treated with the low glycerol rate and lower when treated with the high glycerol rate compared to the control (Figure 15). Analysis of the full model including random effects (plot and subplot) suggests that most of the variability in NO_3^- leaching can be contributed to differences between individual lysimeters. The residual variance ($\sigma^2 = 12.5$) was much greater than subplot and plot variance ($\sigma^2 = 0.3$ and 0.5).

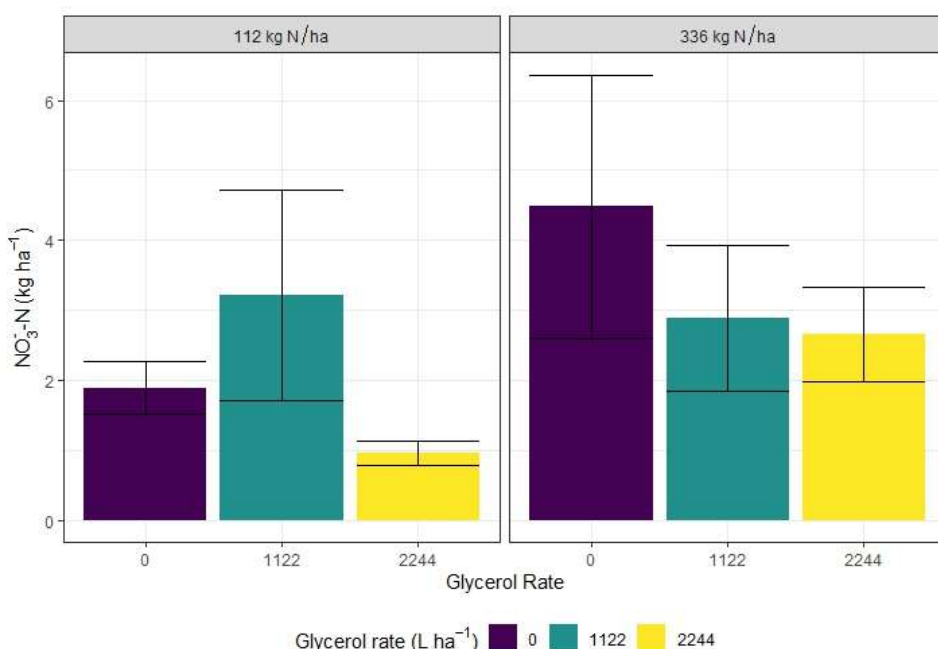


Figure 10. NO₃⁻ leaching at Hancock captured by lysimeters between August 20, 2024, and March 21, 2025. Columns are N fertilizer rate and glycerol rate is indicated by bar color. Error bars are SE (n = 12). The outlier was removed from the dataset.

4.7.2 Farm

The NO₃⁻ leaching at the farm measured in lysimeters installed from May 2024 through Nov 2024 or March 2025 was 22.2 ± 5.6 kg ha⁻¹ (mean and SE) and values ranged from 0.2 to 148.3 kg ha⁻¹. We compared the NO₃⁻ means between the two sampling periods to determine if removal date affected how much NO₃⁻ was captured. The overall means did not significantly differ ($p = 0.55$): 26.8 ± 9.7 kg ha⁻¹ for the November removal, and 19.7 ± 6.9 kg ha⁻¹ for the March removal. Since NO₃⁻ leaching values didn't differ between the removal times, the two datasets were combined for further analysis.

The effect of glycerol on NO₃⁻ leaching differed between fertilizer treatments (Figure 13). Under the reduced fertilizer rate, both glycerol rates tended to have lower nitrate leaching values

than the control (Figure 16). Under the standard fertilizer rate, the higher glycerol rate had a significantly higher average NO_3^- leaching ($45.6 \pm 11.9 \text{ kg ha}^{-1}$) than no glycerol ($p < 0.01$). However, due to the pseudoreplicated design of the on-farm experiment, it is difficult to assess the treatment effects.

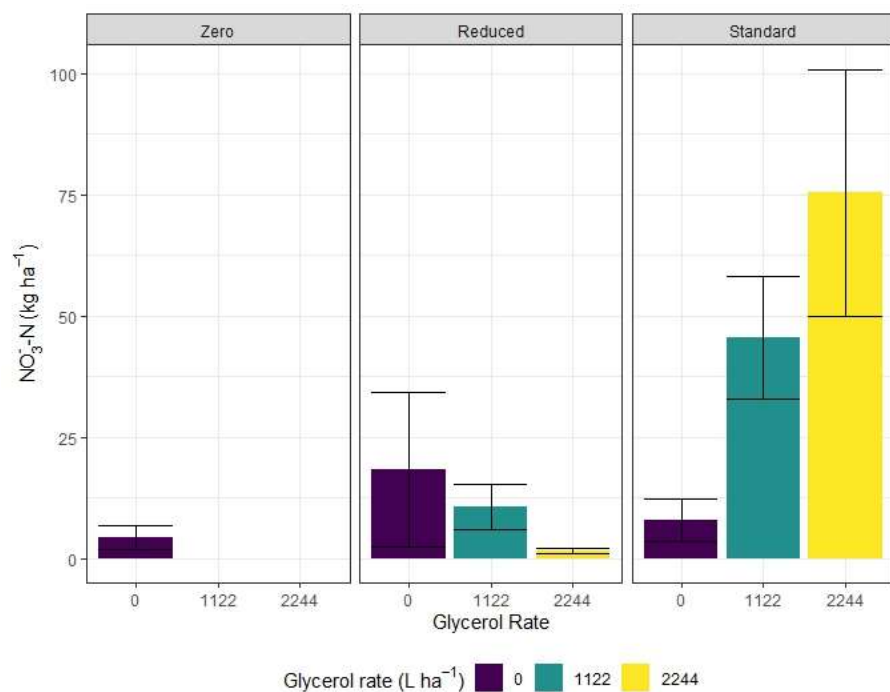


Figure 11. NO_3^- (kg ha^{-1}) leaching at the farm captured by lysimeters between May 10, 2024, and March 28, 2025. Columns are N fertilizer rate and glycerol rate is indicated by bar color. Error bars are SE ($n = 6$).

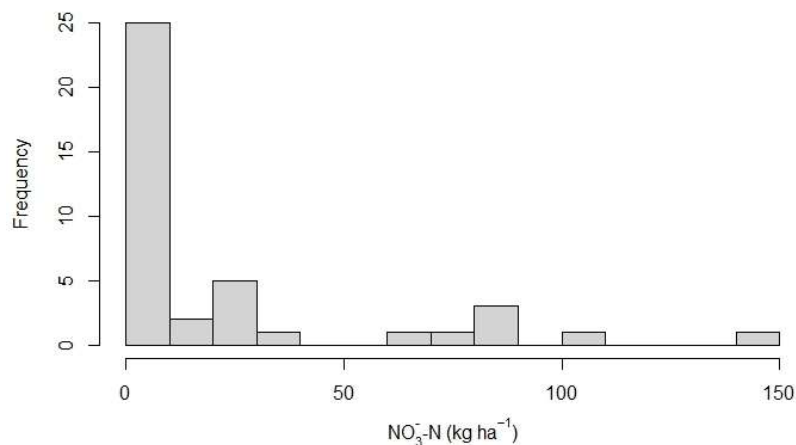


Figure 12. Histogram of NO₃⁻ leaching values (kg ha⁻¹) from the farm. Includes all lysimeters (from both removal dates).

5. Discussion

5.1 Decrease in soil nitrate in the field following glycerol application

The average post-harvest soil NO₃⁻ and inorganic N values of control plots at Hancock (9.8 mg kg⁻¹ as NO₃-N and 12 mg kg⁻¹ as NO₃-N + NH₄-N) are consistent with post-harvest soil measurements in other Upper Midwest commercial potato fields with sandy soil as observed by (Bohman et al., 2020) (7.1 to 8.6 mg NO₃-N kg⁻¹) and (Gunderson, 2025) (8.9 to 17.0 mg kg⁻¹ NO₃-N + NH₄-N). Soil samples taken four weeks after glycerol application at Hancock had lower soil NO₃⁻ in 60 cm (assuming a bulk density of 1.6 g/cm³) when amended with glycerol by 33.4 and 22.3 kg N ha⁻¹ (high and low glycerol rates, respectively), compared to the control. The same trend of low soil NO₃⁻ after glycerol application was observed at Antigo, where glycerol decreased soil NO₃⁻ by 101 and 54.3 kg N ha⁻¹ (high and low rates) compared to the control. This

is an environmentally meaningful amount of NO_3^- reduction. For example, with 25 cm of drainage, glycerol at the high rate could decrease NO_3^- concentrations in drainage by 13 to 40 mg L^{-1} , depending on the study site.. However, six weeks after application at Hancock, there was no significant difference between the glycerol treatments and the control. The difference between the effect of glycerol four weeks after application and six weeks after application could reflect impacts of leaching and/or N mineralization. Since we only have data from these three timepoints and the N could be in different forms at any point, it is difficult to tell if glycerol had a temporary or long-lasting effect. For example, if the NO_3^- that was in the pool at four weeks had leached between then and the next sampling point, six weeks later, the NO_3^- that we measured could be newly mineralized N. At the six-week sampling period, the cover crop was beginning to emerge, so it is possible that the rye was taking up NO_3^- .

One possible explanation for the short-lasting effect on NO_3^- reduction is that glycerol was washed down the soil profile by precipitation. A previous study at Hancock found that 2.5 cm of water can move NO_3^- about 15 to 20 cm down the soil profile (Endelman et al., 1974). We might have expected glycerol to move more slowly in soil than nitrate due to its high viscosity, however it is highly soluble in water (Takamura et al., 2012) and our sorption experiment showed that glycerol did not stick to soil (Figure 11), so it may have been leached. During the glycerol application itself, a very small amount of liquid was added (0.2 and 0.4 mm for the low and high rates), but between glycerol application and the following sampling period four weeks later, Hancock received 4.6 cm of precipitation. This amount of rain could have moved NO_3^- and glycerol 23 to 30 cm down the soil profile following the results of Endelman et al. (1974), reaching the bottom of our topsoil sampling depth. Between the sampling event after glycerol application and two weeks later there was about 0.7 cm of additional precipitation at Hancock,

which may have been enough to move NO_3^- and glycerol past the topsoil sampling depth. While glycerol itself is unlikely to persist in the topsoil, the microbial biomass that it produces, and the N that was immobilized, might persist for longer.

5.2 Loss of N during lab incubation

An unexpected finding was that across all soil incubations conducted during this study, almost all inorganic N was depleted after four weeks, regardless of glycerol rate, N fertilizer rate, or time of sampling at Hancock, the farm, and Antigo (Figure 3 and 5). For example, a previous study measured mostly positive net N mineralization over 28-day incubation of SOM-rich agricultural soils in Iowa, regardless of when samples were collected (Lawrence et al., 2021). While perhaps surprising for Hancock soil, an N-rich agricultural soil with a low C:N, negative N-mineralization has been observed across a variety of soil types and land uses (Weintraub-Leff et al., 2023). In our lab incubations we saw that the glycerol treatments had significantly less immobilization (i.e. less negative net N mineralization) during the incubation compared to soils not amended with glycerol (Figure 3). This can be explained by the low soil NO_3^- in the pre-incubation soils that were treated with glycerol in the field (Figure 4), so the magnitude of N-mineralization was lower compared to the control, which had much higher pre-incubation NO_3^- . Overall, glycerol didn't decrease net N immobilization rates in the lab, and the fact that glycerol had already immobilized N in the field prior to the incubation resulted in low soil NO_3^- values.

Loss of N through denitrification is unlikely to explain our results. The average potential N_2O -N production from the denitrification enzyme assay was $0.17 \text{ mg kg}^{-1} \text{ d}^{-1}$ or $7.1 \text{ ng g}^{-1} \text{ hr}^{-1}$, indicating there were denitrifiers present in the soil. However, these potential denitrification rates are much lower than typically observed in C-rich agricultural soils, such as those from an Iowa, study which found rates $> 100 \text{ ng N g}^{-1} \text{ h}^{-1}$ (Hall et al., 2018). Even if conditions were ideal for

denitrification, which was not the case for our incubation, the depletion of inorganic N ($0.28 \text{ mg kg}^{-1} \text{ d}^{-1}$, on average across all treatments) could not be explained by denitrification alone.

Moreover, the average $\text{N}_2\text{O-N}$ produced during the incubation where glycerol was applied in the lab was only $0.016 \text{ mg kg}^{-1} \text{ d}^{-1}$, so it is unlikely that denitrification contributed significantly to N depletion during the incubation.

A more likely explanation for the decrease of inorganic N during incubations is immobilization of N during decomposition of particulate organic matter (POM): that is, plant material with a higher C:N than the soil as a whole (Lavallee et al., 2020). The presence of POM could explain why all soils, including those without glycerol addition, had negative net N mineralization. After sieving our soil samples, we observed small pieces of plant material that passed through the 2 mm sieve. This material could be fibrous potato vine stems or corn residue in the soil contributing high C:N material for N immobilization.

A study that investigated the effect of glycerol on N-cycling in Iowa found that glycerol decreased net N mineralization, using a lab incubation approach similar to what we used (Potter et al., 2023). In our incubation we were unable to detect any effects of glycerol on N mineralization since the final soil NO_3^- values were so low across all treatments, likely due to a high rate of immobilization by decomposition of POM. In contrast to the high-C Iowa soils, which had much more mineral associated organic matter than POM and had net positive N mineralization (Yi et al., 2025), in our soil N immobilization by POM might have overshadowed any effect of glycerol.

It is unlikely that our methodology strongly influenced our conclusions regarding N mineralization during the incubation. There are many methods for analyzing N mineralization in soil including anaerobic incubation to measure potentially mineralizable N (Liptzin et al., 2023).

For example, another study on soil used for potato production in the Central Sands found potential mineralizable N values from a seven-day anaerobic incubation ranged from 8.6 to 21.2 mg kg⁻¹ or 1.2 to 3.0 mg kg d⁻¹ from soils taken during August to October 2020 (Gunderson, 2025). The difference with our results may be due to the incubation method, because we may not expect to see net N immobilization during an anaerobic incubation due to suppression of POM decomposition (Huang et al., 2020). While some methods incubate soils at field-moisture, we dried the soil before rewetting. Soil drying is commonly used by growers for soil health assays to suppress biological activity while samples are held prior to analysis, and to standardize conditions prior to lab incubation (Leeford et al., 2023). Sandy soils experience frequent drying and rewetting in the field, suggesting the overall condition of our incubation is plausible. Furthermore, drying likely wouldn't inhibit N mineralization, and we might expect a slight increase in mineralization due to the Birch effect, whereby rewetting dried soil causes an influx in microbial respiration (Singh et al., 2023) and N mineralization (Cabrera, 1993). Although we didn't observe net positive N mineralization during the 28-day incubation, longer incubations would be useful to quantify when soils eventually mineralize N. One study found that incubated sandy soils amended with various organic materials had net N immobilization up to 16 weeks of the incubation followed by net N mineralization for the rest of the 40-week incubation (Burgos et al., 2006). The finding of net N immobilization in our study and Burgos et al (2006) may indicate immobilization of N from high-C amendments in the field could be long-lasting. At the commercial farm, we saw consistent soil NO₃⁻ values over sampling periods after an initial spike after fertilization, potentially buffered by N immobilization and later mineralization, although there was no apparent effect of glycerol.

5.3 Unrealistically low NO₃⁻ leaching captured by lysimeters

Although there were some trends of lower NO_3^- leaching under glycerol-treated plots as estimated using the resin lysimeters, none of the differences were significant. The high variability of values between lysimeters within the same subplots outweighed the treatment effects, and the distribution of NO_3^- leaching values at Hancock and the farm was strongly right-skewed (2.8 and 1.9; Figure 17). This finding of strong skew was consistent with a previous study of NO_3^- leaching in finer textured soils measured with resin lysimeters which was also very strongly right skewed (2.8) (Hall et al., 2023). One explanation for the low leaching values could be that we missed the hotspots for high NO_3^- leaching, since there are much fewer of them than the low leaching spots. High variation in lysimeter data could be caused by heterogeneity of soil, a change in preferential flow from the lysimeter, or divergence of flow around the lysimeter due to textural differences. Additionally, water flowing through the soil may follow funnel flow paths, a phenomenon documented in sandy Hancock soils where water moves through the soil based on small changes in the texture, rather than draining through the whole profile uniformly (Kung, 1990). In a separate study in a different field at Hancock, we used the mass balance of chloride (Cl^-) to estimate that only 8% of applied Cl^- was captured in the lysimeters. The Cl^- recovery of our lysimeters was very low compared to unpublished data by Yadav where he measured almost complete recovery of Cl^- from their lysimeters which were installed at Arlington Research Station, Arlington, WI, where the soil is a silt loam (H. Yadav, personal communication). Thus, further corrections for low drainage capture efficiency or greater replication may have been necessary to interpret the NO_3^- leaching data from this study.

The amount of NO_3^- captured by the lysimeters installed at Hancock was very low compared to values at the farm. This difference is likely explained in part by the timing of lysimeter installation. Lysimeters were installed at the farm three months before they were

installed at Hancock. Since there was no difference between the average NO_3^- captured in lysimeters that were removed from the farm in November and those that were removed in March, we infer that most of the leaching occurred from May to November in that particular year. Hancock lysimeters were installed at the end of August, so most of the leaching may have already occurred before then. Typical in-season NO_3^- leaching values in the Central Sands during the growing season (May to September) ranged from 30 to 260 kg ha^{-1} (Campbell et al., 2025). If we corrected our NO_3^- leaching values at the farm for the 8% efficiency, they would be closer to the values measured with equilibrium tension lysimeters by Campbell et al. (2025).

5.4 Sources of mineralized C

Incubated soil from the field did not have a difference in C mineralization among glycerol rates, but soils that were amended with glycerol in the lab tended to mineralize more C compared to unamended soils. This shows us that the addition of glycerol can stimulate microbial activity, but results are not consistent at the field scale, likely due to lack of sorption of glycerol to the soil. We might expect the stable isotope ratio of respired C to decrease when soils were treated with glycerol, which had a very negative $\delta^{13}\text{C}$ value in a previous study (e.g., -34.2‰; (Potter et al., 2023)). However, the addition of glycerol increased the $\delta^{13}\text{C}$ compared to untreated soil. This may be explained by the priming effect (Bingeman et al., 1953), where the introduction of labile carbon stimulates microbes to break down other organic matter in the soil, such as plant material that has a higher $\delta^{13}\text{C}$ value.

6. Conclusion

Although soil NO_3^- decreased substantially (20 to 30 kg N ha^{-1} at Hancock and 54 to 101 kg N ha^{-1} at Antigo) following glycerol application in the field, the glycerol was likely not retained. Even though soil NO_3^- decreased, likely due to immobilization, we don't know its ultimate fate

or when it would be later mineralized. More sampling periods and deeper sampling may be useful to understand the long-term fate of NO_3^- in the field. The goal of glycerol application after potato harvest is to immobilize NO_3^- to prevent leaching during the fallow period, and the observed decrease in NO_3^- indicates that it was effective at immobilizing N, but if glycerol is easily washed down the soil profile, then it won't be effective at immobilizing additional N. The high variability of NO_3^- leaching values and low efficiency of the resin lysimeters in our experiment is something to consider for future experiments measuring NO_3^- leaching in sandy soils. If using resin lysimeters we recommend using Cl^- mass-balance to test the efficiency of the lysimeters and adjust leaching values accordingly. Further, in order to capture leaching hotspots, more lysimeters may be necessary. We also suggest further investigation of the net negative N mineralization during the lab incubation. It may be worth conducting a longer incubation to see when there would be net N mineralization. Even if glycerol isn't the best C amendment, due to it being easily leached, the large initial decrease in NO_3^- indicates that labile C amendments may still be promising if materials that would be more persistent in soil can be identified.

Acknowledgements

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8. Appendix

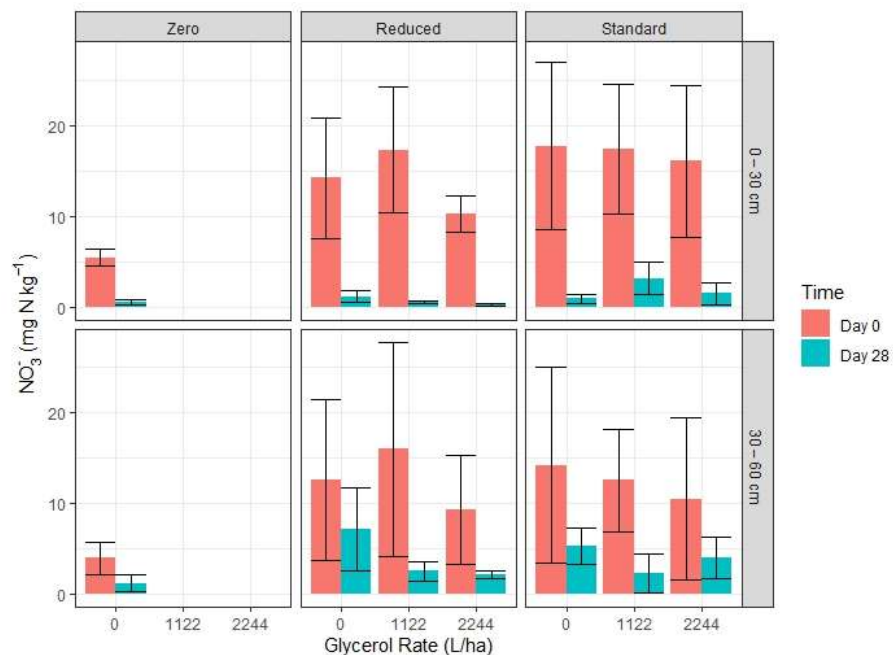


Figure S1. Soil NO_3^- concentrations at the farm before (pink) and after (blue) incubation for 28 days. Bars are means averaged over sampling periods T0, T1, T2, and T3, and error bars are standard error ($n = 4$ for 0–30 cm samples, and $n = 2$ for 30–60 cm samples). N fertilizer rate is in columns and depth is in rows. The x-axis is glycerol rate and before/after incubation is indicated by the bar color.

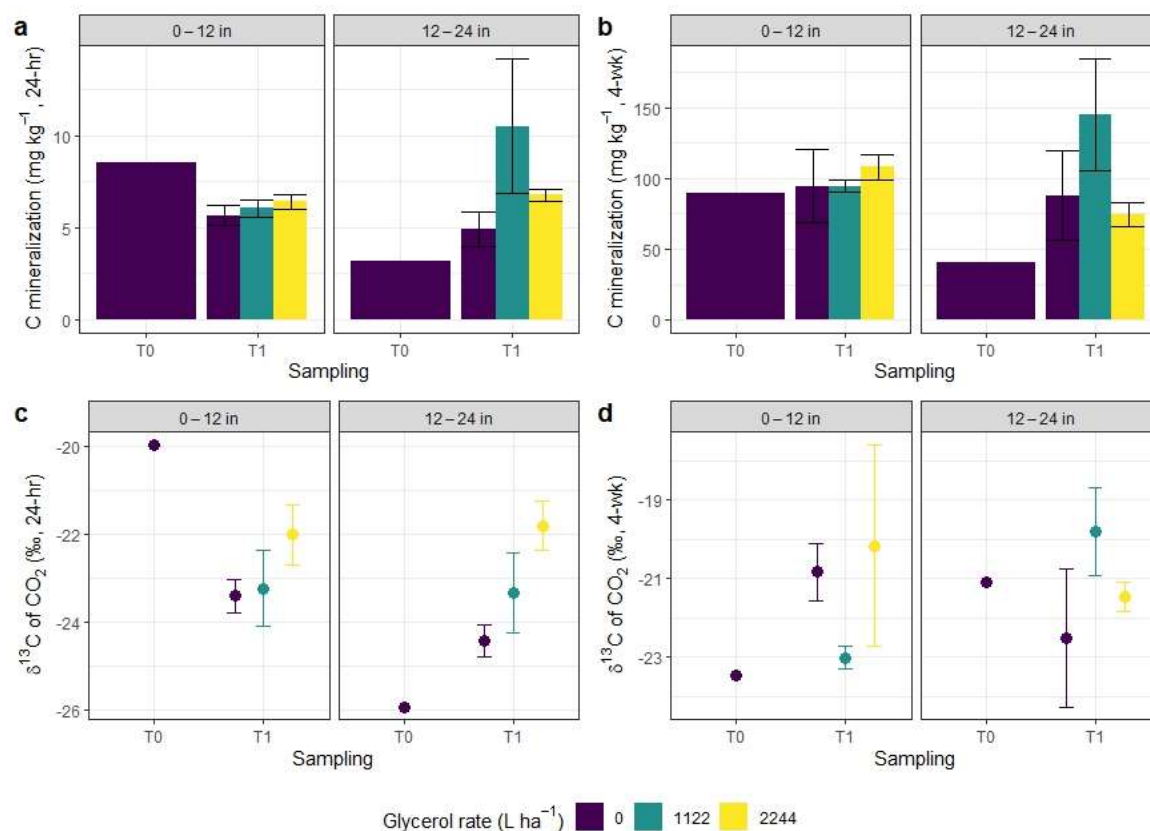


Figure S2. Cumulative C mineralization after 24 hours (a) and 4 weeks (b) and $\delta^{13}\text{C}$ of respired CO₂ after 24 hours (c) and 4 weeks (d) during an incubation of soils from Antigo. Columns are broken up by depth. The x-axis is the sampling period (T0; before glycerol application, and T1; after glycerol application). Glycerol rate is indicated by bar and point colors. Error bars are standard errors ($n = 4$).

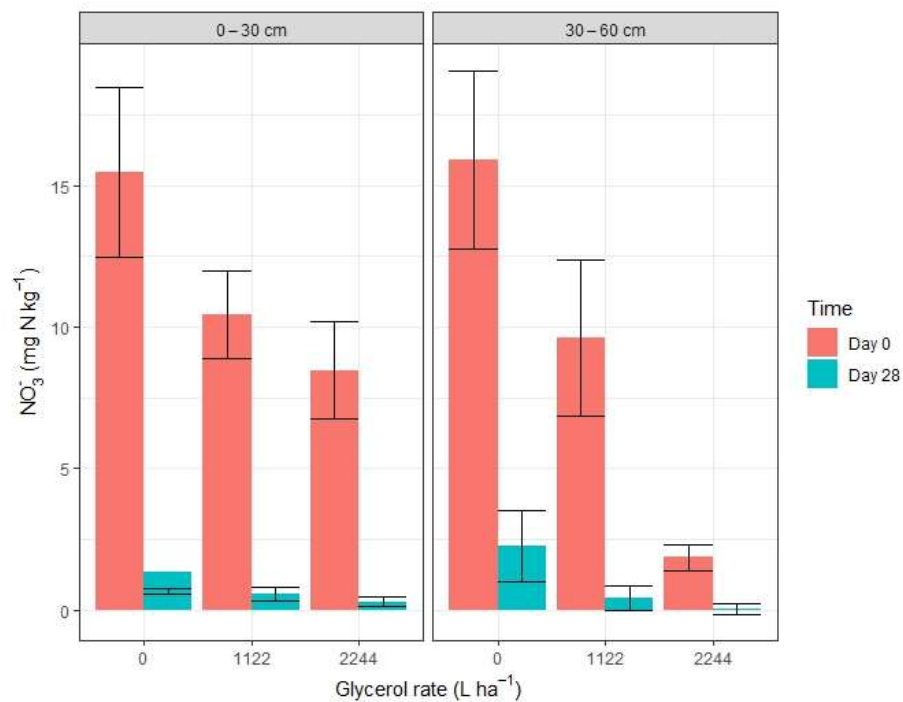


Figure S3. Soil NO_3^- concentrations at Antigo before (Day 0) and after (Day 28) incubation for 28 days. Samples were from after glycerol application. N fertilizer rate is in columns and depth is in rows. The x-axis is glycerol rate and before/after incubation is indicated by the bar color. Error bars are standard errors ($n = 4$).



Figure S4. Photo of a soil sample (2-mm sieved) taken from Hancock Research Station, with particulate organic matter (POM) fragments visible at left.

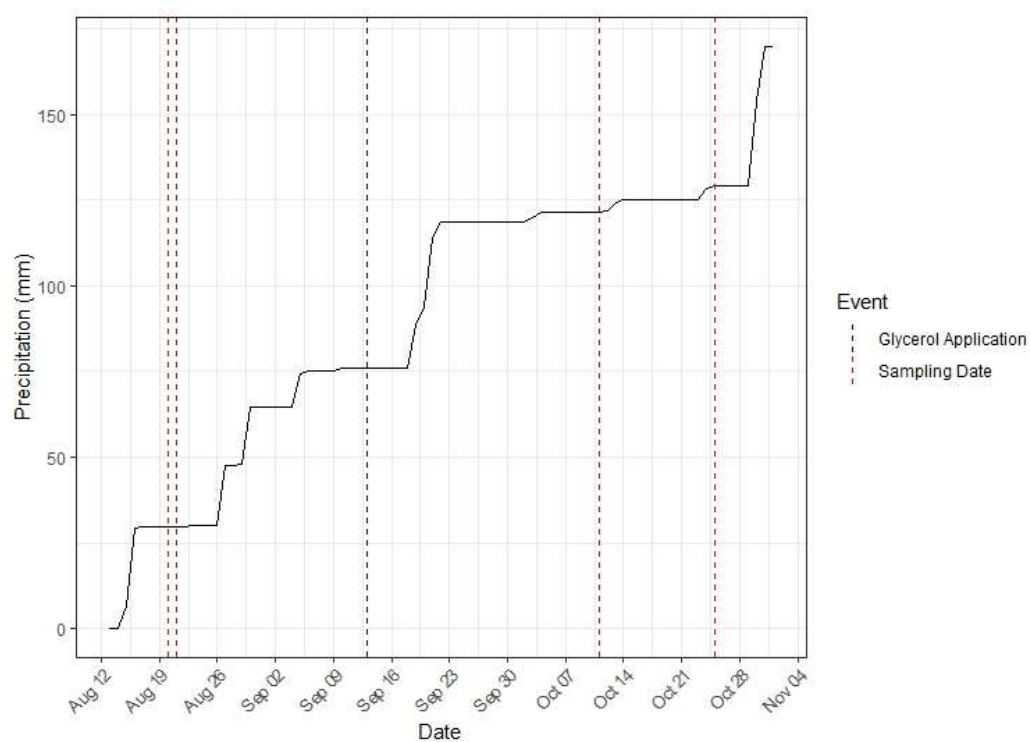


Figure S5. Cumulative precipitation (mm) at Hancock during the time of the experiment (August – October 2024). Red dotted line indicates sampling periods and the blue dotted line is when glycerol was applied.

Chapter 2: Extension Paper

Intercropping, banded fertilizer, and vine removal as potential strategies to reduce NO_3^- leaching in potato production in sandy soil

Nitrate (NO_3^-) leaching is a prevalent issue in the Central Sands Region of Wisconsin due to the high nitrogen (N) fertilizer requirements of the crops grown in this region and the coarse-textured soil. Due to its high mobility in water, NO_3^- is easily leached into the groundwater by precipitation where it causes issues for human and environmental health. While N fertilizer is a main source of soil NO_3^- during the growing season, plant residue contains N that can be mineralized and leached during the fallow period in the fall and winter. In this project we focused on potato vines, which have high N content and are typically mixed with the soil during tuber harvest. By removing the vines from the field, we could remove a source of N that would likely be mineralized to NO_3^- and leached during the fall and winter. Additionally, previous work found that removing potato vines from the field can potentially reduce pathogen pressure for subsequent years of crops (Mol, 1995). In our study we also assessed the potential of winter rye (*Secale cereale* L.) as an intercrop with potato (*Solanum tuberosum* L.) to reduce NO_3^- leaching during the growing season and compared two fertilizer placements: banded or broadcast. The rye was planted in the furrow between potato hills with the goal of reducing N loss from the furrow. The purpose of banded fertilizer is to place N where the plant can use it and keep it out of the furrow where it is more likely to be leached.

This experiment took place at a potato field at Hancock Agricultural Research Station in Hancock, WI during the 2025 field season. All plots received 33 lb N ac^{-1} at planting and 76 lb N ac^{-1} at hilling. The plots had the following treatments: rye planted at potato hilling, rye planted one week after hilling, two weeks after hilling, or no rye intercrop, N fertilizer broadcast applied

at 140 lb ac⁻¹ or N banded on top of hill at 47 lb ac⁻¹ at tuber initiation. The banded fertilizer was applied at a much lower rate since it was placed where the plant can use it, whereas a portion of the broadcasted fertilizer ends up in the furrow where the plant can't get to it. To test the effects of these treatments, we installed three resin lysimeters in each plot (total of 96), 60 cm deep in the hill, shoulder, and furrow. Resin lysimeters capture NO₃⁻ from the water that passes through them, which can later be extracted using a salt solution and measured in the lab. Since our goal was to measure the leaching during the growing season and compare among treatments, the lysimeters were installed on May 22, 2025, directly after hilling, and were removed on September 18, 2025, three days after potato harvest. We removed a 6.5 ft section of potato vines from each plot on September 5, using a knife. To test if the time of vine removal affects how much N would be removed, a subset of vines was removed before desiccation 10 days earlier. All vines were air-dried in a greenhouse before analysis. In addition, we collected soil samples on September 26 to measure residual NO₃⁻, which were kept cool until air-dried at and eventually sieved for analysis. We also measured Cl⁻ in the soil and lysimeter to calculate the efficiency of the lysimeters and adjust the leaching values accordingly. Since the lysimeters only cover a small portion of the field, we want to know what percentage of what is added to the field (N or Cl⁻ fertilizer) is captured in the lysimeters. Based on the Cl⁻ mass-balance, where we use the understanding that what is applied to the field must be accounted for in the soil and leachate, we determined the lysimeters captured about 8% of the Cl⁻ that was applied as fertilizer, so NO₃⁻ leaching values are adjusted (by multiplying by 12) to account for the inefficiency. We describe our lab analysis methods at the end for the interested reader.

Our findings reveal estimated NO₃⁻ leaching was highest when no intercrop was present (Figure 1) and the timing of intercrop planting did not affect NO₃⁻ leaching, but all had lower

leaching than where no intercrop was seeded (Figure 2). When winter rye was present, there was 31.9 lb ac^{-1} less NO_3^- leaching compared to when no rye was present ($p < 0.001$). There were some differences in NO_3^- leaching between the fertilizer placement treatments (broadcast and banded). In general, NO_3^- leaching tended to be higher under the broadcast fertilizer than banded (Figure 3). The mean NO_3^- leaching for the broadcast treatment was $37.5 \pm 6 \text{ lb ac}^{-1}$ (mean and SE) and the mean for banded fertilizer was $28.5 \pm 5 \text{ lb ac}^{-1}$ (mean and SE). If fertilizer is banded instead of broadcasted, our results suggest that NO_3^- concentration in 10 inches of drainage could be decreased by 4 mg L^{-1} . Under broadcast fertilization, more leaching occurred in the furrow than the hill or shoulder, but under the banded treatment, leaching tended to be higher in the hill (Figure 4), which aligns with what we would expect based on the fertilizer placement.

There was no difference in the percentage N and N stock in the vines in the presence or absence of rye. This finding suggests that the intercrop does not reduce the amount of N that ends up in the potato vine. Vine N stock wasn't affected by fertilizer placement and averaged $34 \pm 1.8 \text{ lb N ac}^{-1}$ across all treatments. The post-desiccation vines had a higher N percentage and N stock than the pre-desiccation vines. However, this is likely because the pre-desiccation vines were sampled from the edge row (to avoid overlap with post-desiccation vine sampling), which typically endure harsher conditions from wind and/or pests. In general, we could expect the pre-desiccation vines to have a higher N-content than the post-desiccation vines.

From here on out, we will be discussing the results from the post-desiccation vines only. The vines had a high average N percentage ($1.6 \pm 0.03\%$) and if they were removed from the field, this could prevent 34 lb N ac^{-1} leaching, which is similar to the amount of NO_3^- leaching that could be prevented by the use of rye as an intercrop. In terms of the effect on NO_3^- concentration in water, intercropping or vine removal could decrease NO_3^- in drainage by 15 mg

L⁻¹ if there was 10 inches of drainage. The N percentage and N stock of the vines were 11.1% and 22.9% higher, respectively, with broadcast fertilizer ($p = 0.02$ and $p = 0.05$), but intercropping did not have an effect (Figure 5). Residual soil NO₃⁻ did not differ between fertilizer placements or intercropping (Figure 6), averaging 4.3 ± 0.4 mg kg⁻¹ across all treatments, however, it was 75% lower in the furrow compared to the hill and shoulder ($p < 0.0001$) which had similar soil NO₃⁻ values (Figure 7). This finding suggests the rye in the furrow reduced the amount of NO₃⁻ left in the soil after harvest.

It is also important to consider how these practices might affect potato yields. The overall average yield in 2025 was 562 cwt ac⁻¹ across all treatments. Yield in 2025 was greatest when rye was seeded late (two weeks after hilling) compared to the rest of the seeding times and where no intercrop was seeded but did not differ in 2024. Despite the banded fertilizer rate being a third that of the broadcasted rate, there was no difference in yield. Considering that intercropping and banded fertilizer at a reduced rate didn't reduce potato yield, these practices may be economically viable, in addition to their ability to reduce NO₃⁻ leaching and NO₃⁻ in drainage. These trials will be continued at Hancock Research Station, and we encourage interested growers to contact us.

Chapter 2 Figures

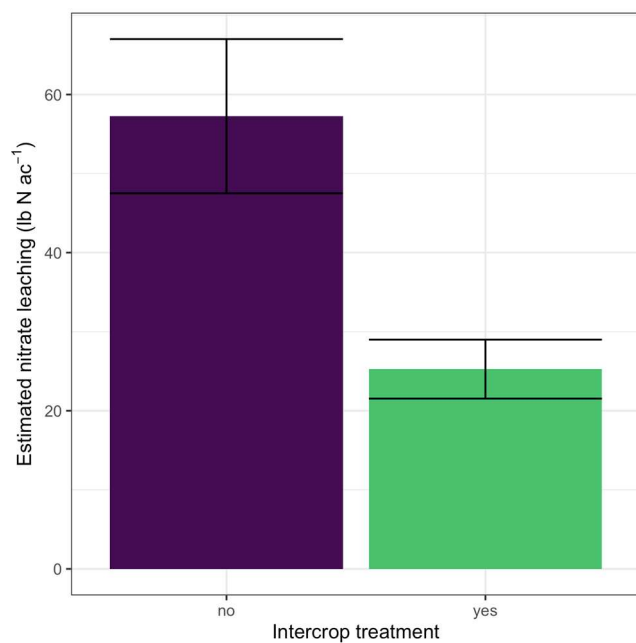


Figure 1. Estimated NO_3^- leaching between no intercrop present (purple) and rye present (green).

The “yes” intercrop treatment is an average of all intercrop planting times. Bars are means and error bars are standard errors.

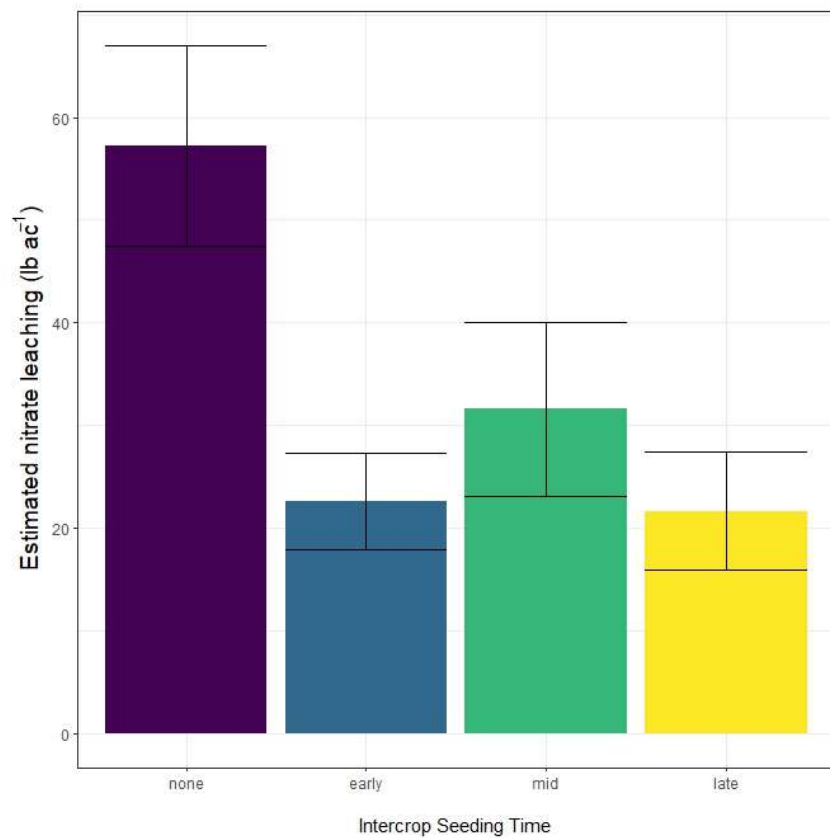


Figure 2. Estimated NO_3^- leaching among intercrop seeding times. Early; at hilling, mid; one week after hilling, and late; two weeks after hilling. Bars are means and error bars are standard errors.

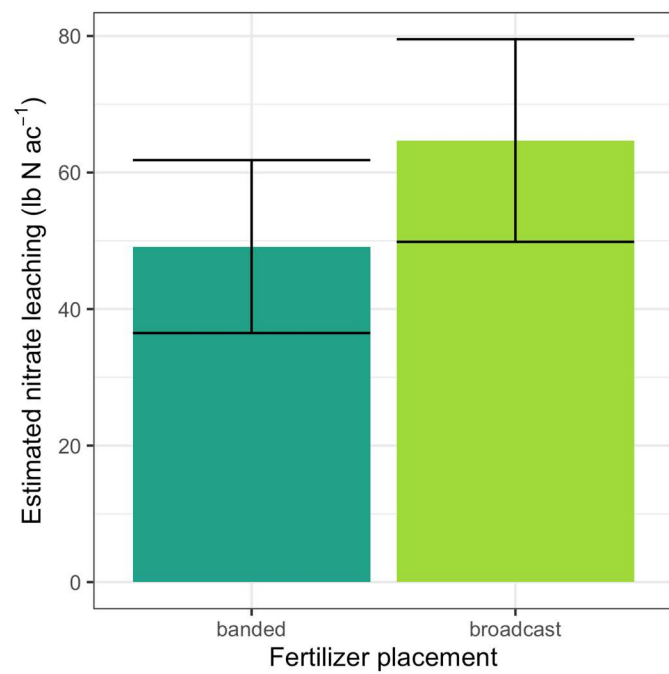


Figure 3. Estimated NO_3^- leaching between fertilizer placements when no intercrop is present.

Bars are means and error bars are standard errors.

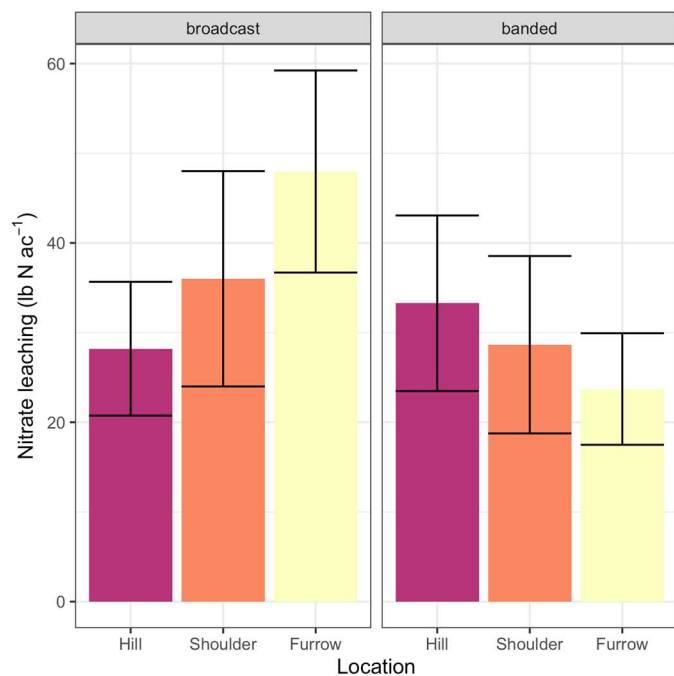


Figure 4. Estimated NO_3^- leaching measured with resin lysimeters at Hancock Research Station. Columns are fertilizer placement, location of lysimeter in the plot is indicated by the bar color, bars are the means and error bars are the standard errors.

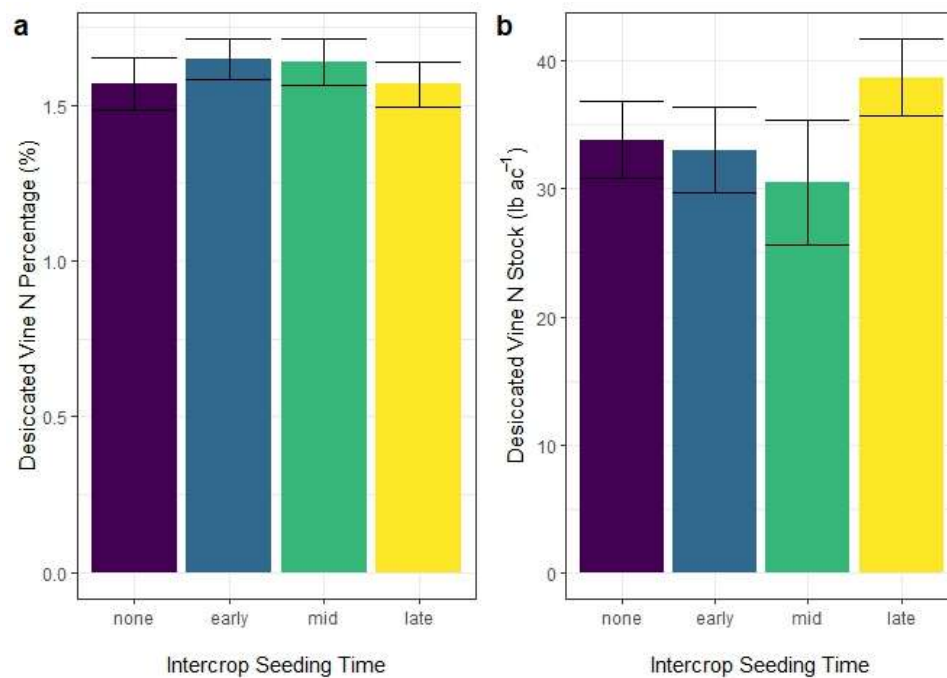


Figure 5. Desiccated vine N percentage (a) and desiccated vine N stock (b) by intercrop seeding time where early; at hilling, mid; one week after hilling, and late; two weeks after hilling. Bars are the means and error bars are the standard errors.

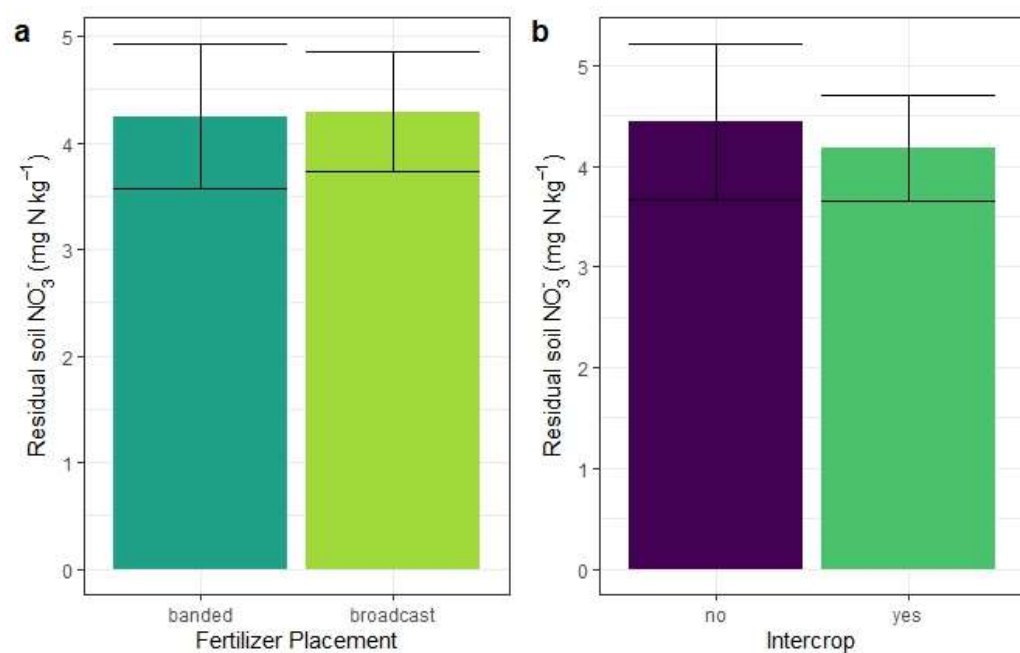


Figure 6. Residual soil NO_3^- from September 26, 2025, by fertilizer placement (a) and intercropping (b). Bars are means and error bars are standard errors.

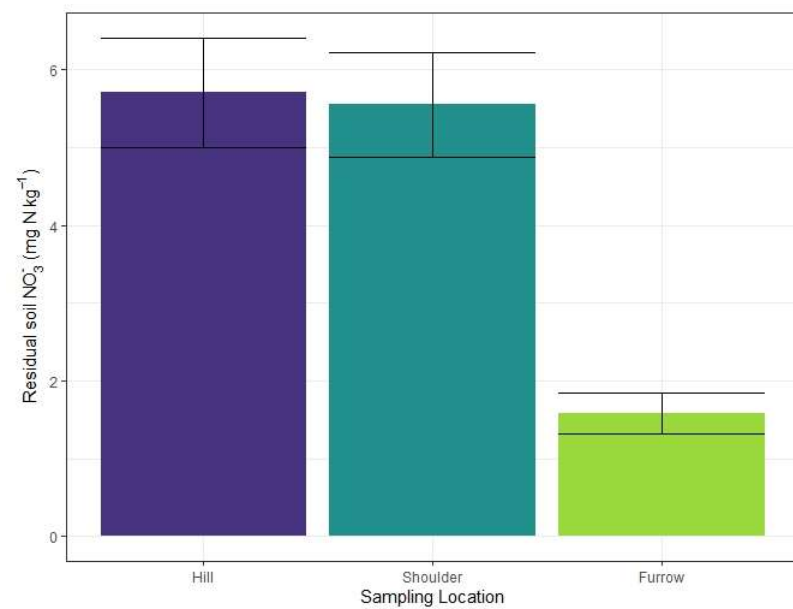


Figure 7. Residual soil NO_3^- from September 26, 2025, by sampling location (hill, shoulder, furrow). Bars are means and error bars are standard errors.

Lab Analysis

Resin from the lysimeters was extracted using 2 M potassium chloride (KCl), and in a subset of 24 lysimeters, the resin was split in half and extracted with potassium sulfate (K_2SO_4) for chloride (Cl^-) analysis. NO_3^- and ammonium (NH_4^+) were measured using colorimetric methods, and Cl^- was measured using an ion-selective electrode and calibrated using a set of standards.

Soil samples were extracted using 2 M KCl and NO_3^- and NH_4^+ were measured using a scanning UV-Vis spectrophotometer and colorimetric methods, respectively. A subset of soil samples was extracted with K_2SO_4 for Cl^- analysis. The N-content of the potato vines was measured using combustion analysis.

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