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Nitrogen Mineralization of Cover Crop Residue Depends on Carbon to Nitrogen Ratio and Soil Temperature

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Core Ideas:

- Nitrogen mineralization data will improve cover crop management and inform policy to reduce N leaching
- At temperatures from 10 to 20 °C, rye C/N ratios predicted N mineralization rates
- Soil temperature had a greater effect on net mineralization or immobilization for high C/N residues
- The risk of leaching from fall-, winter-, or spring-terminated rye declined as the C/N ratio increased
- Doubling the initial soil inorganic N had a negligible effect on the mineralization of the C/N 30 residue

Abbreviations:

C/N, carbon to nitrogen ratio;

d, days;

GWC, gravimetric water content;

MAOM, mineral-associated organic matter;

N, nitrogen;

NH_4^+– N, ammonium nitrogen;

Nmin, nitrogen mineralization;

NO_3^-– N, nitrate nitrogen;

NUE, nitrogen use efficiency;

PAN, plant-available nitrogen;

POM, particulate organic matter;

PVC, polyvinyl chloride;

SOM, soil organic matter;

SPT, sodium polytungstate;

WHC, water holding capacity

ABSTRACT

Groundwater nitrate contamination is largely attributed to fertilizer and intensive livestock manure inputs in agricultural systems. California's Salinas Valley is an area where state policy is aiming to reduce nitrate leaching. Non-legume winter cover crops can help decrease nitrate leaching by scavenging residual soil nitrogen (N) during winter fallow periods following the cropping season. However, the ability of fall-incorporated cover crops to decrease nitrate leaching and recycle N to subsequent cash crops is unknown. We conducted a 112-d laboratory soil incubation experiment using 'Merced' rye (*Secale cereale*) cover crop shoot biomass, with four carbon (C/N) ratios (10, 14, 19, and 30), at three temperatures (10°C, 15°C, and 20°C). Destructive soil sampling was done at six intervals during the incubation to measure plant-available nitrogen (NO_3-N + NH_4-N). Rye biomass with the lowest C/N ratio (10:1) had the highest average nitrogen mineralization (Nmin) rate (56%) at the warmest temperature (20 °C). Conversely, biomass with the highest C/N (30) showed net nitrogen immobilization at 10 °C and 15 °C during the incubation, transitioning to net mineralization only at 20°C. We found a linear correlation between soil temperature and percent Nmin (at 112-d) for higher C/N ratios. Furthermore, doubling the soil mineral nitrogen content had a negligible impact on the percent mineralization of the C/N 30 residue. These results provide useful information to help farmers

and policymakers understand mineralization dynamics from fall-, winter-, or spring-terminated cereal cover crops.

1. Introduction

Nitrate leaching is a major loss pathway of soil nitrogen (N) that degrades surface and groundwater quality (Sutton et al., 2011). Globally, cropland N surplus levels have increased from 16 TgN/y in 1961 to 86 TgN/y in 2010, and N use efficiency (NUE) has decreased from 59% to 46% (Zhang et al., 2021). Thus, in many cases where NUE is low, less than half of the N applied to an agricultural field is removed via the crop harvest, and the rest is prone to multiple loss pathways, including leaching. The Salinas Valley of California is a prime example where nitrate leaching from vegetable farms threatens environmental and human health. Approximately 98% of the population relies on groundwater as a source of drinking water (Boyle et al., 2012), and according to 2023 data, 31% of ranches reported at least one well exceeding the nitrate maximum contaminant level of 10 mg/L NO3-N (https://www.waterboards.ca.gov/centralcoast/water_issues/programs/ilp/dashboard.html). Residual soil mineral N in the top 30 cm of the soil profile following a cash crop, for example broccoli in Salinas Valley, can reach 67-83 μg NO3-N g^{-1} at November cover crop planting (Jackson et al., 1993) or 35-50 μg g^{-1} 70-90 d after cash crop residue incorporation (Smith et al., 2016). In an eight-year organic vegetable trial, (Brennan & Boyd, 2012b) reported NO3-N concentrations ranging between 10 and 40 μg g^{-1} after cash crop harvest. In 2021, the Central Coast Regional Water Board adopted Ag Order 4.0, a regulation to limit N losses from irrigated agricultural fields (Central Coast Region Water Quality Control Board, 2021). This regulation promotes non-legume cover cropping during the winter period when fields are often fallow as a management practice that can reduce N discharge to the environment.

As non-legume cover crops like rye (Secale cereale L.) grow, they take up and store N in their biomass that otherwise is at risk of leaching below the root zone. Cover crop biomass can be thought of as a 'nitrogen sponge', soaking up excess N after the fall cash crop harvest and releasing it at the onset of the spring cash crop. Meta-analyses of cover crop impacts on nitrate leaching illustrate its efficacy in limiting N loss (Abdalla et al., 2019; Nouri et al., 2022; Thapa et al., 2018). Decades ago, non-legume cover crops were shown to reduce leaching in vegetable cropping systems in Salinas Valley by 65 to 70% compared to fallow plots (Wyland, 1996). Furthermore, an eight-year cover cropping and compost application field study in Salinas, CA found that annually planted rye cover crops improved N retention and cycling in vegetable systems (White et al., 2022). However, the effectiveness of cover cropping depends on the timing of crop termination–a prime determinant of residue carbon to nitrogen (C/N) ratio. Furthermore, it is unknown if growing fall-incorporated cover crops (i.e., August-planted and October-terminated) – a management practice that interests many growers – is an effective strategy to reduce N leaching during the winter. In conjunction with soil moisture and temperature, residue C/N ratios determine cover crop decomposition rate, and hence the potential for N loss below the root zone. To improve soil nutrient management and policy recommendations to decrease nitrate pollution, we need to better understand cover crop residue decomposition and N mineralization.

Optimizing the use of non-legume cover crops, particularly by assessing the N mineralization dynamics of rye residues, is essential to develop management practices that mitigate

groundwater pollution caused by nitrate leaching. To receive the highest credit within the local groundwater regulation, Ag Order 4.0, currently cover crops must (1) be non-leguminous (2) be grown over the winter (October-April) for at least 90 days, (3) produce at least ~5,000 kg ha^{-1} of oven-dry shoot biomass, and (4) achieve shoot biomass with a C/N ratio ≥ 20, (Brennan & Smith, 2023). Cover crop adoption in Coastal California has been low (approximately 5%) due to high land rents and because over-winter cover crops can complicate and delay spring planting (Brennan, 2017). Alternatively, summer-planted and fall-terminated cover crops could address the scheduling issues constraint by taking advantage of the fall opening between plantings. Along with recent policy incentives to encourage winter-cover cropping as a nitrogen management practice, fall-terminated cover crops could vastly increase the portion of vegetable land that is cover cropped. Currently, there is a lack of quantitative data to understand if expanding cover crop policy guidelines, for example in Ag Order 4.0, to allow a wider range of management options (i.e., lower C/N ratios, and late summer planted-fall incorporated cover crops) can reduce N leaching throughout the winter rainfall period. Here, we examined the effect of different cover crop C/N ratios from at various stages of development (and thus different termination dates), on the rate of N release from the biomass.

To understand how cover crop biomass C/N ratio, temperature, and inorganic N levels in the soil affect the cover crop decomposition or N mineralization, we ran a 112-d laboratory incubation study. Our research focused on rye, a common cover crop in the Salinas Valley and across the United States. We sought to answer the following questions: (i) How does the C/N ratio of the cover crop shoot biomass influence nitrogen mineralization rates? (ii) How does soil temperature

influence this process? (iii) Does an increased amount of inorganic N in the soil lead to increased mineralization of high C/N cover crop residue?

2. Materials and Methods

2.1 Laboratory Incubations

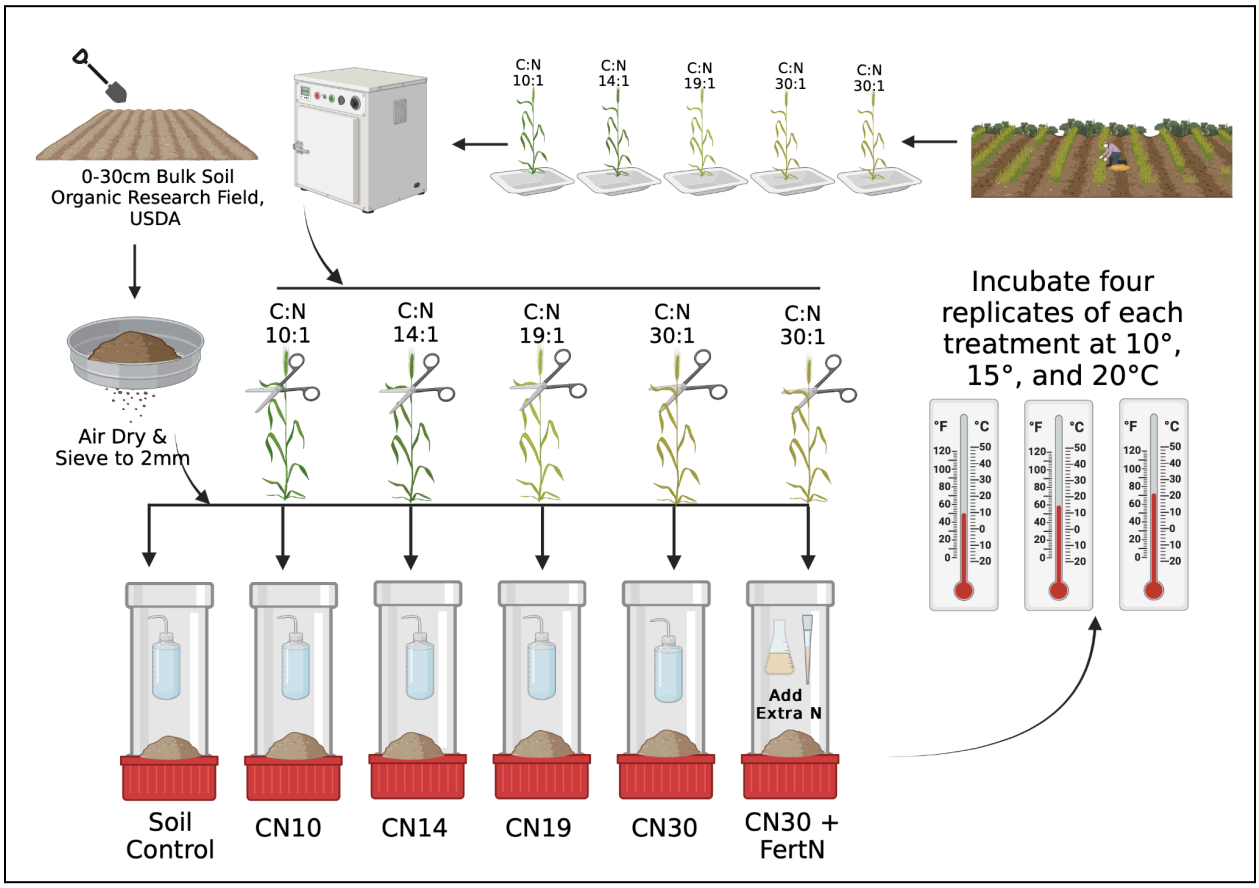

Figure 1. Experimental approach for comparing N mineralization rates between cover crop residue with different C/N ratios and in soil with differing initial inorganic N concentrations under different temperatures.

Soil N mineralization rates were examined using batch incubations (Figure 1). Soil was placed in open polyvinyl chloride (PVC) tubes (5-cm in diameter and 15-cm in height) that were covered with parafilm to maintain moisture while allowing for gas exchange. Each tube contained 250g of (dry weight) soil. All batches were incubated at 50-60% water holding capacity (WHC) and remained aerobic in the dark with temperature held constant through a wired-in regulator device. Cover crop shoot biomass (described below) and DI water were added to the soil (one day after the 10-d pre-incubation at temperature) and incubated for 112-d.

Soil used in the incubation was collected in May of 2023 from the top 30-cm across the USDA Organic Research Field Site in Salinas, CA (36.62° N, 121.547° W). The soil was collected from a field where a winter triticale cover crop had been terminated and soil incorporated several weeks before collection. After collection, the soil was air-dried and passed through a 2 mm sieve; the dried, sieved soil was then refrigerated to minimize microbial activity. Subsamples of the bulk soil were analyzed for the following: gravimetric water content (with oven-drying for 24 h at 105 °C), pH (2:1 MQ water to soil, shaken for 30 min), initial mineral N (5 g soil reacted with 2 M KCl and shaken for 2 h, and then passed through a 42 um filter), total carbon and nitrogen (air dried, ball-milled), texture (air dried, sieved, 2 h hydrometer method), and percent SOM (oven dried, measured by loss on ignition method). Results of these analyses are shown in Table 1. Mineral N extractions were analyzed for concentration of NH_4-N and NO_3-N through colorimetry using a discrete analyzer (SmartChem 200, KPM). Total carbon and nitrogen in the soil samples were determined on an Elemental Analyzer (ECS-8020, NC-Technologies). Soil carbon was categorized as particulate (POM; light-fraction) and mineral-associated (MAOM; heavy-fraction) using a two-part density fractionation method with a 1.65 g/mL sodium polytungstate (SPT) solution to establish the fractionation threshold (Moni et al., 2012; Sollins et al., 2006).

Table 1. Properties of soils used in 112-d incubation. Mean and standard deviation in parenthesis.

% GWC of field moisture	pH	Texture	% OM	C/N Ratio	PAN	% C as POM	% C as MAOM
g H20 g^{-1} soil					µgN g^{-1} dry soil		
23 (1.46)	7.2	Sandy Loam, 79% sand, 13% clay	2.16 (0.07)	12 0.11% N 1.33% C	9.26 (1.29)	16.08 (1.28)	83.92 (1.28)

The three main variables tested over the 112-d incubation were: soil temperature, soil inorganic nitrogen (NO_3- and NH_4+), and biomass C/N ratio as six treatments replicated four times (Table 2). Each batch corresponded to one temperature (10, 15, or 20 °C) and contained six treatments. Temperatures were selected to represent the possible range for cover crops terminated in the fall, winter or spring (Figure 2). The biomass application rate was constant for the different C/N ratio treatments. Hence, there were different nitrogen application rates between the treatments (Table 2). We also examined mineralization rates for the highest C/N (30:1) with additional inorganic nitrogen, all compared to the soil control treatment (no cover crop).

Table 2. Carbon to nitrogen ratios and nitrogen content of cover crop shoots and associated content in soil incubations and field equivalents (FertN= doubled soil (NH_4-N + NO_3-N))

Treatment Name	Cover crop shoot residue		†N added to soil by cover crop shoot residue	
	C/N	%N	µgN g^{-1}	kgN ha^{-1}
Control	-	-	-	-
CN10	10	4.11	263.0	322.5
CN14	14	2.99	191.4	234.6
CN19	19	2.28	145.9	178.9
CN30	30	1.51	96.6	118.5
CN30 + FertN	30	1.51	96.6 (+9.3 from NH_4NO_3)	118.5 (+33 from NH_4NO_3)

†Calculations assume 7846 kg ha^{-1} oven-dry shoots, 30-cm incorporation depth, and a bulk density of 1200 kg/m^3

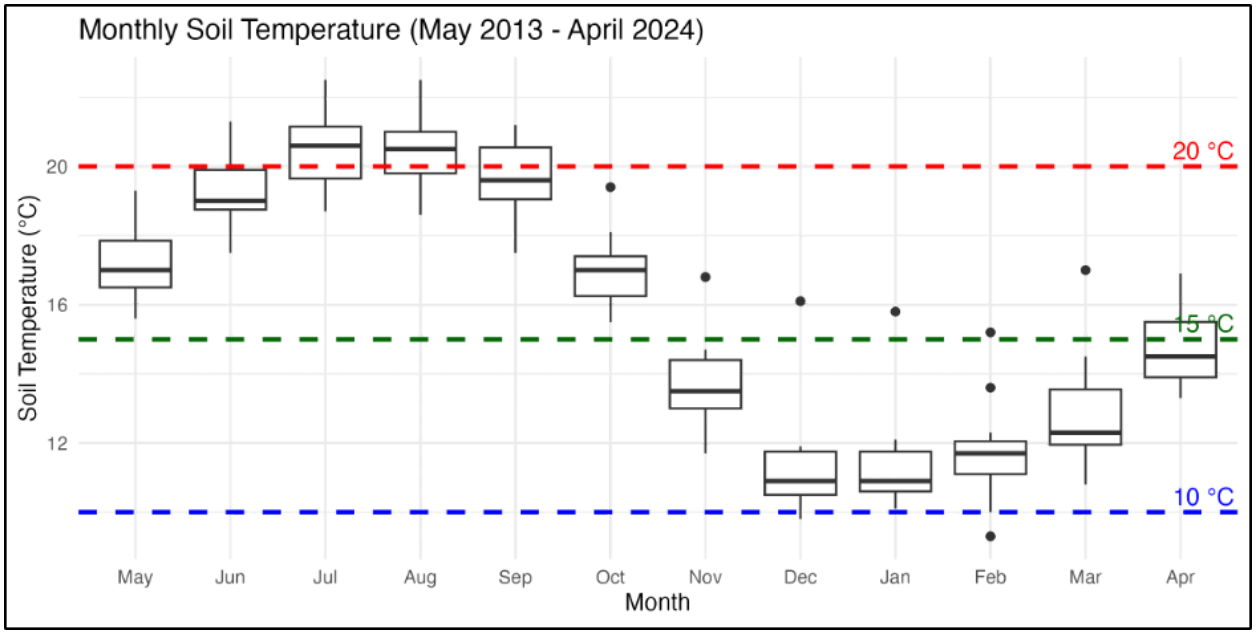

Figure 2. Monthly soil temperature from May 2013 to April 2024. Red, green, and blue dashed lines represent the three different batch temperatures at which cover crop residues were incubated, 20°, 15°, and 10° C, respectively (Data were from the California Irrigation Management Information System (CIMIS) Salinas South Station, #214 which was initiated in May 2013, hence the range of data available; https://cimis.water.ca.gov).

The 'Merced' rye cover crop shoot biomass samples used for this incubation were from a study (Brennan and Smith, 2024 (20099)) at the USDA-ARS in an adjacent field to where the bulk soil was collected. The biomass was oven-dried to 65.6 °C and chopped with a hammer mill with a 12.7 mm diameter screen that produced pieces that were usually less than 2 to 3 cm long; biomass was then stored in paper bags. A subset of each biomass was sent to the UC Davis Analytical Laboratory for analysis of total carbon and nitrogen content. Biomass application rates (oven-dry basis) were constant for all treatments and were calculated to match an oven-dry field application rate of 7846 kg ha^{-1}. The level of rye cover crop shoot biomass was similar to the average season-end biomass of an over-winter rye cover crop in a long-term study with vegetable production in Salinas, CA (Brennan & Boyd, 2012a) but was higher than cover crop minimum biomass (5044 kg ha^{-1} oven-dry) for growers to receive the highest N scavenging

credit in the Ag Order 4.0 regulation. The biomass application rates do not account for any moisture that the oven-dried cover crop residue likely absorbed from the air between the time that it was oven-dried until it was used in the incubation experiments.

Prior to adding biomass and moisture to the soil, tubes of soil were pre-incubated in their respective incubation temperatures for 10 days. For each incubation, 1.6 g of residue across the four C/N ratios was added to 250g of soil. To mimic cover crop incorporation in the field (to a depth of 30 cm), the residue was mixed with the soil. For the CC30+FertN treatment, to double the inorganic nitrogen, the initial level was measured (9.3 μg g^{-1} NO_3-N plus NH_4-N; ~33 kg ha^{-1}), and then a solution of ammonium nitrate representing 9.3 μg g^{-1} (~33 kg ha^{-1}) was added to the soil. Doubled inorganic N treatment (CN30+FertN) was used to address the question of whether higher soil inorganic N content would affect Nmin of the residue. For the treatments (CN10, CN14, CN19, and CN30), half of the soil-cover crop mixture was poured into the PVC tube followed by half of the DI water applied by evenly distributed pipette drips onto the soil surface. Then the second half of the soil-cover crop mixture was added on top, completed with the second half of the DI water, also applied with a pipette. For the CN30+FertN treatment, the residue and moisture were added using the same protocol, only substituting the DI water with the ammonium nitrate solution.

2.2 Mineral Nitrogen Sampling

On day 1, 15, 29, 57, 85, and 112 of the incubation, destructive soil sampling was conducted and a composite ~5 g soil subsample from each incubation tube was utilized. The subsample was extracted with 40 mL of 2 M KCl as follows: vortexed, shaken horizontally at 200 rpm for 2 h,

filtered through 42 µm membrane, and then frozen in 15 mL tubes until thawed and run on the Discrete Analyzer (SmartChem 200, KPM) to measure NO_3^-– N and NH_4^+– N concentrations. On each destructive sampling date, the gravimetric soil moisture content (GWC) was measured (oven-dried at 105° C for 24 h) and then averaged across the six treatments to convert nitrogen concentration to mass ratio. The pH (2:1 MQ water:soil ratio, shaken for 30 minutes) of each treatment was also measured on additional subsamples.

2.3 Data Analysis

Nitrate-N and ammonium-N (µg g^{-1} of dry soil) data were read, analyzed, and plotted in R Studio (Version 2023.12.1+402). On each sampling date, the ammonium-N and nitrate-N values were separately averaged across the four replicates, and the standard errors were calculated. Ammonium-N and nitrate-N values were also added together to give plant-available nitrogen (PAN), and then PAN was averaged across the four replicates. Net nitrogen mineralization values were calculated by averaging the unamended treatment (Soil Control, no cover crop) for each sampling date and subtracting the soil control mineral N from the PAN for each replicate separately (Eq 1.). The net mineral N amounts were averaged, and the standard errors were calculated.

To understand the percentage of organic nitrogen mineralized from each residue with different starting nitrogen values, the percent nitrogen mineralized was calculated. This involved taking the net nitrogen mineralized value and dividing the mineral N (after multiplying by the 250 grams of soil) by the initial organic nitrogen content in the residue added to the tube (Eq. 2).

$$Eq.\,1.\ Net\ Nmin\ =\ (PAN_{treatment}\ -\ Avg\ PAN_{control})$$

$$Eq.\ 2.\ \%\ Nmin\ =\ \frac{(PAN_{treatment} - Avg\ \ PAN_{control})^{*}\ 250g\ soil}{Organic\ N_{residue}}$$

To calculate the percentage of nitrogen mineralized for the CN30+FertN, we first subtracted out 9.2565 ug N g^{-1} that we added to the soil for each replicate on each sampling day, and then subtracted the nitrogen mineralized from the unamended treatment (Soil Control, no cover crop). Lastly, we divided the resulting quantity by the starting organic nitrogen content of the cover crop residue (Eq 2.).

For the percent Nmin measured throughout the incubation, 95% confidence intervals were calculated and plotted. Using the average percentage Nmin at the end of the incubation (112-d), a linear model was calculated for each temperature between the percent Nmin and residue C/N and nitrogen application rate (µg g^{-1}), with the equation and Pearson correlation coefficient (R^2) values printed in R Studio. Additionally, linear regression was used to understand the relationship between the average percent Nmin (112-d) and temperature for each C/N residue treatment.

3. Results

3.1 Cover Crop Residue C/N Predicts N Mineralization

The NO_3-N concentrations on 112-d increased concomitantly with the residue nitrogen (N) content, with the CN10 thus yielding the highest amounts (166.80 to 215.88 µg g^{-1} of NO_3) and CN30 residue the lowest (20.81 to 78.87 µg g^{-1} of NO_3) (Figure 3). As our incubation progressed, nitrate was the predominant form of N measured. By 29-d of the incubation, NH_4-N peaked for all treatments and then remained low throughout the incubation (SI, Figure S1). At its peak concentration, the CN10 treatment (ranging between 33.30 to 47.43 µg g^{-1} of NH_4-N for the three

soil temperatures) had approximately double the NH_4-N of the CN14 treatment (16.72 to 18.82 µg g^{-1} of NH_4-N), which in turn had about double the NH_4-N from the CN19 (7.83 to 8.62 µg g^{-1} of NH_4-N) and CN30 (4.90 to 7.90 µg g^{-1} of NH_4-N) (SI, Figure S1).

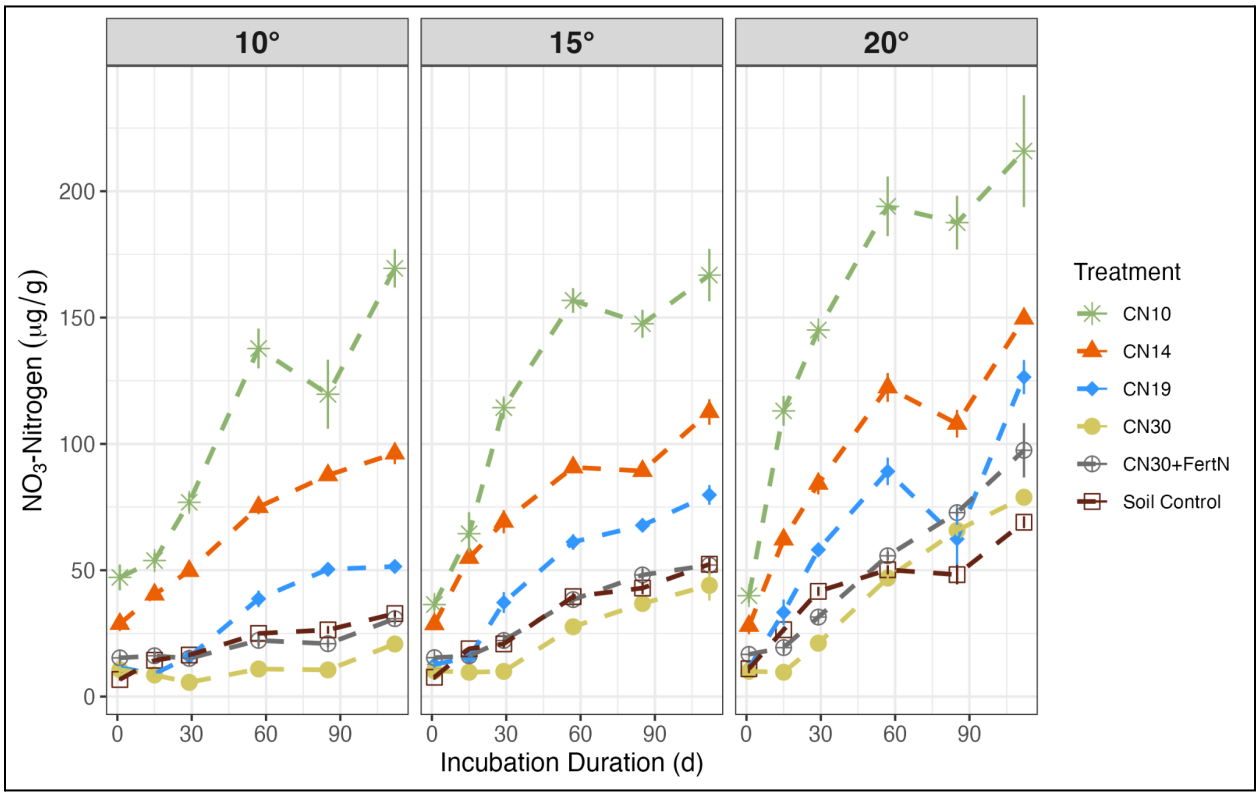

Figure 3. Average NO_3-N (per gram dry soil) with SE bars (n=4) throughout the incubation for each temperature batch (10, 15, and 20 ℃). Treatments included CN10, CN14, CN19, and CN30, which correspond to the four different carbon-to-nitrogen ratios of the residue (10, 14, 19, and 30, respectively). The CN30+FertN treatment had doubled initial NH_4NO_3 and the Soil Control (no cover crop).

Summing the NH_4-N and the NO_3-N values, we calculated the total plant available nitrogen (PAN). Across the three temperatures, the average net N mineralization of the rye cover crop residues at 112-d after subtracting the N mineralized by the unamended soil control treatment, was 114.76 to 147.02 µg g^{-1} for CN10, 63.43 to 80.76 µg g^{-1} for CN14, 18.62 to 57.63 µg g^{-1} for CN19, and -12.13 to 10.53µg g^{-1} for CN30 (Figure 4; negative values indicate net N

immobilization). Our data illustrate a large difference in PAN between residues with differing C/N ratios (Figure 4).

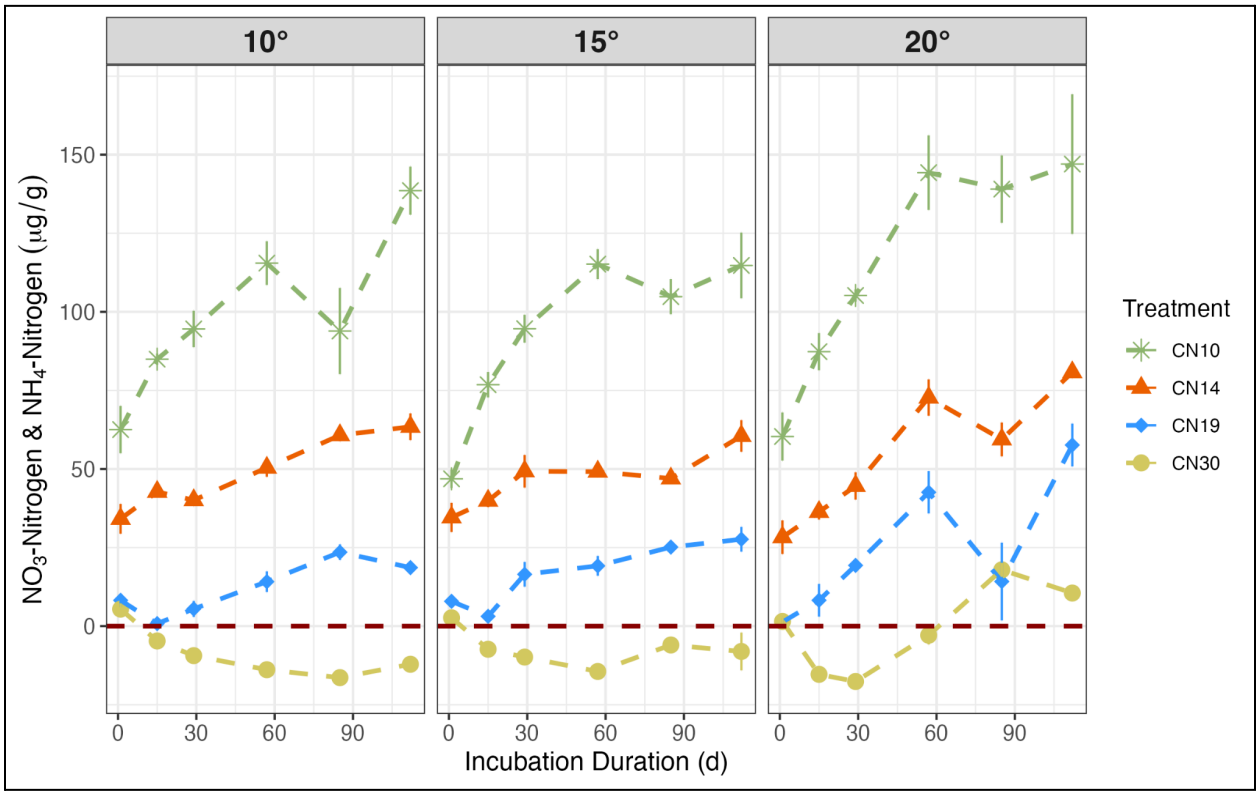

Figure 4. Average net mineral nitrogen (NO_3-N & NH_4-N) with SE bars (n=4). The horizontal dashed red line is set at y=0, representing the threshold between mineralization and immobilization. Treatments included CN10, CN14, CN19, and CN30, which correspond to the four different carbon-to-nitrogen ratios of the residue (10, 14, 19, and 30, respectively).

As expected for nitrification, changes in soil pH followed N mineralization, as the soil became more acidic with higher rates of N mineralization in the residue with the highest N content (i.e., lowest C/N ratio) (SI, Figure S2). At several points in the coldest incubation, the CN10 treatment had the lowest pH following the higher N mineralization, and the CN30 treatment had the highest following the lower N mineralization. Likewise, at the warmer temperatures, the CN10 treatment had the lowest pH; however, the unamended soil control had the highest pH with the remaining treatments in between (SI, Figure S2).

For each temperature, a strong negative linear relationship occurred between N mineralization and residue C/N (Figure 5). Comparing organic N mineralized between the four treatments (excluding the soil control and CN30+FertN), there was a linear trend (R^2 of 0.97, 1, and 0.97 for 10°C, 15°C, and 20°C, respectively) between the fraction (or percent) mineralized and the C/N ratio of the incorporated residue when combining the different incubation temperatures (Figure 5). As the residue C/N increased, the percent N mineralized decreased. There was a consistent cover crop residue application rate for each treatment, therefore, the N application rate increased with a decreased residue C/N ratio (Table 2). N mineralization was highest for the lowest C/N ratio (CN10) and lowest for the highest C/N ratio (CN30). Examining the percentage of N mineralization as a function of temperature (Figure 6) shows that the lowest temperature had the least mineralized N across residue C/N ratios, and CN30 had net immobilization. By the end of the incubation, the largest average percent N mineralized (56%) was for the lowest C/N ratio residue (CN10) at the highest temperature (20 ℃), and the smallest Nmin resulted in net immobilization (-13%) for the highest C/N ratio residue (CN30) at the lowest temperature (10 ℃).

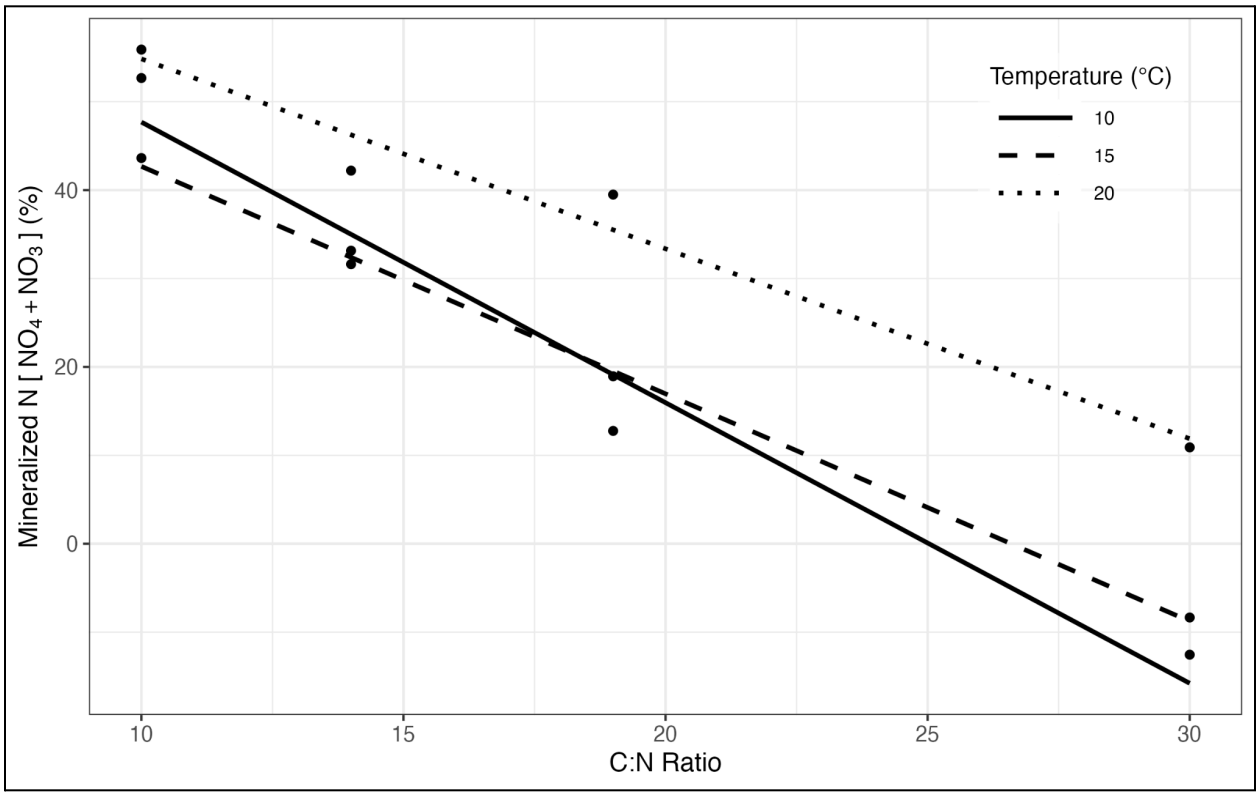

Figure 5. Relationship between residue carbon to nitrogen (C/N) ratio and N mineralized for each temperature at 112-d. Linear fits to the data result in equations as follows: **10°C**: y=79.4−3.17x with an R^2 of 0.97, **15°C**: y=68.4−2.57x with an R^2 of 1.0, and **20°C**: y=76.3−2.15x with an R^2 of 0.97.

For the unamended soil control without cover crop biomass, N was mineralized from the native soil organic matter and increased with soil temperature (SI, Figure S3). For the CN30 residue, the 10 and 15 ℃ incubations showed net immobilization, while the 20 ℃ incubation showed net N mineralization (Figure 6). Temperature had a stronger effect on mineralization for the CN30 and CN19 residues (Figure 7); this is indicated by the slopes that were steeper for the CN30 and CN19 residues. Temperature has no effect on mineralization of the CN10 residue and a relatively small effect on mineralization of the CN14 residue.

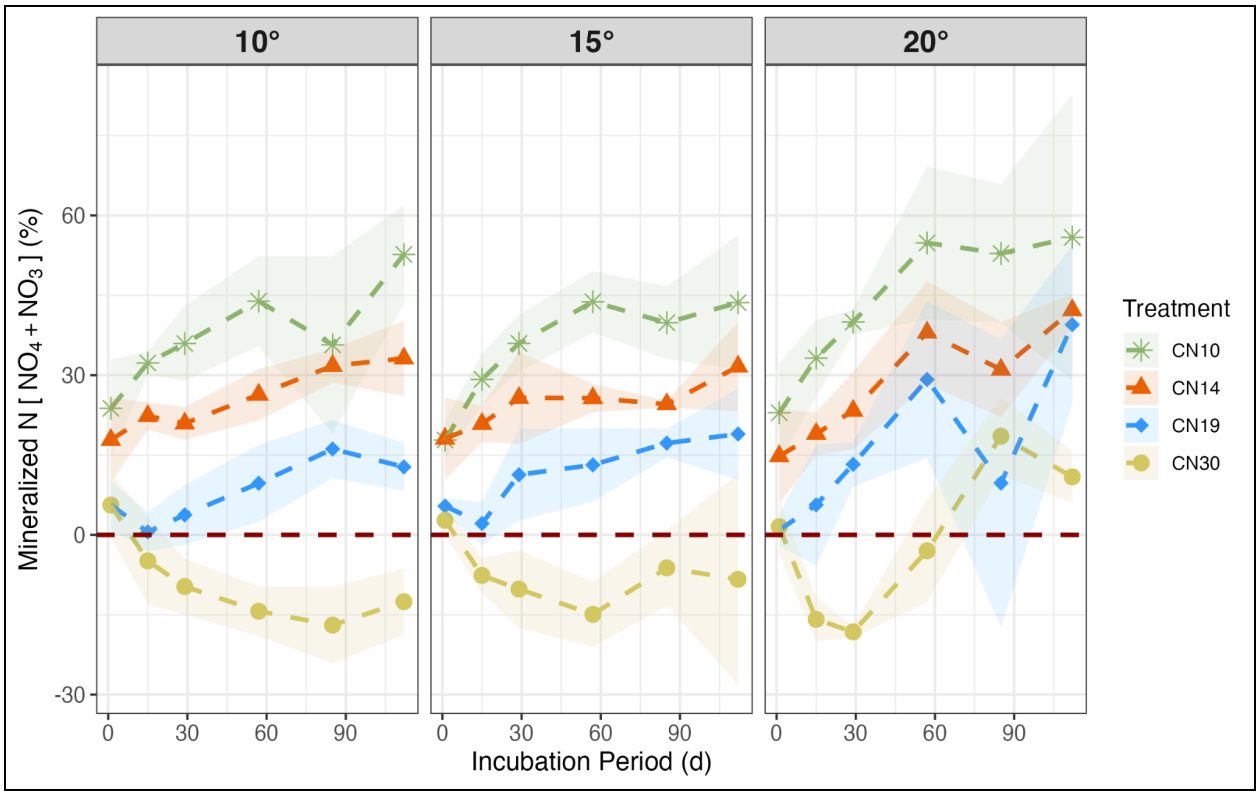

Figure 6. Percentage of nitrogen mineralized across incubation time for residue with C/N ratios of 10 (CN10), 14 (CN14), 19 (CN19), and 30 (CN30) at temperatures of 10 ℃, 15 ℃, and 20 ℃. The shaded areas represent the 95% confidence intervals (n=4); the horizontal dashed red line is set at y=0, representing the threshold between mineralization and immobilization.

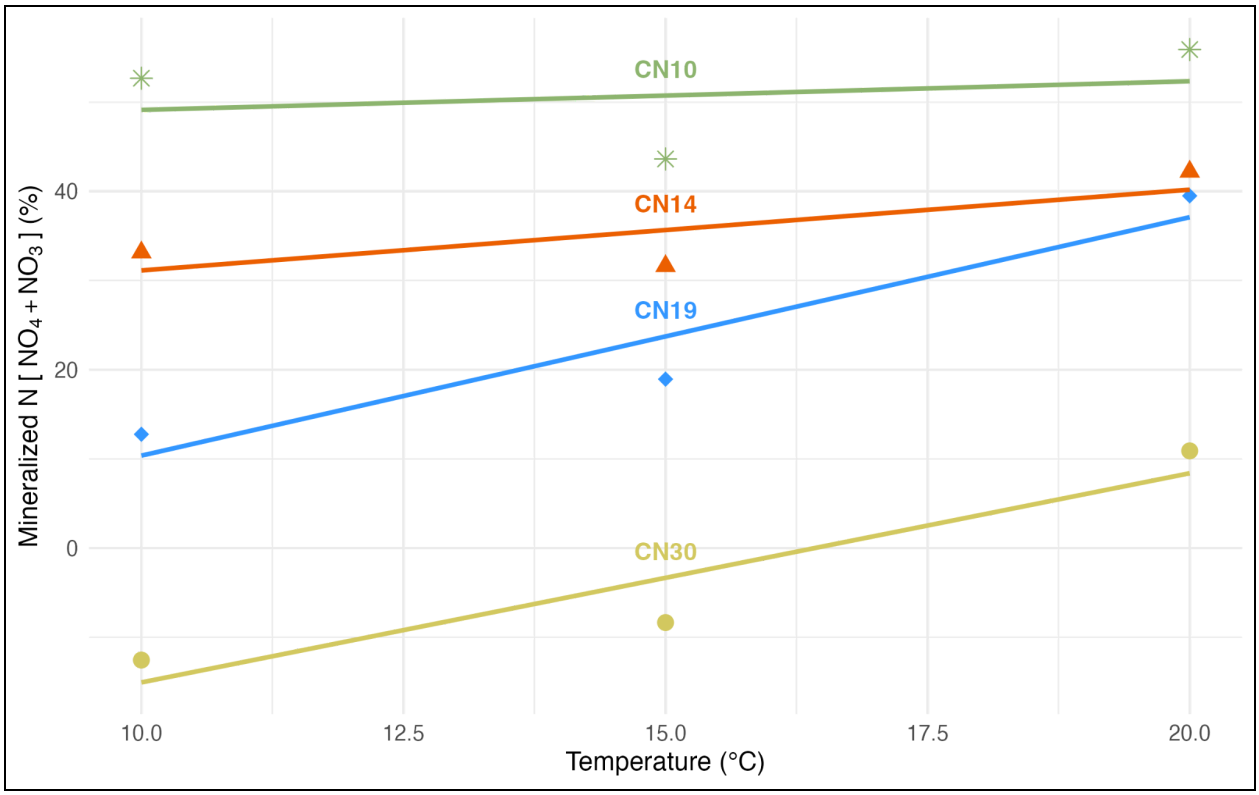

Figure 7. Nitrogen mineralized as a function of soil temperature after 112-d of incubation. Residues with varying C/N ratios were compared: 10 (CN10), 14 (CN14), 19 (CN19), and 30 (CN30). Linear fits to the data result in equations as follows: **CN10:** y = 0.32x + 46 (R^2= 0.06), **CN14:** y = 0.91x + 22 (R^2=0.63), **CN19:** y = 2.7x – 16 (R^2 = 0.91), and **CN30:** y = 2.3x – 39 (R^2=0.88).

3.2 Minimal Difference for Doubled Inorganic N in the Soil

For the highest C/N residue (CN30), we examined the impact of initial inorganic N (i.e., fertilizer addition) on the mineralization rate of the residue by comparing mineralization with soil that had double the initial NH_4NO_3 addition. After 1 day, the soil receiving twice the NH_4NO_3 quantity (CN30+FertN) had a higher NH_4-N average (8.75-10.99 µg g^{-1}) than the treatment receiving only DI water plus residue (CN30) (4.90-7.90 µg g^{-1}) (Figure S1.); however, this was due to the added NH_4NO_3 fertilizer to the FertN treatment. Likewise, at 112-d the residue with the CN30+FertN had a higher average NO3-N (30.82-97.48 µg NO_3-N g^{-1}) than the CN30 treatment (20.81-78.87

µg NO_3-N g^{-1}) (Figure 3). There was minimal difference in N mineralization rates between the soils having doubled initial inorganic N content for any temperature (Figure 8).

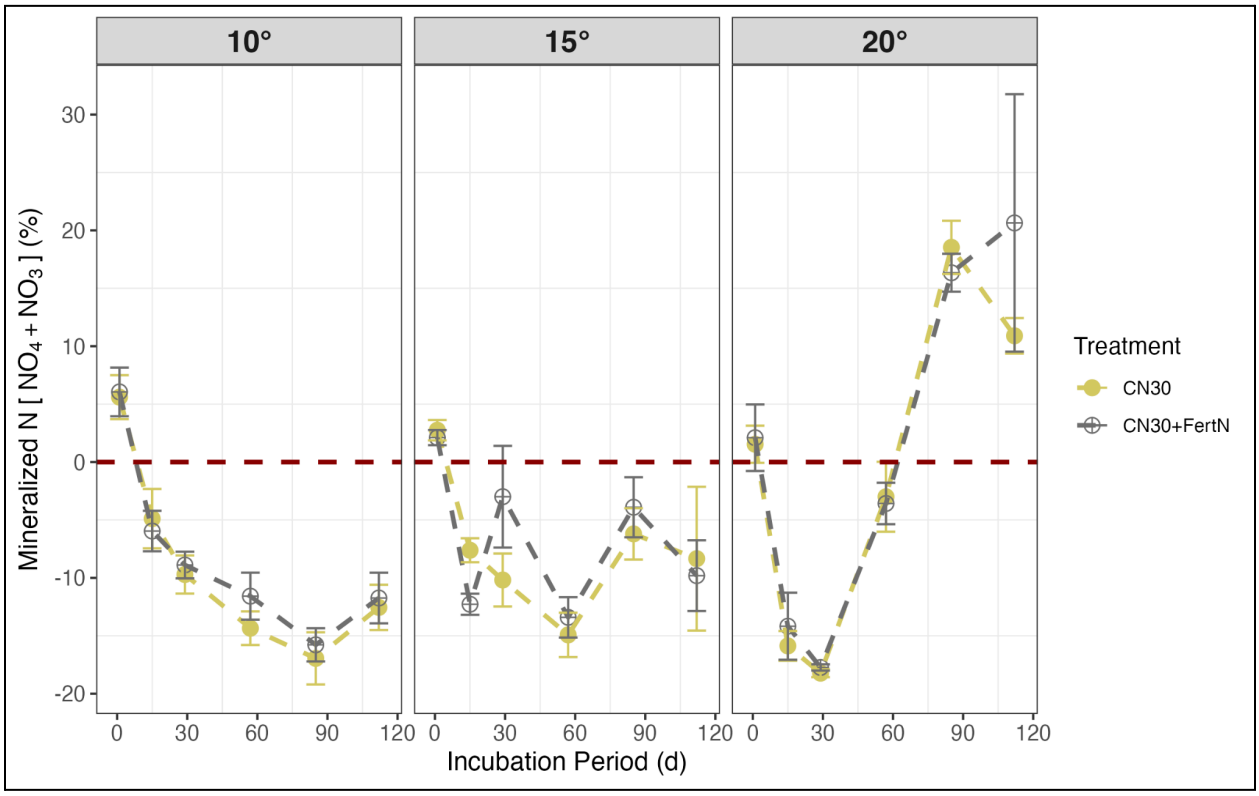

Figure 8. Nitrogen mineralized for the C/N 30 residue (CN30) with and without doubled soil mineral nitrogen (nitrate-N and ammonium-N) (CN30+FertN). The horizontal dashed red line represents the threshold between mineralization and immobilization.

4. Discussion

Cover cropping in high-value vegetable systems is complex and requires a nuanced understanding of factors affecting cover crop residue decomposition. As cereal cover crops mature, the C/N ratio of the shoot biomass increases (i.e. the N concentration decreases) (Brennan et al., 2013), which changes mineralization rates once incorporated into the soil. Our data illustrate a clear relationship between residue C/N ratio and N mineralization (Figure 5), with higher C/N ratios having lower mineralization rates. Our results agree with past incubation studies that also found that C/N ratio was a good predictor of N mineralization from vegetable

residues (Iritani & Arnold, 1960), cover crop residues (Li et al., 2020), and organic fertilizers and composts (Lazicki et al., 2020).

A C/N ratio of 25 is sometimes used as a threshold between net N mineralization (Nmin) and immobilization (Myrold & Bottomley, 2008). Our results for the C/N 19 and 30 treatments are consistent with the 25 threshold for the 10 ℃ and 15 ℃ temperatures; however, at 20 ℃, we also found net mineralization for the C/N 30 residue by the later stage of the incubation (Figure 6). We found that when the biomass C/N ratio of a cover crop residue was low (10 or 14), with sufficient moisture, mineralization occurred at a somewhat similar rate regardless of temperature (a projection for the time of year when the cover crop residue is incorporated) (Figure 7). However, as the C/N ratio increased, the temperature dependence became increasingly pronounced, as illustrated by the steeper slope of the linear temperature dependence for the different C/N ratios (Figure 7). Moreover, the C/N ratio was increasingly important in determining mineralization rates at higher temperatures. For example, in the 20℃ incubation, the CN19 residue mineralized at nearly four times the rate of CN30 (Figure 6). Li et al., (2020) found perennial ryegrass (*Lolium perenne L.*) shoots (C/N of 20) had 18% N mineralization at 100-d at 10 ℃, which is similar to our CN19 residue at 10 ℃ which mineralized 13% at 112-d. A 150-d laboratory incubation with surface-applied rye (C:N 22) at 35℃ and -0.03MPa, reported net N min rates up to 45%, which is similar to the 40% Nmin for the CN19 residue at 20 ℃ in our study (Thapa et al., 2021).

Despite our expectation that N mineralization rate would increase with a higher amount of initial soil inorganic N, we observed no difference in the mineralization rate of the CN30 residue with doubling the initial inorganic N content (adding 33 kg N ha^{-1}). If the amount of nitrogen in the soil is insufficient (relative to catabolic potential), soil microbes dependent on N for anabolic processes will immobilize nitrogen (Robertson & Groffman, 2007), leading to a negative N mineralization value. Even by doubling the soil inorganic N content (CN30 + FertN), we posit that the low total nitrogen content in the soil remained insufficient for altering microbial activity as compared to the CN30 treatment. Investigating the effect of different quantities of inorganic nitrogen in the soil upon residue incorporation may be useful in future studies.

Cover crop residue incubation studies use different approaches between having constant application rates of biomass (Kuo & Sainju, 1998; Li et al., 2020; Roth et al., 2023; Thapa et al., 2021), or varying both nutrient and biomass rate (Lawson et al., 2013; Woli et al., 2016). Our incubation design applied a constant biomass rate (1.6 g residue added to 250 g soil) across the various C/N ratios. This resulted in higher N inputs from cover crop residue for the CN10 (322.5 kg N ha^{-1}) and CN14 (234.6 kg N ha^{-1}) treatments than would typically occur from rye cover crops in the central coast region of California that are between 100 to 150 kg N ha^{-1} (Brennan & Boyd, 2012b; Jackson et al., 1993), but occasionally up to 180 kg N ha^{-1} (Brennan et al., 2011). Furthermore, cover crop biomass levels are typically much lower when N content is high. Thus, our incubation application rate for rye biomass of 7846 kg ha^{-1} would not be possible in the field for the low C/N biomass (i.e. 10 or 14) treatments. For example, in an eight-year field study in Salinas, CA, December sampled rye biomass at a C/N of 12 (Brennan et al., 2013) only reached 2400 kg ha^{-1} (Brennan & Boyd, 2012a). Similarly, in the same study when the rye residue C/N

reached about 18, the biomass was only 4700 kg ha^{-1} and the biomass did not exceed 7000 kg ha until February/March when the C/N reached about 29. A previous incubation study found that residue decomposition rate was inversely related to residue application rate of straw (Broadbent & Bartholomew, 1949). However, their application rates (0.25 – 5 g residue in 100 g soil) were considerably higher than the constant application rate used in our study (1.6 g residue in 250 g soil). In any case, the potential effect of application rates should be addressed in future studies because it suggests that cover crop incubation studies with higher than realistic residue application rates may provide misleading results on N mineralization.

Combining information on the relationships between soil temperature and N mineralization from residues with different C/N ratios can provide practical insights to predict the fate of N in cover crops terminated at various times in the fall, winter, or spring. Our study suggests that in fields where cover crops are terminated in the fall (i.e. mid-October) and then are left bare or fallow over the winter, the risk of N mineralization and thus N leaching during winter periods is highest for cover crops with a low C/N ratio (i.e., 10) and lowest for those with a high C/N ratio. We assume that any N mineralized from the cover crops (Figure 6) would be at risk of leaching in bare fallowed fields. Thus, even with cooler winter temperatures (i.e. 10 ℃), our data suggest that on average, more than 50% of the N in the CN10 residue is at risk of leaching in the first 112-d after termination. Whereas approximately 33% of the cover crop's N is at risk of leaching if the C/N is 14, and even less (~13%) is at risk from residue with a C/N of 19 (at 10 ℃). In contrast, our data suggests essentially no risk of N leaching from a fall-terminated cover crop that has a C/N of 30 or more. Fall-terminated rye cover crops in the Salinas Valley typically are grown to heading when their C/N is between 15 and 20 (Brennan, unpublished data). This ratio is

similar to the CN19 treatment in our study, and also of a rye winter cover crop sampled in January (Brennan et al., 2013). In contrast, rye winter cover crops sown in October or November and terminated in February or March typically reach a C/N of 25 to 30 (Brennan et al., 2013).

Our data suggest that the risk of N leaching from winter- or spring-terminated rye cover crops in our region is extremely low particularly where C/N ratios are near 30, even if rain occurs after cover crop termination and soil temperatures are high (i.e., 20℃). However, it is important to highlight that the discussion here of potential N leaching is based on a laboratory incubation which cannot reflect the complex mineralization dynamics that occur while cover crops are decomposing in a realistic field situation. For example, in the central coast of California, peaked beds are typically formed in fields that are left bare or fallow over the winter. These beds allow for shallow tillage (as the weather permits) to control weeds and hasten bed drying in preparation for subsequent vegetable plantings. This tillage would also likely hasten cover crop residue decomposition in fields where fall-terminated cover crops were grown. However, our laboratory study can provide valuable insight into the relationship between C/N ratio and %Nmin across realistic soil temperatures utilizing the controlled variables of application rate and particle size as well as optimal moisture. Future studies should evaluate cover crop N mineralization dynamics in realistic field conditions with cover crops with different C/N ratios.

Similar to many agricultural regions globally, California's Salinas Valley has been identified as one of the most vulnerable to climate change based on the Agricultural Vulnerability Index (Pathak et al., 2018). Large and intense rain events lead to periods of high soil moisture and

warmer winter temperatures (Figure 2), critical to N mineralization dynamics and loss pathways. Furthermore, warmer soil temperatures in winter could hasten cover crop growth rates and lead to faster changes in C/N ratios. A meta-analysis by Thapa et al. (2018) found that cover crop planting date, shoot biomass, and precipitation impacted the ability of nonlegume cover crops to reduce nitrate leaching, with earlier planting dates and greater shoot biomass leading to the largest effects, particularly during drier years and on coarse-textured soils.

The limitations of our incubation include using dry shoot biomass, rather than fresh, and only aboveground residues (i.e. excluding roots). Santiago & Geisseler (2022) found that soil moisture had a stronger overall effect than residue moisture on mineralization. Moreover, Martinez-Feria et al. (2016) found that even though the roots had a slightly higher C/N than shoots, their results predicted minimal net N immobilization.

5. Conclusions

Nitrogen management through cover cropping requires careful timing of nitrogen release from residues with the uptake by cash crops. Our results highlight the interplay between cover crop residue C/N ratios and soil temperature on N mineralization. We found that lower residue C/N ratios (e.g., 10 and 14) result in higher N mineralization rates regardless of the temperature, provided sufficient moisture is available. Conversely, higher C/N ratios (e.g., 19 and 30) showed greater sensitivity to temperature variations on N mineralization. Our data suggest that summer-planted cover crops that are terminated in the fall and left bare fallow over the winter have the potential to reduce nitrate leaching and that this leaching reduction increases with cover crop C/N ratio. Ideally, these cover crops would produce large amounts of biomass with a

relatively high C/N ratio (i.e., > 20) and would be terminated before viable seed production and winter rains began. We conclude that the biomass C/N ratio and soil temperature are critical for determining mineralization rates and that understanding N mineralization dynamics in conjunction with rainfall patterns can help estimate the effectiveness of cover crops in reducing leaching.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

For access to the raw data and code used for analysis in this study, please visit our GitHub repository (https://github.com/annagomes96/CoverCropIncubation_Nitrogen).

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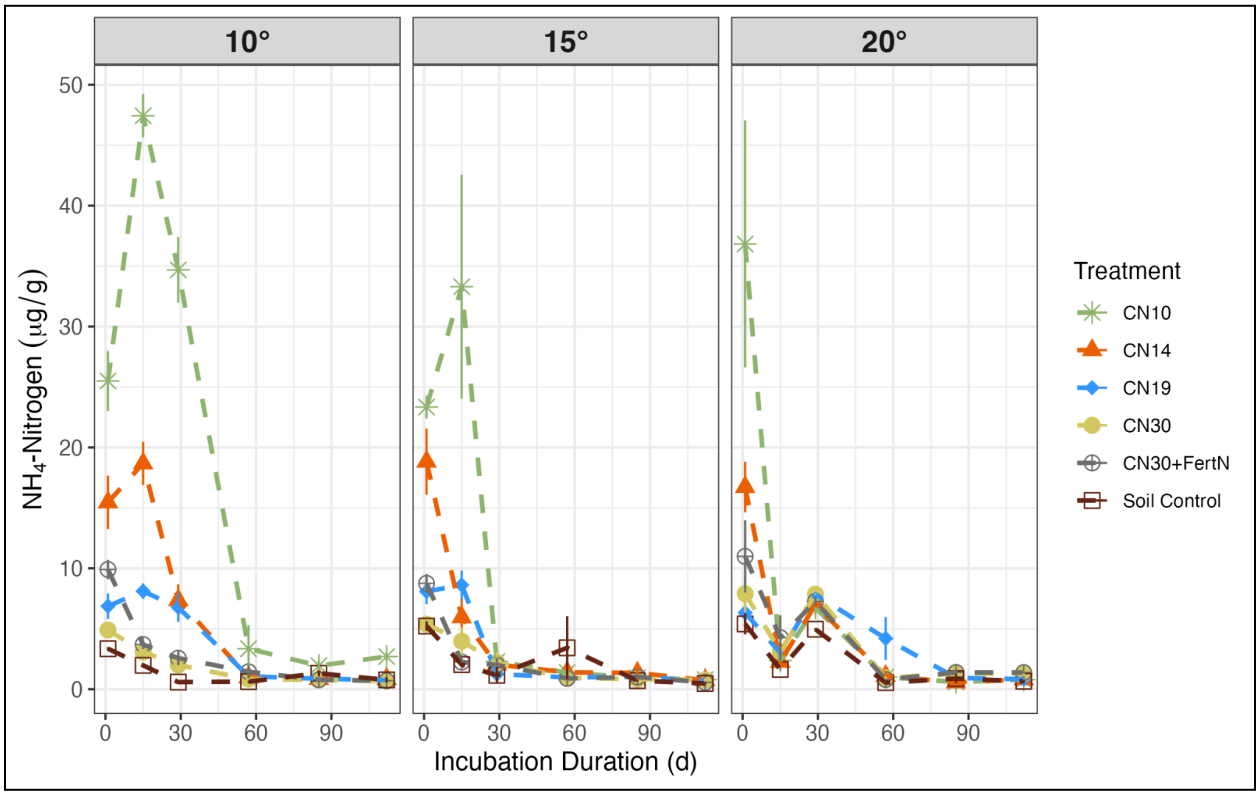
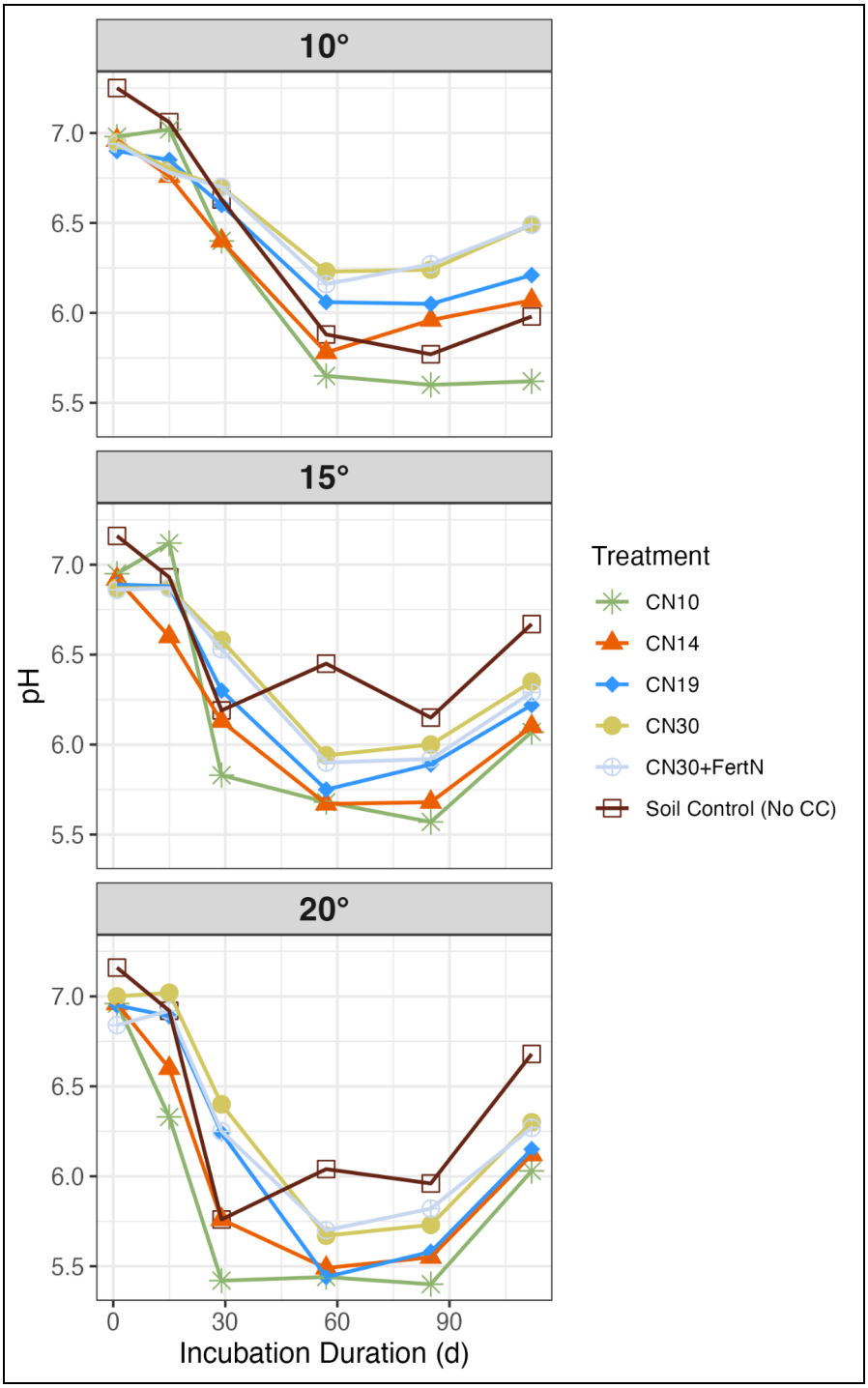
Supplemental Material

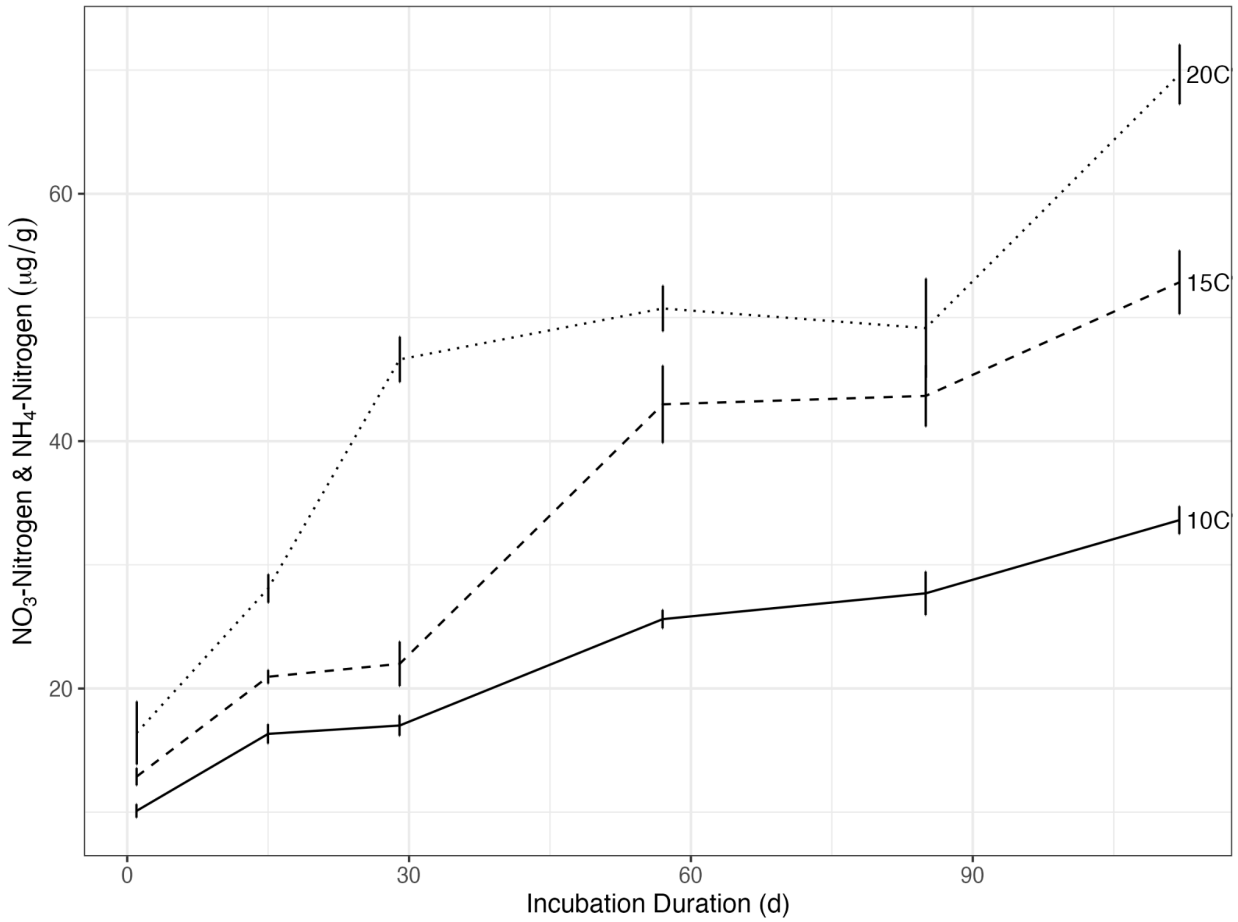
The supplemental material consists of three plots (Figure S1: Ammonium-Nitrogen, Figure S2: Soil pH, and Figure S3: Soil Only N) meant to provide further data for reference.



SI, Figure S1. Average ammonium-N (per gram dry soil) with SE bars (n=4) throughout the incubation for each temperature (10, 15, and 20 ℃). Treatments included CN10, CN14, CN19, and CN30, which correspond to the four different carbon-to-nitrogen ratios of the residue (10, 14, 19, and 30, respectively). The CN30+FertN treatment had FertN (inorganic nitrogen) in the form of doubled initial NH_4NO_3 and the Soil Control (No cover crop) had no cover crop residue.



SI, Figure S2. Temporal changes in soil pH values for varying C/N residue additions for three incubation temperatures (10 ℃, 15 ℃, and 20 ℃).



SI, Figure S3. Soil Control nitrogen mineralization (no cover crop biomass) by soil temperature (bars represent standard error, n=4).