CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
Final Report

Project title: **Nitrogen availability from common cereal cover crops terminated at different growth stages**

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Abstract

The practice of cover cropping plays an important role in soil nitrogen (N) cycling. In addition to being N scavengers which help reduce nitrate leaching, cover crops, when incorporated back into the soil, release nutrients through microorganism-facilitated decomposition of residue-N to plant-available forms. Many factors affect this process, with the carbon to nitrogen (C:N) ratio of the cover crop material being one that greatly influences the availability of N for plant N uptake. This study investigated the effects of varying C:N ratios of cereal cover crop residues, specifically Merced rye (MR) and Pacheco triticale (PT), on N mineralization and immobilization. Soil-incorporated cover crop samples were incubated in the laboratory for 18 weeks at 77 °F and under ideal soil moisture content. A mathematical model was then applied to simulate N availability under California Central Coast soil temperatures. Results demonstrated that, regardless of cover crop species, narrow C:N ratio residues (<20:1) facilitated immediate N mineralization, while wide C:N ratio residues (> 25:1) initially immobilized N before exhibiting remineralization trends. Model simulations highlighted soil temperature's influence on N mineralization and immobilization, with the greatest impact observed in immobilization rates of wide C:N ratio residues. Independent of soil temperature, incorporating residues with C:N ratios above 20:1 would result in temporal net N immobilization, tying up plant-available N and

necessitating careful N fertilization management for subsequent crops. Incorporation of fall-grown cover crops with a wide C:N ratio has the potential to maintain N in the crop residue until the beginning of the planting season in January. The data reveal the importance of considering cereal cover crop residue characteristics and soil temperature in optimizing N availability.

Introduction

Cover crops have multiple effects on soil nitrogen (N) cycling. Cereal winter cover crops can scavenge residual soil nitrate and thus reduce the risk of nitrate leaching with winter rains. This beneficial function of cereal cover crops is the basis of the credit for N scavenging in Ag. Order 4.0 (Brennan, 2022). A sizeable percentage of cover crops are grown in the fall and are typically incorporated by mid-November. Fall-grown cover crops were not allowed a credit in Ag. Order 4.0 because of the lack of data on the amount of N still sequestered in their crop biomass by the beginning of the crop planting season in January/February.

In a previous project supported by the California Leafy Greens Research Program, Eric Brennan and Richard Smith evaluated various methods to easily determine the percent N in cover crop tissue, N uptake of the cover crop and the associated carbon (C) to N ratio of cover crops (Brennan and Smith, 2022). In their project, Brennan and Smith evaluated the two most common cereal cover crops grown in the region, namely Merced rye and Pacheco triticale, in multiple locations and soil types. Cover crop biomass, stem length, and Feekes evaluations were conducted monthly for at least 150 days after planting. Total shoot N uptake by rye and triticale were generally in the range of 100 to 150 lb/acre. Most N was taken up within the first 100 days after seeding, while biomass continued to increase until the final harvest. Consequently, the carbon to nitrogen (C:N) ratio in the biomass increased. While this ratio was below 20 until Feekes growth stage 8, it increased to 40-50 by Feekes growth stage 11. The results showed that for both cereal cover crops, the length of the main stem is a simple, reliable, and robust method for growers to estimate cover crop shoot biomass production (Brennan and Smith, 2023), and that the Feekes growth stage is a good predictor of a cover crop's C:N ratio (Brennan and Smith, 2022).

When cover crop residues are incorporated, soil microorganisms start decomposing them, which results in the release of nutrients contained in the residues. The conversion of residue-N to plant-available ammonium (NH_4^+) is called N mineralization. After being released, ammonium is generally quickly converted to nitrate (NO_3^-) in aerated soils by nitrifying microorganisms. Different factors can affect N mineralization, including temperature, water content, and properties of both the soil and incorporated organic material. The C:N ratio of the material can strongly affect crop N availability (e.g. Lazicki et al., 2020). A narrow C:N ratio of less than 20:1 generally results in net N mineralization, while a wide C:N ratio greater than 20:1 can lead to net N immobilization, the incorporation of mineral N from soil solution into microbial biomass. Nitrogen immobilization temporarily reduces the amount of crop-available soil N.

Therefore, while incorporating cover crop residues terminated at an early growth stage will likely result in net N mineralization, the incorporation of cover crop residues terminated at a late growth stage will likely result in temporary N immobilization. The evaluation of the relationship between residue C:N ratio and N turnover was the objective of this current project.

Objectives

The objective of the study was to determine the amount of N that would be released or tied up when cereal cover crop residues are incorporated into soil. Archived cover crop shoot samples from Brennan and Smith's project were used for the study. The study complemented the results from the field trials by determining the quantity and timing of cover crop N availability.

Procedures

Laboratory incubation

Soil for the incubation was collected from a field in Davis, CA in July 2023 and sieved to 8 mm in the field. The soil was classified as a Yolo Silt Loam with a soil organic matter content of 2.4% and a plant available N content of 17.7 ppm, corresponding to approximately 65 lb/ac in the top foot at the time of sampling. Soil was kept at field moisture and stored in four 5-gallon buckets – one bucket for each replicate – at 40 °F prior to starting the laboratory incubation.

The cover crop incubation occurred from August 2023 to January 2024. The two cover crop treatments utilized – Merced rye (MR; *Secale cereale*) and Pacheco triticale (PT; (× *Triticosecale*) – were planted between October and November 2021 in fields in the Salinas and Pajaro Valleys and the aboveground biomass was harvested every month for up to five months to capture different growth stages (Brennan and Smith, 2022). The samples were oven dried at 60 °C and chopped with a hammer mill using a screen with 19.7 mm holes into approximately 2-3 cm long pieces. Seven samples taken at different growth stages for each of the two crops were utilized for this study, for a total of 14 treatments. A representative subsample was finely ground and analyzed for total C and N. The C:N ratios ranged from 9.1 to 42.9 for PT and from 11.0 to 40.5 for MR (Table 1). Treatments with a C:N ratio ≤20:1, 20:1-25:1, and ≥25:1 will hereafter be referred to as having "narrow", "intermediate", and "wide" C:N ratios, respectively.

At the start of incubation, each soil replicate was analyzed for soil moisture and initial mineral N (ammonium and nitrate) content. Cover crop residues were weighed into labeled 16-oz plastic cups for an equivalent application rate of 100 mg N/kg soil. Soil was weighed into corresponding cups at an equivalent of 300 g oven-dry soil per cup. Cover crop residues and soil were mixed in a plastic bag before being returned to the cup. Soil moisture was adjusted to 60% water holding capacity (WHC) by evenly applying DI water with a side-port needle and syringe. A control with no residue was also included for each replicate and each week of incubation. Cups were covered with punctured plastic wrap to help maintain moisture while still allowing an aerobic environment prior to being incubated at 77 °F (25 °C). Soil moisture was monitored and adjusted weekly with DI water by observing deviation from the documented sample weight prior to incubation.

After 1, 3, 6, 12, and 18 weeks of incubation, samples were removed from the incubator, sieved to 4 mm, and analyzed for ammonium-N and nitrate-N as described below. At each week of incubation and for every replicate, net N mineralization was calculated by subtracting the total mineral N of the unamended control from that of the treated sample.

Soil and cover crop analyses

For ammonium-N and nitrate-N analyses, a 6-g subsample of soil was weighed into a centrifuge tube and shaken with 30 mL of 0.5M K_2SO_4 solution on a reciprocal shaker for 1 hour. Extractant was then filtered through Q5 filter paper and analyzed for mineral N colorimetrically on a spectrophotometer. Soil WHC was measured by draining saturated soil in a filter-paper lined funnel for 1 hour. Soil and cover crop subsamples were finely milled on a paint shaker and analyzed via dry combustion on a Costech Elemental Analyzer to determine total C and N. A subsample from each residue was sent to the UC Davis Analytical Laboratory to determine lignin, cellulose, and hemicellulose contents using reflux methods for the determination of acid detergent fiber and neutral detergent fiber based on Goering and van Soest (1970). Properties of the cover crop samples are reported in Table 1.

Model fitting

The incubation was done at a constant temperature of 77 °F. This temperature is higher than the soil temperature in spring and early summer on the Central Coast. To investigate the availability of N from cereal cover crops under local soil temperatures, a mathematical model with daily time steps was fit to the incubation data. The model fitting was supported by a different grant and is ongoing. The results presented in this report are preliminary.

A detailed description of the model is beyond the scope of this report. Interested readers can find a description in a previously published open access article (Geisseler et al., 2021). Once the model fit was satisfactory, the daily average soil temperatures measured at the Salinas North CIMIS weather station (https://cimis.water.ca.gov/) were used to determine N turnover in the soil after the incorporation of cover crop residues with different C:N ratios. For the simulation, the residues were assumed to be incorporated either on November 15 or on February 15.

Results and Discussion

Laboratory incubation

In all PT treatments with C:N ratios exceeding 20:1, net N immobilization took place initially (Figure 1). Specifically, treatments with an intermediate C:N ratio exhibited N immobilization at Week 1 and it took until Week 6 before the amount of N mineralized exceeded the initial level of N immobilization. Meanwhile, treatments with wide C:N ratios also demonstrated initial N immobilization from Weeks 1 to 3 and it took until Week 12 before the amount of net N mineralized exceeded the level of initial net N immobilization. Despite initial immobilization, all treatments with wide C:N ratios saw positive trends in N mineralization after Week 6 of incubation. In contrast, treatments with narrow C:N ratios exhibited net N mineralization initially at Week 1 and maintained a similar amount of N mineralized when expressed as percent of N in added residues throughout the end of the incubation at Week 18. By Week 18, none of the samples containing intermediate and wide C:N ratio residues had mineralized the amount of N that treatments with narrow C:N ratios had mineralized within the first week of incubation. However, these intermediate and wide C:N ratio residues may have continued a positive trend in net N mineralization, eventually reaching the same N mineralized as that of narrow C:N ratio residues if the incubation persisted for a longer duration. Narrow and wide C:N ratio treatments had significant differences in net N mineralization throughout all weeks of incubation.

In all MR treatments with wide C:N ratios, net N immobilization took place from Weeks 1 to 3 (Figure 1). While these same treatments began to exhibit net N mineralization at Week 6 and saw positive trends in mineralization until the end of incubation at Week 18, it took until Week 12 for the amount of N mineralized from the applied residue to exceed the level of initial net N immobilization; with the exception of the treatment with a C:N ratio of 26:1, where this occurred by Week 9. In all treatments with intermediate and narrow C:N ratios, net N mineralization took place immediately at Week 1. In these treatments, the overall positive mineralization trend continued until the end of incubation, despite C:N ratio 17:1 and 23:1 briefly decreasing in overall N mineralized at Week 3. By Week 18, none of the treatments with a wide C:N ratio had mineralized the amount of N that treatments with intermediate to narrow C:N ratio had mineralized within the first week of incubation, although wide C:N ratio treatments may have continued a positive trend in net N mineralization had the incubation persisted for a longer duration. Like the findings with PT treatments, narrow and wide C:N ratio MR treatments had significant differences in net N mineralization throughout all weeks of incubation.

Regardless of cover crop, there was a strong negative correlation between the cumulative amount of N mineralized from the cover crop at the end of the incubation and the cover crop's C:N ratio, when using logarithmic regression (R^2=0.95, Figure 2). Interestingly, intermediate C:N ratio treatments differed in mineralization behavior between PT and MR, with MR treatments mineralizing immediately and PT treatments immobilizing immediately, the latter not reaching positive net N mineralization until Week 6 of incubation. Both treatments had similar total N content (PT-23: 1.763%; MR-23: 1.817%). When considering other residue properties such as lignin, cellulose, and hemi-cellulose content, the MR treatment with an intermediate C:N ratio (MR-23) had a higher percentage than the intermediate C:N ratio PT treatment (PT-23) across all properties (lignin: 4.6%, cellulose: 32.9%, hemi-cellulose: 27.1% and lignin: 1.7%, cellulose: 19.6%, hemi-cellulose: 20.1%, respectively). Strong negative correlations have been demonstrated between net N mineralization of grass and legume residues and their lignin and hemi-cellulose content (Vahdat et al., 2011). Stronger negative correlations were only seen in PT treatments between final net N mineralization and lignin and cellulose content (r=-0.839 and -0.794, respectively). MR treatments presented weaker negative correlations between final net N mineralization and lignin, cellulose, and hemi-cellulose content (r=-0.499, -0.552, -0.588, respectively). These residue properties may have played a role in the observed differences in N mineralization behavior.

Model simulation

The average soil temperature measured at the Salinas North weather station is 58.7 °F on November 15 and 54.0 °F on February 15. During the months following mid-November, soil temperature decreases, while it increases after mid-February. Overall, the average temperature is lower during the months following a mid-November incorporation than during the months following a mid-February incorporation. Lower soil temperatures reduce microbial activity, including N mineralization and immobilization rates. Nitrogen turnover was simulated for three scenarios: (i) constant soil temperature of 77 °F, (ii) local soil temperature after an incorporation on November 15, and (iii) local soil temperature after an incorporation on February 15.

The comparison of the three scenarios revealed that the effect of temperature on N turnover becomes stronger with increasing C:N ratio of the residues (Figure 3). While the effects of temperature on N mineralization are small when residues with a narrow C:N ratio are

incorporated, temperature effects are especially relevant when wide C:N ratio residues that immobilize N are incorporated. For example, it took 58 days to remineralize the amount of N that was initially immobilized at 77 °F from residues with a C:N ratio of 30, while it took twice as long at local soil temperatures following a mid-February incorporation and 140 days following a mid-November incorporation (Figure 3). Overall, incorporation date had a less pronounced effect on N turnover when compared with a constant temperature of 77 °F.

Combining the simulation data with cover crop biomass-N measured by Brennan and Smith in a field trial in Salinas suggests that more than 30% of the N in a residue with a C:N ratio of 10 is mineralized within the first month after incorporation in mid-February (Table 2). This corresponds to approximately 24-36 lb/ac. Between March 15 and May 15, N continued to be mineralized, but the amount was much smaller than during the first month. Net N mineralization also took place after the incorporation of residues with a C:N ratio of 20. However, the amount was much smaller, reaching 5-7 lb/ac by March 15 and 14-20 lb/ac by May 15. The values were very similar for an incorporation date in mid-November.

Incorporating residue with C:N ratios of 30 and 40 resulted in net N immobilization, meaning that the plant-available N was reduced. The differences between these two residues were small. Net N immobilization reached a maximum of approximately 20% of the residue-N within the first month after incorporation. This corresponded to 19-35 lb/ac. While some of that N became plant-available again during the following two months, the amount of N that was remineralized was less than the amount that was immobilized during the first month. The effect of incorporation date became stronger over time, with the remineralization rate being lower after an incorporation in November, compared to February. In other words, it takes longer until the N that is tied up in the cover crop is released when it is incorporated in November, than in February.

Conclusions

In this study we sought to determine the N mineralization and immobilization rates from two cereal cover crops with a range of C:N ratios. As expected, narrow C:N ratio residues mineralized N initially, whereas wide C:N ratio residues immobilized N prior to gaining net positive mineralization in later weeks of incubation. The results suggest that the threshold C:N ratio above which net N immobilization occurs from cereal cover crop shoot residue is near 20.

Preliminary results from a model simulation revealed that the effect of temperature on N mineralization is small when residues with a narrow C:N ratio are incorporated. In contrast, when residues with a wide C:N ratio are incorporated, net N immobilization can reduce the amount of plant-available N for several months after an incorporation date in November or February. In these situations, the soil residual N level should be monitored to determine whether adjustments to the N fertilization program for the following crop are needed.

Acknowledgements

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References

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Table 1: Properties of the residues used for the incubation. PT and MR refer to Pacheco triticale and Merced rye, respectively. The number refers to the carbon to nitrogen (C:N) ratio.

Sample ID	N (%)	C (%)	C:N Ratio	Lignin - Ash Free (%)	Cellulose (%)	Hemi-cellulose (%)
PT-09	4.5	40.9	9.1	1.7	16.4	19.2
PT-10	3.9	40.2	10.3	1.9	20.1	20.6
PT-23	1.8	40.2	22.8	1.7	19.6	20.1
PT-29	1.4	40.4	28.7	4.1	28.9	23.6
PT-33	1.2	41.0	33.5	4.0	28.3	22.5
PT-35	1.2	41.1	34.8	4.3	30.7	24.9
PT-43	0.9	40.7	42.9	3.5	23.1	18.9
MR-11	3.8	41.5	11.0	<1.0	10.2	14.1
MR-17	2.4	41.2	17.1	4.0	29.3	23.9
MR-23	1.8	41.9	23.1	4.5	32.9	27.1
MR-26	1.6	41.5	26.5	2.6	25.7	23.9
MR-38	1.1	42.7	38.0	5.6	31.0	25.9
MR-40	1.1	42.3	39.8	5.5	29.1	24.6
MR-41	1.0	41.9	40.5	5.8	32.2	25.5

Table 2: Estimated nitrogen mineralization (positive values) or immobilization (negative values) of rye and triticale cover crop at different dates after an incorporation on February 15 (top) or November 15 (bottom). The estimates are based on a model simulation using soil temperatures measured at the Salinas North CIMIS weather station. Nitrogen mineralized/immobilized is expressed in percent of the total N in the cover crop biomass and in lb/ac. Average biomass N and Feekes stage are based on results from a field trial in Salinas by Brennan and Smith.

Incorporation date 02/15

Residue C:N ratio	Approximate Feekes stage	Average N in biomass	Net total N mineralization/immobilization by March 15		April 15		May 15	
		lb/ac	%	lb/ac	%	lb/ac	%	lb/ac
10	5-6	90	33	24 to 36	36	26 to 39	38	28 to 42
20	7-8	100	6	5 to 7	12	9 to 14	17	14 to 20
30	10-11	135	-21	-23 to -35	-16	-18 to -27	-7	-8 to -12
40	11-12	135	-18	-19 to -29	-14	-15 to -23	-6	-6 to -9

Incorporation date: 11/15

Residue C:N ratio	Approximate Feekes stage	Average N in biomass	Net total N mineralization/immobilization by 15-Dec		15-Jan		15-Feb	
		lb/ac	%	lb/ac	%	lb/ac	%	lb/ac
10	5-6	90	33	24 to 36	35	26 to 39	37	27 to 41
20	7-8	100	6	5 to 7	11	8 to 13	15	12 to 18
30	10-11	135	-22	-23 to -35	-17	-19 to -28	-11	-12 to -18
40	11-12	135	-18	-19 to -29	-15	-16 to -24	-9	-10 to -15

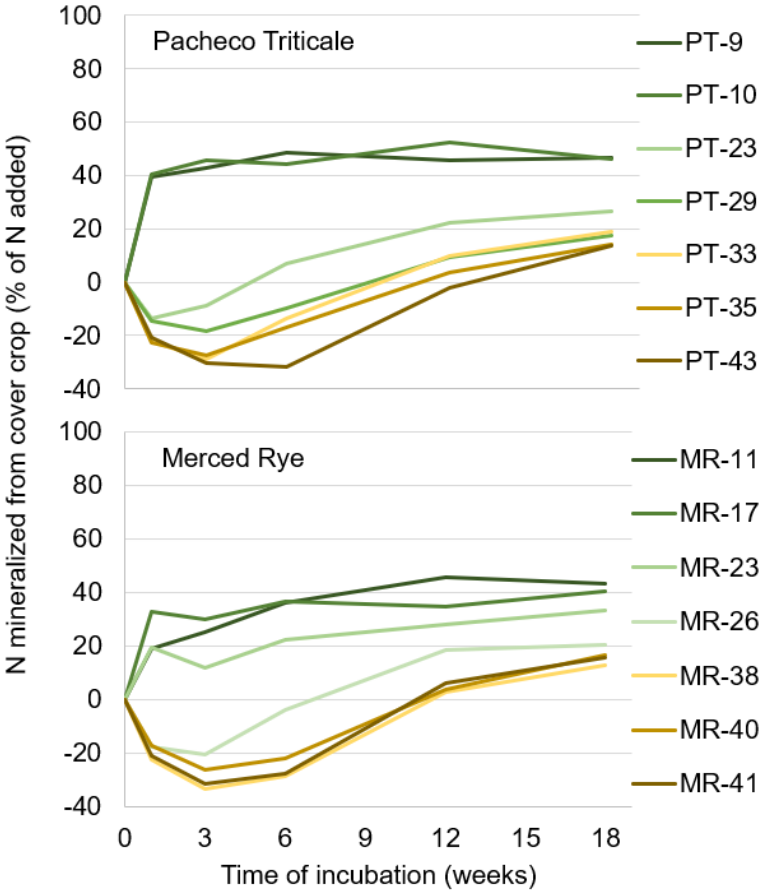

Figure 1: Net nitrogen mineralization and immobilization during an 18-week aerobic incubation of Pacheco triticale (top) and Merced rye (bottom). Samples were incubated at optimal soil moisture content and 77 °F.

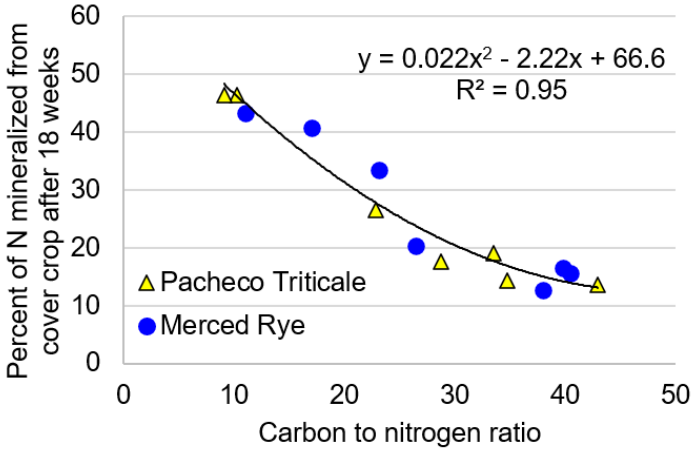

Figure 2: Relationship between the carbon to nitrogen ratio of cereal cover crops and the percentage of the total nitrogen mineralized during an 18-week incubation at 77 °F and optimal soil moisture content.

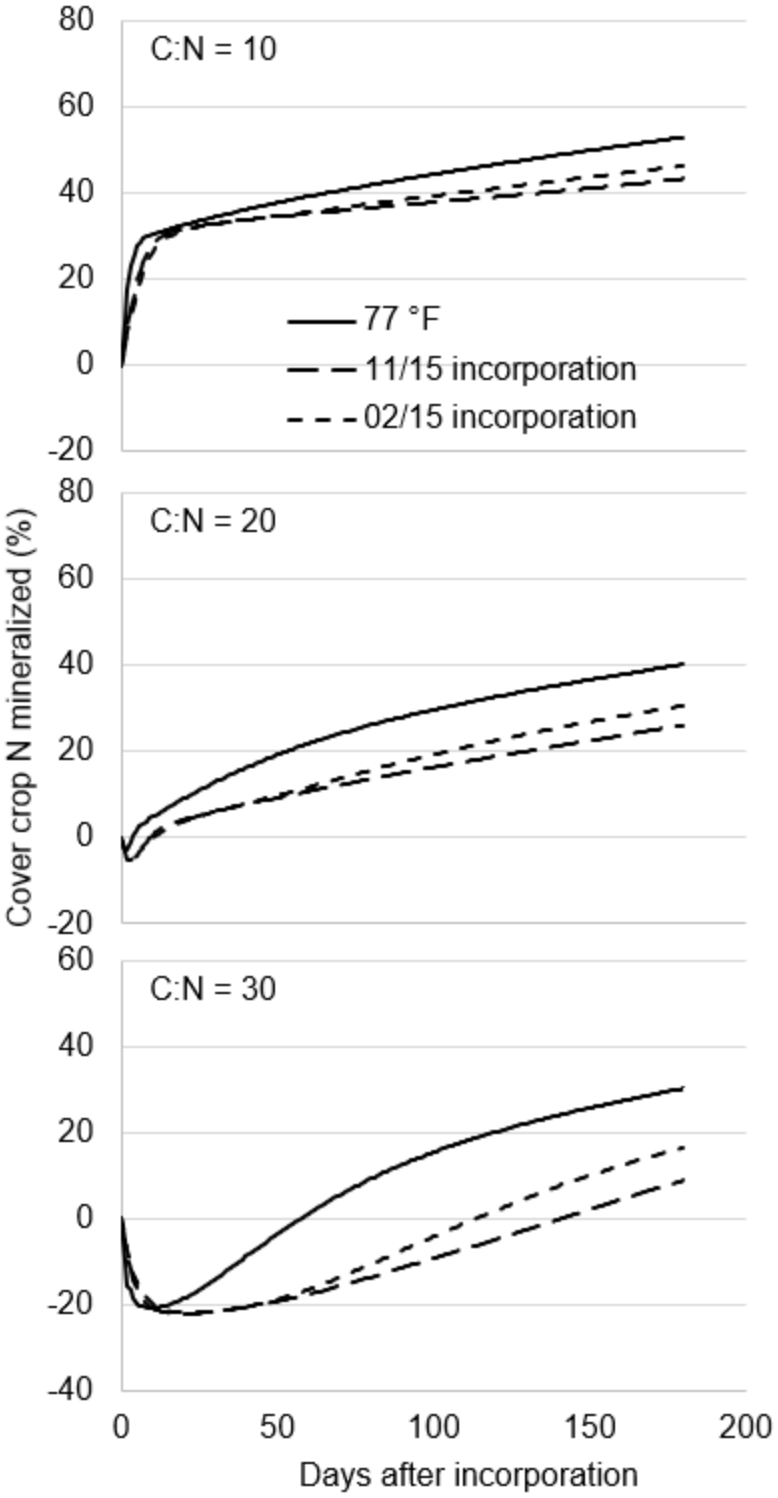

Figure 3: Simulated nitrogen turnover for cereal cover crops with different C:N ratios. Three soil temperature scenarios were simulated: constant temperature of 77 °F, reflecting the conditions during the incubation, and daily average soil temperature measured at the Salinas North CIMIS station with day 0 being either November 15 or February 15. Soil moisture was assumed to be optimal in all scenarios.