


ORIGINAL RESEARCH ARTICLE

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Effects of moisture contents in incorporated residues and soil on net nitrogen mineralization in a laboratory study

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Abstract

In Mediterranean climates, winters are mild, and decomposition of crop residues generally continues during the rainy winter season. The objective of this study was to determine whether letting crop residues dry before incorporation slows down net nitrogen (N) mineralization and reduces the risk of nitrate losses with rain during the winter months. Incubation experiments were performed with processing tomato (*Solanum lycopersicum* L.) and broccoli (*Brassica oleracea* L. var. *italica*) residues. Three residue moisture treatments, ranging from fresh to air-dry residues, and three soil moisture levels were included in the study. Net N mineralization was observed during the first week in the broccoli treatments, whereas tomato residues led to net N immobilization. Increasing moisture of incorporated residues increased net N mineralization or immobilization. An exception was the fresh broccoli residue, which resulted in a lower net N mineralization rate than the drier residues, likely due to anaerobic microsites created by high microbial activity. The moisture content of the residues only had a short-term effect on N turnover. Over the 12 wk of the incubations, the incorporation of both residues resulted in net N mineralization when compared with the unamended control. Nitrogen mineralization increased as soil moisture content increased and approached field capacity. These results indicate that residue moisture at the time of incorporation will not affect N mineralization beyond the initial weeks.

1 | INTRODUCTION

Incorporation of crop residues can reduce pest and disease pressure and recycles plant nutrients (Fu et al., 2021). Residue decomposition rates are mainly determined by the biochemical properties of the residues and environmental factors, such as temperature, moisture, and soil characteristics (Quemada & Cabrera, 1997; Thapa, Tully, Cabrera,

Dann, Schomberg, Timlin, Reberg-Horton, et al., 2021). In Mediterranean climates, most precipitation falls during the winter months, which are also characterized by relatively mild temperatures. Therefore, decomposition of crop residues generally continues during the winter.

The risk of nitrate (NO_3^-) leaching in fallow fields may be increased during the rainy winter season when readily decomposable residues with a high N content are incorporated in fall. One potential approach to reduce the risk of NO_3^- leaching is to allow residues to dry on the soil surface prior to incorporation. The reduction in residue moisture in conjunction

Abbreviations: DI, deionized; EC, electrical conductivity; OD, oven-dry; PWP, permanent wilting point; WHC, water holding capacity.

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with relatively dry soils at the end of the growing season may potentially slow decomposition and thus N mineralization and the accumulation of NO_3^- .

Soil moisture has been well studied in its effects on the rate of decomposition and net N mineralization. In general, increasing soil moisture results in increased decomposition rates and net N mineralization until the soil moisture content approaches anaerobic conditions (Cassman & Munns, 1980; Myers et al., 1982; Quemada & Cabrera, 1997). In contrast, the effects of residue moisture on net N mineralization have received less attention. Most laboratory-based studies investigate residue decomposition and the effects on N mineralization using dried and finely ground residues to improve ease of application and to reduce variability. There is also a growing body of work that uses chopped and rehydrated residues in decomposition studies (Kutlu et al., 2018; Quemada & Cabrera, 1997, 2002; Thapa, Tully, Cabrera, Dann, Schomberg, Timlin, Reberg-Horton, et al., 2021). However, studies using fresh residues are uncommon.

To determine the effects residue and soil moisture content on net N mineralization and their interaction, we conducted incubation studies with processing tomato (*Solanum lycopersicum* L.) and broccoli (*Brassica oleracea* L. var. *italica*) residues. Processing tomatoes and broccoli residues were chosen for this study because they are important vegetables in California and because their residues have a high N content, which is expected to result in net N mineralization and thus an increase in soil NO_3^- after incorporation. We hypothesized that (a) soil moisture will have a stronger effect on net N mineralization than residue moisture and that (b) the effects would be more pronounced with broccoli residues, as they have a narrower carbon (C) to N ratio and higher N mineralization potential.

2 | MATERIAL AND METHODS

Two separate residue incubation experiments were performed. The first was done in fall 2018 with processing tomato residues and the second in fall 2020 with broccoli residues. In late August 2018, just prior to the harvest, tomato residue samples were taken from an experimental field near Davis, CA. The entire tomato vines, with the exception of the large main stems, were collected. The vines were then cut into 1-cm pieces by hand and mixed thoroughly. The residues were divided into three parts of equal weight. One part was placed in plastic bags (one bag per replicate) and frozen immediately, the second part was partially dried at room temperature for 3 d and then frozen, and the third part was air dried until reaching a constant weight. The fresh, partially dried, and air-dried samples had moisture contents of 85, 65, and 7%, respectively. In spring 2020, broccoli residues were collected in a commercial field in Salinas, CA.

Core Ideas

- Moisture of incorporated residues had a short-term effect on N mineralization and immobilization.
- Soil moisture became the dominant factor determining N mineralization over time.
- Decreasing soil and residue moisture led to decreased microbial activity.
- Even in soil at the permanent wilting point, net N turnover took place.

The residues were then treated as described for the tomato vines. The moisture contents for the fresh, partially dry, and air-dry residues were 88, 74, and 7%, respectively.

Subsamples of both residues were ground and analyzed for total C and N by dry combustion on a Costech Elemental Combustion System (ECS 4010; Nelson & Sommers, 1996). Furthermore, a subsample 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). The residue properties are shown in Table 1. Tomato residues had a wider C/N ratio, as well as greater lignin, cellulose, and hemicellulose contents than the broccoli residues.

Soil samples were collected from a field near Davis, CA, for both residue incubations on separate occasions. The soil was classified as a Yolo silt loam (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvents) on 0–2% slopes formed on alluvial flood plains (Custom Soil Resource Report, 2019). Soils were collected from the top 15 cm of the profile prior to starting the incubations, sieved directly in the field to 8 mm, thoroughly mixed, and poured into 18.9-L (5-gallon) buckets. Samples were collected from four plots within the field, which served as replicates for the incubation study. The soil samples were air dried at room temperature (23 °C) in a thin layer and stored in a cold room at 4 °C until incubation initiation. Subsamples were sieved to 2 mm, finely ground, and analyzed for total C and N via dry combustion (Nelson & Sommers, 1996). The total C and N for the soil were 0.91 and 0.10%, respectively. Soil pH and electrical conductivity (EC) in a 2:1 deionized (DI) water/soil suspension were 7.6 and 0.22 dS m^{-1} , respectively.

Each incubation was set up as a two-way factorial, randomized complete block design. The two factors were soil moisture and residue moisture with three levels each. An unamended control was also included for each soil moisture level. The three soil moisture levels were (a) dry soil with a gravimetric moisture content of 0.11 g g^{-1} soil, corresponding to the permanent wilting point (PWP) of a

TABLE 1 Properties of the tomato and broccoli residues used for the incubation study. Values are on a dry matter basis

Residue	Total C	Total N	C/N ratio	Lignin	Cellulose	Hemi-cellulose
	%			%		
Tomato	34.4	1.8	19:1	4.4	24.9	12.1
Broccoli	38.0	3.6	11:1	2.9	21.7	9.4

silt loam (Saxton & Rawls, 2006), equivalent to 24% water holding capacity (WHC); (b) intermediate moisture content (0.17 g g^{-1} soil), which corresponds to 37% WHC; and (c) moist soil at approximately field capacity (0.23 g g^{-1} soil), corresponding to 50% WHC. Water-holding capacity corresponds to the gravimetric water content of a soil sample in a filter-paper-lined funnel that was first saturated with water and then let drain freely for 1 h.

Prior to the incubation, each batch of soil was removed from the cold room and analyzed for moisture content by drying a sample at 105°C for 24 h, as well as for ammonium (NH_4^+) and NO_3^- . Ammonium-N and NO_3^- -N were determined colorimetrically. Briefly, 6-g subsamples of soil were extracted with 30 ml of 0.5 M K_2SO_4 on a reciprocal shaker for 1 h and then strained through Q5 filter paper (Mulaney, 1996). Ammonium-N was analyzed using a method described by Verdouw et al. (1978) with modifications by Forster (1995). Nitrate-N was analyzed using the method by Doane and Horwath (2003). The absorbance of the samples was determined on a Shimadzu UV-1820 spectrophotometer at 650 and 540 nm for NH_4^+ -N and NO_3^- -N, respectively.

The samples for each replicate were prepared in separate batches for the incubation, with each batch corresponding to a replicate. The residues needed for each batch were mixed thoroughly so that the pieces applied to each cup were representative of the whole sample. For each sample, 300 g of oven-dry (OD) soil was weighed in a plastic bag and mixed thoroughly with the correct amount of residues. The tomato residues were applied at an equivalent field application rate of $10 \text{ Mg dry matter ha}^{-1}$, which is the upper limit of vine biomass produced in trials at the sampling site. This resulted in application rates of 10.34 g of fresh residue, 4.36 g of partially dried residue, and 1.65 g of air-dried residue per 300 g of OD soil, which corresponded to an application rate of 92 mg residue N kg^{-1} OD soil. The same amount of N was added with the broccoli residues. This resulted in application rates of 6.49 g of fresh residues, 3.02 g partially dry residues, and 0.84 g air-dry residues per 300 g of OD soil.

After mixing the soil and residues, the samples were filled into pre-labeled 350-ml (16-oz.) plastic cups and packed manually to a bulk density of approximately 1.3 g cm^{-3} . Using a syringe with a 15-cm-long side port needle, DI water was added to reach the target soil moisture content of the different treatments (24, 37, and 50% WHC). To ensure even distribution of the water throughout the cup, the needle was

first pushed into the soil until its tip was approximately 1 cm above the bottom. The water was then injected while pulling up and turning the needle slowly. The total amount of water was applied by repeating this procedure three times in different locations across the surface of the cups. The cups were covered with plastic wrap with air holes and placed into an incubator maintained at 25°C . Soil moisture was monitored weekly by weighing the cups, and DI water was added with a pipette as needed to reach the target moisture content. The tomato residue incubation was carried out with four replicates per treatment, and the broccoli residue incubation was carried out with three replicates. After 1, 3, 6, and 12 wk of incubation, cups were removed, sieved to 4 mm, and analyzed for mineral N (NH_4^+ -N and NO_3^- -N) as described above. Separate cups were used for each time point. Net mineralization of residue N was determined by subtracting the mineral N in the unamended control with the same soil moisture content from the mineral N in the residue-amended sample. Negative values indicated that net immobilization of mineral N took place.

Statistical analyses were performed as a two-way factorial with a completely randomized block design in R-Studio (v. 1.3.1093), using the 'stats,' 'lme4,' and 'emmeans' packages. Analysis of variance was performed to establish significance of the main effects and the interaction, followed by a post-hoc Tukey test to determine treatment effects. Differences were considered significant for $P < .05$. Residuals vs. fitted and normal quantile–quantile plots served to determine if statistical assumptions were met. The datasets for the different dates and residues were analyzed separately. In the absence of a significant interaction between the two factors, the analysis focused on the main effects. With a significant interaction, the effects of a factor were analyzed at each level of the other factor.

3 | RESULTS

3.1 | Effect of moisture contents in tomato residues and soil on N turnover

Net N immobilization took place in all treatments with tomato residues during the first week. Increasing residue moisture resulted in increased net N immobilization (Figure 1). Fresh residues immobilized significantly more N than partially dry residues. The amount of mineral N in the fresh residue treatment remained significantly lower through Weeks 3 and 6

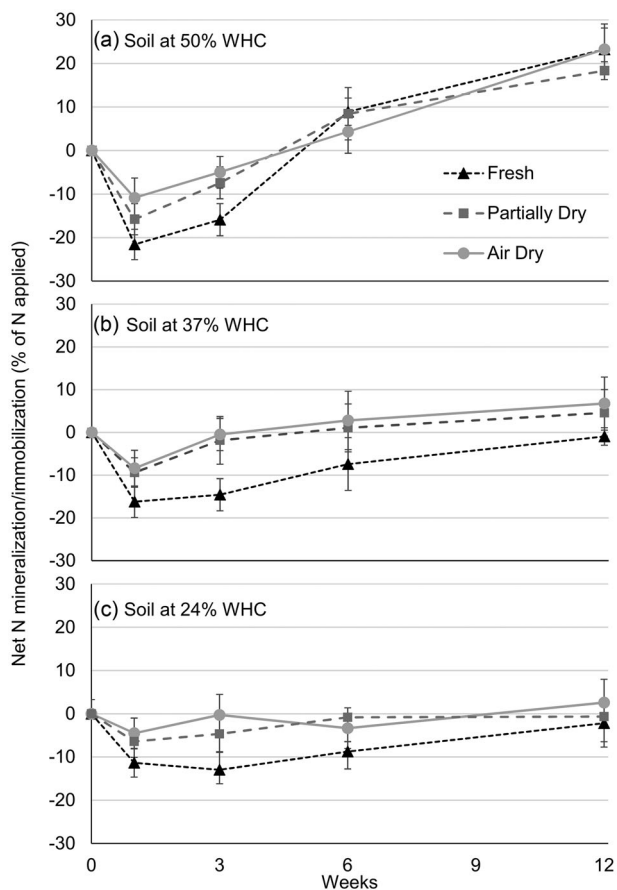


FIGURE 1 Net nitrogen (N) mineralization and immobilization in soil amended with tomato residues and incubated at 25 °C for 12 wk at three different soil moisture contents. A water holding capacity (WHC) of 50% reflects optimal conditions for N mineralization, whereas 24% WHC corresponds to the permanent wilting point. The three residue moisture contents were 7% (air dry), 65% (partially dry), and 85% (fresh). Error bars indicate standard error ($n = 4$). Units are in percent of applied residue N mineralized. Negative values indicate net N immobilization of soil N

(Table 2). However, the effect of residue moisture became less pronounced over time and was no longer significant at 12 wk.

Increasing soil moisture also led to increased net N immobilization during the first week (Figure 1). After the first week, however, net N mineralization took place in the soils at 50 and 37% WHC, a trend that continued throughout the 12-wk incubation. In the dry soil at 24% WHC, residue N tended to be mineralized as well after the first week; however, the rates were low and less consistent. Except for Week 3, soil moisture had a significant effect on mineral N concentrations throughout the incubation (Table 2).

A significant interaction was detected between residue moisture and soil moisture after 6 wk. The fresh residues mineralized significantly more N at 50% WHC than the treatments with a lower soil moisture content, whereas this increase was much less pronounced with the other two residue treatments.

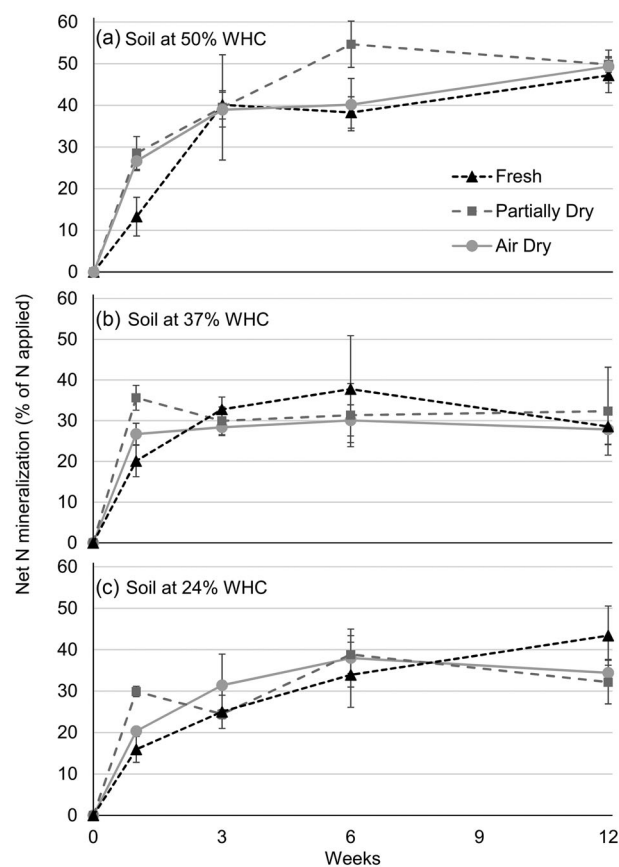


FIGURE 2 Net nitrogen (N) mineralization in soil amended with broccoli residues incubated at 25 °C for 12 wk at three different soil moisture contents. A water holding capacity (WHC) of 50% reflects optimal conditions for N mineralization, whereas 24% WHC corresponds to the permanent wilting point. The three residue moisture contents were 7% (air dry), 74% (partially dry), and 88% (fresh). Error bars indicate standard error ($n = 3$). Units are in percent of applied residue N mineralized

After 12 wk, the amount of N mineralized increased with increasing soil moisture. Averaged across the three residue moisture treatments, the addition of tomato residues increased soil mineral N by 20 mg N kg⁻¹ soil in the soil at 50% WHC over the 12-wk incubation, which corresponds to 21.6% of the applied residue N. In the soil held at 24% WHC, which corresponded to the PWP, the soil mineral N levels of the amended treatments only reached the level of the control by the end of the incubation (Figure 1). Therefore, it took until Week 12 to mineralize the same amount of N that was immobilized initially.

3.2 | Effect of moisture contents in broccoli residues and soil on N turnover

All broccoli residue treatments began mineralizing N immediately (Figure 2). During the first week, only residue

TABLE 2 Statistical analysis of net nitrogen (N) mineralization and immobilization in soil amended with tomato residues

Treatment	Net N mineralization/immobilization			
	Week 1	Week 3	Week 6	Week 12
	% of residue N			
50% WHC	−16.1a	−9.4a	7.2b	21.6b
37% WHC	−11.3b	−5.7a	−1.2a	3.5a
24% WHC	−7.4c	−6.0a	−4.3a	−0.1a
Fresh residue	−16.4a	−14.5a	−2.4a	6.7a
Partially dry residue	−10.5b	−4.7b	2.9b	7.4a
Air-dry residue	−7.9b	−1.9b	1.3b	10.9a
	P values from ANOVA			
Soil moisture (S)	<.001	.1952	<.001	<.001
Residue moisture (R)	<.001	<.001	<.01	.167
S × R interaction	.553	.912	<.01	.448

Note. Treatments included different residue moisture contents and soil kept at different moisture contents, expressed as percentage water holding capacity (WHC). The dataset was analyzed as a two-factor ANOVA with four replicated blocks. Each week was analyzed separately. Different letters indicate significant differences.

TABLE 3 Statistical analysis of net nitrogen (N) mineralization in soil amended with broccoli residues

Treatment	Net N mineralization			
	Week 1	Week 3	Week 6	Week 12
	% of residue N			
50% WHC	22.8a	39.5a	44.4a	50.2b
37% WHC	27.5a	30.4a	33.1a	30.8a
24% WHC	22.1a	27.0a	36.9a	38.1ab
Fresh residue	16.4a	32.6a	36.7a	41.3a
Partially dry residue	31.4b	31.3a	41.6a	39.2a
Air-dry residue	24.6b	32.9a	36.1a	38.7a
	P values from ANOVA			
Soil moisture (S)	.679	.059	.237	<.01
Residue moisture (R)	<.001	.943	.650	.860
S × R interaction	.752	.911	.665	.726

Note. Treatments included different residue moisture contents and soil kept at different moisture contents, expressed as percentage water holding capacity (WHC). The dataset was analyzed as a two-factor ANOVA with three replicated blocks. Each week was analyzed separately. Different letters indicate significant differences.

moisture had a significant effect on net N mineralization (Table 3). Significantly less N was mineralized in the treatments with fresh residues than with partially dry and air-dry residues, whereas most N was mineralized in the treatment with partially dry residue. After the first week, however, the effect of residue moisture was no longer significant (Table 3).

During the first 6 wk, soil moisture had no significant effect on net N mineralization. After 12 wk, however, significantly more N had been mineralized at 50% WHC than at 37% WHC, with the 24% WHC treatment being in between and not significantly different from the other two treatments (Table 3). No significant interactions between residue moisture and soil moisture were found.

Averaged across the three residue moisture treatments, the addition of broccoli residues increased soil mineral N by 44 mg N kg^{−1} soil in the soil at 50% WHC, corresponding to 50.2% of the applied residue N being mineralized.

4 | DISCUSSION

Residue and soil moisture had both an effect on N turnover. Initially, residue moisture was the dominant factor. Over time, the effect of residue moisture decreased, and soil moisture became more important. By the end of the 12-wk incubation, soil moisture was the dominant factor affecting net N mineralization. Therefore, our first hypothesis that soil moisture will

have a stronger effect on net N mineralization than residue moisture is not supported by the results from the first weeks of the incubation but is supported by the longer term data.

Tomato and broccoli residues resulted in distinctively different N turnover patterns. Whereas broccoli residues led to net N mineralization during the first week of incubation, tomato residues initially resulted in net N immobilization. With a C/N ratio of 19:1, tomato residues were not expected to cause net N immobilization. Organic materials with C/N ratio below 20:1 generally mineralize N immediately (Frankenberger & Abdelmagid, 1985). Our data suggest that this threshold may be too high. After the first week, however, net N mineralization took place in most treatments. By the end of the incubation, more N had mineralized in soil amended with broccoli residues, which had a narrow C/N ratio of 11, than in soil with tomato residues. In the soil with an optimal moisture content of 50% WHC, the amount of N mineralized corresponded to 50.2% of broccoli N and 21.6% of tomato N. The effects of soil moisture were numerically more pronounced with tomato residues compared to broccoli residues. The same was true for the effects of residue moisture, with the exception of Week 1. Therefore, we reject our hypothesis that the effects of moisture treatment are more pronounced with broccoli residues.

4.1 | Effect of soil moisture

The observed increase in net N immobilization in soil amended with tomato residues with increasing soil moisture is in line with other studies. Thapa, Tully, Cabrera, Dann, Schomberg, Timlin, Reberg-Horton, et al. (2021) found that at the highest water potential treatment (-0.03 MPa), early N immobilization was greatest compared with all other water potential treatments (ranging from -10.0 to -0.03 MPa) for rye residues (*Secale cereale* L.), which had a C/N ratio of 87.7. The N immobilized in rye-amended soil was later released and by the end of their trial after 24 wk, net N mineralization corresponded to 11% of rye residue N. We observed the same trend in our study with tomato residues during the 12-wk incubation.

Even though the difference was not significant, a surprising result was that less N was mineralized during Week 1 in the soil with broccoli residues at 50% WHC compared with the soil at 37% WHC. This effect was especially pronounced for the fresh residue, which had the highest moisture content. Increased residue and soil moisture was expected to result in increased microbial activity and higher residue N mineralization. A potential explanation for the reduced apparent net N mineralization is that high microbial activity depleted oxygen, resulting in anaerobic conditions in microsites, which in turn led to denitrification losses. This effect was likely temporary, as it was no longer observed after the first week. Quemada

and Cabrera (1997) also observed a decrease in N mineralization with crimson clover residues (*Trifolium incarnatum* L.) when the soil moisture content approached -0.03 MPa. This decrease was only observed at the highest temperature treatment of 35°C . The authors hypothesized that the effect was due to denitrification in anaerobic microsites under conditions that favored microbial activity. In our study, increasing soil moisture led to increased microbial activity with both residues. Initially, this led to more net N immobilization in the soil with tomato residues, whereas more N was mineralized in soil with broccoli residues and possibly denitrified.

By the final week of the incubation, there was a significant difference in the amount of residue N mineralized across soil moisture treatments. The effect of soil moisture on N mineralization is in line with previous research (Cassman & Munns, 1980; Klemmedtsson et al., 1988; Myers et al., 1982; Quemada & Cabrera, 1997; Stanford & Epstein, 1974).

With both residues, N mineralization was significantly reduced but did not stop in the soil maintained at the PWP. These results imply that even at very low moisture contents, soil microorganisms continue to mineralize N, though slower than at higher soil moisture contents. This is supported by a study by Dirks et al. (2010), who found that decomposer microorganisms remained active even at extremely low relative humidity contents. In their study, water vapor in the form of fluctuating atmospheric relative humidity was the only source of moisture for their litterbag decomposition study in a Mediterranean shrubland. They observed that during the dry season, local litter lost between 4 and 18% of its mass over a rainless 4-mo period. Furthermore, Thapa, Tully, Cabrera, Dann, Schomberg, Timlin, Reberg-Horton, et al. (2021) found that even as water potential fell below -10.0 MPa, microbial decomposition continued to occur.

4.2 | Effect of residue moisture

Our results suggest that residue moisture may play a role in short-term N turnover, but that soil moisture ultimately determines long-term N availability. Our study was conducted at 25°C . During the fall and winter, soil temperatures in the field are generally cooler, slowing down microbial activity. Therefore, the duration of the short-term effects would be different in the field and depend on soil temperature. A potential explanation for the diminishing effect of residue moisture on N turnover over time is that the air-dry and partially dry residues were absorbing moisture from the surrounding soil and eventually reached similar moisture contents as the fresh residues. This is due to what Kutlu et al. (2018) referred to as the “sponge effect,” wherein the strong capillary forces within the residue absorb moisture from the surrounding soil. In their study, air-dried residues absorbed enough moisture from the soil (which was at 30% water-filled pore-space) until they reached full

saturation. Water absorption characteristics and thresholds varied between corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] residues. Kutlu et al. (2018) stated that in dry soil, residues that have absorbed moisture from the surrounding soil serve as microsites for decomposition and nutrient cycling, which was likely the case in our study as well. A similar trend was observed in a study by Quemada and Cabrera (2002), where oat straw (*Avena sativa* L.) exhibited higher water retention characteristics than wheat straw (*Triticum aestivum* L.). The authors of both studies hypothesized that differences in residue quality, such as lignin and carbohydrate contents, determine the capacity of residues to absorb water by influencing the matric potential of the residues. This conclusion was supported by Dirks et al. (2010), who found that higher lignin/N ratios reduced a residue's ability to absorb water vapor. Thapa, Tully, Cabrera, Dann, Schomberg, Timlin, Gaskin, et al. (2021) also reported a negative correlation between water retention by cover crop residues and their lignin content. They also found that water retention decreased when the lignin content increased over time due to decomposition. In our study, the tomato residues had a lignin/N ratio of 2.4, which was higher than the ratio of 0.8 in the broccoli residues. Therefore, tomato residues may have absorbed water at a lower rate than broccoli residues. The difference in water absorption capacity could explain why the tomato residue moisture had a prolonged significant effect on N mineralization. In contrast, residue moisture was only significant in the broccoli treatments for the first week, as those residues may have absorbed moisture more quickly from the surrounding soil, leading to rapid decomposition in all residue moisture treatments.

5 | CONCLUSIONS

This study was carried out to determine whether letting crop residues with a high N content dry on the soil surface before incorporation is a management practice that slows down N mineralization and accumulation of NO_3^- in the soil and thus reduces the risk of NO_3^- leaching with winter rains. Our results showed that residue moisture only has a short-term effect on N mineralization. The duration of these short-term effects will depend on soil temperature, as microbial activity decreases in cooler soils. Therefore, letting residues dry on the soil surface before incorporation does not seem to be an effective practice to reduce the amount of residual soil NO_3^- present during the winter months.

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AUTHOR CONTRIBUTIONS

Suzette Santiago: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, and Writing – original draft, Writing – review & editing. Daniel Geisseler: Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Visualization; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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