

Short-term Nitrogen Mineralization Potential in Soils of Biofuel Cropping Systems

Yili Meng,¹ Teresita Chua-Ona,² and Michael L. Thompson³

Abstract: Plant-available N in soil originates both from fertilizer N amendments and from mineralization of soil organic matter and crop residues. This study compared the N mineralization potential of soil in five biofuel cropping systems in Iowa (continuous corn [*Zea mays*], continuous corn with a winter rye [*Secale cereale*] cover crop, perennial prairie, N-fertilized perennial prairie, and corn-soybean [*Glycine max*] rotation). Net N mineralization potential was measured by a 4-week incubation and leaching method. To evaluate the impact of plant residues on mineralization, net N mineralization potential was measured with and without macroscopic in situ residues. To explore the effect of freezing and thawing on net N mineralization, some soil samples were frozen and thawed before incubation. Cropping systems had a significant effect ($P < 0.05$) on net N mineralization rate, in the following order: N-fertilized perennial prairie \approx continuous corn with winter rye \geq corn-soybean rotation \geq continuous corn \geq perennial prairie. The freezing and thawing treatment increased the 30-day net N mineralization rate approximately two-fold. The presence of plant residues alone did not affect the N mineralization rate, but N mineralization was significantly and positively correlated with the amount of plant residue N in the soil.

Key Words: freezing, N mineralization potential, plant residues

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Nitrogen fertilizer is usually a major cost in production of non-leguminous crops, but not all N that is applied to the soil is taken up by plants. Uptake of N fertilizer by agricultural crops is seldom more than 50% of the amount applied (Raun and Schepers, 2008; Singh and Ryan, 2015). Nitrogen applied as ammonium (NH_4^+) cations may be adsorbed to clay and organic matter in the soil, but with increasing soil pH and temperature, ammonia volatilization is likely, especially in soils of low cation exchange capacity. Soil NH_4^+ can also be transformed to NO_3^- through nitrification, but nitrate can be lost because of leaching (Almasri and Kaluarachchi, 2004; Puckett et al., 2011; Williams et al., 2015). Excess applied fertilizer N can also be lost to the atmosphere if nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by denitrifiers, and the nitrite is further reduced to gaseous forms such as nitric oxide, nitrous oxide, or N_2 . In contrast, N that is incorporated into soil organic matter—either as crop residues or humified organic matter—may be slowly mineralized to a plant-available form, reducing N loss by leaching. In this study, we sought to

investigate N mineralization at the cropping systems level, specifically considering annual and perennial cropping systems that are designed to produce biofuel crops.

Cropping systems can affect N mineralization by their impact on soil chemical and biological properties. For example, N mineralization potential is related to the amount of crop residues returned to the soil. Christenson and Butt (1997) measured N mineralization potentials under four cropping systems (crop rotations that included corn, sugar beet, navy bean, and oat) in a silty clay soil in Michigan. They reported that variations among the cropping systems in the amount of residues returned to the soil led directly to variations in N mineralization potential. Using an exponential model, they also reported that the N mineralization potential increased by 1 mg kg^{-1} for each 0.33 Mg ha^{-1} of crop residue in the soil. Laird and Chang (2013) investigated N mineralization potentials of soils in fields cropped to corn and soybean in Minnesota. They found that removal of all crop residues led to reductions in soil organic C, total N, N mineralization potential, and cation exchange capacity in the upper 15 cm of the soil.

Cropping systems that include perennial species are expected to add C and N to the soil organic pool because perennial species build considerable belowground biomass (Davis et al., 2010; Davis et al., 2013). Additional belowground biomass increases the potential for N mineralization by enriching the biologically active N pools in soils (Deng and Tabatabai, 2000; Carpenter-Boggs et al., 2000). Moreover, winter cover crops such as cereal rye may be used to scavenge residual soil inorganic N after the growing season, so that N may be released at an early growth stage of the subsequent crop. Therefore, cropping systems that include winter cover crops may have higher N mineralization rates than those systems without winter cover crops (Ranells and Waggoner, 1996; Hu et al., 1997; Dabney et al., 2010).

Besides the amount of crop residues and belowground biomass, the chemical composition of these residues may also exert an important influence on N mineralization in soils. The N concentration and the C/N ratio of crop residues regulate N release and have been used to predict the net effects of crop residues on soil mineral N dynamics (Vigil and Kissel, 1991; Trinsoutrot et al., 2000; Seneviratne, 2000). Net N immobilization often prevails in plant residues with N concentration up to approximately 1.7% to 1.8% N ($17\text{--}18 \text{ g kg}^{-1}$) (Constantinides and Fownes, 1994). It has been reported that net N mineralization usually occurs when the C/N ratios of residues are less than 25 (e.g., Trinsoutrot et al., 2000; Kumar and Goh, 2003), although the C/N ratio of cover crop residues may not regulate N mineralization over the first few weeks of decomposition (O'Connell et al., 2015).

The experiments described here are part of the long-term Comparison of Biofuel Cropping Systems (COBS) project at Iowa State University, which is designed to compare lignocellulosic biomass production and environmental impacts in several cropping systems: continuous corn grown for both grain and stover removal (with and without a rye cover crop), reconstructed multispecies prairie grown for whole plant removal (with and without N fertilizer), and a conventional corn-soy grain system, which is used as a baseline cropping system. Studies associated

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with the COBS project have documented the impact of annual and perennial cropping systems on aboveground and belowground biomass production (Jarchow et al., 2015), temporal dynamics of N uptake by biofuel feedstock crops (Dietzel et al., 2015), field-scale CO₂ efflux (Daigh et al., 2014), root decomposition rates in laboratory conditions (Rivas et al., 2013), export of N and phosphorus in tile drainage water (Daigh et al., 2015), the structure and activity of soil microbial communities (Bach and Hofmockel, 2015), and variation in ethanol yields among the annual and perennial cropping systems (Nichols et al., 2014). In the present study, we sought to assess the impact of cropping systems on N mineralization potential.

In this context, we hypothesized that crop residues would vary in quantity and quality among the cropping systems and that these variations could regulate N mineralization potential. The decomposition of crop residues has been documented by numerous studies in which a measured amount of fresh or dried crop residues was mixed with soil and then incubated under laboratory conditions (Constantinides and Fownes, 1994; Kumar and Goh, 2003; Hadas et al., 2004; Senwo and Tabatabai, 2005; Nakhone and Tabatabai, 2008). However, amending soil samples with fresh crop residues does not necessarily reproduce the mineralization potential during key periods in the growing season when N is most needed by plants. In this experiment, we looked at the effect of partly decomposed crop residues on N mineralization. Mineralization potential was measured in soil samples as collected (with partly decomposed residues) and in soil samples from which visible crop residues had been removed. In addition, the chemical characteristics of the partly decomposed residues were measured. Thus, we hoped to more realistically characterize potential rates of N mineralization in the soil in the early part of the crop-growing season.

Soils in midlatitude climates such as that of Iowa may be subjected to freeze-thaw cycles in late winter and early spring. Freezing of soils could decrease living microbial biomass (Larsen et al., 2002), influencing the turnover of soil organic matter and thus affecting N mineralization (Matzner and Borken, 2008). It has been reported that N mineralization rates increase shortly after freezing and thawing cycles (Deluca et al., 1992; Herrmann and Witter, 2002). To improve the assessment of N mineralization potential in these cropping systems, we also investigated the effect of freezing in this study.

MATERIALS AND METHODS

Site Description and Cropping System Treatments

The COBS site is located in Boone County, Iowa (41°55' N, 93°45' W). The 9-ha experimental site was in a corn-soybean rotation before 2007. The predominant soils at the site are Webster silty clay loam (a fine-loamy, mixed, superactive, mesic Typic Endoaquoll) and Nicollet loam (a fine-loamy, mixed, superactive, mesic Aquic Hapludoll) distributed on a generally flat slope of mostly less than 1%, with 2% to 3% in some small areas (Andrews and Dideriksen, 1981). The site is composed of 24 plots (61 m × 27 m each), and the experimental design includes five cropping system treatments in four replications, arranged as a randomized complete block design. The five cropping systems, with treatment abbreviations shown in parentheses, are (1) continuous corn grown for both grain and stover (CC); (2) continuous corn grown for grain and stover with rye as a winter cover crop (CCW); (3) corn-soybean annual rotation (CS); (4) reconstructed, multispecies prairie grown for aboveground biomass (P); and (5) N-fertilized reconstructed, multispecies prairie grown for aboveground biomass (PF). In 2012 (the year before our

sampling), each CC plot received a total (preplant plus side dress) N application of 199 kg ha⁻¹, each P plot received a total N application of 84 kg ha⁻¹, whereas each CCW plot received a total N application of 221 kg N ha⁻¹ as liquid UAN (32% N, i.e., 320 g N kg⁻¹). In 2012, the crop grown in the sampled CS plots was soybean, and N fertilizer was not applied that year, so the residues collected with the soil samples included soybean residues, as well as any residual corn residues from previous years. The preplant N fertilizer application rate was determined by the harvest removal of N. The side-dress N fertilizer application rate was determined by the late spring soil nitrate test (Blackmer et al., 1989).

Soil Collection, Sample Preparation, and Characterization

Soil samples were collected in June 2013, after the annual crops were planted, but before side-dressed N was applied in the corn treatments. Crop plants were approximately 15 cm tall when the soil samples were collected. Twenty cores per plot were collected from the 0- to 15-cm depth by using a 1.7-cm-inner-diameter steel step probe. The cores were combined in an 18-L plastic bucket and mixed by hand before subsamples were withdrawn and stored in a 4-L resealable plastic bag. The bags were kept cool until ready for transport to the laboratory.

Upon arrival in the laboratory, the field-moist soil samples were passed through a 4-mm sieve, and materials larger than 4 mm were removed. Each sample was split into two bags; one bag was designated as a treatment with residue (WR), that is, the soil material as sampled, and the other bag was designated as a treatment without residue (WOR). For the WOR sample, all visible fresh and partly decomposed roots and plant materials were handpicked from the soil. A subsample of each soil sample was air dried and ground to pass a 2-mm sieve for chemical characterization. Moisture contents were determined in both WR and WOR soil samples. Additional details about sample preparation are presented in the supplemental data (Supplemental Digital Content 1, <http://links.lww.com/SS/A44>).

By using the procedure of Kettler et al. (2001), the particle size distribution of soil samples was determined without pretreatment to remove organic matter. Exchangeable N (NH₄⁺ and NO₃⁻) was determined after equilibration of a field-moist soil sample, equivalent to 10 g of oven-dried soil, with 100.0 mL of 2 M KCl for 1 h. The solution was analyzed for inorganic nitrate + nitrite (cadmium reduction method) and ammonium (salicylate-hypochlorite method) using an automated continuous flow analyzer (Mulvaney, 1996). Total C and N were measured in soil samples with plant residues (WR), in soil samples without plant residues (WOR), and in the residues themselves by dry combustion of samples that had been ground to pass a 0.1-mm sieve and using an automated vario MICRO cube CN analyzer (Elementar, Hanau, Germany). All measurements were conducted in duplicate. Because samples from all the plots had similar pH values, ranging from 6.5 to 6.9, inorganic C was assumed to be negligible, and total C was assumed to represent organic C. Soil organic N was calculated by subtracting the sum of ammonium-N and nitrate-N from the total soil N. The calculation was performed separately for the WR soil samples and the WOR soil samples.

N Mineralization Study

Nitrogen mineralization was investigated using a method similar to that of the Stanford and Smith (1972) procedure, as modified by Chae and Tabatabai (1986). Soil samples with and without residues (WR and WOR) were stored at 4°C before setting up the first batch of the mineralization study. The first batch

of the mineralization study used 15 soil samples from three field replicates (five cropping systems each) in the WOR treatment and five samples (each cropping system) from one field replicate in the WR treatment. After the first run of the mineralization-incubation study, the WR and WOR soil samples were frozen at -4°C for approximately 2 weeks before the second run of the mineralization study. The second batch of the mineralization study used 15 soil samples from three field replicates (five cropping systems each) in the WR treatment and five samples (each cropping system) from one field replicate in the WOR treatment. In the second batch, the frozen samples were thawed at room temperature for 1 day before being used in the incubation experiments, which are detailed below. In both batches, samples from all cropping system and residue treatments were run in duplicate.

Soil material was incubated in an open-top glass tube (3.5-cm diameter and 15 cm long), the bottom of which had been fitted with an open, narrow, glass stem that was 0.6 cm in diameter and 5 cm long. A field-moist soil sample (ground to pass a 4-mm sieve) equivalent to 30 g (oven-dry basis) was mixed with 30 g of acid-washed, 0.84-mm sand and 15 g of acid-washed glass beads (approximately 2–3 mm in diameter). The soil-sand-glass bead mixture was packed into the glass tube on top of a 1-cm-thick glass wool bottom support. The soil columns had an average height of 10 cm. A glass wool fiber plug (approximately 1 cm in thickness) was placed over the soil column to inhibit soil dispersion when the leaching solution was added. A glass wool plug (approximately 2 cm long) was also inserted into the tip of the 5-cm-long glass stem to prevent any loss of soil materials from the leaching tip. Before incubation at 35°C , the tubes were covered with paraffin film with a single 5-mm-diameter borehole.

At the end of each equilibration period, the soil columns were leached with 100 mL of 5 mM CaCl_2 in five increments. When the last CaCl_2 increment was added, the glass tube was placed on a 250-mL suction flask, and a suction of 80 kPa was applied to remove the remaining CaCl_2 . The CaCl_2 leaching was followed by adding 20 mL of a nutrient solution that contained no N. This nutrient solution was made up of 5 mM MgSO_4 and 5 mM KH_2PO_4 , adjusted to pH 7.2 with KOH or H_2SO_4 (Carpenter-Boggs et al., 2000), to provide the nutrients other than N for soil microbial growth. Then a suction of 80 kPa was applied again to remove excess nutrient solution and to equalize the soil water potential of all samples before incubation. The gravitational water content after 80 kPa suction was approximately 250 g kg^{-1} . The moisture contents of the columns were adjusted by weighing the columns every 2 days and by adding deionized water to maintain a constant weight. The leaching procedure was repeated approximately every 5 days for 4 weeks. The incubation-leaching procedure was extended up to 90 days for selected samples. The incubation was terminated by leaching the soil column with 100 mL of 2 M KCl instead of 5 mM CaCl_2 .

The leachate collected was made up to 100 mL with deionized water before it was filtered through a $0.45\text{-}\mu\text{m}$ membrane filter paper. The filtered leachates were frozen for later analysis for NO_3^- and NH_4^+ , using a microplate method based on Hood-Nowotny et al. (2010) and described in detail in the supplemental data (Supplemental Digital Content 1, <http://links.lww.com/SS/A44>). After the initial leaching event, NH_4^+ levels in the later leachates were negligible or below the detection limit, so in the subsequent leachates only nitrate was analyzed.

First-Order Kinetics Versus Zero-Order Kinetics

Potentially mineralizable N refers to the amount of N that can be mineralized under optimum and constant environmental conditions (Stanford and Smith, 1972). Many laboratory experiments concerning N mineralization are incubated at 35°C and at approximately

80% of field water-holding capacity often considered the ideal temperature and matric potential for N mineralization (e.g., Deng and Tabatabai, 2000; Carpenter-Boggs et al., 2000). Nitrogen mineralized and measured in the laboratory represents a useful index of the potential for N mineralization in soil.

In experiments where the incubation period lasts longer than 20 weeks, the rate of soil organic N mineralized over time may start to decrease, and the cumulative mineralized N may begin to level off. In these instances, the first-order kinetic model of Stanford and Smith (1972) is often used to determine N mineralization potential. The first-order kinetic model relates cumulative mineralized N, N_m , to time t to solve for N mineralization potential, N_o , and the first-order rate constant k (Stanford and Smith, 1972; Stanford et al., 1974): $N_m = N_o [1 - e^{-kt}]$. However, in the present short-term study, the net N mineralization rates did not decrease during the incubation period, perhaps because there were relatively large pools of mineralizable N present initially or because the most readily mineralizable N had already been mineralized by the time of soil sampling, either during the previous fall or the early spring. Consequently, we used a zero-order kinetics model ($N_m = kt$) to determine potential N mineralization rates (Addiscott, 1983).

Calculations and Statistical Analyses

Nitrate-N values were summed after each leaching to express the cumulative mineralized N, except that the nitrate-N obtained in the initial leaching event was not included in the cumulative mineralized N. Nitrate-N obtained in the initial 5 mM CaCl_2 leaching closely paralleled the 2 M KCl-extractable nitrate-N before incubation (Fig. S1, Supplemental Digital Content 1, <http://links.lww.com/SS/A44>). Mulvaney et al. (2016) have recently reported that dilute solutions of CaCl_2 may not displace all exchangeable ammonium in N mineralization studies of some temperate-region soils. Here, in samples from the 16 N-fertilized plots, KCl-extractable ammonium accounted for an average of only 2.9% of the total KCl-extractable N. Therefore, we assumed that the initial leaching process effectively removed both extractable ammonium and nitrate in the soil and that ammonium in subsequent leachings could be discounted without significant error (see further discussion in the supplemental data, Supplemental Digital Content 1, <http://links.lww.com/SS/A44>).

The net N mineralization rate is the slope of the zero-order kinetics plot of cumulative mineralized N versus incubation time, and the 30-day cumulative mineralized N was found by multiplying the daily rate by 30 days. The fraction of soil organic N mineralized was calculated by dividing the 30-day cumulative mineralized N by soil organic N. An analysis of main effects and interactions was completed by using generalized linear mixed models (GLIMMIX) in SAS 9.4 (SAS Institute Inc, Cary, NC). The significance of the treatments was determined by using the analysis of variance and least significant difference analysis. In the statistical analysis, block effects were considered when comparing the treatments.

RESULTS AND DISCUSSION

Soil Properties

The characteristics of soil samples collected from the cropping systems plots are given in Table 1. Soil organic C and total N were not statistically different among the cropping systems (based on four-field replications per treatment). Extractable NH_4^+ was low ($\leq 1 \text{ mg N kg}^{-1}$) in all cropping systems. Extractable NO_3^- in the two reconstructed prairie cropping systems (0.7 mg N kg^{-1} in the P treatment and $11.8 \text{ mg N kg}^{-1}$ in the PF treatment) was

TABLE 1. Chemical and Physical Characteristics of Soil (0–15 cm) in the Cropping Systems Plots

Cropping Systems	Organic C	Total N	Inorganic N		pH	Clay	Sand
			NH ₄ ⁺	NO ₃ ⁻	1:1 Water		
	— g kg ⁻¹ —		— mg kg ⁻¹ —				— g kg ⁻¹ —
CS	25.8a*	2.0a	0.49a	21.9ab	6.8a	276a	375a
	(±0.2)	(±0.0)	(±0.05)	(±3.3)	(±0.2)	(±24)	(±35)
CC	24.2a	1.9a	0.50a	28.7a	6.5a	282a	362a
	(±0.2)	(±0.0)	(±0.04)	(±7.1)	(±0.2)	(±16)	(±25)
CCW	22.0a	1.7a	0.59a	30.8a	6.6a	244a	423a
	(±0.2)	(±0.0)	(±0.09)	(±7.0)	(±0.2)	(±23)	(±31)
P	24.3a	1.8a	0.77a	0.7c	6.9a	269a	404a
	(±0.3)	(±0.0)	(±0.15)	(±0.4)	(±0.2)	(±30)	(±48)
PF	27.6a	2.1a	0.61a	11.8bc	6.7a	282a	373a
	(±0.2)	(±0.0)	(±0.07)	(±1.0)	(±0.2)	(±16)	(±30)

Values in parentheses are SE.
*Means in a column that are followed by the same letter are not significantly different at $P = 0.05$.

significantly less than in the CC (28.7 mg N kg⁻¹) and CCW (30.8 mg N kg⁻¹) cropping systems.

Nitrogen Mineralization Potential

In this study, the cumulative (30-day) mineralized N was linearly related to the incubation time, with R^2 values of almost 1.0. Figure 1 is an illustrative example of the mineralization data with plant residues intact (WR) for one block of the field plots, that is, with all cropping systems present. Each point represents a mean value for two laboratory incubation replicates. We also found that the cumulative mineralized N was linearly related to time over an extended incubation period of 3 months (approximately 90 days) (exemplary data and discussion are presented in the supplemental data, Supplemental Digital Content 1, <http://links.lww.com/SS/A44>). The slopes of the lines of cumulative mineralized N versus time over 4 weeks in Fig. 1 represent the net N mineralization rate. (One exception to the linear rates occurred in a single replicate of the prairie [P] treatment in which N mineralization was slightly delayed at the beginning of the incubation period.) Because not all leachates were collected after exactly 30 days, we used the

linear net N mineralization rate to extrapolate a consistent value for the cumulative N mineralized to 30 days for the five cropping systems.

Net Nitrogen Mineralization Rate

The three treatments were (1) the five cropping systems, (2) whether the soil samples were frozen or not frozen before incubation, and (3) whether the samples were incubated with (WR) or without (WOR) plant residues. The overall analysis of variance of all the observations explored the effects of these three treatments on net N mineralization rate (analysis of variance, $n = 80$). While the presence of plant residue treatment (WOR and WR) alone did not significantly affect net N mineralization rate ($P = 0.31$), the cropping systems ($P = 0.0038$) and the freezing treatment ($P < 0.0001$) did have significant effects on net N mineralization rate. With this overview of the treatment effects in mind, in the following discussion, we present detailed data and statistical comparisons of the interactions of the cropping systems, freezing, and residue treatments.

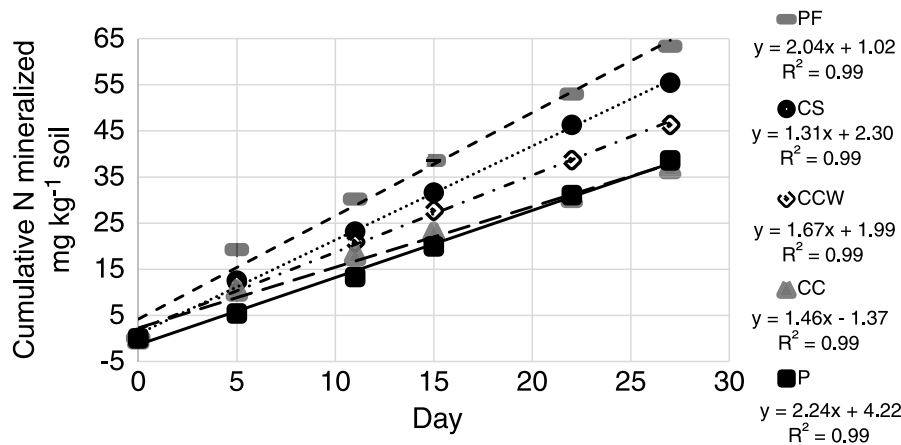


FIG. 1. Example of cumulative mineralized N with plant residues intact (WR) and without freezing for one of the four field blocks of plots with all cropping systems present. Each point represents a mean value for two laboratory incubation replicates. The five cropping systems are: CC, CCW, CS, P, and PF.

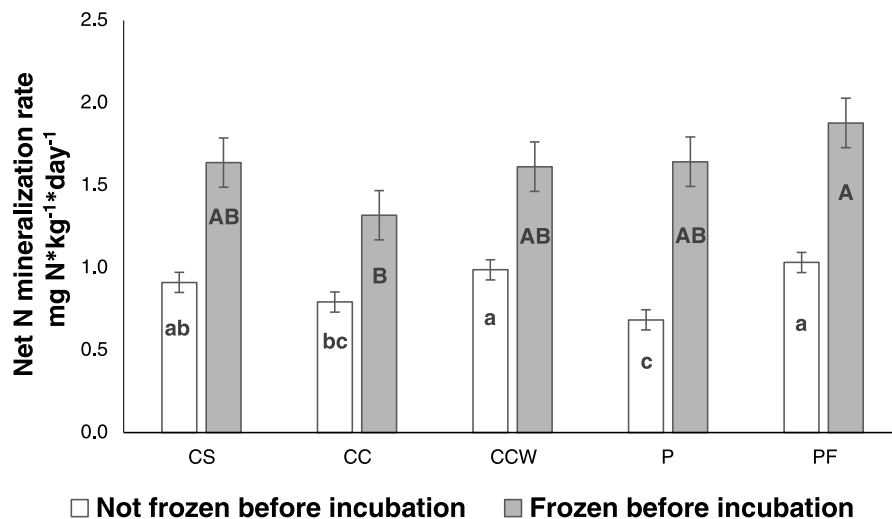


FIG. 2. Net N mineralization rates among cropping systems under no-freezing and freezing treatments. Because there was no statistical impact of the residue treatment on N mineralization, data from the residue treatments were pooled to provide more statistical power. Lowercase letters indicate differences among cropping systems under the no-freezing treatment. Uppercase letters indicate differences ($P < 0.05$) among cropping systems under the freezing treatment. Error bars represent SEM. The five cropping systems are: CC, CCW, CS, P, and PF.

Cropping Systems

Over time, cropping systems based on perennial grass build considerable mineralizable belowground biomass, and they are expected to have greater N mineralization rates than corn-based systems (Davis et al., 2013). For example, N was mineralized at a faster rate from the N-fertilized prairie cropping system (PF) than from the N-fertilized CC cropping system under both freezing and no-freezing treatments (Fig. 2, in which data from the residue treatments have been pooled). The absence of N fertilization in a cropping system can also lower the N mineralization rate. For example, the N mineralization rate of the prairie system that received no N fertilizer (P) was significantly less than the mineralization rates of three of the fertilized cropping systems (PF, CS, and CCW) and somewhat smaller than the rate of the CC cropping system when the soil was not frozen before incubation (Fig. 2).

Some studies have reported that inclusion of a legume in a crop rotation may increase net N mineralized (Carpenter-Boggs et al., 2000; Deng and Tabatabai, 2000). However, in the present study, there was not a significant difference in N mineralization potential between the CS (corn-soybean rotation) and the CC cropping systems (Fig. 2). Perhaps the greater frequency of fertilizer N application in the CC treatment would have stimulated mineralization sufficiently to make it similar to that of the CS treatment. On the other hand, our observations matched the expectation that row-crop systems that include a winter cover crop may have a higher N mineralization rate than cropping systems without a winter cover crop (Ranells and Waggoner, 1996; Hu et al., 1997). In the absence of the freezing treatment, the N mineralization rate of the cropping system of CCW was significantly larger than the rate of CC cropping system (Fig. 2). The N stored in winter cover rye residue may be released by mineralization in the spring, considerably increasing the net N mineralization rate compared with row-crop systems with no cover crop.

Effect of Cropping Systems and Freezing

The impact of cropping systems on the N mineralization rate was investigated separately for samples that had been frozen before incubation and for samples that were not frozen (Fig. 2).

Without freezing, the net N mineralization rate varied significantly among cropping systems, ranging from $0.68 \text{ mg N kg}^{-1} \text{ d}^{-1}$ in the prairie (P) systems to $1.03 \text{ mg N kg}^{-1} \text{ day}^{-1}$ in the PF systems. The net N mineralization rates in the PF and the CCW systems were significantly larger than the rates in the CC and P systems. After the freezing treatment, the average net N mineralization rate also varied among the cropping systems, ranging from $1.3 \text{ mg N kg}^{-1} \text{ day}^{-1}$ in the CC systems to $1.9 \text{ mg N kg}^{-1} \text{ day}^{-1}$ in the PF systems (Fig. 2). After freezing, the net N mineralization rate in the PF was significantly larger than the rate in the CC system. Overall, the variation among cropping systems in the freezing treatment was less differentiated than the variation among cropping systems in the treatment without freezing. Among the cropping systems, freezing the samples before incubation increased the net N mineralization rate the most in the prairie (P) system (Fig. 2).

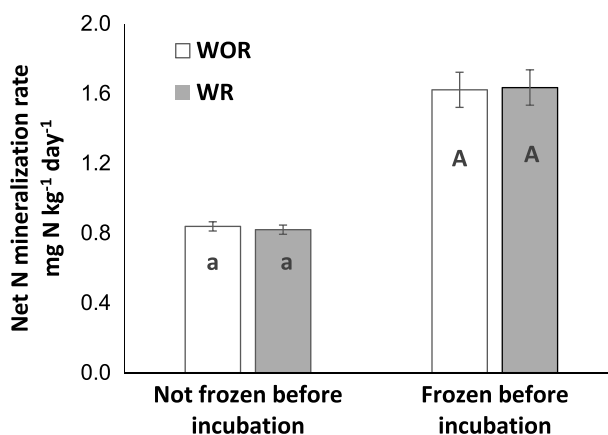


FIG. 3. Effect of plant residues on net N mineralization rate in the freezing and the no-freezing treatments. Lowercase letters indicate the difference ($P < 0.05$) between treatments with (WR) and without (WOR) plant residues under the no-freezing treatment. Uppercase letters indicate the difference ($P < 0.05$) between treatments with (WR) and without (WOR) plant residues under the freezing treatment. Error bars represent SEM.

TABLE 2. Effect of Soil Characteristics on N Mineralization in Samples Without Plant Residues (WOR) and Without Freezing (n = 30)

Soil Characteristics	Net N Mineralization Rate	Fraction of Soil Organic N Mineralized Over 30 d
	<i>P</i>	
Clay content	0.005	0.0004
Soil total N	0.04	NA*
Soil organic C	0.07	NA
Soil C/N ratio	0.1	NA
Preincubation exchangeable NO ₃ ⁻	0.2	0.10
Preincubation exchangeable NH ₄ ⁺	0.6	0.09

*NA = not applicable. Because the concentration of total N in the soil was strongly correlated with organic C, the fraction of N mineralized would also be correlated with organic C.

Overall, the freezing treatment resulted in a significant increase in the N mineralization rate. Deluca et al. (1992) also found that, when soil samples from Iowa were frozen and then thawed before incubation at 25°C, the N mineralization increased up to twofold and remained at a higher rate up to 20 days. Herrmann and Witter (2002) studied soil samples from Sweden in modeling the freeze-thaw cycles in late winter and early spring before laboratory incubation, and they also found that freezing and thawing increased N mineralization twofold to threefold in 40 days. Our results are therefore consistent with those reported by Deluca et al. (1992) and Herrmann and Witter (2002).

Freezing and thawing of soil can cause biological and physical perturbations that can release soluble organic materials and rupture microbial cells (Morely et al., 1983). The organic material released could be more easily decomposable after a freezing-thawing cycle. Ivarson and Sowden (1966, 1970) showed that the concentration of free amino acids and sugars in soils increased as a result of freezing to -14°C, but the source of the increased free amino acids and sugars was not well understood. Freezing also decreases soil water potential, and soil organic matter that is associated with clay minerals may be dehydrated and dissociated from the clay minerals while freezing. We speculate that this process would increase the surface area of soil organic matter, perhaps making it more readily bioavailable.

The decomposition of killed microbial cells by freezing could also be a source of the increase in N mineralization rate

(Schimel and Klein, 1996; Herrmann and Witter, 2002). The microbial community that survives freezing may quickly recover after thawing and feed on the dead microbial cells, releasing inorganic N. Typically, soil microbial biomass N is approximately 4% of total soil N, ranging from approximately 2% to 6% (Brookes et al., 1985). In our study, the average N mineralized was approximately 1.2% of total soil N (12 g mineralized N kg⁻¹ soil N) under the no-freezing treatment, whereas it was approximately 2.6% (26 g mineralized N kg⁻¹ soil N) under the freezing treatment (Table 3). Therefore, the 1.4% increase in mineralized N due to freezing did not exceed the likely amount of microbial biomass N. We suggest that the killed microbial cells could have been an important source of organic N that was available for mineralization after the freezing-thawing cycle. However, it is thought that the increase in mineralization is a short-term phenomenon, and the increase in mineralized N after thawing is usually small in relation to the expected annual mineralization potential (Herrmann and Witter, 2002; Matzner and Borken, 2008).

Effects of the Presence of Plant Residues and Freezing

By pooling the cropping systems, the impact of plant residues on the net N mineralization rate was investigated for samples that had been frozen before incubation and for samples that were not frozen (Fig. 3). In the not-frozen treatment, the net N mineralization rate was not different (*t* test, n = 20, *P* = 0.38) between the

TABLE 3. Means (and SEM) of the Fraction of Soil Organic N Mineralized in 30 Days Across Cropping Systems (WR and WOR Treatments Pooled)

Cropping System	30-d Cumulative Mineralized N		Fraction of Soil Organic N Mineralized in 30 d	
	Not Frozen Before Incubation	Frozen Before Incubation	Not Frozen Before Incubation	Frozen Before Incubation
	— mg kg ⁻¹ of Soil —		—g N kg ⁻¹ of Soil Organic N—	
CS	27.2 (±1.3)	50.7 (±2.1)	12.4 (±0.9)ab*	25.5 (±2.2)ab
CC	23.9 (±1.0)	41.4 (±0.8)	11.3 (±0.8)ab	22.1 (±1.3)b
CCW	29.6 (±0.5)	50.1 (±1.5)	13.8 (±0.6)a	29.4 (±2.5)a
P	20.1 (±1.3)	47.9 (±3.5)	9.7 (±1.5)b	27.2 (±3.3)ab
PF	30.6 (±1.0)	57.3 (±2.5)	12.9 (±0.9)ab	26.8 (±1.6)ab

Overall, n = 80; each mean value represents eight incubations per treatment. Values in parentheses are SE.

*Means within a column that are followed by the same letter are not significantly different at *P* = 0.05.

TABLE 4. Characteristics of Belowground Residues (Isolated From the WOR Soil Samples; $n = 20$)

Cropping System	Total C	Total N	Residue C:N	Residue Concentration	Residue N in Soil
	— g kg ⁻¹ Residues —			— g kg ⁻¹ Soil —	mg N kg ⁻¹ Soil
CS	355a*	12.4a	28b	0.87b	11.0b
	(10)	(0.2)	(1)	(0.01)	(0.1)
CC	288b	12.9a	22c	0.46c	6.0e
	(5)	(0.1)	(1)	(0.01)	(0.1)
CCW	297b	13.3a	22c	0.58c	7.7d
	(5)	(0.1)	(1)	(0.02)	(0.1)
P	365a	6.7b	55a	1.30a	8.8c
	(9)	(0.2)	(1)	(0.02)	(0.1)
PF	377a	12.4a	31b	1.41a	17.0a
	(6)	(0.6)	(1)	(0.02)	(0.1)

Values in parentheses are SE.

*Means within a column that are followed by the same letter are not significantly different among cropping systems at $P = 0.05$.

WOR (overall mean of 0.84 mg N kg⁻¹ d⁻¹) and WR (overall mean of 0.82 mg N kg⁻¹ d⁻¹) treatments. For the frozen samples, the net N mineralization rate of the WOR treatment was also not significantly different from that of the WR (both had overall mean rates of 1.6 mg N kg⁻¹ d⁻¹) treatment (t test, $n = 20$, $P = 0.83$). On the other hand, when WOR and WR treatments were lumped together, the samples that were frozen before incubation had a net N mineralization rate approximately two times larger than that of the samples that were not frozen (t test, $P < 0.00001$).

Effect of Soil Characteristics on N Mineralization

We sought to isolate the effects of soil characteristics only on N mineralization by considering data for samples without plant residues (WOR) and without freezing (Table 2). In this case, the net N mineralization rate was strongly and negatively related to clay content ($P = 0.005$, $r = -0.54$), which may be attributed to the potential for clay to adsorb and protect organic matter and colloidal plant residues from enzymatic attack (Hassink, 1992). The net N mineralization rate was weakly related to the soil C/N ratio ($P = 0.1$, $r = -0.41$), weakly related to soil organic C ($P = 0.07$, $r = -0.35$), and moderately related to soil total N ($P = 0.04$, $r = -0.30$). In contrast, there was no statistical relationship ($P > 0.1$) between the net N mineralization rate and preincubation exchangeable NO₃⁻ or NH₄⁺ in the soils, but the fraction of soil organic N mineralized in 30 days was weakly related to the preincubation exchangeable NO₃⁻ ($P = 0.1$, $r = 0.29$) and NH₄⁺ ($P = 0.09$, $r = 0.36$) (Table 2).

Because the presence of plant residues did not affect the potential N mineralization rate, we pooled the WR and WOR residue treatments to determine if the fraction of soil organic N mineralized in 30 days varied among the cropping systems (Table 3). When the samples were not frozen, the continuous corn with winter rye treatment had the highest fraction of soil N mineralized, and the unfertilized prairie had the lowest fraction. When the samples had been frozen first, the highest fraction of soil N that was mineralized also occurred in samples from the continuous corn with winter rye, and the lowest fraction of soil N that was mineralized was found in samples from the continuous corn treatment.

Ros et al. (2011) determined mineralizable N over 126 days of incubation for 98 agricultural soils with a wide range of properties and reported that soil total C, soil total N, and the soil C/N ratio were positively related to mineralizable N ($P < 0.001$). Over

the fairly limited range of organic C and total N values in the soils of the present study, we did not find such strong relationships. Indeed, by themselves, soil C, N, and C/N might not always be reliable predictors of N mineralization. Other researchers have recommended that combined indices can improve predictions of mineralizable N (Schomberg et al., 2009; Ros et al., 2011). On the other hand, the negative relationship between clay content and N mineralization rate that we observed was consistent with and even more significant than the relationship reported by Ros et al. (2011) ($P < 0.005$ vs. $P < 0.01$), perhaps because the mineralogy of the clay fraction in our soils was quite uniform (dominantly smectite; unpublished data) and thus more likely to induce a consistent effect on mineralization.

Effect of Plant Residues on N Mineralization

Concentration and Chemical Characteristics of Plant Residues

The mass of plant residues removed by hand from the soil samples varied considerably among the cropping systems (Table 4). The two prairie systems produced significantly larger amounts of plant residues (1.30 g kg⁻¹ in the unfertilized prairie (P) and 1.41 g kg⁻¹ in the PF than the other systems did. However, over all treatments, the analysis of variance demonstrated that the net N mineralization rate did not depend on the concentration of plant

TABLE 5. Effects of Plant Residue Concentration and Characteristics on Net N Mineralization Rate in Samples With Plant Residues (WR) Under the Freezing Treatment ($n = 20$)

Properties of Plant Residues	Effect on Net N Mineralization Rate
	P
Concentration of plant residues in the soil	0.61
Total C of plant residues	0.96
Total N of plant residues	0.51
C/N ratio of plant residues	0.89
Total N * concentration of plant residues	<0.001

residues in the soil ($P = 0.61$, Table 5). This result, coupled with the lack of treatment significance in the WR versus WOR comparison described above, suggests that little N was being mineralized from residues that were large enough to identify and physically isolate from the soil samples. We speculate that the apparent lack of impact of plant residues on N mineralization rates in this study reflects slow mineralization from macroscopic residues.

The chemical characteristics of the plant residues that were separated from the soil samples are also shown in Table 4. The total C of the plant residues ranged from 288 g kg⁻¹ in the CC system to 377 g kg⁻¹ in the PF system. Total N ranged from 6.7 g kg⁻¹ in the P system to 13.3 g kg⁻¹ in the CCW system. The residue C/N ratio among the five cropping systems had a wide range, from 22 in CC and CCW to 55 in P. The analysis of variance (Table 5) explored the effects of the chemical characteristics of the plant residue on net N mineralization rate. There were no significant effects of total C, N, or C/N ratio of the plant residues on net N mineralization rate. These results contrast with most authors' conclusions that when the C/N ratio of plant residues is less than 25, net N mineralization is likely, and when the C/N ratio is greater than 30, immobilization occurs (e.g., Trinsoutrot et al. 2000; Kumar and Goh, 2003). Like those of O'Connell et al. (2015), our results indicate that the C/N ratios of macroscopic plant residues would not be effective predictors of short-term N mineralization rates in these cropping systems.

Interactions of the Quantity and Quality of Plant Residues

The plant residue N per unit soil mass (Table 4) was calculated by multiplying the total N of plant residues by the residue concentration, so it is a parameter that represents both the quantity and chemical characteristics of the residues. The plant residue N ranged from 6.0 mg N kg⁻¹ soil in continuous corn (CC) to 17.0 mg N kg⁻¹ soil in N-fertilized prairie (PF). The least significant difference analysis showed that the plant residue N was significantly different in each cropping system (Table 4). The analysis of variance for the samples with residues (WR, Table 5) showed that there was a significant effect of plant residue N in the soil on net N mineralization rate ($P < 0.001$). The net N mineralization rate was positively related to macroscopic plant residue N in the soil, as shown in Fig. 4 ($r = 0.65$). We conclude that in this experiment the rate of N mineralization was significantly

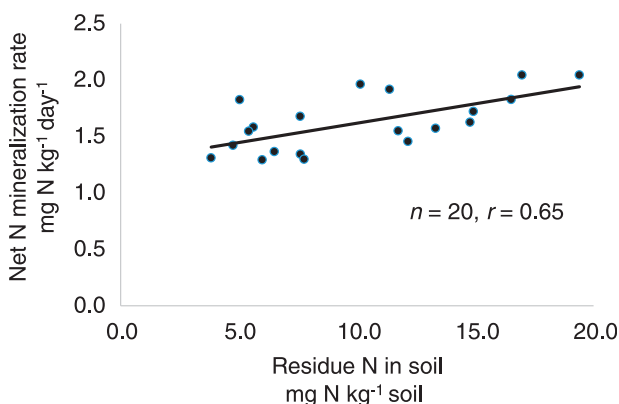


FIG. 4. Correlation of net N mineralization rate of soil samples with plant residues (WR) and under the freezing treatment versus plant residue N. A color version of this figure is available in the online version of this article. A color version of this figure is available in the online version of this article. [full color](#)

TABLE 6. Illustrative Calculations to Compare Potentially Mineralizable N (30-Day PMN) to N_{\max} Uptake in the 2011 Growing Season in Three Cropping Systems

Cropping System	PMN (This Study)	N_{\max} Uptake (Fitted by Dietzel et al., 2015)	PMN as Fraction of N_{\max} Uptake
		kg N ha ⁻¹	%
CC	50	189	26
P	43	88	49
PF	59	173	34

affected by the interactions of quantity and quality of the partly decomposed plant residues.

Potentially Mineralizable N and Crop Uptake

In a study related to the present investigation, Dietzel et al. (2015) determined and modeled the uptake of N into the biomass of the corn cropping systems and perennial prairie systems in the Comparison of Biofuel Systems project for the 2011 growing season. Total N uptake (aboveground and belowground) by the crops (to the point of maximum aboveground biomass accumulation) ranged from 88 (unfertilized prairie system) to 189 (continuous corn systems) kg ha⁻¹ (Table 5). To illustrate the potential for mineralization to supply N for uptake, we used our laboratory incubation results, the sampling depth of 15 cm, and measured bulk densities for the soils in each of the COBS research plots to estimate the potentially mineralizable N at that depth for those cropping systems. On this basis, N mineralized from the crop residues and soil organic matter in the upper 15 cm of the soil could have supplied 26% and 34% of the total N demand in the continuous corn system and fertilized prairie system, respectively, and 49% of the total N demand in the unfertilized prairie system (Table 6). These values are comparable to the degree of apparent in-crop N mineralization reported by St. Luce et al. (2016) for no-till wheat and canola cropping systems.

CONCLUSIONS

In this study, the cumulative amount of mineralized N soils of biofuel cropping systems was linearly related to time of incubation over a 30-day period. Cropping systems had a significant effect on the net N mineralization rate; that is, the fertilized perennial prairie system had a higher net N mineralization rate than did corn-based systems. Inclusion of a winter cover crop in the continuous corn system also increased the net N mineralization rate by releasing the N from the cover crop residue.

Freezing and thawing of soil samples increased the net N mineralization rate approximately twofold. Freezing may change the characteristics of soil organic matter, making it more easily decomposable. Microorganisms killed by freezing might also be an important source for N mineralization. We speculate that, after thawing, the remaining microbial community quickly recovered and released N to the soil inorganic N pool by decomposition of the dead microbial biomass.

In this experiment, an analysis of variance indicated that soil total N and clay content were related to N mineralization. But because of the narrow range of soil total N and clay content among our samples, a correlation was not clearly expressed between those characteristics and the mineralization rate. Individually, the presence, the concentration, and the chemical characteristics of plant residues did not affect the net N mineralization rate. But the concentration of plant residue N per unit mass of soil did

significantly increase the N mineralization rate, indicating that interactions between the chemical characteristics and the quantity of the plant residues regulated N mineralization.

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