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Jason Clark

Kristen S. Veum

Fabián G. Fernández

Newell R. Kitchen

James J. Camberato

*See next page for additional authors*

Authors
Jason Clark, Kristen S. Veum, Fabián G. Fernández, Newell R. Kitchen, James J. Camberato, Paul Carter, Richard Ferguson, David W. Franzen, Daniel E. Kaiser, Carrie A. M. Laboski, Emerson Nafziger, Carl J. Rosen, John E. Sawyer, and John F. Shanahan

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NUTRIENT MANAGEMENT & SOIL & PLANT ANALYSIS

# Soil sample timing, nitrogen fertilization, and incubation length influence anaerobic potentially mineralizable nitrogen

Jason D. Clark[1] | Kristen S. Veum[3] | Fabián G. Fernández[2] | Newell R. Kitchen[3] | James J. Camberato[4] | Paul R. Carter[5] | Richard B. Ferguson[7] | David W. Franzen[6] | Daniel E. Kaiser[2] | Carrie A. M. Laboski[8] | Emerson D. Nafziger[9] | Carl J. Rosen[2] | John E. Sawyer[10] | John F. Shanahan[11]

[1]South Dakota State University, 1148 Medary Ave., Brookings, SD, 57007

[2]University of Minnesota, 1991 Upper Buford Circle, St. Paul, MN, 55108

[3]USDA-ARS Cropping Systems and Water Quality Research Unit, Columbia, MO, 65211

[4]Purdue University, 915 W. State St., West Lafayette, IN, 47907

[5]Independent Agronomist, 13801 Summit Dr., Clive, IA, 50325

[6]North Dakota State University, PO Box 6050, Fargo, ND, 58108

[7]University of Nebraska, Keim 367, Lincoln, NE, 68583

[8]University of Wisconsin-Madison, 1525 Observatory Dr., Madison, WI, 53706

[9]University of Illinois, 1102 S. Goodwin, Urbana, IL, 61801

[10]Iowa State University, 3208 Agronomy Hall, 716 Farm House Lane, Ames, IA, 50011

[11]Soil Health Institute, 6807 Ridge Rd., Lincoln, NE, 68512

**Correspondence**
J.D. Clark, South Dakota State University, 1148 Medary Ave., Brookings, SD 57007
Email: Jason.D.Clark@sdstate.edu

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**Abstract**

Understanding the variables that affect the anaerobic potentially mineralizable N ($PMN_{an}$) test should lead to a standard procedure of sample collection and incubation length, improving $PMN_{an}$ as a tool in corn (*Zea mays* L.) N management. We evaluated the effect of soil sample timing (preplant and V5 corn development stage [V5]), N fertilization (0 and 180 kg ha$^{-1}$) and incubation length (7, 14, and 28 d) on $PMN_{an}$ (0–30 cm) across a range of soil properties and weather conditions. Soil sample timing, N fertilization, and incubation length affected $PMN_{an}$ differently based on soil and weather conditions. Preplant vs. V5 $PMN_{an}$ tended to be greater at sites that received < 183 mm of precipitation or < 359 growing degree-days (GDD) between preplant and V5, or had soil C/N ratios > 9.7:1; otherwise, V5 $PMN_{an}$ tended to be greater than preplant $PMN_{an}$. The $PMN_{an}$ tended to be greater in unfertilized vs. fertilized soil in sites with clay content > 9.5%, total C < 24.2 g kg$^{-1}$, soil organic

**Abbreviations:** AWDR, Abundant and well-distributed rainfall; GDD, Growing degree-day; $PMN_{an}$, Anaerobic potentially mineralizable N; SDI, Shannon diversity index; SOM, Soil organic matter.

matter (SOM) < 3.9 g $kg^{-1}$, or C to N ratios < 11.0:1; otherwise, $PMN_{an}$ tended to be greater in fertilized vs. unfertilized soil. Longer incubation lengths increased $PMN_{an}$ at all sites regardless of sampling methods. Since $PMN_{an}$ is sensitive to many factors (sample timing, N fertilization, incubation length, soil properties, and weather conditions), it is important to follow a consistent protocol to compare $PMN_{an}$ among sites and potentially use $PMN_{an}$ to improve corn N management.

## 1 | INTRODUCTION

Nitrogen mineralization can supply 20 to 100% of crop N needs depending on several factors (Khan, Mulvaney, & Hoeft, 2001; Ros, Temminghoff, & Hoffland, 2011; Yost, Coulter, Russelle, Sheaffer, & Kaiser, 2012). Knowledge of the N supplied from soil organic matter (SOM) mineralization may improve N fertilizer guidelines. The N mineralization estimated from the $PMN_{an}$ test was used, along with preplant- and presidedress-nitrate tests, to improve N management decisions in Argentina (Orcellet, Reussi, Sainz Rozas, Wyngaard, & Echeverría, 2017; Sainz Rozas, Calvino, Echeverría, Barbieri, & Redolatti, 2008). The use of the $PMN_{an}$ test also improved the predictability of N needs of winter wheat (*Triticum aestivum* L.) in the U.S. Pacific Northwest and corn in the U.S. Southeast (Christensen & Mellbye, 2006; Williams, Crozier, White, Sripada, & Crouse, 2007). Therefore in the U.S. Midwest, the use of the $PMN_{an}$ test may also be able to improve N guidelines for corn. However, we need to consider various sampling and methodological conditions in order to determine a standardized $PMN_{an}$ protocol that will optimize the utility of the $PMN_{an}$ test in predicting corn N requirements in the U.S. Midwest.

First, most soil samples collected for $PMN_{an}$ analysis are obtained early in the spring when limited mineralization has taken place. These mineralization rates increase through the spring as temperatures increase and change throughout the remainder of the growing season (Culman, Snapp, Green, & Gentry, 2013; Fernández, Fabrizzi, & Naeve, 2017; Kuzyakova, Turyabahika, & Stahr, 2006; Sierra, 1996). However, the differences between early and later season soil and weather conditions and their influence on $PMN_{an}$ have not been investigated. Another important aspect of soil sample timing to consider in the U.S. Midwest is that N mineralized early in the season (April to approximately mid-June) is susceptible to loss (denitrification or leaching) because of greater spring precipitation and limited N uptake by young corn (Randall & Vetsch, 2005; Struffert, Rubin, Fernández, & Lamb, 2016). Moving $PMN_{an}$ soil sampling to later in the season when N loss potential is less and corn N uptake is increasing may improve the accuracy of the N amount that will be available to the corn crop, potentially improving the ability to predict corn N needs.

**Core Ideas**
- Soil parameters and weather influence how sampling time, N fertilization, and incubation length affect N mineralization.
- Nitrogen mineralization at preplant > in-season timing 27% of the time; in-season timing > preplant 23% of the time.
- Nitrogen fertilization reduced N mineralization 31% of the time and increased it 7% of the time.
- Sites with fine-textured soils and higher SOM had the greatest change in N mineralization from extended incubations.

Second, most soil samples collected for $PMN_{an}$ analysis are obtained before spring N fertilizer application. However, the application of N fertilizer before soil sampling results in greater variability of N mineralization (Fernández et al., 2017; Kuzyakova et al., 2006; Ma, Dwyer, & Gregorich, 1999). Understanding the influence of N fertilizer application on N mineralization has important practical implications because most agricultural fields receive some N fertilizer before or at planting to optimize corn yield. The greater variability of N mineralization after N fertilizer application in the spring may be partially attributed to the interaction of N fertilizer with the quality of soil organic matter (i.e., C to N ratio) (Chen et al., 2014; Conde et al., 2005; Hamer & Marschner, 2005). The rate of mineralization early in the season may be more influenced by N fertilization in soils with high C to N ratios compared to soils with C to N ratios that are already low enough to promote mineralization without additional N inputs. Because of the potential for N fertilizer to influence N mineralization, mineralization estimates obtained from soil before spring N fertilization might result in an inaccurate estimate of how much N the soil can supply to a crop. Therefore, the measurement of the effect of early-season N fertilization on $PMN_{an}$ requires further research. Increasing our understanding of the effect of N fertilizer on $PMN_{an}$ would also likely improve N management guidelines.

Third, the standard incubation length for the $PMN_{an}$ test is 7 d. Extending the incubation allows for more mineralization and often results in greater $PMN_{an}$ (Angus, Ohnishi, Horie, & Williams, 1994; Smith, McNeal, Owens, & Klock, 1981). Increasing the anaerobic incubation beyond 7 d may be difficult for commercial soil testing labs that prefer high-throughput analytical methods, unless the benefits outweigh the extra costs associated with longer incubations. Clark et al. (2019) showed that $PMN_{an}$ from longer than 7-d incubations (e.g., 14 or 28 d) related better to soil properties such as SOM, total N, and clay content in soils that have been fertilized with N and other studies observed improved correlations with crop biomass and N uptake of rice (*Oryza sativa*) with $PMN_{an}$ from longer than 7-d incubations (Russell, Dunn, Batten, Williams, & Angus, 2006). While limited at present, those studies hint that longer incubation lengths may be more representative of N mineralization in the field, which could improve the accuracy of fertilizer-N guidelines. The potential for longer incubations to explain the variability of N mineralization in relation to contrasting soil properties and weather conditions deserves further inquiry. Therefore, the objective of this paper was to evaluate the effect of soil sample timing, N fertilization, and incubation length on $PMN_{an}$ across a range of soil properties and weather conditions in the U.S. Midwest. Specific research findings regarding the relationships between $PMN_{an}$ from different sampling methodologies and $PMN_{an}$ incubation lengths with plant available N, N uptake, and yields will come in future papers.

# 2 | MATERIALS AND METHODS

## 2.1 | Experimental design

This study was conducted as a coordinated effort with uniform treatments and measurement methodology across eight U.S. Midwestern states (Illinois, Indiana, Iowa, Minnesota, Missouri, Nebraska, North Dakota, and Wisconsin). Kitchen et al. (2017) contains information regarding general soil characteristics and precipitation and temperature patterns across the study region along with specific details of experimental site descriptions, agronomic practices, and research protocols. Briefly, two sites were selected in each state in 2014 and 2015 for 32 site-years total that varied in soil properties and weather conditions (Table 1). An unfertilized check and an N rate that was considered representative of the optimal N rate (180 kg N $ha^{-1}$) was selected in this study for measuring $PMN_{an}$. Ammonium nitrate (340 g N $kg^{-1}$) was broadcast applied on the soil surface at planting. As stated in Kitchen et al. (2017), ammonium nitrate was used because it was expected to perform more similarly across the environmental conditions represented in the study region, provide a uniform broadcast application that would allow for soil $NO_3$–N and $NH_4$–N evaluation shortly after application, and be suitable for surface application.

## 2.2 | Soil sampling and analysis

Soil characterization was performed before spring tillage and planting at each experimental site by obtaining two, 120-cm deep soil cores (3.8 to 4.0 cm i.d.) from every replicate and dividing them by horizons to measure physical and chemical properties including a taxonomic description; bulk density (bulk density-measured), soil texture, total C, total organic C, SOM, total N, cation exchange capacity, and pH (1:1 soil/water) as described in Kitchen et al. (2017). Saxton bulk density (bulk density-Saxton) was also calculated using the soil texture and SOM measurements (Saxton & Rawls, 2006). Weighted averages were calculated for the top 30 cm using the depth of each horizon within the 0- to 30-cm soil depth.

The preplant soil samples were obtained each spring 2 to 4 wk before planting and fertilization using a ten core (1.9 to 4.0 cm i.d.) composite soil sample from each replication at 0- to 30-, 30- to 60-, and 60- to 90-cm soil depths. In addition, a six-core composite (1.9-cm i.d.) soil sample (0- to 30- and 30- to 60-cm depth) was obtained at the V5 corn development stage from the 0 and 180 kg N $ha^{-1}$ treatments. All soil samples were dried ($\leq$ 32°C) and ground to pass through a 2-mm sieve. Soil $NO_3$–N was extracted using 0.2 mol $L^{-1}$ KCl (Saha, Sonon, & Biswas, 2018) and quantified by the cadmium reduction method (Gelderman & Beegle, 2012) with a modified Technicon AutoAnalyzer (SEAL Analytical, Inc., Fareham, UK). For $PMN_{an}$ analysis, only the surface soils (0–30 cm in this study) were analyzed to maintain consistency with depth used when the $PMN_{an}$ test was originally calibrated (Bundy & Meisinger, 1994). Anaerobic potentially mineralizable N was quantified by combining 4.0 g of dried soil with 20 ml of ultrapure water in 50 ml Falcon tubes (Corning Inc., Corning, NY), capped, and subjected to an incubation length of 7, 14, and 28 d at 40°C (Keeney & Bremner, 1966). After incubation, 20 ml of 4 mol $L^{-1}$ KCl was added for a final extractant concentration of 2 mol $L^{-1}$ KCl and samples were shaken for 30 min. Next, the solution was passed through a washed 0.45-μm syringe filter disk and stored in a microtube at −80°C to await $NH_4$–N analysis. Extracted $NH_4$–N was determined by the Berthelot method (Rhine, Mulvaney, Pratt, & Sims, 1998) using a Glomax-Multi Detection System plate reader (Promega Biosystems, Inc., Sunnyvale, CA, USA). An initial $NH_4$–N value was determined for each soil sample following the above extraction procedure with 2 mol $L^{-1}$ KCl and subtracted from the incubation results to obtain net $NH_4$–N produced or $PMN_{an}$.

**TABLE 1** Minimum, maximum, mean, standard deviation, and coefficient of variation of soil properties and weather conditions across 32 site-years

| Property[a] | Min. | Max. | Mean | SD | CV |
|---|---|---|---|---|---|
| Soil properties | | | | | |
| Sand, g $kg^{-1}$ | 20 | 930 | 260 | 250 | 950 |
| Silt, g $kg^{-1}$ | 40 | 790 | 500 | 190 | 390 |
| Clay, g $kg^{-1}$ | 20 | 610 | 240 | 110 | 470 |
| BD-measured, g $cm^{-3}$ | 1.0 | 1.7 | 1.4 | 0.1 | 9.8 |
| BD-Saxton, g $cm^{-3}$ | 1.1 | 1.6 | 1.3 | 0.1 | 10.0 |
| TC, g $kg^{-1}$ | 4.4 | 55.5 | 14.6 | 7.6 | 51.8 |
| TOC, g $kg^{-1}$ | 4.4 | 47.8 | 14.2 | 6.9 | 48.5 |
| SOM, g $kg^{-1}$ | 7.7 | 71.0 | 25.7 | 10.0 | 38.9 |
| TN, g $kg^{-1}$ | 0.4 | 4.3 | 1.4 | 0.6 | 41.8 |
| C to N ratio | 7.2 | 12.7 | 10.0 | 1.0 | 10.4 |
| CEC, $cmol_c$ $kg^{-1}$ | 3 | 44 | 20 | 9 | 46 |
| pH-salt | 4.4 | 7.8 | 6.1 | 0.8 | 13.6 |
| pH-water | 5.1 | 8.8 | 6.7 | 0.8 | 11.4 |
| Soil-N at $PP_{0N}$, mg $kg^{-1}$ | | | | | |
| $NH_4$–N 0–30 cm | 3 | 19 | 8 | 4 | 44 |
| $NO_3$–N 0–30 cm | 1 | 18 | 6 | 3 | 53 |
| $NO_3$–N 0–60 cm | 1 | 12 | 5 | 2 | 42 |
| $NO_3$–N 0–90 cm | 1 | 9 | 4 | 2 | 40 |
| Soil-N at $V5_{0N}$, mg $kg^{-1}$ | | | | | |
| $NH_4$–N 0–30 cm | 1 | 14 | 7 | 3 | 47 |
| $NO_3$–N 0–30 cm | 3 | 27 | 8 | 4 | 58 |
| $NO_3$–N 0–60 cm | 2 | 21 | 7 | 4 | 49 |
| Soil-N at $V5_{180N}$, mg $kg^{-1}$ | | | | | |
| $NH_4$–N 0–30 cm | 2 | 34 | 9 | 5 | 63 |
| $NO_3$–N 0–30 cm | 7 | 75 | 32 | 12 | 38 |
| $NO_3$–N 0–60 cm | 9 | 58 | 24 | 9 | 35 |
| Precipitation, Preplant-V5 | | | | | |
| Max precipitation, mm | 19 | 95 | 39 | 18 | 46 |
| Sum of precipitation, mm | 85 | 331 | 175 | 68 | 39 |
| Mean precipitation, mm | 2 | 5 | 3 | 0.6 | 17 |
| SDI | 0.5 | 0.7 | 0.6 | 0.1 | 8 |
| AWDR | 47 | 242 | 110 | 47 | 43 |
| Temperature, Preplant-V5 | | | | | |
| Mean max temperature, °C | 19 | 27 | 22 | 2 | 8 |
| Mean min temperature, °C | 6 | 13 | 10 | 2 | 1 |
| Mean temperature, °C | 13 | 20 | 16 | 2 | 10 |
| GDD | 228 | 543 | 347 | 84 | 24 |

[a]BD, bulk density; TC, total carbon; TOC, total organic carbon; SOM, soil organic matter; TN, total nitrogen; CEC, cation exchange capacity; SDI, Shannon diversity index; AWDR, abundant and well-distributed rainfall; GDD, growing degree-day.

## 2.3 | Weather

Weather data was collected at each experimental site with a HOBO U30 automatic weather station (Onset Computer Corporation, Bourne, MA, USA). Precipitation and temperature measurements were recorded every five min. These measurements were used to determine the daily minimum, maximum, and mean temperatures, and the daily cumulative precipitation. These daily weather measurements were quality checked by comparing the weather station measurements against interpolated temperature data from Multi-Radar/Multi-Sensor rainfall data (National Severe Storms Lab, NOAA). Outliers

**TABLE 2** Weather variables used and their definitions

| Weather parameter | Definition |
|---|---|
| Mean min temperature | Tmin = Minimum daily temperature |
| Mean max temperature | Tmax = Maximum daily temperature |
| Mean temperature | MeanTemp = (Tmax + Tmin)/2 |
| Growing degree-days | GDD = [(Tmax + Tmin)/2] − 10°C, where Tmax = Tmax if 10 ≤ Tmax ≤ 30, if Tmax ≤ 10 then Tmax = 10, if Tmax ≥ 30 then Tmax = 30; Tmin = the minimum daily temperature if Tmin ≥ 10, if Tmin ≤ 10 then Tmin = 10; all temperatures were measured in degrees Celsius, °C |
| Sum of precipitation | SP = Σ(Rain), where rain is the daily precipitation (mm) |
| Mean precipitation | MP = SP/n, where n is the number of days in that period. |
| Max precipitation | MP = Maximum amount of rain in a single day in that period |
| Shannon diversity index | SDI = [−Σpi ln(pi)]/ln(n), where pi = rain/SP is the fraction of daily precipitation relative to the total precipitation in a given time period and n is the number of days in that period. SDI = 1 implies complete evenness (i.e., equal amounts of precipitation in each day of the period); SDI = 0 implies complete unevenness (i.e., all rain in 1 d) |
| Abundant and well-distributed rainfall | AWDR = SP(SDI) |

**TABLE 3** Minimum, maximum, mean, standard deviation, and coefficient of variation of anaerobic potentially mineralizable N ($PMN_{an}$) as influenced by soil sample timing, N fertilization and incubation length across 32 site-years

| | $PMN_{an}$ | | | | |
|---|---|---|---|---|---|
| Property[a] | Min. | Max. | Mean | SD | CV |
| | mg kg$^{-1}$ | | | | |
| $PP_{0N}$, 7 d | 0.7 | 84.0 | 26.7 | 15.1 | 56.8 |
| $PP_{0N}$, 14 d | 2.4 | 94.5 | 37.8 | 18.9 | 50.0 |
| $PP_{0N}$, 28 d | 6.0 | 125.3 | 48.9 | 25.4 | 51.9 |
| $V5_{0N}$, 7 d | 0.2 | 99.9 | 28.3 | 15.0 | 53.1 |
| $V5_{0N}$, 14 d | 2.1 | 122.7 | 37.0 | 17.4 | 47.0 |
| $V5_{0N}$, 28 d | 4.0 | 136.7 | 48.5 | 23.2 | 47.8 |
| $V5_{180N}$, 7 d | 0.9 | 92.2 | 23.2 | 15.2 | 65.4 |
| $V5_{180N}$, 14 d | 6.9 | 109.9 | 32.4 | 17.5 | 53.9 |
| $V5_{180N}$, 28 d | 8.1 | 130.7 | 43.1 | 23.6 | 54.7 |

[a]$PP_{0N}$, $PMN_{an}$ from preplant soil sampling with 0 kg N ha$^{-1}$; $V5_{0N}$, $PMN_{an}$ from V5 corn development stage with 0 kg N ha$^{-1}$; $V5_{180N}$, $PMN_{an}$ from V5 corn development stage with 180 kg N ha$^{-1}$ applied at planting.

and/or missing values were replaced by the interpolated temperature or Multi-Radar/Multi-Sensor rainfall estimates (Kitchen et al., 2017). The daily measurements were then used to calculate growing degree-days (GDD), mean precipitation, Shannon diversity index (SDI) of daily cumulative precipitation following Bronikowski and Webb (1996), and abundant and well-distributed rainfall (AWDR) following Tremblay et al. (2012) for the time period between the two soil sample timings (preplant to V5). These weather parameters were calculated using equations contained in Table 2. Water provided as irrigation in four of the 32 experimental sites was treated as natural precipitation in these calculations. These weather measurements were used to evaluate the effect of weather on $PMN_{an}$ from the two sample timings.

## 2.4 | Statistical analysis

The effect of soil sample timing, N fertilization, and incubation length on $PMN_{an}$ were evaluated using the MIXED procedure of SAS (SAS Institute Inc.). The experimental design was a randomized complete block design with four replications (blocks). Residuals within each experimental unit showed normality and constant variance assumptions were met. Block was considered a random effect. Experimental site, sample timing and N rate, incubation length, and their interactions were considered fixed effects. Least squares means were calculated for each effect and their interactions using the LSMeans statement and the differences between them were determined using Tukey's adjustment for multiple comparisons when needed. Within the three sample timing and N fertilization treatments, contrasts were used to determine the significance ($P \leq .05$) of the effect of soil sample timing (preplant vs. V5 with no N fertilization), N fertilization (0 vs. 180 kg N ha$^{-1}$ applied at planting and soil sampled at V5), and their interaction with site on $PMN_{an}$ (Crossa et al., 2015). When the site by fixed effects interactions were significant, sites were evaluated individually. Soil sample timing was evaluated at only 30 sites due to missing preplant soil samples. All 32 sites were used to evaluate the effect of N fertilization and incubation length (except at two sites where incubation length was evaluated only using the V5 soil samplings due to missing preplant soil samples).

The effect of soil properties and weather conditions on the site-year to site-year differences in the effect of soil sample timing, N rate, and incubation length on $PMN_{an}$ were evaluated using covariate analysis in the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Soil properties, weather measurements, sample timing and N rate, incubation length, and their interactions were considered fixed effects with

**TABLE 4** Statistical analysis of fixed and random effects and their interactions for anaerobic potentially mineralizable N ($PMN_{an}$) across 32 site-years

| | Fixed effects | | | |
|---|---|---|---|---|
| **Covariance parameters** | **Numerator df** | **Denominator df** | **F-value** | **Pr > F** |
| Site | 31 | 96 | 14.3 | <.0001 |
| Sample timing and N rate (STNR) | 2 | 750 | 34.8 | <.0001 |
| Incubation length (Inc.) | 2 | 750 | 383.8 | <.0001 |
| Site × STNR | 60 | 750 | 6.6 | <.0001 |
| Site × Inc. | 62 | 750 | 3.9 | <.0001 |
| STNR × Inc. | 4 | 750 | 0.8 | 0.5200 |
| Site × STNR × Inc. | 120 | 750 | 0.5 | 1 |
| **Contrasts** | | | | |
| Preplant (PP) vs. V5 | 1 | 720 | 1.1 | 0.3000 |
| 0 kg N $ha^{-1}$ vs. 180 kg N $ha^{-1}$ | 1 | 750 | 45.8 | <.0001 |
| Site × (PP vs. V5) | 29 | 720 | 8.2 | <.0001 |
| Site × (0 kg N $ha^{-1}$ vs. 180 kg N $ha^{-1}$) | 31 | 480 | 4.7 | <.0001 |
| | Random effects | | | |
| | Estimate | Standard error | Z value | Pr > Z |
| Block (Site) | 44 | 8.1 | 5.4 | <.0001 |
| Residual | 106 | 5.5 | 19.4 | <.0001 |

block, site, and site by fixed effect interactions as random effects. This analysis method allowed us to determine what soil properties and weather conditions were likely responsible for the site-year to site-year variations of the effect of soil sample timing, N fertilization, and incubation length on $PMN_{an}$. The slope and intercept coefficients from regressing soil characteristics and weather measurements against each $PMN_{an}$ treatment combination were also determined using this covariate analysis. These coefficients were then used to determine the critical value of the soil or weather variables at which $PMN_{an}$ from the preplant sample timing became greater or less than the V5 sample timing where no N fertilizer was applied, and $PMN_{an}$ at V5 from the unfertilized soil became greater or less than the soil fertilized with 180 kg N $ha^{-1}$. The intercept and slope coefficients were also used to compare the effect of soil and weather variables on $PMN_{an}$ as incubation length increased from 7 to 14 and 28 d.

## 3 | RESULTS AND DISCUSSION

The wide range in soil properties and weather conditions (Table 1) across all sites prior to soil sample collection led to a wide range of $PMN_{an}$ values (0.2 to 137 mg N $kg^{-1}$) (Table 3). The 7 d $PMN_{an}$ incubation results of this study (0.7 to 100 mg N $kg^{-1}$) were similar to other reported $PMN_{an}$ values (12 to 87 mg N $kg^{-1}$) in Pennsylvania and western Oregon, USA (Christensen & Mellbye, 2006; Fox & Piekielek, 1984) and generally lower than $PMN_{an}$ values from Argentina (71 to 222 mg N $kg^{-1}$) (Reussi, Rozas, Echeverría, & Berardo, 2013). Lower mean $PMN_{an}$ in our study may be related to our overall smaller mean SOM value (25.7 g $kg^{-1}$) from a greater range of lower SOM values (7 to 71 g $kg^{-1}$) or deeper soil samples (30 cm) relative to the SOM values (44 to 68 g $kg^{-1}$) and shallower sampling depth (20 cm) of the Argentina study.

### 3.1 | Soil sample timing effect on $PMN_{an}$

The effect of soil sample timing on $PMN_{an}$ varied from site-year to site-year (Table 4). Time of soil sampling did not affect $PMN_{an}$ in 15 of the 30 sites evaluated (50%) (Figure 1; Supplemental Table S1). In the 15 sites where $PMN_{an}$ was affected by soil sample timing, eight sites (27%) had greater $PMN_{an}$ at V5 than preplant while in the other seven sites (23%), $PMN_{an}$ from preplant was greater than V5 (preplant vs. V5 contrast analysis, $P \leq .05$). Soil properties and early season weather conditions influenced the effect of soil sample timing on $PMN_{an}$, namely precipitation amount and evenness of distribution, temperature, C to N ratio, and V5 soil $NO_3$–N concentration (Figure 2). The strength of the relationships between $PMN_{an}$ from preplant and V5 with soil properties and early weather conditions shown in Figure 2 were significant but not strong (mean $R^2 = 0.05$). However, these relationships help determine how soil properties and weather conditions likely influenced the effect of sample timing on $PMN_{an}$.

Preplant and V5 $PMN_{an}$ were likely to be similar at sites that received approximately 183 mm of precipitation, rainfall distribution of 0.6 SDI or 115 AWDR, or accumulated

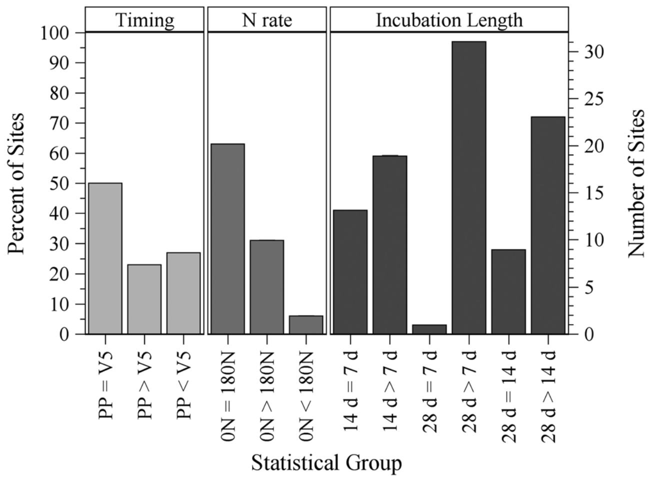


**FIGURE 1** Percentage and number of sites where anaerobic potentially mineralizable N ($PMN_{an}$) was affected ($P \leq .05$) by soil sample timing in the 0 kg N ha$^{-1}$ treatment (preplant [PP] vs V5), fertilizer-N rate applied at planting and soil sampled at V5 (0 [0N] vs. 180 kg N ha$^{-1}$ [180N]), and incubation length (7, 14, and 28 d) when averaged across all treatments

359 GDD between the preplant and V5 sample timings (Figure 2a–d). These were the critical values where (1) above these threshold values, $PMN_{an}$ from V5 tended to be greater than preplant, or (2) below these threshold values, $PMN_{an}$ from preplant tended to be greater than at V5. Greater than normal early season temperatures and more evenly distributed precipitation between the preplant and V5 soil samplings likely increased the breakdown of organic materials into more easily decomposable materials by the V5 sample timing (Cabrera, Kissel, & Vigil, 2005; Culman et al., 2013; Fernández et al., 2017; Goulding et al., 1998; Kuzyakov, Friedel, & Stahr, 2000; Ma & Wu, 2008). This greater abundance of easily decomposable material available at V5 sampling likely led to the increase of V5 $PMN_{an}$ over preplant $PMN_{an}$.

The C to N ratio and V5 soil $NO_3$–N concentration also influenced the effect of sample timing on $PMN_{an}$ (Figure 2e,f). The critical values where $PMN_{an}$ from preplant and V5 were similar were 9.7:1 for C to N ratio and 8.2 mg kg$^{-1}$ for V5 soil $NO_3$–N. Specifically, the $PMN_{an}$ from preplant tended to be greater than V5 at C to N ratios and V5 soil $NO_3$–N values above these critical values and $PMN_{an}$ from preplant tended to be greater than V5 below

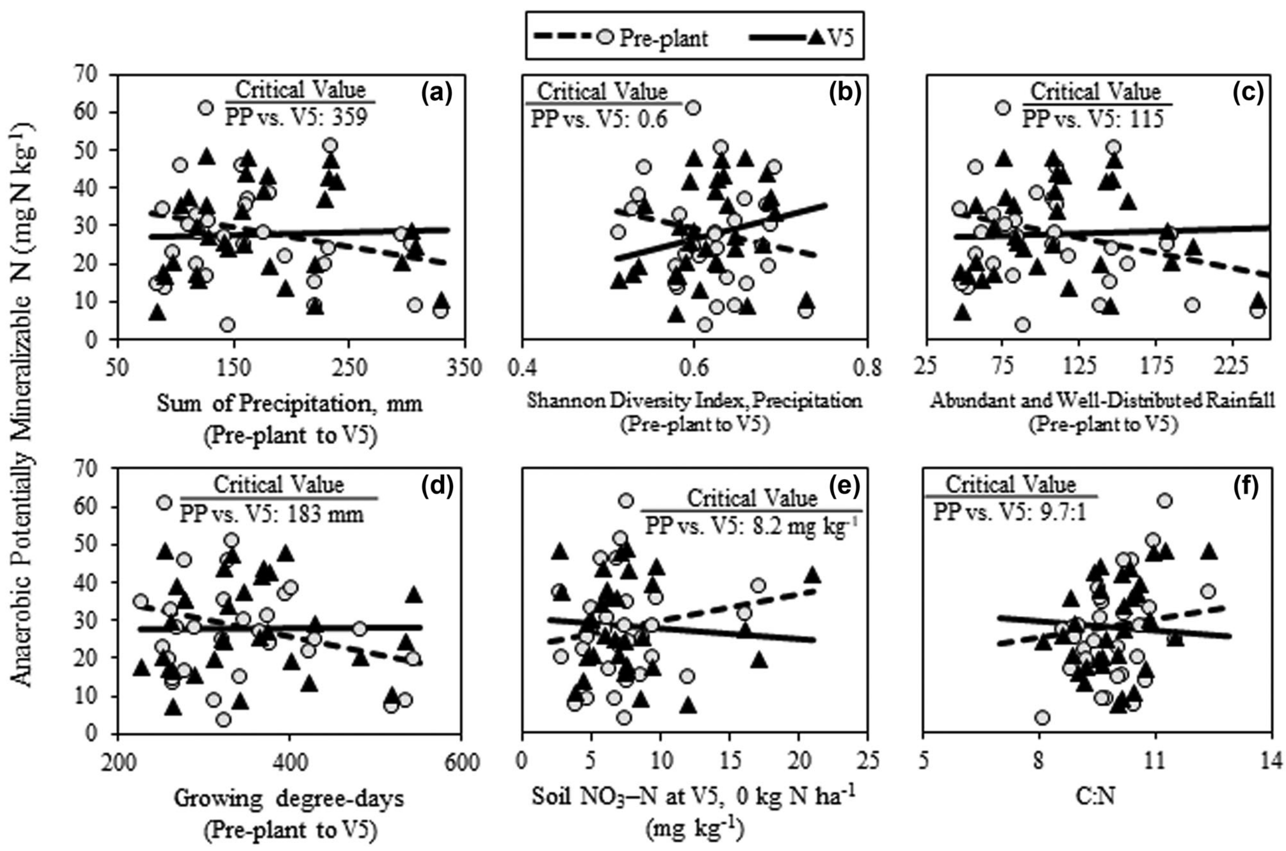


**FIGURE 2** Anaerobic potentially mineralizable N ($PMN_{an}$) from a 7-d incubation that was soil sampled before planting (PP) and at the V5 corn development stage as a function of soil properties (a) and weather conditions (b to e). Critical values represent the intersection point where $PMN_{an}$ from the preplant and V5 sample timing became greater or less than the other. Only those weather conditions and soil properties that had a significant interaction ($P \leq .05$) with soil sample timing were included

these critical values. These results indicate $PMN_{an}$ is not consistent throughout the growing season and that soil and weather conditions influence the effect soil sample collection timing has on $PMN_{an}$. Therefore, the timing of obtaining soil samples to complete $PMN_{an}$ analysis should remain consistent from year to year to make appropriate comparisons. In addition, because $PMN_{an}$ is sensitive to sample timing, further research is needed to determine the timing of soil sampling that best relates to crop N requirement before a standard protocol can be recommended.

## 3.2 | Nitrogen fertilization effect on $PMN_{an}$ at the V5 corn development stage

The effect of N fertilization on $PMN_{an}$ varied from site-year to site-year (Table 4). Nitrogen fertilization did not affect $PMN_{an}$ in 20 of the 32 sites evaluated (63%) (Figure 2; Supplemental Table S1). In the 12 sites where N fertilization affected $PMN_{an}$, 10 sites (31%) had greater $PMN_{an}$ from unfertilized compared to fertilized soil while in the other two sites (6%), $PMN_{an}$ from fertilized soil was greater than unfertilized soil (0 vs. 180 kg N $ha^{-1}$ applied at planting and soil sampled at V5 contrast analysis, $P \leq .05$). These results indicate that N fertilization does not consistently influence $PMN_{an}$ and when it does, it most often reduces $PMN_{an}$. The variable effect of N fertilization on $PMN_{an}$ in this study is similar to the findings of others (Fernández et al., 2017; Kuzyakova et al., 2006; Ma et al., 1999). Furthermore, soil properties influenced the effect of N fertilization on $PMN_{an}$, namely total C, total organic C, SOM, C to N, clay content, and V5 soil $NO_3$–N concentration (Figure 3). The strength of the relationships between $PMN_{an}$ from fertilized and unfertilized soil with soil properties shown in Figure 3 were significant but not strong (mean $R^2 = 0.16$). However, similar to the N timing evaluations, these relationships help determine how soil properties likely influenced the effect of N fertilization on $PMN_{an}$.

The $PMN_{an}$ from unfertilized soil was generally greater than fertilized soil at sites with low amounts of total C, total organic C, SOM, or C to N ratio (Figure 3a–d). The reduction in $PMN_{an}$ from fertilized relative to unfertilized soil is likely the result of the N fertilizer stimulating mineralization of the labile organic matter in the soil and depleting the amount of SOM available for mineralization by the V5 sample timing (Chen et al., 2014; Conde et al., 2005; Hamer & Marschner, 2005; Kuzyakov et al., 2000). The differences in $PMN_{an}$ due to N fertilization became less pronounced as total C, total organic C, SOM, or C to N ratio increased toward the high end of the range measured across the sites. The similarity in $PMN_{an}$ values from fertilized and unfertilized soil with these characteristics is likely the result of a reduction in the stimulation of N mineralization from the addition of N fertilizer as soil C content increased, as reported in other studies (Chen et al., 2014; Conde et al., 2005). Since only two sites had statistically greater $PMN_{an}$ from fertilized compared to unfertilized soils, it is difficult to establish what soil parameters or critical values may help explain this response. We observed only a trend, suggesting that $PMN_{an}$ from fertilized relative to unfertilized soil became greater when total C, total organic C, SOM, or the C to N ratio increased above 24.2 g $kg^{-1}$, 21.1 g $kg^{-1}$, 37.9 g $kg^{-1}$, 11.0:1, respectively.

The clay content and V5 soil $NO_3$–N concentrations also influenced the similarities and differences between $PMN_{an}$ from unfertilized and fertilized soil (Figure 3e,f). The $PMN_{an}$ from unfertilized relative to fertilized soil was generally greater at those sites with the greatest amounts of clay content and V5 soil $NO_3$–N concentrations. The $PMN_{an}$ from unfertilized and fertilized soils became similar as clay content and V5 soil $NO_3$–N decreased toward the low end of the range measured in our study. These results indicate N fertilization can affect $PMN_{an}$ and that soil properties influenced the effect N fertilizer application has on $PMN_{an}$. Therefore, soil samples collected for $PMN_{an}$ analysis should always be obtained before or after N fertilization from year to year to make appropriate comparisons. In addition, because $PMN_{an}$ is sensitive to N fertilization, further research is needed to determine whether $PMN_{an}$ from fertilized or unfertilized soil best relates to crop N requirements before a standard protocol can be recommended.

## 3.3 | Incubation length effect on $PMN_{an}$

Extending the incubation length beyond 7 d generally increased $PMN_{an}$ at all sites (Figure 1; Supplemental Table S1), which is similar to the findings of Angus et al. (1994). The magnitude of the increase in $PMN_{an}$ with longer incubations varied from site to site (Table 4), depending on soil properties such as silt and clay content, cation exchange capacity, total C, total organic C, SOM, total N, or preplant $NH_4$–N concentration (30-cm depth) (Table 5). The greater $PMN_{an}$ from longer incubations increased (greater slope and intercept values) as these soil properties increased across the sites. In contrast to this result, $PMN_{an}$ increased at a reduced rate (reduced slope but greater intercept values) with longer incubation lengths as sand content or bulk density values increased across the sites. Precipitation and temperature did not impact the effect of incubation length on $PMN_{an}$. Cation exchange capacity, total C, total organic C, SOM, total N, and bulk density were the soil measurements that interacted with incubation length and accounted for the greatest variation in $PMN_{an}$ (mean F-value of 13) (Table 5). These soil properties also reduced the estimate of variance the most for site (mean decrease = 73) and the site by incubation length interaction (mean decrease = 12) (Supplemental Table S3). All other significant interactions between incubation length and soil variables had a weaker influence on $PMN_{an}$ (mean F-value of 4.7) (Table 5). These results indicate that cation exchange

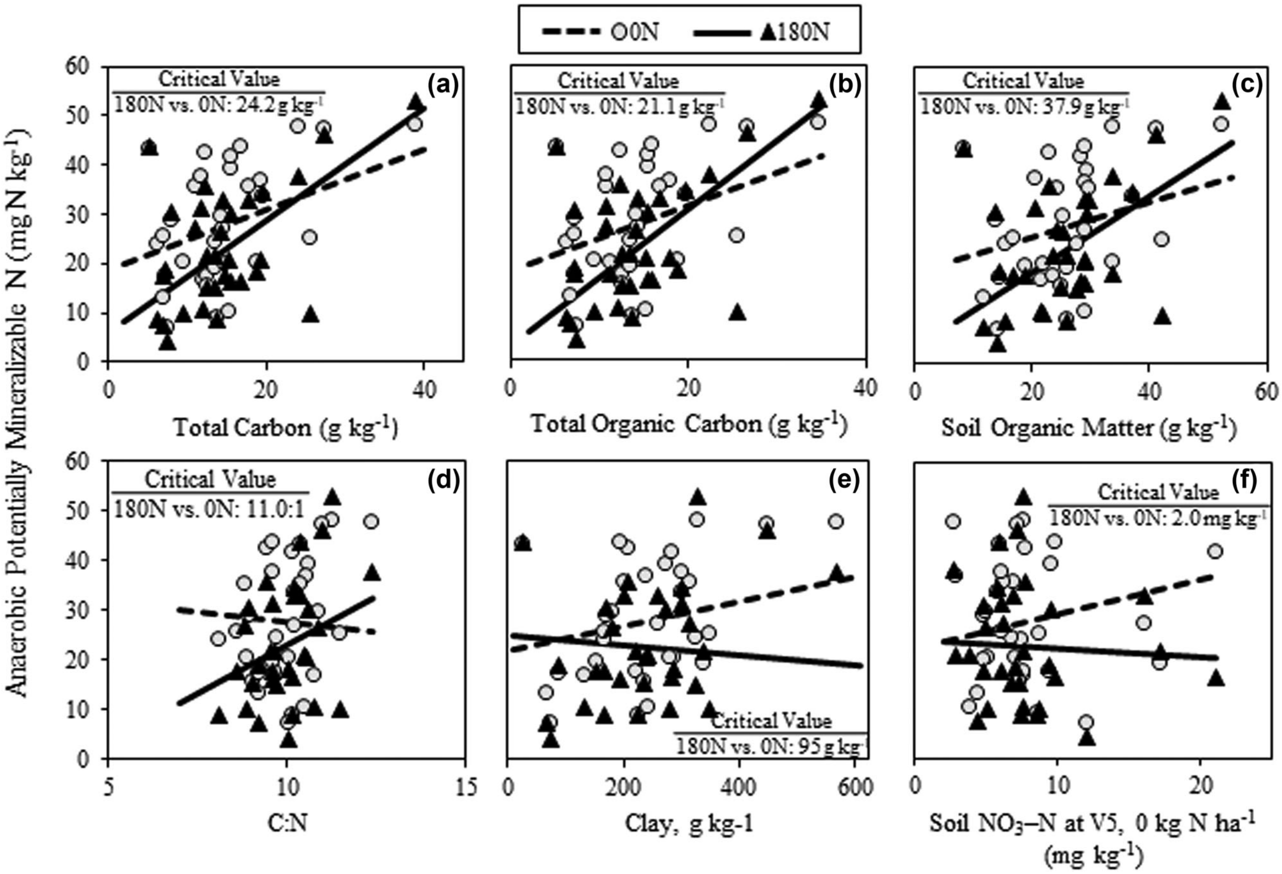


**FIGURE 3** Anaerobic potentially mineralizable N ($PMN_{an}$) from a 7-d incubation that was soil sampled at the V5 corn development stage where 0 (0N) or 180 kg N $ha^{-1}$ (180N) was applied as a function of soil properties (a to f). Critical values represent the intersection point where $PMN_{an}$ from the unfertilized and fertilized soil became greater or less than the other. Only those soil properties that had a significant interaction ($P \leq .05$) with N fertilization were included

**TABLE 5** Change in slope and intercept of anaerobic potentially mineralizable N ($PMN_{an}$) as a function of soil properties when incubation length increased from 7 to 14 and 7 to 28 d. Only those soil properties that had a significant interaction ($P \leq .05$) with incubation length were included

| | Change in slope coefficient from 7-d incubation | | Change in intercept from 7-d incubation | | |
|---|---|---|---|---|---|
| Variable[a] | 14 d | 28 d | 14 d | 28 d | F-value |
| Sand, g $kg^{-1}$ | −0.01[b] | −0.02* | +12* | +27* | 9* |
| Silt, g $kg^{-1}$ | +0.01 | +0.02* | +4 | +10* | 4* |
| Clay, g $kg^{-1}$ | +0.02 | +0.05* | +6 | +10* | 8* |
| BD-measured, g $cm^{-3}$ | −8.23 | −33.50* | +21 | +67* | 9* |
| BD-Saxton, g $cm^{-3}$ | −19.89* | −51.73* | +36* | +90* | 16* |
| TC, g $kg^{-1}$ | +0.23 | +0.62* | +6* | +12* | 8* |
| TOC, g $kg^{-1}$ | +0.28 | +0.79* | +6* | +10* | 11* |
| SOM, g $kg^{-1}$ | +0.22 | +0.60* | +4 | +5 | 14* |
| TN, g $kg^{-1}$ | +3.48 | +9.28* | +5 | +8* | 11* |
| CEC, $cmol_c$ $kg^{-1}$ | +0.22 | +0.63* | +5 | +9* | 10* |
| $PP_{0N}$ $NH_4$–N, 0–30 cm | +0.14 | +1.00* | +8* | +12* | 4* |

*Significant at $P \leq .05$.

[a]BD, bulk density; TC, total carbon; TOC, total organic carbon; SOM, soil organic matter; TN, total nitrogen; CEC, cation exchange capacity.

[b]Change in slope coefficient and intercept when moving from 7 to 14 or 28 d of incubation. (Sand content example: 7 d $PMN_{an}$ = (slope coefficient)(sand content) + intercept. When $PMN_{an}$ incubation length moves from 7 to 14 d, the slope coefficient decreases by 0.01 and the intercept increases by 12.

capacity, total C, total organic C, SOM, total N, and bulk density were likely the soil properties that were driving most of the differences in the increase of $PMN_{an}$ with longer incubations from site to site.

## 4 | CONCLUSIONS

Soil properties (especially cation exchange capacity, soil C content, SOM, total N, and bulk density) and early season weather conditions (especially evenness of early season precipitation) had a large influence on the effect of soil sample timing, N fertilizer application, and incubation length on $PMN_{an.}$ Therefore, careful consideration as to the time of soil sampling, N fertilization status, and incubation length should be made when comparing $PMN_{an}$ values among sites. Producers and scientists should follow a consistent protocol when obtaining soil samples and analyzing them for $PMN_{an}$ to make comparisons related to N mineralization capacity of soils and for guiding fertilizer-N rates. There are tradeoffs with the sampling methodologies and incubation lengths evaluated in this study. For example, commercial soil testing labs may not want to incubate soil samples for 28 d because they prefer high-throughput analytical methods that reduce costs and provide rapid results to producers. Therefore, a better understanding of the relationship between crop N availability, N uptake, yields, and $PMN_{an}$ from these different soil sampling methodologies and incubation lengths are needed before we can determine the protocol that best relates to crop N management.

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## ORCID

*Jason D. Clark* https://orcid.org/0000-0001-7793-6411
*Kristen S. Veum* https://orcid.org/0000-0002-6492-913X
*David W. Franzen* https://orcid.org/0000-0003-4862-8086
*Daniel E. Kaiser* https://orcid.org/0000-0002-8478-701X
*John E. Sawyer* https://orcid.org/0000-0003-4080-9616

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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**Soil Sample timing, Nitrogen Fertilization, and Incubation Length Influence Anaerobic Potentially Mineralizable Nitrogen**

## SUPPLEMENTAL MATERIAL

Supplemental material includes two detailed tables containing the effect of soil sample timing, fertilizer-N rate, and incubation length on $PMN_{an}$ for each of the 32 site-years and one comparing the significance levels of site and its interactions with fixed variables as soil and weather parameters were individually added as fixed effects.

Supplemental Table S1. Mean comparisons of anaerobic potentially mineralizable N ($PMN_{an}$) affected by soil sample timing and N rate and incubation length of 32 site-years.

| | | | Sample timing and N rate† | | | Incubation length‡ | | |
|---|---|---|---|---|---|---|---|---|
| Year | State | Site§ | $PP_{0N}$¶ | $V5_{0N}$# | $V5_{180N}$†† | 7-d | 14-d | 28-d |
| | | | mg kg$^{-1}$ | | | | | |
| 2014 | IA | Ames14 | 22.0a[e] | 17.7ab | 12b | 10.8b | 16.7b | 24.2a |
| 2014 | IA | MasonCity14 | 36.7a | 31.5a | 29.8a | 20.4b | 29.5b | 48.0a |
| 2014 | IL | Brownstown14 | 15.3b | 37.0a | 18.7b | 12.1c | 23.3b | 35.6a |
| 2014 | IL | Urbana14 | 49.8a | 58.4a | 47.4a | 32.5c | 51.8b | 71.3a |
| 2014 | IN | Loam14 | 18.5a | 20.9a | 11.8b | 13.6b | 17.8a | 19.8a |
| 2014 | IN | Sand14 | 18.5a | 11.1b | 8.3b | 8.7b | 13.3ab | 15.9a |
| 2014 | MN | NewRichland14 | 73.0a | 64.9a | 67.8a | 48.0b | 57.1b | 81.4a |
| 2014 | MN | StCharles14 | 46.8a | 25.4b | 29.2b | 24.6b | 30.5b | 46.2a |
| 2014 | MO | Bay14 | 38.2a | 31.9a | 16.1b | 19.2b | 29.9a | 37.7a |
| 2014 | MO | Troth14 | 28.0b | 33.9a | 35.6a | 27.8c | 33.1b | 36.4a |
| 2014 | ND | Amenia14 | 28.1b | 46.4a | 31.9b | 25.6c | 36.3b | 44.4a |
| 2014 | ND | Durbin14 | 48.3a | 55.9a | 53.7a | 41.5c | 52.6b | 64.5a |
| 2014 | NE | Brandes14 | 30.1a | 21.3b | 13.8c | 14.2c | 21.9b | 29.0a |
| 2014 | NE | SCAL14 | 53.4a | 33.2b | 36b | 26.4c | 42.5b | 53.7a |
| 2014 | WI | Steuben14 | 57.7a | 44.2b | 42.3b | 38.0c | 48.2b | 58.0a |
| 2014 | WI | Wauzeka14 | 45.4a | 25.8b | 27.1b | 19.7b | 36.1a | 42.5a |
| 2015 | IA | Boone15 | 17.3b | 17.0b | 31.2a | 12.9b | 19.2b | 33.4a |
| 2015 | IA | Lewis15 | 14.1b | 33.1a | 19.1b | 16.0b | 21.4ab | 28.8a |
| 2015 | IL | Brownstown15 | 35.4a | 36.0a | 23.2b | 23.5b | 32.6a | 38.6a |
| 2015 | IL | Urbana15 | 40.3a | 32.7a | 38.1a | 30.4b | 38.7ab | 41.9a |
| 2015 | IN | Loam15 | 43.9a | 34.8b | 30.8b | 29.7c | 36.4b | 43.4a |
| 2015 | IN | Sand15 | 25.1a | 22.1a | 22.9a | 18.6c | 23.5b | 28.0a |
| 2015 | MN | NewRichland15 | 40.7a | 37.6a | 33a | 20.0c | 36.7b | 54.6a |
| 2015 | MN | StCharles15 | 32.0b | 48.7a | 42.4ab | 34.1b | 40.5ab | 48.5a |
| 2015 | MO | LoneTree15 | 25.6b | 44.0a | 36.7ab | 26.4a | 37.8a | 42.0a |
| 2015 | MO | Troth15 | 38.3b | 44.3a | 37.7b | 33.1c | 41.5b | 45.7a |
| 2015 | ND | Amenia15 | 15.2b | 25.7a | 26.6a | 15.4b | 21.2b | 31.0a |
| 2015 | ND | Durbin15 | 73.4a | 60.1ab | 56.9b | 48.1b | 58.0b | 84.3a |
| 2015 | NE | Brandes15[f] | - | 45.9b | 49.1a | 43.5b | 48.5a | 50.5a |
| 2015 | NE | SCAL15[f] | - | 59.0a | 41.2b | 29.1c | 45.0b | 76.2a |
| 2015 | WI | Belmont15 | 52.5a | 54.4a | 21.5b | 31.9b | 42.0b | 54.6a |
| 2015 | WI | Darlington15 | 69.8a | 58.9a | 60.9a | 37.9c | 59.5b | 92.2a |

† The $PMN_{an}$ values were averaged across the three incubation lengths when comparing soil sample timing and N rates.

‡ The $PMN_{an}$ values were averaged across soil sample timing and N rates when comparing incubation lengths.

§ Site name is name of site followed by last two digits of year.

¶ $PP_{0N}$, $PMN_{an}$ from pre-plant soil sampling with 0 kg N ha$^{-1}$ $V5_{0N}$, $PMN_{an}$ from V5 corn development stage with 0 kg N ha$^{-1}$ $V5_{180N}$, $PMN_{an}$ from V5 corn development stage with 180 kg N ha$^{-1}$ applied at planting.

# Within row of sample timing and N rate and incubation length variables, means followed by the same letter are not different ($P \geq 0.05$).

†† Comparisons of soil sample timing $PMN_{an}$ values were not available for the Brandes15 and SCAL15 sites and their incubation length comparisons were made only with the $V5_{0N}$ and $V5_{180N}$ soil sample timing and N rates.

Supplemental Table S2. Comparison of the random effects on anaerobic potentially mineralizable N ($PMN_{an}$) and their significance level when site and its interactions were evaluated as random effects and their change when soil properties, precipitation, and temperature variables were added as individual fixed effects. Only those soil and weather variables that had a significant interaction ($P \leq 0.05$) with sample timing and N rate (STNR) or incubation length (Inc.) were included.

| | Variance estimates of random effects | | | | | | |
|---|---|---|---|---|---|---|---|
| Soil/Weather Variable (SW)† | Site | Site (Block) | Site* STNR | Site* Inc. | Site* STNR*Inc. | Residual | AIC |
| No SW | 158 | 44 | 54 | 32 | -13 | 106 | 8868 |
| **Soil Physical Characteristics** | | | | | | | |
| Sand | 127 | 40 | 55 | 24 | -13 | 106 | 8843 |
| Silt | 113 | 46 | 63 | 23 | -13 | 105 | 8842 |
| Clay | 160 | 39 | 56 | 29 | -13 | 106 | 8856 |
| BD-measured | 108 | 35 | 56 | 21 | -13 | 107 | 8783 |
| BD-Saxton | 74 | 39 | 58 | 18 | -13 | 106 | 8766 |
| **Soil Chemical Characteristics** | | | | | | | |
| TC | 79 | 35 | 60 | 22 | -13 | 105 | 8807 |
| TOC | 76 | 35 | 64 | 20 | -13 | 104 | 8797 |
| SOM | 86 | 37 | 60 | 17 | -13 | 106 | 8807 |
| TN | 78 | 34 | 59 | 19 | -13 | 106 | 8779 |
| C:N | 136 | 44 | 55 | 30 | -13 | 105 | 8824 |
| CEC | 91 | 43 | 57 | 22 | -13 | 106 | 8832 |
| pH-water | 157 | 45 | 54 | 32 | -13 | 106 | 8841 |
| **Inorganic Nitrogen** | | | | | | | |
| $PP_{0N}$ $NH_4$–N 30 cm | 139 | 45 | 53 | 24 | -14 | 107 | 8461 |
| $V5_{0N}$ $NH_4$–N 30 cm | 151 | 44 | 55 | 32 | -13 | 106 | 8853 |
| $V5_{180N}$ $NH_4$–N 30 cm | 162 | 44 | 55 | 31 | -13 | 106 | 8857 |
| $V5_{0N}$ $NO_3$–N 30 cm | 154 | 44 | 61 | 31 | -13 | 104 | 8846 |
| $V5_{0N}$ $NO_3$–N 60 cm | 156 | 45 | 58 | 33 | -13 | 109 | 8179 |
| **Precipitation and Temperature**‡ | | | | | | | |
| Sum | 163 | 44 | 52 | 33 | -13 | 106 | 8881 |
| SDI | 165 | 44 | 47 | 33 | -13 | 106 | 8806 |
| AWDR | 163 | 44 | 50 | 33 | -13 | 106 | 8875 |
| GDD | 160 | 44 | 52 | 33 | -13 | 106 | 8882 |

* All values were significant at $P \leq 0.05$.

† AIC, Akaike information criterion; CEC, cation exchange capacity; BD, bulk density; BD-Saxton; SDI, Shannon diversity index; AWDR, Abundant and well-distributed rainfall; GDD, growing-degree-day; $PMN_{an}$ Anaerobic potentially mineralizable N; $PP_{0N}$, $PMN_{an}$ from preplant soil sampling with 0 kg N $ha^{-1}$; $V5_{0N}$, $PMN_{an}$ from V5 corn development stage with 0 kg N $ha^{-1}$; $V5_{180N}$, $PMN_{an}$ from V5 corn development stage with 180 kg N $ha^{-1}$ applied at planting.

‡ Weather variables used were calculated from the preplant to V5 period.