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# Predicting Economic Optimal Nitrogen Rate with the Anaerobic Potentially Mineralizable Nitrogen Test

Jason D. Clark
*South Dakota State University*, jason.d.clark@sdstate.edu

Fabian G. Fernandez
*University of Minnesota - Twin Cities*

Kristen S. Veum
*USDA, Agricultural Research Service*

James J. Camberato
*Purdue University*

Paul R. Carter
*DuPont Pioneer*

***See next page for additional authors***

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

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## ABSTRACT

Estimates of mineralizable N with the anaerobic potentially mineralizable N ($PMN_{an}$) test could improve predictions of corn (*Zea mays* L.) economic optimal N rate (EONR). A study across eight US midwestern states was conducted to quantify the predictability of EONR for single and split N applications by $PMN_{an}$. Treatment factors included different soil sample timings (pre-plant and V5 development stage), planting N rates (0 and 180 kg N ha$^{-1}$), and incubation lengths (7, 14, and 28 d) with and without initial soil $NH_4$–N included with $PMN_{an}$. Soil was sampled (0–30 cm depth) before planting and N application and at V5 where 0 or 180 kg N ha$^{-1}$ were applied at planting. Evaluating across all soils, $PMN_{an}$ was a weak predictor of EONR ($R^2 \leq 0.08$; RMSE, ≥67 kg N ha$^{-1}$), but the predictability improved (15%) when soils were grouped by texture. Using $PMN_{an}$ and initial soil $NH_4$–N as separate explanatory variables improved EONR predictability (11–20%) in fine-textured soils only. Delaying $PMN_{an}$ sampling from pre-plant to V5 regardless of N fertilization improved EONR predictability by 25% in only coarse-textured soils. Increasing $PMN_{an}$ incubations beyond 7 d modestly improved EONR predictability ($R^2$ increased ≤0.18, and RMSE was reduced ≤7 kg N ha$^{-1}$). Alone, $PMN_{an}$ predicts EONR poorly, and the improvements from partitioning soils by texture and including initial soil $NH_4$–N were relatively low ($R^2 \leq 0.33$; RMSE ≥ 68 kg N ha$^{-1}$) compared with other tools for N fertilizer recommendations.

## Core Ideas

- Anaerobic potentially mineralizable N ($PMN_{an}$) is a weak predictor of economic optimal N rate (EONR).
- Predictability of EONR by $PMN_{an}$ improves when accounting for soil texture.
- For coarse-textured soils, $PMN_{an}$ at V5 improves EONR predictability.
- Increasing incubation length does not substantially improve EONR predictability.
- $PMN_{an}$ alone is not a reliable management tool for N rate determination.

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Nitrogen is the nutrient that most often limits grain yield and needs to be supplied by application of fertilizer, manures, or other nutrient sources. In commercial agriculture, the two main sources of N for corn are N derived from mineralization of soil organic matter and N derived from synthetic N fertilizers (Mikha et al., 2006; O'Leary et al., 2002). Corn plants normally take up 110 (25% quartile) to 225 kg N ha$^{-1}$ (75% quartile) (mean, 170 kg N ha$^{-1}$) during the growing season (Ciampitti and Vyn, 2012). The process of mineralization can contribute 20 to 100% of this yearly crop N requirement, with the remainder needing to be supplied by another source, such as synthetic fertilizers (Broadbent and Hauck, 1984; Khan et al., 2001; Roberts et al., 2011; Ros et al., 2011; Yost et al., 2012). However, predicting the amount of N that will be mineralized, to aid in determining how much N fertilizer to apply, is challenging because this process is influenced by many factors that are difficult to predict. For example, weather is an influential factor for which we have no control and poses a substantial challenge for proper N management. Nitrogen management guidelines could be greatly improved by increasing our ability to estimate the contribution of N to a corn crop from mineralization.

The daily N mineralization rate can be as high as 10 kg N ha$^{-1}$, with an average of 1.19 kg N ha$^{-1}$ in the early part of the growing season (Fernández et al., 2017). However, this mineralization rate decreases as the season progresses while N uptake by the crop increases (Ritchie et al., 1996). If there is

J.D. Clark, South Dakota State Univ., 1148 Medary Ave., Brookings, SD 57007; F.G. Fernández, D.E. Kaiser, C.J. Rosen, Univ. of Minnesota, 1991 Upper Buford Circle, St. Paul, MN 55108; K.S. Veum, N.R. Kitchen, USDA–ARS Cropping Systems and Water Quality Research Unit, Columbia, MO 65211; J.J. Camberato, Purdue Univ., Lilly 3-365, West Lafayette, IN 47907; P.R. Carter, DuPont Pioneer, 7100 NW 62nd Ave., P.O. Box 1000, Johnston, IA 50131; D.W. Franzen, North Dakota State Univ., PO Box 6050, Fargo, ND 58108; R.B. Ferguson, Univ. of Nebraska, Keim 367, Lincoln NE 68583; C.A.M. Laboski, Univ. of Wisconsin-Madison, 1525 Observatory Dr., Madison, WI 53706; E.D. Nafziger, Univ. of Illinois, W-301 Turner Hall, 1102 S. Goodwin, Urbana, IL 61801; J.E. Sawyer, Iowa State Univ., 3208 Agronomy Hall, Ames, IA 50011; J.F. Shanahan, Fortigen, 6807 Ridge Rd, Lincoln, NE 68512. Received 25 Mar. 2019. Accepted 17 June 2019.
*Corresponding author (Jason.D.Clark@sdstate.edu).

**Abbreviations:** EONR, economic optimal N rate; $EONR_{single}$, economic optimal N rate using a single N application; $EONR_{split}$, economic optimal N rate using a split N application; $NH_4$–$N_{inc}$, $NH_4$–N after the soil was incubated for 7, 14, or 28 d; $NH_4$–$N_{initial}$, initial $NH_4$–N in the soil; $PMN_{an}$, anaerobic potentially mineralizable N; $PP_{0N}$, pre-plant soil sampling with 0 kg N ha$^{-1}$ applied at planting; $V5_{0N}$, V5 soil sampling with 0 kg N ha$^{-1}$ applied at planting; $V5_{180N}$, V5 soil sampling with 180 kg N ha$^{-1}$ applied at planting.

not enough N in the soil from mineralization, then N fertilizer is needed for optimal grain yield to be achieved. However, the N use efficiency of corn and profitability for the grower decreases when N fertilizer is overapplied. Excess N fertilizer is also susceptible to environmental losses that can cause negative environmental effects, including contamination of drinking water, eutrophication of surface waters (Helmers et al., 2012; McCasland et al., 2012; Mitsch et al., 2001; Ribaudo et al., 2011), reduced air quality, and global warming (Cavigelli et al., 2012; USEPA, 2018). Conversely, corn grain yield and grower profit are reduced if insufficient N is applied. Despite these significant consequences, the rate of N fertilizer applied to corn fields in the US Midwest is often determined by N guideline tools developed by Land Grant Universities that do not include input for estimates of potential soil N mineralization but rather indirectly include mineralization effects on optimal N rate. Such N management tools include the pre-sidedress soil nitrate test (Andraski and Bundy, 2002; Binford et al., 1992; Fox et al., 1989; Magdoff et al., 1984), the maximum return to N approach (Sawyer et al., 2006), or yield goal formula (Lory and Scharf, 2003; Stanford, 1973). Public and private model approaches, such as HybridMaize (Yang et al., 2004), Encirca (DuPont Pioneer Johnston, IA), Climate FieldView (The Climate Corp., St. Louis, MO), and Adapt-N (Yara International ASA Oslo, Norway), are being evaluated and include estimates of mineralization and other soil processes. The strength and weaknesses of a number of these tools were recently compared (Morris et al., 2018). Although these approaches and tools differ, better predictability of mineralization could help improve N management tools that result in improved N fertilizer use efficiency, increased grower profits, and reduced environmental impact.

Many field and laboratory tests have been developed that measure N mineralization (Bundy and Meisinger, 1994; Hart, 1994; Kolberg et al., 1997; Raison et al., 1987; Stanford and Smith, 1972), each with its advantages and disadvantages. The anaerobic potentially mineralizable N ($PMN_{an}$) test has been considered the most promising because of its ease of use and reliability (Waring and Bremner, 1964). The $PMN_{an}$ test is simple and rapid because it can be conducted with both air-dried or field-moist soils, no amendments or preliminary analyses are needed to determine the amount of water required for incubation, and only $NH_4$–N measurements are needed (Keeney and Bremner, 1966). The $PMN_{an}$ test also does not require aeration of samples or long incubation periods because mineralization is more rapid in anaerobic conditions (Waring and Bremner, 1964). These advantages make the $PMN_{an}$ test a potentially useful N management tool to account for N derived from mineralization.

Additional work is needed to contribute to the limited number of studies that have looked at improving predictions of crop responses by including $PMN_{an}$ as a predictive variable. Some of the findings to this point include the 7-d $PMN_{an}$ test correlating with N uptake of ryegrass (*Lolium multiflorum*) (Keeney and Bremner, 1966) and rice (*Oryza sativa* L.) (Angus et al., 1994). The response of winter wheat (*Triticum aestivum* L.) to different N rates has also been well correlated ($R^2 = 0.87$) with $PMN_{an}$ (Christensen et al., 1999). However, in relating $PMN_{an}$ to economic optimal N rate (EONR) of corn, the correlations have been much weaker ($R^2 = 0.33$) (Williams et al., 2007). Potential ways to improve these correlations may be accomplished by including initial $NH_4^+$ in the soil with $PMN_{an}$ and by grouping soils by their geographic location because including these factors have improved the correlations between $PMN_{an}$ and aerobic mineralization (Bushong et al., 2007; Mariano et al., 2013). Grouping soils in the southeastern United States by their physical characteristics has also led to improvements in the correlations between $PMN_{an}$ and corn EONR (Williams et al., 2007). However, studies are lacking on whether similar improvements occur in the US Midwest by grouping soils by soil physical properties and/or including initial soil $NH_4$–N. Other important variables that may improve the correlation of $PMN_{an}$ to EONR, such as time of soil sampling, sampling soil after N application, and increasing the incubation length, should be explored.

The most common soil sample timing used for $PMN_{an}$ analysis is within 2 wk of planting. In the US Midwest, N mineralized early in the season is susceptible to loss due to limited corn N uptake and excessive rainfall. For example, early spring rainfall in Minnesota results in >60% of the annual water drainage and $NO_3$–N lost to subsurface drainage or leached below the root zone (MPCA, 2013; Randall and Vetsch, 2005; Randall et al., 2003a, 2003b; Struffert et al., 2016). Delaying $PMN_{an}$ soil sampling to closer to when corn N uptake increases and N loss potential (from mineralization and fertilizer) decreases might improve the correlation between EONR and $PMN_{an}$. Others have reported that soil sample timing can affect results of N-mineralization indices (Arrobas et al., 2012; Clark et al., 2019; Culman et al., 2013). The predictability of EONR with mineralizable N estimates from later soil samplings have not been evaluated.

Measuring $PMN_{an}$ before fertilizer applications can pose difficulties. These difficulties occur because N fertilizer can decrease N mineralization from soil organic matter and stimulate crop residue decomposition, which results in greater amounts of N mineralization (Chen et al., 2014; Conde et al., 2005; Hamer and Marschner, 2005; Kuzyakov et al., 2000; Raun et al., 1998; Steinbach et al., 2004). This potential increase in N mineralization from the N fertilizer application might reduce the predictability of EONR with the $PMN_{an}$ test when sampling is performed before fertilizer application. Nitrogen fertilizer applications have reduced $PMN_{an}$ of in-season soil samples in some sites and increased it in others relative to $PMN_{an}$ measured before fertilization, depending on soil and weather conditions (Clark et al., 2018).

The relationship of $PMN_{an}$ after N fertilization has not yet been related to EONR. Applying N as a single pre-plant application or splitting it with some N applied pre-plant and the rest while corn is growing can result in changes in EONR (Gehl et al., 2005; Kablan et al., 2017; Rasse et al., 1999; Tremblay et al., 2012; Walsh et al., 2012; Xie et al., 2013). These differences in EONR due to N application timing may also affect the predictability of EONR with $PMN_{an}$. The relationship between $PMN_{an}$ and EONR of single N applications has been evaluated in the climates of the northeastern and southeastern United States (Fox and Piekielek, 1984; Williams et al., 2007) but not in the midwestern United States. Furthermore, work is lacking relating $PMN_{an}$ to EONR of split N applications.

A 7-d incubation has been part of the standard method when relating $PMN_{an}$ to EONR. However, extending the incubation length of the $PMN_{an}$ test may improve the correlation with EONR. There is evidence that extending the incubation length beyond 7 d increases $PMN_{an}$ more as the silt, clay, and soil organic matter content increases and that correlations between $PMN_{an}$ and soil and weather parameters are stronger with longer incubation lengths (Clark et al., 2019). These varying amounts of greater $PMN_{an}$ from longer incubation lengths, depending on soil physical properties, may help separate the ability of different soils to supply N to corn and increase the predictability of EONR with $PMN_{an}$. For example, there was an improvement in the correlation between $PMN_{an}$ and biomass and N uptake of rice when incubation length was extended to 21-d in Australia (Russell et al., 2006). Studies relating EONR to $PMN_{an}$ from incubations longer than 7 d are lacking for corn in the US Midwest.

Given these points and the need to improve corn EONR predictions, research was conducted (i) to evaluate the $PMN_{an}$ test as a tool to predict EONR of single and split N applications across varying soil and weather conditions in the US Midwest and (ii) to determine the effect of different variables (soil sample timings, N fertilizer rates, incubation lengths, soil texture, and initial soil $NH_4$–N) on improving the prediction of EONR with the $PMN_{an}$ test.

## MATERIALS AND METHODS

### Experimental Design

This study was conducted in the following US Midwest states: Illinois, Indiana, Iowa, Minnesota, Missouri, Nebraska, North Dakota, and Wisconsin. Two experimental sites were established in each state in 2014 and 2015, resulting in 32 site-years of data. Detailed descriptions of experimental sites, agronomic practices, and research protocol are provided in Kitchen et al. (2017). Briefly, a standard protocol for the experimental design was used across all experimental sites that included N fertilizer source, rate, and application timing; plant and soil sample collection method and timing; and weather data collection. A randomized complete block design was used with four replications at each site. Eight N rates (0–315 kg N $ha^{-1}$ in 45 kg N $ha^{-1}$ increments) were applied as single N applications or split N applications to establish grain yield response curves. Single N applications were performed at planting, and split applications included 45 kg N $ha^{-1}$ applied at planting with the remainder applied at the V9 ± 1 corn development stage (2015 North Dakota sites received N between V5 and V8) (Ritchie et al., 1996). Ammonium nitrate (34% N) was broadcast on the soil surface.

### Soil Sampling and Analysis

At each site, a soil characterization to 120 cm was performed before planting. Soil cores were divided by horizons and evaluated for soil texture, total N, and soil organic matter as described in Kitchen et al. (2017). Weighted averages were calculated for the various soil measurements using the depth of each horizon within the 0- to 30-cm soil depth. Additionally, each year, a 10-core composite soil sample from each replication was obtained 2 to 4 wk before planting and fertilization [pre-plant soil sampling with 0 kg N $ha^{-1}$ applied at planting ($PP_{0N}$)], and a six-core (1.9 cm i.d.) composite soil sample was taken at the V5 ± 1 corn development stage from the 0 ($V5_{0N}$) and 180 ($V5_{180N}$) kg N $ha^{-1}$ treatments. These soil samples were obtained to a depth of 30 cm and dried (≤32°C) and ground to pass through a 2-mm sieve. Anaerobic potentially mineralizable N was quantified by determining the extractable $NH_4$–N in the soil before incubation ($NH_4$–$N_{initial}$) and subtracting it from the extractable $NH_4$–N after the soil was incubated for 7, 14, or 28 d ($NH_4$–$N_{inc}$) (i.e., $PMN_{an}$ = $NH_4$–$N_{inc}$ - $NH_4$–$N_{initial}$) (Bundy and Meisinger, 1994). The $NH_4$–$N_{initial}$ was determined by combining 4.0 g of soil with 20 mL of 2 M KCl in 50-mL Falcon tubes (Corning Inc., Corning, NY), shaking for 30 min, filtering the solution through a washed 0.45-µm syringe filter disk, and storing the solution in a microtube at −80°C until $NH_4$–N analysis could be completed using the Berthelot method (Rhine et al., 1998) with a Glomax-Multi Detection System plate reader (Promega Biosystems, Inc., Sunnyvale, CA). The $NH_4$–$N_{inc}$ was determined by combining 4.0 g of soil with 20 mL of ultrapure water in 50-mL Falcon tubes. The Falcon tubes were then capped and incubated for 7, 14, and 28 d at 40°C (Keeney and Bremner, 1966). Next, 20 mL of 4 M KCl was added to the solution to obtain a final extractant concentration of 2 M KCl. The same extraction method to determine $NH_4$–$N_{initial}$ was also used to determine extractable $NH_4$–N of $NH_4$–$N_{inc}$.

### Plant Sampling

Six corn plants were collected per experimental unit from the center two crop-rows at physiological maturity (R6) by clipping at the soil surface. The ears were shucked and separated from the stover (stalks plus leaves). All plant materials were dried at 60°C to constant mass. Ears were then shelled, and grain and cob samples were weighed separately to determine dry matter yield. Grain yield was determined by harvesting the middle two rows of each experimental unit and adjusting grain weight to 155 g $kg^{-1}$ moisture and including the moisture-adjusted weight of the grain collected at R6 from each experimental unit.

### Statistical Analysis

SAS software version 9.4 (SAS Institute Inc., Cary, NC) was used to complete all statistical analyses. The means and standard deviations of $PMN_{an}$, $NH_4$–N, EONR, and soil characteristics were determined using the PROC MEANS procedure. Using PROC REG and PROC NLIN procedures, the linear, linear-plateau, quadratic, and quadratic-plateau models were used to determine corn N response to total N applied separately for single and split N applications (Cerrato and Blackmer, 1990; Sawyer et al., 2006; Scharf et al., 2005). Models were compared using the metrics of model probability significance, coefficient of determination, and RMSE. The quadratic-plateau model performed the best in most sites. There were a few sites where the linear-plateau or quadratic models had slightly better metrics, but the improvement in $R^2$ values was ≤0.03 in all sites. Because of the small change in $R^2$ and for simplification, the quadratic-plateau model was used. The EONR for single and split N applications was calculated using an N price of US\$0.88 $kg^{-1}$ (US\$0.40 $lb^{-1}$) and a corn grain price of US\$0.158 $kg^{-1}$ (US\$4.00 $bu^{-1}$). Sites were identified as nonresponsive, and their EONR was set at 0 kg N $ha^{-1}$ when no plateau was reached; the quadratic-plateau model had an $\alpha$ value >0.10. The EONR was set to the maximum N rate applied (315 kg N $ha^{-1}$) if no plateau was reached and a linear model best described the N response. Four of the 32 experimental sites received irrigation. If the irrigation water had nitrate-N concentrations >10 mg $L^{-1}$, that contribution (41–42 kg N $ha^{-1}$ for two of the four sites) was added to the calculated EONR.

Table 1. Economic optimum N rate (EONR) for single ($EONR_{single}$) and split N applications ($EONR_{split}$) and soil characteristics across 32 site-years and partitioned by soil texture (coarse, medium, and fine).

| Texture | $EONR_{single}$ | $EONR_{split}$ | Sand | Silt | Clay | SOM† | Total N |
|---|---|---|---|---|---|---|---|
| | kg N ha$^{-1}$ | | % | | | g kg$^{-1}$ | |
| All soils | 180 ± 78‡ | 167 ± 65 | 26 ± 25 | 50 ± 19 | 24 ± 11 | 25.7 ± 10 | 1.39 ± 0.58 |
| Coarse | 218 ± 66 | 186 ± 20 | 67 ± 14 | 24 ± 10 | 10 ± 5 | 15.6 ± 5.5 | 0.87 ± 0.29 |
| Medium | 172 ± 80 | 157 ± 74 | 19 ± 17 | 60 ± 17 | 21 ± 3 | 24.1 ± 6.2 | 1.30 ± 0.36 |
| Fine | 169 ± 77 | 167 ± 69 | 12 ± 10 | 53 ± 12 | 35 ± 8 | 33.3 ± 9.9 | 1.79 ± 0.64 |

† Soil organic matter.

‡ Values are means ± SD.

To evaluate differences in EONR of single and split N applications, the N rates where the profit would be ±US\$2.47 of EONR were determined (excluding the sites where there was no response to N and where the response was linear). The difference between the upper and lower N limits were then averaged across experimental sites and N applications. This approach resulted in significant differences between the EONR of single and split N applications at ±10 kg N ha$^{-1}$.

The predictability of EONR rate using a single N application ($EONR_{single}$) and EONR using a split N application ($EONR_{split}$) by $PMN_{an}$, $NH_4$–$N_{inc}$, and $NH_4$–$N_{initial}$ with $PMN_{an}$ as two separate variables was determined using the PROC REG procedure. Residuals within experimental units showed that normality and constant variance assumptions were met. Linear and quadratic models were evaluated. The highest-order model with an $\alpha < 0.05$ was selected. The $R^2$ and RMSE values were the metrics used to compare the predictability of $EONR_{single}$ and $EONR_{split}$ with $PMN_{an}$ from different soil sample timings, N fertilizer rates, and incubation lengths. The same procedure was used to determine the best explanatory variable(s) with all sites in one category and when soils were separated into coarse-, medium-, and fine-texture categories. The grouping of the soils into the three texture categories followed the approach used by Tonitto et al. (2006) and Tremblay et al. (2012); coarse textures included sandy loam, loamy sand, sandy clay loam, sandy clay, and sand soils; medium textures included loam, silt loam, and silt soils; and fine textures included clay, silty clay, silty clay loam, and clay loam soils. Using this grouping, there were 26 replications in the coarse-, 54 in the medium-, and 48 in the fine-textured soil groupings for predicting EONR with $PMN_{an}$. However, due to missing samples there were four fewer replications in the coarse- and fine-textured soils groups for the $PP_{0N}$ sample timing.

## RESULTS AND DISCUSSION

The EONR for single and split N applications across the 32 sites was highly variable, ranging between 0 and 315 kg N ha$^{-1}$, with a mean of 180 kg N ha$^{-1}$ for $EONR_{single}$ and 167 kg N ha$^{-1}$ for $EONR_{split}$ (Table 1). The $EONR_{single}$ was less than $EONR_{split}$ in eight sites (12–115 kg N ha$^{-1}$ less with a mean of 42 kg N ha$^{-1}$), $EONR_{split}$ was less than $EONR_{single}$ in 17 sites (13–52 kg N ha$^{-1}$ less with a mean of 37 kg N ha$^{-1}$), and in seven sites there was no statistical difference (difference between EONRs was less than or equal to ±10 kg N ha$^{-1}$). The wide range in EONR for single and split N applications, along with differences in $PMN_{an}$ due to sample timing, N rate, and incubation length in this study (Table 2), provide an ideal dataset to evaluate the relationship between $PMN_{an}$ and the EONR of different N application timings.

### Predicting Economic Optimal N Rate with $PMN_{an}$

Statistically significant relationships between $PMN_{an}$ and $EONR_{single}$ and $EONR_{split}$ were observed at the pre-plant sample timing ($R^2$ = 0.04–0.08; RMSE = 68–78 kg N ha$^{-1}$) when evaluated across all sites for the 7-, 14-, and 28-d incubations (Tables 3 and 4). Despite statistical significance, the $R^2$ values were all very small at <0.10 with large RMSEs (>65 kg N ha$^{-1}$) and indicate a poor relationship between $PMN_{an}$ and EONR. Delaying soil sampling for $PMN_{an}$ analysis to V5 regardless of N fertilizer application rate ($V5_{0N}$ and $V5_{180N}$) produced no significant relationships with $EONR_{single}$ and $EONR_{split}$ except for predicting $EONR_{split}$ ($R^2$ = 0.07; RMSE = 70 kg N ha$^{-1}$) using samples from the no-N control plots collected at V5 when incubated for 28 d. Delaying soil sampling until after early-season N losses may have occurred (planting to V5 corn development stage) and measuring $PMN_{an}$ from fertilized soil ($V5_{180N}$) did not improve the predictability of EONR with the $PMN_{an}$ test when evaluated across all sites. This may be because the mineralizable N pool changes as the growing season progresses (Arrobas et al., 2012; Culman et al., 2013) depending on cropping systems, management practices, and the influence of environmental conditions such as soil temperature and moisture (Kuzyakov, 2002; Cabrera et al., 2005; Conde et al., 2005; Kuzyakova et al., 2006; Wu et al., 2008). Nitrogen fertilizer applications also reduced mean $PMN_{an}$ ($V5_{180N}$ vs. $V5_{0N}$), as observed in a related study (Clark et al., 2018), reduced mineralization of soil organic matter (Mahal et al., 2019), and often increase variability in N mineralization (Fernández et al., 2017; Kuzyakova et al., 2006; Ma et al., 1999). The convergence of these factors also likely contributed to the greater variability in $PMN_{an}$ associated with delayed soil sample timing and N fertilization in our study and led to the reduction in predictability of EONR by $PMN_{an}$.

Soil texture can influence N mineralization and the ability of $PMN_{an}$ to relate to EONR (Bushong et al., 2007; Mariano et al., 2013; Six et al., 2002). For example, clay particles can form aggregates with organic matter that protect it from mineralization (Bloem et al., 1994; Kuzyakova et al., 2006; Shen et al., 1989), and $NH_4^+$ produced during $PMN_{an}$ analysis can be fixed by clay particles that are abundant in fine-textured soils (Russell et al., 2006). For these reasons, we partitioned the soils in our study into three major texture categories (coarse, medium, and fine).

The predictability of $EONR_{single}$ with $PMN_{an}$ improved for coarse-textured soils compared with the analysis across all sites but only when using the V5 sample timing (Tables 3 and 4). Using the $PP_{0N}$ sample timing did not improve predictions of $EONR_{single}$ because it may have overestimated plant available N from mineralization because some of the mineralized N was lost to leaching. Collection of soil samples at V5 is typically after the time period when early season N losses can occur, resulting in a more accurate estimation of mineralizable N available for the crop and therefore providing an improved prediction of $EONR_{single}$ in coarse-textured soils. Values of $R^2$ improved at the V5 sample timing regardless of N rate (0 and 180 kg N $ha^{-1}$) and were greatest with the 7- and 14-d incubations. Anaerobic potentially mineralizable N in coarse-textured soils was unable to predict $EONR_{split}$ because there were no significant relationships between $EONR_{split}$ and $PMN_{an}$ regardless of the incubation length. This result indicates that, for coarse-textured soils where split N applications are often used, the $PMN_{an}$ test alone cannot be used to predict $EONR_{split}$ reliably.

The predictability of $EONR_{single}$ and $EONR_{split}$ with $PMN_{an}$ improved for medium-textured soils relative to analysis across all sites, especially for $PMN_{an}$ predictions from the $PP_{0N}$ sample timing (Tables 3 and 4). These results were similar regardless of incubation length ($R^2$ = 0.23–0.25; RMSE = 69–76 kg N $ha^{-1}$). The V5 sample timing also had significant relationships with $EONR_{split}$ but only for the 7-d incubation in the $V5_{0N}$ sampling ($R^2$ = 0.10; RMSE = 75 kg N $ha^{-1}$) and for the 14-d incubation in the $V5_{180N}$ sampling ($R^2$ = 0.06; RMSE = 77 kg N $ha^{-1}$). The later V5 sample timings regardless of at planting N fertilization reduced $R^2$ by 0.16, on average. As with the evaluation across all sites, these results show that delaying soil sampling to V5 in medium-textured soils has a minimal ability to improve the predictability of EONR.

There was no relationship between $EONR_{single}$ or $EONR_{split}$ and $PMN_{an}$ for fine-textured soils (Tables 3 and 4). This result indicates that, regardless of $PMN_{an}$ sample timing, N rate, and incubation length, $PMN_{an}$ alone should not be used to predict EONR of fine-textured soils. Fine-textured soils have greater clay content, organic matter, and $PMN_{an}$ compared with coarse- and medium-textured soils (Tables 1 and 2). The larger $PMN_{an}$ (greater $NH_4^+$ in the soil solution) may have suppressed mineralization and fixed more $NH_4^+$ within smectitic soil clays (Russell et al., 2006). Organic matter is also more protected in fine-textured soils because of the complexation of organic matter with clay particles that reduce the mineralization potential (Sierra, 1997). The smaller pore sizes in soils with greater clay content might have also led to more water saturated conditions during the wetter-than-normal conditions in some of our study sites and thus decreased N mineralization and increased denitrification losses during the season. This highlights the difficulty of predicting EONR when only a small portion of the weather conditions over the growing season can be accounted for at the time of sample collection for $PMN_{an}$ analysis.

## Predicting Economic Optimal N Rate with $NH_4$–$N_{inc}$ and $NH_4$–$N_{initial}$ with $PMN_{an}$

We also examined a second simplistic model where the $NH_4$–$N_{initial}$ was not subtracted from $NH_4$–$N_{inc}$ and a multivariate model where $NH_4$–$N_{initial}$ with $PMN_{an}$ were used to predict EONR as separate variables. Both approaches produced similar results ($R^2$ = 0.06–0.08; RMSE = 68–79 kg N $ha^{-1}$) as those described with using only $PMN_{an}$ to predict $EONR_{single}$ and $EONR_{split}$ when analysis was completed across all sites (Tables 3 and 4). Once soils were partitioned by texture categories, differences were found between the effectiveness of the three models.

In coarse-textured soils, the model using $NH_4$–$N_{inc}$ generally performed better than the multivariate model ($NH_4$–$N_{initial}$ with $PMN_{an}$) but only when using $PMN_{an}$ from the V5 samplings and predicting $EONR_{single}$. Nonetheless, these significant relationships were never better than $PMN_{an}$ alone (Tables 3 and 4). For medium-textured soils, the multivariate model ($NH_4$–$N_{initial}$ with $PMN_{an}$) improved $R^2$ values for both EONR relationships relative to $NH_4$–$N_{inc}$ and $PMN_{an}$ alone when using $PMN_{an}$ from the pre-plant sample timing regardless of incubation length, but RMSE values were only minimally improved. The same was true when comparing these models using $PMN_{an}$ from the $V5_{180N}$ sampling that was incubated for 7 and 14 d to predict $EONR_{single}$ and for 14 and 28 d to predict $EONR_{split}$. However, using $PMN_{an}$ in the models from these later V5 $PMN_{an}$ sample timings with or without N fertilizer at planting reduced the $R^2$ by 0.17, on average, relative to using $PMN_{an}$ from the pre-plant sample timing. These results indicate that the pre-plant sample timing was still the best time to obtain soil samples to test for $PMN_{an}$ and to use to predict EONR in medium-textured soils regardless of the model used to predict EONR. In coarse- and medium-textured soils, the differences in predicting $EONR_{single}$ and $EONR_{split}$ among the three $PMN_{an}$ models ($PMN_{an}$, $NH_4$–$N_{inc}$, and $NH_4$–$N_{initial}$ with $PMN_{an}$) were small ($\Delta R^2$ = ±0.16 and ΔRMSE = ±3 kg N $ha^{-1}$). This similarity suggests that the simpler, less expensive model ($NH_4$–$N_{inc}$) would suffice to predict $EONR_{single}$ and $EONR_{split}$ in coarse- and medium-textured soils. In that regard, the $NH_4$–$N_{inc}$ model would be the simplest for routine analysis and the least expensive because there is no need to quantify $NH_4$–$N_{initial}$, as with the $PMN_{an}$ and multivariate models ($NH_4$–$N_{initial}$ with $PMN_{an}$).

There were no significant relationships with $EONR_{single}$ or $EONR_{split}$ using the simpler models ($PMN_{an}$ and $NH_4$–$N_{inc}$) for fine-textured soils (Tables 3 and 4). The multivariate model ($NH_4$–$N_{initial}$ with $PMN_{an}$) produced significant and similar relationships ($R^2$ = 0.20–0.21; RMSE = 71 kg N $ha^{-1}$) when

Table 2. Ammonium N concentration before incubation and anaerobic potentially mineralizable N ($PMN_{an}$) concentration at different soil sample timings and N rate treatments ($PP_{0N}$, $V5_{0N}$, and $V5_{180N}$) incubated for 7, 14, and 28 d across 32 site-years and partitioned by soil texture (coarse, medium, and fine). Mean values ± standard deviation.

| Soil sampling† | Incubation period | Soil texture category: All soils | Coarse | Medium | Fine |
|---|---|---|---|---|---|
| | d | mg N $kg^{-1}$ soil | | | |
| | | Initial $NH_4$–N | | | |
| $PP_{0N}$ | 0 | 8 ± 4‡ | 6 ± 4 | 4 ± 3 | 5 ± 4 |
| $V5_{0N}$ | 0 | 7 ± 3 | 10 ± 10 | 8 ± 7 | 11 ± 9 |
| $V5_{180N}$ | 0 | 9 ± 5 | 8 ± 7 | 7 ± 6 | 9 ± 7 |
| | | Anaerobic potentially mineralizable N | | | |
| $PP_{0N}$ | 7 | 27 ± 15 | 17 ± 8 | 25 ± 13 | 34 ± 17 |
| | 14 | 38 ± 19 | 23 ± 12 | 36 ± 15 | 48 ± 20 |
| | 28 | 49 ± 25 | 28 ± 13 | 45 ± 19 | 63 ± 29 |
| $V5_{0N}$ | 7 | 28 ± 15 | 20 ± 13 | 28 ± 13 | 33 ± 16 |
| | 14 | 37 ± 17 | 25 ± 12 | 37 ± 15 | 44 ± 19 |
| | 28 | 49 ± 23 | 29 ± 13 | 47 ± 19 | 62 ± 24 |
| $V5_{180N}$ | 7 | 23 ± 15 | 17 ± 14 | 22 ± 12 | 27 ± 18 |
| | 14 | 32 ± 17 | 23 ± 15 | 30 ± 14 | 40 ± 19 |
| | 28 | 43 ± 24 | 27 ± 17 | 39 ± 16 | 56 ± 27 |

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

‡ Values are mean ± SD.

Table 3. Coefficient of determination averaged across all soils and when partitioned by soil texture (coarse, medium, and fine) for the regression of economic optimum N rate (EONR) of single- and split-N applications against three $NH_4$–N based models at different soil sample timings (pre-plant and V5 development stage), at planting N rates (0 and 180 kg N $ha^{-1}$), and incubation lengths (7, 14, and 28 d). The models were 1) anaerobic potentially mineralizable N ($PMN_{an}$) as a single explanatory variable, 2) $NH_4$–N from incubated samples ($NH_4$–$N_{inc}$) as a single explanatory variable, and 3) $PMN_{an}$ with initial soil $NH_4$–N as separate explanatory variables ($NH_4$–$N_{initial}$ with $PMN_{an}$).

| | | Soil texture category | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All soils | | | Coarse | | | Medium | | | Fine | | |
| Variable | EONR | 7 d | 14 d | 28 d | 7 d | 14 d | 28 d | 7 d | 14 d | 28 d | 7 d | 14 d | 28 d |
| | | | | | | | $PP_{0N}$† | | | | | | |
| $PMN_{an}$ | Single | 0.05** | 0.04** | 0.06** | <0.01 | <0.01 | <0.01 | 0.25*** | 0.25*** | 0.24*** | <0.01 | <0.01 | 0.02 |
| $NH_4$–$N_{inc}$ | | 0.05** | 0.04** | 0.06** | 0.01 | 0.02 | <0.01 | 0.21*** | 0.22*** | 0.20*** | <0.01 | <0.01 | 0.04 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 0.05* | 0.04* | 0.06** | 0.02 | 0.03 | 0.02 | 0.28*** | 0.27*** | 0.29*** | 0.21** | 0.2** | 0.20** |
| $PMN_{an}$ | Split | 0.07** | 0.05** | 0.08** | <0.01 | <0.01 | <0.01 | 0.23*** | 0.23*** | 0.25*** | <0.01 | <0.01 | 0.04 |
| $NH_4$–$N_{inc}$ | | 0.06** | 0.05** | 0.07** | 0.02 | <0.01 | 0.03 | 0.19** | 0.19*** | 0.21*** | 0.02 | 0.01 | 0.05 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 0.07** | 0.05** | 0.08** | 0.16 | 0.15 | 0.17 | 0.27*** | 0.25*** | 0.31*** | 0.06 | 0.06 | 0.08 |
| | | | | | | | $V5_{0N}$‡ | | | | | | |
| $PMN_{an}$ | Single | <0.01 | <0.01 | 0.02 | 0.14* | 0.33** | 0.09 | 0.03 | <0.01 | 0.01 | <0.01 | <0.01 | 0.01 |
| $NH_4$–$N_{inc}$ | | <0.01 | <0.01 | 0.01 | 0.14* | 0.32** | 0.08 | 0.02 | <0.01 | 0.01 | <0.01 | <0.01 | <0.01 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | <0.01 | <0.01 | 0.02 | 0.14 | 0.19* | 0.10 | 0.03 | 0.02 | 0.02 | 0.04 | 0.05 | 0.04 |
| $PMN_{an}$ | Split | <0.01 | <0.01 | 0.07** | <0.01 | <0.01 | 0.02 | 0.10** | <0.01 | <0.01 | 0.03 | 0.02 | 0.05 |
| $NH_4$–$N_{inc}$ | | <0.01 | <0.01 | 0.06** | <0.01 | <0.01 | 0.02 | 0.02 | <0.01 | <0.01 | 0.05 | 0.03 | 0.04 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | <0.01 | <0.01 | 0.07** | 0.04 | 0.04 | 0.06 | 0.03 | 0.02 | 0.02 | 0.17* | 0.08 | 0.09 |
| | | | | | | | $V5_{180N}$§ | | | | | | |
| $PMN_{an}$ | Single | 0.02 | <0.01 | <0.01 | 0.28** | 0.17** | 0.12* | <0.01 | 0.05 | 0.02 | 0.03 | <0.01 | <0.01 |
| $NH_4$–$N_{inc}$ | | 0.02 | <0.01 | <0.01 | 0.26** | 0.14* | 0.09 | 0.13** | 0.08** | 0.04 | 0.04 | <0.01 | <0.01 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 0.02 | <0.01 | <0.01 | 0.15 | 0.19* | 0.16 | 0.15** | 0.01* | 0.07 | 0.04 | <0.01 | <0.01 |
| $PMN_{an}$ | Split | 0.01 | <0.01 | <0.01 | 0.02 | 0.02 | <0.01 | <0.01 | 0.06* | 0.04 | 0.05 | <0.01 | <0.01 |
| $NH_4$–$N_{inc}$ | | 0.02 | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.03 | 0.09** | 0.06* | 0.06 | <0.01 | <0.01 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 0.02 | 0.01 | <0.01 | 0.06 | 0.07 | 0.06 | 0.07 | 0.11* | 0.09* | 0.06 | 0.01 | 0.01 |

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Pre-plant soil sampling with 0 kg N $ha^{-1}$ applied at planting.
‡ V5 corn development stage with 0 kg N $ha^{-1}$ applied at planting.
§ V5 corn development stage with 180 kg N $ha^{-1}$ applied at planting.

predicting $EONR_{single}$ using $NH_4$–N values from any of the three $PP_{0N}$ incubation lengths. The significant relationship with $EONR_{single}$ of fine-textured soils likely developed because adding $NH_4$–$N_{initial}$ accounted for some of the inorganic N that was present in the soil at the time of sampling, which the $PMN_{an}$ alone model did not take into consideration. Adding $NH_4$–$N_{initial}$ separately to the model at V5 ($V5_{0N}$ and $V5_{180N}$) likely did not provide benefits because the majority of inorganic N at the later V5 sample timing is $NO_3^-$–N (Bronson, 2008), which was not considered in these models. However, there was a significant but weaker relationship ($R^2 = 0.17$; RMSE = 75 kg N $ha^{-1}$) at the 7-d incubation length when $PMN_{an}$ from $V5_{0N}$ sample timing was used in the multivariate model to predict $EONR_{split}$.

## $PMN_{an}$ Incubation Length and Predicting Economic Optimal N Rate

The $R^2$ and RMSE minimally improved ($R^2$ improvement <0.07 and RMSE <2 kg N $ha^{-1}$) when incubation length was increased beyond 7 d regardless of $PMN_{an}$ sample timing, at planting N fertilization, model used ($PMN_{an}$, $NH_4$–$N_{inc}$, and $NH_4$–$N_{initial}$ with $PMN_{an}$), and texture category evaluated to predict $EONR_{single}$ and $EONR_{split}$ (Tables 3 and 4). The one exception to this was for coarse-textured soils, where increasing the incubation length of the $V5_{0N}$ sample timing from 7 to 14 d improved $R^2$ by 0.19 for the 0 kg N $ha^{-1}$ rate and reduced RMSE by 7 kg N $ha^{-1}$. However, the improved predictability was not substantial enough to justify the longer incubation length with a soil sample taken at a time in the growing season (V5) where timely N application decisions are needed. Based on these data, the 7-d incubation should be used because of simplicity.

Partitioning soils by texture, separately accounting for $NH_4$–$N_{initial}$ and $PMN_{an}$ in the prediction model, and increasing the incubation length generally improved the predictability of EONR, but the $R^2$ values were still poor. Similar poor correlations between $PMN_{an}$ and EONR ($R^2 = 0.09$–$0.10$) were reported by Fox and Piekielek (1984). Other soil tests, such as the soil $NO_3$–N test obtained at the V5-V6 corn development stages ($R^2 = 0.28$–$0.76$) (Bundy and Andraski, 1995; Nyiraneza et al., 2010) and the gas pressure test ($R^2 = 0.38$) (Williams et al., 2007), have been reported to have stronger relationships with EONR compared with $PMN_{an}$. On the other hand, there are soil tests that have produced mixed results. For example, the Illinois soil N test (Khan et al., 2001) showed a good correlation with EONR ($R^2 = 0.81$) in the study by Williams et al. (2007), but others found no correlation (Barker et al., 2006; Laboski et al., 2008; Osterhaus et al., 2008; Sawyer and Barker, 2011). This inconsistent result from using the Illinois soil N test to predict EONR demonstrates the difficulty of finding soil tests that will consistently relate well to EONR and other crop responses regardless of soil and weather conditions.

Table 4. Root mean square error averaged across all soils and when partitioned by soil texture (coarse, medium, and fine) for the regression of economic optimum N rate (EONR) of single- and split-N applications against three $NH_4$–N based models at different soil sample timings (pre-plant and V5 development stage), at planting N rates (0 and 180 kg N $ha^{-1}$), and incubation lengths (7, 14, and 28 d). The models were: anaerobic potentially mineralizable N ($PMN_{an}$) as a single explanatory variable, $NH_4$–N from incubated samples ($NH_4$–$N_{inc}$) as a single explanatory variable, and $PMN_{an}$ with initial soil $NH_4$–N as separate explanatory variables ($NH_4$–$N_{initial}$ with $PMN_{an}$).

| | | Soil texture category | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All | | | Coarse | | | Medium | | | Fine | | |
| Variable | EONR | 7 d | 14 d | 28 d | 7 d | 14 d | 28 d | 7 d | 14 d | 28 d | 7 d | 14 d | 28 d |
| | | RMSE (kg N $ha^{-1}$) | | | | | | | | | | | |
| | | $PP_{0N}$† | | | | | | | | | | | |
| $PMN_{an}$ | Single | 78 | 78 | 78 | 69 | 69 | 69 | 76 | 76 | 76 | 78 | 78 | 78 |
| $NH_4$–$N_{inc}$ | | 78 | 78 | 78 | 69 | 69 | 69 | 78 | 78 | 78 | 78 | 78 | 77 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 78 | 79 | 78 | 70 | 70 | 70 | 75 | 76 | 75 | 71 | 71 | 71 |
| $PMN_{an}$ | Split | 68 | 68 | 67 | 46 | 46 | 46 | 69 | 70 | 69 | 71 | 71 | 69 |
| $NH_4$–$N_{inc}$ | | 68 | 69 | 68 | 46 | 46 | 45 | 71 | 71 | 71 | 70 | 71 | 69 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 68 | 69 | 68 | 43 | 43 | 43 | 68 | 69 | 67 | 70 | 70 | 69 |
| | | $V5_{0N}$‡ | | | | | | | | | | | |
| $PMN_{an}$ | Single | 85 | 85 | 85 | 72 | 65 | 74 | 86 | 87 | 87 | 88 | 88 | 88 |
| $NH_4$–$N_{inc}$ | | 85 | 85 | 85 | 72 | 65 | 74 | 86 | 87 | 87 | 88 | 88 | 88 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 86 | 86 | 85 | 73 | 71 | 75 | 86 | 87 | 87 | 87 | 87 | 87 |
| $PMN_{an}$ | Split | 73 | 73 | 70 | 44 | 44 | 43 | 75 | 78 | 78 | 79 | 79 | 78 |
| $NH_4$–$N_{inc}$ | | 73 | 73 | 71 | 44 | 44 | 43 | 78 | 79 | 79 | 78 | 79 | 78 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 73 | 73 | 71 | 44 | 44 | 43 | 78 | 79 | 79 | 75 | 77 | 77 |
| | | $V5_{180N}$§ | | | | | | | | | | | |
| $PMN_{an}$ | Single | 85 | 85 | 85 | 67 | 71 | 73 | 87 | 85 | 86 | 87 | 88 | 88 |
| $NH_4$–$N_{inc}$ | | 85 | 85 | 85 | 68 | 72 | 74 | 82 | 84 | 85 | 87 | 88 | 88 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 85 | 85 | 86 | 73 | 71 | 72 | 82 | 84 | 85 | 88 | 89 | 89 |
| $PMN_{an}$ | Split | 72 | 73 | 73 | 43 | 43 | 44 | 78 | 77 | 77 | 78 | 80 | 80 |
| $NH_4$–$N_{inc}$ | | 72 | 73 | 73 | 44 | 43 | 44 | 78 | 75 | 76 | 77 | 80 | 80 |
| $NH_4$–$N_{initial}$ with $PMN_{an}$ | | 72 | 73 | 73 | 43 | 43 | 43 | 77 | 75 | 76 | 78 | 80 | 80 |

† Pre-plant soil sampling with 0 kg N $ha^{-1}$ applied at planting.
‡ V5 corn development stage with 0 kg N $ha^{-1}$ applied at planting.
§ V5 corn development stage with 180 kg N $ha^{-1}$ applied at planting.

One possible explanation for the poor capacity of $PMN_{an}$ to predict EONR may be that we only evaluated the top 30 cm of soil, but ~40% of the N taken up by corn can be in soil below 30 cm (Gass et al., 1971). Accounting for N mineralization deeper in the soils may improve the predictability of EONR. For example, accounting for mineralization in the top 50 cm had $R^2$ values from 0.61 to 0.67 when relating $PMN_{an}$ to N uptake in wheat, whereas the $R^2$ was only 0.21 when $PMN_{an}$ was calculated only from the top 20 cm (Börjesson et al., 1999). Also, it may be necessary to split the soil sampling depth into smaller increments because N mineralization decreases with depth as the C/N ratio normally increases due to less organic N content in deeper layers of the soil (Paul et al., 2001; Purnomo et al., 2000a, 2000b). The lower organic N content and changing C/N ratio as soil depth increases may have diluted our deeper (30 cm) $PMN_{an}$ samples, causing them to be lower than other studies that sampled the top 20 cm (Börjesson et al., 1999; Orcellet et al., 2017; Williams et al., 2007).

Another reason for the generally poor correlations of the $PMN_{an}$ test to EONR may be that the $PMN_{an}$ test is only an index of how much N mineralization is possible in a growing season. Actual N mineralization in the field depends on the interaction of soil characteristics, weather, and management that can be influenced by soil moisture and the accessibility of microorganisms to organic-bound N across the entire season (Beyaert and Voroney, 2011; Cabrera et al., 2005; Kuzyakova et al., 2006; Mikha et al., 2006; Rice and Havlin, 1994; Sierra, 1992; Wu et al., 2008). Further, the N that is mineralized from soil organic matter or added from fertilizers is subject to loss processes (leaching, denitrification, volatilization, and immobilization) that affect the availability of N to the corn crop, which are not quantified by the $PMN_{an}$ test. Finally, the culmination of various biophysical stressors over the season define the health of the crop and its yield potential, thus affecting the crop N need and use of the available N to produce yield. All these factors make corn EONR prediction difficult with a single N management tool. Future studies should focus on a more integrated approach where EONR predictions with $PMN_{an}$ are evaluated together with more components of the N cycle and weather conditions that most influence plant N availability.

## CONCLUSIONS

Anaerobic potentially mineralizable N is a weak predictor of EONR ($R^2 \le 0.08$ and RMSE ≥67 kg N $ha^{-1}$) when evaluated across all soils. Predictions of EONR by $PMN_{an}$ improved (15%) when analysis was completed after sites were grouped by soil texture. Sample timing and N fertilization generally had a greater impact on the ability of $PMN_{an}$ models to predict EONR compared with increasing the $PMN_{an}$ incubation length and the use of different $PMN_{an}$ models. At the V5 soil sample timing, $PMN_{an}$ similarly predicted EONR regardless of N fertilizer rate applied at planting (0 vs. 180 kg N ha$^{-1}$), demonstrating that sampling after fertilization does not improve the ability of $PMN_{an}$ to predict EONR. Soil texture influenced when soil samples should be collected for $PMN_{an}$ analysis to best predict EONR. For coarse-textured soils, soils should be sampled at V5 and medium- and fine-textured soils sampled prior to planting. Increasing the length of $PMN_{an}$ incubation affected $PMN_{an}$ but did not improve EONR predictability (≤18% improvement) or reduce RMSE (decreased ≤7 kg N ha$^{-1}$) enough to justify the extra time required to complete the longer incubation lengths; thus, incubation length should remain at 7 d. When determining $PMN_{an}$, subtracting $NH_4$–$N_{initial}$ from $NH_4$–$N_{inc}$ had a minimal impact on optimizing the predictability of EONR in coarse-, medium-, and fine-textured soils. Discontinuing the measurement of $NH_4$–$N_{initial}$ as part of the $PMN_{an}$ test would lower analysis cost and increase the potential for the $PMN_{an}$ test to be commercially available to farmers as an N management tool. Although N fertilization as a single application at planting or split application affected EONR, there was minimal influence on the ability of $PMN_{an}$ to predict either EONR. Overall, the relationships between EONR and $PMN_{an}$ models were poor regardless of the improvements from partitioning soils by texture and including $NH_4$–$N_{initial}$ with $PMN_{an}$ ($R^2 \leq 0.33$ and RMSE ≥ 68 kg N ha$^{-1}$). These results indicate that $PMN_{an}$ models alone should not be used to predict either $EONR_{single}$ or $EONR_{split}$, and other factors influencing EONR need to be investigated.

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