THE ANAEROBIC POTENTIALLY MINERALIZABLE NITROGEN TEST AS A TOOL FOR NITROGEN MANAGEMENT IN THE MIDWEST

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ABSTRACT

The anaerobic potentially mineralizable nitrogen (PMN_{an}) test is a tool that can improve estimations of mineralizable nitrogen (N) and enhance nitrogen use efficiency. This tool may also help improve predictions of N uptake, grain yield, and the economic optimum nitrogen rate (EONR) of corn (*Zea mays L.*). A 32 site-year study across eight US Midwestern states was conducted to 1) compare the effect of incubation length (7-, 14-, 28-d), soil sampling timing, N fertilizer rate, and their interactions on PMN_{an}, and 2) quantify the predictive power of PMN_{an} values at different soil sampling timings and N fertilizer rates in determining EONR, yield, and plant N uptake for single and split N applications. Soil was sampled from 0-30 cm before planting and N fertilizer application (PPNT) or at the V5 leaf stage where 0 ($V5_{0N}$) or 180 kg-N ha^{-1} ($V5_{180}$) were applied at planting. Overall, PMN_{an} ranged from 0.19 to 136 mg-N kg^{-1} with a mean of 36 mg-N kg^{-1}. There was no consistent pattern in PMN_{an} with time of sampling. The PMN_{an} was larger in nine site-years at PPNT (0.74–125 mg-N kg^{-1}) and ten site-years at $V5_{0N,}$ (0.19–136 mg-N kg^{-1}) with no difference in the other eleven site-years. There was also no consistent pattern in response to the addition of N fertilizer. The $V5_{180}$ PMN_{An} (0.86–122.47 mg-N kg^{-1}) was lower than the $V5_{0N}$ in ten site-years, greater in one site-year, and no different in 19 site-years. The PPNT soil sampling timing had the best correlation to EONR for single (R^2=0.023) and split (R^2=0.053) N applications. These coefficients of determination are low (similar to many studies) but are significant (P<0.05). The $V5_{0N}$ sample timing improved the correlation of plant N uptake and grain yield for split N applications. The 7-d incubation for the $V5_{180N}$ sampling best predicted N uptake (R^2=0.09) and yield (R^2=0.12) when 180 kg-N ha^{-1} were applied at planting. On average, all site-years increased in PMN_{an} as laboratory incubation length increased. However, increasing the incubation length beyond 7-d was not justifiable as the maximum increase in R^2 values was ≤ 4% across all agronomic variables.

INTRODUCTION

Nitrogen (N) is the fourth most abundant element in corn (*Zea mays L.*) and is involved in metabolic processes and plant structural components (Hawksford et al., 2012). Because of the high amount of N needed by corn, it often limits grain yield. The two main sources of N for corn are mineralization of organic materials, and synthetic fertilizers (O'Leary et al., 2002; Mikha et al., 2006). The process of mineralization can contribute 20 to 80% of corn N needs in a given season (Broadbent and Hauck, 1984; Steinbach et al., 2004). The amount of actual mineralization is dependent on the size and quality of the organic N pool, moisture, accessibility to the organic N, and temperature (Sierra, 1992; Cabrera et al., 2005; Kuzyakova et al., 2006; Mikha et al., 2006; Wu et al., 2008; Beyaert and Paul Voroney, 2011). These characteristics are influenced by the carbon to nitrogen ratio and lignin in the residue and organic matter in the soil, soil texture, weather, and management practices such as tillage (Rice and Havlin, 1994). The amount of N needed by the plant to obtain optimum yields that is not supplied through mineralization of organic matter is often supplemented by synthetic N fertilizers.

When N fertilizer is over applied the N use efficiency of corn and the profitability of the grower decrease. The N fertilizer not used by the plant has the potential to be lost to denitrification or leaching. This N loss can result in negative environmental effects such as contamination of drinking water and eutrophication of surface waters (Mitsch et al., 2001; Ribaudo et al., 2011; Helmers et al., 2012; Woolverton, 2015). If not enough N is applied corn grain yields will decrease and also lower the economic profit of a grower. The rate of N fertilizer applied to fields in the US Midwestern Cornbelt is often determined by a N recommendation tool such as the pre-sidedress soil nitrate test (PSNT) (Magdoff et al., 1984; Fox et al., 1989; Binford et al., 1992; Andraski and Bundy, 2002), maximum return to N (MRTN) formula (Sawyer et al., 2006), or yield goal formula (Stanford, 1973; Lory and Scharf, 2003). These N management tools and others do not explicitly account for the potential of a soil to mineralize N. Developing a N management tool that explicitly accounts for the potential of the soil to mineralize N can improve N fertilizer use efficiency increasing grower profits and lessening negative environmental effects.

Lab and field tests have been developed that measure how much N a soil can mineralize. Some of the common soil tests done in the field are the buried bag method (Hart, 1994), the PVC tube method described by Raison et al. (1987), and the PVC method with ion exchange resins (Kolberg et al., 1997). The difficulty of these tests is that they take a lot of time and labor in the field burying bags or placing PVC tubes, analyzing many soil samples, and waiting for a full growing season before the mineralization potential of the soil is known. Two of the common and most reliable lab procedures are the aerobic and anaerobic N mineralization tests (Bundy and Meisinger, 1994). The aerobic mineralization test (Stanford and Smith, 1972) requires careful measurement of water that must be added to the soil to keep it moist over the duration of the test, which is normally 30 weeks. It is also very costly in time, labor, and equipment. The anaerobic potentially mineralizable N (PMN_{an}) test (Keeney and Bremner, 1966) has several advantages. One of the main advantages is that it is simple and rapid. Air-dried and field-moist soils can be used. No amendments or preliminary analyses are needed to determine the amount of water required for incubation because the same amount is used for each sample. The anaerobic conditions do not result in the formation of NO_3^--N or NO_2^--N so only NH_4+-N needs to be measured. Aeration of samples and long durations of incubation are also not needed to gain a good potential mineralization measurement because mineralization is more rapid in anaerobic conditions (Waring and Bremner, 1964). These advantages make the PMN_{an} test a good candidate to be used as a soil test that account for the potential of soil to mineralize N.

The 7-d PMN_{an} test has been successfully correlated to N uptake of ryegrass (Keeney and

Bremner, 1966) and rice (Angus et al., 1994). When looking at soil samples (0-30 cm) taken in the winter for a spring wheat crop, the 7-d PMN_{an} test correlated poorly to N uptake and yield in plots with no N (R^2 < 0. 20 and 0.07, respectively) and plots with added N (R^2 < 0.09) (Christensen and Mellbye, 2006). However, when 0 to 20-cm soil samples were used the correlation of PMN_{an} to wheat grain yield increased to an R^2 of 0.41 (Reussi Calvo et al., 2013). For N uptake of corn Fox and Piekielek (1984) tested the 7-d PMN_{an} test in 67 Pennsylvanian soils and found that its correlation to corn N uptake was low (R^2 = 0.09). The correlation of corn to EONR was low (R^2 = 0.33) and to other yield measurements (delta yield, and yield with 0-N) there was no significant relationship (Williams et al., 2007). Some possible way to improve these low correlations of the PMN_{an} test to N uptake and yield variables may be to change the timing of soil sampling, sampling soil where N has been added, and increasing the length of incubation used.

The timing of soil sampling may be important because of when corn takes up the most water and N from the soil. Corn is planted from mid-April to mid-May in the Cornbelt. In the beginning of the growing season (April to June), plant growth above and belowground is slow, the water and nutrients required by corn is low, and precipitation normally exceeds demand.

During this period of high rainfall and low water and nutrient uptake by corn, precipitation often exceeds evapotranspiration (ET) and soil water storage capacity leading to approximately 62% of the annual water drainage and 70% of the NO_3^--N lost to subsurface drainage (Randall et al., 2003a; b; Randall and Vetsch, 2005; MPCA, 2013). Because of this high chance of N loss during the early part of the season, much of the mineralized N from the soil could be lost to denitrification or leaching. This loss, when the PMN_{an} test is done before planting, can result in inaccurate predictions of EONR, N uptake, or yields compared to a test done closer to when corn N uptake increases and N loss decreases.

Nitrogen fertilizer can affect the rate and timing of N mineralization. The addition of N fertilizer decreases the N mineralization from humic substances and stimulates residue decomposition, which can result in higher amounts of N being mineralized (Kuzyakov et al., 2000; Steinbach et al., 2004; Conde et al., 2005; Hamer and Marschner, 2005; Chen et al., 2014). N fertilizer is most often applied to corn fields as a single or split application. Split applications involve applying a small amount of fertilizer early in the season when N loss potential is high and corn N uptake is low. The remainder of the fertilizer is applied when the rate of corn N uptake is high and leaching potential is low. It is unknown how the PMN_{an} test is influenced when the soil sample is taken from an area that has been recently fertilized or how the correlation of PMN_{an} to N uptake and yield variables is influenced if single or split N applications are used.

The incubation length used in the PMN_{an} test influences its value and its potential to predict EONR, N uptake, and grain yield. When relating PMN_{an} to corn the 7-d incubation has been the standard length. However, a longer incubation period has the potential to increase the correlation of PMN_{an} values to EONR, N uptake, and yield variables. For example, a study by Angus et al. (1994) involving rice used 7-, 14-, and 28-d PMN_{an} incubations to predict net mineralization. The amount of N mineralized increased linearly from 7- to 14-d and then the slope lessened from 14- to 28-d. The 28-d PMN_{an} incubation led to the best results in their model when relating laboratory incubations to actual measured mineralization in the field. Further testing of how incubation lengths influence PMN_{an} in corn systems and their subsequent relationship to EONR, N uptake, and yield variables is needed.

The objectives of this research were to 1) compare the effect of incubation length, soil sampling timing, N fertilizer rate, and their interactions on PMN_{an}, 2) Quantify the predictive power of PMN_{an} values at different soil sampling timings and N fertilizer rates in determining EONR, yield, and plant N uptake for single and split N applications.

MATERIALS AND METHODS

Experimental Design

This study was conducted across eight US Midwestern states (North Dakota, Minnesota, Wisconsin, Nebraska, Iowa, Illinois, Indiana, and Missouri). Two sites were selected in each state in 2014 and 2015 for a total of 32 site-years. A standard protocol for the experimental design; N fertilizer source, rate, and application timing; plant and soil sample collection method and timing; and weather data collection was used across all sites. The experimental design was a randomized complete block design with four replications at each site. Fourteen N application treatments created a complete grain yield response to single and split N applications by applying eight N rates from 0 to 314 kg-N ha^{-1} in increments of 45 kg-N ha^{-1} (Table 1). Single applications were done at planting and split applications had 45 kg-N ha^{-1} at planting with the remainder applied at the V9 corn development stage. Ammonium nitrate was used as the N source and was spread by hand across each plot.

Three soil cores (1.875-cm inside diameter) composite from the 0-15 cm depth increment were taken before planting from each replication. These samples were analyzed for phosphorous, potassium, pH, and organic matter. Nutrient and pH deficiencies were then corrected before planting. All tillage, seedbed preparations and planting were done by the grower/cooperator. Weeds, pests, and disease were controlled using appropriate chemicals following local university recommendations. A well-adapted DuPont Pioneer® hybrid for each region was planted in 56- or 76-cm row spacing at 86,420 seeds ha^{-1}.

Soil Sampling

Soil characterization was done before planting at each site. A 4.76-cm inside diameter hydraulic soil sampler (Giddings Machine Company, Windsor, CO; Model #5-UV / MGSRPSUV) was used to obtain two, 120-cm deep cores within 60 cm of each other from every replication. A taxonomic description of each set of soil cores was done following Natural Resources and Conservation Society guidelines (Schoeneberger et al., 2012), specifically horizon designation, horizon depth start and end, and any other visible characteristics were noted. Both cores were cut at each horizon and stored in a sealed plastic bag. One of the soil cores was used to determine soil water content (Topp and Ferre, 2002) and bulk density using the core method (Grossman and Reinsch, 2002). The other core was used to determine pH (Thomas, 1996), texture by the pipette method (Gee and Or, 2002), total carbon and total organic carbon (TOC) by the dry combustion method (Nelson and Sommers, 1996), cation exchange capacity (CEC) by the summation method (Sumner and Miller, 1996), and total N by the dry combustion method (Bremner, 1996). Organic matter (OM) was determined by using a Thermo Gravimetric Analyzer with 150 ^{o}C drying temperature and 360 ^{o}C burn off temperature. Weighted averages of these measurements by horizons were then calculated for the 0-30-cm soil depth.

Pre-plant soil samples were taken each spring before planting and fertilization. Ten cores were taken randomly in each replication and composited within three depths (0- to 30-, 30- to 60-, and 60- to 90-cm.). In addition, soil samples were collected at the V5 corn development stage from treatments one through eight (Table 1). Six soil cores (1.875-cm inside diameter) from the 0- to 30- and 30- to 60-cm soil depth were taken between rows two and three. All soil samples were dried and ground to pass through a 2-mm sieve and soil NO_3^--N was extracted using 0.01 *M* KCl and quantified by the cadmium reduction method (Gelderman and Beegle, 1998).

The surface 0 to 30 cm soil samples from the pre-plant (PPNT) and the V5 soil sampling timings [0 ($V5_{0N}$) and 180 ($V5_{180N}$) kg N ha^{-1} plots] were used for the anaerobic potentially mineralizable nitrogen (PMN_{an}) test. Four g of soil with 20 ml of ultrapure water were placed in Falcon tubes and incubated for 7-, 14-, and 28-d at 40 ^{o}C (Keeney and Bremner, 1966). After incubation, 20 ml of 4 *M* KCl was added and samples were shaken for 30 min. Next, the solution was passed through a washed 0.45 um syringe filter disk and stored in a microtube at -80 ^{o}C until NH_4^+-N analysis could be done. Ammonium-Nitrogen produced was determined by the Berthelot method (Rhine et al., 1998). An initial NH_4^+-N value was determined for each treatment and subtracted from the incubated samples to obtain net NH_4^+-N produced or PMN_{an} (Bundy and Meisinger, 1994).

Plant Sampling

Plant samples were taken at the R6 development stages to evaluate corn plant N uptake, plant biomass, and grain yield. At R6, six plants were collected per plot from the center two rows. The ears were shucked and separated from the stover (stalks plus leaves). All stover, cob, and grain samples were dried at 60 ^{o}C until constant mass was achieved. Corn ears were shelled. The grain, cob, and stover samples were then weighed separately to determine dry matter yield. The weight of this grain was added to the final grain yield harvested for each plot. Final yield was determined by harvesting the middle two rows of each plot and adjusting to 15.5% moisture. All samples were ground to pass a 1-mm sieve. The N content of the stover and grain was determined using the dry combustion method (Bremner, 1996; Nelson and Sommers, 1996; Wortmann et al., 2011). Nitrogen uptake of the stover and grain was calculated to a kg N ha^{-1} basis using biomass production and N content. The N content of the cob was determined by multiplying the sum of grain and stover N uptake by 0.048 (J. Sawyer, 2016, personal communication). Total plant N uptake was determined by summing the N content of the stover, grain, and cob.

Weather

Weather data were collected during the growing season for each site by using a Hobo (Onset Computer Corporation, Bourne, MA) U30 Automatic Weather Station at each site. Precipitation and temperature measurements were recorded every five minutes. These measurements were used to determine the minimum and maximum temperatures, total precipitation and calculate growing degree days (GDD) using the following formula for each day.

$$GDD = \frac{Tmax + Tmin}{2} - 10°C$$

Where Tmax = the maximum daily temperature if 10 ≤ Tmax ≤ 30, if Tmax ≤ 10C 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 (^{o}C)

For the PPNT soil sampling the cumulative growing degree days (GDD) and precipitation and the mean of the maximum (Tmax) and minimum (Tmin) air temperatures were calculated for the 30 d period before sampling. For the $V5_{0N}$ and $V5_{180N}$ soil samplings GDD was calculated for the 30 d period before sampling. The mean Tmax and Tmin and the cumulative precipitation was calculated from the pre-plant soil characterization sampling to the time of the V5 soil sampling.

Statistical Analysis

Anaerobic potentially mineralized N was evaluated using the MIXED procedure of SAS 9.4 (SAS Institute Inc, Cary, NC). Analysis was done using a randomized complete block design with 32 site-years, four blocked replicates, three samplings (PPNT, $V5_{0N}$, and $V5_{180N}$), and three incubation lengths. Residual plots did not show violations of normality and constant variance assumptions. Site-year, block, and any interaction with these factors were considered random effects. Soil sampling date, incubation length, and their interactions were considered fixed effects. Least square means were calculated for each effect and their interactions using the LSMeans statement and the differences between them were determined using the Tukey method to adjust for multiple comparisons when needed.

The economic optimum nitrogen rate (EONR) was determined using PROC NLIN of SAS 9.4 (SAS Institute Inc, Cary, NC). A quadratic-plateau regression model (Cerrato and Blackmer, 1990; Scharf et al., 2005) was used to fit the grain yield data from treatments one through eight for the single at planting N applications and treatments one, two, and nine through fourteen for the split N applications. A price ratio was calculated using a N price of \$0.88 kg^{-1} and a corn grain price of \$ 0.03 kg^{-1}. If no plateau was reached, the highest N rate applied (315 kg-N ha^{-1}) was set to be the maximum value for EONR.

Regression analysis was done using PROC REG of SAS 9.4 (SAS Institute Inc, Cary, NC) to determine what site characteristics and weather measurements had a significant relationship with PMN_{an}. Regression was also used to determine how well PMN_{an} explained EONR, yield at EONR, Relative yield, and Plant N uptake in single and split N applications. Linear and quadratic models were evaluated. The highest order model with a P-value below 0.05 was selected. If neither model was significant ($\alpha = 0.05$) then the model with the lowest P-value was selected. The R^2 values of these models were compared to determine if they increased within each sampling as incubation length increased and if different samplings affected how well PMN_{an} explained the different agronomic variables.

RESULTS AND DISCUSSION

Anaerobic Potentially Mineralizable Nitrogen

Across all soil sampling times and incubation lengths PMN_{an} ranged from 0.19 to approximately 137 mg-N kg^{-1} with a mean of 36 mg-N kg^{-1} (Table 2). Averaged over all sites PMN_{an} increased as incubation length increased for each soil sampling time. The PPNT and $V5_{0N}$ sampling times were on average within 2 mg-N kg^{-1} of each other and the $V5_{180N}$ sampling was on average less than 6 mg-N kg^{-1} less than the PPNT and $V5_{0N}$ sampling times (Table 2).

Incubation Length

Thirty-two site-years were evaluated to determine the effect of incubation length on PMN_{an} for the three soil sampling times. The random interaction of site-year by soil sampling by incubation length was significant. Because the interaction included site-year, each site-year was evaluated separately. When evaluating each site individually, there was no significant interaction between soil sampling timing and incubation length. The LoneTree15 site was the only one with no differences between the 7-, 14-, and 28-d incubations ($P \leq 0.05$). However, at the $P \leq 0.1$ level of significance the PMN_{an} at 28-d was greater than the 7-d. In the other 31 site-years, incubation length had a significant effect on PMN_{an}. In 14 of the site-years PMN_{an}

increased as incubation length increased. In the remaining 17 site-years, the 28-d incubation resulted in the greatest PMN_{an} while the 7-d incubation was the lowest. The 14-d incubation in these 17 site-years was similar to the 7-d incubation in 8 site-years, similar to the 28-d incubation in five site-years, and similar to both in 4 site-years. The mean PMN_{an} value in all site-years increased as incubation time lengthened from 7- to 14- to 28-d. These results follow Angus et al. (1994) who reported that the longer 28-d incubation correlated better with the amount of mineralization found in the field when growing rice. Our preliminary results show a promising opportunity for PMN_{an} values from the longer incubation lengths to increase the correlation of PMN_{an} to EONR, N uptake and grain yield for corn. We are currently conducting further analyses.

Sampling Timing

Thirty site years were evaluated on the effect of soil sampling timing (PPNT vs. $V5_{0N}$) and incubation length on PMN_{an}. The random interactions of site-year by sampling time and site-year by incubation length were significant ($P \leq 0.05$). Because these interactions included site-year, most likely due to their soil and climate differences, each site-year was evaluated separately. Brownstown14 had a significant interaction between sampling time and incubation length. The $V5_{0N}$ treatment with the 28-d incubation had greater PMN_{an} than the PPNT treatment for all three incubation lengths and all three incubations of the $V5_{0N}$ were greater than the 7-d PPNT treatment. However, the 7- and 14-d incubations of the $V5_{0N}$ treatment were similar to one or both of the 28- and 14-d incubations of the PPNT treatments. Sampling timing had a significant effect on PMN_{an} in 18 sites. In nine of those sites, the PPNT timing had greater PMN_{an} than the $V5_{0N}$ timing while in the other nine sites the $V5_{0N}$ timing had greater PMN_{an} than the PPNT timing. No difference in PMN_{an} was found between samplings in the remaining 11 sites.

Coarse textured soils normally have less organic matter, but typically warm up and begin to mineralize N faster in the spring compared to medium and fine textured soils. This can result in less organic N available to mineralize by the $V5_{0N}$ sampling. This may help explain greater PMN_{an} at PPNT compared to $V5_{0N}$ at three of the five coarse textured soils in this study. The two coarse textured soils that had greater PMN_{an} at $V5_{0N}$ had more organic matter and clay compared to the other coarse textured soils, which may be why they had higher PMN_{an} at $V5_{0N}$ compared to PPNT. These PMN_{an} differences due to soil sampling timing provide the potential for improving the ability of PMN_{an} to predict EONR, N uptake, and grain yield if soil sampling is postponed until after the high leaching potential period, but before high corn N uptake begins.

Nitrogen Fertilizer

Thirty-two site-years were evaluated to compare the effect of the addition of N at the time of planting on PMN_{an} for soil samples taken at the V5 corn development stage. The random interaction of site-year by N treatment by incubation length was significant ($P \leq 0.05$). Because these interactions included site-year, each site-year was evaluated separately. Within each site-year there was no interaction between N treatment and incubation length. Nitrogen fertilizer addition had a significant effect on PMN_{an} in thirteen sites. In 11 of those sites, the $V5_{0N}$ treatment had greater PMN_{an} than the $V5_{180N}$ treatment while in the other two sites the $V5_{180N}$ treatment had greater PMN_{an} than the $V5_{0N}$ treatment. No difference in PMN_{an} was found in the remaining 19 sites.

The lower PMN_{an} for the $V5_{180N}$ sampling in 11 sites may have occurred because the N fertilizer increased the rate of mineralization of the easily decomposable organic matter and left the more recalcitrant organic materials to be mineralized at the V5 sampling (Conde et al., 2005; Chen et al., 2014). Brandes15 was one of two sites where the addition of N increased PMN_{an} at the $V5_{180N}$ treatment. This was likely the result of a small standard error (1.8 mg-N kg^{-1}), since the mean difference between the $V5_{0N}$ and $V5_{180N}$ treatments was only 3 mg-N kg^{-1}. This difference is similar to the mean difference between the $V5_{0N}$ and $V5_{180N}$ treatments for those site-years where there were no statistical differences in PMN_{an} (average difference = 3.8 mg-N kg^{-1}) and much lower compared to those sites with a statistical difference (average difference = 12.2 mg-N kg^{-1}). These results illustrate that the addition of N can alter PMN_{an}. This may indicate that PMN_{an} values produced from soil samples that have been fertilized could improve the correlation of PMN_{an} with EONR, N uptake, and corn grain yield.

Correlation of PMN_{an} to Agronomic Variables

Delaying soil sampling from PPNT to the V5 corn development stage and the addition of N fertilizer influenced PMN_{an} values. Also, lengthening the incubation period increased PMN_{an} values. These changes in PMN_{an} values have the potential to increase the correlation between PMN_{an} and EONR, N uptake, and corn grain yield.

EONR

None of the PMN_{an} values for the different soil sampling times or incubation lengths predicted EONR when all of the N was applied at planting ($EONR_{AP}$) ($P \leq 0.05$). However, at $P \leq 0.1$ the PMN_{an} value of the PPNT soil sampling time and 7-d incubation was correlated (R^2=0.023) to $EONR_{AP}$. At $P \leq 0.05$, the PPNT soil sampling time with 7-, 14-, and 28-d incubations produced PMN_{an} values that were correlated to EONR of the split N applications ($EONR_{V9}$) with the 28-d incubation having the highest correlation (R^2=0.056). Moving the soil sampling from PPNT to the V5 corn development stage or the addition of N fertilizer did not improve PMN_{an} correlation to EONR for single or split N applications. Increasing the incubation length from 7- to 14- or 28-d also did not increase the correlation of PMN_{an} to EONR for single N applications. However, moving from 7- to 28-d incubations improved the correlation (R^2) of PMN_{an} to EONR for split N applications by 0.0035 and 0.018 for PPNT and $V5_{0N}$ soil sampling timings, respectively.

Plant N Uptake

The PMN_{an} values for the PPNT sampling time were correlated to plant N uptake of the 0-N treatment regardless of incubation length, but the 14-d incubation was the best (R^2=0.055). The PMN_{an} values for the 28-d incubation of the $V5_{0N}$ sampling timing was also correlated to plant N uptake (R^2=0.035). When predicting N uptake of the 0-N treatment, delaying soil sampling time from PPNT to V5 did not improve the correlation with PMN_{an}. However, increasing the incubation length did improve the predictive power of PMN_{an} by 0.0097 and 0.029 for PPNT and $V5_{0N}$ soil sampling time, respectively.

The PMN_{an} values from the PPNT soil sampling time were not correlated to plant N uptake when 180 kg-N ha^{-1} were applied as a single or split application. The 7- and 14-d incubation lengths for the $V5_{0N}$ soil sampling correlated to plant N uptake, but the 180 kg-N ha^{-1} split application with the 7-d incubation provided the best coefficient of determination (R^2=0.064). The 7-d incubation of the $V5_{180N}$ soil sampling time was the only one to produce PMN_{an} values that were correlated to plant N uptake when 180 kg-N ha^{-1} was applied as a single application

(R^2=0.098). Moving the soil sampling from PPNT to the V5 corn development stage improved the correlation of PMN_{an} to N uptake for single and split N applications. Increasing the incubation length beyond 7-d did not improve the correlation for single or split N applications.

Grain Yield

The PMN_{an} values for PPNT in all three incubation lengths were correlated to the relative yield of the 0-N treatment for single and split N applications with the 14-d incubation (R^2= 0.054 and 0.077, respectively) being the best for both applications. The 28-d incubation length of the $V5_{0N}$ soil sampling timing was the only other PMN_{an} value correlated to the relative yield of the 0-N treatment for single and split N applications (R^2= 0.046 and 0.062, respectively). Delaying soil sampling from PPNT to the V5 corn development stage did not improve the correlation of PMN_{an} to the relative yield of single or split N applications. Increasing the incubation length did improve the correlation for the PPNT (14-d was the best) and for $V5_{0N}$ (28-d was the best) soil sampling timings.

None of the PMN_{an} values of the PPNT sampling time, regardless of incubation length, predicted yield when 180 kg-N ha^{-1} were applied as a single application ($P \geq 0.05$). However, when 180 kg-N ha^{-1} was split applied, the PMN_{an} from the PPNT soil sampling time with the 7-d incubation correlated to yield (R^2=0.04). The PMN_{an} from the $V5_{0N}$ soil sampling timing at all three incubations correlated to grain yield when 180 kg-N ha^{-1} was split applied, but the 7-d incubation had the best correlation (R^2=0.095). When 180 kg-N ha^{-1} was applied at planting, the PMN_{an} from the 7- and 14-d incubations of the $V5_{180N}$ sampling time correlated to grain yield with the correlation of the 7-d incubation being the best (R^2=0.105). Delaying soil sampling timing from PPNT to V5 improved the correlation of PMN_{an} to grain yield. However, increasing the incubation length from 7- to 14- or 28-d did not improve the correlation when N was applied as a single or split application regardless of sampling time.

PRELIMINARY CONCLUSIONS

On average, the PMN_{an} for all three soil sampling times increased as incubation length increased. The timing of soil sampling resulted in PMN_{an} differences in 19 sites. Coarse textured soils normally had higher PMN_{an} at the $V5_{0N}$ sampling, but no specific trends were observed for the fine- and medium-textured soils. The addition of N fertilizer at planting most often lowered PMN_{an} when samples were taken at the V5 corn development stage.

Delaying soil sampling from PPNT to V5 did not improve the predictive power of PMN_{an} in determining EONR for single and split N applications. However, the later sampling at V5 did improve the predictability of plant N uptake and grain yield when N fertilizer was split applied. Delaying soil sampling to the V5 corn development stage improved the correlation of PMN_{an} to plant N uptake and grain yield for the $V5_{180N}$ treatment. When predicting N uptake of the 0-N plot and the relative yield of the 0-N plot for single and split N applications, delaying the sampling time to V5 did not improve correlations. While incubation length increased R^2 values in a few instances, the increase was minimal, making it unjustifiable to extend the incubations period past 7-d. The correlations of PMN_{an} to these agronomic variables were improved in this study, but overall these correlations are still very low. At present PMN_{an} measurements do not provide strong evidence that the test can be used as a N management tool to predict EONR, N uptake, or grain yield. Currently, we are evaluating whether adding other soil and weather parameters could help improve the PMN_{an} test as a potential management tool.

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Table 1. Nitrogen (N) treatments used for single and split N applications.

Treatment	Nitrogen Application Timing		
	At Planting	V9	Total N
	-------------------------kg-N ha^{-1}-------------------------		
1	0	0	0
2	45	0	45
3	90	0	90
4	135	0	135
5	180	0	180
6	225	0	225
7	270	0	270
8	315	0	315
9	45	45	90
10	45	90	135
11	45	135	180
12	45	180	225
13	45	225	270
14	45	270	315

Table 2. Minimum, maximum, and mean anaerobic potentially mineralizable nitrogen values for each soil sampling and incubation length.

Soil Sampling	Incubation Length	Anaerobic Potentially Mineralizable Nitrogen		
		Minimum	Maximum	Mean
	Days	----------------mg-N kg^{-1} soil---------------		
PPNT	7	0.74	84.02	26.65
PPNT	14	2.38	94.53	37.83
PPNT	28	5.98	125.27	48.85
$V5_{0N}$	7	0.19	99.92	28.29
$V5_{0N}$	14	2.10	122.73	36.97
$V5_{0N}$	28	4.02	136.72	48.45
$V5_{180N}$	7	0.86	65.04	22.76
$V5_{180N}$	14	6.89	78.66	31.98
$V5_{180N}$	28	8.07	122.47	42.50

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