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Abstract
Soil tests can help optimize nitrogen (N) fertilizer rates, thereby improving farmer profitability and environmental performance. In US Midwest maize (Zea mays) production, however, most soil N tests have limited accuracy to predict N fertilizer requirements. Here we tested the individual and combined ability of 30 soil tests (12 rapid N extractions, seven biological carbon or N tests, six long-term incubation kinetic parameters, and five other routine soil tests), as well as environmental and management data, to predict maize response to N fertilizer across 56 site-years in the US Midwest. Out of 30 soil tests, and across all site-years, a 14-d aerobic incubation best predicted whether maize responded to N fertilizer, and a 5-min tetraphenyl borate extraction best predicted agronomic optimum N rate. We combined these two tests to evaluate their ability to predict N fertilizer response against the most commonly used soil N test in the US Midwest, the pre-sidedress or late-spring nitrate test (PSNT or LSNT). The combination of soil tests nearly doubled the ability to predict nonresponsive sites compared to PSNT, and on average resulted in a 40% reduction in over-application and 37% reduction in under-application of N fertilizer. Weather and management variables marginally improved the prediction of maize N response. Our results indicate that a simple combination of biological N mineralization (14-d aerobic incubation) and chemical extraction (5-min tetraphenyl borate) assays could improve current N fertilizer recommendations.

1 | INTRODUCTION

Soil tests have had a critical role in the field of soil fertility and plant nutrition for over half a century (Feller, Blanchart, Bernoux, Lal, & Manlay, 2012; Schröder, JNeeteson, Oenema, & Struik, 2000; Tisdale & Nelson, 1956). Over that time, soil testing has contributed to increased crop productivity (Cassman et al., 2006; Duvick & Cassman, 1999) and more efficient use of fertilizers preventing excess fertilizer from reaching surface waters (Jaynes et al., 2004; Sharpley et al., 2010).
Nitrogen (N) is perhaps the most difficult-to-predict nutrient due high crop requirement, complex internal soil cycling, and high environmental losses. There are two goals for a soil N test: (i) predict whether or not a crop–soil combination requires N fertilization, and if so (ii) how much fertilizer N is required. Most current soil N tests are based on salt extraction of nitrate (NO$_3^-$) in the surface 15- or 30-cm depth of soil. This approach alone seems to be a poor predictor of crop response to fertilizer N in many soils (Andraski & Bundy, 2002; Schröder et al., 2000), and thus comes as no surprise since these tests are only ‘snapshots’ of plant-available N during the growing season. Efforts have long been made to avoid these ‘snapshots,’ and to measure more seasonally-integrated measures of ‘N-supplying power’ or net N mineralization potential (Jarvis, Stockdale, Shepherd, & Powlson, 1996; Stanford & Smith, 1972; Waksman & Starkey, 1924). But despite the long-term research interest, these soil N tests have yet to be adopted widely.

More recently, simple and rapid soil tests have been developed to approximate the potentially mineralizable N supply through a variety of methods. These recent tests typically fall under one of three categories (Curtin et al., 2017): (i) measurement of an actual biological process (e.g., 7-d anaerobic net N mineralization; Keeney & Bremner, 1966; or carbon dioxide burst, Franzluebbers, 2018), (ii) extraction of an organic form of N thought to be readily mineralized over the growing season (e.g., Illinois Soil Nitrogen Test; Khan et al., 2001; or glucan extraction; Hurisso et al., 2018), or (iii) quantify labile soil organic matter fractions (e.g., permanganate extractable carbon; Culman, Snapp, Green, & Gentry, 2013). But even these tests have limited ability to predict crop N response. The soil N tests that have been studied in N response trials do not reliably estimate whether or not a crop will respond to N, and if crops do respond, typically do not predict the critical value or agronomic optimum N rate well (AONR; Laboski et al., 2008; Lory & Scharf, 2003; Osterhaus, Bundy, & Andraski, 2008)

An ideal soil N test should meet the following criteria: (i) be based on robust correlation and calibration for a region; (ii) be a close proxy for plant-available N supply, thus predicting at minimum whether a crop will or will not respond to applied N, and ideally also predict the AONR at a regional level; (iii) be a simple and rapid procedure to allow for high-throughput of samples to give producers quick turn-around on application recommendations; and (iv) be relatively inexpensive to encourage adoption. Better prediction of when a crop–soil combination will respond to N fertilizer and the AONR will save producers money on N inputs and reduce potential for environmental N loss (Sims, 1998; Vanotti & Bundy, 1994).

We used 30 different soil tests across 56 site-years of maize N response trials to answer the following two major questions. First, what soil test (or combination of tests) best predict whether a maize–soil combination shows a response to N fertilizer (RN; Figure 1a), and if there was no maize response to N fertilization, was it a high-yielding (NRHY) or low-yielding (NRLY) site-year? We predict that a NRHY soil will have a greater soil N test than the RN soils (Figure 1b), but that NRLY will be much more variable and low yielding because some other factor than N could be limiting yield. Second, what other soil, weather, and management factors can help explain the differences in N responses? We predict that using weather, management and soil properties will help predict maize N responses.

**Core Ideas**
- We evaluated 30 soil tests and weather to predict maize response to fertilizer N.
- Best test combination: 14-d aerobic incubation with 5-min tetraphenyl borate extraction.
- This combination greatly improved N response compared to PSNT (or LSNT).
- Weather and management information somewhat improved prediction.

**FIGURE 1** (a) Conceptual graph showing three different categories of N response curves: responds to N (RN), nonresponsive to N with a high yield (NRHY), and nonresponsive to N with a low yield (NRLY). (b) Conceptual graph showing how the hypothesized range in a soil N test under the three N response categories. An ideal soil N test should discriminate between RN and NRHY, but NRLY may show a wide range of soil N test values because something other than N would be limiting response to N or crop yield.
2 | MATERIALS AND METHODS

2.1 | Site description and experimental design

Nitrogen response trials included 56 site-years across seven US Midwestern states (Supplemental Figure S1). Most of the N response trials took place in Nebraska, Wisconsin, and Minnesota between 1995–1998. Each trial used a randomized complete block design with 3–6 replicates of each N rate, and 91% of trials had four or more replicates. Nitrogen rates ranged from 0–310 kg N ha$^{-1}$, applied in five increments on average (Range is 3–8), and at 34–100 kg N ha$^{-1}$ in each increment. Fertilizer N was broadcast as urea, and incorporated in the spring before planting. Maize was grown using the local management practices for each region and time period. There was large variation in management practices including irrigation, tillage, crops grown for previous 7 yr, manure addition, and crop rotation. We accounted for these management practices as categorical variables (Supplemental Table S1, S2). Weather data was also collected from nearby weather stations and used to predict N response, including cumulative maize growing degree days, soil degree days (at 10-cm depth), day of year when maize reached V6 growth stage, and precipitation (and irrigation).

2.2 | Soil and plant collection and analysis

Composite soil samples (eight cores) from the zero N plots (0N) were collected from a depth of 0–30 cm just prior to planting (pre-plant), just before V6 (pre-sidedress), and prior to maturity (pre-maturity). Soils were homogenized and brought back to lab. Soils were analyzed for 30 soil tests including (Table 1): six kinetic parameters for a 300-d incubation using the Stanford and Smith (1972) method, 12 rapid soil N extractions, seven biological carbon (C) or N tests, and five routine soil tests. The five routine soil measurements included: pH in water and 0.01 CaCl$_2$ (soil/solution ratio of 1:2.5, w/w), Mehlich III extractable phosphorus (soil test P or STP), Mehlich III extractable K (STK), and bulk density. We used a 300-d aerobic incubation as the benchmark for estimated net N mineralization, as long-term incubations have been found to be the most accurate in predicting N-supplying power of soils (Campbell, Ellert, & Jame, 1993; Franzluebbers, Pershing, Crozier, Osmond, & Schroeder-Moreno, 2018; Stanford, Legg, & Smith, 1973). We hypothesized that this test would best predict N response type, but realize this length of an incubation is not feasible for a soil N test, so we wanted to look at which rapid N tests of plant-available N (Table 1) would correlate with the 300-d incubation test. In addition to the soil tests studied, ancillary soil characteristics such as soil A horizon textural class, topographic position, and parent material were collected and used as categorical variables. Maize yield was collected at every site-year from the center two rows (18.2-m length) between late-October through mid-November after plant maturity. Grain yields are reported at 15.5 g kg$^{-1}$ moisture. Total N in grain and aboveground plant material were determined by digestion and N analysis (Nelson & Sommers, 1973). Total plant N uptake was calculated as the sum of grain and aboveground plant material.

2.3 | Data analysis and statistics

A series of data analyses and criteria were followed to determine maize N response (v.8 SAS Institute, Cary, NC; sensu Andraski & Bundy, 2002; Supplemental Figure S2). First, an analysis of variance (ANOVA) was performed on the response to N fertilizer rate; and if there was no significant effect of N rate ($P > .10$) then the AONR was deemed to be 0 kg N ha$^{-1}$. If there was a significant N rate effect then four regression analyses were performed (linear, quadratic, linear-plateau, and quadratic-plateau), and the best-fit N response curve was chosen and determined by highest $R^2$ and lowest standard error. If the $R^2$ was $> .25$ then AONR was calculated from the regression model. If the $R^2$ was $< .25$, then the AONR was calculated as the lowest N rate in the highest t-grouping (least significant difference of .10). To differentiate ‘High-yielding’ from ‘Low-yielding’ nonresponsive sites, we split at the median value (10.92 Mg ha$^{-1}$). In addition to the AONR calculation, we also calculated relative yield and N use efficiency (NUE). Relative yield is simply the 0N (control) yield divided by the maximum yield (mean of any N rate) with fertilizer N added. Nitrogen use efficiency was calculated as the apparent grain recovery efficiency or the N uptake in maximum maize yield grain minus the N uptake in the 0N grain, divided by the amount of fertilizer added at maximum yield. This NUE can be underestimated, at least for soils that showed linear responses, since the ‘true’ maximum is possibly out of the range of N rates we used.

We compared performance of the best soil test(s) against the current standard, a 1-h 2 M KCl extract of soil (NO$_3^-$)– N (0–30 cm) at pre-sidedress at approximately the V6 stage (PSN; Table 1). This current standard test, known as the pre-sidedress soil nitrate test (PSNT) in some regions but also known as the late-spring nitrate test (LSNT) in others, is commonly used in the US North Central Midwest (Magdoff, Ross, & Amadon, 1984; Shapiro, Ferguson, Hergert, Dobermann, & Wortmann, 2008). We used the critical soil nitrate N level at 0–30 cm from Bundy, Walters, and Olness (1999) of 16.8 mg N kg$^{-1}$ (dry soil) to determine Type I and Type II failures. Type I error is where fertilizer N would not be recommended based on the soil N test, but maize actually responded to N addition (or where a farmer is missing yield potential, i.e. economic risk); and Type II error is where fertilizer N would be recommended by the soil test, but maize did not respond (or
**TABLE 1** Soil tests, their abbreviations, method description, units, and reference for method

| Test category and abbreviation | Method description | Units | Most similar reference |
|-------------------------------|--------------------|-------|-------------------------|
| **Benchmark of potentially mineralizable N** – 300-d aerobic incubation modeled with equation \( N_t = N_0(1 - \exp^{-kt}) \) | | | |
| LTN0 300-d incubation first-order nitrogen \((N_0)\) | \(\text{mg N kg}^{-1}\) | Stanford and Smith (1972) |
| kNTP 300-d incubation, first-order rate constant for N in temperature | \(\circ ^{\circ} \text{C}^{-1}\) | Stanford and Smith (1972) |
| kN1 300-d incubation, first-order rate constant for N in time | \(\text{d}^{-1}\) | Stanford and Smith (1972) |
| LTC0 300-d incubation first-order carbon \((C_0)\) | \(\text{mg C kg}^{-1}\) | Stanford and Smith (1972) |
| kCTP 300-d incubation, first-order rate constant for carbon in temperature | \(\circ ^{\circ} \text{C}^{-1}\) | Stanford and Smith (1972) |
| kCTM 300-d incubation, first-order rate constant for carbon in time | \(\text{d}^{-1}\) | Stanford and Smith (1972) |
| **Rapid tests of plant-available N (Extractions)** | | | |
| PTB4 4-min \(\text{PO}_4--\text{B}_2\text{O}_3: 4\text{ g (field moist) distilled with 40 ml of 0.26 M Na}_3\text{PO}_4 + 0.066 M \text{Na}_2\text{B}_2\text{O}_3 (pH = 11.2)}\) | \(\text{mg N kg}^{-1}\) | Gianello and Bremner (1986) |
| PTB8 8-min \(\text{PO}_4--\text{B}_2\text{O}_3: 4\text{ g (field moist) distilled with 40 ml of 0.26 M Na}_3\text{PO}_4 + 0.066 M \text{Na}_2\text{B}_2\text{O}_3 (pH = 11.2)}\) | \(\text{mg N kg}^{-1}\) | Gianello and Bremner (1986) |
| KCI4 4-min 2 M KCl: 4 g was distilled with 20 ml of 2 M KCl and 0.25 g MgO, \(\text{NH}_4^+ + (\text{NO}_3^-)\) | \(\text{mg N kg}^{-1}\) | NA |
| KCI8 8-min 2 M KCl: 4 g was distilled with 20 ml of 2 M KCl and 0.25 g MgO, \(\text{NH}_4^+ + (\text{NO}_3^-)\) | \(\text{mg N kg}^{-1}\) | NA |
| STB4 4-min \(\text{Na}_2\text{B}_2\text{O}_3: 4\text{ g of field moist sample distilled with 40 ml of 0.066 M Na}_2\text{B}_2\text{O}_3 (pH = 11.2)}\) | \(\text{mg N kg}^{-1}\) | Gianello and Bremner (1986) |
| STB8 8-min \(\text{Na}_2\text{B}_2\text{O}_3: 4\text{ g of field moist sample distilled with 40 ml of 0.066 M Na}_2\text{B}_2\text{O}_3 (pH = 11.2)}\) | \(\text{mg N kg}^{-1}\) | Gianello and Bremner (1986) |
| FIXN Fixed \(\text{NH}_4--\text{N}: 5\text{ M HF} + 1\text{ M HCl}\) | \(\text{mg N kg}^{-1}\) | Silva and Bremner (1966) |
| TPB5 5-min \((\text{C}_6\text{H}_5\text{Na})_2\)Ba extraction of \(\text{NH}_4\) | \(\text{mg N kg}^{-1}\) | Cox et al. (1996) |
| TPB7 7-d \((\text{C}_6\text{H}_5\text{Na})_2\)Ba extraction of \(\text{NH}_4\) | \(\text{mg N kg}^{-1}\) | Cox et al. (1996) |
| PPN 1-h 2 M KCl extraction of pre-plant soils, \(\text{NO}_3\) | \(\text{mg N kg}^{-1}\) | Blackmer et al. (1989) |
| PSN 1-h 2 M KCl extraction of pre-sidedress soils, \(\text{NO}_3\) | \(\text{mg N kg}^{-1}\) | Blackmer et al. (1989) |
| PRN 1-h 2 M KCl extraction of pre-maturity soils, \(\text{NO}_3\) | \(\text{mg N kg}^{-1}\) | Blackmer et al. (1989) |
| **Biological C or N tests (assessing carbon or biological sources of N)** | | | |
| SOC Soil organic C measured with combustion on elemental analyzer (LECO CHN) | % | NA |
| TN Total N measured with combustion on elemental analyzer (LECO CHN) | % | NA |
| MBC Microbial biomass C via chloroform-fumigation extraction | \(\text{mg C kg}^{-1}\) | Vance, Brookes, and Jenkinson (1987) |
| MBN Microbial biomass N via chloroform-fumigation extraction | \(\text{mg N kg}^{-1}\) | Horwath and Paul (1994) |
| AEIM 14-d aerobic incubation: \(~10 \text{ g soil, plus 30 g sand, at } 30^\circ \text{C, } \text{NH}_4^+ + (\text{NO}_3^-)\text{N extracted with 2 M KCl}\) | \(\text{mg N kg}^{-1}\) | Keeney and Bremner (1967) |
| ANIM 14-d anaerobic incubation: 4 g soil/12.5 ml DI \(\text{H}_2\text{O at } 30^\circ \text{C, } (\text{NH}_4^+)\text{N measured with steam distillation}\) | \(\text{mg N kg}^{-1}\) | Waring and Bremner (1964) |
| ANID 14-d anaerobic incubation: 4 g soil/12.5 ml DI \(\text{H}_2\text{O at } 30^\circ \text{C, } (\text{NH}_4^+)\text{N measured with steam distillation}\) | \(\text{mg N kg}^{-1}\) | Waring and Bremner (1964) |
| **Routine soil measurements** | | | |
| pH \(_{\text{water}}\) Soil pH measured in water, with electrode (1:1 w/w) | unitless | Thomas (1996) |
| pH \(_{\text{CaCl}}\) Soil pH measured in 0.01 M CaCl\(_2\), with electrode (1:1 w/w) | unitless | Thomas (1996) |
| STP Mehlich III P (or soil test P) | \(\text{mg P kg}^{-1}\) | Mehlich (1984) |
| STK Mehlich III K (or soil test K) | \(\text{mg K kg}^{-1}\) | Mehlich (1984) |
| \(D_b\) Bulk density with core method | \(\text{Mg m}^{-3}\) | NA |
FIGURE 2 (a) Maize yield response to N rate for the 56 site-years. Data points at each N rate within line graphs are means from each site \((n = 3–6)\). (b) Box and whisker plots of 0N (control) maize yield and maximum yield at highest N rate (Max. Yield) with mean (black line), median (line with respective box color), 10th percentile (top whisker), 25th percentile, 75th percentile, and 90th percentile (bottom whisker). For abbreviations, see Figure 1 or manuscript abbreviation list. See Table 2 for more information and statistics for each site-year.

We also used a combination of traditional statistics and a decision-tree machine learning tool, to determine differences among response types and to predict the response types (i.e., RN, NRHY, and NRLY). Data were checked for normality and heterogeneity of variances in R (v3.4.3, the R Foundation for Statistical Computing, Vienna, Austria) using Q-Q plots (\texttt{qqnorm}), Shapiro test (\texttt{shapiro.test}), and Barlett test (\texttt{bartlett.test}), and outliers were removed if \(P < .05\). Data did not need transformation based on these tests. The ANOVAs between N response types were performed with the R package \texttt{aov} and comparison of means with \texttt{TukeyHSD} (\(\alpha = .05\)). Nonparametric decision-tree analysis was used to understand the variables, and their interactions, that best explained the variations in maize yield response to N (Breiman, 2001). The \texttt{randomForest} function, an R package (Liaw & Wiener, 2002), was used on the imputed data with the control parameters \(ntree = 500\) (number of trees) and \(mtry = 3\) (number of variables considered for splitting at each node). We chose to use two decision trees to predict N response: (i) decision tree using only soil test data that separated out the three N response categories, and (ii) decision tree that included all available data (i.e., soil tests, ancillary soil variables, weather, and management data). Additionally, given the importance of management history and climate in determining maize yield response to N, we also used a multivariate logistic regression model to determine the overall best predictors of N response. We used SigmaPlot (v.13, Systat Software, San Jose, CA) for linear and nonlinear correlations among variables and visualization of data.

3 | RESULTS AND DISCUSSION

3.1 | Soil test(s) that best predict maize response to nitrogen

Maize showed a wide variety of responses to N rate (Figure 2a; Table 2); with 59% \((n = 33)\) of the soils responding to N, and from these the 0N yields ranged from 4.33–16.13 Mg ha\(^{-1}\) (average of 7.95 Mg ha\(^{-1}\)). Bundy et al. (1999) across similar sites in the US Midwest (between 1988–1992) found a similar percentage of responsive sites (56%), but had a less strict definition of responsive as just \(\leq 90\%\) relative
TABLE 2  Site information and N response characteristics

| Site # | Site ID | Surface soil texture | Cropping systema | Tillageb | Current and/or previous manure history | Yield at 0N (and SD)c | Effect of N rate P-value | Equationd | AONR kg N ha−1 | N response category |
|--------|---------|----------------------|------------------|----------|----------------------------------------|-----------------------|--------------------------|------------|----------------|------------------|
| 1      | NE951   | silt                 | ccc              | r        | 1 yr + 2 yr                            | 13.37 (1.59)          | .97 ns                   | 0 NRHY     |                |                  |
| 2      | NE953   | silt                 | ccc              | d        | study yr + 2 yr                         | 8.47 (1)              | .23 ns                   | 0 NRLY     |                |                  |
| 3      | NE954   | silt                 | ccc              | d        | none                                   | 7.85 (0.89)           | <.01 LRP                 | 45 RN      |                |                  |
| 4      | NE955   | silty clay           | ccc              | d        | 1 yr                                   | 5.77 (0.9)            | .51 ns                   | 0 NRLY     |                |                  |
| 5      | WI951   | silt                 | cr               | cp       | none                                    | 10.04 (0.48)          | <.01 Quad                | 121 RN     |                |                  |
| 6      | WI952   | silt                 | ccc              | nt       | none                                    | 8.03 (0.58)           | <.01 Quad                | 136 RN     |                |                  |
| 7      | IL961   | silty clay           | cs               | cp       | none                                    | 6.03 (0.34)           | <.01 QRP                 | 148 RN     |                |                  |
| 8      | IL962   | silt                 | cs               | d        | none                                    | 9.29 (0.62)           | <.01 QRP                 | 120 RN     |                |                  |
| 9      | MI961   | sandy clay           | ccs              | mp       | none                                    | 8.47 (0.67)           | <.01 QRP                 | 169 RN     |                |                  |
| 10     | MNW961  | clay loam            | cca              | mp       | 1 yr                                    | 10.36 (0.54)          | .09 LSD                  | 30 RN      |                |                  |
| 11     | MNW962  | clay loam            | ccc              | mp       | 2 yr                                    | 7.66 (0.4)            | <.01 Quad                | 123 RN     |                |                  |
| 12     | MNW963  | clay loam            | ccc              | mp       | 2 yr                                    | 10.42 (0.61)          | .02 LRP                  | 29 RN      |                |                  |
| 13     | MNW964  | clay loam            | ccc              | mp       | 1 yr                                    | 11.49 (0.43)          | .73 ns                   | 0 NRHY     |                |                  |
| 14     | NE961   | silty clay           | cs               | nt       | none                                    | 6.78 (0.82)           | <.01 Linear              | 178 RN     |                |                  |
| 15     | NE962   | silty clay           | cs               | nt       | 1 yr + 2 yr                             | 6.65 (1.14)           | <.01 Linear              | 178 RN     |                |                  |
| 16     | NE963   | silty clay           | cs               | nt       | 1 yr + 2 yr                             | 10.42 (0.85)          | .79 ns                   | 0 NRLY     |                |                  |
| 17     | NE965   | silt                 | ccc              | r        | none                                    | 13.93 (0.92)          | .21 ns                   | 0 NRHY     |                |                  |
| 18     | NE966   | silty clay           | ca               | nt       | none                                    | 16.13 (2.53)          | .15 ns                   | 0 NRHY     |                |                  |
| 19     | NE967   | silty clay           | cs               | d        | none                                    | 9.35 (1.13)           | .71 ns                   | 0 NRLY     |                |                  |
| 20     | WI961   | silt                 | ca               | none     | none                                    | 8.03 (0.55)           | .1 LRP                   | 45 RN      |                |                  |
| 21     | WI962   | silt                 | cw               | cp       | none                                    | 10.92 (0.54)          | .11 ns                   | 0 NRHY     |                |                  |
| 22     | WI963   | silt                 | ccc              | nt       | none                                    | 5.21 (0.45)           | <.01 Quad                | 173 RN     |                |                  |
| 23     | WI964   | silt                 | cs               | nt       | none                                    | 9.73 (0.73)           | .01 Quad                | 118 RN     |                |                  |
| 24     | IL971   | silty clay           | cs               | cp       | none                                    | 9.85 (0.87)           | .08 Quad                | 175 RN     |                |                  |
| 25     | IL972   | silty clay           | cs               | d        | none                                    | 5.84 (0.67)           | <.01 LRP                | 156 RN     |                |                  |
| 26     | MI971   | sandy clay           | ccs              | nt       | none                                    | 4.33 (0.58)           | <.01 Linear              | 200 RN     |                |                  |
| 27     | MNW971  | clay loam            | ccc              | mp       | 2 yr                                    | 6.84 (0.38)           | <.01 LRP                | 106 RN     |                |                  |
| 28     | MNW972  | clay loam            | ccc              | mp       | 1 yr                                    | 11.42 (0.56)          | .06 Linear              | 90 RN      |                |                  |
| 29     | MNW973  | silt                 | ccc              | mp       | 2 yr                                    | 11.24 (0.51)          | .13 ns                   | 0 NRHY     |                |                  |
| 30     | NE971   | silty clay           | ccc              | r        | 1 yr + 2 yr                             | 11.42 (1.07)          | .36 ns                   | 0 NRHY     |                |                  |
| 31     | NE972   | silty clay           | ccc              | r        | none                                    | 12.37 (0.94)          | .41 ns                   | 0 NRHY     |                |                  |
| 32     | NE973   | silty clay           | cc               | d        | study yr                                | 5.4 (0.46)            | .16 ns                   | 0 NRLY     |                |                  |
| 33     | NE974   | silt                 | cs               | d        | study yr + 1 yr + 2 yr                   | 5.15 (1.96)           | .61 ns                   | 0 NRLY     |                |                  |
| 34     | NE975   | silty clay           | ca               | nt       | none                                    | 8.47 (1.81)           | .57 ns                   | 0 NRLY     |                |                  |
| 35     | NE976   | silty clay           | cs               | d        | study yr                                | 10.55 (0.61)          | .35 ns                   | 0 NRLY     |                |                  |
| 36     | NE977   | silty clay           | cs               | d        | none                                    | 10.55 (0.88)          | .49 ns                   | 0 NRLY     |                |                  |
| 37     | NE978   | silty clay           | ccs              | d        | study yr                                | 13.87 (0.8)           | .26 ns                   | 0 NRHY     |                |                  |
| 38     | NE979   | silty clay           | ccs              | d        | none                                    | 11.61 (1.03)          | <.01 LRP                | 117 RN     |                |                  |
| 39     | WI971   | silt                 | ccs              | cp       | 1 yr                                    | 9.85 (0.59)           | <.01 Quad                | 173 RN     |                |                  |
| 40     | WI972   | silt                 | ccs              | cp       | 2 yr                                    | 10.42 (0.86)          | .77 ns                   | 0 NRLY     |                |                  |
| 41     | WI973   | silt                 | cs               | nt       | none                                    | 6.78 (0.68)           | <.01 LRP                | 99 RN      |                |                  |
| 42     | MI981   | clay                 | cs               | nt       | none                                    | 7.47 (0.46)           | <.01 QRP                | 83 RN      |                |                  |
| 43     | MI982   | sandy clay           | ccs              | mp       | none                                    | 7.47 (0.27)           | <.01 LRP                | 125 RN     |                |                  |

(Continues)
yield. Nevertheless, more recent studies find that the majority of sites respond to N fertilizer addition (Kablan et al., 2017; Laboski et al., 2008; Woli et al., 2016; Yost et al., 2018). We split the 23 nonresponsive site-years into two groups: (i) 12 site-years were deemed high-yielding (NRHY) with average 0N yield of 12.75 Mg ha\(^{-1}\), and 11 site-years low-yielding (NRLY) with average 0N yield of 8.19 Mg ha\(^{-1}\). The mean, median, and ranges of 0N yield from these three N response types is consistent with our RN, NRHY, and NRLY categories. While at first it may seem arbitrary to separate nonresponsive soils since there is nothing a producer can do (with regards to N fertilizer) about a nonresponsive soil; we did this because we were interested in the underlying soil fertility mechanisms that may be responsible for these low-yielding, yet non-responsive, soils.

There were four individual soil tests that resolved differences between the three maize N response categories (Table 3; Figure 3): (i) 8-min 2 M KCl extract for nitrate (KCl8) resolved NRHY and NRLY, (ii) soil organic carbon (SOC) was able to resolve RN from NRLY, (iii) 14-d aerobic incubation with moist soils (AEIM) resolved RN from nonresponsive soils, and (iv) STP resolved RN and NRHY. The SOC and AEIM highlight the importance of soil organic matter to maize yields (Oldfield, Bradford, & Wood, 2019), but in different and not always straightforward ways. For example, the most N-responsive sites had the highest SOC. Nearly 40% of the soils had SOC > 2%, and those with higher SOC were more responsive than low SOC. This seems counterintuitive, as any increases in SOC would be expected to be coupled with increases in potentially mineralizable N as well as other soil health benefits (Li et al., 2019; Mahal, Castellano, & Miguez, 2018; Oldfield et al., 2019). However, SOC and AEIM were not related across these 56 site-years and soils with the highest SOC actually responded to N more often than soils with low SOC. There are two likely explanations for this counterintuitive finding. First, SOC may just indirectly reflect soil texture (Augustin & Cihacek, 2016; Plante, Conant, Stewart, Paustian, & Six, 2006), meaning soils with higher SOC are also finer-textured, and finer-textured soils have been shown by some to be more responsive to N fertilizer for reasons still unknown (Alotaibi, Cambouris, St. Luce, Ziadi, & Tremblay, 2018; Spackman, Fernandez, Coulter, Kaiser, & Paiao, 2019; Tremblay et al., 2012). Second, soils with greater SOC may be more responsive to fertilizer N because of labile C and N stoichiometry. In other words, soils with high SOC also have greater labile C, perhaps relative to potentially mineralizable N, driving N immobilization that needs to be overcome by fertilizer N addition. Although these dynamics are not supported by our soil microbial biomass C and N data, others have found microbial biomass to relate to potentially mineralizable N (Li et al., 2019; Mahal, Castellano, & Miguez, 2018; Oldfield et al., 2019). Mostly regardless of SOC, differences between responsive and nonresponsive soils were best predicted by AEIM alone (Figure 3).

Soils with greater potentially mineralizable N (assessed via AEIM) had greater yields, N uptake, and required less fertilizer N (P ≤ .03; Figure 4). The negative correlation with

### Table 2 (Continued)

| Site # | Site ID | Surface soil texture | Cropping systema | Tillageb | Current and/or previous manure history | Yield at 0N (and SD)c | Effect of N rate | Equationd | AONR (kg N ha\(^{-1}\)) | N response category |
|--------|--------|----------------------|------------------|----------|----------------------------------------|----------------------|----------------|------------|---------------------|-------------------|
| 44     | NE981  | silty clay           | ccc               | d        | 1 yr                                   | 7.72 (0.83)          | <.01           | LRP        | 153 RN              |
| 45     | NE982  | silty clay           | ccc               | d        | none                                   | 5.96 (0.63)          | <.01           | Quad       | 170 RN              |
| 46     | NE983  | silt loam            | ca                | nt       | none                                   | 5.59 (1.43)          | .88            | NS         | 0 NRLY              |
| 47     | NE984  | silt                 | cs                | d        | none                                   | 7.09 (1.21)          | <.01           | LRP        | 175 RN              |
| 48     | NE985  | sandy loam           | ccs               | d        | study yr                               | 12.49 (0.9)          | .37            | ns         | 0 NRHY              |
| 49     | NE986  | sandy loam           | ccs               | d        | none                                   | 6.28 (0.7)           | .03            | QRP        | 75 RN               |
| 50     | NE987  | sandy loam           | ccs               | nt       | study yr                               | 10.92 (0.82)         | .15            | ns         | 0 NRHY              |
| 51     | IL981  | clay loam            | nd                | nd       | nd                                     | 2.39 (0.32)          | <.01           | LRP        | 197 RN              |
| 52     | MNW981 | clay loam            | ccc               | mp       | 2 yr                                   | 9.67 (0.95)          | <.01           | Linear     | 150 RN              |
| 53     | MNW982 | clay loam            | ccc               | mp       | none                                   | 7.72 (0.37)          | <.01           | LRP        | 121 RN              |
| 54     | MNW983 | clay loam            | ccc               | mp       | none                                   | 9.73 (0.38)          | <.01           | Quad       | 175 RN              |
| 55     | WI981  | silt loam            | cs                | nt       | none                                   | 9.79 (0.54)          | <.01           | Quad       | 190 RN              |
| 56     | WI982  | silt loam            | ccs               | cp       | 1 yr                                   | 14.31 (0.79)         | .68            | ns         | 0 NRHY              |

### Notes

aCropping System abbreviation with current year, then previous one (or two) years of crops. ca, maize–alfalfa; cca, maize–maize–alfalfa; ccc, continuous maize; cs, maize–soybean; ccs, maize–maize–soybean; cw, maize–wheat; cr, maize–rye; nd, no data.
bPrimary tillage type. mp, moldboard plow; cp, chisel plow; d, disk; r, ridge till; nt, no-till.
cSD, standard deviation; AONR, agronomic optimum nitrogen rate; NRHY, maize nonresponsive to N fertilizer but high-yielding site-year; NRLY, maize nonresponsive to N fertilizer but low-yielding site-year; RN, maize responsive to N fertilizer; LSD, least significant difference.
dBest fit nitrogen response equation. LRP, linear to plateau; NS, not significant; QRP, quadratic to plateau; Quad, quadratic.
NUE can be explained by the inefficiency of adding N to soils that already have high N-supplying power, emphasizing the need for a soil test that incorporates potentially mineralizable N. Using only the sites that were responsive, or had AONR > 0, the TPB5 test best correlated with AONR as well as some of the rate constants for N release in 300-d incubation (Figure 4; Supplemental Figure S3). The (C₆H₅)₄–BNa in the 5-min tetraphenyl borate extraction of ammonium (TPB5) test quickly binds with NH₄⁺ fixed in clay interlayers, and reflects fixed NH₄⁺ that could be made available over a longer period of time (Cox, Joern, & Roth, 1996), such as N in a long-term incubation or over the growing season. Private soil testing laboratories, and research laboratories, rarely use (C₆H₅)₄–BNa for soil extractions. But for these US
Midwestern soils, the TPB5 best predicted the AONR within those soils that responded to N.

We separately correlated soil tests with relative yield and 0N yield to elucidate mechanisms driving baseline yield potential among N responsive and nonresponsive soils (Table 4). It is interesting to note that SOC, soil microbial biomass carbon (MBC), and anaerobic net N mineralization assays with moist and dry soils best correlated with responsive soils’ relative yield and 0N yield. Within nonresponsive soils, however, the C kinetic parameters in the 300-d incubation negatively correlated with relative yield, and pre-plant nitrate correlated positively with relative yield; but no test related well to 0N yield in nonresponsive soils. Labile SOC, measured with short-term incubations, has been shown to
FIGURE 4  Maize N dynamics regressed against potentially mineralizable N from 14-d aerobic incubation (AEIM; a–d) and 5-min tetraphenylborate extraction of NH$_4^+$ (TPB5; e). Dependent variables are (a) maize yield from 0N (or control) plots, (b) relative yield (0N divided by Maximum Yield, times 100) (c) total N uptake in 0N (or control) plots including grain and stover, and (d) N use efficiency (NUE) as measured by apparent recovery efficiency (RE; RE = ($U - U_0$)/$F$, where $U$ = total N uptake in aboveground maize in non-N-limiting plot, $U_0$ = total N uptake in maize in 0N plot, and $F$ = fertilizer N rate applied). (e) agronomic optimal nitrogen rate (AONR) calculated using best fit models (See Table 2). For more details on all regressions see Supplemental Table S3

be positively related to maize yield and yield response to N, and likely reflects a labile pool of potentially mineralizable N (Culman et al., 2013; Franzluebbers, 2018). The lack of relationship between yield (0N or relative) and potential net N mineralization in nonresponsive soils, yet strong negative relationship with long-term incubation kinetics, likely indicates that short- and long-term tests are measuring different pools of potentially mineralizable N, or that labile C dynamics may be different for nonresponsive soils compared to responsive ones. Availability of soil-labile C can shift microbial supply and demand of inorganic N through favoring net mineralization or immobilization (Hart, Nason, Myrold, & Perry, 1994; McDaniel & Grandy, 2016).

Despite the number of soil tests we examined, no one test could predict N response and AONR well. Even the 300-d aerobic incubation, which we assumed would be the benchmark for potential net N mineralization, was unable to resolve maize response to N fertilizer (Table 3; Figure 3). While there were significant correlations between the rapid N tests with 300-d incubation kinetic parameters (Supplemental Table S4), it was also quite surprising that 14-d aerobic incubations did not correlate with the 300-d incubation kinetics. The PO$_4$–B$_4$O$_7$ and Na$_2$B$_4$O$_7$ extraction tests, both at 4- and 8-min extractions, best and most consistently correlated with 300-d incubation N kinetics. Previous studies, on some of the same soils, have shown that sodium borate extractions related strongly to arylamidase activities (Dodor & Tabatabai, 2007; Ekenler & Tabatabai, 2004), and that biological catalysts for amides can comprise a large fraction of organic N in soils (15–25%; Sowden, 1958). These inconsistencies, even among a 300-d potential net N mineralization and other N tests (Supplemental Table S4), provide further evidence for the need of more than one soil test to predict maize response (Curtin et al., 2017; Ros, Temminghoff, & Hoffland, 2011; Schomberg et al., 2009).

3.2 | A combined soil test to predict nitrogen response

One of our goals was to use the extensive variety of soil tests (Table 1) to evaluate combining two or more tests to best predict maize response to fertilizer N, and determine AONR. To do this we used randomForest regression tree analysis to derive the best combination of tests from rapid N tests, biological, and routine soil tests to predict the three N response categories (Figure 1). We chose not to include the 300-d incubation parameters, even though it moderately predicted AONR (Supplemental Figure S3), because it would be unrealistic for a soil test lab to use such a long incubation, and this test was poor at predicting whether or not maize responded to N fertilizer (Figure 3; Table 3). The best combination of soil tests
## TABLE 4  Soil test Pearson correlation coefficients from linear correlations* with relative yield or 0N yield

| Test abbreviation | Responds to N (RN) |  |  |  | Nonresponsive to N (both High and Low yields – NRHY, NRLY) |  |  |
|-------------------|--------------------|---|---|---|------------------|---|---|
|                   | n | Relative yield | 0N Yield | Relative yield | 0N Yield | n | Relative yield | 0N Yield |
|                   |   | r | P-value | r | P-value |   | r | P-value | r | P-value |
| LTN0              | 28 | ns | ns | ns | ns | 21 | ns | ns | ns | ns |
| kNTP              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| kNTM              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| LTC0              | 28 | .41 | .022* | .45 | .011* | 23 | −.47 | .023* | ns | ns |
| kCTP              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| kCTM              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| PTB4              | 28 | .34 | .059 | .33 | .069 | 23 | ns | ns | ns | ns |
| PTB8              | 28 | .39 | .029* | .33 | .069 | 23 | ns | ns | ns | ns |
| KCl4              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| KCl8              | 28 | .39 | .029* | .33 | .069 | 23 | ns | ns | ns | ns |
| STB4              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| STB8              | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| FIXN              | 28 | .30 | .098 | ns | ns | 20 | ns | ns | ns | ns |
| TPB5              | 18 | ns | ns | ns | ns | 8 | ns | ns | ns | ns |
| TPB7              | 18 | ns | ns | ns | ns | 8 | ns | ns | ns | ns |
| PPN               | 28 | ns | ns | .32 | .077 | 23 | .46 | .028* | .38 | .076 |
| PSN               | 28 | ns | ns | 0.30 | 0.099 | 23 | ns | ns | ns | ns |
| PRN               | 28 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| SOC               | 28 | .43 | .016* | .46 | .009** | 23 | .36 | .090 | ns | ns |
| TN                | 28 | .37 | .043* | .36 | .045* | 23 | ns | ns | ns | ns |
| MBC               | 28 | .36 | .048* | .41 | .022* | 20 | ns | ns | ns | ns |
| MBN               | 28 | ns | ns | ns | ns | 19 | ns | ns | ns | ns |
| AEIM              | 28 | .31 | .095 | .37 | .043* | 23 | ns | ns | ns | ns |
| ANIM              | 28 | .51 | .003** | .47 | .008** | 23 | ns | ns | ns | ns |
| ANID              | 28 | .42 | .019* | .33 | .069 | 23 | ns | ns | ns | ns |
| pH<sub>water</sub> | 26 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| pH<sub>acif</sub> | 26 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| P                 | 26 | ns | ns | ns | ns | 23 | ns | ns | ns | ns |
| K                 | 28 | −.36 | .049* | −.04 | .027* | 20 | ns | ns | ns | ns |
| D<sub>b</sub>     | 28 | .35 | .057 | .34 | .064 | 22 | ns | ns | ns | ns |

*Significant at the .05 probability level.
**Significant at the .01 probability level.
*Values at least marginally significant (P < .10), otherwise non-significant (ns).

Aerobic incubation (AEIM) and KCl8 (Figure 5; Supplemental Figure S4), with an out-of-bag or total bootstrapped error estimate of 35%. With this test combination, there were still errors, where 87% of RN, 73% of NRLY, and 53% of NRHY predicted correctly. Using only the AEIM, there would be a 13% chance of committing a Type I Error or where N would be applied to a nonresponsive soil that year.

Predicting responsiveness (whether maize will or will not respond) is only part of our goal. Ultimately, a combination of soil tests should predict responsiveness and N rate required for responsive soils to reach maximum potential maize yield. Accomplishing this might require a two-phase test: (i) separate responsive from nonresponsive site-years—in our case AEIM served this purpose, and (ii) determine AONR based only on responsive soils—in our case the TPB5 best correlated with AONR among responsive soils (Figure 5; Supplemental Figure S3). We compared the AEIM–TPB5 combination test with the most common soil N test used in the Midwest for N recommendations, the PSNT, with
FIGURE 5 Combination of regression tree analysis predicting N response categories, and the best-correlated rapid N test with agronomic optimal N rate (AONR) in the responsive soils. Nitrogen response categories are: responds to N (RN, blue); nonresponsive to N, and a high yield (NRHY, red); nonresponsive to N, and a low yield (NRLY, green). This model predicted the N response type with an out-of-bag estimate of error rate at 35%. To read the tree, at each branch, follow the answer to the corresponding rapid N test—yes, move left at the intersecting node or no, move right at the intersecting node. The terminal nodes show the total number of site-years (n) predicted by the randomForest model, the distribution of their N response categories in pie graphs, and boxplots from site-years control (0N) and maximum yield at highest N rate (Max.) Box and whisker plots show mean (white line) median (black middle line), 10th percentile (top whisker), 25th percentile, 75th percentile, and 90th percentile (bottom whisker). The scatter plot at the bottom is the rapid N test that best correlated with AONR—the 5-min tetraphenylborate extraction of NH₄⁺ (Figure 4; Supplemental Figure S3).

FIGURE 6 Comparison of N recommendations predicted using pre-sidedress nitrate test (PSNT) from Shapiro et al. (2008) and new combined tests (14-d aerobic incubation, AEIM; and 5-min tetraphenylborate extraction, TPB5). Presented are data for each site-year; the difference between the predicted N rate and actual agronomic optimal N rate (AONR). Positive values are over-application of N fertilizer (susceptible to environmental loss), negative are under-applied N fertilizer (economic loss). (a) Histogram showing the frequency of under- and over-applied N for each 50 kg N ha⁻¹ category. (b) Boxplots showing under- and over-applied fertilizer rates (left and right boxes respectively). Box and whisker plots show mean (dashed line), median (solid line), 10th percentile (left whisker), 25th percentile, 75th percentile, and 90th percentile (right whisker).

Using the combination of tests also decreased the number of site-years with over application (from 18 to 15) and under application (from 27 to 21). This resulted in a net average 37% decrease in under-applied and 40% decrease in over-applied fertilizer N.

Other studies investigated soil test accuracy, or Type I and II failures (Andraski & Bundy, 2002; Bundy et al., 1999; Mulvaney, Khan, & Ellsworth, 2006); however, our study was the first to use a two-soil-test approach. Granted, a soil test or...
even combinations of tests, will always have limited predictive ability due to variation in maize response to N driven by weather. Probably the best evidence for this comes from a study of 101 field trials in Wisconsin in the 1990s that showed spring temperatures drastically changed predictive capability of the PSNT (Andraski & Bundy, 2002). When Wisconsin spring air temperatures were below average, the PSNT rarely predicted optimal N fertilization rates correctly (37% correct and 59% with over-applied N), but when at or above average spring temperature, PSNT correctly predicted 76% of sites. This study, and others, highlight the potential need for forecasting and weather modeling to help inform N fertilizer recommendations.

3.3 | Other factors regulating the maize response to nitrogen

Using soil tests in combination with weather, management, and ancillary soil data slightly improved the out-of-bag error estimate from 35 to 38% (Supplemental Figure S5, S6). The AEIM was again the largest discriminating factor, with surprisingly few other factors explaining the N response types. However, other discriminating variables included pH$_{\text{CaCl}_2}$, soil organic matter, and the only weather variable to make the model, soil growing degree days between planting and maturity, which resolved RN and NRHY sites ($n = 5$ and 8 site-years, respectively).

Very little weather, and no management variables, improved prediction of maize yield response to N fertilizer with machine learning (Supplemental Figure S6). This might be explained by the overarching effect of climate and management on maize yield at every N rate at each individual site. In other words, calculating whether or not a maize–soil combination responds to N, and the AONR of that combination, is highly soil specific (Vanotti & Bundy, 1994). Past studies have shown how AONR, or EONR, for maize is often well-predicted solely by potentially mineralizable N (Curtin et al., 2017; Franzluebbers, 2018; Ros et al., 2011), yet there is still a large amount of variability within those studies. This is not to say there is no interaction between weather and N response, but rather we did not observe a strong effect here with our limited weather data. The importance of weather in regulating N response has clearly been shown by the Wisconsin study mentioned previously (Andraski & Bundy, 2002), but also more recently by others, including how rainfall is distributed over a growing season or part of a growing season (Kablan et al., 2017; Tremblay et al., 2012; Xie et al., 2013). Interactions between soil N-supplying power and current-year weather interact in complex ways and remains poorly understood by agronomists.

For many producers in the US Midwest, N fertilizer rates are derived from research-based N rate calculators (e.g., Maximum Return to N, http://cnrc.agron.iastate.edu/) or yield goals. We applied a ‘no-soil-test-required’ approach to predict N responsiveness based on management and soil properties (Table 5). The best logistic regression model included manure application, soil drainage class, growing degree days, and total precipitation over the growing season (Supplemental Table S5). This model more poorly predicted nonresponsive sites (6% lower accuracy), but better predicted responsive sites (3% greater), than the AEIM test. Even though this model is nearly as effective as taking a soil sample, at least with predicting responsiveness, it is not practical. While producers may have management history and soil drainage class before planting maize, they will not have weather data for the current growing season. Therefore, application of models like this are better for explaining past maize response, rather than forecasting or predicting responsiveness.

4 | CONCLUSIONS

Out of 30 soil tests, weather data, management, and ancillary soil properties, only two tests emerged as the best predictors of maize response to N. The AEIM best predicted whether (or not) a maize–yield–soil combination responded to N fertilizer. However, for practical reasons, this test is not used at private or public soil test laboratories. A 14-d aerobic incubation requires too much time and effort for a private (or university) soil test laboratory. Further research is needed to better approximate plant-available N over a growing season. Whether that might involve shorter incubations, or extractions of organic N pools that are likely to be mineralized, these ‘N-supplying power’ soil tests must be able to be performed quickly and reliably (low analytical
variability). While the AEIM best predicted responsiveness to N fertilizer, to make an agronomic N rate recommendation, a second test was needed. Here we found the TPB5, or 5-min (C_6H_4)_2BNa extraction, best correlated with AONR within responsive soils. The combination of these two tests better predicted N response than the most commonly used US Midwest soil N test (PSNT), and could potentially increase yields in under-applied fields and save producers money on fertilizer N in over-applied fields (and also reduce effects of N loss to the environment). A rapid and inexpensive, yet scientifically robust test(s) for potential net N mineralization is needed to be a viable option for producers to enhance N fertilizer recommendations.

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