

## ORIGINAL ARTICLE

## Soil Biology &amp; Biochemistry

# An evaluation of nitrogen indicators for soil health in long-term agricultural experiments

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**Abbreviations:** ACE, autoclavable citrate extractable protein; EU, experimental unit; NAG, *N*-acetyl- $\beta$ -*D*-glucosaminidase; NAPESHM, North American Project to Evaluate Soil Health Measurements; PMN, potentially mineralizable nitrogen; SOC, soil organic carbon; STIR, standard tillage intensity rating; TN, total soil nitrogen; WEON, water-extractable organic nitrogen.

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#### Abstract

Various soil health indicators that measure a chemically defined fraction of nitrogen (N) or a process related to N cycling have been proposed to quantify the potential to supply N to crops, a key soil function. We evaluated five N indicators (total soil N, autoclavable citrate extractable N, water-extractable organic N, potentially mineralizable N, and *N*-acetyl- $\beta$ -*D*-glucosaminidase activity) at 124 sites with long-term experiments across North America evaluating a variety of managements. We found that 59%–81% of the variation in N indicators was among sites, with indicator values decreasing with temperature and increasing with precipitation and clay content. The N indicators increased from 6%–39% in response to decreasing tillage, cover cropping, retaining residue, and applying organic sources of nutrients. Overall, increasing the quantity of organic inputs, whether from increased residue retention, cover cropping, or rotations with higher biomass, resulted in higher values of the N indicators. Although N indicators responded to management in similar ways, the analysis cost and availability of testing laboratories is highly variable. Further, given the strong relationships of the N indicators with carbon (C) indicators, measuring soil organic C along with 24-h potential C mineralization could be used as a proxy for N supply instead of measuring potentially mineralizable N or any other N indicator directly.

## 1 | INTRODUCTION

Nitrogen (N) cycling is a key soil function related to soil health because the mineralization of soil organic N is essential for agricultural production and the soil biota (Doran & Zeiss, 2000). Thus, measurements that capture N cycling are a component of quantifying soil health. The organic N from dead plant or microbial biomass or from compounds exuded by plant roots and microbes is converted into inorganic forms of N by microbes. The rates of these N transformations depend on microclimate, substrate availability and composition, and the microbial community (Li et al., 2019). While these inorganic forms can be taken up by organisms, there are also microbes that can convert nitrate ( $\text{NO}_3^-$ ) to gaseous forms, including nitrous oxide ( $\text{N}_2\text{O}$ ), that are emitted from soils (Davidson et al., 2000). The movement of water through the soil has the potential to leach soil  $\text{NO}_3^-$ , another form of N loss from soils. Applying N fertilizer is a way to eliminate the need for the mineralization of organic N and provide inorganic N to crops, but this fertilizer N is also subject to the same pro-

cesses leading to N losses. In theory, if N can be retained and supplied from the cycling of the soil organic N pool, then less fertilizer would be needed.

The overall goal of N management should be to supply the nutrients necessary for the crop yields while retaining N in the soils and plants of the agroecosystem. Historically, soil N measurements have differed for soil fertility and soil health. Soil fertility tests guide nutrient management across the globe, but these tests can vary regionally and are based almost exclusively on extractions of inorganic N, mainly  $\text{NO}_3^-$  (Schröder et al., 2000). The short-term goal for N management in annual cropping systems is to optimize the rate, timing, placement, and source of fertilizer application that maximizes yield and minimizes costs (van Grinsven et al., 2022). However, nutrient management that minimizes N losses, especially  $\text{NO}_3^-$  leaching and the  $\text{N}_2\text{O}$  emissions, is also desirable. In a soil health context, indicators measuring organic N pools, N processes, or proxies for N pools/processes all intend to measure the capacity of a soil to supply N rather than the snapshot of bioavailable N with traditional soil fertility tests. Soil health

principles, with the goals of minimizing soil disturbance and maximizing living plant cover, should retain N in living and dead biomass, effectively increasing the pool of organic N that could be mineralized. It has been suggested that merging traditional pre-plant or pre-sidedress fertility testing and soil health assessment of biological process rates could result in more accurate fertilizer recommendations (Clark et al., 2020; Franzluebbers, 2016; McDaniel et al., 2020; Yost et al., 2018). Although the common methods for nutrient testing for fertilization are well established, it is imperative to identify which N indicators are most appropriate for quantifying soil health.

Several assays have been proposed to provide insight into soil health in terms of N cycling capacity. One way to quantify N cycling is with laboratory assays of potential N mineralization (Schomberg et al., 2009). In addition, other laboratory assays of potential activity, such as *N*-acetyl- $\beta$ -*D*-glucosaminidase (NAG) enzyme activity, have been linked to N mineralization rates (Muruganandam et al., 2009). The autoclavable citrate extractable protein (ACE) method liberates many proteins that are likely to mineralize over a growing season (Hurisso et al., 2018) and has been found to be highly correlated with laboratory net N mineralization (Geisseler et al., 2019). Organic N compounds that are in the soil solution, or so weakly sorbed to the exchange surface that they can be dislodged while shaking with water, have been suggested to be easily transformed by soil microbes to plant available N forms (Haney et al., 2018). Finally, total soil N (TN) may provide an assay of the amount of N that can be mineralized as it encompasses all the potential substrate for N mineralization, especially at large spatial scales. In general, N indicators are thought to have a 'more is better' relationship to soil health (Andrews et al., 2004). Although all these measurements relate to N cycling, they have never been compared across a range of soils and climates to determine their potential effectiveness as soil health indicators.

Soil health carbon (C) indicators may also be useful for predicting N cycling. TN is well known to be highly correlated with soil organic C (SOC), one of the most commonly measured soil health indicators. The water-extractable C and N pools have also been found to be highly correlated (Haney et al., 2012). Further, rates of potential C and N mineralization have also been shown to be related (Franzluebbers & Pershing, 2020; Haney et al., 2012). Thus, it may be possible to understand both soil C and N cycling with a more limited number of soil health indicators. These C and N indicators are known to be related to soil functions, but in order to be useful, they must also meet the following criteria: be responsive to management, be easy and inexpensive to collect and measure, and be interpretable by land managers (Doran & Zeiss, 2000). These N indicators cannot capture every reaction related to N cycling, but they could provide insight into the soil N cycling capacity.

### Core Ideas

- Nitrogen indicators of soil health vary predictably across North America with climate and soil texture.
- The response to soil health practices is similar across all indicators.
- There are strong correlations among nitrogen indicators, but also between carbon and nitrogen indicators.
- Soil organic carbon and 24-h carbon mineralization potential can be used to capture nitrogen cycling.

These indicators of pools and potential activity related to N have been studied at individual sites, or at a handful of sites, in some cases for decades. In some cases, multiple N indicators have been studied at the same site. It can be difficult to compare across studies because different soil collection or laboratory analysis methods were used in different studies or different depths were collected. Comparisons can also be complicated by the ephemeral and site-specific nature of N cycling. The goal of the present study was to identify soil health indicators best suited to characterize N cycling at a continental scale using soil data collected with the same field and laboratory methods as part of the North American Project to Evaluate Soil Health Measurements (NAPESHM). The first objective was to determine if absolute values of the soil N indicators varied predictably across North America based on inherent site factors (climate, soil texture, and pH). The second objective was to determine if the soil N indicators were responsive to management. The final objective was to evaluate the relationships among the soil N indicators and between soil N and soil C indicators to determine if a more limited suite of soil health indicators could effectively quantify soil health.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental design and data collection

The experimental design, sites, treatments, and sampling strategy for NAPESHM have been described previously (Liptzin et al., 2022; Norris et al., 2020). The indicator selection process began in 2013, as part of the "Soil Renaissance" initiated by the Samuel Roberts Noble Foundation and the Farm Foundation. Over multiple years, experts from the public and private sector participated in series of workshops and surveys to come up with a list of effective soil health indicators

based on whether the indicators (1) responded to management and (2) provided insight into soil function. From this list, a panel of soil health experts was commissioned by the Soil Health Institute to determine the most appropriate laboratory method for each indicator. The site selection began when scientists volunteered to have their long-term agricultural experiments included in the project. An expert panel reviewed the submissions based on experimental design and geographic distribution, resulting in the selection of 124 long-term agricultural research sites in the major agricultural regions of Mexico, United States, and Canada. Each site had at least two experimental treatments with one to six replicates of each treatment. We collected soils from 2032 experimental units (EUs) at 124 sites, but 20 EUs at two sites lacked sufficient management data to include in the statistical analysis. The 2012 EUs with management data represented 688 treatments. We collected soils from February 2019 to July 2019, aiming to sample just prior to planting in annual-cropping sites, except for six sites that could not be sampled until September 2019. Composite soil samples were collected with a soil knife to a 15-cm depth from three sides of a hole dug with a flat spade. Soils were collected from four to six locations depending on the size of the plot scattered in a zig-zag pattern across the EU. Samples were kept in a cooler or refrigerator until shipping and usually arrived at the laboratory within 3 days of sampling. Following sample collection, we compiled a thorough management history for each treatment: tillage timing and equipment; the timing, amount, and type of nutrients applied; the presence or absence of cover crops; and the yield for each crop.

Mean annual temperature and precipitation were calculated for each site from Daymet using the daily weather data from 2010 to 2019 (Thornton et al., 2016). The sites spanned 36° of latitude and 59° of longitude with mean annual temperatures and precipitation ranging from 3°C to 25°C and 178 to 1773 mm year<sup>-1</sup>, respectively. Irrigation, when present, was quantified on an annual basis and added to mean annual precipitation.

## 2.2 | Analytical methods of nitrogen soil health indicators

All soils were air dried and sieved to 2 mm prior to analysis. Total C and N were analyzed on oven dried (55°C) and ground soils by dry combustion in a NC2100 (CE Instruments) soil analyzer (Nelson & Sommers, 1996). To quantify ACE protein, air dried soil was shaken with a sodium citrate solution, autoclaved at 121°C for 30 min and centrifuged. The concentration of proteins in the supernatant was quantified by colorimetric reaction with bicinchoninic acid (Wright & Upadhyaya, 1996). The potential N mineralization was measured as potentially mineralizable N (PMN): the concentration

of ammonium (NH<sub>4</sub><sup>+</sup>) after a 7-day anaerobic incubation at 40°C (Bundy & Meisinger, 1994). After incubating, the soils were extracted with 2 M KCl and the concentration of NH<sub>4</sub><sup>+</sup> was measured on a Lachat (Hach) flow injection autoanalyzer (Bundy & Meisinger, 1994). While N mineralization in situ often occurs in oxic conditions, an incubation with an N<sub>2</sub> headspace makes the soil environment more homogeneous and limits transformation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> or other forms of N (Drinkwater et al., 1997). *N*-acetyl-β-*D*-glucosaminidase is a hydrolytic enzyme that breaks the glycosidic bonds in the chitin polymer *N*-acetylglucosamine resulting in the release of amino sugars. The NAG assay (Deng & Popova, 2011) is a colorimetric determination of *p*-nitrophenol released during the incubation of field moist soil with *p*-nitrophenyl-*N*-acetyl-β-*D*-glucosaminide in an acetate buffer at pH 5.5.

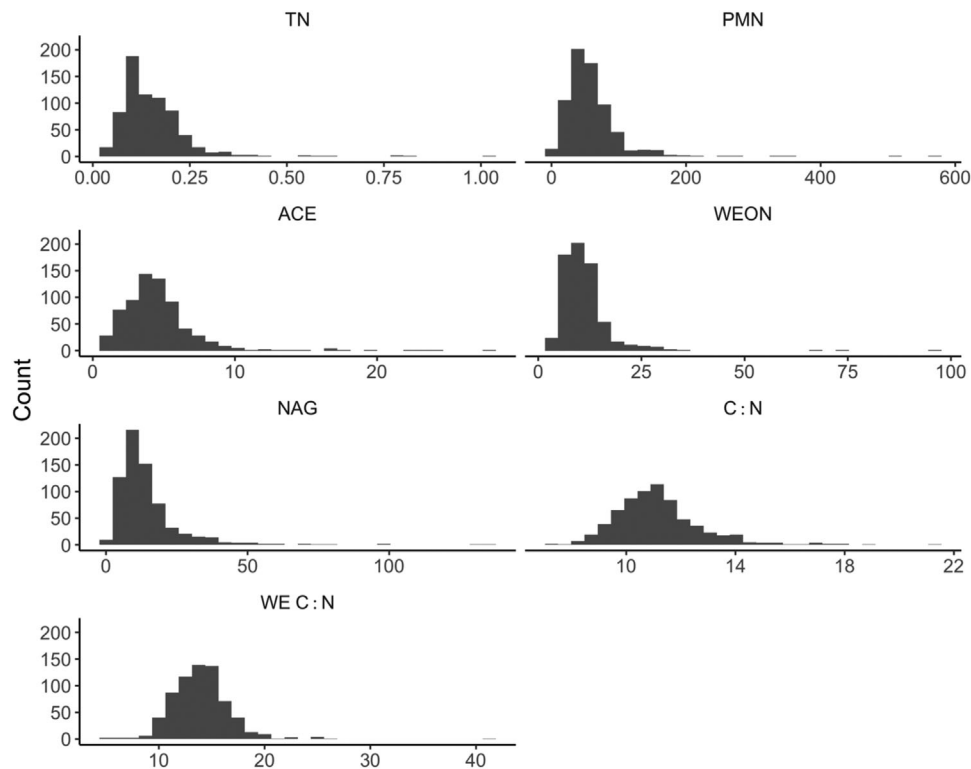
Dissolved forms of N were quantified with two extracts: H3A or water. Subsamples of soil (4 g) were shaken for 5 min with 40 mL of water and H3A in 50-mL centrifuge tubes (Haney et al., 2006). Both extracts were centrifuged and decanted prior to analysis for inorganic N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) on a flow injection analyzer (Seal Analytical) (Bundy & Meisinger, 1994). Water-extractable organic N (WEON) was measured with an Apollo 9000 (Teledyne Tekmar) with WEON calculated as the difference between total extractable N and inorganic N (Haney et al., 2012).

The SOC, TN, and PMN were analyzed by the Soil Water and Environmental Laboratory at Ohio State University, ACE was measured at the Cornell Soil Health Laboratory, and WEON and NAG were measured at Ward Laboratory in Kearney, NE.

## 2.3 | Data analysis

The data analysis was performed in RStudio Version 2021.09.1 (R studio), using base functions unless specified. Statistical significance was considered at  $p < 0.05$ . The variation in the dataset was explored in two ways using data from individual EUs. To explore the variation of each indicator, a nested model was used to partition the variance among sites, among treatments within sites, and among field replicates within treatments, using the lme4 package (Bates et al., 2015). Using the 63% of treatments with at least three field replicates, within treatment variation was assessed with the coefficient of variation.

Prior to any further data analysis, the distribution of the N indicators was explored with histograms (Figure 1). Based on the distributions, all the soil health indicators were log 10 transformed for correlation and regression analyses. We explored the relationships among the N indicators with a matrix of the Pearson correlation coefficients for the log-transformed treatment means. To explore the relationship



**FIGURE 1** Histograms of nitrogen indicators. ACE, autoclavable citrate extractable protein ( $\text{mg protein g}^{-1}$ ); Soil C:N, ratio of soil organic carbon to total soil nitrogen; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase ( $\text{mg pNP kg}^{-1} \text{ h}^{-1}$ ); PMN, potentially mineralizable nitrogen ( $\text{mg N kg}^{-1}$ ); TN, total soil nitrogen (%); WE C:N, ratio of water-extractable organic carbon to water-extractable organic nitrogen; WEON, water-extractable organic nitrogen ( $\text{mg N kg}^{-1}$ ).

between the site means of the N indicators and inherent site characteristics, we used a multiple regression model approach. We predicted the log transformed site means of each of the indicators using five site characteristics: clay content, sand content, pH, temperature, and precipitation (+irrigation). All five predictors were included in the final model regardless of their significance. We quantified the relationships among similar C and N indicators using linear regression. The analytical methods for the C indicators (SOC, water-extractable organic C,  $\beta$ -glucosidase, and 24-h potential C mineralization) can be found in Liptzin et al. (2022).

To determine the response of the N indicators to management, we used a meta-analysis approach. We compared pairs of treatments within sites whose management was identical except for one of six soil health promoting practices: decreased tillage, organic nutrients, cover cropping, crop count, rotation diversity, and residue retention. The type and frequency of the tillage equipment was cataloged for each treatment, and a standard tillage intensity rating (STIR) value for each operation that disturbed the soils was assigned (USDA-ARS, 2022). Decreased tillage (160 treatment pairs, 51 sites) was a comparison of treatments that differed only in the maximum STIR value or the sum of the STIR values for that rotation. Cover crop presence (21 treatment pairs, 10 sites) was a comparison between a treatment with at least one

cover crop (a crop that persisted for less than 1 year and was not harvested) in the rotation relative to a treatment with no cover crops. Organic nutrient addition (31 treatment pairs, 12 sites) was a comparison of treatments where biosolids, compost, or manure were used to supply the N, phosphorus, or both versus commercially available fertilizer. Crop count (199 treatment pairs, 33 sites) was a comparison between rotations with the same crop grown every year (e.g., continuous corn) to rotations with at least two different harvested crops (e.g., corn–soybean) across all years of the rotation. Rotation diversity (63 treatment pairs, 24 sites) was a comparison between rotations with only grains (e.g., continuous corn, wheat–sorghum) to a rotation with other types of crops, typically legumes, but also canola, safflower, or cotton. Treatments with fallow years were excluded. Residue retention (54 treatment pairs, 14 sites) compared treatments where the amount of residue retained was greater in at least 1 year of the rotation.

At some sites, more than one treatment pair was included for a soil health practice (e.g., two crop rotations that each had tillage treatments). Controlling for site as a random factor, we tested if there was a significant difference in each soil health indicator from the adoption of each of the six soil health practices compared to the conventional practice. In addition to the five N indicators, we also tested the SOC to TN ratio (soil

**TABLE 1** Percentage of variance for log transformed nitrogen indicators among sites, among treatments within sites, and within treatments.

Nitrogen indicator	Among sites (%)	Among treatments (%)	Within treatments (%)
TN	81	9	10
PMN	67	21	12
ACE	78	15	7
WEON	66	18	16
NAG	59	24	17

Abbreviations: ACE, autoclavable citrate extractable protein; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WEON, water-extractable organic nitrogen.

C:N) and the water-extractable C:N ratios. The meta-analysis was performed with the metafor package using log response ratios as the metric on untransformed treatment means and variances (Viechtbauer, 2010). The soil health treatments (the numerator of the log response ratio) were decreased tillage, cover cropping, organic nutrients, more than one crop for crop count, a rotation with crops other than grains, and more residue retained. To further compare the responses of the N indicators to decreased tillage, we used a principal component analysis on the site-averaged log response ratios using the prcomp function and the correlation matrix in the vegan package (Oksanen et al., 2019). We also used the same meta-analysis approach to examine the effects of fertilization on N indicators. We evaluated eight treatment pairs at five sites using organic nutrients and 39 treatment pairs at 18 sites using commercial fertilizer that compared treatments with zero N controls to typical fertilization rates.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Variability

Site was the dominant source of variation for all the N indicators, accounting for 59% of the variance in NAG up to 81% of the variance in TN (Table 1). For the within-site variance, the variance was typically about the same or slightly lower among replicate plots within treatments compared to the variance among treatments (Table 1). Exploring the within treatment variation further, the absolute amount of variation differed among the N indicators. The coefficient of variation was lowest for the indicators of N pools (ACE: 11%; TN: 12%, and WEON: 15%) and was higher for the indicators of N processes (PMN: 18% and NAG: 26%) (Figure 2).

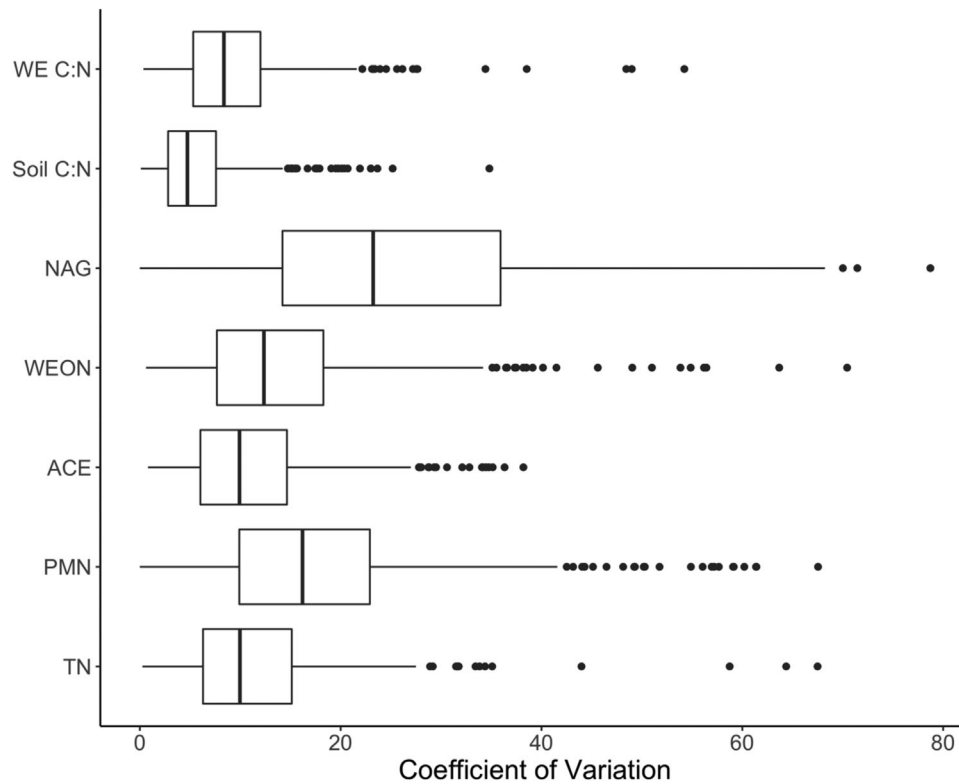
The multiple regression models predicting N indicators from the suite of inherent site factors were always significant with  $R^2$  values ranging from 0.20 for NAG up to 0.59 for ACE (Table 2). Temperature was the most consistent predictor, with

all the N indicators increasing with decreasing temperature. Three of the five N indicators were positively related to clay (TN, WEON, and NAG) and precipitation (TN, ACE, and PMN), and only one indicator was negatively related to sand (PMN) and pH (ACE).

Given that soil organisms and biological processes are sensitive to temperature, moisture, and pH, it is not surprising that site was the dominant variance component (Fierer & Jackson, 2006; Lützow & Kögel-Knabner, 2009). Nor is it surprising that inherent site factors could predict site-level means of the N indicators (Table 2). An analysis of C indicators in the NAPESHM dataset resulted in similar conclusions: site-level variance was the largest variance component and a negative relationship with temperature was the most consistent relationship with indicators (Liptzin et al., 2022). Global studies have found that TN matches patterns in SOC, but even taking this relationship into account, other site factors like texture and climate are also significant predictors (Glendining et al., 2011; Post et al., 1985). There are no global datasets for ACE, but in the United States, significant differences in ACE were reported among classes of soil texture (Fine et al., 2017). This differs from the NAPESHM dataset which found no linear relationship between either clay or sand content and ACE. Although Fine et al. (2017) reported significant differences among regions of the United States, there was no explicit test of any climate variables. Soil organic matter, temperature, and pH were significant predictors of NAG at the global scale, but soil texture was not tested as a predictor (Sinsabaugh et al., 2008). In the NAPESHM dataset, temperature and clay were significant predictors. In the multiple regression models for NAPESHM, sand, temperature, and precipitation were significant predictors of PMN. Precipitation, but not clay or temperature, was found to be correlated with PMN in a global meta-analysis (Li et al., 2019). While the significant site-level predictors of N indicators in the NAPESHM dataset were not identical to those reported in the literature, in part because the suite of predictors in the models varied across studies, these inherent site characteristics account for a large fraction of the variation in N indicators across sites.

#### 3.2 | Management

The N indicators generally increased in response to soil health practices except for cash crop diversity (Figure 3). All five N indicators significantly increased in response to the use of organic nutrients (22%–28%) and residue retention (13%–38%), all but PMN significantly increased in response to decreased tillage (6%–11%), and all but WEON increased significantly in response to cover cropping (12%–47%). While there have been many meta-analysis studies on the response of C indicators to management (Liptzin et al., 2022), there are few studies including multiple sites for N indicators. One



**FIGURE 2** Coefficient of variation for nitrogen (N) indicators calculated for within treatment variability in treatments with at least three replicates. ACE, autoclavable citrate extractable protein; C:N, ratio of soil organic carbon to TN; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WE C:N, ratio of water-extractable organic carbon to water-extractable organic nitrogen; WEON, water-extractable organic nitrogen. The box represents the 25th to 75th quartiles and the line within the box is the median. Whiskers represent 1.5 times the interquartile range and the circular symbols are shown for treatments above 1.5 times the interquartile range.

**TABLE 2** Relationship between inherent site characteristics and log transformed nitrogen indicators.

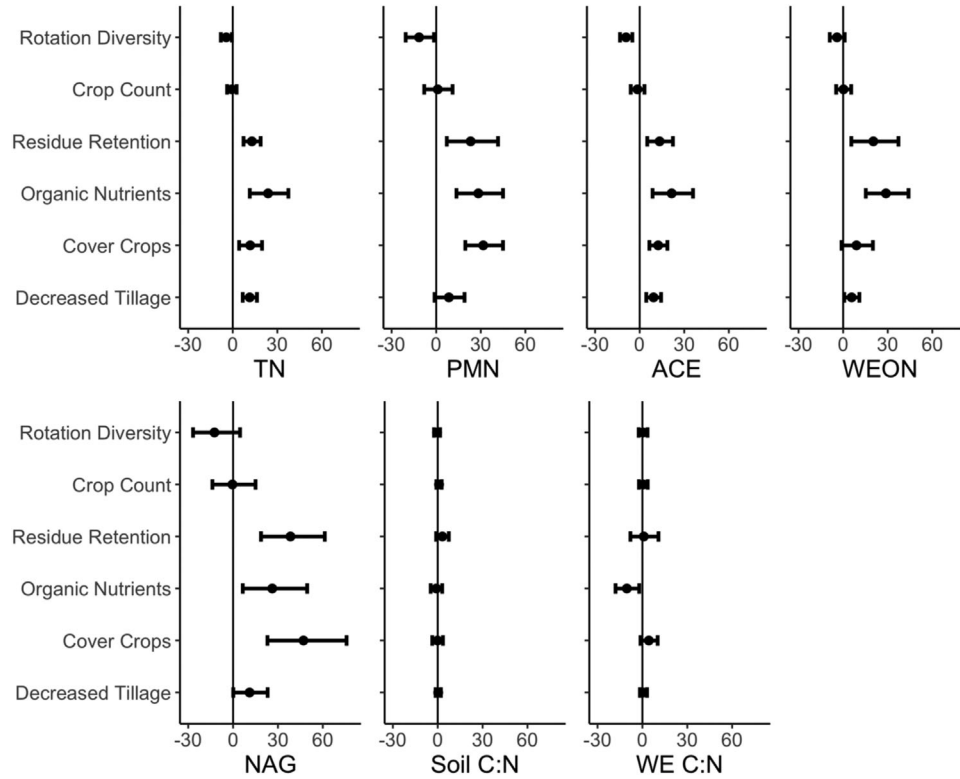
Nitrogen indicator	Sand	Clay	pH	Temperature	Precipitation	Adjusted $R^2$
TN		+		-	+	0.55
PMN	-			-	+	0.39
ACE			-	-	+	0.59
WEON		+		-		0.29
NAG		+		-		0.20

*Note:* A plus and minus symbol indicate a significant positive and negative effect, respectively, in a multiple regression model. The adjusted  $R^2$  is for the multiple regression model with all five site characteristics regardless of whether they were significant.

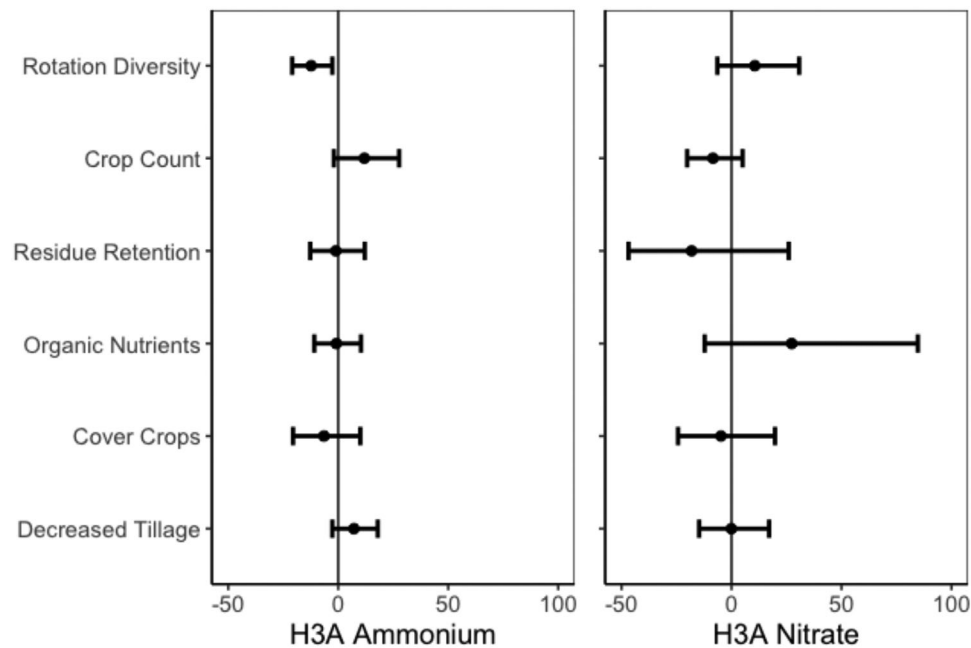
Abbreviations: ACE, autoclavable citrate extractable protein; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WEON, water-extractable organic nitrogen.

exception is that increases in PMN in response to reductions in tillage and use of cover crops have been reported (Mahal et al., 2018). Although pre-plant  $\text{NO}_3^-$  is one of the most common soil measurements and a useful indicator of fertility needs (Clark et al., 2020), the H3A extractable inorganic N measurements did not respond to the four practices considered and would not be useful as soil health indicators (Figure 4). None of the N indicators responded to crop count, which compared monocultures to rotations with at least two

cash crops of any kind. Rotation diversity, which compared rotations of only annual grain crops to rotations with grain crops and other annual crops, had a significant negative effect on TN (-4%), PMN (-12%), and ACE (-9%). Mahal et al. (2018) found that PMN increased with crop diversity, but only in rotations with three or more monoculture cash crops compared to a single species grown every year. None of the soil health practices affected soil C:N and only organic nutrients altered water-extractable C:N. The responses of the N

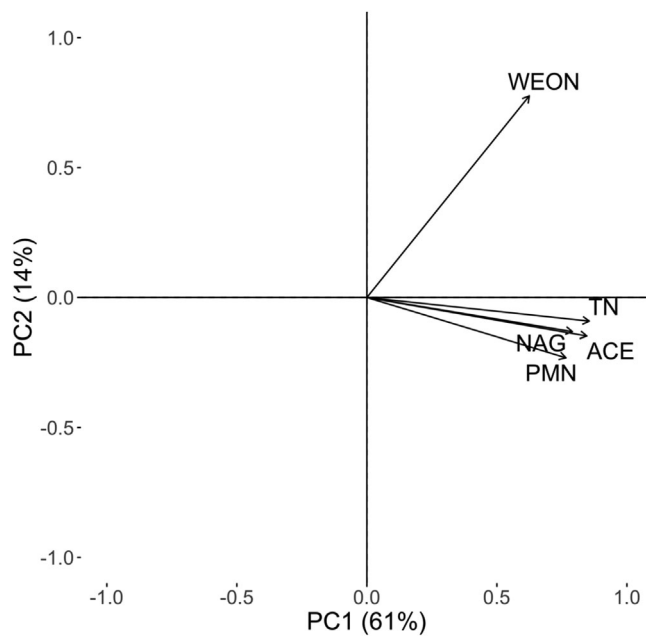


**FIGURE 3** Percent change in soil health indicator in response to each management. Black symbols are means and whiskers represent 95% confidence limits. ACE, autoclavable citrate extractable protein; Soil C:N, ratio of soil organic carbon to total soil nitrogen; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WE C:N, ratio of water-extractable organic carbon to water-extractable organic nitrogen; WEON, water-extractable organic nitrogen.



**FIGURE 4** Percent change in H3A-extractable nitrate and ammonium in response to each management. Black symbols are means and whiskers represent 95% confidence limits.





**FIGURE 5** Principal components analysis of the relationship among response ratios for nitrogen indicators to tillage. ACE, autoclavable citrate extractable protein; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WEON, water-extractable organic nitrogen.

indicators to decreased tillage were highly consistent as all five N indicators had positive loadings on the first axis (61% of the variance) of the principal components analysis (Figure 5)

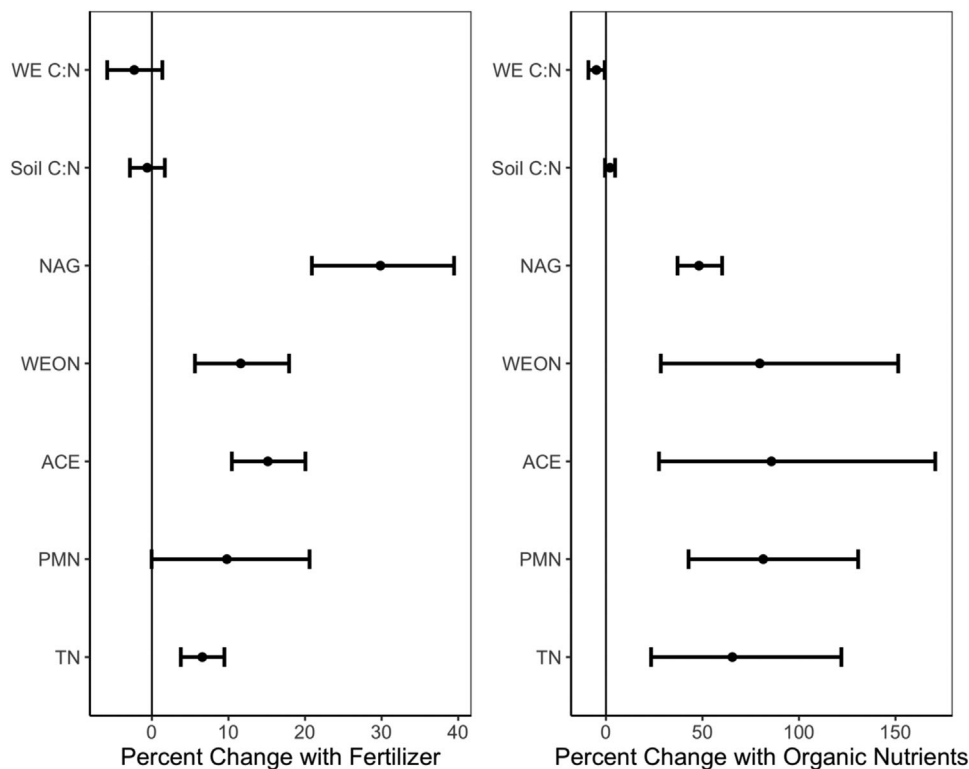
There were no positive responses to either of the tests of cash crop diversity. None of the N indicators showed any response to crop count, suggesting that having more than one species in a rotation was not sufficient to cause change in the N indicators. The most common comparison for rotation diversity, that is, a more diverse rotation with at least one non-grain annual crop versus an annual grain crop, was corn–soybean compared to continuous corn. For rotation diversity, the responses to management were more mixed: TN, ACE, and PMN were significantly lower with rotation diversity, but WEON and NAG did not exhibit a significant response (Figure 3). Several studies reporting data from multiple sites have found that corn–soybean rotations resulted in lower SOC compared to continuous corn (Liptzin et al., 2022; West & Post, 2002), which aligns with our findings that TN also decreased and soil C:N remained constant. Difference in litter composition between corn and soybeans have been suggested as drivers of the declines in C and N pools because of changes in microbial activity; the N-rich soybean litter increases microbial biomass leading to greater capacity to decompose the N-poor corn litter (Hall et al., 2019). There is also a long history of quantifying differences in N dynamics in corn–soybean systems compared to continuous corn, but the typical result is that N mineralization is greater after the soy-

bean crop in field measurements (Gentry et al., 2001). Perhaps some of the difference in N dynamics is due to the lower N fertilization rates and the lower biomass produced in the soybean year of a corn–soybean rotation along with the greater residue in continuous corn rotations (Poffenbarger et al., 2017). There are also likely effects of the differences in the chemistry of the residue between corn and soybean (Green & Blackmer, 1995). The overall effect of rotation on N indicators is equivocal, but rotation may affect some N pools and processes.

While the dominant source of variation of the N indicators was among sites, there were detectable within-site effects associated with most of the soil health practices. Most N indicators increased in response to decreasing tillage, cover cropping, retaining residue, and using organic sources of nutrients (Figure 3). The magnitude of the effect size from management for the N indicators (i.e., signal) was generally lower for the N indicators of pools (TN, ACE, and WEON) and higher for measures of microbially regulated processes (PMN and NAG). However, the 95% confidence limits of the response ratios (i.e., noise), similar to the coefficient of variation within a treatment, were also higher for the microbial processes as well. In other words, process-based measurements were noisier but showed greater signal in response to management. Thus, all N indicators were capable in detecting change from management, the sensitivity of which is a balance between signal and noise.

The N indicators also increased in plots receiving typical rates of inorganic fertilizer or organic fertilizer compared to unfertilized control plots, with the exception of PMN for commercial fertilizer, as the 95% confidence limits just barely overlapped zero (Figure 6). All indicator responses were smaller for inorganic fertilizer (7%–27% higher) compared to organic nutrients (48%–86% higher). The NAG assay showed the largest response to inorganic fertilizer, which is somewhat surprising as a meta-analysis found that enzymes associated with N acquisition, like NAG, did not have a significant response to N fertilization in natural ecosystems or farmland, perhaps because N fertilization alleviated N limitation (Jian et al., 2016). Perhaps the significant effect observed in the NAPESHM dataset is because the increase in biomass production resulting from fertilization counteracts the increased soil N. Fertilization did not affect the soil C:N, but the control plots had 5% lower water-extractable C:N compared to organic nutrients.

While it is not surprising that fertilization was found to affect N dynamics, as the treatments receiving nutrient management from manure or commercial fertilizer generally exhibited significantly greater values of soil N indicators compared to the unfertilized controls, it is not obvious why sites with more N input as commercial fertilizer would also have greater PMN. Mahal et al. (2018) suggested that the increased plant biomass inputs associated with fertilization at the recommended rates could lead to greater PMN. Increased



**FIGURE 6** Percent difference in fertilized compared to unfertilized controls for commercial fertilizer and organic nutrients. Black symbols are means and whiskers represent 95% confidence limits. ACE, autoclavable citrate extractable protein; Soil C:N, ratio of soil organic carbon to total soil nitrogen; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WE C:N, ratio of water-extractable organic carbon to water-extractable organic nitrogen; WEON, water-extractable organic nitrogen.

N fertilizer has also been known to increase net N mineralization regardless of changes in plant biomass inputs in a phenomenon known as N priming (Jenkinson et al., 1985). However, gross ammonification has been shown to be inhibited by N fertilization (Mahal et al., 2019). The cause of the lower PMN observed in the corn–soybean compared to continuous corn rotations and the unfertilized compared to fertilized crops may have the same underlying cause. While it is well established that corn yields are greater in corn–soybean systems than in continuous corn systems, that is, the corn yield penalty, continuous corn does add more residue to the soil. That is, while corn stover would be higher in the corn year of the corn–soybean system, a less productive corn crop in continuous corn still produces more residue, especially with optimal fertilization, than a soybean crop (Jarchow et al., 2015; Poffenbarger et al., 2017). The residue retention, cover cropping, and organic nutrient treatments also result in greater organic matter inputs to soils and were associated with greater PMN. While the C:N ratio of these added organic materials varies, they all led to increase soil carbon without a change in soil C:N ratio. Decreasing tillage does not necessarily add more organic matter to the soil, but it does cause vertical stratification of soil organic matter, with more organic matter in the surface soil where soil health measurements are typically made (Franzluebbers, 2002). The observed increase in PMN

from fertilization is consistent with the hypothesis of Mahal et al. (2018) and the results of the meta-analysis in this study that PMN increases in response to increased organic matter inputs to soils.

One other puzzling result of the treatment comparisons was the lack of significant effects of soil health practices on soil C:N and water-extractable C:N (Figures 3 and 6). With the exception of a decrease in water-extractable C:N in the comparisons of organic nutrients and unfertilized controls, there were no significant effects of added nutrients on these ratios in any comparison. There are global patterns in soil C:N driven by climate and biome (Cleveland & Liptzin, 2007). Similarly, changing the vegetation type by planting forests on non-forest land was found to change the mineral soil C:N (Shi et al., 2016). Studies at one or a few sites have shown that cover cropping (Hubbard et al., 2013) and decreased tillage (Lou et al., 2012) can result in changes to soil C:N, but these studies only examined the top few cm of soil. There are fewer examples of large datasets, but in a study with 11 sites comparing tillage treatments, only four showed a significant change in soil C:N (Blanco-Canqui & Lal, 2008). Export of dissolved organic C and N is different in agricultural land than forest land, (Mattsson et al., 2009), and the water-extractable C:N has been found to vary in response to crop rotation (Xu, Wilson et al., 2013), but much less is known about how

**TABLE 3** Correlation matrix for log transformed treatment means of nitrogen indicators.

Nitrogen indicator	TN	PMN	ACE	WEON	NAG
TN	1				
PMN	0.79	1			
ACE	0.74	0.69	1		
WEON	0.61	0.54	0.32	1	
NAG	0.61	0.63	0.50	0.62	1

Note: All correlations are significant at  $p < 0.001$ .

Abbreviations: ACE, autoclavable citrate extractable protein; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN, potentially mineralizable nitrogen; TN, total soil nitrogen; WEON, water-extractable organic nitrogen.

management can affect the water-extractable C:N in cropland soils. There is still much to learn about the sources and roles of WEON in the soil as it represents a mixture of compounds like amino acids produced by extracellular enzyme activity that are taken up by plants and microbes and complex molecules that are not readily metabolized (Neff et al., 2003). In summary, soil health practices, except those related to cash crop diversity, resulted in significant increases in N indicators, but not changes in either of the C:N ratios.

Finally, there is great interest in understanding how changes in soil health indicators due to the adoption of soil health management are related to yield. These relationships are complicated as soil health practices have been found to increase both yields and PMN without a correlation between yields and PMN (Mahal et al., 2018). At present, there is not even a consensus on whether the adoption of soil health practices should result in changes in yields or yield stability (Miner et al., 2020). It has also been suggested that the adoption of soil health practices increases farm profitability regardless of any yield effects (Bagnall et al., 2021). While these relationships could be explored with the Napehsm database, such an analysis is beyond the scope of this study.

### 3.3 | Relationship of C and N indicators

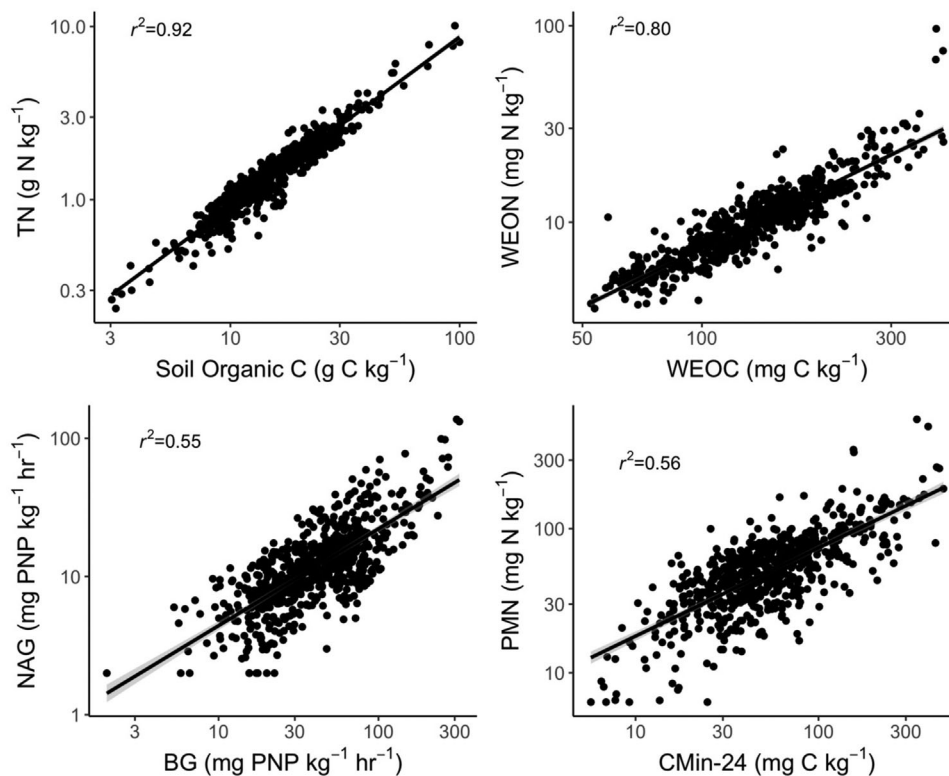
The N indicators were moderately to strongly linearly related with each other. The weakest relationship was between ACE and WEON ( $r = 0.32$ ), and the strongest was between PMN and TN ( $r = 0.79$ ) (Table 3). The indicators of N pools were strongly associated with parallel C indicators. More than 90% of the variance in TN could be predicted from SOC, and 80% of the variance in WEON could be predicted from water-extractable organic C (Figure 7). Similarly, indicators of N processes were also associated with indicators of C processes: 55% of the variance in NAG could be predicted from  $\beta$ -glucosidase (BG) activity, and 56% of the variance in PMN could be predicted from 24-h potential C mineralization.

The Napehsm results highlight how strongly associated the C and N indicators of soil health are across broad spatial scales. That SOC and TN are strongly correlated should not come as a surprise as there are extensive global datasets demonstrating this relationship (Xu, Thornton et al., 2013). While BG and NAG activity are suggested to be more related to C and N acquisition, respectively, and respond to different drivers of substrate availability, they are typically correlated when examined across multiple sites (Waring et al., 2014) or when management within a site varies (Kanté et al., 2021). Much less is known about WEON and WEON in cropland soils across sites, but the limited evidence suggests they are correlated (R. Haney et al., 2012). There are many examples of strong relationships between potential C and N mineralization rates (Franzluebbers, 2018; R. L. Haney et al., 2008; Pehim Limbu & Franzluebbers, 2022). In the Napehsm dataset, 24-h C mineralization on its own could predict 56% of the variance in PMN, but if SOC or TN were also included in a multiple regression model, about 70% of the variance in PMN could be predicted. This matches the findings of Schomberg et al. (2009) that more rapid and widely available indicators, like C mineralization and TN, could predict PMN.

While N (and C) indicators of soil health capture emergent properties of the C and N dynamics of these systems, there is still much to learn about the mechanisms that are driving these observed changes. Most N cycling is happening where plants, microbes, and soil minerals meet suggesting that analysis at the microscale is needed (Daly et al., 2021). Further examination of the breakdown of plant and microbially derived organic N and the sorption–desorption reactions of these molecules on mineral surfaces should provide insights into how and why these N indicators are changing. Similarly, metagenomic approaches can provide a window into which organisms and genes relevant to N cycling are responding to soil health management (Hu et al., 2021). Finally, isotopic, molecular, and metagenomic techniques may help clarify the unexplained variation in soil health indicators across sites and treatments by measuring rates of individual processes or abundances of particular genes.

## 4 | CONCLUSIONS

This continental scale-assessment provides guidance to stakeholders on how to choose N indicators related soil health. These indicators had been selected for testing because they had been previously identified to be directly or indirectly related to the soil function of N cycling. All the N indicators varied predictably with climate and soil texture, meaning that the absolute values of these indicators are context dependent. Further work across the diversity of soils of North America is needed to interpret the absolute values of the indicators. However, the consistent response to soil health management



**FIGURE 7** Log-log relationships between nitrogen indicators and related carbon indicators with  $r^2$  for the regression of log transformed variables. ACE, autoclavable citrate extractable protein; BG,  $\beta$ -glucosidase; Cmin-24, 24-h potential carbon mineralization; NAG, *N*-acetyl  $\beta$ -*D*-glucosaminidase; PMN is potentially mineralizable nitrogen; TN is total soil nitrogen; WEOC is water-extractable organic carbon; WEON, water-extractable organic nitrogen.

suggests that changes in the relative values of the N indicators can be interpreted over time in a field or across fields with similar soils and climates. Given the similarity of the indicator responses to inherent factors and management, choosing a recommended N indicator for producers to measure at scale would depend more on the other criteria, that is, easy to use, cost to collect and measure at commercial laboratories, and interpretability by land managers (Doran & Zeiss, 2000). For example, the most direct indicator of a soil's capacity to cycle N, PMN, requires a relatively lengthy anaerobic incubation and is not the most widely available measurement at commercial laboratories. There may be examples, like testing soil health N indicators combined with fertility measurements to improve nutrient management, where measuring one or multiple N soil health indicators is required. However, PMN, the most direct indicator of the capacity for N cycling, can be predicted from SOC combined with potential C mineralization, the recommended C indicators from NAPESHM (Liptzin et al., 2022). Given the strong relationships between all the indicators of C and N pools and processes, measuring N indicators in addition to these two C indicators would be redundant for evaluating soil health. By limiting the recommended measurements needed to capture C and N dynamics to SOC and potential C mineralization, the cost of quanti-

fying soil health would be much more affordable for land managers.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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