Evaluating rapid plant tissue analysis method for nitrogen diagnostics in corn (Zea mays L.) production

Thomas Wilbur Davis

tdavis31@huskers.unl.edu

University of Nebraska–Lincoln

Mritunjoy Barman

University of Nebraska–Lincoln

Subham Dutta

Bidhan Chandra Krishi Viswavidyalaya

Method Article

Keywords: nitrogen management, physiology, leaf firing, Picketa system, laboratory tissue sampling

Posted Date: December 23rd, 2025

DOI: https://doi.org/10.21203/rs.3.rs-8371845/v1

Additional Declarations: No competing interests reported.

Abstract

Background

Accurate assessment of plant nitrogen status is essential for optimizing fertilizer inputs, increasing productivity, and ensuring environmental quality. This study compared three nitrogen status assessment in corn (*Zea mays* L.): visual assessment method, a novel real-time nutrient estimation using the Picketa LENS™ system, and the conventional laboratory tissue analysis as a reference method. To evaluate the accuracy of the Picketa LENS™ system, a field experiment with four nitrogen treatments (0% nitrogen (control), 80% nitrogen, 100% nitrogen, and 100% nitrogen + stabilizer) and four replications was conducted in York County, Nebraska.

Results

Visual assessment detected treatment differences, with the 0% nitrogen plots showing severe chlorosis and a progressive decline (63.4% reduction) in healthy leaves below the ear over five weeks, whereas 100% nitrogen maintained consistently higher healthy leaf counts (only a 7.4% reduction). However, visual assessment showed limited ability to distinguish between 80% nitrogen and 100% nitrogen + stabilizer treatments until weeks four and five. Both quantitative methods did not detect treatment differences due to the sampled leaf position. The Picketa system (2024 corn model) reported higher absolute nitrogen concentrations (approximately 4.4–4.6%) than laboratory analysis (2.9–3.2%) across all treatments and did not detect significant treatment effects. Conventional laboratory analysis detected only a modest increase in the 100% nitrogen treatment compared with the 0% control. For macronutrients, the Picketa system measured concentrations higher than conventional tissue sampling for phosphorus, potassium, and calcium, with potassium showing approximately 40% higher values and calcium showing 40–50% higher values, while magnesium and sulfur showed close agreement between methods. Micronutrient analysis revealed that the Picketa system consistently reported higher concentrations than conventional tissue sampling for iron (45% higher), manganese (approximately 4-fold higher), copper (90% higher), and zinc (33% higher), but reported significantly lower boron concentrations (67% lower). Despite these absolute value differences, both methods demonstrated similar patterns of detection across treatments.

Conclusions

Visual assessment effectively detected treatment differences, while the Picketa System and the conventional method did not, but maintained similar patterns. These findings highlight the promise of Picketa LENS and the importance of matching sample positions and timing to diagnostic objectives. Integrating real-time sensing with conventional methods can improve nitrogen management decisions.

Background

Effective nitrogen management decisions allow farmers to optimize fertilizer use, improve plant health and productivity, and reduce environmental impact [1]. Since nitrogen is the top yield limiting nutrients in corn production, effectively assessing plant nutrient status is key to improving fertilizer use efficiency, crop productivity, and environmental sustainability. Approximately 95% of plant dry matter consists of carbon, hydrogen and oxygen, while the remaining 5% comprises essential macro and micronutrients such as nitrogen that must be properly managed for optimal plant growth and development [2]. In production systems, plant nutrient status assessment guides precision application technologies that ensure nutrients are delivered exactly when and where they are needed [3]. Thus, plant nutrient assessment is a cornerstone of modern precision agriculture and a fundamental component of evidence-based agronomic decision-making systems worldwide.

Nitrogen management in corn production relies on several assessment methods, including visual assessment and plant tissue analysis. Visual assessment techniques are widely used by farmers for their simplicity, immediacy, and ability to provide qualitative evaluation based on leaf coloration and plant vigor but may lack precision compared to quantitative methods [4]. Plant tissue analysis is also an indispensable tool for evaluating crop nutritional status and serves as a critical decision-support mechanism in commercial agriculture [5–7]. As a decision-support tool, plant tissue analysis provides quantitative data for developing nitrogen management models and enables farmers to make timely interventions that optimize input costs while maximizing yields. Currently, plant tissues can be analyzed in the laboratory using conventional methods or in the field, in real-time.

Conventional methods of plant tissue analysis have evolved from basic laboratory methods to sophisticated laboratory techniques involving multi-elemental analysis with complex sample preparation and analytical procedures [6, 2, 8–11]. Early techniques relied heavily on time-consuming wet digestion procedures followed by single-element colorimetric determinations, which limited their throughput and analytical scope [8]. Atomic absorption spectroscopy was developed in the late 1950s [12], but the introduction of inductively coupled plasma (ICP) spectrometry revolutionized plant tissue analysis by enabling simultaneous detection of multiple elements while also offering greater sensitivity and a wider dynamic range [3]. Modern ICP optical emission spectroscopy (ICP-OES) and ICP mass spectrometry (ICP-MS) now detect elements ranging from less than 0.1 to over 50,000 µg/g with detection limits in the sub-parts-per-trillion (ppt) range [5, 2]. However, this method typically involves using meticulous sampling, proper tissue handling, particle-size reduction, and standardized analytical procedures that take between 3 and 7 days to ensure reliable results. Despite their comprehensiveness and precision, and role as accepted reference methods, these conventional laboratory methods remain time consuming, and demand specialized equipment and technical expertise, which results in relatively high costs per sample [11].

Recent technological advances have led to the development of real-time plant tissue analysis systems, such as the Picketa Leaf Evaluated-Nutrient System (Picketa LENS™, hereafter called Picketa system). Picketa system offers instant nutrient status assessment without the need for complex sample preparation or laboratory analysis [13]. The Picketa system operates by capturing spectral reflectance

data from fresh leaf tissue each time a scan is taken and instantly transmitting it to a cloud-based platform for processing. Machine learning models trained on thousands of laboratory-calibrated samples interpret these spectral signatures to estimate nutrient concentrations for elements such as nitrogen, potassium, magnesium, and sulfur, providing real-time, in-field nutrient analysis. This system addresses the limitations of conventional methods by providing immediate results that can inform timely management decisions [14]. However, in terms of accuracy, the relationship between these rapid real-time assessment technologies and established conventional laboratory methods remains inadequately understood, particularly for nitrogen status assessment in corn production systems [15]. Moreover, despite the critical importance of nitrogen management, few studies have compared the accuracy of different nitrogen assessment methods such as visual assessment, laboratory analysis, and real-time sensing technologies [16]. Without a direct comparison, agricultural practitioners cannot select the most accurate tool to diagnose and subsequently manage nitrogen status with certainty. Finally, the validation of newer rapid assessment technologies against established methods is essential for their widespread adoption in commercial agriculture.

To provide evidence-based support for selecting the most accurate and efficient nitrogen assessment tools, a comparative analysis of three distinct methods for nitrogen status assessment in corn (Zea mays L.) was conducted. Specifically, visual assessment, real-time sampling using the Picketa system, and conventional laboratory tissue analysis were systematically evaluated to compare their accuracies in assessing the status of nitrogen and other nutrients. The findings from this study may support improved management of nitrogen and other essential nutrients to enhance fertilizer use efficiency, sustain crop productivity and reduce environmental impact.

Methods

To evaluate three distinct methods for assessing corn nitrogen status under varying nitrogen application rates, a field experiment was conducted in York County, Nebraska (40°54'54.05"N, 97°39'16.60"W). A non-irrigated research trial field containing 16 plots was used to plant corn (Hybrid LG 63C82113 (113 CRM)) from LG Seeds. Each plot consisted of four rows with 0.76 m (30 in) row spacing and plants were spaced approximately 0.19 m (7.5 in) apart within rows, resulting in a total plant population of approximately 70,000 plants ha^{-1}. A randomized complete block design with four nitrogen treatments (0% nitrogen control (no added fertilizer), 80% nitrogen (120 lb N/ac + 52 lb P_2O_5/ac), 100% nitrogen (150 lb N/ac + 52 lb P_2O_5/ac), and 100% nitrogen (150 lb N/ac + nitrification inhibitor + 52 lb P_2O_5/ac)) was used. The fertilizer application was made up of urea (46–0–0) and monoammonium phosphate (11–52–0). Fertilizers were broadcast and incorporated into the soil before planting. To delay the conversion of ammonium to nitrate in the soil, a nitrification inhibitor containing dicyandiamide (DCD, 6.7 lb ac^{-1}) was used as the stabilizer in the 100% nitrogen + stabilizer treatment. Each treatment was replicated four times. Plant sampling was conducted at the early R1 (silking) stage when differences in leaf firing severity became observable among treatments. The R1 stage was selected because it coincides with peak nitrogen uptake and clear visual differences among treatments. It provided a reliable point to

compare assessment methods while plants are still responsive enough for late-season management decisions [17].

The soil type was predominantly Hastings silty clay loam, a deep, moderately well-drained soil and typical of the Loess Hills extending into south-central Nebraska [18]. Before planting, a soil analysis revealed that the surface layer (0–20 cm) contained 2.1% organic matter, a pH of 7.3, and a cation exchange capacity (CEC) of 14.9 meq/100g (Table 1). The soil fertility parameters, including phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca), were measured only in the topsoil (0–20 cm). These nutrients are mostly concentrated in the upper soil layer, are relatively immobile, and primarily influence root development near the surface [19]. Nitrate-nitrogen was measured in both the upper (0–20 cm) and subsurface (20–91 cm) layers due to its high mobility and susceptibility to leaching [19]. This approach was critical for evaluating plant response to the different nitrogen application rates in this study. In the surface layer, the soil contained moderate levels of P (Bray-1 P: 17 ppm; Olsen P: 10 ppm) and high levels of K (237 ppm), Mg (319 ppm), and Ca (2328 ppm; Table 1). Nitrate-nitrogen levels were 13 ppm in the surface layer (0–20 cm) and 12 ppm in the subsurface layer (20–91 cm; Table 1). These soil nitrate-nitrogen levels were below the 20–30 ppm threshold considered adequate for high-yielding corn [20], thereby providing adequate conditions for comparing different assessment methods for detecting corn nitrogen status at different application rates.

Table 3.1
Soil nutrient status and characteristics before treatments applications and planting.

Parameter	Surface Sample (0–20 cm depth)		Subsurface Sample (20–91 depth)	
	Value/Concentration	Unit	Value/Concentration	Unit
Organic Matter	2.1 [L]	%	-	-
Phosphorus (P)	8 [L]	ppm	-	-
P (Bray-1 P)	17 [L]	ppm	-	-
Olsen P	10 [L]	ppm	-	-
Potassium (K)	237[VH]	ppm	-	-
Magnesium (Mg)	319 [VH]	ppm	-	-
Calcium (Ca)	2328 [H]	ppm	-	-
pH	7.3	-	-	-
CEC	14.9	meq/100g	-	-
K	4.1	%	-	-
Mg	17.8	%	-	-
Ca	78.1	%	-	-
H	0.0	%	-	-
Nitrate-nitrogen	13	ppm	12	ppm

[1]Values followed by superscripts **L**, **H**, and **VH** indicate Low, High, and Very High concentrations, respectively, according to laboratory soil test interpretations. Surface samples were collected from 0–20 cm depth, and subsurface samples from 20–91 cm depth. **Dashes** (-) indicate parameters not measured for the subsurface sample. **CEC** = Cation Exchange Capacity; **meq/100g** = milliequivalents per 100 grams of soil; **ppm** = parts per million.

Visual Assessment of Nitrogen Deficiency

To estimate the severity of nitrogen deficiency and quantify leaf firing symptoms, visual leaf evaluation was systematically performed once each week for five consecutive weeks using a standardized guide (Fig. 1). These evaluations were initiated at the R1 stage on July 22, 2024. Proper identification of nitrogen deficiency symptoms relies on distinguishing between healthy leaves showing normal dark green coloration (Fig. 1A) and various stress phenotypes including phosphate, potassium, nitrogen, and

magnesium deficiencies as well as disease stress and chemical injury (Fig. 1). Specifically, phosphate deficiency is characterized by reddish-purple discoloration in young plants (Fig. 1B). Potassium deficiency exhibits necrosis (browning) along tips and edges of lowest leaves (Fig. 1C). Nitrogen deficiency in corn manifests visually as leaf firing, a condition characterized by premature yellowing progressing from the leaf tip down the midrib (Fig. 1D). Magnesium deficiency appears as whitish interveinal strips with purplish coloration on the underside of lower leaves (Fig. 1E). Disease stress, particularly from Helminthosporium blight, presents as spreading spots (Fig. 1F). Finally, chemical injury results in burned tissue with whitecap formation (Fig. 1G).

To ensure representative sampling and avoid border effects while determining leaf firing severity, ten plants from rows 2 and 3 of each 4-row plot were randomly selected for sampling at a location midway through the rows. Leaf firing severity indicates the extent of nitrogen stress experienced by the plant, with more severe cases showing greater numbers of affected lower leaves. To quantify this severity, we counted the number of green leaves remaining below the ear for each of the ten selected plants, with fewer green leaves indicating greater nitrogen deficiency. The assessment relies on the knowledge that corn plants typically develop a total of 18 and 20 leaves [21].

Nutrient Assessment Using the 2024 Corn Model of Real-time Picketa System

Real-time nutrient assessment was performed using advanced scanning technology with the Picketa system (Picketa Systems, Fredericton, NB, Canada). To ensure accurate and consistent results, all analyses were performed according to the manufacturer's guidelines. Briefly, the corn leaf that was directly below the ear (hereafter called "ear leaf") was harvested for analysis from each plot. This position was selected because it is directly associated with developing tissue and reflects the current nitrogen status of the plant for grain production [22]. Leaf harvesting was conducted on July 22, 2024. In each plot, four ear leaves were randomly sampled and immediately scanned in the field using the Picketa system. During scanning, the device captured spectral reflectance data from the leaf surface and transmitted it to Picketa's cloud-based analytical platform. This process produced nutrient concentration data in real time without the need for sample drying, grinding, or chemical digestion as required for laboratory assays.

Nutrient Assessment Using Conventional Tissue Analysis

To provide a validated reference method for nutrient assessment, conventional laboratory procedures for plant tissue analysis were used [7, 10]. The same four ear leaves sampled for the Picketa assessment in each plot on July 22, 2024 were composited into a single sample and sent to Midwest Laboratories (Omaha, NE), a commercial plant tissue sampling facility, for analysis of macro- and micronutrients. A complete nutrient profile was determined using inductively coupled plasma optical emission spectrometry (ICP-OES), which serves as the accepted reference method for plant tissue analysis [5, 2].

The samples were received intact on July 24, 2024, and processing was completed on July 26, 2024, consistent with the recommended processing time of 3–7 days when using this conventional laboratory method [11].

Statistical Analysis

Initial data processing and organization were conducted using a standard spreadsheet application. Treatment effects were analyzed using analysis of variance (ANOVA) in Minitab 21.1.1.0 applying a general linear model with nitrogen application treatment as the fixed factor. No covariates were included in the model, because soil parameters were relatively uniform across the experimental site. The ANOVA generated F-values to test for overall treatment effects, and where significant differences were detected, Tukey's HSD test was used for pairwise mean comparisons. A significance threshold of $\alpha = 0.05$ was applied for all statistical tests. Data visualization was performed using GraphPad Prism 10.1.0 and JASP 0.9.3.0.

Results

Visual Assessment of Nitrogen Deficiency

Visual assessment of corn plants revealed distinctive nitrogen deficiency symptoms among treatments that began severe overtime (Fig. 2). Nitrogen deficiency became more visible in week 3 when plants in the 0% nitrogen treatment exhibited severe nitrogen deficiency symptoms, characterized by pronounced chlorosis (yellowing) that began at the leaf tips and progressed downward along the midrib in a V-shaped pattern (Fig. 2A). These plants also displayed stunting compared to all other treatments. Plants treated with 80% nitrogen showed moderate nitrogen deficiency symptoms, with partial chlorosis visible primarily in older leaves while newer growth maintained green coloration (Fig. 2B). These plants showed improved vigor compared to the 0% treatment but still displayed visible stress. Plants receiving the full 100% nitrogen application maintained very good to excellent health with deeper green foliage, strong stalks, and no deficiency symptoms (Fig. 2C). Similarly, plants receiving 100% nitrogen + stabilizer exhibited good plant health characteristics (Fig. 2D).

For leaf firing quantification, a distinctive pattern was observed over the five-week period during which plants were assessed (Fig. 3). The 0% nitrogen treatment plants showed significantly lower healthy leaf counts compared to all other treatments ($p < 0.05$), while plants receiving the 100% nitrogen treatment maintained consistently higher counts throughout the observation period ($p < 0.05$).

Overall, the average number of healthy leaves for the plants treated with 0% nitrogen decreased progressively from approximately 4.1 leaves per plant in the first week to 1.5 leaves per plant by the fifth week, representing a 63.4% reduction (Fig. 3). In contrast, the plants treated with 100% nitrogen maintained relatively stable healthy leaf counts, ranging from about 6.8 in the first week to 6.3 in the fifth week, demonstrating only a 7.4% reduction (Fig. 3). Specifically, from the first to the third weeks, plants

treated with 80% nitrogen and those treated with 100% nitrogen + stabilizer showed statistically similar leaf firing scores ($p > 0.05$) but diverged thereafter. Interestingly, the 100% nitrogen + stabilizer treatment showed a more pronounced decline (about 1.0 to 1.5 fewer leaves) in healthy leaf counts during the fourth and fifth week, and significant differences ($p < 0.05$) were only observed between the plants treated with 80% nitrogen and the plants treated with 100% nitrogen + stabilizer during these last two weeks (Fig. 3). This result suggests that corn plants entering the silking stage, and therefore requiring more nutrients, may not have been adequately supported by the 100% nitrogen + stabilizer treatment.

Comparison of Real-time and Conventional Laboratory Methods

Nitrogen Analysis

Nitrogen concentration measurements were generally similar across all treatments for both methods (Fig. 4). Only a slight increase was observed for the 100% nitrogen treatment compared to the 0% control in the laboratory tissue analysis. The Picketa system analysis (Fig. 4A) showed higher nitrogen concentrations across all treatments compared to conventional laboratory tissue sampling; however, no significant differences were observed between treatments which ranged from 4.4–4.6% ($F_{3,12} = 0.45$, $p = 0.72$, Fig. 4A). Conventional laboratory analysis demonstrated significantly lower nitrogen levels of approximately 2.9–3.2% with only the 100% nitrogen treatment significantly higher than the 0% nitrogen treatment ($F_{3,8} = 4.69$, $p = 0.036$, Fig. 4B).

Macronutrient Analysis

To evaluate whether real-time plant tissue analysis could accurately measure macronutrient content compared to conventional laboratory methods, we examined phosphorus, potassium, calcium, and magnesium concentrations across the four nitrogen application treatments. Assessment of macronutrient detection capabilities revealed consistent detection patterns between methods despite often having different absolute values (Fig. 5).

For phosphorous, concentrations of approximately 0.4–0.5% were observed with the Picketa system (Fig. 5A), while conventional analysis showed statistically similar values in the range of 0.3–0.4% ($p > 0.05$, Fig. 5B). Potassium concentrations were highest among all measured macronutrients, with values of approximately 3.0-3.1% observed with the Picketa system (Fig. 5A), and conventional analysis showing lower values of 2.1–2.2% (Fig. 5B). This represents a difference of approximately 40% between analysis methods. Neither method showed significant differences between P or K levels across all nitrogen treatments (Picketa: $F_{3,16} = 0.002$, $p > 0.99$; Lab: $F_{3,16} = 0.003$, $p > 0.99$, Fig. 5).

Calcium levels were found to be approximately 1.0% with the Picketa system analysis and 0.6–0.7% by conventional laboratory analysis, representing a 40–50% difference between the methods (Fig. 5A, 5B). Magnesium concentrations were consistently found to be one of the lowest of all measured macronutrients, with statistically similar concentrations of approximately 0.2–0.3% with the Picketa system analysis and conventional laboratory analysis ($p > 0.05$, Fig. 5A, 5B). Similarly, sulfur

concentrations were approximately 0.2% with both the Picketa system analysis and conventional laboratory analysis (Fig. 5A, 5B). No differences were seen between the nitrogen treatments for Ca, Mg, or S levels. Overall, the Picketa system analysis generally reported higher absolute values for most macronutrients, particularly for potassium and calcium (Fig. 5A), but neither method detected significant differences across nitrogen treatments (Picketa: $F_{3,16} = 0.002$, $p > 0.99$; Lab: $F_{3,16} = 0.003$, $p > 0.99$).

Micronutrient Analysis: To compare the accuracy of real-time and conventional laboratory methods in detecting micronutrient concentration, iron (Fe), manganese (Mn), boron (B), copper (Cu), and zinc (Zn) concentrations across the four nitrogen application treatments were analyzed. The two methods produced similar overall trends in terms of relative concentrations among micronutrients, though substantial differences in absolute values were observed between methods (Picketa: $F_{3,16} = 0.001$, $p > 0.99$; Lab: $F_{3,16} = 0.066$, $p = 0.98$, Fig. 6). Neither method detected significant differences among nitrogen treatments for any micronutrient measured.

Iron showed the highest concentrations among measured micronutrients for both methods but with different absolute values. The Picketa system reported Fe concentrations of approximately 440–484 ppm across all nitrogen treatments with minimal variation between treatments (Fig. 6A). In contrast, conventional laboratory analysis showed lower Fe concentrations (257–388 ppm), though the apparent trend of higher values in the 100% nitrogen and 100% nitrogen + stabilizer treatments (388 ppm) compared to the 0% nitrogen treatment (257 ppm) was not statistically significant ($p > 0.05$, Fig. 6B). Similarly, the Picketa system also reported higher concentrations for Mn (271–276 ppm) across all treatments compared to the conventional laboratory analysis, which measured substantially lower concentrations (47–68 ppm) (Fig. 6A, 6B). This represents an approximately four-fold difference between methods. The conventional laboratory method showed a numerical trend with the plants that received 100% nitrogen showing the highest Mn concentration (68 ppm) and the 0% nitrogen treatment showing the lowest (47 ppm), but this difference was not statistically significant ($p > 0.05$, Fig. 6B). Boron measurements showed an opposite trend, with Picketa system analysis reporting lower concentrations (4.5–5 ppm) compared to conventional laboratory analysis (13.3–15.3 ppm).

For Cu and Zn analysis, the Picketa system output maintained higher values, measuring Cu concentrations at 19.8–21.3 ppm by Picketa system and 10–11.6 ppm by conventional laboratory analysis, representing a two-fold difference. Zn concentrations were detected at approximately 40 ppm by Picketa system analysis and 30 ppm by conventional laboratory analysis. No significant treatment effects were detected for Cu or Zn by either method ($p > 0.05$).

Discussion

This study compared three methods for assessing nitrogen status in corn. These are visual assessment, real-time analysis using the Picketa LENS™ system, and conventional laboratory tissue analysis. Each method provided unique insights into plant nitrogen status but also revealed important limitations. Visual assessment effectively identified severe nitrogen deficiency (without yield impact assessment)

through characteristic leaf firing symptoms. However, both quantitative methods showed limited sensitivity in distinguishing between nitrogen treatments, particularly at the higher application rates. The conventional laboratory method detected some treatment differences that the Picketa system did not, though both methods showed similar overall trends in nutrient concentrations.

Visual assessment proved most effective for identifying severe nitrogen deficiency in the 0% nitrogen control. Plants exhibited pronounced chlorosis beginning at leaf tips and progressing downward along the midrib in a characteristic V-shaped pattern (Fig. 2A). This distinctive symptom results from nitrogen remobilization from older leaves to support new growth which is a well-documented plant response to nitrogen limitation [23]. The progressive decline in healthy leaf counts, from approximately 4.1 leaves in week one to 1.5 leaves by week five was a 63.4% reduction in the untreated plants ($F_{3,76} = 66.48$, $p < 0.001$, Fig. 3). This dramatic loss of photosynthetic tissue clearly indicated severe nitrogen stress.

Visual assessment showed important limitations for detecting moderate nitrogen deficiency. Differences between the 80% nitrogen and 100% nitrogen + stabilizer treatments were not visually apparent until the third, fourth and fifth weeks of observation (Fig. 3). This delayed detection is a critical drawback for management decisions. Yield potential may already be compromised before deficiency becomes visually apparent [24]. The subtle differences between moderate deficiency and sufficiency were not consistently distinguishable through visual assessment alone. This limitation could lead to suboptimal fertilization decisions when nitrogen stress is developing but not yet severe [25]. Since tissue sampling for both Picketa and lab analyses was conducted during week one, the result does not reflect the nutritional status of the plants during the entire study period. However, it provides important information on the accuracy of using the real time Picketa system which is the objective of this study. Another important limitation of visual assessment is that chlorosis and leaf yellowing can result from multiple nutrient deficiencies, not exclusively nitrogen deficiency. While well-trained agronomists and researchers can distinguish between different deficiency symptoms using diagnostic guides (Fig. 1), field scouts and producers often misdiagnose the underlying cause of leaf discoloration. This diagnostic uncertainty is a significant contributor to nitrogen over-application in production systems, as producers apply nitrogen fertilizer in response to any yellowing symptoms regardless of the actual causal factor. Quantitative tissue analysis methods, whether real-time or laboratory-based, provide more definitive nutrient status information that can prevent such misdiagnosis.

The declining performance of the 100% nitrogen + stabilizer treatment in later weeks warrants attention. Plants receiving this treatment showed more pronounced decline in healthy leaf counts during weeks four and five, with approximately 1.0 to 1.5 fewer healthy leaves compared to earlier weeks (Fig. 3). This result suggests that corn plants entering the silking stage may not have been adequately supported by the stabilizer treatment. The R1 (silking) stage is a period of peak nitrogen demand. Plants require substantial nutrient availability to support reproductive development and grain fill [26].

While visual assessment distinguished treatment effects (Fig. 3), neither the Picketa system nor conventional laboratory analysis detected comparable differences in leaf nitrogen concentration (Fig. 4).

The Picketa system measured nitrogen concentrations of approximately 4.5% across all treatments with no statistical differences ($F_{3,12} = 0.45$, $p = 0.72$). The conventional laboratory method showed slightly lower values ranging from 2.9% to 3.2%, with only the 100% nitrogen treatment significantly higher than the 0% control ($F_{3,8} = 4.69$, $p = 0.036$).

This may be explained by the sampling strategy employed in this study. Tissue samples were collected from the ear leaf, which is the leaf directly below the ear. This sampling position was selected based on established protocols indicating that the ear leaf reflects current nitrogen status most relevant for grain production [22]. The ear leaf is directly associated with developing reproductive tissue and provides the most reliable indicator of nitrogen availability during critical grain-filling stages. However, nitrogen deficiency symptoms manifest first in lower, older leaves as plants remobilize nitrogen from senescing tissues to support newer growth and reproductive development [23].

At the time of sampling (R1 silking stage), the ear leaf position in the middle to upper canopy still contained adequate nitrogen even in deficient plants. The visual symptoms of leaf firing observed in lower leaves (Fig. 2) had not yet progressed upward to affect the ear leaf. This may explain why neither analytical method was able to detect differences between treatments despite clear visual evidence of deficiency in lower canopy positions (Fig. 2). The findings point out an important consideration in plant tissue sampling which dictates that the tissue sampled must match the diagnostic objective. For detecting existing deficiency, lower leaves may be more appropriate. For assessing nitrogen status relevant to current grain production, the ear leaf remains the standard position despite potentially masking deficiency that is already affecting lower canopy photosynthetic capacity.

The Picketa system consistently reported nitrogen concentrations approximately 1.5% higher than conventional laboratory analysis across all treatments (Fig. 4). This systematic overestimation likely reflects both calibration differences between analytical methods and the fundamental difference in sample handling. The Picketa system analyzes fresh tissue immediately after sampling, capturing nitrogen in all forms present in the living leaf. Conventional laboratory analysis involves sample drying, grinding, and extraction over several days. During this processing, some nitrogen forms may volatilize or undergo chemical transformation [7]. Additionally, the Picketa system's machine learning models were trained on datasets that may differ from the calibration standards used in ICP-OES analysis. The observed baseline offset may also reflect differences in calibration datasets across analytical laboratories. The Picketa system's machine learning models were initially trained using data from multiple laboratories, including A&L Canada Laboratories (Ontario) for early corn trials and subsequently Midwest Labs for US trials. Since conventional laboratory methods themselves show inter-laboratory variation in baseline values, comparing absolute nutrient concentrations between methods requires consideration of lab-specific calibration differences. Understanding this baseline offset between laboratories and their correlation with spectral data will be necessary for a better understanding of the two methods. As with traditional laboratory analysis, interpreting nutrient trends over time within a single analytical platform provides more reliable information than comparing absolute values across different analytical systems.

Importantly, the conventional laboratory method demonstrated greater sensitivity in detecting treatment differences. It showed a statistically significant increase in the 100% nitrogen treatment compared to the 0% control ($F_{3,8}$ = 4.69, p = 0.036, Fig. 4B). The Picketa system failed to detect any treatment differences ($F_{3,12}$ = 0.45, p = 0.72, Fig. 4A), while conventional laboratory detected just one ($F_{3,8}$ = 4.69, p = 0.036; Fig. 4B). This is a critical limitation for both the first-generation Picketa model used in this study and the ICP-OES analysis for detecting subtle nitrogen concentrations under field condition. It is important to clearly state that the technology was in its initial deployment for corn and likely lacked corn-specific calibration refinement. Subsequent versions of the Picketa system have been updated with expanded training datasets and improved algorithms designed to enhance sensitivity for detecting subtle differences in nutrient status. Independent blind case studies from Kansas and Ontario agricultural sites, using data not included in model training, have validated these improvements [27]. Nevertheless, even with this early model, the Picketa system showed similar overall trends to laboratory analysis.

An important advantage of the Picketa system over older sensor technologies is its capability to assess all 12 nutrients typically measured in conventional laboratory analysis simultaneously in real-time. While earlier field-based sensing technologies were limited to measuring only nitrogen, phosphorus, and potassium, the Picketa system's spectral analysis and machine learning algorithms enable comprehensive nutrient profiling including both macro- and micronutrients. This multi-nutrient capability provides producers with immediate access to complete nutritional status information rather than requiring separate analyses or sensor technologies for different nutrients [28]. Assessment of macronutrient concentrations revealed variable agreement between the Picketa system and conventional laboratory analysis (Fig. 5). Some nutrients showed reasonable concordance while others exhibited substantial differences. Neither method detected significant differences among nitrogen treatments for any macronutrient (Picketa: $F_{3,16}$ = 0.002, p > 0.99; Lab: $F_{3,16}$ = 0.003, p > 0.99), which was expected since only nitrogen supply varied across treatments. With this context, the Picketa–laboratory comparisons provide meaningful validation for the field-measured nutrient patterns observed in both systems. Magnesium and sulfur demonstrated the closest values between methods. Magnesium concentrations were approximately 0.25% with Picketa analysis and 0.22% with laboratory analysis, which is a 14% difference. Sulfur showed even closer agreement at approximately 0.20% and 0.19%, respectively, a difference of only 5%. These similarities suggest that the Picketa system's spectral algorithms are well-calibrated for detecting these particular nutrients.

Phosphorus showed moderate agreement, with Picketa measuring approximately 0.45% compared to 0.35% by laboratory analysis, a 29% difference. While this is a notable discrepancy, both methods detected similar patterns across treatments with no significant treatment effects observed. This consistency in relative measurements suggests that Picketa could reliably track phosphorus status changes even if absolute values require calibration adjustment.

In contrast, potassium and calcium showed substantial differences between methods. Potassium concentrations measured approximately 3.05% with Picketa but only 2.15% with laboratory analysis, which is a 42% overestimation. Calcium showed even larger discrepancies, with Picketa measuring

approximately 1.0% compared to 0.65% by laboratory analysis, a 54% difference. These disparities highlight the importance of method-specific calibration when interpreting nutrient data. Direct comparison of absolute values between methods could lead to incorrect conclusions about nutrient status. However, both methods showed consistent patterns across treatments, which suggest that within-method comparisons remain valid for evaluating relative nutrient status.

Micronutrient analysis revealed the largest discrepancies between methods (Fig. 6). The Picketa system overestimated most micronutrients compared to laboratory analysis. Iron concentrations averaged approximately 465 ppm with Picketa and 320 ppm with laboratory analysis, a 45% overestimation. Manganese showed even more dramatic differences, measuring approximately 273 ppm with Picketa but only 57 ppm with laboratory analysis, which is a 379% overestimation.

Copper and zinc also showed consistent overestimation by the Picketa system. Copper measured approximately 20.5 ppm with Picketa and 10.8 ppm with laboratory analysis (90% higher). Zinc measured approximately 40 ppm with Picketa and 30 ppm with laboratory analysis (33% higher). Interestingly, boron showed the opposite pattern, with Picketa underestimating concentrations at approximately 4.7 ppm compared to 14.3 ppm by laboratory analysis, which is a 67% underestimation.

Despite the differences in absolute values between methods, neither the Picketa system nor the laboratory method detected statistically significant differences among nitrogen treatments for any micronutrient measured (Picketa: $F_{3,16} = 0.001$, $p > 0.99$; Lab: $F_{3,16} = 0.066$, $p = 0.98$, Fig. 6). The laboratory method showed numerical trends suggesting higher iron and manganese concentrations in plants receiving 100% nitrogen and 100% nitrogen + stabilizer compared to the 0% nitrogen treatment, but these differences did not reach statistical significance. This lack of detectable treatment effects for micronutrients is not unexpected, as only nitrogen supply varied across treatments and the study was not designed to create micronutrient deficiencies. The absence of significant differences in both methods indicates that the varying nitrogen treatments did not substantially alter micronutrient uptake or accumulation patterns in the ear leaf tissue sampled. The findings from this comparative analysis provide important guidance for nitrogen and other nutrients management in corn production. Visual assessment remains valuable as an initial screening tool for identifying severe deficiency. Its immediacy and low cost make it accessible to all producers. However, visual symptoms appear only after substantial physiological impairment has occurred which limits its use for preventive management [23].

Conventional laboratory analysis provides the most accurate measurements and serves as the essential reference standard. Its ability to detect the subtle treatment difference in nitrogen concentration that the Picketa system missed demonstrates its continued importance. However, the 3–7-day processing time limits applicability for time-sensitive decisions during critical growth stages [11]. By the time results are available, optimal intervention timing may have passed, particularly during rapid growth phases like vegetative development and grain fill.

The Picketa system is an important technological development that addresses the timing gap between sampling and results. Providing quantitative nutrient data within seconds of sampling makes possible

responsive management during critical growth windows. However, the substantial calibration discrepancies for the first-generation model observed in this study indicate that the technology requires further refinement before it can serve as a standalone assessment tool. The comparable overall trends shown in this study by the first-generation Picketa model to the lab-based results indicates promise for use and future development.

Conclusions

This comparative analysis revealed that each assessment method offers unique capabilities and limitations for nitrogen management in corn. Visual assessment effectively identifies severe deficiency but lacks sensitivity for moderate stress and provides only qualitative information. The conventional laboratory method demonstrated superior sensitivity and accuracy, detecting a significant treatment difference in nitrogen concentration that the Picketa system could not resolve. However, processing delays limit applicability for time-sensitive decisions. The Picketa system showed promise for rapid assessment but exhibited substantial calibration discrepancies in its first-generation form.

The disconnect between visual deficiency symptoms and ear leaf nitrogen concentrations highlights the importance of matching sampling position with diagnostic objectives. While the ear leaf appropriately reflects nitrogen status for grain production, it may not reveal deficiency affecting lower canopy photosynthesis. Future versions of rapid assessment technologies with improved calibration may offer valuable tools for responsive nitrogen management. However, current evidence suggests that an integrated approach combining multiple methods provides the most comprehensive strategy for optimizing nitrogen management in corn production systems.

Future research should prioritize several key areas to advance rapid nutrient assessment technology. First, expanded calibration datasets specific to corn across diverse growth stages, environments, and cultivars are essential. The overestimation observed in this study may be corrected through improved training data and algorithm refinement. Secondly, validation studies across multiple growing seasons and geographic regions should increase the reliability and consistency of rapid assessment methods under variable conditions.

Additionally, research examining the optimal integration of multiple assessment methods within decision support frameworks would help farmers maximize the value of available tools. Understanding when each method provides maximum utility and how to interpret results within an integrated management system is an important knowledge gap. Finally, studies examining the relationship between sampling position, growth stage, and nutrient diagnostic accuracy would refine sampling protocols to optimize the match between tissues sampled and management objectives.

Abbreviations

CEC
Cation Exchange Capacity

DCD
Dicyandiamide
Fe
Iron
ICP-MS
Inductively Coupled Plasma Mass Spectrometry
ICP-OES
Inductively Coupled Plasma Optical Emission Spectrometry
LENS
Leaf Evaluated-Nutrient System
Mn
Manganese
N
Nitrogen
ppm
Parts per million
R1
Reproductive stage 1 (silking)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Not applicable.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contribution

T.W.D. designed the study, conducted field experiments, performed data analysis, and drafted the manuscript. M.B. and S.D. contributed to data analysis, data interpretation, and manuscript preparation. All authors read and approved the final manuscript.

Acknowledgement

Special thanks to Jess Spotaski, Dr. Zachary Rystrom and the technical staff at Midwest Research Inc, in York, NE for providing the experimental site, research materials and technical assistance.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Tei F, De Neve S, de Haan J, Kristensen HL. Nitrogen management of vegetable crops. Agric Water Manag. 2020;240:106316.
2. Hansen TH, Laursen KH, Persson DP, Pedas P, Husted S, Schjoerring JK. Micro-scaled high-throughput digestion of plant tissue samples for multi-elemental analysis. Plant Methods. 2009;5:12.
3. Ammann AA. Inductively coupled plasma mass spectrometry (ICP MS): A versatile tool. J Mass Spectrom. 2007;42:419–27.
4. de Jesus Matos Viégas I, Cordeiro RAM, de Almeida GM, Silva DAS, da Silva BC, Okumura RS, et al. Growth and Visual Symptoms of Nutrients Deficiency in Mangosteens. Am J Plant Sci. 2018;9:1014–28.
5. Dimkpa C, Bindraban P, McLean JE, Gatere L, Singh U, Hellums D. Methods for Rapid Testing of Plant and Soil Nutrients. In: Drechsel JJ, Heffer PP, Magen H, editors. Improving potassium recommendations for agricultural crops. Springer; 2017. pp. 1–43.
6. Dong Y, Li B, Malitsky S, Rogachev I, Aharoni A, Kaftan F, et al. Sample preparation for mass spectrometry imaging of plant tissues: A review. Front Plant Sci. 2016;7:60.
7. Hansen TH, De Bang TC, Laursen KH, Pedas P, Husted S, Schjoerring JK. Multielement plant tissue analysis using ICP spectrometry. Methods Mol Biol. 2013;953:121–41.

8. Jones JB. Sampling, Handling, and Analyzing Plant Tissue Samples. In: Westerman RL, editor. Soil testing and plant analysis. 3rd ed. Madison: Soil Science Society of America; 1990. pp. 389–427.
9. Jones JB. Plant Tissue Analysis in Micronutrients. In: Mortvedt JJ, Cox FR, Shuman LM, Welch RM, editors. Micronutrients in agriculture. 2nd ed. Madison: Soil Science Society of America; 1991. pp. 477–521.
10. Jones JB, Wallace A. Sample preparation and determination of iron in plant tissue samples. J Plant Nutr. 1992;15:2085–108.
11. Kalra YP, editor. Handbook of reference methods for plant analysis. Boca Raton: CRC; 2019.
12. Isaac RA. Atomic Absorption Methods for Analysis of Soil Extracts and Plant Tissue Digests. J Assoc Off Anal Chem. 1980;63:788–91.
13. Thompson LJ, Ferguson RB, Kitchen N, Frazen DW, Mamo M, Yang H, et al. Model and sensor-based recommendation approaches for in-season nitrogen management in corn. Agron J. 2015;107:2020–30.
14. Burton L, Dave N, Fernandez RE, Jayachandran K, Bhansali S. Smart Gardening IoT Soil Sheets for Real-Time Nutrient Analysis. J Electrochem Soc. 2018;165:B3157–62.
15. Colaço AF, Bramley RGV. Do crop sensors promote improved nitrogen management in grain crops? Field Crops Res. 2018;218:126–40.
16. Muñoz-Huerta RF, Guevara-Gonzalez RG, Contreras-Medina LM, Torres-Pacheco I, Prado-Olivarez J, Ocampo-Velazquez RV. A review of methods for sensing the nitrogen status in plants: Advantages, disadvantages and recent advances. Sensors. 2013;13:10823–43.
17. Donovan TC, Comas LH, Schneekloth J, Schipanski ME. Nitrogen and water availability affect soil nitrogen mineralization and maize nitrogen uptake dynamics. Nutr Cycl Agroecosyst. 2025;130:387–405.
18. Kuzila MS. Identification of multiple loess units within modern soils of Clay County, Nebraska. Great Plains Res. 1995;5:183–201.
19. Jobbágy EG, Jackson RB. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. Biogeochemistry. 2001;53:51–77.
20. Shapiro CA, Ferguson RB, Wortmann C, Maharjan B. Nutrient Management Suggestions for Corn (EC155). Lincoln: University of Nebraska-Lincoln Extension; 2019. https://extensionpubs.unl.edu/publication/9000016363764/. Accessed 15 Nov 2024.
21. Nielsen RL. Corn growth & development: What goes on from planting to harvest? West Lafayette: Purdue University Cooperative Extension Service; 2000. https://www.agry.purdue.edu/ext/corn/news/timeless/Corngrowth.html. Accessed 20 Oct 2024.
22. Aliyu KT, Huising J, Kamara AY, Jibrin JM, Mohammed IB, Nziguheba G, et al. Understanding nutrient imbalances in maize (Zea mays L.) using the diagnosis and recommendation integrated system (DRIS) approach in the Maize belt of Nigeria. Sci Rep. 2021;11:15896.

23. Townsend AJ, Retkute R, Chinnathambi K, Randall JWP, Foulkes J, Carmo-Silva E, et al. Suboptimal acclimation of photosynthesis to light in wheat Canopies. Plant Physiol. 2018;176:1233–46.
24. Scharf PC, Kitchen NR, Sudduth KA, Davis JG. Spatially Variable Corn Yield is a Weak Predictor of Optimal Nitrogen Rate. Soil Sci Soc Am J. 2006;70:2154–60.
25. Fox RH, Walthall CL. Crop Monitoring Technologies to Assess Nitrogen Status. In: Schepers JS, Raun WR, editors. Nitrogen in agricultural systems. Madison: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America; 2008. pp. 647–74.
26. Mueller SM, Vyn TJ. Maize plant resilience to N stress and post-silking N capacity changes over time: A review. Front Plant Sci. 2016;7:53.
27. Blind Case Studies. Independent evaluation of updated Picketa nutrient calibration models in Kansas and Ontario agricultural sites. Fredericton: Picketa Systems; n.d. (Unpublished report).
28. Picketa Systems Inc. Plant tissue analysis with LENS™. Fredericton: Picketa Systems; n.d. https://www.picketa.com. Accessed 10 Dec 2024.

Figures

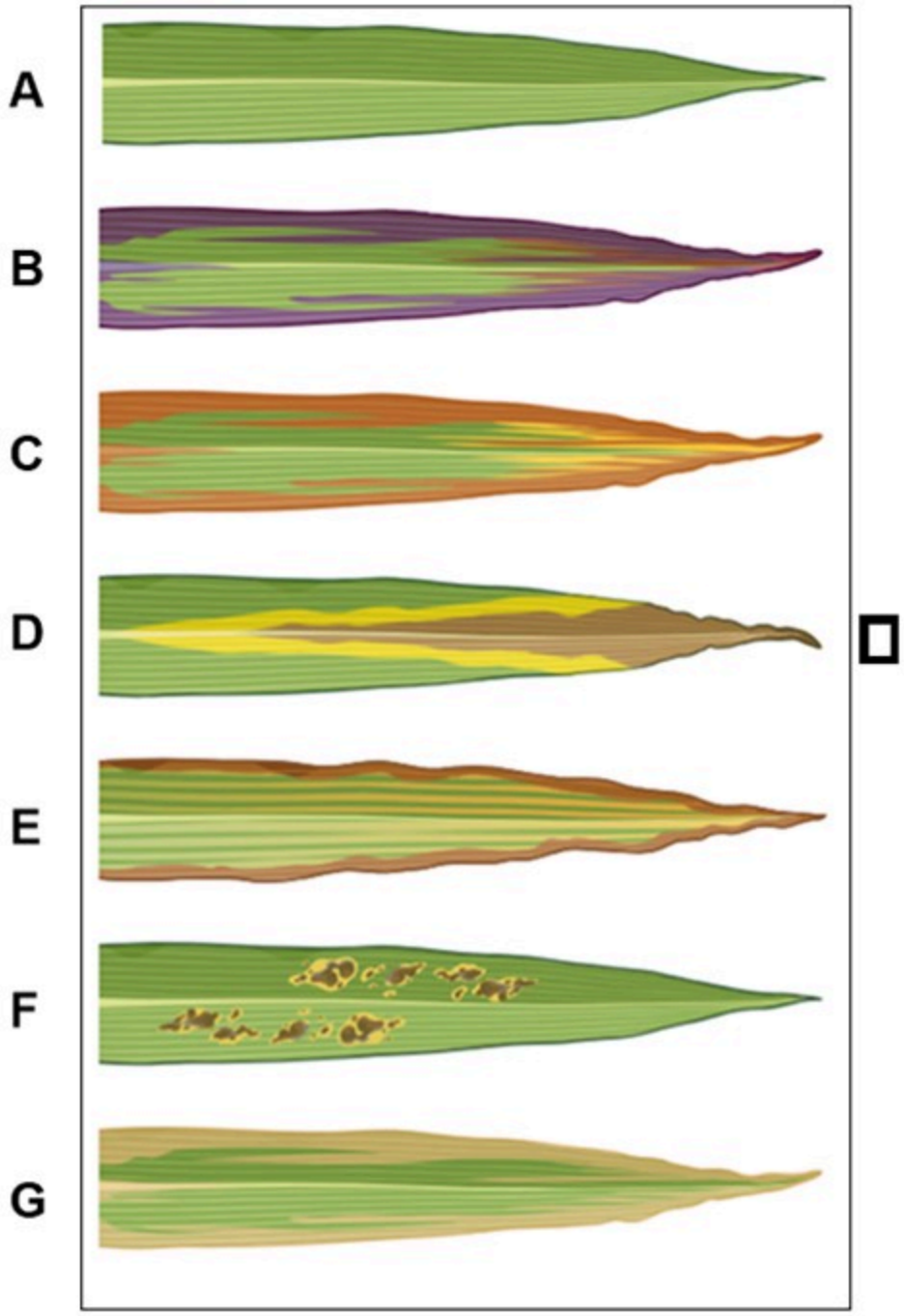

Figure 1

Figure 3.1. Identification guide for various nutrient deficiencies and stress symptoms in Corn leaves. **(A)** Healthy leaf showing normal dark green coloration; **(B)** Phosphate deficiency characterized by reddish-purple discoloration, particularly in young plants; **(C)**Potassium (Potash) deficiency exhibiting necrosis (browning) or drying along the tips and edges of lowest leaves; **(D)** Nitrogen deficiency manifested as yellowing beginning at the leaf tip and progressing along the midrib (typical symptom targeted in this study); **(E)**Magnesium deficiency appearing as whitish interveinal strips with possible purplish coloration on the underside of lower leaves; **(F**) Disease stress caused by Helminthosporium blight, presenting as

small spots that gradually spread across the leaf; **(G)** Chemical injury resulting in burned leaf tips, edges, and contact points with subsequent tissue death and whitecap formation.

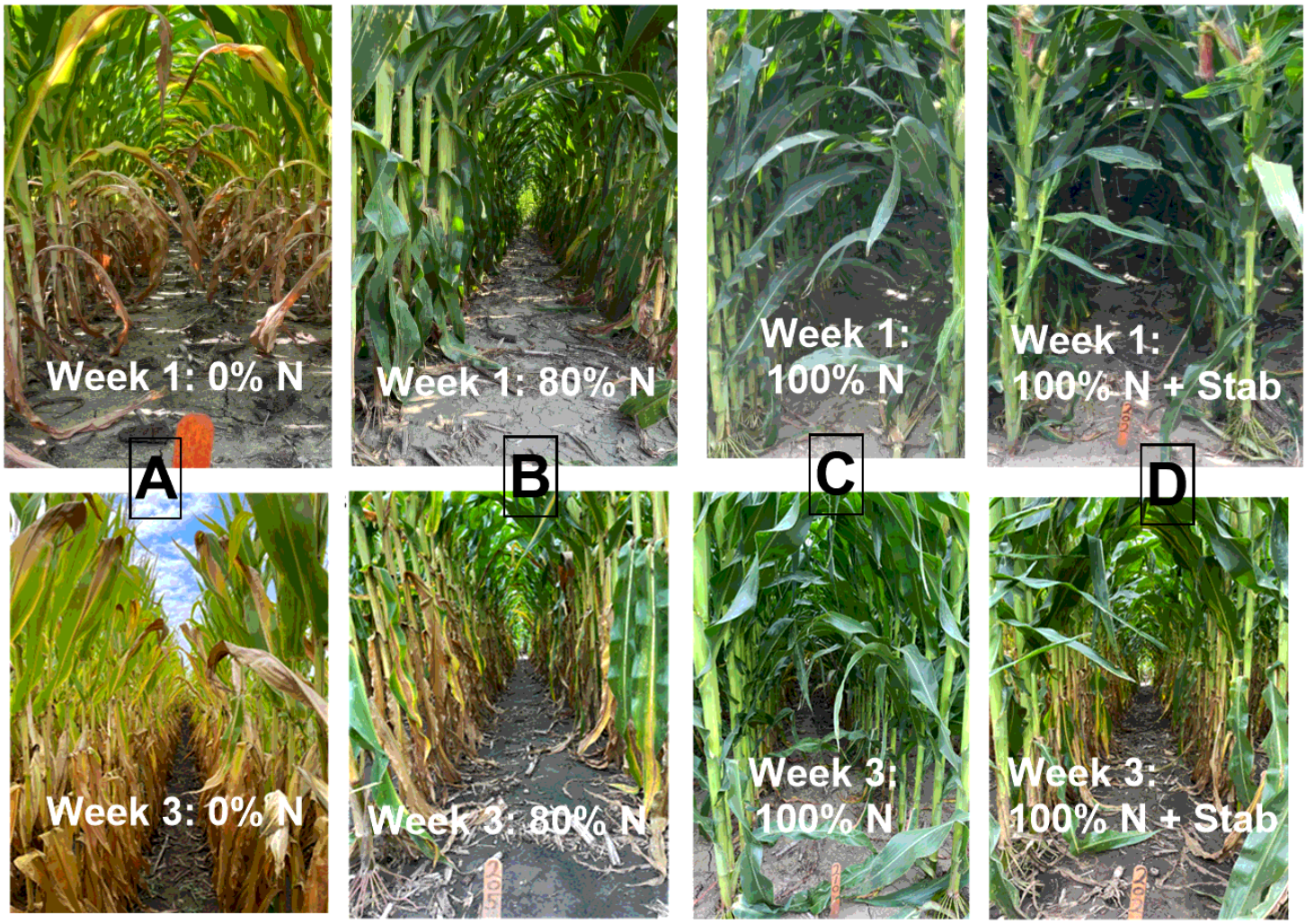

Figure 2

Figure 2. Visual comparison of corn plant health under different nitrogen treatments during week 3 of the study period. Panels show representative plots from **(A)** 0% N treatment exhibiting severe nitrogen deficiency symptoms characterized by yellowing that starts at the tip and moves down along the middle of the leaf, **(B)** 80% N treatment showing moderate N deficiency, **(C)** 100% N treatment showing no N deficiency, and **(D)** 100% N + Stabilizer treatment showing low to no N deficiency

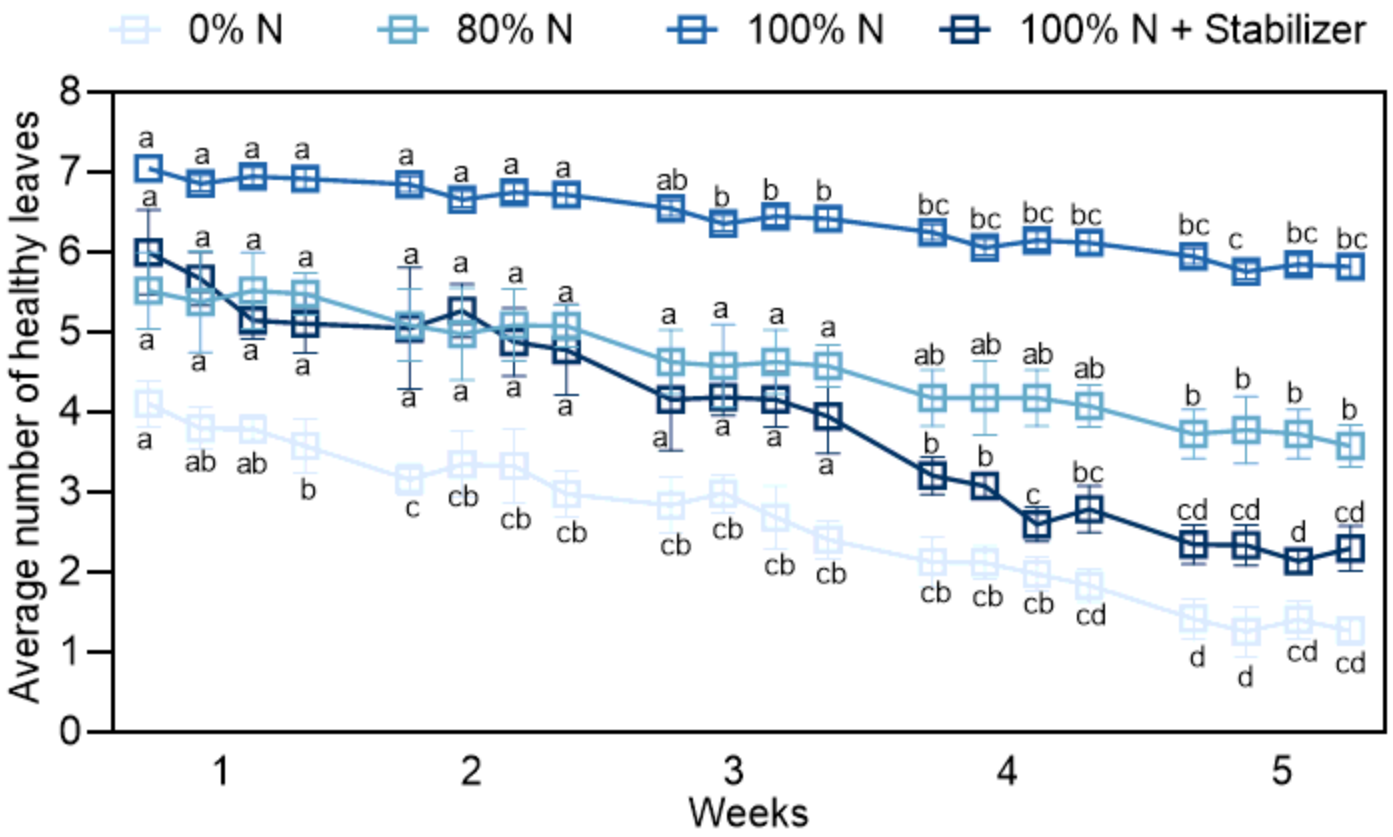

Figure 3

Figure 3. Temporal progression of leaf firing symptoms across nitrogen treatments over five weeks. The line graphs show the average number of leaves below the ear that did not display the characteristic premature yellowing which is associated with leaf firing. Each point represents the average number of leaves per plant (n = 40 plants) for the 0% N (light blue), 80% N (medium light blue), 100% N (medium dark blue), and 100% N + stabilizer (dark blue) treatments. Statistical significance was calculated using Turkey's HSD test. Different letters represent significant differences ($p < 0.05$) across sampling weeks within a treatment. Error bars represent (mean ± SD).

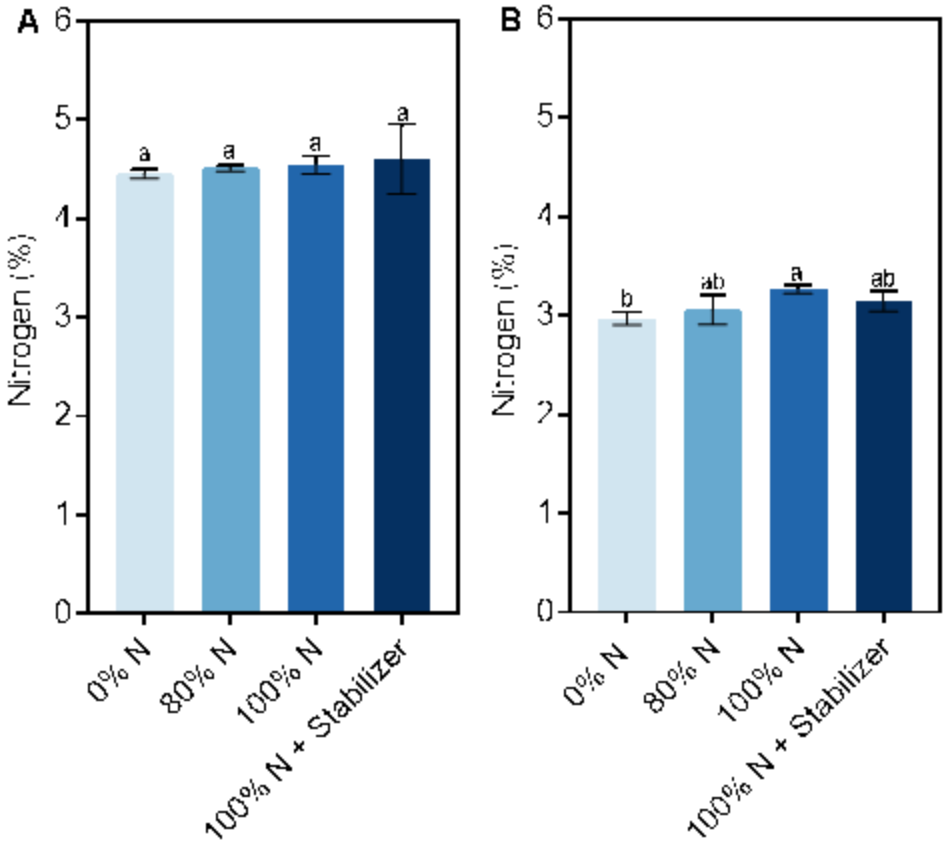

Figure 4

Figure 4. Comparison of the accuracy of two methods of corn leaf nitrogen content measurements. **(A)** real-time Picketa System and **(B)** Conventional laboratory tissue sampling method (inductively coupled plasma (ICP) spectrometry). Treatments included: 0% N (light blue), 80% N (medium light blue), 100% N (medium dark blue), and 100% N + stabilizer (dark blue). Statistical significance was calculated using Turkey's HSD test. Different letters represent significant differences ($p < 0.05$). Error bars represent (mean ± SD).

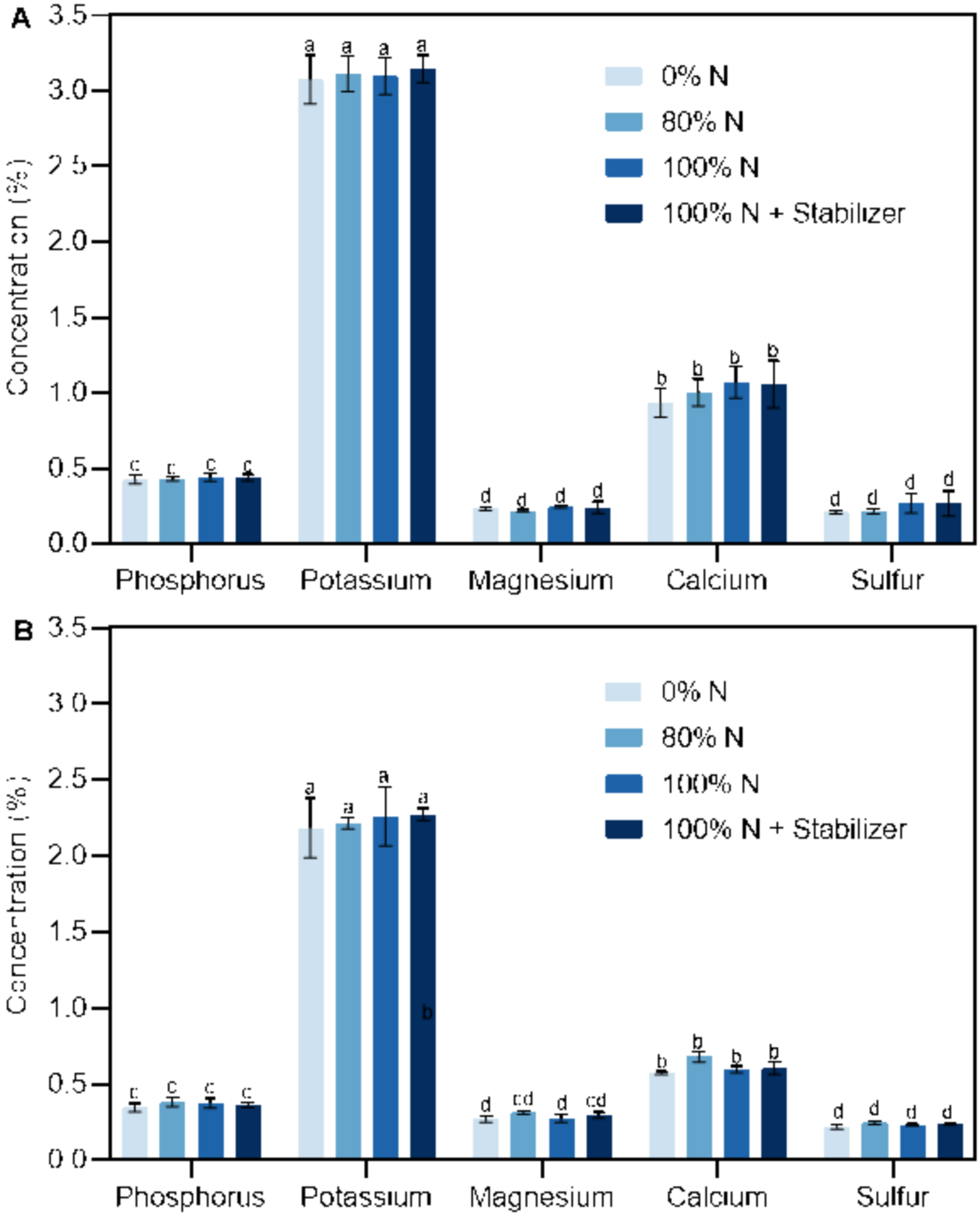

Figure 5

Figure 5. Comparison of the macronutrient measurement accuracy using two assessment methods: **(A)** Picketa real-time analysis results for macronutrient concentrations, **(B)** Conventional tissue sampling analysis results for macronutrient concentrations. Treatments included: 0% N (light blue), 80% N (medium light blue), 100% N (medium dark blue), and 100% N + stabilizer (dark blue). Statistical significance was calculated using Turkey's HSD test. Different letters represent significant differences ($p < 0.05$). Error bars represent (mean ± SD).

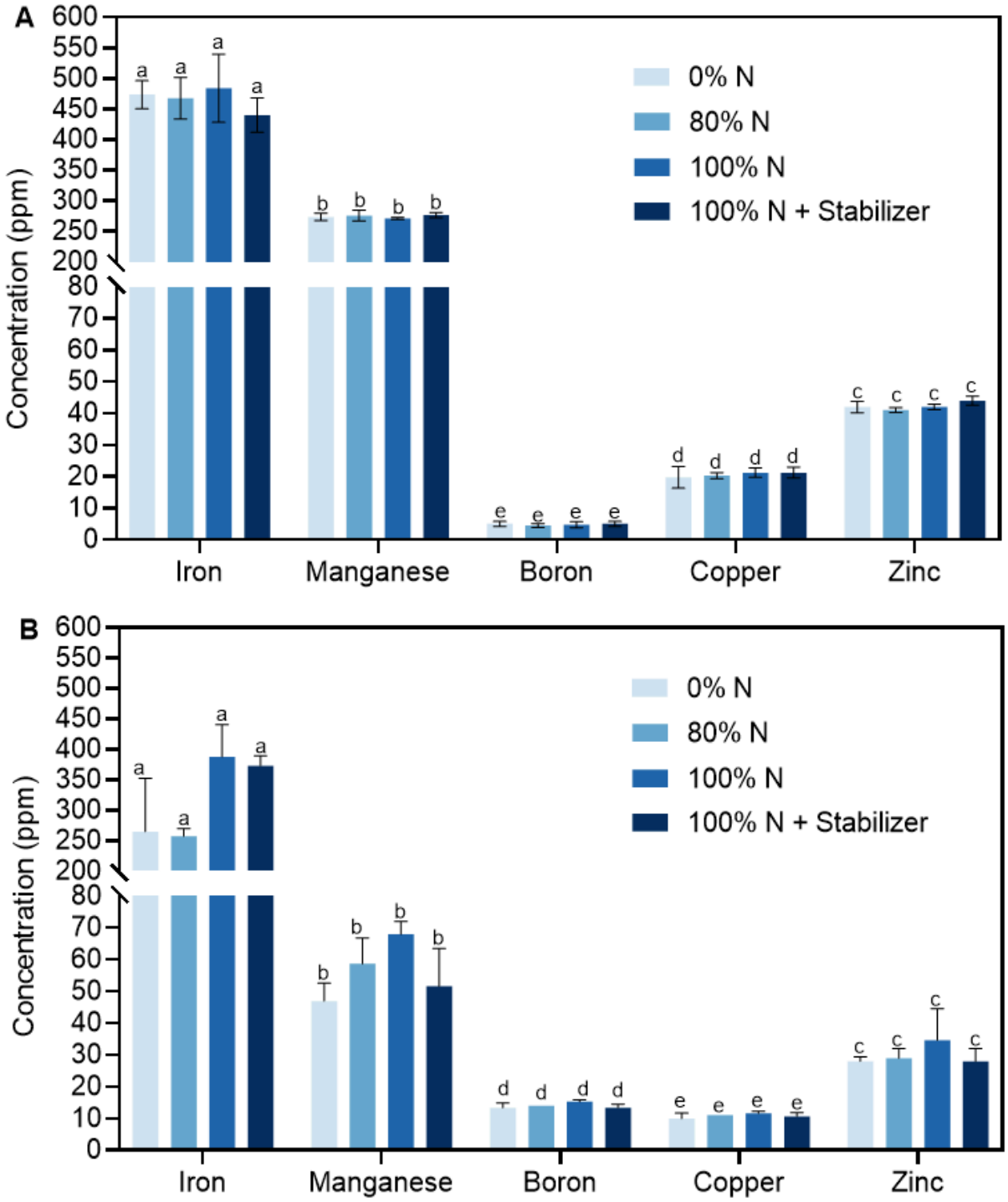

Figure 6

Figure 6. Micronutrient measurement accuracy comparison using two assessment methods: **(A)** Picketa real-time analysis of micronutrient levels, **(B)** Conventional tissue sampling analysis of micronutrients. Treatments included: 0% N (light blue), 80% N (medium light blue), 100% N (medium dark blue), and 100% N + stabilizer (dark blue) treatments. Statistical significance was calculated using Turkey's HSD test. Different letters represent significant differences ($p < 0.05$). Error bars represent (mean ± SD).