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Stalk sap nitrate test as a potential tool for nitrogen fertilizer recommendations for maize

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ABSTRACT

Context or problem: Nitrogen (N) fertilizer is among the costliest inputs to maize (*Zea mays* L.) production, and the most challenging input to predict the optimum application for enhanced productivity while preventing loss to the environment.
Objective: This study aimed to determine if late spring maize stalk sap nitrate-N concentrations measured during vegetative growth stages can be used to guide in-season N fertilizer input decisions.
Methods: Maize stalk sap nitrate-N concentrations were measured at the seven to nine-leaf (V7-V9) developmental stage across eight sites (location-crop rotation-year) in Iowa. Each site received four to eight pre-plant N fertilizer rates.
Results: At each site, the stalk nitrate-N concentration consistently increased with the N fertilization rate. Relative grain yield was positively related to sap nitrate concentration. In addition, there was a positive relationship between sap nitrate concentration, tissue total N concentration, late spring soil nitrate test, and end-of-season maize stalk nitrate test. Overall, across all sites, the sap nitrate-N concentration that indicated N sufficiency (i.e., N supply sufficient to achieve the highest relative yield) spanned a relatively narrow range (715–893 mg N L^{-1} sap) compared to the full observed range (22–1478 mg N L^{-1} sap).
Conclusion: Observations from this multi-site-year study suggest that stalk sap nitrate concentration has the potential to aid in-season N fertilizer application recommendations. Thus, it deserves further study considering other environmental and management factors that potentially affect the sap nitrate N concentrations.
Implications or significance: A stalk sap nitrate test along with rapid, reliable, and low-cost nitrate sensors can create unprecedented databases to optimize soil fertility and plant nutrition.

1. Introduction

Careful management of nitrogen (N) fertilizer is necessary to achieve optimum crop yield while minimizing N losses to the environment. The balance of crop N demand, soil N supply, and environmental N loss controls the N fertilizer requirement. The average N balance in the US is a surplus of 36 kg ha^{-1} (Ludemann et al., 2024). However, this balance varies within fields and across years. As a result, there is no relationship between maize (*Zea mays* L.) grain yield at the optimum N fertilizer rate and the optimum N fertilizer rate (Lory and Scharf, 2003). Hence, the optimum N fertilizer input is difficult to predict and varies with genetics, environment, and management (Caviglia et al., 2014; Puntel et al., 2016; Puntel et al., 2018).

The goal of N fertilizer recommendation systems is to estimate the gap between N supplied by the soil and N required by the plant after accounting for environmental losses. The late spring soil nitrate test (LSNT) or pre-sidedress soil nitrate test (PSNT) is a common tool used to optimize the N input to maize (Magdoff, 1984; Blackmer and Schepers, 1995). The LSNT (or PSNT) measures the concentration of nitrate in the soil 0–30 cm depth immediately prior to sidedress N input when maize is approximately at the six-leaf (V6) developmental stage (Magdoff, 1984); the soil nitrate concentration is calibrated to N response and used to

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determine the appropriate N input. This test is an indicator of N supplying capacity of the soil rather than the N status of the plant (Blackmer and Schepers, 1995). The end-of-season maize stalk nitrate test (CSNT) is another tool used to improve N fertilizer use efficiency in maize production systems. However, in contrast to the LSNT, the CSNT is a post-season evaluation that helps to determine if N fertilizer input was insufficient, sufficient, or excessive. At crop maturity; a section of the basal stalk is cut, dried, ground, and extracted for nitrate (Binford et al., 1992). The stalk nitrate concentration provides an index of crop N status but does not aid in-season N management decision for future years (Morris et al., 2018).

There have been attempts to transform end-of-season performance evaluations into an in-season decision making aid. Iversen et al. (1985), suggested that nitrate concentration in the basal stalk of young maize plants was affected by N fertilizer rate and the relative grain yield was positively associated with the stalk nitrate-N concentration. McClenahan and Killorn (1988) also observed a positive correlation between maize grain yield and basal maize stem nitrate-N concentration and reported a critical range of 0.9–1.78% (dry weight basis) at V6 maize developmental stage. However, Fox et al. (1989), found poor correlations between stalk nitrate at the V5-V6 developmental stage and relative grain yield. These inconsistent results may be due to methodological limitations as well as the effects of genetics, environment, and management (Morris et al., 2018; Justes, 1997). These studies measured in extractions of dried, and ground plant materials.

Regardless of whether there is a relationship between stalk sap nitrate concentration and maize N sufficiency, these historical measurements of stalk nitrate concentration were made using labor-intensive extractions of maize stems and reported on a dry matter basis. Moreover, these studies followed different protocols for plant sampling and methods to determine stalk nitrate concentration. Stalk nitrate was extracted with $(NH_4)_2SO_4$ (Iversen et al., 1985), buffer solution (McClenahan and Killorn, 1988), and hot distilled water (Schepers et al., 1990). However, labor intensity is a major limitation of these extraction-based analyses because extensive sample preparation procedures in the laboratory including drying, grinding and extraction create significant cost and significant delays between field sampling, lab analysis, and on-farm N fertilizer management.

Another approach using plant nitrate concentration for N fertilizer recommendations is to directly measure sap nitrate concentration without an extraction process or scaling to a dry matter basis (Gilbert et al., 1927; Esteves et al., 2021). This method is commonly used for leafy vegetable crops. However, no research in the Midwest US, which is the largest maize producing region in the world, has investigated the potential for direct measurements of maize stalk sap nitrate concentration to aid in-season N fertilizer rate decision making.

The goal of this study is to determine the potential for developing a new direct sap nitrate-N concentration test for in-season maize fertilization. If sap nitrate-N concentration and grain yield response to N fertilizer are correlated, the maize stalk sap nitrate test could make a useful tool to monitor crop N status and aid in-season N fertilizer recommendations. The availability of a stalk sap nitrate test would provide a low-cost, fast, and convenient method to detect the N status of the crop for in-season fertilizer applications. This study hypothesizes that there will be a positive significant relationship between sap nitrate-N concentrations with fertilizer N rate, grain yield, tissue total N concentration, LSNT, and CSNT. The objectives of this study were to investigate: (i) the relationships of sap nitrate-N concentration with N fertilizer rate and relative grain yield, (ii) the relationships of tissue total aboveground N concentration, LSNT, and CSNT with relative grain yield and sap nitrate-N concentration, and (iii) establish a critical sap nitrate-N concentration at V7-V9 maize developmental stage to trigger N fertilizer application.

2. Materials and methods

2.1. Experimental locations and treatments

The study was conducted across five locations and eight site-years in Iowa, encompassing a wide variety of growing conditions and management practices (Table 1, Supplementary Table 1). In 2017, there were three locations with maize following maize; in 2018, there were two locations with both maize following maize and maize following soybean [*Glycine max* (L.) Merr.] and one location with only maize following maize (Supplementary Figure 1). Across the site-years, three to eight different N fertilizer rates were applied, and plots ranged from 0.01 to 0.4 ha (Table 2, Supplementary Table 1). At all sites, N fertilizer rate was the only experimental treatment and experimental designs were randomized complete block designs with 2–4 replications. The 2017_Wellman_MM location received a combination of ammonium sulfate (different N rates) in fall and spring, liquid swine manure (same rate to all treatments, except 0 N treatment) in spring, and starter UAN application (different application rates) at planting, added to the total N application rates (see Supplementary Table 1 for details). The 2017 Crawfordsville site N rates were urea-ammonium nitrate solution (UAN) injected preplant in the spring whereas other sites had granular urea preplant surface broadcast (no incorporation) in the spring. Except for N fertilizer, all management followed local best management practices. All other nutrients were maintained at optimum agronomic levels.

2.2. Plant and soil analyses

Stalk sap nitrate test and LSNT were performed in spring at the time corresponding to late spring N fertilizer application that is seven to nine leaf (V7-V9) vegetative developmental stage. At this stage, the growth point in maize is aboveground for stalk sap nitrate test, and sidedress N fertilization can be performed without damaging the maize crop.

At the V7-V9 developmental stage, five stalk samples (0–15 cm length from the soil surface) in 2017 and ten stalk samples in 2018 were collected randomly within each plot at each site. Stalk samples were kept in a cooler while transferred to the lab where they were stored at 4^0 C for < 24 h. Leaves and leaf sheaths were removed, and stalks were rinsed with deionized water to avoid nitrate leaching from the sample. Sap was extracted using a mini hand juicer (Kai DH3011ENG Select 100 Mini Hand Juicer). Due to high nitrate-N concentrations, the sap was diluted 10–300 times with deionized water in 50 ml centrifuge tubes. The diluted samples were analyzed the same day for nitrate-N (NO_3^--N) concentration using colorimetry (Hood-Nowotny et al., 2010).

During plant sampling, soil was collected for the LSNT. Ten 2.5 cm dia. x 30 cm depth soil cores were collected in each plot. Samples were taken from random locations capturing different parts of the maize row and inter-row areas within each plot and bulked to represent one sample for each plot. Soil moisture content was determined from a 10 g subsample. Nitrate-N concentration (mg NO_3^--N kg^{-1} dry soil) was determined by extracting another 10 g subsample with 50 ml 2 M potassium chloride (KCl) and subsequent measurement with colorimetry (Hood-Nowotny et al., 2010).

In 2018 only, ten whole-plant samples per plot were collected by cutting at the soil surface at the same time as stalk sap nitrate samples for total N analysis. The whole plant samples were weighted immediately and then dried in forced-air dryers at 60 °C for 10 days. Oven-dried plant samples were finely ground for total N determination by dry combustion elemental analysis using a LECO CHN-2000 analyzer (LECO Corp., St. Joseph, MI).

Stalk samples for the conventional maize stalk nitrate test (CSNT) were collected at the end of the season (Binford et al., 1990, Sawyer and Mallarino, 2018). After maize reached full maturity, 12 stalk samples (20 cm length, 15–35 cm from the soil surface) were collected from each plot. Leaves, along with leaf sheath, were removed from stalks before drying. Samples were dried in forced-air dryers at 60 °C for 24 hr. and

Table 1
Soil properties and climatic conditions at each experimental site.

Site	Year	Predominant Soil Series	Soil Texture	Mean Annual Temperature (°C)	Annual Cumulative Precipitation (mm)	Total precipitation during crop period (mm)
Ames	2017	Nicollet, Webster, Clarion	Loam, Clay loam	10.6	796	499
Crawfordsville	2017	Kalona, Taintor	Silty clay loam	11.5	699	300
Wellman	2017	Nevin, Bremer, Wiota	Silty clay loam	11.5	699	300
Ames	2018	Nicollet, Webster, Clarion	Loam, Clay loam	9.2	1209	840
Nashua	2018	Floyd, Clyde, Readlyn	Loam	7.6	1432	1035
Sutherland	2018	Primghar, Galva	Silty clay loam	7.3	914	607

Temperature and precipitation data from the Iowa Environmental Mesonet of Iowa State University (IEM, 2022).

Table 2
Fertilizer N rate treatments at each site and crop rotation.

Site	N fertilizer	N fertilizer rates (kg N ha^{-1})	Number of Replicates
2017_Ames_MM*	Urea	0, 67, 135, 202, 269, 336	3
2017_Crawfordsville_MM	UAN***	0, 56, 112, 168, 224, 280, 336, 392	2
2017_Wellman_MM	Manure†, ammonium sulfate††, UAN††	0, 140, 211, 280†††	4
2018_Ames_MM	Urea	0, 67, 135, 202, 269, 336	3
2018_Ames_SM**	Urea	0, 67, 135, 202, 269, 336	3
2018_Nashua_MM	Urea	0, 67, 135, 202, 269, 336, 403	3
2018_Nashua_SM	Urea	0, 67, 135, 202, 269, 336, 403	3
2018_Sutherland_MM	Urea	0, 67, 135, 202, 269, 336, 403	3

*MM represents maize after maize. **SM represents maize after soybean. ***Urea ammonium nitrate solution. †Same rate of swine manure was applied to all treatments except the 0 N rate in Spring. †† different rates of ammonium sulfate were applied in the Fall and Spring, and different rates of UAN for each treatment were applied at planting. †††Total N application rate (total of manure, ammonium sulfate, and UAN) in fall and spring.

ground to pass 2 mm screen. Nitrate was extracted from a 0.5 g sample with 50 ml 2 M KCl and measured with colorimetry.

The middle two rows of each individual plot were hand- or machine-harvested after the crop reached physiological maturity. Grain yield was adjusted to 155 g kg^{-1} moisture content.

2.3. *Statistical analyses*

An analysis of variance (ANOVA) for each site included the fixed effect of fertilizer N rate for maize yield and sap nitrate-N concentration. Replication was considered as a random effect. Sites with a significant effect of N fertilizer rate at $p \leq 0.10$ were considered responsive.

Two segmented polynomial regression models: linear plateau (LP) (Eq. 1) and quadratic-plateau (QP) (Eq. 2) models were fitted to maize grain yield response to N fertilizer rate to estimate agronomic and economic optimum N rates (AONR and EONR, respectively) for each site.

$$\text{Linear-plateau model: } y = a + bx \text{ if } x < xs;\ y = Ymax \text{ if } x > xs \quad (1)$$

$$\text{Quadratic-plateau model: } y = a + bx + cx\hat{}2 \text{ if } x < xs;\ y = Ymax \text{ if } x > xs(2)$$

Where, y = dependent factor, a = intercept, b = slope, c = quadratic, x = independent factor, xs = join-point (x-value where y reaches plateau), Ymax = value of y at the plateau.

The AONR was defined as the minimum fertilizer rate at which no yield increase is expected if the rate is increased and EONR as the rate at which crop yield increase is not large enough to pay for additional N fertilizer. The best fit model was chosen according to their Akaike Information Criterion (AIC values) (Miguez and Poffenbarger, 2022 and Archontoulis and Miguez, 2015). For individual sites, AONR was considered equal to the break point of the best fit model. Yield at AONR (y-value at plateau) was considered as maximum yield for the site. Economic optimum N rates for each site were calculated by setting the first derivative of the N response curve equal to the N fertilizer to grain price ratio of 5.6:1 (US\$ kg $^{-1}$ N and US\$ kg^{-1} grain) during the study years (Cerrato and Blackmer, 1990). Linear (Eq. 3), quadratic (Eq. 4), linear-plateau (Eq. 1) and quadratic plateau (Eq. 2) models were fitted for correlation of sap nitrate-N concentration with N fertilizer rate at individual site.

$$\text{Linear model: } y = a + bx \quad (3)$$

$$\text{Quadratic model: } y = a + bx + cx\hat{}2 \quad (4)$$

Where, y = dependent factor, a = intercept, b = slope, c = quadratic, x = independent factor

To develop the correlations across sites, the yield was expressed as relative yield (%) for each site using the quotient of mean yield in each treatment and maximum yield (i.e., yield at the AONR). Quadratic-plateau (Eq. 2) and LP (Eq. 1) regression models were fitted to correlate (i) relative yield with sap nitrate-N concentration, total aboveground N concentration, LSNT, and CSNT, (ii) sap nitrate-N concentration with fertilizer N rate, total aboveground N, LSNT, and CSNT.

A Cate-Nelson analysis was performed to estimate the critical sap nitrate test (CSTV) and critical soil nitrate test values for 95% relative yield (Cate and Nelson, 1965). Also, two segmented polynomial regression models i.e., LP (Eq. 1) and QP (Eq. 2), were used to determine the optimum range of stalk sap nitrate-N concentration and late spring soil nitrate-N concentration to maximize the relative yield from N fertilizer applied (Stammer and Mallarino, 2018). The join-point (xs) of the LP model represented the lower limit, and the join-point (xs) of the QP model represented the upper limit of the sufficiency range.

All Statistical analyses were done using R software version 3.3.2 (R Core Team, 2017).

3. Results

3.1. *Yield and sap nitrate response to N fertilizer rate at each site*

All sites showed significant effects of N fertilizer rate on grain yield and sap nitrate-N concentration and therefore all sites were considered responsive (Supplementary Table S2). Maize grain yield across treatments and sites ranged from 2563 to 15,410 kg ha^{-1}. The relationship between grain yield and N fertilizer rate at seven of the eight site-years

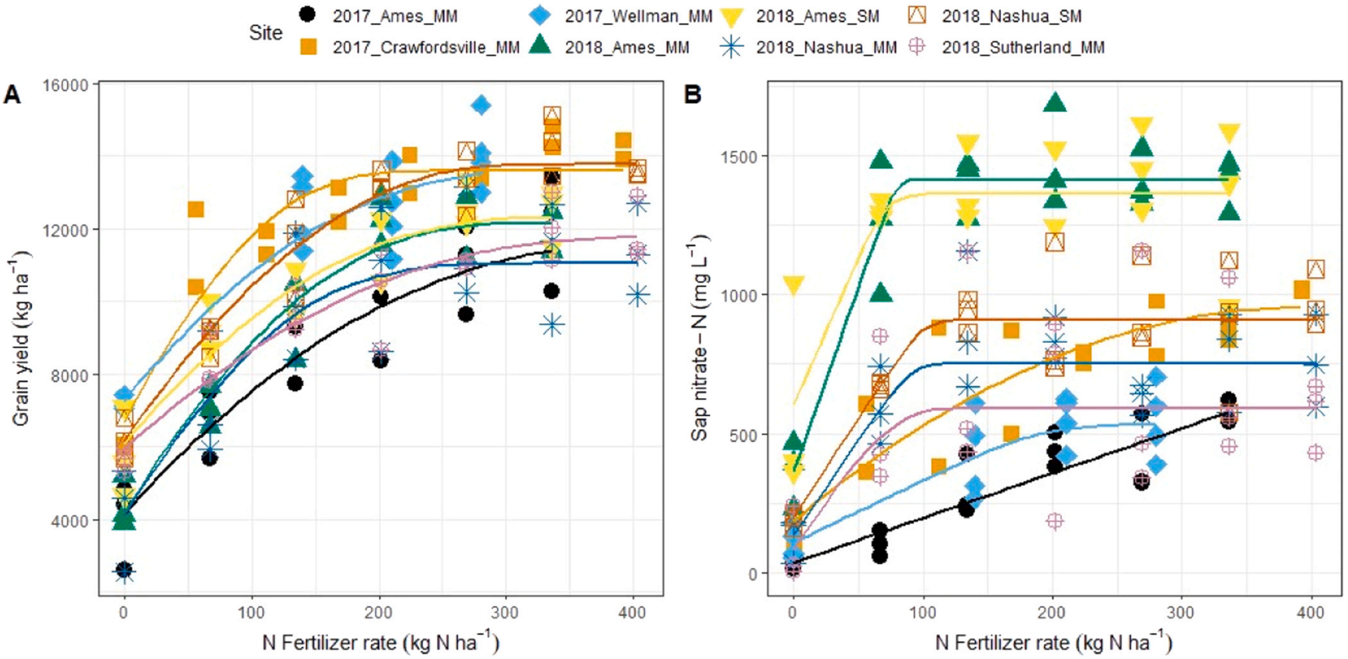

Fig. 1. (A) Maize grain yield (kg ha^{-1}) and (B) sap nitrate-N concentration (mg N L^{-1}) response to N fertilizer rate (kg N ha^{-1}) at each site. MM in site names represents maize after maize and SM represents maize after soybean crop rotation. Parameters of best fit model for grain yield at each site are given in Table 3. Parameters for model comparison for sap nitrate-N at each site are given in Table 4.

Table 3

Estimated Agronomic Optimum Nitrogen Rate (AONR) (kg N ha^{-1}), Economic Optimum Nitrogen Rate (EONR) (kg N ha^{-1}), information criteria AIC for LP and QP models, and best fit model parameters (in bold text) for each site yield response.

Site	AONR	EONR	Model Information Criteria		Best fit model parameters				Ymax
	(kg N ha^{-1})	(kg N ha^{-1})	LP[†] AIC	QP[††] AIC	a	b	c	xs	(kg ha^{-1})
2017_Ames_MM*	398	336	315.6	**310.3**	4138.1	39.6	-0.05	397.8	12013
2017_Crawfordsville_MM	210	193	277.0	**271.2**	6676.1	67.9	-0.16	210.1	13807
2017_Wellman_MM	306	266	273.6	**272.0**	7262.9	43.1	-0.07	306.1	13852
2018_Ames_MM	193	193	**292.4**	297.1	4426.1	39.9		193.5	12151
2018_Ames_SM**	306	265	288.1	**284.1**	6070.7	41.9	-0.07	305.8	12482
2018_Nashua_MM	253	228	370.5	**370.4**	4116.5	57.1	-0.11	252.7	11326
2018_Nashua_SM	299	267	342.5	**341.9**	6142.2	51.4	-0.09	299.1	13832
2018_Sutherland_MM	379	311	353.0	**345.3**	5956.9	31.2	-0.04	378.5	11868

*MM, maize after maize; **SM, maize after soybean.
[†]LP, Linear-plateau model.
[††]QP, Quadratic-plateau model.
Where, y = yield (kg ha^{-1}); x = N fertilizer rate (kg N ha^{-1}); a = intercept, b = slope, c = quadratic; xs = join-point;
Ymax = yield at plateau (xs).

was best fit by the QP model (Fig. 1 A, Table 3). However, at the 2018_Ames_MM site, it was best described by the LP model. Among sites, the AONR ranged from 193 to 398 kg N ha^{-1} and the EONR ranged from 193 to 336 kg N ha^{-1} (Table 3).

The relationship between sap nitrate-N concentration and N fertilizer rate was best explained by the linear plateau model at six of the eight site-years. However, at the other two site-years, the linear and quadratic models best explained the sap nitrate response (2017_Ames_MM and 2017_Crawfordsville_MM, respectively; Fig. 1B, Table 4). At the 2017_Ames_MM site with the linear relationship, the QP join point is above the highest N rate applied therefore the estimated AONR was higher than the highest N fertilizer rate in the trial.

3.2. Relative yield and stalk sap nitrate-N concentration across sites

Across all sites, there was a significant relationship between relative grain yield and sap nitrate-N concentration, as well as between sap nitrate-N concentration and N fertilizer rate. The sufficiency range was 715 – 893 mg nitrate-N L^{-1} as the LP model reached plateau at a sap nitrate-N concentration level of 715 mg nitrate-N L^{-1} (R^2=0.58), and for the quadratic plateau regression model, it reached a plateau at 893 mg nitrate-N L^{-1} (R^2=0.59) (Fig. 2 A). Similarly, sap nitrate-N had a significant positive relationship with N fertilizer rate with a plateau with the LP model at 85 kg N ha^{-1} and with the QP model at 135 kg N ha^{-1}(Fig. 2B).

3.3. Sap nitrate-N concentrations vs aboveground biomass total N concentration, LSNT, and CSNT

Across all sites, the relationship between relative yield and total plant N concentration in aboveground biomass was linear as the LP and QP models did not converge to get the sufficiency range (Fig. 3 A). However, there was a significant relationship between sap nitrate-N concentration and aboveground total N concentration; it was best described by a LP model (R^2 = 0.64; AIC 180) (Fig. 3B). Total N concentration was highest (40 mg kg^{-1}) at 885 mg nitrate-N L^{-1} sap with the LP and 1210 mg nitrate-N L^{-1} sap with the QP model.

Table 4
Regression models for sap nitrate-N correlation with N fertilizer rate. Text in bold represents best fit model.

Site	Linear (R^2, AIC)	Quadratic (R^2, AIC)	LP† (R^2, AIC)	QP†† (R^2, AIC)
2017_Ames_MM*	**$y = 35 + 1.6x$ (0.83, 216)**	$y = 5.6 + 2.27x + -0.002x^2$ (0.84, 217)	$y = -1.9 + 2.15x$ if $x \leq 228$; $y = 489$ if $x>228$ (0.83, 219)	$y = 5.6 + 2.27x + -0.002x^2$ if $x \leq 579$; $y = 664$ if $x > 579$ (0.84, 217)
2017_Crawfordsville_MM	$y = 296 + 1.96x$ (0.72, 213)	**$y= 184 +3.95x + -0.005x^2$ (0.78, 212)**	$y = 884 + 3.43x$ if $x \leq 203$; $y = 884$ if $x > 203$ (0.74, 214)	$y = 181 + 4.03x + -0.005x^2$ if $x \leq 379$; $y = 945$ if $x > 379$ (0.78, 212)
2017_Wellman_MM	$y = 143 + 1.67x$ (0.70, 203)	$y = 104 + 3.17x + -0.006x^2$ (0.75, 202)	**$y = 107 + 2.25x$ if $x \leq 196$;** **$y = 547$ if $x > 196$ (0.76, 201)**	$y = 104 + 3.17x + -0.006x^2$ if $x \leq 285$; $y = 555$ if $x > 285$ (0.75, 202)
2018_Ames_MM	$y = 806 +2.44x$ (0.47, 262)	$y =488 + 9.55x + -0.020x^2$ (0.82, 245)	**$y = 363 + 13.22x$ if $x \leq 80$;** **$y = 1422$ if $x > 80$ (0.90, 233)**	$y = 363 + 18.87x + -0.084x^2$ if $x \leq 112$; $y = 1422$ if $x > 112$ (0.90, 233)
2018_Ames_SM**	$y = 951 + 1.7x$ (0.32, 247)	$y =695 + 7.62x + -0.018x^2$ (0.63, 239)	**$y = 602 + 10.45x$ if $x \leq 75$;** **$y = 1387$ if $x > 75$ (0.69, 236)**	$y = 602 + 15.80x + -0.080x^2$ if $x \leq 99$; $y = 1387$ if $x > 99$ (0.69, 236)
2018_Nashua_MM	$y = 443 + 1.07x$ (0.29, 293)	$y = 237 + 4.76x + -0.009x^2$ (0.57, 284)	**$y = 127 + 6.91x$ if $x \leq 94$;** **$y = 778$ if $x > 94$ (0.71, 276)**	$y = 124 + 9.43x + -0.034x^2$ if $x \leq 138$; $y = 776$ if $y > 138$ (0.71, 276)
2018_Nashua_SM	$y = 487 + 1.48x$ (0.47, 291)	$y = 285 + 5.09x + -0.009x^2$ (0.70, 281)	**$y = 191 + 7.17x$ if $x \leq 103$;** **$y = 927$ if $x > 103$ (0.78, 274)**	$y = 188 + 9.34x + -0.029x^2$ if $x \leq 159$; $y = 929$ if $x > 189$ (0.78, 274)
2018_Sutherland_MM	$y = 370 + 0.91x$ (0.14, 305)	$y = 177 + 4.36x + -0.008x^2$ (0.31, 303)	**$y = 89 + 6.70x$ if $x \leq 83$;** **$y = 648$ if $x > 83$ (0.35, 302)**	$y = 89 + 9.32x + -0.039x^2$ if $x \leq 120$; $y = 648$ if $x > 120$ (0.35, 302)

*MM represents maize after maize; **SM represents maize after soybean.
†LP = Linear plateau model; ††QP = Quadratic plateau model.
x = N rate (kg ha^{-1}); y = sap nitrate-N (mg L^{-1}).

The relationship between the LSNT and relative yield was best described by the LP model (Fig. 4 A). The sufficiency range described by LP and QP models was 15–25 mg nitrate-N kg^{-1} dry soil as the maize relative yield reached a plateau of 97% relative yield at 15 mg nitrate-N kg^{-1} dry soil using the LP model and 99% relative yield at 25 mg nitrate-N kg^{-1} dry soil with the QP model. Similarly, the LP model had the best fit for the relationship between sap nitrate-N concentration and LSNT (Fig. 4B). Sap nitrate-N concentration reached a plateau (1032 mg nitrate-N L^{-1}) at 12.6 mg nitrate-N kg^{-1} soil LSNT with LP model and 1036 mg nitrate-N L^{-1} plateau at 18 mg nitrate-N kg^{-1} soil LSNT with QP model indicating the sufficiency range.

There was a significant relationship between maize stalk nitrate-N concentration at the end of the growing season (i.e., the CSNT) and relative yield. On average across all sites, relative grain yield reached a plateau (99%) at 140 mg nitrate-N kg^{-1} aboveground dry matter stalk nitrate-N concentration with LP model and at 197 mg nitrate-N kg^{-1} dry matter with QP model (Fig. 5 A) describing the sufficiency range.

There was a significant relationship between end-of-season stalk nitrate-N concentration (CSNT) and sap nitrate concentration at V7-V9 developmental stage, but the correlation was poor (Fig. 5B). The sap nitrate-N concentration was highest (933 mg nitrate-N L^{-1} sap) at 793 mg nitrate-N kg^{-1} CSNT with LP model and was 944 mg nitrate-N L^{-1} sap at 1373 mg nitrate-N kg^{-1} CSNT with the QP model representing the sufficiency range.

3.4. *Critical sap nitrate-N and late spring soil nitrate-N concentrations*

Using the relative yield and sap nitrate-N LP and QP model join points (Fig. 2 A), the lower limit of a potentially critical sap nitrate-N concentration range was 715 mg L^{-1} and the upper limit was 893 mg L^{-1} – at 93% relative yield. A potential critical sap nitrate-N concentration using the Cate-Nelson analysis at 95% relative yield, was 709 mg L^{-1} (Fig. 6 A). Using relative yield and LSNT LP and QP model join points (Fig. 4 A), the lower limit of a potential critical soil nitrate-N concentration range was 15 mg kg^{-1} and the upper limit was 25 mg kg^{-1} – at 97% relative yield. Using the Cate-Nelson analysis, the critical soil nitrate-N concentration at 95% relative yield was 21 mg kg^{-1} (Fig. 6 B).

4. Discussion

From the results across eight site-crop rotation-years, we provide support for the hypothesis that sap nitrate-N concentration of basal maize stalk at the V7-V9 plant developmental stage has the potential to determine N deficiency in maize at an early growth stage. The LSNT (mg nitrate-N kg^{-1} soil), which is the most widespread soil test used to adjust N fertilizer input in Midwest US maize, explained approximately 12–15% more variation in percent relative yield than sap nitrate concentration (Figs. 3 and 5; R^2 0.71–0.73 vs. 0.58–0.59). That is, performed better than the stalk sap testing in regard to response fit and more importantly reduced incorrect identification of deficit/excess situations. However, the LSNT benefits from decades of research that has helped to refine the deployment of the test (e.g., determination of optimum sampling depth and number of samples per field, etc.). As far as we know this is the first attempt to correlate early season maize stalk sap nitrate concentration and relative grain yield in the US Maize Belt. The relationships reported herein may be further improved with additional site-years and geographic area research. There is potential to improve the relationship by: 1) better control for methodological factors such as number of samples, maize developmental stage, and position of sampling on the stalk (and for use at later growth stages), 2) information from a more robust dataset, and 3) account for genetics, environment, and management practices.

Moreover, due to the use of spatially concentrated (i.e., banded) N fertilizer applications that interfere with soil sampling requirements and accurate soil nitrate measurement, there is a growing interest in alternative N tests that avoid the challenges associated with collecting a representative soil sample. Concentrated N applications create a major challenge for the implementation of the LSNT. For example, Mitchell et al. (2013) observed that soil nitrate concentrations at approximately the V6 maize developmental stage were ~30-fold higher in soils that received the fertilizer band vs. adjacent soils that did not. Accidental mixing of soil from the inside and outside of the fertilizer band will decrease certainty in the mean soil nitrate concentration for the overall field and thus increase soil sampling requirements (Mueller et al., 2018). In contrast, the use of sap nitrate may address this concern because the sap test is an indicator of plant N status i.e., actual N uptake by the plant

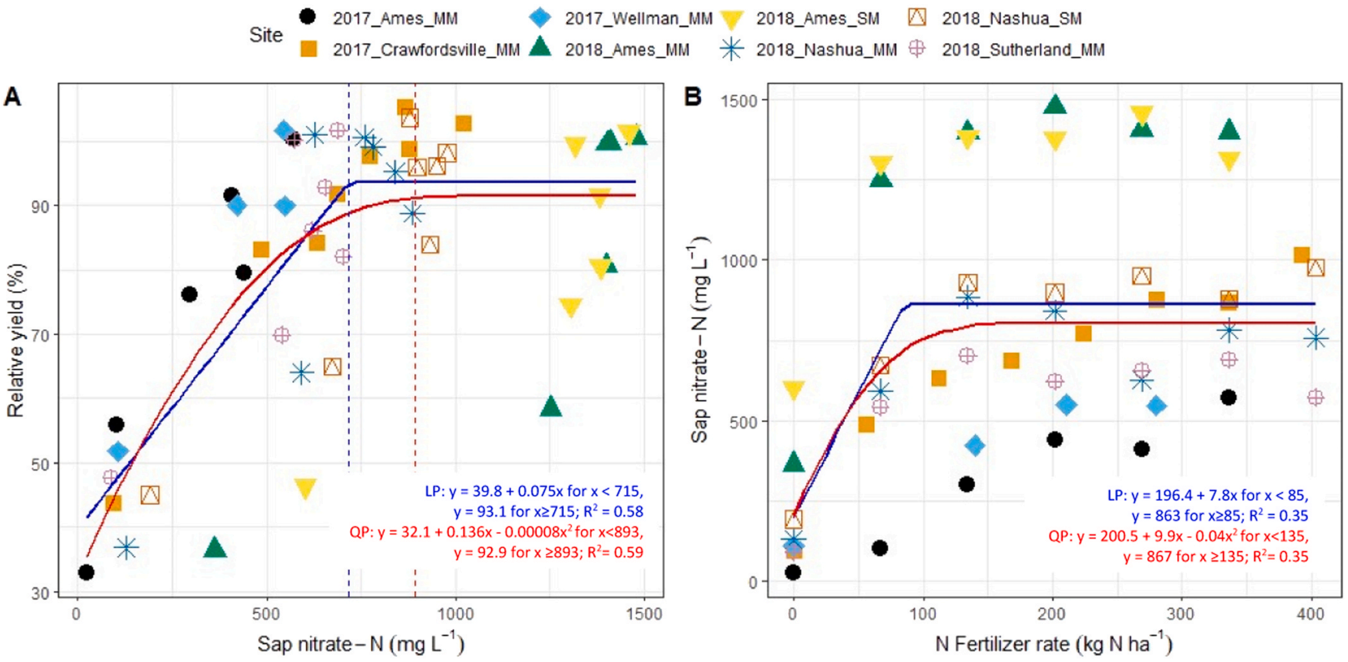

Fig. 2. (A) Relative yield (%) response to stalk sap nitrate-N concentration (mg N L^{-1}) and (B) sap nitrate-N concentration (mg N L^{-1}) to fertilizer N rate (kg ha^{-1}) across all sites. The lower limit of critical sap nitrate-N concentration level at the maximum relative yield is indicated by the blue dotted line (join-point of LP regression model, blue solid line) and the upper limit of critical level is indicated by red-dotted line (join-point of the QP regression model, red solid line) in (A). MM in site names represents maize after maize and SM represents maize after soybean. LP represents linear-plateau model and QP quadratic-plateau model.

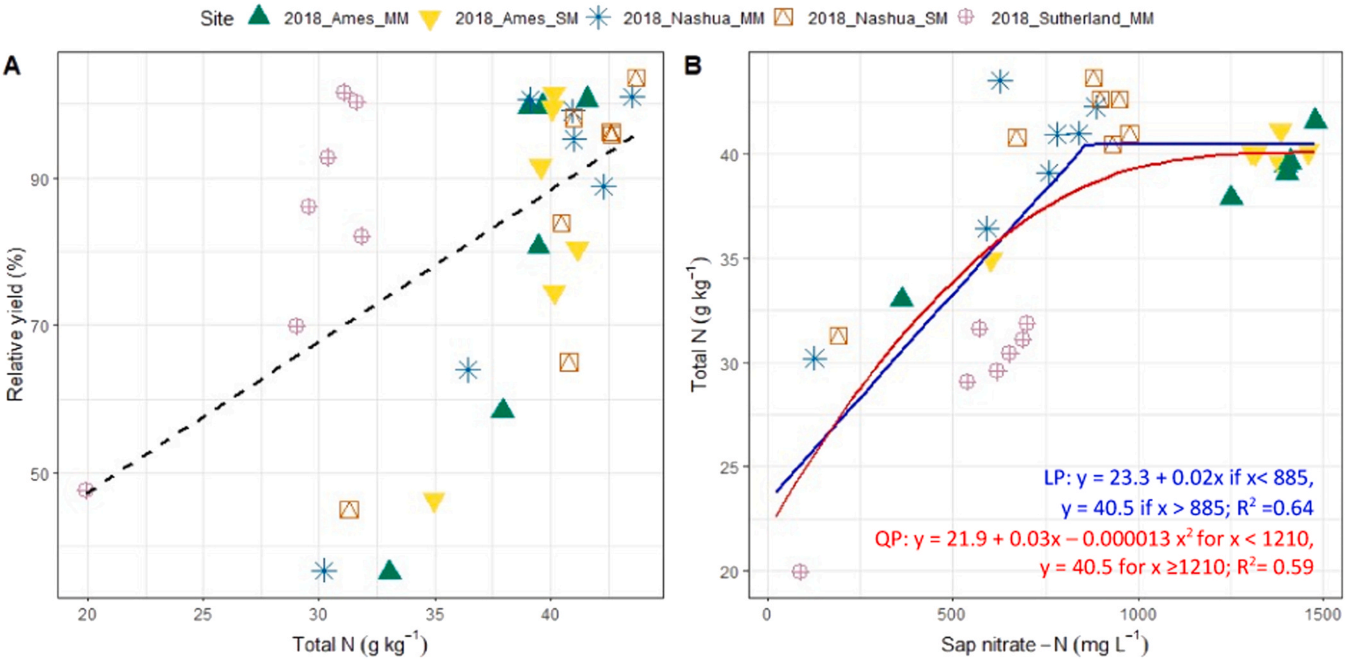

Fig. 3. (A) Relative yield (%) response to aboveground biomass total N concentration (g N kg^{-1} aboveground dry matter) and (B) total N concentration (g N kg^{-1} aboveground dry matter) response to stalk sap nitrate-N concentration (mg N L-1) across sites in 2018. MM in the site name represents maize after maize and SM represents maize after soybean. LP represent linear-plateau model and QP as quadratic-plateau model. Black dashed line represents linear model, blue solid line LP model and red solid line QP model.

instead of N supplying capacity of soil as in the case of LSNT. Future research should explore the potential effect of fertilizer application time and placement on stalk nitrate concentration.

Total plant N concentration, which is another potential indicator of crop N status, had a positive significant relationship with sap nitrate N concentration (Fig. 3). Total N concentration reached a peak at 885–1210 mg nitrate-N L^{-1} sap, which suggests luxury N uptake by the plant above 1210 mg nitrate-N L^{-1} stalk sap concentration, at which point the plant no longer reduces nitrate into organic compounds. It is worth noting that LP and QP models did not converge to determine the sufficiency range using tissue total N concentration. The LSNT, which is a soil-based test, suggested a sufficiency range of 1032–1036 mg nitrate-

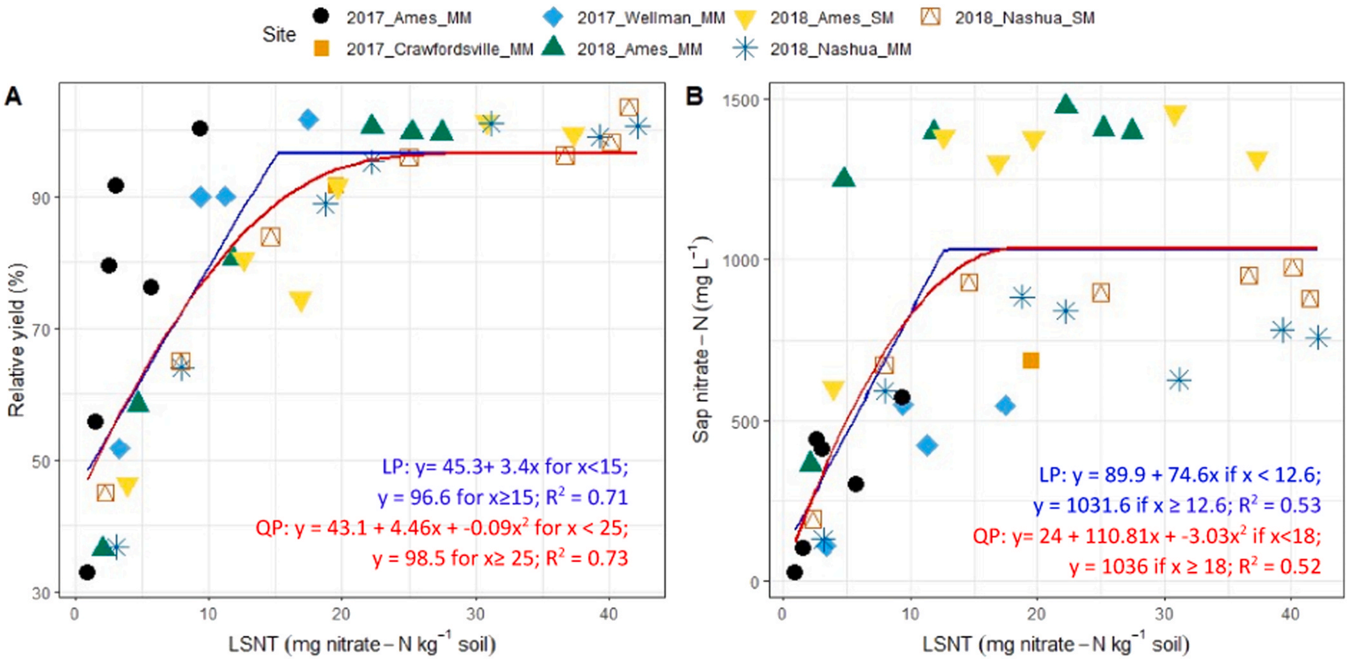

Fig. 4. (A) Relative yield (%) response to the late spring soil nitrate-N test values (mg nitrate-N kg^{-1} soil) and (B) sap nitrate-N concentration (mg nitrate-N L^{-1}) relationship to the late spring soil nitrate-N concentration (mg nitrate-N kg^{-1} soil) across all sites. Solid blue and red lines represent LP and QP regression models, respectively. LP represents linear-plateau model and QP as quadratic-plateau model. MM represents maize after maize and SM represents maize after soybean.

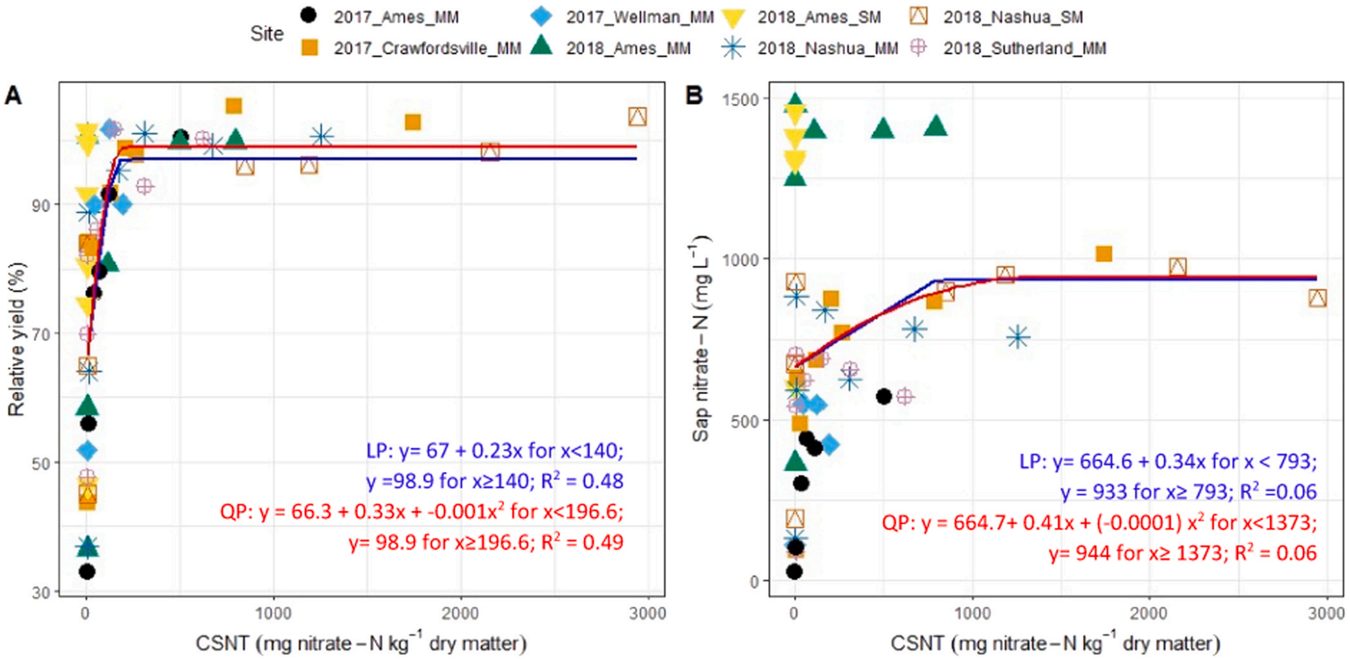

Fig. 5. (A) Relative grain yield relationship with the end of season maize stalk nitrate-N test (CSNT) values and (B) sap nitrate-N concentration relationship with the end of season maize stalk nitrate-N test (CSNT) values across all sites. Blue line represents LP regression model and red line represents QP model. LP represents linear-plateau model and QP as quadratic-plateau model. MM represents maize after maize and SM represents maize after soybean.

N L^{-1} at 12.6 – 18 mg nitrate-N kg^{-1} soil, indicating values above this range reflect excessive N application to the soil. The CSNT taken at crop maturity also indicated sap concentration above 944 mg nitrate-N L^{-1} at the V7-V9 developmental stage as luxury N uptake by the plant. Overall, these results are comparable to the sufficiency range of 715 –893 mg nitrate-N L^{-1} developed using N fertilizer rate and sap nitrate correlations (i.e., above approximately, 900 mg nitrate-N L^{-1} there is luxury uptake by the plant).

The high variability of sap nitrate concentration within N rates appears to be a challenge. The variability in these relationships could be due to a number of factors, including diurnal patterns in stalk nitrate (as observed in preliminary unpublished studies). The variability in sap concentration was similar to site-to-site variability observed by McClenahan and Killorn (1988) in stalk nitrate-N concentration measured on a dry weight basis at the V6 developmental stage. We also observed variability across site-years, yet this variability could not be explained

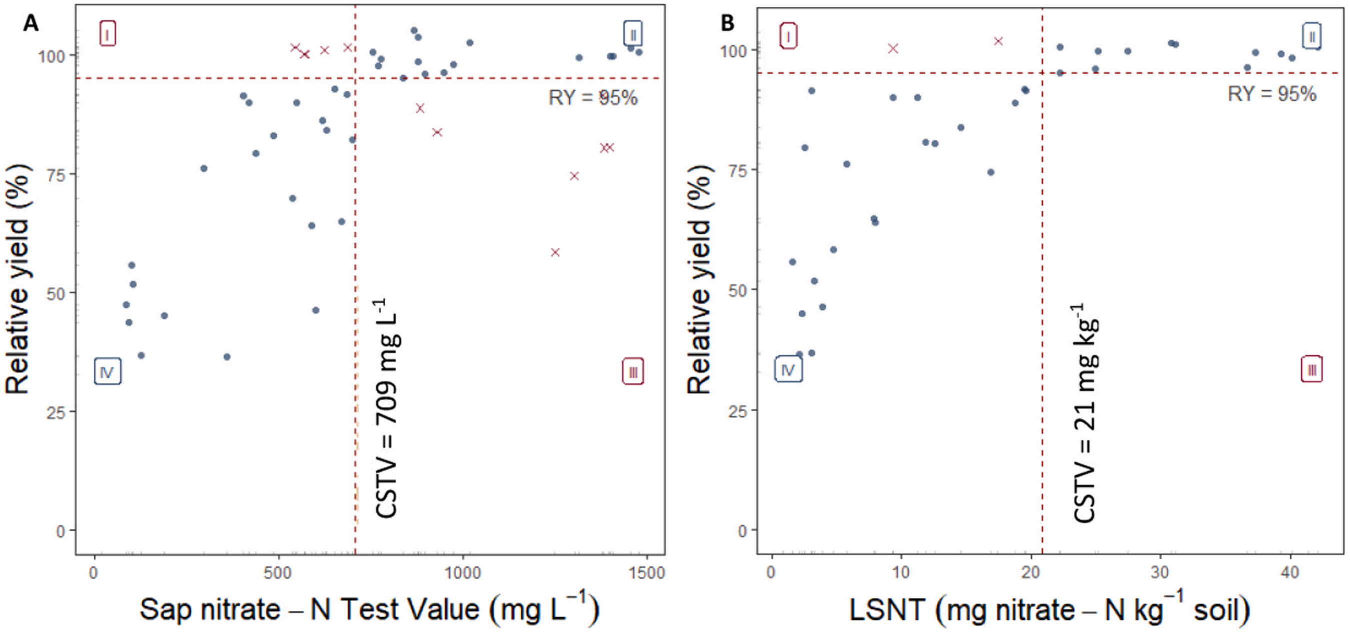

Fig. 6. Cate-Nelson analysis plot. The dashed horizontal line represents 95% relative yield and the vertical dashed line represents critical soil nitrate test value (CSTV; mg L^{-1}) with (A) sap nitrate test and (B) late-spring nitrate test (LSNT). Cate-Nelson analysis divides data into 4 quadrants:(A) I, sap nitrate – N < CSTV & relative yield > target relative yield; II, sap nitrate – N >= CSTV & relative yield >= target relative yield; III, sap nitrate – N >= CSTV & relative yield < target relative yield; IV, sap nitrate – N < CSTV & relative yield < target relative yield.; (B) I, LSNT < CSTV & relative yield > target relative yield; II, LSNT >= CSTV & relative yield >= target relative yield; III, LSNT >= CSTV & relative yield < target relative yield; IV, LSNT < CSTV & relative yield < target relative yield.

by soil type alone. Other factors such as genotype, N application time, N source, N application method, soil nutrient status, soil moisture status, topography, local weather conditions, and plant growth rate (assimilation of nitrate into vegetative tissue) may also contribute to the variability. It is worth noting that each site had different management practices. For example, 2017_Wellman_MM received a combination of organic and inorganic N fertilizer at different times i.e., in fall, spring, and at planting, Crawfordsville received UAN injection at planting, while other sites received Urea as surface broadcast in spring. Therefore, each site must differ in terms of N losses, total N availability for plant uptake, and time of N availability, as well as variability in sap nitrate concentration among sites. Moreover, this study did not account for the precipitation, soil moisture, and rooting depth. Overall, these observations suggest local calibration or large data sets might be required to improve the sap test. However, the ability to directly and instantaneously measure sap nitrate concentration with new low-cost, instantaneous electrochemical sensors (e.g., Ali, 2017) promises rapid *in situ* measurement that eliminates long time lags between sample collection, analysis, and on-farm fertilizer management. Hence, it may be possible to assemble large datasets that enable new data analytics and modeling approaches towards traditional plant and soil fertility tests.

A major challenge with laboratory analysis of sap nitrate analysis is the requirement for sample dilution. Electrochemical sensing can address this challenge. The concentration ranges that we measured in stalk sap required 10–300 times dilution for analysis with colorimetry (data published herein). This creates a time-consuming analytical challenge due to the large dilution of a small sample volume. However, electrochemical sensing has a much wider dynamic range (e.g., 100–10,000 ppm).

Sap nitrate testing has proven to be a useful indicator of plant nutrient status for several horticultural and agronomic field crops (Hochmuth, 1994, Raynal and Cousin, 1996, Smith et al., 1998). Similarly, our work indicates that maize stalk sap nitrate concentration deserves further study as a tool to recommend or adjust N fertilizer rate, or more simply to determine if sidedress N fertilizer is required. Relative grain yield, N fertilizer rate, and sap nitrate concentration were positively correlated. Stalk sap nitrate was also positively associated with total N, LSNT, and CSNT. In the future, yield response to sap nitrate measurements data along with new low-cost, instantaneous plant and soil nitrate sensors offer the potential to create new tests and unprecedented databases that can be used to optimize soil fertility and plant nutrition. To further develop the utility of early season maize stalk nitrate testing, instantaneous plant and soil placed nitrate sensors could provide the needed rapid feedback on maize N status (for sidedress decisions). Such methods will need to be developed and tested with robust in-field N rate response research.

5. Conclusion

The results from this study suggest that it may be possible to use maize stalk sap nitrate-N concentration as an indicator of N fertilizer requirement and make in-season fertilization decisions. Across all sites, there was a significant positive relationship between sap nitrate-N concentration and N fertilizer rate, grain yield, aboveground total N, LSNT, and CSNT. The sap nitrate-N sufficiency range was 715–893 mg nitrate-N L^{-1} across all sites. However, there was high variability among sites, which suggests a need for further research to collect more robust multi-site, multi-crop rotation, and muti-year in-field N rate response data for correlation/calibration to develop in-season sidedress fertilizer recommendations.

CRediT authorship contribution statement

Navreet Kaur Mahal: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fernando E Miguez:** Writing – review & editing, Data curation, Methodology, Formal analysis. **John E Sawyer:** Writing – review & editing, Methodology, Formal analysis. **Liang Dong:** Writing – review & editing. **Patrick S Schnable:** Writing – review & editing. **Michael J Castellano:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michael Castellano reports financial support was provided by ARPA-e. Michael Castellano reports a relationship with EnGeniousAg LLC that includes: board membership, employment, and equity or stocks. Liang Dong reports a relationship with EnGeniousAg LLC that includes: board membership, employment, and equity or stocks. Patrick Schnable reports a relationship with EnGeniousAg LLC that includes: board membership, employment, and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109330.

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