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## Re-Evaluation of Soil Nitrogen Sampling Strategy Effects on Statistical Power

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### ABSTRACT

Soil sampling may be used as a decision-making tool for late-vegetative stage nitrogen (N) fertilizer applications in corn (*Zea mays* L.). Recommended sampling strategies following banded fertilizer applications commonly suggest taking cores from both on the fertilizer band (B) and off the band (O-B), however we hypothesized that soil nitrate concentrations ( $\text{NO}_3^-$  ppm) in the O-B were not influenced by N application rate. Analyzing samples from six experiments, we found there was a strong relationship between  $\text{NO}_3^-$  ppm and applied N rate in the B, but not the O-B position. Power analysis revealed that finding significant differences in applied N rates was only likely when sampling on the B and the difference in N rate was greater than  $110 \text{ kg N ha}^{-1}$ . This demonstrates that soil N sampling is not sensitive to small differences in applied N, and that O-B soil cores may only dilute the ability to detect these differences.

**Abbreviations:** B, on N fertilizer band; O-B, halfway between the corn row and the N fertilizer band;  $\text{NO}_3^-$  ppm, log-transformed nitrate-N concentration (ppm);  $\text{NH}_4^+$  ppm; ammonium-N concentration (ppm); D1, 0–30 cm depth; D2, 30–60 cm depth; C-220, contrast of N rates differing by 220  $\text{kg N ha}^{-1}$ ; C-110, contrast of N rates differing by 110  $\text{kg N ha}^{-1}$ ; C-55H, contrast of N rates differing by 55  $\text{kg N ha}^{-1}$  at high N rates; C-55L, contrast of N rates differing by 55  $\text{kg N ha}^{-1}$  at low N rates; A:N, ratio of non-transformed ammonium-N to nitrate-N concentrations; ON, unfertilized treatment; CV, coefficient of variation; SE, standard error.

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Banded fertilizer application; nitrogen; power analysis; soil N concentration; soil sampling

## Introduction

Banded fertilizer applications are common in modern corn (*Zea mays* L.) cropping systems, but determination of an appropriate soil sampling scheme that accurately assesses soil nutrient availability in and surrounding the band is inherently difficult. The challenge of sampling in banded fertilizer applications is the drastic increase in the heterogeneity of applied nutrients across an agricultural field perpendicular to the fertilizer band. Previous literature has resulted in several recommended strategies for soil sampling in banded applications of nitrogen (N) (Blackmer, Voss, and Mallarino 1997; Brouder and Mengel 2003; Clay et al. 1995; Mahler and Tindall 1994; Zebarth et al. 1999), phosphorus (P) (Fernández and Schaefer 2012; James and Hurst 1995; Kitchen, Westfall, and Havlin 1990; Stecker and Brown 2001), or organic fertilizers (Assefa and Chen 2007; Tewolde et al. 2013). Although some authors suggested random sampling (Mahler 1990) or systematic sampling that avoids the fertilizer band (Clay et al. 1995; Mahler and Tindall 1994), most researchers proposed a specified ratio of on-the-band (B) and off-the-band (O-B) soil cores per composite

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sample ranging from as many as 1:20 (Kitchen, Westfall, and Havlin 1990) to as few as 1:1 (Zebarth et al. 1999). These systematic composite samples typically follow a transect perpendicular to the fertilizer band where the soil cores are taken at even intervals. A modification of this method was promoted by Blackmer, Voss, and Mallarino (1997) in which the soil cores are taken from predetermined distances from the band, but are collected from random locations within the field or plot. Several researchers have also suggested that a continuous slice of soil perpendicular to the fertilizer band would be ideal; however, most concede the necessary equipment is impractical and therefore this method is unlikely to become widely adopted (Ashworth, Crépin, and Repski 1994; Mahler and Tindall 1994; Tewolde et al. 2013).

An important consideration in choosing a soil sampling strategy in banded fertilizer applications is deciding what parameter should represent the “true” average soil test value. Definitions of “true” soil test values (primarily for N or P) have included: average of all cores taken from a transect (Charlesworth 1996; Zebarth et al. 1999), fitting an exponential decay function and considering values of the integrated exponential equations with sampling distance (Kitchen, Westfall, and Havlin 1990), soil test values of a broadcast fertilizer application (Fernández and Schaefer 2012), or the normalized value of the N content in a given core in the transect to the N content in the entire transect-slice (Clay et al. 1995). Additionally, several studies have chosen sampling strategies based on minimizing required core number for a given confidence level and precision (Assefa and Chen 2007; Clay et al. 1997; James and Hurst 1995; Stecker and Brown 2001). These various definitions of “true” soil test value demonstrate that it is very difficult, if not impossible, to estimate the total nutrient availability in the soil for uptake by a growing crop when banded fertilizers have been applied recently without subsequent tillage operations.

Current equipment technologies can supply N fertilizer to corn during the late-vegetative or early-reproductive growth stages which may improve N recovery efficiency relative to traditional sidedress application timing (Mueller et al. 2017). Therefore, there is increased interest in soil sampling during the growing season to determine mineral N still available from prior basal fertilizer application(s). Estimation of N availability is particularly difficult because of its mobility in the soil in the nitrate ( $\text{NO}_3^-$ ) form (Harmsen and Kolenbrander 1965). The distribution of  $\text{NO}_3^-$  concentration ( $\text{NO}_3^-$  ppm) with time, depth, and distance from the fertilizer band is complex (Zebarth et al. 1999), further adding to the difficulty of identifying accurate soil sampling strategies. The movement of  $\text{NO}_3^-$  from the fertilizer band is primarily vertical below the applied band (Clay et al. 1995; Nelsen 1953). Lateral movement also occurs and has been reported as less than 10–15 cm (Nelsen 1953; Omonode and Vyn 2013; Terry and McCants 1974) under field conditions.

The movement and quantity of  $\text{NO}_3^-$  is of greater interest than ammonium ( $\text{NH}_4^+$ ) because the rapid nitrification of  $\text{NH}_4^+$  under field conditions during the growing season results in  $\text{NO}_3^-$  being the dominant source of inorganic N accumulation in corn (Magdoff 1991). Additionally, N in the  $\text{NH}_4^+$  form has a much lower risk of loss via leaching because its positive charge allows it to adhere to the cation exchange sites in the soil, reducing its mobility. Given warm soil temperatures and adequate moisture, spring-banded N in the form of either anhydrous ammonia or urea-ammonium nitrate solution (UAN) is typically completely converted to  $\text{NO}_3^-$  within 4 weeks after application (Omonode and Vyn 2013; Touchton, Hoelt, and Welch 1978).

The most commonly recommended sampling depth for soil N status is 0–30 cm (Blackmer, Voss, and Mallarino 1997; Brouder and Mengel 2003; Magdoff 1991; Mahler and Tindall 1994). Studies investigating the appropriate sampling depth for the pre-sidedress N test (PSNT) have concluded that the  $\text{NO}_3^-$  ppm in the 0–30 cm depth is reflective of the deeper 30–60 cm depth and, therefore, adequate when corn is 15 to 30 cm tall (Blackmer et al. 1991; Brouder and Mengel 2003). Others have found poor correlations among the  $\text{NO}_3^-$  ppm values at the depths of 0–30, 30–60, and 60–90 cm (Ehrhardt and Bundy 1991; Ferrera et al. 2003). Blackmer et al. (1989) reported that sampling only to 0–15 cm instead of 0–30 cm increased variability and, therefore, suggested 0–30 cm as a more appropriate sampling depth.

Previous research on sampling strategies following banded N fertilizer applications have focused on a given strategy's ability to reflect true "average"  $\text{NO}_3^-$  ppm despite the presence of highly concentrated N fertilizer bands. However, the interpretation of "average"  $\text{NO}_3^-$  ppm available to corn roots is questionable given the extreme soil heterogeneity resulting from recent banded fertilizer applications. We suggest there is more to be gained by focusing on the bands themselves. Theoretically, an ability to detect soil  $\text{NO}_3^-$  ppm differences in bands from previously-applied N fertilizer rates should assist in soil N status determination for decision making about whether further late-season N applications are warranted. This study was conducted to test the hypotheses that: 1) O-B soil  $\text{NO}_3^-$  ppm are independent of  $\text{NO}_3^-$  ppm in the fertilizer band, but these two positions become more homogenous over the growing season, and 2) significant differences in applied fertilizer rates are more detectable when sampling only in the B position. The ability to detect known differences in applied N rates serves as a proxy for increasing our understanding of how sampling position affects the interpretation and sensitivity of soil N sampling to inform in-season N application decisions.

## Materials and methods

### Field experiments

Field experiments were conducted at six sites in Northwestern, West-Central, and East-Central Indiana in 2016. Management varied across sites (Table 1), but all experiments included at least two N fertilizer rates and all N fertilizer was band-applied. Corn was planted at moderate plant densities ( $\sim 8$  plants  $\text{m}^{-2}$ ) in 76-cm wide rows at all sites and most experiments used starter fertilizer placed 5 cm to the side and 5 cm below the seed. Dates of soil N sampling following banded sidedress N application and number of treatments sampled varied according to treatments specific to each experiment. Either three or four replications were sampled in each experiment at each sampling date. The soil type of each site was obtained using USDA Web Soil Survey (<https://websoilsurvey.sc.egov.usda.gov>).

Composite soil samples consisted of 12–15 cores taken either on the B or O-B. The O-B position was half-way between the fertilizer band and the corn row, approximately 19 cm from the B sampling position. Collecting the O-B samples 19 cm from the corn row ensured that the starter

**Table 1.** Soil type, crop rotation, N rates, N source, time of N applications, time of soil sampling, number of composite samples per soil sampling date, and cumulative precipitation following N application in 6 experimental sites in 2016. C-S, corn-soybean rotation; C-C, continuous corn; UAN, 28% urea anhydrous ammonia solution; DAP, days after planting; DAA, days after N application; D1, sampling depth of 0–30 cm; D2, sampling depth of 30–60 cm.

Site	Soil Type	Crop Rotation	N Rates ( $\text{kg ha}^{-1}$ )	N Source	N Timing (DAP)	Soil Sampling (DAA/DAP)	Sample Number (D1/D2)	Cum. Precip. (mm)
1	Tracy Sandy Loam	C-S	0, 110, 155, 200, 245	UAN	V5 (41)	V10 (22/63)	V10 (30/0)	V10 (28)
2	Gilford Fine Sandy Loam	C-C	0, 110, 165, 220, 275	UAN	Pre- Emergence (1)	V11 (63/66) VT (77/78) R2 (92/93) R6 (167/168)	V11 (40/40) VT (30/30) R2 (30/30) R6 (40/40)	V11 (196) VT (225) R2 (312) R6 (504)
3A	Drummer	C-S	0, 220	UAN	V3 (30)	V10 (46/76)	V10 (46/46)	V10 (159)
3B	Drummer	C-C	0, 220	UAN	V3 (30)	V10 (46/76)	V10 (46/46)	V10 (159)
4	Chalmers Silty Clay Loam	C-S	0, 55, 165, 220	UAN	V4 (21)	R1 (41/62) R6 (118/139)	R1 (32/32) R6 (16/16)	R1 (211) R6 (507)
5	Pewamo Silty Clay Loam	C-S	77, 132, 187	UAN	V3 (13)	V10 (28/41) R6 (123/136)	V10 (24/24) R6 (24/24)	V10 (76) R6 (486)
6	Brookston Silty Clay	C-S	121, 165, 209, 253	Anhydrous Ammonia	V2 (21)	V11 (29/50) R6 (124/145)	V11 (32/32) R6 (32/32)	V11 (163) R6 (503)

fertilizer band (placed 5 cm from the corn row) was not sampled. All sites were sampled to two depths: 0–30 cm (D1) and 30–60 cm (D2) except Site 1 where only D1 was sampled. Samples were collected with standard 2 cm inner-diameter soil probes. After collection, all samples were air-dried, ground, and analyzed for exchangeable  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Site 2 was analyzed by a commercial lab (Solum Laboratory, Ames, IA). All other sites were analyzed by extracting a 2 g subsample using 20 mL of 2 M potassium chloride and a 1 hour shaking time. Concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were determined color metrically with a Discrete AQ2 Analyzer (SEAL Analytical, Mequon, Wisconsin).

### Statistical analysis

All  $\text{NO}_3^-$  and  $\text{NH}_4^+$  values were transformed to the common logarithm for statistical analysis. All data and results are presented as log-transformed values unless otherwise noted. Correlation between B and O-B samples, as well as the relationship between B or O-B  $\text{NO}_3^-$  ppm and applied N rate, were determined using R Studio (R Development Core Team 2014). Descriptive statistics were obtained using the R package “psych”.

To determine whether significant differences in  $\text{NO}_3^-$  ppm could be detected among different levels of N application between V10 and R1 corn growth stages, four contrasts, which could be applied to most experiments, were tested: a difference in applied N rate of about 220 (C-220), 110 (C-110), or 55 kg N ha<sup>-1</sup> (actual rates presented in Tables 2 and 3). The contrast of 55 was further divided into 55 high (C-55H) and 55 low (C-55L) in order to investigate whether the ability to detect differences among N treatments differed between low and high N rates. To avoid duplication, only the first sampling date from site 2 (63 days after N application) was used in these comparisons. Use of contrasts allowed for comparison across sites despite each site having unique N rates. All references to N rate refer only to the rate applied in a single, banded N application in the form and timing described in Table 1.

A linear mixed effects model was fitted to the data from each site separately using PROC MIXED in SAS 9.3 (SAS Institute, 2012). At all sites except Site 3, N rate, sampling position, and sampling

**Table 2.** Summary of contrasts for D1 (0–30 cm). C-220 and C-110 represent contrasts with differences in N rate of about 220 or 110 kg N ha<sup>-1</sup>, respectively. C-55H and C-55L represent contrasts with a difference of 55 kg N ha<sup>-1</sup> using high and low total N rates, respectively. Effect Size, difference in the means (log[NO<sub>3</sub><sup>-</sup> concentration]); SE, standard error; Df, degrees of freedom; Critical Effect Size, effect size where Power > 0.8; Sig, significance indicated by \* when p < 0.05, \*\* when p < 0.01, \*\*\* when p < 0.001, and ns when p > 0.05.

Site	Contrast	N Rates	Band				Off-Band					
			EffectSize	SE	Df	Sig	Effect Size	SE	Df	Sig	Critical Effect Size	
1	C-220	245–0	1.16	0.1	18	***	0.30	0.11	0.1	18	ns	0.30
	C-110	245–110	0.16	0.1	18	ns	0.30	–0.05	0.1	18	ns	0.30
	C-55H	245–200	0.06	0.1	18	ns	0.30	–0.13	0.1	18	ns	0.30
	C-55L	155–110	0.05	0.1	18	ns	0.30	–0.10	0.1	18	ns	0.30
2	C-220	220–0	1.24	0.1	48	***	0.30	0.40	0.14	53	**	0.45
	C-110	220–110	0.76	0.13	48	***	0.30	0.35	0.20	53	ns	0.45
	C-55H	220–165	0.37	0.1	48	***	0.30	0.13	0.14	53	ns	0.45
	C-55L	165–110	0.39	0.13	48	**	0.30	0.22	0.20	53	ns	0.45
3A	C-220	220–0	0.58	0.07	8	***	0.25	0.27	0.18	15	ns	0.55
3B	C-220	220–0	1.51	0.20	9	***	0.65	0.38	0.10	15	**	0.30
4	C-220	220–0	1.05	0.09	11	***	0.30	0.16	0.16	12	ns	0.50
	C-110	165–55	0.52	0.09	11	***	0.30	–0.11	0.16	12	ns	0.50
	C-55H	220–165	0.28	0.09	11	**	0.30	0.13	0.16	12	ns	0.50
5	C-55L	55–0	0.25	0.09	11	*	0.30	0.13	0.16	12	ns	0.50
	C-110	187–77	0.05	0.14	9	ns	0.45	0.23	0.14	9	ns	0.50
	C-55H	187–132	–0.01	0.14	9	ns	0.45	0.07	0.14	9	ns	0.50
6	C-55L	132–77	0.06	0.14	9	ns	0.45	0.16	0.14	9	ns	0.40
	C-110	253–121	3.85	1.06	11	**	3.25	1.72	0.44	6	**	1.50
	C-55H	253–209	1.83	1.06	11	ns	3.25	1.26	0.44	6	*	1.50
	C-55L	165–121	1.76	1.03	11	ns	3.25	0.94	0.44	6	ns	1.50

**Table 3.** Summary of contrasts for D2 (30–60 cm). C-220 and C-110 represent contrasts with differences in N rate of about 220 or 110 kg N ha<sup>-1</sup>, respectively. C-55H and C-55L represent contrasts with a difference of 55 kg N ha<sup>-1</sup> using high and low total N rates, respectively. Effect Size, difference in the means (log[NO<sub>3</sub><sup>-</sup> concentration]); SE, standard error; Df, degrees of freedom; Critical Effect Size, effect size where Power > 0.8; Sig, significance indicated by \* when p < 0.05, \*\* when p < 0.01, \*\*\* when p < 0.001, and ns when p > 0.05.

Site	Contrast	N Rates	Band					Off-Band				
			Effect Size	SE	Df	Sig	Critical Effect Size	Effect Size	SE	Df	Sig	Critical Effect Size
1	-											
2	C-220	220-0	0.33	0.10	53	***	0.30	0.39	0.12	53	**	0.35
	C-110	220-110	0.33	0.14	53	*	0.30	0.12	0.17	53	ns	0.35
	C-55H	220-165	0.15	0.10	53	ns	0.30	0.15	0.12	53	ns	0.35
	C-55L	165-110	0.18	0.14	53	ns	0.30	-0.03	0.17	53	ns	0.35
3A	C-220	220-0	1.07	0.23	7	**	0.80	0.11	0.16	15	ns	0.55
3B	C-220	220-0	0.87	0.34	6	*	1.20	0.09	0.07	12	ns	0.25
4	C-220	220-0	0.70	0.15	12	***	0.50	0.25	0.09	6	*	0.30
	C-110	165-55	0.27	0.15	12	ns	0.50	0.12	0.09	6	ns	0.30
	C-55H	220-165	0.34	0.15	12	*	0.50	-0.04	0.09	6	ns	0.30
	C-55L	55-0	0.10	0.15	12	ns	0.50	0.17	0.09	6	ns	0.30
5	C-110	187-77	0.45	0.14	9	*	0.50	0.58	0.22	9	*	0.70
	C-55H	187-132	0.19	0.14	9	ns	0.50	0.37	0.22	9	ns	0.70
	C-55L	132-77	0.26	0.14	9	ns	0.50	0.21	0.22	9	ns	0.70
6	C-110	253-121	1.58	0.44	2	ns	2.65	0.10	0.54	6	ns	1.85
	C-55H	253-209	0.92	0.44	2	ns	2.65	0.40	0.54	6	ns	1.85
	C-55L	165-121	0.03	0.44	2	ns	2.65	-0.30	0.54	6	ns	1.85

depth were considered main effects and block was considered a random effect. Site 3 was a long-term crop rotation and tillage experiment that included both continuous corn and corn-soybean rotation. In order to avoid the potentially confounding effect of crop rotation, this site was split into Site 3A (corn-soybean) and Site 3B (continuous corn) for statistical analysis. There were also four tillage systems present (moldboard plow, chisel plow, no-till, and strip till). Tillage was included as a fixed effect in addition to N rate, sampling position, and sampling depth; however, the tillage main effect was not significant in either the corn-soybean rotation ( $p$ -value = 0.76) or continuous corn ( $p$ -value = 0.57). Therefore, data for this site is presented as the mean of all tillage systems. At all other sites, there were no sub-treatments sampled.

For each sampling position, the NO<sub>3</sub><sup>-</sup> ppm values were modeled as repeated measures on depth with the unstructured variance-covariance matrix. The option GROUP = sample position was specified, which allowed for different covariances at different positions, resulting in unique contrast standard error and degrees of freedom for each depth x position combination within each site. Contrasts were tested by site using LSMESTIMATE statement of PROC MIXED. Power curves for  $t$ -tests were then calculated using the standard error and degrees of freedom from each site x depth x position contrast using methods similar to those described by Littell et al. (2006).

## Results

### Overview of the data

The final data set included 285 samples collected during the 2016 growing season and 112 samples collected at or after corn physiological maturity. Because movement of inorganic N in the soil is complex and impacted by many soil and environmental factors, it is beyond the scope of this research to postulate what the ideal soil-sampling depth is and, therefore, D1 and D2 will be discussed separately unless otherwise noted.

Measured NH<sub>4</sub><sup>+</sup> ppm's were much smaller compared to NO<sub>3</sub><sup>-</sup> ppm. The median of the untransformed ratio of NH<sub>4</sub><sup>+</sup> ppm to NO<sub>3</sub><sup>-</sup> ppm (A:N) was similar between the 0N and non-0N

treatments when averaged over sites and depths except for B samples during the growing season (Table 4). The similarity of the A:N between unfertilized (0N) and fertilized treatments indicates that the levels of  $\text{NH}_4^+$  in the unfertilized treatments were representative of background soil  $\text{NH}_4^+$  ppm and were not highly influenced by N fertilizer application. The lower A:N observed in B samples during the growing season indicated that the majority of the applied N fertilizer was in the  $\text{NO}_3^-$  form, regardless of the dominant form at the time of application. Therefore, our analysis focuses only on  $\text{NO}_3^-$  ppm.

Averaged over all sites and depths,  $\text{NO}_3^-$  ppm's in the B position were more than five-fold higher in the fertilized compared to the unfertilized (0N) treatments during the growing season, and were still more than double post-harvest (Table 4). In contrast, soil  $\text{NO}_3^-$  ppm's in the O-B position with N fertilizer application were only 30–50% greater than those with 0N both in-season and post-harvest (Table 4).

Because standard deviation is influenced by sample number and mean, and the values of the latter varied widely within each group (Table 4), the impacts of N treatment, time of sampling, and position on standard deviation were compared using coefficient of variation (CV) and standard error (SE). Although there was a large range in CV for both B and O-B, there was no clear pattern of CV impacts resulting from sampling position. There was also little difference among SE when comparing B and O-B for each N group (fertilized or unfertilized) either in-season or post-harvest (Table 4).

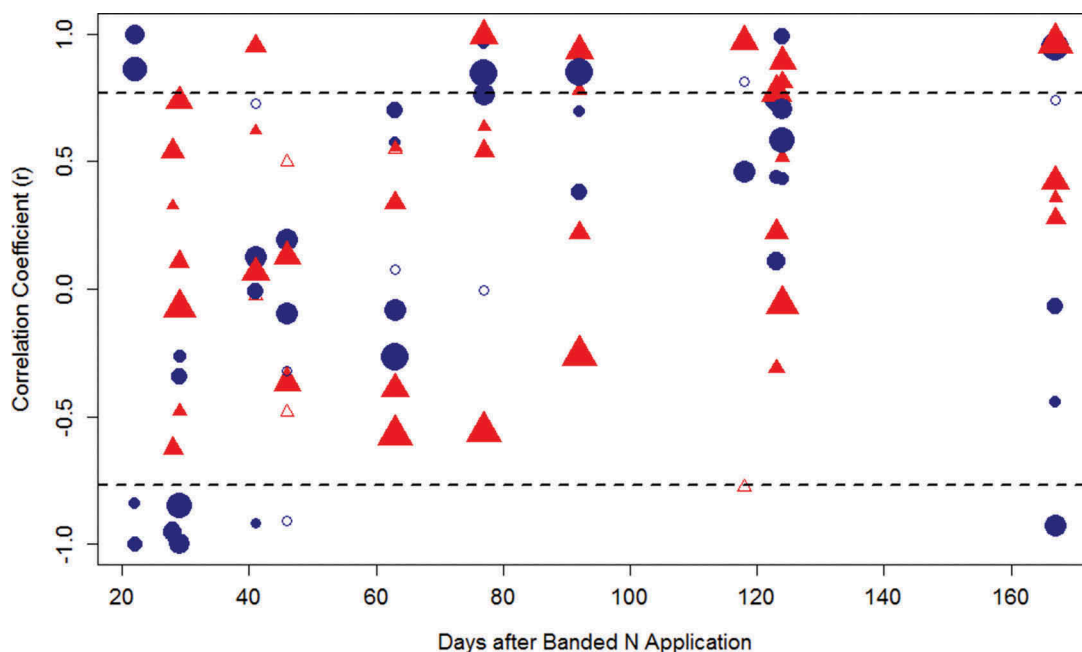
### Relationship between band and off-band

In order to test the hypothesis that O-B is not influenced by the applied N in the B position, the Pearson correlation ( $r$ ) of B and O-B was calculated for each site  $\times$  date  $\times$  depth  $\times$  N rate combination. It stands to reason that if O-B is independent of B, they will be uncorrelated. Additionally, we hypothesized that the O-B to B correlation would increase over time as the N fertilizer dispersed out of the band and/or was accumulated by the growing crop. Correlation coefficients of  $r \geq \pm 0.77$ , equivalent to  $R^2 = \pm 0.6$ , were considered highly correlated. Among the non-0 N rates within D1, 17 of the 40 site  $\times$  date  $\times$  depth  $\times$  N rate combinations (43%) achieved  $r \geq \pm 0.77$  (Figure 1). Depth 2 had less correlation with only 8 out of 36 combinations (22%) above the threshold of  $r \geq \pm 0.77$ . Furthermore, there was no pattern of  $r$  changing over time or evidence of  $r$  values being related to N rate at either sampling depth (Figure 1). Surprisingly, the relationship between B and O-B was also poor for the 0N treatments; only 3 out of 8 combinations were highly correlated at both D1 and D2 (Figure 1).

To further explore the degree to which O-B versus B sampling reflected applied N rates,  $\text{NO}_3^-$  ppm was regressed against the applied N rate for the earliest sampling date from each site, representing corn growth stages V10-R1 (only V12 was used from site 2) (Figure 2). The N rates used in this analysis include only the N that had been applied in the fertilizer band. The strong relationship between  $\text{NO}_3^-$  ppm and applied N rate in the B samples, but not the O-B samples, again

**Table 4.** Summary statistics for Band and Off-Band log-transformed  $\text{NO}_3^-$  concentrations. In-season refers to all samples collected prior to corn maturity, post-harvest includes all samples collected after corn maturity. SE, standard error; CV, coefficient of variation; IQR, inter-quartile range; A:N, median of the un-transformed ratio of ammonium-N to nitrate-N concentrations.

	Sampling Time	N		Mean	Median	SE	CV	IQR	A:N
		Treatment	N						
Band	In-Season	Unfertilized	50	0.22	0.24	0.07	2.27	0.55	0.31
	In-Season	Fertilized	235	1.25	1.27	0.03	0.42	0.84	0.15
	Post-Harvest	Unfertilized	16	0.34	0.41	0.11	1.24	0.39	0.24
	Post-Harvest	Fertilized	96	0.72	0.73	0.03	0.47	0.40	0.35
Off-Band	In-Season	Unfertilized	59	0.29	0.28	0.06	1.55	0.48	0.26
	In-Season	Fertilized	229	0.57	0.58	0.03	0.79	0.56	0.21
	Post-Harvest	Unfertilized	15	0.38	0.38	0.07	0.74	0.40	0.31
	Post-Harvest	Fertilized	96	0.55	0.53	0.03	0.53	0.38	0.37



**Figure 1.** Pearson correlation coefficient ( $r$ ) between Band and Off-Band  $\text{NO}_3^-$  concentrations for each site-sampling date-depth-N rate combination. Open circles and open triangles represent ON treatments at D1 (0–30 cm) and D2 (30–60 cm), respectively. Filled circles and triangles represent non-ON treatments at D1 and D2, respectively. Symbol size reflects N rate with larger symbols denoting larger N rates. Dotted black line represents  $r = \pm 0.77$ .

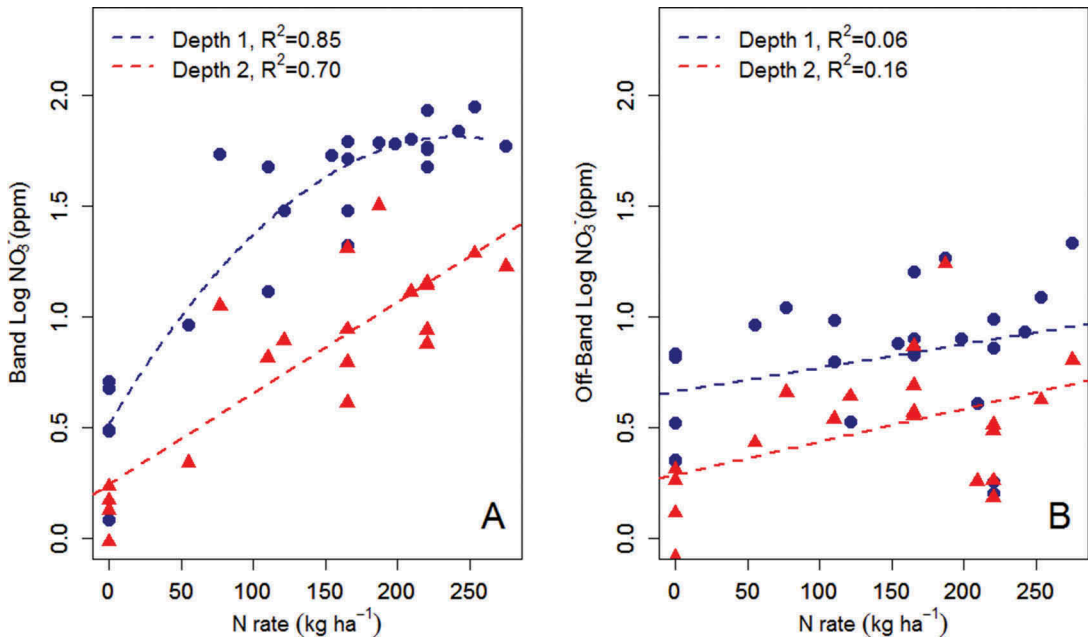
supports the hypothesis that O-B is independent of applied N rate. Band  $\text{NO}_3^-$  ppm increased with increasing N rate in either a quadratic (D1,  $R^2 = 0.85$ ,  $p$ -value < 0.001) or linear (D2,  $R^2 = 0.70$ ,  $p$ -value < 0.001) (Figure 2A) fashion, but there was no relationship between  $\text{NO}_3^-$  ppm and N rate in the O-B samples (D1,  $R^2 = 0.06$ ,  $p$ -value = 0.12 and D2,  $R^2 = 0.16$ ,  $p$ -value = 0.04, Figure 2B). The quadratic (and not linear) relationship between N rate and the B  $\text{NO}_3^-$  ppm in D1 was likely caused by the complex relationship between  $\text{NO}_3^-$  movement down the soil profile combined with the simultaneous crop N accumulation.

### **Impact of sampling position on statistical power**

In the present experiment, contrasts were used at a significance level of  $\alpha = 0.05$  to demonstrate whether significant differences in applied N rate could be detected during the growing season. As emphasized by Hoenig and Heisey (2001), there is little value in post-hoc power calculations because observed power has a 1:1 relationship with the  $p$ -value. However, by treating the present data set as a pilot study to inform future research, we can use power analysis to understand the impact of sampling strategy on the likelihood of detecting significant differences in  $\text{NO}_3^-$  ppm among different applied N rates. In order to analyze how often B and O-B samples were able to detect significant differences in applied N rates, four contrasts, which could be applied to most experiments, were explored.

At D1, sites 1–4 showed similar responses in effect size (the difference in treatment means) and, as expected, effect size decreased as the difference in applied N rate decreased (Table 2). Site 6 followed a similar pattern, but the effect sizes were much larger ranging from 0.9 to 3.8 compared to a range of 0.1–1.5 for sites 1–4. At all sites except Site 5, the effect size was consistently larger for B than O-B. At the Site 5 exception, the effect size was larger for O-B than B, and there was no relationship between effect size and difference in N rate. This is likely explained by the fact that at Site 5, 55 kg N ha<sup>-1</sup> of a 28% UAN source was broadcast-applied uniformly across the field prior to planting, increasing the  $\text{NO}_3^-$  ppm in the





**Figure 2.** Relationships between applied N rate and NO<sub>3</sub><sup>-</sup> concentrations in the Band (A) and Off-Band (B) positions for the first sampling date at all sites. Regression equations for A are Depth 1  $y = 0.52 + 0.01x - 0.00002x^2$ ,  $p$ -value < 0.001; Depth 2  $y = 0.24 + 0.004x$ ,  $p$ -value < 0.001. Regression equations for B are Depth 1  $y = 0.66 + 0.001x$ ,  $p$ -value = 0.12; Depth 2  $y = 0.33 + 0.001x$ ,  $p$ -value = 0.04.

O-B position. Depth was also important: effect size was almost always (i.e. with exception of Site 5 and 3A in the B position) smaller at D2 compared to D1 (Tables 2 and 3).

Generalizing across sites and depths, significant differences were detected in 100% of cases for C-220 and in 6 out of 9 (67%) cases for C-110 when sampling in the B position (Table 2). The ability to detect differences among N rates was lowered when sampling O-B and only 45% (5 out of 11) and 22% (2 out of 9) of contrasts found significant differences for C-220 and C-110, respectively. Contrasts with only a difference of 55 kg N ha<sup>-1</sup> were unlikely to detect significant differences at either depth or position (Tables 2 and 3). Furthermore, there was no apparent influence of low versus high N rates on effect size for the C-55H and C-55L contrasts.

The observed pattern of B position sampling being more likely to detect significant differences in applied N rates, but only when large differences of 220 or 110 kg N ha<sup>-1</sup> are present, can be explained through power analysis. For all sites (excluding site 6), the critical effect size needed to reach a power of 0.8 at D1 ranged from 0.25 to 0.65, with a median of 0.45. At D2 there was a greater range of critical effect sizes (0.25–1.2) but the median was similar at 0.43. There was little difference in critical effect size among the positions because of the minor differences in sample size or standard error (Tables 2 and 3). As previously mentioned, Site 6 had much higher standard errors, effect sizes, and critical effect sizes. This may be attributed to both high spatial variability found in that experiment as well as the use of anhydrous ammonia as the N source. Critical effect size for site 6 ranged from 1.50 to 3.25.

## Discussion

The lack of correlation for NO<sub>3</sub><sup>-</sup> ppm between the B and O-B positions (Figure 1), as well as the strong relationship between N rate and B, but not O-B sampling (Figure 2), suggests that O-B is not influenced by applied N and therefore does not provide useful information about how much N fertilizer is present in the soil during the growing season. However, the hypotheses that correlation

between B and O-B would increase with days after N fertilizer application, and that B and O-B samples would be highly correlated in 0N plots, proved incorrect. The lack of influence of either N rate, time of sample collection, or days after N application on the correlation between B and O-B  $\text{NO}_3^-$  ppm emphasizes the additional challenges, beyond the ever-present spatial variability, of accurately describing the ever-changing soil N pools following banded N applications in actively growing corn.

Using these data as a pilot study, we can increase our understanding of how sampling position affects the likelihood of detecting differences among N application rates. Power analysis is usually promoted for determining the necessary sample number to achieve a desired level of statistical power. Statistical power for a given test at a specified significance level is influenced by expected effect size and sample size. Because, all else being equal, greater sample size results in lower standard error, standard error may also be considered a factor in determining power. If we assume that we are not willing to increase sample size because soil sampling is laborious and time-consuming, we can instead use power analysis to determine if our sampling strategy will yield the necessary effect size to allow detection of different N rates, and therefore, provide useful information for in-season N fertilizer decisions. Our data indicate a critical effect size of approximately 0.45 given the standard errors observed in Sites 1–5. The high critical effect size found for Site 6 points to the negative implications of high spatial variability and may also indicate that the sampling strategies need to be unique to the source of banded N fertilizer used (UAN versus anhydrous ammonia).

Because there was little influence of sampling position on the standard error of the contrasts (Tables 2 and 3), and given our stated assumption that we are not willing to increase sample number, the only way to increase power through sampling strategy is to maximize effect size. As demonstrated in Tables 2 and 3, large enough effect sizes to result in significant contrasts only occurred consistently when sampling on the B and only under very large differences in applied N rates (C-220 and C-110).

We readily acknowledge that limiting soil  $\text{NO}_3^-$  ppm sampling to the B position alone drastically overestimates the  $\text{NO}_3^-$  ppm in the entire rooting zone. It is for this reason sampling strategies that include both B and O-B positions have been the most commonly recommended in previous research (Blackmer, Voss, and Mallarino 1997; Brouder and Mengel 2003; Kitchen, Westfall, and Havlin 1990; Zebarth et al. 1999). However, using the present data set as a pilot study, we have demonstrated that there was little influence of position (B or O-B) on standard error, on the steepness of the power curve, and therefore, on the critical effect size. If sampling position does not influence the factors that determine critical effect size, composite samples that include both B and O-B soil cores can reasonably be expected to have similar critical effect sizes to those demonstrated in this research. If critical effect size remains near 0.45 regardless of sampling strategy, including O-B samples will only “dilute” the  $\text{NO}_3^-$  ppm found in the B, decreasing the ability to detect differences in  $\text{NO}_3^-$  ppm due to N fertilizer rate. In this research, we used the ability to detect known differences in applied N rate as a proxy of the overall sensitivity of soil N sampling and how sampling position may affect interpretation.

In conclusion, if interested in sampling in-season for the purpose of making decisions about the potential effectiveness of additional N application, sampling on the B is superior to O-B or, by extension, composite samples that include both B and O-B soil cores. Additionally, significant differences are unlikely to be detected when true differences in applied N rate are less than  $110 \text{ kg N ha}^{-1}$ . Given these limitations of in-season soil N sampling in banded fertilizer applications, future research should focus on plant-based methodologies for determining additional crop N needs near late-vegetative growth stages.

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