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Nitrogen source affects in-season nitrogen availability more than nitrification inhibitor and herbicide in a fine-textured soil

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Abstract

Nitrogen (N) fertilizer management continues to be challenging due to potential nitrogen losses under variable weather conditions. This study aimed to evaluate the performance of nitrification inhibitors, nitrogen sources, and herbicides on in-season nitrogen availability and agronomic indicators. A 2 site-year field experiment was conducted in silty-clay loam soil in the maize (Zea mays L.) phase of a maizesoybean rotation in Central Nebraska. The study included two herbicide treatments (Acuron and Resicore) and four nitrogen treatments: (1) anhydrous ammonia with a nitrification inhibitor, (2) anhydrous ammonia without a nitrification inhibitor, (3) urea with a nitrification inhibitor, and (4) urea without a nitrification inhibitor. Nitrogen sources had a more significant effect on NH₄⁺-N retention (300%–340% higher in anhydrous ammonia vs. urea) than nitrification inhibitors (14%-50% higher with inhibitor vs. without inhibitor) and herbicides. Similarly, nitrogen sources significantly affected NO₃⁻-N formation (58%–64% lower in anhydrous ammonia vs. urea) compared with nitrification inhibitors (7%-27% lower with inhibitor vs. without inhibitor) and herbicides. Nitrification inhibitors did not affect agronomic indicators. However, anhydrous ammonia increased grain yield by 1.1 Mg ha⁻¹, partial factor productivity by 6 kg grain kg⁻¹ N, agronomic efficiency by 5.5 kg grain kg⁻¹ N, aboveground biomass N-uptake by 34 kg N ha⁻¹, grain N-uptake by 15 kg N ha^{-1} , nitrogen recovery efficiency by 33%, and residual total inorganic N by 6– 40 kg N ha⁻¹ compared to urea. These findings suggest that using the right fertilizer source, followed by nitrification inhibitor and herbicide, can be an effective strategy for conserving nitrogen and improving nitrogen use efficiency in maize.

INTRODUCTION 1

Balancing maize (Zea mays L.) nitrogen requirements while maintaining proper stewardship of land, air, and water resources is one of the major challenges facing maize producers in Nebraska. Though the nitrogen use efficiency (NUE) of maize production in Nebraska has continuously improved over time (Ferguson, 2015), there remain significant challenges in managing nitrogen. Only one-third to half of N fertilizer input is recovered in the harvested product (Morris et al., 2018; Mueller et al., 2017), while the unrecovered N is lost to air and water resources. The unrecovered N

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Abbreviations: NUE, nitrogen use efficiency; AA, anhydrous ammonia.

losses cause a range of environmental problems, such as water contamination, biodiversity loss, and greenhouse gas emissions. These N losses are evidenced in groundwater nitrate-N concentrations frequently exceeding the 10-ppm EPA drinking water standard in several wellhead protection areas of Nebraska (Nebraska Department of Environmental Quality, 2018). This higher level of nitrate leaching to groundwater is likely due to poor synchrony between N fertilizer applications and crop N demand, excessive nitrogen inputs, and heavy rainfall events during spring fallow periods. Nitrogen losses to groundwater tend to be greatest during wet or warm conditions during the April-June period, when soil nitrate is present without actively growing crops (Bowles et al., 2018). Thus, N applied before maize planting can be lost either to groundwater through nitrate leaching or to air through gaseous emissions. Therefore, protection of nitrogen input is needed to reduce nitrogen losses during early spring.

One strategy to reduce potential nitrogen losses after fertilizer nitrogen application is the use of nitrification inhibitors. These chemical compounds slow down soil nitrification, a biological process responsible for the transformation of ammonium (NH4⁺-N) to nitrate (NO3⁻-N) (Martens-Habbena et al., 2009). The NO₃⁻-N form is volatile and can easily be lost through either denitrification or nitrate leaching if not intercepted by crop roots. Therefore, decreasing nitrification is an important practice to reduce nitrogen losses during the early season (Yu et al., 2018). Numerous studies have tested the effect of nitrification inhibitors on nitrogen losses and crop nitrogen uptake (Cahill et al., 2007, 2010; Noellsch et al., 2009; Wang & Alva, 1996). Nitrapyrin [2chloro-6-(trichloromethyl)-pyridine] is one compound that has been successfully used to reduce nitrification and, thus, nitrogen losses (Wolt, 2000). However, the performance of nitrapyrin is affected by site-specific weather conditions (Maharjan et al., 2017).

Nitrogen fertilizer type and placement can also significantly affect the nitrification process. For example, anhydrous ammonia (AA), when injected in a band below the soil surface, can stabilize nitrogen and improve NUE. On the other hand, urea, the most commonly used fertilizer, has the potential to lead to substantial N losses through various nitrogen transformation pathways (e.g., ammonia volatilization, nitrification, and denitrification) when broadcast over the soil surface. Consequently, the performance of the surface broadcasted fertilizer N can substantially vary compared to in-band fertilizer N application (Touchton & Hargrove, 1982). However, the effects of conventional fertilizer nitrogen sources compared with nitrification inhibitors on soil nitrification have not yet been tested.

Chemical herbicides that are widely used to kill harmful weeds have also been confirmed to affect soil nitrification in previous studies (Li et al., 2008; Mahía et al., 2008; Zhang et al., 2018). These herbicides vary in their toxicity level to affect microorganisms and the rate of soil nitrification. Some

- Nitrogen source conserved nitrogen more than nitrification inhibitors and herbicides.
- Anhydrous ammonia retained four times higher soil NH₄⁺-N than urea.
- Nitrification inhibitors did not affect agronomic indicators and maize grain yield.
- Anhydrous ammonia improved agronomic indicators and maize grain yield more than urea.
- Right nitrogen source can improve NUE, followed by nitrification inhibitors and herbicides.

of these are more toxic than others to nitrifiers (Debona & Audus, 1970). For example, atrazine and acetochlor have been found to show inhibitory effects on nitrifying bacteria (Li et al., 2008; Mahía et al., 2008). Chen et al. (2015) found that atrazine has both stimulatory and inhibitory effects on soil nitrification. In another study, a higher level of atrazine inhibited nitrification, while a lower level of atrazine increased nitrification (Laursen & Carlton, 1999). In these studies, the effect of herbicides on nitrification has mainly been studied in laboratories; however, to our knowledge, only some studies have evaluated the effects of herbicides on nitrification at a field scale (e.g., Hernandez et al., 2011).

In this study, we aimed to compare the effect of nitrification inhibitors, nitrogen sources, and herbicides on nitrification, NUE, and crop yield. We hypothesized that the integrated use of nitrification inhibitor, nitrogen source, and herbicide can improve the synchronization of nitrogen release and crop nitrogen uptake and lead to greater crop yield and less potential nitrogen loss. Thus, the objective of this study was to compare the effect of nitrification inhibitors, herbicides, and nitrogen sources on in-season N availability, NUE, maize grain yield, and postharvest soil nitrogen.

2 | MATERIALS AND METHODS

2.1 | Experimental site

The experiment was conducted at the South-Central Agricultural Laboratory (SCAL; 40.540° N; 98.084° W; 538 m elevation) near Clay Center, Nebraska, over 2 years (2020 and 2021) on different sites each year. Both sites were located within 100 m of each other. The soil at both site-years was Hastings silt clay loam (*Udic Argiustoll*) with moderately well-drained to well-drained classification. The sites were under linear irrigation and no-till management. The sites have a sub-humid climate with a 20-year annual average

TABLE 1 Mean (± standard error) of selected soil chemical and physical properties of both site-years (2020 and 2021).

Property	2020	2021
Soil pH (1:1 soil to water)	6.1 ± 0.1	5.9 ± 0.1
Soil organic matter (%)	2.7 ± 0.03	3.2 ± 0.04
NO_3 -N (mg kg ⁻¹)	12 ± 1	6 ± 1
Mehlich-III P (mg kg ⁻¹)	50 ± 6.56	46.5 ± 4.09
Mehlich-III K (mg kg ⁻¹)	336 ± 10	332 ± 23
$SO_4 (mg kg^{-1})$	8 ± 1	9 ± 1
Ca (mg kg ⁻¹)	2400 ± 80	2100 ± 60
$Mg (mg kg^{-1})$	385 ± 25	272 ± 3
Na (mg kg ^{-1})	39 ± 5	37 ± 1
Sum of cations (me 100 g^{-1})	20.3 ± 0.8	18.3 ± 0.4
Soil texture	Silty clay loam	Silty clay loam

temperature of 10.38°C and average precipitation of 514.5 mm year⁻¹, with significant interannual variability.

Prior to treatment establishment, soil samples were taken at 0- to 20-cm soil depth to determine the basic chemical and physical properties of the soil. Specific soil properties for each site-year are listed in Table 1. Precipitation data were collected from the nearest weather station in the High Plains Regional Climate Center network.

2.2 | Experimental design and agronomic management

The experiment was a split-plot design with four replications. The main plot consisted of two preemergence herbicides and a no-preemergence herbicide plot, while the subplots contained four nitrogen treatments and a control with no nitrogen. Each subplot was 3-m wide by 15-m long. During each site-year, the treatments were applied in the maize phase of a maizesoybean rotation. Two herbicide treatments representing common preemergence herbicide programs for Nebraska growers were used: (1) Acuron (a premix of atrazine/bicyclopyrone/smetolachlor/mesotrione), and (2) Resicore (a premix of acetochlor/clopyralid/mesotrione). These preemergence herbicides were applied at 6.4 L ha⁻¹. Four nitrogen treatments with two nitrogen sources (AA and urea) with and without nitrification inhibitors were used as follows: (1) AA-I (anhydrous ammonia with nitrification inhibitor of N-serve); (2) AA-No (anhydrous ammonia without nitrification inhibitor of N-serve); (3) Urea-I (urea with nitrification inhibitor of Instinct or guardian DL); and (4) Urea-No (urea without nitrification inhibitor of Instinct or guardian DL). A control with no nitrogen was included in each set of subplot treatments. In each site year, the N-serve product from Corteva was used with AA at a rate of 2.6 L ha^{-1} . N-serve is a nitrification inhibitor product that contains nitrapyrin as an active ingredient to inhibit nitrification and improve NUE (Di & Cameron,

2016; Goring, 1962). Different nitrification products were used with urea during each year depending on product availability. In 2020, Guardian-DL was used to impregnate urea at the rate of $1.95 \text{ L} \text{ ha}^{-1}$, while in 2021, Instinct NXTGEN from Corteva was used at the recommended rate of $1.95 \text{ L} \text{ ha}^{-1}$. Guardian-DL is a nitrification inhibitor product that contains dicyandiamide (DCD) as an active ingredient, while Instinct NXTGEN contains nitrapyrin as an active ingredient to inhibit nitrification. AA with and without a nitrification inhibitor was injected below the soil surface at 15-cm depth between the corn rows at 76-cm spacing. The urea with and without a nitrification inhibitor was manually broadcasted over the soil surface.

All nitrogen treatments except the control received one application rate (168 kg N ha⁻¹ in 2020 and 169 kg ha⁻¹ in 2021) based on the University of Nebraska (UNL) nitrogen algorithm. All treatments, including herbicide and nitrogen application, occurred on the same day at each site year (April 23, 2020, and April 28, 2021). In 2020, the site received 0.5 in. of rain within 1 week following fertilizer application, while in 2021, 1 in. of irrigation was applied within 24 h following treatment application to incorporate urea and limit ammonia volatilization. Each year, monoammonium phosphate (MAP) at a rate of 152 kg ha⁻¹ was applied during the winter months to meet phosphorus demands of the maize crop, resulting in the addition of 18 kg N ha^{-1} in all plots. Maize with a 110day relative maturity (RM) was no-till planted into soybean residue at a targeted rate of 81,000 seed ha⁻¹ on April 23, 2020, and April 28, 2021. A postemergence (POST) herbicide application of a premix of dicamba/tembotrione at a rate of 1.6 L ha⁻¹ was made on all plots (June 13, 2020, and June 1, 2021) to reduce weed uptake of N and impact on maize yield. Each year, maize was irrigated based on the soil moisture percentage, resulting in 282 mm of irrigation in 2020 and 155 mm in 2021. Management decisions such as hybrid selection and irrigation scheduling were made at the discretion of SCAL farm management.

2.3 | Soil sampling and analysis

To evaluate the effect of treatments on soil nitrification following treatment application, weekly soil samples were collected at 0- to 20-cm soil depth during May and June of each year. Different soil sampling strategies were used to collect soil samples from the injected AA and broadcast urea plots. In AA plots, six soil cores were collected with a 4cm diameter probe in the in-band and in-row positions, and kept and analyzed separately for NH₄⁺-N and NO₃⁻-N. The final NH4⁺-N and NO3⁻-N values in the anhydrous plots were determined using a weighted average proportional to the lateral dimension of the area within and without the band, as used in our previous studies (e.g., Archontoulis et al., 2020). In the broadcasted urea plots, six cores were collected from the equivalent positions in each plot and composited. The soil samples were transported in a cooler from the field to the laboratory and were refrigerated until analysis to determine soil NH₄⁺-N and NO₃⁻-N. Soil samples were extracted with 2 M KCl solution (5:1 solution to soil ratio) after shaking for 1 h at 180 rpm. Extracts were subsequently filtered using pre-leached Whatman #1 filter paper and analyzed for $NO_3^-+NO_2^--N$ (hereafter NO_3^--N) and soil NH_4^+-N in microplates using the Griess-Ilosvay reaction with vanadium (III) chloride as a reducing agent and the Berthelot reaction, respectively (Hood-Nowotny et al., 2010).

To determine residual NH₄⁺-N and NO₃⁻-N, three soil cores between anhydrous bands (using the same position for urea broadcast treatment) in each plot at a depth of 120 cm were collected using a Gidding hydraulic probe (Giddings) after the crop harvest each year. Soil samples were split into depth increments of 0–10 cm, 10–20 cm, 20–40 cm, 40–60 cm, 60–90 cm, and 90–120 cm. The soil samples were transported in a cooler from the field to the laboratory and were refrigerated until analysis to determine soil NH₄⁺-N and NO₃⁻-N (as mentioned above).

2.4 | Plant sampling and analysis

At physiological maturity in the year 2021, six plant samples were randomly harvested in each plot by cutting the stalk at ground level to determine nitrogen concentration in grain and stover (stalk, leaves, and cobs). The plants were separated into ears and stovers and weighed. The stover was shredded using a portable woodchipper. Ears and subsamples of chopped maize stover were weighed and dried at 71°C to determine moisture content. Ears were shelled to separate grain and cobs. Grain and stover were milled and analyzed for total nitrogen using the dry combustion method at Ward Lab (Kearney, NE). Hand harvest grain yield at 15.5% moisture, nitrogen concentration in grain and stover, and plant population were used to calculate total aboveground biomass N uptake, nitrogen recovery efficiency (NRE), and nitrogen harvest index (NHI) as follows:

Aboveground biomass N uptake (kg N/ha)	
= [Grain N % × Average grain mass per plant]	
+ [Stover N % \times Average stover mass per plant]	
\times plant population	(1)

NRE (%)

= Nuptake in fertilized plot – Nuptake in unfertilized plot Total N applied

 $\times 100$

$$= \frac{\text{Grain N uptake}}{\text{Aboveground biomass N uptake}} \times 100 \quad (3)$$

At harvest, a two-row combine harvester was used to harvest the middle two rows of each plot. The final grain yield was adjusted to 15.5% grain moisture. The combined harvest yield was used to calculate partial factor productivity (PFP) and agronomic efficiency (AE) as follows:

$$PFP (kg grain/kg N) = \frac{Grain yield}{Total N applied}$$
(4)

AE (kg grain/kg N)

2.5 | Statistical analysis

The data were analyzed using SAS version 9.4 (SAS Institute) for each site-year separately because there were significant site-year × treatment interactions. Analysis of variance was performed using the PROC GLIMMIX procedure. Herbicide and the fertilizer treatments were considered fixed effects, while replication was considered random in the linear model to test the effect of N fertilizer and herbicide on grain yield, PFP, AE, residual NO₃⁻-N and NH₄⁺-N, and NUE indicators, including grain N concentration and uptake, aboveground biomass N uptake, NRE, and NHI. An Analysis of variance (ANOVA) was performed for the weekly measurements of

(2)

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TABLE 2	Probability values (PROC GLIMMIX procedure) for the main effect of herbicide (H), fertilizer (F), time, and their interaction	on
in-season soil N	IO_3 -N and NH ₄ -N concentrations (mg kg ⁻¹ soil) measured during May–June of 2020 and 2021.	

	2020		2021	
Effect/year	NO ₃ -N	NH ₄ -N	NO ₃ -N	NH ₄ -N
Н	0.0282	0.3891	0.7625	0.5011
F	<0.0001	<0.0001	<0.0001	<0.0001
Time	<0.0001	<0.0001	<0.0001	<0.0001
$H \times F$	0.0854	0.6594	0.8574	0.545
$H \times time$	0.0448	0.1491	0.1941	0.6006
$F \times time$	<0.0001	<0.0001	<0.0001	<.0001
$H \times F \times time$	0.0777	0.3583	0.3926	0.9863

Note: Significant effects are shown in bold.

TABLE 3 Treatment means and significance for soil NO_3^{-} -N and NH_4^{+} -N concentrations (mg kg⁻¹ soil) measured during May–June of 2020 and 2021 as affected by herbicide and fertilizer treatment.

	2020		2021	
Treatment	NO ₃ ⁻ -N	NH4 ⁺ -N	NO ₃ ⁻ -N	NH4 ⁺ -N
No-PEH	29	13	30	10
Acuron	34	14	30	12
Resicore	35	13	31	12
AA-I	16	26	14	19
AA-No	22	18	18	17
Urea-I	43	6	43	5
Urea-No	48	4	46	4
	p > F			
No-PEH vs. acuron	**	NS^{\dagger}	NS	NS
No-PEH vs. resicore	***	NS	NS	NS
Acuron vs. resicore	NS	NS	NS	NS
AA vs. urea	***	***	***	***
Urea-I vs. Urea-No	*	NS	NS	NS
AA-I vs. AA-No	**	***	*	NS

Abbreviations: AA, anhydrous ammonia; AA-I, anhydrous ammonia with nitrification inhibitor of N-serve; AA-No, anhydrous ammonia without nitrification inhibitor of N-serve; no-PEH, no preemergence herbicide; Urea-I, urea with nitrification inhibitor of Instinct or guardian DL; Urea-No, urea without nitrification inhibitor of Instinct or guardian DL.

*, **, and *** denote significance at p < 0.05, 0.01, and 0.001, respectively.

[†]NS, not significant.

in-season soil NO₃⁻⁻N and NH₄⁺⁻N, considering sampling dates as repeated measurements. Data for in-season and fall residual soil NO₃⁻⁻N and NH₄⁺⁻N were analyzed and presented by their sampling date and sampling depth, respectively. To analyze a complete factorial design (4 N sources × 3 herbicides), data from the zero N treatment were left out of ANOVA results presented in Tables 2–4. Means from the zero N treatment were not included in the main effects to determine significant differences among the treatments. Specific pairwise comparisons among the treatments of interest were made using the CONTRAST statement, and means were compared using the least significant difference at $p \le 0.05$.

3 | RESULTS

3.1 | Climate

Total seasonal precipitation from March 1 to October 31 was 298 mm in 2020 and 386 mm in 2021 (Figure 1). Compared to the Clay County 30-year average precipitation of 632 mm in the same period, 2020 and 2021 precipitation was low by 53% and 39%, respectively. It is worth noting that the total seasonal water inputs (precipitation plus irrigation) from March 1 through October 31 of 580 mm in 2020 and 541 mm in 2021 were lower than the 30-year average precipitation of 632 mm.



FIGURE 1 Daily precipitation, irrigation, and cumulative precipitation in each site-year of the study.

May precipitation (the first month following fertilizer application) was 35% and 17% lower in 2020 and 2021, respectively, than the Clay County 30-year May average. The same trend was found in the second month (June) following fertilizer application, when June precipitation was 48% and 58% lower in 2020 and 2021, respectively, than the Clay County 30-year June average (Figure 1).

3.2 | In-season soil nitrogen availability

3.2.1 | Soil NH₄⁺-N concentration

In-season weekly soil NH₄⁺-N and NO₃⁻-N concentrations varied significantly over time for both years (Figure 2; Table 2). Herbicide did not have a main or interaction effect on NH_4^+ -N concentration in either year (Tables 2 and 3). However, fertilizer treatments significantly affected NH₄⁺-N concentrations during both years (Table 2). Within the fertilizer treatments, nitrification inhibitors had a variable effect on NH_4^+ -N across the two nitrogen sources (AA and Urea) and years. For example, AA-I versus AA-NO contrast on NH_4^+ -N was significant in 2020 only, while there was no significant effect between Urea-I and Urea-No in both years (Figure 2; Table 3). AA-I retained significantly higher NH_4^+ -N than AA-No on five of eight sampling dates in 2020, while the difference was significant on only two of eight sampling dates in 2021 (Figure 2). When averaged across the entire sampling period, AA-I retained 44% and 14% higher NH₄⁺-N than AA-No in 2020 and 2021, respectively (Table 3). On the other hand, Urea-I retained significantly higher NH₄⁺-N than Urea-No on one of eight sampling dates in 2020, while no significant difference was found on any sampling date in 2021 (Figure 2). During the entire sampling period, Urea-I retained 50% and 25% higher NH4⁺-N than Urea-No in 2020 and 2021, respectively. When averaged across all sampling dates, AA retained significantly higher NH₄⁺-N concentration by 340% and 300% compared to urea in 2020 and 2021,

respectively (Table 3). Notably, nitrogen source had a more significant effect on NH_4^+ -N retention (300%–340% higher in AA vs. urea) compared to nitrification inhibitor with either nitrogen source (14%–50% higher with inhibitor vs. without inhibitor).

3.2.2 | Soil NO₃⁻-N concentrations

The herbicide had a significant main effect on soil NO₃⁻-N in 2020 only (Table 2). However, no significant interaction of herbicide and fertilizer was observed on soil NO₃⁻-N in both years (Table 2). Across all fertilizer treatments, Acuron and Resicore had 17% and 21% higher soil NO3--N than No-PEH, respectively (Table 3). Furthermore, soil NO₃⁻-N was significantly influenced by the main effects of fertilizer in both years. Within fertilizer treatments, nitrification inhibitors had a variable impact on NO3⁻-N across the two nitrogen sources (AA and Urea) and years. For example, AA-I had significantly higher NO₃⁻-N than AA-NO in both years, while Urea-I had significantly higher NO₃⁻-N than Urea-No in 2020 only (Figure 2; Table 3). During the entire sampling period, AA-I had significantly lower NO₃⁻-N concentration compared to AA-No on four of eight sampling dates in 2020 and one of eight sampling dates in 2021 (Figure 2). When averaged across all sampling dates, AA-I had 27% and 22% lower NO₃⁻-N concentrations than AA-No in 2020 and 2021, respectively. Similarly, Urea-I had significantly lower NO₃⁻⁻ N concentration in two of eight sampling dates each year. Across all sampling dates, Urea-I had 10% and 7% lower NO₃⁻-N than Urea-No in 2020 and 2021, respectively. When averaged across all sampling dates, AA had 58% and 64% lower NO₃⁻-N concentrations than urea in 2020 and 2021, respectively. It is worth mentioning that nitrogen source had a more significant effect on NO₃⁻-N production (58%-64% lower in AA vs. urea) compared to nitrification inhibitor with either nitrogen source (7%-27% lower with inhibitor vs. without inhibitor).



FIGURE 2 Weekly soil NH₄⁺-N (upper two panels) and NO₃⁻-N (lower two panels) concentration in soil sampled at 0- to 20-cm depth for 2 months (May and June) of 2020 and 2021. Asterisks indicate significant mean comparison between anhydrous ammonia with inhibitor (AA-I) and anhydrous ammonia without inhibitor (AA-No) at each sampling date within each site-year as determined by least-square means (* \leq 0.05, ** \leq 0.01, *** \leq 0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U-I) and urea without inhibitor (U-No) at each sampling date within each site-year as determined by least-square means (* \leq 0.001). Significant *p* values using repeated measure PROC GLIMMIX procedure for the differences of nitrification inhibitor and nitrogen source are shown in Table 3.

3.3 | Agronomic responses

The effects of fertilizer treatments, herbicides, and their interactions on agronomic responses are given in Tables 4-6. Agronomic responses to fertilizers, herbicides, and their interactions varied across both years. In 2020, fertilizer and herbicide significantly interacted with grain yield, PFP, and AE (Table 4). Within fertilizer treatments, nitrification inhibitors across both N sources did not significantly affect grain yield, PFP, or AE (Table 5). Though not significant, these three parameters were slightly lower with a nitrification inhibitor than without a nitrification inhibitor in 2020, while the opposite trend was observed in 2021, where grain yield, PFP, and AE were slightly higher with an inhibitor than without an inhibitor (Table 5). Furthermore, different fertilizer responses on grain yield, PFP, and AE were observed each year (Figure 3; Table 5). For example, AA had a 1.1 Mg ha⁻¹ higher grain yield compared with urea in 2020, while there was no significant grain yield response in 2021. Similarly, AA had a significantly higher PFP (6 kg grain kg⁻¹ N) and AE (5.5 kg grain kg⁻¹ N) compared to the urea in 2020. There were no significant differences between AA and urea for grain yield, PFP, or AE in 2021.

The herbicide had a variable main effect on grain yield, PFP, and AE each year (Table 4). For example, in 2020, herbicides had a marginally significant impact on grain yield and PFP (Table 4). Acuron and Resicore had 1.4 and 1.6 Mg ha⁻¹ higher grain yields than no-PEH, respectively (Table 5). This increase in grain yield was accompanied by an increase of 8.0 kg grain kg⁻¹ N PFP with both Acuron and Resicore, compared with no-PEH. A significant but opposite effect of herbicide on AE was found in 2020, where AE of Acuron and Resicore was 12 and 6.5 kg grain kg⁻¹ N lower compared with No-PEH (Table 5). In 2021, no significant effect of herbicide on grain yield, PFP, and AE was observed.

Nitrogen indicators, including grain N concentration, grain N uptake, aboveground biomass N uptake, NRE, and NHI, were measured in 2021 only. The effects of fertilizer treatment, herbicide, and their interactions on the nitrogen indicators are given in Tables 4 and 6. Though no significant main or interaction effects of fertilizer and herbicide were observed on grain N concentration or nitrogen uptake, AA had 15 kg N ha⁻¹ higher grain N uptake compared to urea (Table 6). Resicore had 7.5 kg N ha⁻¹ and 6 kg N ha⁻¹ higher grain N uptake than Acuron and No-PEH treatment, respectively (Table 6). All the nitrogen indicators and their interactions had insignificant effects on aboveground biomass N uptake. Though insignificant, AA increased aboveground biomass N uptake by 34 kg N ha⁻¹ compared to urea (Table 6). Similarly, AA significantly increased NRE by 33% compared to urea. In contrast, urea had a 3% higher NHI compared to AA.



FIGURE 3 The effect of nitrogen fertilizer and herbicide on maize grain yield in 2020 and 2021. The values are mean \pm standard error of mean. Different letters above bars indicate significant mean differences between treatments as determined by least-square means. No-PEH indicates no preemergence herbicide.



FIGURE 4 Postharvest soil NH₄⁺-N (left two panels) and NO₃⁻-N (right two panels) concentration in soil sampled at 0- to 120-cm depth in 2020 and 2021. Asterisks indicate significant mean comparison between anhydrous ammonia with inhibitor (AA-I) and anhydrous ammonia without inhibitor (AA-No) at each sampling depth within each site-year as determined by least-square means (* ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U-I) and urea without inhibitor (U-No) at each sampling depth within each site-year as determined by least-square means (* ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.0001). Caret symbols indicate significant mean comparison between urea with inhibitor (U-I) and urea without inhibitor (U-No) at each sampling depth within each site-year as determined by least-square means (* ≤ 0.05 , ** ≤ 0.001). Significant *p* values using PROC GLIMMIX procedure for the differences of N Inhibitor and N source are shown in each panel.

3.4 | Residual soil NH_4^+ -N and NO_3^- -N

Across both years, fertilizer and herbicide did not have a significant main or interaction effect on residual NH_4^+ -N except for a significant main effect of fertilizer in 2021 (Figure 4; Tables 4 and 5). Within the fertilizer treatments, the nitrification inhibitor did not affect residual NH_4^+ -N across both sources and years (Table 5). However, within fertilizer treatments in 2021, AA had 174% (63 kg N ha⁻¹ at 0- to 1.2-m depth) higher residual soil NH₄⁺-N than urea (23 kg N ha⁻¹ at 0- to 1.2-m depth) (Figure 4; Table 5). Furthermore, residual NH₄⁺-N did not differ by soil depth in 2020, but it significantly increased with increasing soil depth in 2021 (Figure 4, depth statistics not shown). When totaled across the entire soil

Sources of			2020			2021									
effect	GY	PFP	AE	NO ³ N	NH4 ⁺ -N	GY	PFP	AE	NO ₃ N	NH4+-N	GNC	GNU	ABN	NRE	IHN
Н	0.056	0.0590	0.0312	0.739	0.616	0.241	0.265	0.894	0.457	0.881	0.880	0.804	0.379	0.178	0.220
Ц	<0.0001	< 0.0001	<0.001	0.221	0.642	0.280	0.277	0.289	0.724	0.004	0.609	0.479	0.237	0.263	0.035
$H \times F$	0.004	0.0049	<0.001	0.369	0.273	0.937	0.949	0.953	0.814	0.948	0.774	0.818	0.507	0.546	0.124

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profile at 0–1.2 m, AA and urea had 12 and 13 kg $\mathrm{NH_4^+}$ -N ha^{-1} in 2020 and 63 and 23 kg $\rm NH_4^+\text{-}N$ ha^{-1}in 2021 (Figure 4; Table 5).

No significant main or interaction effects of fertilizer and herbicide on residual soil NO3⁻-N occurred in either year (Table 4). Soil NO_3^{-} -N was significantly higher in the upper than in the lower soil layer in 2020, but no differences across depth were found in 2021 (Figure 4). When totaled across the entire soil profile, AA and urea had 33 and 27 kg NO₃⁻-N ha^{-1} in 2020, and 0.3 and 0.5 kg NO_3^{-} -N ha^{-1} in 2021. Overall, across both years, AA had 6-40 kg N ha⁻¹ higher total inorganic nitrogen than urea.

DISCUSSION 4

The effect of nitrification inhibitor, 4.1 nitrogen source, and herbicide on in-season N availability

This study compared the effect of nitrification inhibitors, nitrogen sources, and herbicides on early-season soil nitrogen availability. Nitrification inhibitors containing nitrapyrin and DCD are known to reduce nitrification and delay the conversion of NH₄⁺-N to NO₃⁻-N (Franzen, 2017; Peng et al., 2015) during the early season before the crop can actively take up nitrogen during the early season to mid season. Generally, potential N losses are more likely during the heavy rainfall period in early spring (Loecke et al., 2017; Van Metre et al., 2016). However, in both site-years of this study, early season and cumulative seasonal precipitation were lower than the 30-year average precipitation, suggesting a lower probability for nitrogen loss through nitrate leaching. Regardless of weather conditions, nitrification inhibitors can temporarily inhibit nitrification and delay the conversion of NH₄⁺-N to NO₃⁻-N for several weeks after fertilizer application, as reported in previous studies (Franzen, 2017). This study found variable effects of nitrification inhibitors on nitrogen availability during the early season across 2 years, where nitrification inhibitors had 14%-50% higher NH₄⁺-N and 7%–27% lower NO₃⁻-N compared to without inhibitors across two N sources (AA and urea). During the May and June soil sampling periods across both years, NH₄⁺-N concentration decreased over time across both nitrogen sources as nitrification gradually increased as expected. At the end of June, assuming no nitrogen loss, we expected similar values of soil NO₃⁻-N concentration between AA and urea through nitrification, as both of these treatments received the same N rate; however, the NO₃⁻-N concentration was much lower in AA compared to urea. This might be partly due to an underestimation of the NO₃⁻-N release from the anhydrous band because soil sampling was conducted within the anhydrous band and the row (representing the area outside the band) and

	2020					2021				
Treatment	GY (Mg ha-1)	PFP (kg grain _{Lo} -1 N)	AE (kg grain t _{ed} -1 N)	NO ₃ N (kg N ha- ¹)	NH4 ⁺ -N (kg N ha- ¹)	GY (Mg ha ⁻¹)	PFP (kg grain kg ⁻¹ N)	AE (kg grain Lo ⁻¹ N)	NO ₃ N (kg N h ₂ -1)	NH ₄ +-N (kg N ha-1)
No-PEH	11.5	62 57)	23	26	13	16.0	86	21 21	0.3	45
Acuron	12.9	70	11	35	11	16.2	87	22	0.6	46
Resicore	13.1	70	16	30	12	15.7	85	21	0.4	39
I-AA-I	13.1	70	19	41	13	16.2	87	22	0.2	68
AA-No	13.0	70	19	26	11	16.0	86	21	0.4	59
Urea-I	11.8	63	12	27	12	16.1	86	22	0.6	25
Urea-No	12.1	65	15	27	13	15.6	84	19	0.4	21
	p > F									
No-PEH vs. acuron	***	***	* *	NS⁺	NS	NS	NS	NS	NS	NS
No-PEH vs. resicore	***	* *	*	NS	NS	SN	NS	NS	NS	NS
Acuron vs. resicore	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
AA vs. urea	*	*	*	NS	NS	NS	NS	NS	NS	**
Urea-I vs. Urea-No	NS	NS	NS	NS	NS	SN	NS	NS	NS	NS
AA-I vs. AA-No	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vote: Maize grain vields are expressed	l at 155 g kg ⁻¹ moi	sture.								

Abbreviations: AA, anhydrous ammonia; AA-I, anhydrous ammonia with nitrification inhibitor of N-serve; No-PEH, no preemergence herbicide; Urea-I, urea with nitrification inhibitor of Instinct or guardian DL; Urea-No, urea without nitrification inhibitor of Instinct or guardian DL.

*, **, and *** denote significance at p < 0.05, 0.01, and 0.001, respectively.

[†]NS, not significant.

			Aboveground biomass N		
	Grain N concentration	Grain N uptake	uptake	NRE	NHI
Treatment	(%)	(1	kg N ha ⁻¹)	(%)
No-PEH	1.3	216	313	71	69
Acuron	1.3	217	310	60	70
Resicore	1.3	223	338	77	66
AA-I	1.3	228	341	79	67
AA-No	1.3	224	334	75	68
Urea-I	1.3	208	301	57	69
Urea-No	1.3	215	306	59	71
	p > F				
No-PEH vs. acuron	NS [†]	NS	NS	NS	NS
No-PEH vs. resicore	NS	NS	NS	NS	*
Acuron vs. resicore	NS	NS	NS	*	**
AA vs. urea	NS	NS	NS	*	*
Urea-I vs. Urea-No	NS	NS	NS	NS	NS
AA-I vs. AA-No	NS	NS	NS	NS	NS

TABLE 6 Treatment means and significance for grain nitrogen (N) concentration, grain N uptake, aboveground biomass N uptake, nitrogen recovery efficiency (NRE), and nitrogen harvest index (NHI) as affected by herbicide and fertilizer treatment in 2021.

Note: Maize grain yields are expressed at 155 g kg⁻¹ moisture.

Abbreviations: AA, anhydrous ammonia; AA-I, anhydrous ammonia with nitrification inhibitor of N-serve; AA-No, anhydrous ammonia without nitrification inhibitor of N-serve; No-PEH, no preemergence herbicide; Urea-I, urea with nitrification inhibitor of Instinct or guardian DL; Urea-No, urea without nitrification inhibitor of Instinct or guardian DL.

*, **, and *** denote significance at p < 0.05, 0.01, and 0.001, respectively.

[†]NS, not significant.

did not include the available NO_3^- -N between the band and the row, as nitrate would diffuse away from the band over time (Khengre & Savant, 1977).

Regardless, nitrogen sources had a more significant effect on inhibiting nitrification compared to nitrification inhibitors, as AA had 300%-340% higher NH4+-N and 58%-64% lower NO₃⁻-N than urea across both site-years. This lower nitrification of AA compared with urea could be attributed to the knife injection of nitrogen beneath the soil surface, as concentrated ammonia band changes soil pH and inhibits microbial activity for several weeks after anhydrous injection (Biederbeck et al., 1996; Stehouwer & Johnson, 1990). These results were consistent with previous studies, where band application of nitrogen stabilized nitrogen compared to surface broadcast nitrogen application (Biederbeck et al., 1996; C. Shapiro et al., 2016). However, the comparative effect of nitrification inhibitor versus nitrogen source has not been previously focused on field crops (Redding et al., 2020). The findings from this study indicate that selecting the right nitrogen source has a higher probability of inhibiting nitrification and protecting against potential N loss compared with nitrification inhibitors during the spring period, especially as more wet springs are predicted in the future (Dai et al., 2016; Hatfield et al., 2011). If the potential for nitrogen losses through nitrate leaching and denitrification were higher during this study, urea would have lost more nitrogen than AA (Stehouwer &

Johnson, 1990). The absence of a wet year during this study precludes the possibility of evaluating the same treatments for years with different weather conditions. Regardless, the potential of N loss can be minimized by selecting a combination of the right nitrogen source and nitrification inhibitors, as AA with a nitrification inhibitor retained 427%–600% more NH_4^+ -N than urea without a nitrification inhibitor (Figure S1). This combined nitrification inhibitor and source effect on nitrification was higher than the nitrogen source effect of 300%–340%, as reported above (Table 3).

Though herbicides did not have a consistent effect on inhibiting nitrification across both sources and site-years, Acuron retained 8% higher NH4+-N than No-PEH at the start of the season in 2020, while both herbicides (Acuron and Resicore) had 20% higher NH₄⁺-N than No-PEH in 2021, indicating some potential for reducing soil nitrification. These findings are consistent with previous lab studies where herbicides containing atrazine and acetochlor inhibited nitrification (Li et al., 2008; Mahía et al., 2008). Herbicides containing atrazine produce non-target effects on the microbial community by decreasing soil microbial biomass (Mahía et al., 2008) and altering ammonia-oxidizing archaea and ammoniaoxidizing bacteria amoA gene abundances, which are related to the soil nitrification process (Caffrey et al., 2007; Zhang et al., 2018). To our knowledge, this is the first study demonstrating the effect of herbicide on nitrification under field

conditions. The herbicides provided an additive nitrification effect to nitrogen source and nitrification inhibitor in the AA-I treatment in both years, as both herbicides in AA-I had 500%–625% higher NH_4^+ -N than the Urea-No treatment with No-PEH, which retained the least NH_4^+ -N among all nitrogen treatments in both site-years (Figure S1). This indicates that the application of the right source with nitrification inhibitor and herbicide can have the cumulative effect of stabilizing and protecting nitrogen when conditions become susceptible to N losses. However, compared to herbicide effect on NH_4^+ -N, herbicides had an inconsistent effect on NO_3^- -N during both years. Significantly higher NO₃⁻-N concentration with Acuron and Resicore compared to No-PEH in 2020 (Table 3) was likely because of N uptake by weeds, as this effect was mainly found from mid-May to the end of June, when more weeds were observed in the No-PEH treatment (visual observation) due to delayed postemergence herbicide application in 2020. While weed presence can reduce soil NO_3^{-} -N up to 50% by the pollination stage (Lindquist et al., 2010), in the same year, it was interesting to note that within fertilizer treatments, weeds did not affect nitrate in the AA treatment, as the weeds might not be able to exploit nitrogen in the concentrated anhydrous band compared to available nitrogen at the surface broadcast urea, where weeds presence considerably reduced NO₃⁻-N in No-PEH compared to Acuron and Resicore (Figure S1). In 2021, timely postemergence herbicide application on June 1 resulted in no weeds (visual observation), and thus no considerable effect of herbicide within the fertilizer source on NO₃⁻-N was observed (Figure S1).

4.2 | The effect of nitrification inhibitor, nitrogen source, and herbicide on agronomic responses

Though nitrification inhibitors conserved nitrogen by reducing nitrification during the early part of the growing season, this did not significantly affect agronomic indicators across both site-years. The lack of response of the nitrification inhibitors can be due to several factors. First, below-normal precipitation during the growing season might have lowered the nitrate-leaching potential by reducing the effectiveness of the nitrification inhibitors. Second, previous studies have suggested that nitrapyrin and DCD are required in higher concentrations than the labeled rate to produce a crop yield response (Franzen, 2017). Third, NH₄⁺-N concentrations in AA are often higher and persist longer at higher rates, thereby diminishing the efficacy of nitrification inhibitors (Hughes & Welch, 1970; Stehouwer & Johnson, 1990). These results are consistent with previous studies, where no effects of nitrification inhibitors on agronomic indicators were found under drier conditions (Franzen, 2017; Sassman, 2014). In this study, N supply from the high N fertilizer rate and soil

organic matter mineralization might have compensated for the advantage of nitrification inhibitors against no nitrification inhibitors.

Compared to nitrification inhibitors, nitrogen sources significantly affected grain yield and PFP in 2020 (Table 5). Though AA had significantly higher grain yield, PFP, and AE than urea, this effect was mainly due to nitrogen fertilizer interaction with the herbicides, where No-PEH in urea with and without nitrification inhibitors had significantly lower grain yield than No-PEH in AA with and without inhibitors. This was possibly because the nitrogen from the broadcast urea resulted in more weeds (visual estimate) due to a late postemergence herbicide application in 2020 that likely led to higher nitrogen uptake by weeds and resulted in less nitrogen availability for the maize crop (Lindquist et al., 2010). Meanwhile, in AA with and without a nitrification inhibitor, weeds might not have been able to access the nitrogen from the concentrated anhydrous band to proliferate weed growth in the early season, resulting in reduced N uptake by weeds. In 2021, the postemergence herbicide was applied 2 weeks earlier following planting than in 2020 (June 13, 2020 compared with June 1, 2021) and resulted in fewer weeds with no interaction effect of nitrogen fertilizer and herbicide on either grain yield, PFP, or AE. Nevertheless, findings from the current study indicate the advantage of band placement in improving maize yields, thus supporting previous evidence of higher maize yield with band than broadcast N application (Howard & Tyler, 1989; Lamond et al., 1991; Stecker et al., 1993).

Plant nitrogen uptake is another indicator that can be used to evaluate the performance of nitrification inhibitors and nitrogen sources. Nitrogen analysis of grain and stover at the black layer showed no significant difference in grain N uptake with the fertilizer treatments. Still, it did show the difference in NRE with nitrogen source in 2021. AA had 15 kg N ha⁻¹ higher grain N uptake, 34 kg N ha⁻¹ higher aboveground biomass N uptake, and 33% higher NRE compared to urea, showing the advantage of band over broadcast N application. This also provides evidence that inhibiting nitrification with the right source can prolong nitrogen availability in the surface soil, better synchronize soil nitrogen availability with crop nitrogen uptake, and enhance nitrogen recovery efficiency (Stehouwer & Johnson, 1990). This is similar to findings from C. Shapiro et al. (2016), who reported a 20 kg ha⁻¹ increase in aboveground biomass uptake with band versus broadcast nitrogen treatments. Other studies also reported higher aboveground biomass N uptake with injected than surface broadcast nitrogen (Mengel et al., 1982; Stehouwer & Johnson, 1990). This favorable effect of band versus broadcast corresponds to the lower nitrification rate of AA than urea during the early growing season. The higher aboveground biomass N with AA did not lead to higher grain yield in 2021, likely because nitrogen might be available in excess amounts, while maize plants tend to partition more nitrogen from grain to stalks when excess nitrogen is available (C. Shapiro

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et al., 2016). This also explains why urea treatments had significantly higher NHI, as AA and urea had statistically similar grain yield but comparatively higher aboveground biomass N with AA than with urea. Regardless, comparatively higher aboveground biomass N and NRE in AA than in urea can be beneficial, as it leads to more plant N uptake and less seasonal potential N loss through nitrate leaching or denitrification.

4.3 | The effect of nitrification inhibitor, nitrogen source, and herbicide on residual soil nitrogen

Nitrogen conservation with nitrification inhibitors and nitrogen sources during the growing season is often implied to better synchronize nitrogen availability with crop nitrogen uptake. However, any nitrogen left after the crop harvest is not useful, as it can be lost during winter (C. Shapiro et al., 2016). Previous research has shown that enhanced efficiency fertilizers with nitrification inhibitors can leave higher postharvest soil nitrate nitrogen when used at higher nitrogen rates (Maharjan et al., 2016; C. Shapiro et al., 2016). In this study, residual nitrate nitrogen across all treatments was lower (0.4–30 kg N ha⁻¹) than the generally accepted normal residual NO₃⁻-N concentration of 50 kg NO₃⁻-N in the fine-textured soils of Nebraska (C. A. Shapiro et al., 2008). However, we found higher residual NH₄⁺-N (12-43 NH₄⁺ kg N ha⁻¹) than residual NO₃⁻⁻N (0.4–30 kg N ha⁻¹) across all treatments. This could be partly due to drier than normal conditions during both site-years, which left more NH₄⁺-N due to lower N movement in the soil profile. Higher residual NH₄⁺-N values from AA than from urea in 2021 further indicate more nitrogen conservation with band placement than with broadcast, as reported for in-season N availability earlier. It was interesting to observe higher residual NH_4^+ -N in deeper soil layers than in upper soil layers in 2021, suggesting some unknown processes to produce NH₄⁺-N in deeper soil layers. This trend is similar to the results of a study reported by a colleague who found higher NH₄⁺-N in deeper soil layers than in the topsoil layer (unpublished results). Nevertheless, these results suggest that, in addition to residual NO₃⁻-N, NH₄⁺-N, especially under dry conditions, should be considered when accounting nitrogen credits for nitrogen requirements for the subsequent cash crop. Furthermore, nitrogen conservation from the nitrogen sources and nitrification inhibitors should be considered when analyzing the value of the nitrogen placement and nitrification inhibitor.

5 | CONCLUSION

This study compared the effects of nitrification inhibitors, nitrogen sources, and herbicides on early-season soil nitro-

gen availability and agronomic indicators. Though this study experienced below-normal precipitation during both years, we still observed some significant nitrification inhibitor and source effects on nitrogen indicators. Nitrogen sources had a more substantial effect on in-season nitrogen availability than nitrification inhibitors. AA had 300%-340% higher NH₄⁺-N and 58%-64% lower NO3⁻-N production than urea in both years, indicating the potential of higher nitrogen conservation with band placement than broadcasted nitrogen. Compared to nitrogen sources, nitrification inhibitors had a smaller effect on nitrogen conservation, retaining 14%-50% higher NH_4^+ -N and producing 7%–27% lower NO_3^- -N than without inhibitors. The herbicide also had a smaller effect on in-season nitrogen availability and retained 8%-20% higher NH₄⁺-N than No-PEH. Furthermore, the anhydrous application showed significant advantages over urea, as it increased grain yield by 1.1 Mg ha⁻¹, partial factor productivity by 6 kg grain kg⁻¹ N, agronomic efficiency by 5.5 kg grain kg⁻¹ N, aboveground biomass N uptake by 34 kg N ha⁻¹, grain N uptake by 15 kg N ha⁻¹, and nitrogen recovery efficiency by 33%. These improved agronomic indicators might be attributed to higher nitrogen conservation with AA than with urea. In addition, residual total soil N was higher by 6-40 kg N ha⁻¹ in AA compared to urea, indicating the potential for nitrogen stabilization with nitrogen application in the band rather than broadcast. These results suggest that nitrogen management in maize can be improved by banding compared to broadcasting nitrogen in the soil. Though nitrification inhibitors and herbicides had a smaller effect on nitrogen conservation than nitrogen sources, using the right combination of nitrogen source, nitrification inhibitor, and herbicide can provide an additive effect in conserving soil nitrogen and improving NUE in maize.

AUTHOR CONTRIBUTIONS

William Neels: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft. Amit Jhala: Conceptualization; investigation; methodology; supervision; writing—review and editing. Swetabh Patel: Conceptualization; formal analysis; investigation; writing—review and editing. Glen Slator: nevestigation; writing—review and editing. Javed Iqbal: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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