Soil pH Effects on Nitrification of Fall-Applied Anhydrous Ammonia

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ABSTRACT

Soil temperature at the time of application has been the primary factor used to predict rates of nitrification and assess the risks associated with losses of N applied in the fall as anhydrous ammonia in the Corn Belt. We report studies assessing the importance of soil pH as a factor affecting nitrification rates and losses of this N before corn (Zea Mays L.) begins rapid growth in June. Data were collected in a series of field studies conducted during 4 yr. Anhydrous ammonia was applied in the fall after soils had cooled to <8°C, and soils were sampled before corn plants emerged in the spring. Soil pH ranged from <6.0 to >7.5. Significant relationships between soil pH and percentage nitrification were observed each year. Means of measurements made in mid-April (when planting begins) indicated 89% nitrification of fertilizer N in soils having pH > 7.5 and 39% nitrification of this N in soils having pH < 6.0. The finding that soil pH influenced when nitrification occurred helps to explain why the effects of nitrification inhibitors have been variable in this region. Significant relationships between soil pH and recovery of fertilizer N as exchangeable NH$_4$ and NO$_3$ were observed in years with above-average rainfall before samples were collected in April. The effects of soil pH on nitrification, therefore, influenced the amounts of NO$_3$ lost by denitrification or leaching during spring rainfall. The observed effects of pH on nitrification rates suggest that economic and environmental benefits of delaying application of fertilizer N may be greater in higher-pH soils than in lower-pH soils.

Anhydrous ammonia is the most widely used form of fertilizer N in the Corn Belt and is applied to many fields in October or November for corn to be planted in April or May. Fertilizing some fields in the fall and others in the spring creates a wider period for fertilizer application and enables more efficient use of the relatively expensive facilities and equipment needed to store this gaseous form of N, transport it to farms, and inject it into soils (Kurtz and Smith, 1966; Pesek et al., 1971; Aldrich, 1980; Black, 1984; Peterson and Voss, 1984; Randall et al., 1985). Fall applications also reduce the number of field operations that must be done within a short period in the spring and thereby make it practical for farmers to manage more hectares planted to corn. Ample time is available for fall applications because corn usually is preceded by soybean [Glycine max (L.) Merr.], which normally is harvested by mid-October. Anhydrous ammonia usually cannot be applied after November or before April because soils either are frozen or too wet.

It has been recommended that fall applications of N be delayed until soil temperature has decreased to 10°C at a depth of 10 to 15 cm (Nelson and Hansen, 1968; McVickar and Walker, 1978; Follett et al., 1981). The underlying assumption is that nitrification is strongly inhibited by low temperatures during winter months, so fertilization in late fall instead of early spring does not significantly increase the potential for losses of the fertilizer N by leaching or denitrification of NO$_3$ when excess water is present during the fall-through-spring period. A review by Schmidt (1982), for example, indicates that nitrifying organisms are essentially inactive in cold soils and that such limitations prevail until temperatures increase to 4 or 5°C. However, nitrification has been observed in frozen soils (Nyborg and Malhi, 1979), and there is evidence that the cumulative effect of relatively slow nitrification in cold soils could be important during the fall-to-spring period (Frederick, 1956; Sabey et al., 1959; Anderson and Boswell, 1964; Frederick and Broadbent, 1966; Sabey, 1969; Campbell et al., 1973; Gomes and Loynachan, 1984; Haynes, 1986). More information concerning rates of nitrification during the fall-through-spring period is needed because Balkcom et al. (2003) showed that March-through-May rainfall is a major factor affecting losses of N from fertilized cornfields to rivers before plants begin rapid growth in June.

Soil pH usually is not considered to be an important factor affecting rates of nitrification of fall-applied N because agricultural soils of the Corn Belt usually have pH values between 5.5 and 8. Reviews relevant to fertilized agricultural soils (Pesek et al., 1971; Russell, 1973; Schmidt, 1982) report that nitrification rates in soils are little affected by soil pH within this range. Reviews relevant to nitrification in nonfertilized soils (Grant, 1994; Stark and Firestone, 1996; Norton, 2000) do not discuss the effects of pH on nitrification as an independent step in the overall process of N mineralization. It is well established, however, that nitrification is relatively slow at pH values < 5.5 (Alexander, 1965; Sahrawat, 1982; Schmidt, 1982).

There is some evidence that soil pH in the range of 6 to 8 could influence nitrification rates in soils. The pH of artificial growth media is known to strongly influence the activity of nitrifying microorganisms, with maximum growth at pH values in the range of 7.5 to 8.2 (Waksman and Starkey, 1931; Alexander, 1965; Norton, 2000). Effects of soil pH on nitrification in the range of 6 to 8 have been observed (Eagle, 1961; Morrill and Dawson, 1967; Dancer et al., 1973; Focht and Verstraete, 1977; Kissel et al., 1985; Tlustos and Blackmer, 1992). Part of this effect may be due to the presence of carbonates found at the higher pH values (Schmidt, 1982; Kin-
sbursky and Saltzman, 1990). Carbonates can provide a source of CO₂ needed for growth of the autotrophic organisms involved. However, these effects have not been linked to problems related to nitrification and losses of fall-applied N.

In this article, we describe a series of field studies that assess the importance of soil pH as a factor affecting the rates at which N from fall-applied anhydrous ammonia is nitrified in soils and the potential for losses of this N before plants grow. The studies began with an initial survey to assess percentage nitrification and recovery of fall-applied anhydrous ammonia-N in fields after a relatively warm winter and wet spring. This was followed by 2 yr of spring observations in fields where anhydrous ammonia had been applied in the fall with and without a nitrification inhibitor. A follow-up study was conducted to observe rates of nitrification during the first month after fall application. The effects of nitrification inhibitors were studied because these compounds are often used to delay nitrification of fall-applied anhydrous ammonia and thereby reduce losses of fertilizer N before crops grow (Hendrickson et al., 1978; Keeney, 1980; Hoeft, 1984; Hauck, 1985; Mengel and Rehm, 2000).

MATERIALS AND METHODS

Studies were conducted in fields within the Clarion–Nicollet–Webster and Canisteo–Nicollet–Webster soil associations of central Iowa. These soils were developed on calcareous glacial till that was deposited about 12 000 yr ago (Prior, 1991). The landscape of this area is flat to gently rolling, divided into fields (usually 400 by 800 m) for management, and dominated by a corn–soybean cropping system. A noteworthy characteristic of this region is that soils often range from relatively acid (pH < 6.0) to highly calcareous (CaCO₃ equivalent often exceeds 10%) within the same field. Major soil series in the fields studied were Clarion (fine-loamy, mixed, superactive, mesic Typic Hapludolls), Nicollet (fine-loamy, mixed, superactive, mesic Aquic Hapludolls), Webster (fine-loamy, mixed, superactive, mesic Typic Endoaquolls), Harps (fine-loamy, mixed, superactive, mesic Typic Calciaquolls), and Canisteo (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls).

The first phase of the study (an initial survey) involved sampling 11 fields in mid-April of 1998 to assess recovery of fall-applied N following a relatively warm fall and relatively large amounts of rainfall in early spring (Fig. 1 and 2). Farmers had applied anhydrous ammonia to each field the previous fall, followed by 2 yr of spring observations in fields where anhydrous ammonia had been applied in the fall with and without a nitrification inhibitor. A follow-up study was conducted to observe rates of nitrification during the first month after fall application. The effects of nitrification inhibitors were studied because these compounds are often used to delay nitrification of fall-applied anhydrous ammonia and thereby reduce losses of fertilizer N before crops grow (Hendrickson et al., 1978; Keeney, 1980; Hoeft, 1984; Hauck, 1985; Mengel and Rehm, 2000).

The second phase of the study (the main part) involved fall application of fertilizer treatments in strips going the lengths of six fields (3 in 1998 and 3 in 1999) and sampling soils to assess fertilizer recovery the next spring. The treatments included anhydrous ammonia with and without a nitrification inhibitor applied in adjacent strips (Fig. 3). The inhibitor was nitrapyrin [2-chloro-6-(trichloromethyl)-piridine], which is marketed as N-Serve by Dow AgroSciences (Indianapolis, IN). The anhydrous ammonia was applied to a depth of about 20 cm by using a state-of-the-art applicator (DMI Nutri-placér, model 6000, DMI Inc., Goodfield, IL) used in production agriculture. It fertilized a swath 12.2 m wide (16 knives spaced 76 cm apart) and could inject nitrapyrin (0.56 kg a.i. ha⁻¹) into the fertilizer as needed. It was equipped with radar and a Raven 750 (Raven Industries Inc., Sioux Falls, SD) monitor to adjust flows of ammonia for changes in ground speed and desired rates of N application. Calibrations were established as the applicator was used across hundreds of hectares that were fertilized before the research trials. Uniformity of N applications across the width of the applicator (i.e., among knives) was verified by remote sensing of corn canopy reflectance (Blackmer and White, 1998) each year the applicator was used. The ammonia was applied to three fields at a constant rate within each field. The rates ranged from 140 to 156 kg N ha⁻¹ for the fields fertilized in the late November of 1998 and 112 kg N ha⁻¹ for the fields fertilized in late November of 1999. Soil temperature at 1000 h on days of applications was always <8°C at a depth of 15 cm.

Follow-up studies (the third phase) involved sampling three 36-ha fields on 20 Dec 2001 after anhydrous ammonia (without inhibitor) was applied on 18 November. Fertilizer was applied at a rate of 140 kg N ha⁻¹. This follow-up study was prompted by unusually warm fall weather conditions and by observations that fall-applied N in the preceding years had nitrified more rapidly than initially expected.

None of the fields were tiled between fertilization and sampling, so tracks left by knives of the applicator could be identified in the spring. Soil samples in the second phase of the study were collected in April of 1999 and 2000 from 8 to 15 matched pairs (one with the inhibitor, one without the inhibitor) of test areas within each field as illustrated in Fig. 3. The matched pairs of test areas (3.5 × 6.2 m) were positioned so that apparent soil variability (i.e., elevation, soil series from survey maps, landscape position, surface roughness, and residue cover) within each pair of test areas was as small as possible, but apparent soil variability among test areas represented the widest possible range in landscape positions within the fields.

The soil in each test area was sampled within and between fertilizer bands at specified sampling points (Fig. 3) by using an auger 30 cm in diam. and powered by a gasoline engine. The sampling depth (45 cm) was selected to include fertilizer-derived NO₃⁻ that had moved downward from the fertilizer band but remained within the plant-rooting zone. Sampling to greater depths was not possible because fluctuating water tables often were found in the surface meter when the soils were sampled. The auger removed all soil from holes to the depth sampled. 25-kg samples of this soil were placed in tubs, and a small gas-powered rototiller was used to mix this soil. Two composite samples (500 g) were taken to the laboratory from each test area, one was derived from five sampling points
that centered on fertilizer bands and the other was derived from three sampling points collected between fertilizer bands (Fig. 3).

Soil sampling methods used in Phases 1 and 3 were similar to those used in Phase 2, but sampling in Phase 1 was done within single test areas rather than matched test areas. Other exceptions were that the sampling depth was 30 cm in Phase 1 and sampling time was mid-December in Phase 3.

Portions of the field-moist composite samples were extracted with 1 M KCl, and the extracts were analyzed for NO\textsubscript{3}\textsuperscript{-}\textsuperscript{−} and exchangeable NH\textsubscript{4}\textsuperscript{+} by using steam distillation as described by Keeney and Nelson (1982). Other portions were dried for determination of moisture and pH. Soil pH was determined on the soil samples collected between bands by using an ion selective electrode after adding deionized water (2.5 mL water g\textsuperscript{-1} soil).

Recovery (R) of anhydrous-ammonia-derived N (kg ha\textsuperscript{-1}) during sampling was calculated as

\[ R = KD(F_i - C_i), \]

where \( F_i \) refers to mg kg\textsuperscript{-1} of (NO\textsubscript{3}\textsuperscript{-} plus NH\textsubscript{4}\textsuperscript{+})-N in samples that included fertilizer bands and \( C_i \) refers to mg kg\textsuperscript{-1} of (NO\textsubscript{3}\textsuperscript{-} plus NH\textsubscript{4}\textsuperscript{+})-N in samples collected between fertilizer bands. The coefficient \( K \) is needed to convert concentrations in the area actually sampled (by the 30-cm auger) to the area (30 by 76 cm) assumed to be fertilized by the 30-cm segment of a band. \( D \) converts mg kg\textsuperscript{-1} of (NO\textsubscript{3}\textsuperscript{-} plus NH\textsubscript{4}\textsuperscript{+})-N to quantities of N (kg ha\textsuperscript{-1}) within the layer of soil sampled (6.72 when soils were sampled to 45 cm, and 4.48 when soils were sampled to 30 cm). Bulk density was estimated to be 1.45 g cm\textsuperscript{-3} on the basis of earlier measurements. Samples collected between bands were used as controls to distinguish fertilizer N from N already in soils because many studies (Blue and Eno, 1954; McIntosh and Frederick, 1958; Cochran et al., 1973; Hogg and Henry, 1982; Robbins and Voss, 1989) have shown that little NH\textsubscript{4}\textsuperscript{+} or NO\textsubscript{3}\textsuperscript{-} derived from anhydrous ammonia moves more than 10 cm laterally from the point of injection within the depths sampled.

Percentage recovery (\( P \)) of applied-N was calculated as

\[ P = 100R/A, \]

where \( R \) is defined in Eq. [1] and \( A \) is the rate (kg N ha\textsuperscript{-1}) of fertilizer-N application.

Percentage nitrification (\( Q \)) of fertilizer N was calculated as

\[ Q = 100KD(F_N - C_N)/R, \]

where \( F_N \) refers to concentrations of NO\textsubscript{3}\textsuperscript{-}N (mg kg\textsuperscript{-1}) collected in samples that included fertilizer bands and \( C_N \) refers to concentrations of NO\textsubscript{3}\textsuperscript{-}N (mg kg\textsuperscript{-1}) in samples collected between fertilizer bands. \( K, D, \) and \( R \) are defined as in Eq. [1].

The effects of soil pH on percentage recovery of N and percentage nitrification of fertilizer N were studied by simple regression analyses using a SAS statistical package (SAS Institute, 1996). Mean monthly air temperature and precipitation for the Central Iowa (measured at two locations) and mean 30-yr weather data (1971–2000) were obtained from National Climatic Data Center, Asheville, NC (available at: http://bws.ncdc.noaa.gov/oa/climate/onlineprod/drought/xmgrg1.html; verified 25 Nov. 2003).

RESULTS AND DISCUSSION

Percentage Nitrification

Percentage nitrification of fertilizer N between application and sampling increased linearly with increased soil pH in each of the 4 yr (Fig. 4). These relationships provide compelling evidence that rates of nitrification were influenced by soil pH in the range of 6 to 8.

A noteworthy exception to the linear pH effects occurred in 1999 when nitrification was essentially complete where the inhibitor was not applied. This exception illustrates that linear relationships between soil pH and percentage nitrification should be expected only when measurements are made within a specific period; nonlinear relationships should be expected if measurements are made slightly earlier or later than optimal. Even great effects of soil pH on nitrification rates cannot be detected if measurements are made too early or too late.

The follow-up study in the fall of 2001 (Fig. 4d) was prompted by recognition of the critical importance of sampling time when assessing the effects of soil pH on nitrification rates. It seemed likely that April of 1999
December were considerably above normal (mean temperatures were 9°C for November and 0.4°C for December). Observations made about a month after fall application confirmed that the effects of pH in the range of 6 to 8 were clearly expressed on nitrification if measurements were made at an appropriate time.

Substantial variation in percentage nitrification was observed within a narrow range of pH values in some years (Fig. 4). Such variation should be expected because soil pH values were measured on samples derived from all depths rather than the specific depth where nitrification occurred. Marked variation at high pH values in Fig. 4d, for example, can be explained if the sampling mixed highly calcareous materials from lower depths with relatively acidic soil at the depth of fertilizer injection. Such mixing was observed at some sampling points. Although problems associated with the mixing of soil materials from different depths undoubtedly reduced $r^2$ values for the relationships observed, these problems do not invalidate the conclusion that soil pH had important effects on rates of nitrification.

Important effects of pH on nitrification in the range of 6 to 8 may have gone undetected in the past because measurements were not made on time scales that could reveal these effects. The studies of Sahrawat (1982), for example, were conducted on a time scale appropriate for relatively slow nitrification in soils having pH $< 5$. Nitrification in soils having pH $> 6.0$ was essentially complete at the first sampling time, so any effects of pH on nitrification in the range of 6 to 8 could not have been detected.

There clearly is greater potential for expression of pH effects on nitrification in soils having fertilizer-derived NH$_4^+$ than in soils not having such NH$_4^+$. In our study, the mean concentration of NH$_4^+$ in samples collected between bands (0.9 mg N kg$^{-1}$) was much less than the mean concentration (13.2 mg N kg$^{-1}$) in samples that contained fertilizer bands. The mean concentration of NO$_3^-$ in samples collected between bands (6.5 mg N kg$^{-1}$) was much less than the mean concentration (36.0 mg N kg$^{-1}$) in samples that contained fertilizer bands. Unlike in recently fertilized soils, supplies of NH$_4^+$ generated by mineralization of soil organic matter often present the primary limitation on rates of nitrification in soils not recently fertilized. Reports that soil pH has no apparent effect on nitrification in nonfertilized soils (Grant, 1994; Stark and Firestone, 1996; Norton, 2000), therefore, do not contradict evidence that significant effects of pH occur in the soils where NH$_4^+$ is added.

The observed effects of pH on nitrification in the range of pH from 6 to 8 are consistent with observations of nitrifier growth in artificial cultures (Waksman and Starkey, 1931; Alexander, 1965; Norton, 2000). The amount of care taken to ensure that nitrifier activity is not limited by NH$_4^+$ may be a key factor explaining why the observed effects of pH on microbial growth in artificial cultures have seemed different than those observed during nitrification in soils. Abundant supplies of NH$_4^+$ and CO$_2$ in moist soils buffered at optimal pH values could be expected to essentially maximize rates of nitrification at any given temperature. The effects of temper-
ature still are important, however, and probably explain much of the variability in extent of nitrification among years.

**Nitrification Inhibitor Effects**

The effects of nitrapyrin were much greater in 1999 than in 2000 (Fig. 4). A likely reason for the difference between these years is that relatively cold weather in December preceding the 2000 crop resulted in less nitrification in the fall (Fig. 1). Delaying nitrification from fall to spring should be expected to reduce the effectiveness of nitrapyrin because this inhibitor gradually hydrolyzes to inactive forms in soils (Bremner et al., 1978; Hauck, 1980). By influencing when nitrification occurred in the soils, therefore, soil temperature influenced the effectiveness of the inhibitor. Analysis of variance showed that the effects of the nitrification inhibitor were significant ($p < 0.05$) even though regression analyses indicated that the effects of the nitrification inhibitor were less than the effects of half a pH unit for the data collected in 2000 (Fig. 4).

In 1999, the observed effects of the nitrapyrin were smaller in the higher-pH soils than in the lower-pH soils (Fig. 4). Hendrickson and Keeney (1979) observed similar effects of soil pH on inhibitor efficacy and suggested that the smaller effect of the nitrification inhibitor in high-pH soils could be attributed to rapid nitrifier recovery and growth after the inhibitor became inactive in the soil. This explanation is reasonable because nitrapyrin has been shown to be effective in high-pH soils (Bundy and Bremner, 1974; Blackmer et al., 1980). The smaller observed effects of inhibitor in the higher-pH soils, therefore, can be explained by recognizing that measurements were made too late to detect the effects of the inhibitor in the higher-pH soils even though they were not too late to detect these effects in the lower-pH soils.

The preceding discussion indicates that the effects of pH on rates of nitrification complicate the task of assessing efficacy of nitrification inhibitors. The observation that effects of inhibitors are more consistent in soils of the eastern than of the western Corn Belt (Mengel and Rehm, 2000) could be explained by recognizing that some of the soils in the western Corn Belt have higher pH values, the presence of $\text{CO}_2$, and higher rates of nitrification with and without nitrapyrin. There is need to explore the possibility that the effects of nitrification inhibitors would be more consistent if the inhibitors were used and evaluated with a greater appreciation for the effects of pH on nitrification rates.

**Recovery of Fertilizer Nitrogen**

Significant relationships between soil pH and recovery of fertilizer N as $(\text{NO}_3^- + \text{exchangeable NH}_4^+)$-N were observed for the data collected in 1998 and 1999 (Fig. 5a,b) but not for the data collected in 2000 and 2001 (Fig. 5c,d). Much of this difference among years is probably due to amounts of rainfall that occurred and, therefore, the amounts of NO$_3^-$ lost by leaching and denitrification. The amounts of rainfall that occurred between fertilizer application and soil sampling were greater in the first 2 yr than the second 2 yr.

Data for the 1999 cropping season are of special interest because, as shown in Fig. 4, the nitrification inhibitor greatly reduced percentage nitrification of fertilizer N.
in the lower-pH soils. Data presented in Fig. 5 show that this reduction in percentage of nitrification resulted in increased recovery of fertilizer N in this season, which had weather conducive for losses of NO$_3^-$ by leaching or denitrification. These observations illustrate that more rapid nitrification, whether due to higher pH or lack of inhibitor, increases the potential for losses of NO$_3^-$ by leaching or denitrification during spring rainfall.

Large amounts of rainfall tend to occur in May and June in this region (Fig. 2), so the effects of nitrification inhibitors can be masked by rainfall that occurs after nitrification is complete with the inhibitor. A key underlying problem is that nitrification inhibitors only delay nitrification in soils and the delays often are not long enough to prevent N losses. Because of differences in rates of nitrification, this seems to be a greater problem in the higher-pH soils than in the lower-pH soils.

Relationships analogous to those presented in Fig. 5 but based solely on recovery of fertilizer N as NH$_4^+$ (data not shown) clearly support the conclusion that nitrification rates were influenced by soil pH. Unlike data presented in Fig. 5, however, some of the relationships could be incorrectly interpreted by assuming that all effects of pH could be explained by greater volatilization of NH$_3$ in higher-pH soils. This observation illustrates that recovery of fertilizer N as NO$_3^-$ was necessary to show that soil pH had effects on nitrification in the soils.

Recoveries of fertilizer N as NO$_3^-$ and exchangeable NH$_4^+$ were highly variable within a narrow range of soil pH. It is not possible to determine the extent to which this variability was because of (i) nonuniform applications of fertilizer, (ii) errors associated with sampling, (iii) other factors affecting losses of N from soils, or (iv) transformations of N to forms other than NO$_3^-$ or exchangeable NH$_4^+$. The finding that large amounts of variability cannot be explained by the methods used, however, does not negate evidence that soil pH should be considered an important factor affecting nitrification and losses of fall-applied anhydrous ammonia.

CONCLUSIONS

Soil pH within the range of 6 to 8 must be considered an important factor affecting the risks and benefits associated with fall applications of anhydrous ammonia under climatic conditions found in the Corn Belt. Relatively rapid nitrification in the higher-pH soils increases the potential for early season losses of fertilizer N by leaching and denitrification of NO$_3^-$ before plants begin rapid growth and uptake of N in June. Although these effects of pH have not been generally recognized, they are consistent with observations made in studies of nitrifier growth in artificial media and in soil studies where the effects of pH were not limited by depletion of NH$_4^+$. Effects of pH within this range seem to have passed undetected because experimental methods have been designed to accommodate relatively slow rates of nitrification under more acid conditions. The previously unrecognized effects of soil pH on nitrification may help to explain the observed variability in the effectiveness of nitrification inhibitors in regions that have high-pH soils. The observed effects of pH on nitrification rates suggest that the economic and environmental benefits of delaying application of fertilizer N may be greater in higher-pH soils than in lower-pH soils.

ACKNOWLEDGMENTS

This study was made possible by grants from the Iowa Corn Promotion Board, the U.S. Department of State NIS Secondary School Partnership Program, and the NATO Science Fellowship Program of Spain.

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