

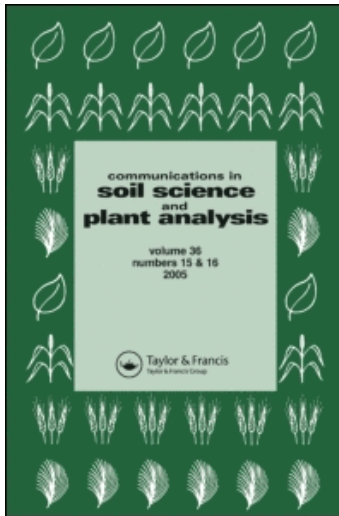
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Soil Acidity Changes in Bulk Soil and Maize Rhizosphere in Response to Nitrogen Fertilization

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Abstract: The capacity of nitrogen (N) fertilizers to acidify the soil is regulated principally by the rate and N source. Nitrogen fertilizers undergo hydrolysis and nitrification in soil, resulting in the release of free hydrogen (H⁺) ions. Simultaneously, ammonium (NH₄⁺) absorption by roots strongly acidifies the rhizosphere, whereas absorption of nitrate (NO₃⁻) slightly alkalizes it. The rhizosphere effects on soil acidity and plant growth in conjunction with N rate are not clearly known. To assess the impact of these multiple factors, changes in the acidity of a Typic Argiudol soil, fertilized with two N sources (urea and UAN) at two rates (equivalent to 100 and 200 kg N ha⁻¹), were studied in a greenhouse experiment using maize as the experimental plant. Soil pH (measured in a soil–water slurry), total acidity, exchangeable acidity, and exchangeable aluminum (Al) were measured in rhizospheric and bulk soil. Plant biomass and foliar area (FA) were also measured at the V₆ stage. Nitrogen fertilization significantly reduce the pH in the bulk soil by 0.3 and 0.5 units for low and high rates respectively. Changes in the rhizosphere (the “rhizospheric effect”) resulted in a significant increase in soil pH, from 5.9 to 6.2. The rhizospheric effect × N source interaction significantly increased exchangeable acidity in the rhizosphere relative to bulk soil, particularly when UAN was added at a low rate. Only total acidity was significantly increased by the fertilizer application rate. In spite of the bulk soil acidification, no significant differences in exchangeable aluminum were detected. Aerial biomass and FA were significantly increased by the higher N

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rate, but N source had no effect on them. Although changes in acidity were observed, root biomass was not significantly affected.

Keywords: Maize, nitrogen fertilization, rhizosphere, soil acidity

INTRODUCTION

Nitrogen (N) is the mineral element that plants require in the greatest amounts and whose availability often limits plant productivity in natural and managed ecosystems (Marschner 1995). Most plants obtain the vast majority of their nitrogen (N) through root absorption of the inorganic ions ammonium (NH_4^+) and nitrate (NO_3^-) from the soil solution (Bloom, Frensch, and Taylor 2003). One way these ions are provided to the plants is by N fertilizers. However, the use of N fertilizers can cause soil acidification (Hinsinger et al. 2003).

The problem of soil acidification resulting from the application of fertilizers has been known for many years. The number of locations where this problem has become an issue has risen substantially with the global expansion of synthetic fertilizer use (Calba et al. 2004). The acidification can result in negative impacts to root metabolism and decrease plant growth and yields when soil pH falls to less than critical thresholds (usually less than 5.5), which leads to increased activity of aluminum (Al) and manganese (Mn) (Tarkalson et al. 2006).

Nitrogen fertilizers containing ammonium or amida groups contribute to soil acidification because they undergo hydrolysis and nitrification in soil, resulting in the release of free hydrogen (H^+) ions. Although acidity generated by this process is usually not enough to change the pH in calcareous soils, it can cause pH-neutral soils to become acidic (Peryea and Burrows 1999). If fertilizers are applied exactly as needed by crops, they will have minimum effect on soil pH, but frequently, too much fertilizer is applied and soil acidification can occur (Zasoski et al. 1992). Many authors found decreases in soil pH after the addition of high quantities of urea to maize (Hoffmann et al. 1994; Rodríguez, Taboada, and Cosentino 1999).

The acidification capacity of N fertilizers is characterized using the calcium carbonate equivalent (CCE), usually expressed as pounds of pure calcium carbonate required to neutralize 100 pounds of N in a particular fertilizer (California Fertilizer Association 1995). The CCE is often considered a constant value. It has been established that ammonium sources produce acidity in the soil equivalent to 3.57 calcium carbonate (CaCO_3) neutralization capacity kg N^{-1} . However, the behavior of fertilizer that elicits a change in soil pH does not always follow the

pattern described in fertilizer handbook charts because it is affected by several factors (Peryea and Burrows 1999).

The N fertilizer most used worldwide is urea, and in some countries, urea–ammonium nitrate (UAN) is also heavily used. Although their CCE values are similar each other, the formulations are different. Urea provides N in an organic form, whereas UAN formulation is half organic and half inorganic. This different formulation can affect rhizosphere pH, which varies mainly because of ions related to mineral uptake (Durand, Bellon, and Jaillard 2001). The “acid-growth hypothesis,” originally postulated to explain auxin-induced growth, may apply to N-induced growth (Rayle and Cleland 1992). Plant N metabolism alters rhizosphere pH: NH_4^+ assimilation releases protons, whereas NO_3^- assimilation produces hydroxide ions. Plants supplied with NH_4^+ as the N source strongly acidify the rhizosphere and those supplied with NO_3^- slightly alkalize it. According to this, Hoffmann et al. (1994) observed that rhizosphere pH decreased after $\text{NH}_4\text{-N}$ application by 0.1 to 0.5 units as compared to the bulk soil, whereas in those fertilized with $\text{NO}_3\text{-N}$, pH increased by 0.1 to 0.5 units. Significant differences, therefore, can be expected between rhizosphere and the bulk soil when different N sources are added.

From an ecological standpoint, the acidity changes are highly relevant because of the importance of soil pH in many biotic and abiotic activities. The rhizosphere pH changes may be responsible for the differential patterns of root growth observed under NH_4^+ vs NO_3^- . Root development responds not only to the quantity of inorganic N in the rhizosphere but to N forms NH_4^+ or NO_3^- . Root extension and mass of maize seedlings were greater in nutrient solutions containing NH_4^+ than in those containing NO_3^- (Bloom, Frensch, and Taylor 2003). Then, interactions between N formulations and rates may influence the rhizospheric and bulk soil pH, thus affecting the plant growth. The objective of the current study was to explore the impact of N source and rate on soil acidity in rhizospheric and bulk soil and maize growth.

MATERIALS AND METHODS

Soil, Plant, and Experimental Design

A greenhouse experiment was developed in the College of Agronomy of the Buenos Aires University (Buenos Aires, Argentina). The soil was the A horizon (0–20 cm) of a Typic Argiudoll, which is characterized by a loam texture, pH (1:2.5 soil–water ratio) of 5.3, 1.8% total carbon (C), and 0.15% total N. The soil was distributed to 3-dm³ plastic pots (3.6 kg per pot), which were placed in a greenhouse for the study.

The test plant was maize (*Zea mays L.*). Seeds were pregerminated in sterile conditions until the root appeared. Three germinated seeds were then placed in each pot at a depth of 0.03 m. Maize plants were grown during the southern hemisphere spring months under ambient daylight. Pots were watered daily to maintain the soil water content at field capacity (33 Kpa), which was determined before test initiation. Upon emergence (V_E), two plants were removed from each pot.

The experiment was conducted according to a factorial arrangement (CRD) with three factors and two levels for each: N sources (urea, 46% N, and UAN, 30% N), N rates (N 100 and N 200 equivalent to 100 and 200 kg N ha⁻¹, respectively), and soil [rhizospheric (RS) and bulk soil (BS)]. Urea is a source of organic N [CO(NH₂)₂] whereas UAN is a solution with both organic and inorganic N [50% urea, 50% ammonium nitrate (NH₄NO₃)]. The CCE for urea and UAN (as assumed by the fertilizer industry) is 84 and 58 kg of CaCO₃ per 100 kg of fertilizer, respectively (Soil Improvement Committee 1984).

Soil Determination

At the V6 stage, the maize plants were gently removed from the pots. Rhizospheric soil (RS) was considered the fraction adhered to the surface roots. It was collected for analysis by gently shaking the roots. The remaining soil in the pot was considered bulk soil (BS). In both soils, (i) actual pH (1:2.5 soil–water) (Sparks 1996), (ii) exchangeable acidity (EA) and exchangeable Al (EAl) [potassium chloride (KCl) 1 M as described by Thomas (1982)], and (iii) total acidity with trietanolamine [TEA–barium chloride as described by Peech (1965)] were determined.

Plant Determination

Root and aerial biomass samples were dried at 60 °C until constant weight. Foliar area (FA) of each treatment was also determined using the LI-3100 area meter (LI-COR, Inc., Nebraska, USA).

Proton Balance Calculation

Proton balance was calculated using the following equation (Bouman et al. 1995):

$$\frac{\text{M. acidity (A)}}{\text{Th. acidity}} = \frac{\text{M. acidity (A)}}{\text{Pot acid (B) + Prot plant(C) - Ex. bases(D)}}$$

where (A) measured acidity is exchangeable acidity values expressed as $\text{cmol}_c \text{kg}^{-1}$, (B) Potential acidity is potential acidity (A) due to the addition of N sources calculated by assuming that all of the N fertilizer was transformed to nitrate at a 1:1 ratio. Volatilization was discarded. Thus, 1 mol of nitrate yielded 1 mol of N and released 1 mol of H^+ ($\text{N mol}/\text{H}^+ \text{ mol}$), (C) Protons neutralized by the plant is quantity of H^+ released from the root in response to the N-NO_3^- absorbed by the plant and estimated from maize biomass and its N content, (D) Excess of bases is acidity caused by the excess of absorbed cations, considered negligible.

Statistical Analysis

The data were analyzed with the Statistix program (Analytical Software, Tallahassee, Florida, USA). We used analysis of variance (ANOVA) to distinguish the significant effects of rate, sources, and rhizospheric effect on the measured parameters ($P < 0.05$). The least significant differences (LSD) test was used to compare the means when the F-test indicated significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

The three factors (rate, source, and rhizospheric effect) influenced actual pH and exchangeable and total acidity (Table 1).

The RS actual pH (mean 6.2) was significantly higher than BS pH (mean 5.9) (Figure 1). These results indicate that, in the rhizospheric zone, hydroxyl (OH^-) ions released by the roots equilibrated with the soil

Table 1. ANOVA results for actual acidity, exchangeable acidity, and total acidity

Source of variation	ANOVA (Pr > F)		
	Actual acidity	Exchangeable acidity	Total acidity
Rate (R)	0.170	0.076 *	0.013 **
Source (S)	0.330	0.430	0.64
Rhizospheric effect (RE)	0.005 ***	0.015 **	0.37
R × S	0.560	0.780	0.46
R × RS	0.200	0.480	0.93
S × RE	0.600	0.045 **	0.27
R × RE × S	0.780	0.900	0.23

*, **,***Significant differences at $P < 0.10$, $P < 0.05$, and $P < 0.005$, respectively.

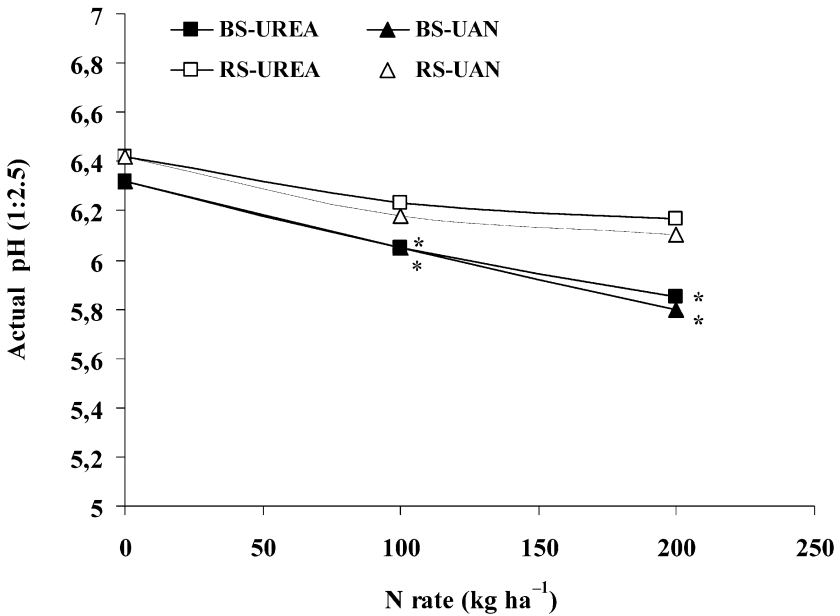


Figure 1. Changes of the actual pH measured in the V6 stage in rhizospheric (RS) and bulk soil (BS) of maize after the addition of N 100 and N 200 as urea and UAN sources. An asterisk (*) indicates significant differences between treatments for the same N rate ($P < 0.05$).

chemistry and caused a 0.3 pH rise. In contrast, Calba et al. (2004) observed that 14 days after ammonium fertilization, soil pH decreased 0.5, 0.9, and 1 units, respectively, in the maize rhizosphere of three different soils. Such decreases were due to H^+ release from the root in response to cationic absorption [calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+})], which is typical in crops such as maize where more cations than anions are absorbed (Wallace 1994). However, in our experiment, the acidity generated by the treatments was equilibrated by the release of OH^- in response to the nitrate absorption. As a result, in the rhizospheric zone, the application of the equivalent of 100 and 200 kg N ha⁻¹ of urea and UAN at sowing time did not cause a pH drop in the soil solution (Figure 1).

In bulk soil, actual pH decreased relative to the control by 0.3 and 0.5 units for N 100 and N 200, respectively. No significant differences between rates or sources were observed, indicating that the different NO_3^-/NH_4^+ of each fertilizer did not alter soil pH.

Whereas pH in a soil solution is frequently used to evaluate soil acidity, the exchangeable acidity is useful in estimating acidification potential. The exchangeable acidity values observed in the experiment are shown in Figure 2.

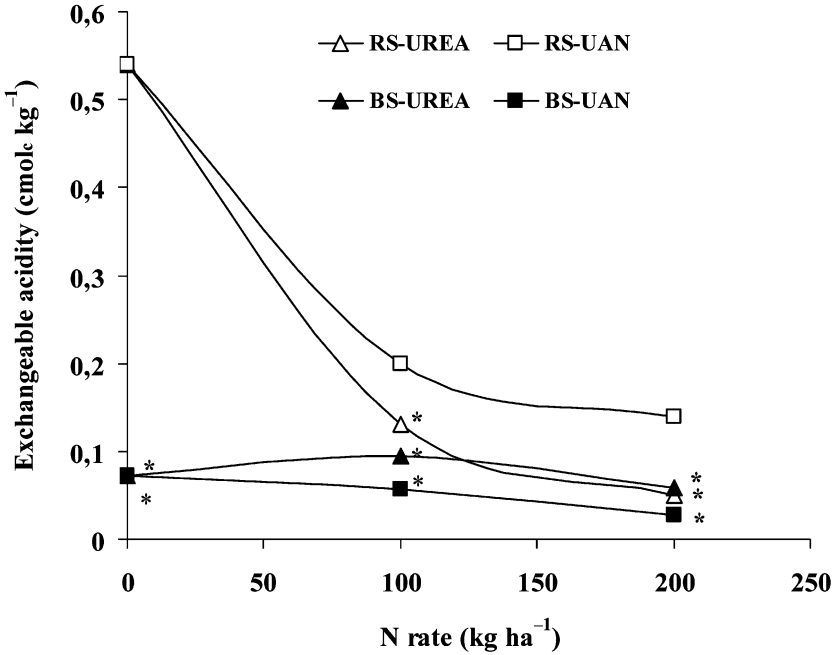


Figure 2. Exchangeable acidity values for different N rates measured in rhizospheric (RS) and bulk soil (BS). An asterisk (*) indicates significant differences between treatments for the same N rate ($P < 0.05$).

The EA values varied from 0.07 to 0.12 $\text{cmol}_c \text{kg}^{-1}$ and were significantly affected by the interaction source \times rhizospheric effect (Table 1). In general, the EA values in the RS samples were higher than in the BS samples, which suggests that the rhizospheric effect provoked a decrease in soil pH in the exchangeable complex due to a flux of nonneutralized H^+ as a consequence of nitrification by the fertilizers. This difference occurred despite the release of OH^- from the root, which was insufficient to equilibrate all the H^+ derived from the oxidation of N sources, thus increasing the exchangeable acidity. The soil treated with UAN resulted in more acid in the RS treatments compared to BS. In contrast, the urea treatment did not alter the exchangeable pH in either the RS or BS samples. Although there were significant differences between values, all of them were less than 0.1 $\text{cmol}_c \text{kg}^{-1}$ (0.027 – 0.094 $\text{cmol}_c \text{kg}^{-1}$), which supports the conclusions of Curtin, Campbell, and Messer (1996) and Fabrizzi et al. (1998), who reported similar pH values.

Total acidity were significantly affected by the N application rate ($P = 0.013$). Mean total acidity increased from 6.57 to 8.21 at N 100 and N 200, respectively. Neither soil type (RS or BS) nor N source appeared to impact acidity (Figure 3).

These data did not correspond with the results of the proton balance (Table 2). The measured acidity was lower than the theoretical one in almost all the treatments, a trend observed by other authors (Bouman et al. 1995). In soils of the same region, Fabrizzi et al. (1998) found that measured acidity represented only the 4.7% of theoretical acidity. This difference was attributed to N losses associated with two processes, denitrification and volatilization, both of which would help to balance the acidifying effect of the ammonium. In our experiment, the measured acidity represented 70.6% and 58.3% of the theoretical acidity for N 100 and N 200 treatments, respectively, indicating that theoretical acidity overestimates the impact of N fertilizers, particularly at a high dose, and thus is not a good predictor of in situ effects on soil acidity.

Even though the measured acidity values were all lower than the theoretical ones, we observed differences between treatments. The UAN application resulted in more acidification, especially in RS samples at a rate of N 100 (Figure 3), probably because of the NH_4^+ contribution in the fertilizer. This trend may explain the decrease in rhizospheric soil pH,

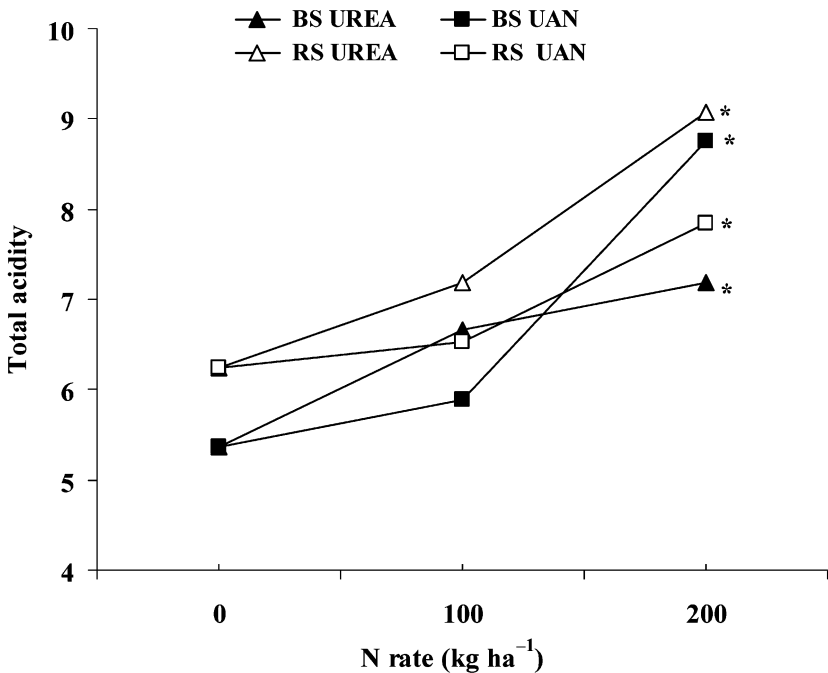


Figure 3. Effect of rate of fertilizer on total acidity in rhizospheric soil samples fertilized with urea and UAN (RS-urea and RS-UAN respectively) and bulk soil samples fertilized with urea and UAN (BS-urea and BS-UAN respectively). An asterisk (*) indicates significant differences between treatments for the same N rate ($P < 0.05$).

Table 2. Proton balance calculated for the rhizospheric and bulk soil samples (RS and BS respectively) fertilized with urea and UAN with the equivalent of 100 and 200 kg N ha⁻¹

Treatment	Potential acidity	Excess of bases	Theoretical acidity	Measured acidity	Ratio
N source	Rate	(cmol _c kg ⁻¹)	(cmol _c kg ⁻¹)	(cmol _c kg ⁻¹)	(D / C) × 100
	A	B	C = A - B	D	
Urea	100	0.47	0.30 ^a	0.13 (RS)	76.50
				0.09 (BS)	52.90
	200	0.93	0.45 ^b	0.05 (RS)	10.40
				0.06 (BS)	12.50
UAN	100	0.47	0.30 ^a	0.20 (RS)	117.60
				0.06 (BS)	35.30
	200	0.93	0.45 ^a	0.14 (RS)	29.20
				0.03 (BS)	6.25

^aQuantity of OH⁻ released by the maize plants during the period sowing—V6 for N 100 (corresponded to the absorption of the 10% of the total N requirement for the total cycle).

^bQuantity of OH⁻ released by the maize plants during the period sowing—V6 for N 200 (corresponded to the absorption of the 10% of the total N requirement for the total cycle).

as the impact of the N ions is of sufficient strength to partially overcome the neutralizing effect of OH⁻ released from the plant. Fertilization-related acidity would be completely neutralized if all of the added nitrate is absorbed by the plants (Bolan, Hecley, and White 1991). Instead, the plants only absorbed a portion of the total amount.

Although in our experiment, the observed decrease in pH did not appear to be correlated with incremental fluctuations in exchangeable aluminum, other researchers have identified that connection, attributing it to the dissolution of labile forms of Al (Calba et al. 2004). However, even in those cases, a drop in pH was insufficient to provoke the release of Al from more recalcitrant aluminosilicate structures.

The aerial biomass and the foliar area of maize varied significantly (P < 0.02) with the fertilizer application rate. The incremental changes were independent of the N source. Conversely, root biomass was incremented by the N fertilization, but no differences between rates, sources, or the observed variations in acidity were detected (Table 3).

Bloom, Frensch, and Taylor (2006) observed that the presence of NH₄⁺ stimulated root elongation and accumulation of root biomass to a greater extent than that of NO₃⁻. The lower energy requirement for NH₄⁺ assimilation may permit cells to maintain higher elongation rates and to accumulate more biomass. Although urea and UAN differ in their NH₄⁺ and NO₃⁻ content, no differences in root biomass were observed.

Table 3. Root and aerial biomass and foliar area (FA) of maize plants fertilized with urea and UAN with the equivalent of 100 and 200 kg N ha⁻¹ (urea 100, UAN 100; UREA 200, and UAN 200 respectively)

Treatments	Root biomass (g plant ⁻¹)	Aerial biomass (g plant ⁻¹)	Foliar area (FA) (cm ² plant ⁻¹)
Control	6.73 a	5.62 a	767.55 a
Urea 100	8.57 a	8.57 abc	1159.67 b
UAN 100	8.29 a	7.09 bc	935.03 b
Urea 200	8.26 a	11.45 c	1416.38 c
UAN 200	8.93 a	10.17 ab	1430.75 c

Note. Different letters mean significant differences between treatments ($P < 0.05$).

Because no rhizosphere pH changes were observed as a result of urea and UAN application, no differential patterns of root growth should be expected.

In summary, the results of this study indicate that no differences in the rhizosphere pH should be expected if urea or UAN are used as N fertilizers in the maize crop. The differences in their NH₄⁺ and NO₃⁻ content are not large enough to generate differences in the root biomass accumulation. However, a significant soil pH decrease was observed, particularly in the bulk soil, thus reflecting the rhizosperic effect on the soil pH. The proton balance data demonstrated that the theoretical acidity overestimated the effect that N fertilization would have on soil acidification, particularly at a high dose. Therefore, collecting empirical data on the different forms of acidity in the rhizosphere and bulk soil is necessary to establish the real impact of N fertilization on acidification risk.

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