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PHYSIOLOGICAL AND PRACTICAL IMPLICATIONS OF DIFFERENT FORMS
OF NITROGEN NUTRITION IN MAIZE

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Physiological and practical implications of different forms of nitrogen nutrition in maize

by

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For the Graduate College

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INTRODUCTION

Corn (Zea mays L.) can absorb and utilize moderate concentrations of both ammonium ($\text{NH}_4^+$) and nitrate ($\text{NO}_3^-$) ions (Warncke and Barber, 1973; Blair et al., 1970; Bennett et al., 1964; DeKock and Kirkby, 1969; Schrader et al., 1972). At high concentrations, $\text{NH}_4^+$ can be toxic to plants. Nitrate, in contrast, is the safer nitrogen (N) source but under field conditions it is subjected to losses by leaching (due to its mobility) and denitrification. The major site of $\text{NH}_4^+$ assimilation in plants is the roots, and the N is exported to the shoots as a mixture of amino acids and amides (Yoneyama and Kumazawa, 1974). There may be some free $\text{NH}_4^+$ in the xylem (Weissman, 1972). Nitrate assimilation can occur in the roots and/or shoots (Pate, 1973); consequently, the transport product can be amino acids, amides or free nitrate. Assimilation of N involves the production of little more than one $\text{H}^+$ per N when $\text{NH}_4^+$ is the N source and one $\text{OH}^-$ per N when $\text{NO}_3^-$ is the N form (Raven and Smith, 1976). Because of differences in assimilation and charge, the form of N absorbed has significant effects in plant nutrition and metabolism; moreover, under field conditions, the predominant form of N has important implications with respect to the efficiency of N fertilization.

The general objective of this work was to study the
physiological and practical implications of different forms of N nutrition in corn. The specific objectives were, among others, to study: (1) the effect of nitrate-N/ammonium-N (NO$_3^-$-N/NH$_4^+$-N) ratio and form of N in general on N uptake, growth, percentage NO$_3^-$ uptake, percentage NH$_4^+$ uptake, and nitrate reductase activity of corn plants growing in soil; (2) the mineral composition of corn plants as affected by the form of N in the soil; (3) the effect of ions other than N on N uptake; (4) the cytoplasmic and vacuolar pH of excised corn root tips as affected by the form of N under normal and hypoxic conditions; (5) internal NO$_3^-$ and NH$_4^+$ concentration and organic acid content in roots as a function of the N form; (6) effect of the form of N and split N applications on the efficiency of N fertilization in the field.

The results are interpreted with respect to the models presented in the literature.
Warncke and Barber (1973) reported that their greatest dry matter yields of corn (grown in nutrient solution) were recorded with 0.94 to 4.24 ppm concentrations of N regardless of the NH$_4$-N to NO$_3$-N ratio. As N concentration increased above 4.24 ppm, yield was lower with the higher NH$_4^+$ to NO$_3^-$ ratios. Blair et al. (1970) grew corn equally well in nutrient solution with either NH$_4^+$ or NO$_3^-$ as long as the N concentration was kept low. With higher N concentration, NO$_3^-$ plants yielded more than NH$_4^+$-N plants. Schrader et al. (1972) reported that fresh and dry weight per corn plant grown in nutrient solution generally increased more rapidly when plants were provided 100 ppm of N as a combination of NO$_3^-$-N and NH$_4$-N than when 100 ppm of either NO$_3$-N and NH$_4$-N was supplied alone. They also found that uptake of NO$_3^-$ was not retarded by the presence of NH$_4^+$ but assimilation of NO$_3^-$ into organic N was retarded by NH$_4^+$. So, NH$_4^+$ was used preferentially for synthesis of amino acids and protein.

Dibb and Welch (1976) reported that yield of corn plants grown in soil was not negatively affected by form of N absorbed when the meq ratio of applied N:K was 2:1. They suggested that the critical NH$_4^+$:K interaction was probably inside the plant rather than competition for uptake and that increased K is necessary as more of the N was absorbed as NH$_4^+$. 
There also are data on other species. Cox and Reisenauer (1971) found higher yields of wheat (\textit{Triticum aestivum} L.) with a combination of \text{NH}_4^+ and \text{NO}_3^- than with either source alone in a continuous flow culture system. Weissman (1964) reported that dry weight, total protein content, protein concentration, and protein as a percentage of total N were all higher in leaves from sunflower (\textit{Helianthus annus} L.) plants grown on \text{NH}_4^+ plus \text{NO}_3^- than on either source alone. Gamborg (1970) reported that soybean (\textit{Glycine max} (L.) Merr.) cell suspension cultures did not grow on \text{NO}_3^- unless supplemented with \text{NH}_4^+ or glutamine. McElhannon and Mills (1977) reported greater dry matter production in snapbean (\textit{Phaseolus vulgaris} L.), lima bean (\textit{Phaseolus lionensis} L.) and southern field pea (\textit{Vigna sinensis} L.) seedlings grown in a solution for 14 days with a mixture of \text{NH}_4^+ and \text{NO}_3^- as the source of N than with either source alone. Tomato plants (\textit{Lycopersicum esculentum} L.) grown under constant pH in a continuous flow solution containing 50/50% mixture of \text{NH}_4^-N/\text{NO}_3^-N showed the most growth and dry matter production (Ganmore-Neumann and Kafkafi, 1980a,b). Gashaw and Mugwira (1981) found that triticale (\textit{X Triticasecale}, Wittmack), wheat and rye (\textit{Secale cereale} L.) seedlings grown in nutrient solutions consisting of 25/75, 50/50, and 75/25 \text{NH}_4^-N/\text{NO}_3^-N ratios produced the greatest dry matter.

Hiatt and Leggett (1974) concluded that ion absorption
by system II (which is the absorption system that is functional when the external ion concentration exceeds approximately 1 mM) is strongly influenced by the rate of absorption of the counter ion and requires that cations and anions be absorbed in equivalent quantities. Consequently, the form of N absorbed may influence the uptake of other ions. Many experiments report increased P uptake when NH$_4^+$ replaces NO$_3^-$ as the N source (Mattson, 1966; Harada et al., 1968). Dijkshoorn and Van Wijk (1967) suggested that plants supplied with NH$_4^+$ salts should contain a greater amount of sulfate than those given NO$_3^-$. Blair et al. (1970) found higher plant levels of P and S in the NH$_4^+$ treatment and higher Ca and Mg in the NO$_3^-$ treatment, indicating a predominantly cation-anion balance effect. They did not find differences in yields of tops or roots between the two N sources. Kirkby and Hughes (1970) reported that plants supplied with NH$_4^+$ usually contained lower concentrations of Ca, Mg, and K than when NO$_3^-$ was the N source. Dibb and Welch (1976) found that the cation-anion relationship within the plant was altered dependent on the form of N absorbed. Their results suggest that the use of nitrification inhibitors may affect the cation concentration of corn plants. Wilcox et al. (1973) suggested that maintaining N in the NH$_4^+$ form would reduce Mg uptake by plants. Other studies in soil reveal little effect of high NH$_4^+$ concentrations on the uptake of Mg (DeKock and
Kirkby, 1969). Results from Kirkby and Mengel (1967) and Kirkby and Knight (1977) show for tomato plants that anion uptake decreases and cation uptake increases as more of the N is supplied as NO$_3^\text{-}$. Magalhaes and Wilcox (1983a,b) also working with tomato plants found that NH$_4^+ \text{ nutrition suppressed}$ cation accumulation in tissue and increased P content when compared to NO$_3^\text{-}$ nutrition.

Warncke and Barber (1973) reported that, at low total N concentration (less than 67 $\mu$m), NH$_4^+$ and NO$_3^-$ were absorbed at nearly the same ratio as they were supplied in nutrient solution. As N concentration increased, the ratio of NH$_4^+$-N/NO$_3^-$-N absorbed by the corn seedlings moved toward 1.0 as compared to the ratio in the solution. Hence, corn seedlings presumably prefer a balanced uptake of NH$_4^+$ and NO$_3^-$. The data presented by Schrader et al. (1972) indicate that total N uptake per unit of root fresh weight was higher when N was supplied as a combination of NO$_3^-$ and NH$_4^+$ than with either source alone.

In general, the literature indicates that plants prefer a balanced NH$_4^+$ and NO$_3^-$ nutrition (with better growth and more nitrogen uptake) and that the inorganic cation-anion balance is affected by the form of nitrogen. Many models in the literature attempt to explain the mechanisms of ion uptake and how they are affected by the N form.

If uptake of anions by the roots exceeds cation uptake,
HCO$_3^-$ or OH$^-$ must be transported outwardly from the cells to satisfy the charge difference. On the other hand, when cation uptake is in excess, H$^+$ must be released from the roots (Hiatt, 1967). As a consequence of this, the cytoplasmic pH would be altered. The activity of several enzymes would be influenced by this change in pH in a way leading to organic acid synthesis or organic acid breakdown in order to neutralize the effect of the unbalanced cation-anion nutrition. This "pH stat" mechanism would operate with two enzymes involved in CO$_2$ dark fixation and organic acid metabolism as follows (Smith and Raven, 1976; Lütge and Higinbotham, 1979):

\[
\begin{array}{ccc}
\text{high pH} & \text{low pH} \\
\text{PEP} & \text{Malic enzyme} & \text{PEP} \\
\text{carboxylase} & \text{CO}_2 & \text{Malate} \\
\text{OAA} & \text{Pyruvate} & \text{CO}_2
\end{array}
\]

Ben-Zioni et al. (1971) postulated that, as NO$_3^-$ is being taken up, it is translocated with cations to the shoots where assimilation takes place. The reduction of NO$_3^-$ stimulates the synthesis of organic anions (malate) which are moved down to the roots (with cations as counter ions) and oxidized yielding HCO$_3^-$.

The generated HCO$_3^-$ is exchanged for NO$_3^-$ allowing further N uptake. According to this scheme, assimilation of NO$_3^-$ in the shoots controls or regulates its uptake by the roots.

Raven and Smith (1976) presented a complete review of N assimilation and transport in plants, with emphasis on aspects
of intracellular pH regulation. For the case of NH₄⁺ assimilation, they presented a model in which the incorporation of NH₄⁺ into organic compounds in the roots produces a H⁺ which is exchanged for external NH₄⁺ maintaining constant charge and pH in the roots. There may be some free NH₄⁺ in the xylem sap (Weissman, 1964, 1972); in this case, the pH stat mechanism would operate in shoots and roots. In roots, organic acid synthesis would produce H⁺ to exchange for NH₄⁺ in the soil solution; NH₄ malate would move to the shoot where malate is broken down to neutralize the acidity produced during NH₄⁺ assimilation in shoots. Based on measurements of vacuolar and phloem pH, they concluded that vacuolar storage or transport to the roots of the H⁺ generated during NH₄⁺ assimilation is impossible. In conclusion, all the H⁺ generated during NH₄⁺ assimilation would be lost to the soil solution.

Nitrate assimilation can occur in roots or shoots (Pate, 1973). According to Raven and Smith (1976), assimilation of NO₃⁻ in roots generates OH⁻ groups that are exchanged for NO₃⁻ from the soil solution or neutralized by the pH stat mechanism. The organic acids produced would move to the vacuole together with the cations that accompanied NO₃⁻ uptake. When NO₃⁻ is assimilated in the shoots, the situation is different; NO₃⁻ could move up to the shoot with a cation as a counter ion (K) and the OH⁻ produced during NO₃⁻ assimilation
would be balanced by organic acid synthesis. The organic acid would move together with the cation into the vacuole. This model does not involve OH\textsuperscript{−} excretion to the rooting medium. In corn, half of the OH\textsuperscript{−} generated is excreted out of the roots (Coic, 1971; Dijkshoorn, 1971). A model involving K recirculation (KNO\textsubscript{3} moving up and K malate down), organic acid breakdown in roots and organic acid synthesis in shoots would explain these observations. According to the model, some of the malate generated in shoots is transported to the roots with K as counter ion. In roots, a NO\textsuperscript{−}−OH\textsuperscript{−} exchange across the plasmalemma tends to produce an acidification of the cytoplasm. Then, the pH stat mechanism breaks down the organic acids coming from the shoots, generating OH\textsuperscript{−}. This balances the pH and allows further NO\textsubscript{3}\textsuperscript{−} uptake. This model is similar to that proposed by Ben Zioni et al. (1971). Blevins et al. (1978) concluded that K malate is cycled from the tops to the roots. This finding supports the previous model. Moreover, Kirkby and Armstrong (1980) presented evidence suggesting that NO\textsubscript{3}\textsuperscript{−} uptake by roots is regulated by NO\textsubscript{3}\textsuperscript{−} assimilation in the shoots. This would involve K recirculation. Previously, Armstrong and Kirkby (1979) calculated for tomato plants that about 20% of the upward flux of K in the xylem results from K recirculation. Nitrate transport to the shoots requires the presence of a counter ion. Frost et al. (1978) demonstrated that wheat
seedlings preloaded with cations showed enhanced NO\textsubscript{3}\textsuperscript{−} uptake and NO\textsubscript{3}\textsuperscript{−} reduction even though the absorption of the cations and NO\textsubscript{3}\textsuperscript{−} were separated in time. Moreover, Rufty et al. (1981) reported that the presence of K stimulated NO\textsubscript{3}\textsuperscript{−} translocation to the shoots and reduced the amount of NO\textsubscript{3}\textsuperscript{−} assimilated in the roots.

Dejaegere et al. (1981) presented a model for ion uptake. They proposed that cation uptake is driven by the electrochemical gradient resulting from H\textsuperscript{+} extrusion (ATPase). The HCO\textsubscript{3}\textsuperscript{−} ions left over by the H\textsuperscript{+} efflux are either used for organic acid synthesis or exchanged for anions. Thus, inside the plant, the cations are neutralized by organic acids and by inorganic anions. In relation to nitrogen nutrition, they postulated that there are just as many HCO\textsubscript{3}\textsuperscript{−} ions produced inside the cell as NH\textsubscript{4}\textsuperscript{+} ions absorbed and that all the HCO\textsubscript{3}\textsuperscript{−} is used when the NH\textsubscript{4}\textsuperscript{+} is assimilated. They also stated that NO\textsubscript{3}\textsuperscript{−} sustains its own uptake since the HCO\textsubscript{3}\textsuperscript{−} generated in the reduction of NO\textsubscript{3}\textsuperscript{−} in roots is used for further NO\textsubscript{3}\textsuperscript{−} uptake. This model would not be appropriate for plants that reduce NO\textsubscript{3}\textsuperscript{−} in shoots and/or accumulate NO\textsubscript{3}\textsuperscript{−} or NH\textsubscript{4}\textsuperscript{+} in roots. The consequences of this model would be that NH\textsubscript{4}\textsuperscript{+} uptake does not bring about any cation uptake and that NO\textsubscript{3}\textsuperscript{−} is the only anion whose absorption does not depend on cation uptake.

Smith (1973), working with excised barley roots, found that NO\textsubscript{3}\textsuperscript{−} uptake was greater from KNO\textsubscript{3} than from Ca(NO\textsubscript{3})\textsubscript{2}. 
He speculated that absence of K uptake would reduce the activity of the K/H\(^+\) exchange system and, via feedback from change in cytoplasmic pH, reduce anion (NO\(^-\)) uptake. However, if organic acid content of the root is high, breakdown of organic acids by the pH stat mechanism would produce HCO\(_3^-\) which would allow NO\(^-\) influx to proceed.

Feedback regulation of ion uptake has been proposed by several researchers and could have an important role in the uptake processes. It was already mentioned that Smith (1973) proposed a feedback regulation of ion uptake based on cytoplasmic pH changes. Cram (1973) showed that the main factor determining the decrease in active Cl influx during salt accumulation is the concentration of Cl plus NO\(^-\) in the vacuole. Net NO\(^-\) influx is similarly reduced by high Cl concentration in the vacuole. He speculated that the negative feedback could be due to (1) a direct or 2nd messenger allosteric feedback on the pumps, (2) electropotential and (3) control of energy supply for active uptake. Moreover, Glass (1975) found that plasmalemma K influx was inversely correlated with internal K concentration, indicating the possibility of the existence of a feedback mechanism.

Recent studies (Deane-Drummond and Glass, 1983) using \(^{36}\)ClO\(^-\) as tracer for NO\(^-\) indicate that NO\(^-\) influx appears to be independent of NO\(^-\) concentration inside the cells, whereas NO\(^-\) efflux is strongly correlated with internal NO\(^-\)
concentration of the roots. This form of control is referred to as pump and leak (Glass, 1983). Some models (for barley roots and algae) explain in a very simple way the reduction in ion uptake when its internal concentration is high; the dissociation of the carrier-ion at the cytoplasmic phase is reduced because of the high cytoplasmic concentration of that ion (Glass, 1983).

Warncke and Barber (1973) reported that the ratio of \( \text{NH}_4^- \)/\( \text{NO}_3^- \) absorbed by corn seedlings moved toward 1.0 as compared to the ratio in the solution. Hence, corn seedlings presumably prefer a balanced uptake of \( \text{NH}_4^+ \) and \( \text{NO}_3^- \). The feedback and pump and leak models (Cram, 1973; Glass, 1975; Smith, 1973; Deane-Drummond and Glass, 1983) would explain this observation. It has been documented that net \( \text{NO}_3^- \) influx is reduced by prior accumulation of \( \text{NO}_3^- \) in roots (MacKown et al., 1981; Jackson et al., 1976; Smith, 1973). Another explanation would be that excess \( \text{NO}_3^- \) nutrition creates internal and external conditions that favor \( \text{NH}_4^+ \) uptake and, conversely, excess \( \text{NH}_4^+ \) nutrition creates internal and external conditions that favor \( \text{NO}_3^- \) uptake.

A combination of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) seems to be better for plant growth and N uptake. Moreover, a balanced \( \text{NO}_3^- \)/\( \text{NH}_4^- \) ratio could be optimum in the sense of maintaining a good balance of all the other ions.

Raven and Smith (1976) said that when both \( \text{NH}_4^+ \) and \( \text{NO}_3^- \)
are assimilated simultaneously, the pH stress in the cytoplasm of the assimilating cells can be less than when a single N source is employed (provided that NO$_3^-$ and NH$_4^+$ are assimilated in the same cells). Cation-anion unbalances would produce similar pH stresses in the cytoplasm of root cells; however, Roberts et al. (1981, 1982) using nuclear magnetic resonance as a way to measure cytoplasmic and vacuolar pH (Roberts, 1984; Roberts et al., 1980; Shulman, 1983) found that intracellular pH is tightly regulated when excised corn root tips absorb an unbalanced cation-anion ratio. This result is against the pH stat mechanism hypothesis which proposes that changes in cytoplasmic pH induce organic acid synthesis or breakdown.

Ammonium at high concentrations is toxic to plants, while NO$_3^-$ may exist as a free anion with no apparent negative influences on metabolism (Epstein, 1972). Assimilation of NH$_4^+$, however, could save energy to the plants since this form of N is already reduced (Cox and Reisenauer, 1973). This could be another reason explaining why a 50:50 combination of the two N sources is optimum for plants. Reproductive sink capacity is commonly a limiting factor for grain yield in maize in temperate and subtropical regions. Grain sink size, expressed as number of kernels per plant, declines considerably during the flowering period and this sink capacity is determined by assimilate supply to the ear during
this critical period. Grain yield improvement may be achieved with those treatments or factors which increase at that specific moment the assimilate supply to the ear (Tollenaar, 1977). The presence of \( \text{NH}_4^+ \) reduced \( \text{NO}_3^- \) assimilation (Schrader et al., 1972). It is possible, then, that having some \( \text{NH}_4^+ \) available to the leaves could save some energy (because the N is already reduced) which could be available to the developing ear, increasing sink capacity and, consequently, yield. However, Andrade (1983) showed that grain yield of field grown plants did not show any response to the different \( \text{NO}_3^- \)-N/\( \text{NH}_4^- \)-N ratios available during the flowering period.

When the uptake of anions and cations is unbalanced, the roots must excrete \( \text{OH}^- \) or \( \text{H}^+ \) in order to maintain electrical neutrality (Raven and Smith, 1976; Mengel et al., 1983). This produces changes in the external environment of the roots affecting the relative uptake of cations and anions (Riley and Barber, 1969) and the mineral nutrition of the plant in general (Clarkson and Hanson, 1980).

Plants growing under field conditions are always subjected to a combination of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) where \( \text{NO}_3^- \) usually predominates. In the field, the \( \text{NO}_3^- \)-N/\( \text{NH}_4^- \)-N ratio has other important implications. If N is applied and kept as \( \text{NH}_4^+ \), leaching and denitrification should be reduced (Gomes and Loynachan, 1984; McCormick et al., 1983). This can be achieved with the use of nitrification inhibitors (nitrapyrin).
Nitrapyrin inhibits the cytochrome oxidase involved in ammonia oxidation to hydroxylamine by *Nitrosomonas* (Hooper and Terry, 1973; Campbell and Aleem, 1965). Nitrapyrin, in low concentration, acts as a copper-chelating agent on the cytochrome oxidase system that is involved in ammonia oxidation. Soil type affects the effectiveness of nitrapyrin. Organic matter decreases the effectiveness by affecting the sorption and decomposition rates of the chemical (Goring, 1962a,b; Redemann et al., 1964; Bundy and Bremner, 1973; Hendrickson and Keeney, 1979). The form of N fertilizer affects the effectiveness of nitrapyrin. Nitrapyrin is more effective with \((\text{NH}_4)_2\text{SO}_4, \text{NH}_4\text{NO}_3\) or \((\text{NH}_4)_2\text{HPO}_4\) than with urea (Goring, 1962a). Bundy and Bremner (1973) obtained a similar result and suggested that the hydrolysis of urea by soil urease increases the soil pH which would render the chemical less effective. Nitrapyrin is more effective at low temperatures because of the slow degradation and/or volatilization of the chemical as well as the low nitrification activity under cooler temperatures (Bundy and Bremner, 1973). Management practices in applying nitrapyrin affect its effectiveness. The time of application alters the bioactivity of the chemical according to the soil temperature. Briggs (1975) and Meikle et al. (1978) reported that surface application of nitrapyrin was very inefficient and extremely variable due to volatilization losses, photolysis and minimal penetration.
into the soil. In sandy soils, the effectiveness of nitrapyrin may be decreased because it may become separated from \( \text{NH}_4^+ \) when leaching conditions prevail (Hendrickson et al., 1978; Rudert and Locascio, 1979).

Fertilizer N applied in the fall or early spring is often used less efficiently than N applied closer to the time of maximum N demand by the plants. Nitrification inhibitors make it possible to delay nitrification of applied \( \text{NH}_4^- \)N in the soil and thereby reduce overwinter and early spring N loss from leaching and denitrification.

Sandy soils of the southeastern coastal plain have a low water-holding capacity and are normally exposed to leaching conditions (Terry and McCants, 1970). Leaching of \( \text{NO}_3^- \) below the rooting zone results in low N fertilizer efficiency and \( \text{NO}_3^- \) pollution of the subsurface drainage waters (Grambrell et al., 1975). Therefore, N fertilization of corn on sandy coastal plain soils often involves split N application due to the possibility of a large loss of \( \text{NO}_3^- \) by leaching.

The effects of nitrapyrin on corn response have been variable. Sewezey and Turner (1962), Warren et al. (1975), Hergert et al. (1978), and Malzer et al. (1979) found yield increases due to nitrapyrin application. On the other hand, Hendrickson et al. (1978) and Touchton et al. (1979) observed little or no effect. Ong (1982) reported that nitrapyrin significantly increased total leaf N concentration in corn.
leaves. Where increased yields have been observed with nitrapyrin, these can be attributed to the effective inhibition of nitrification with the consequent reduction of N loss. The absence of any response to nitrapyrin could be the result from either ineffective inhibition of nitrification or to the lack of N loss from the rooting zone. Chancy and Kamprath (1982) found that leaching conditions in 1978 resulted in large losses of inorganic N from the rooting zone and, when nitrapyrin was added with urea, losses of N were significantly reduced, causing an increase in leaf N concentration, grain yield, plant N accumulation, and N fertilizer recovery. In contrast, in 1977, inclusion of nitrapyrin with urea had little effect due to small losses of soil inorganic N by leaching. Warren et al. (1975) reported that addition of nitrapyrin to fall-applied NH₄⁺ significantly increased grain yield and grain protein in loam, sandy loam, and silty clay loam soil types. They did not find a yield response to nitrapyrin with spring-applied NH₃, which could be attributed to the limited loss of spring-applied N under the conditions of these experiments.

Nitrification inhibitors under field conditions can effectively reduce N losses without adversely affecting the uptake of other minerals or the mineral composition of corn leaf tissue. Warren et al. (1980) concluded that, although the uptake of cations may be influenced by the major form of
N assimilated by plants, the degree of that influence is determined by the specific environment. They found that nitrapyrin did not affect the elemental composition of corn. Their results are based on the application of NH$_3$ with and without nitrapyrin under field conditions, whereas results showing competitive inhibition of cation uptake are from studies with nutrient solutions in the greenhouse. The failure of stabilized NH$_4^-$-N to markedly alter the elemental composition of corn leaves in these field studies may indicate that:

1. The concentration of NH$_4^+$ and K$^+$ ions were balanced for maximum utilization of both ions (Dibb and Welch, 1976).

2. Both NH$_4^+$ and NO$_3^-$ ions were available in the field.

3. Effective uptake of NH$_4^+$ and other cations possibly occurring in the stabilized NH$_4^-$-N band were nullified by NO$_3^-$ uptake from the remaining soil mass.

There is evidence that independently of the leaching effect, the NO$_3^-/NH_4^+$ ratio also affects yield under field conditions. In a two-year field experiment on irrigated sandy soils, yields were increased by 13% when ammonium nitrate or urea was compared with KNO$_3$ as a side-dressing treatment (Jung et al., 1972). These experiments suggest that corn does not obtain enough N for maximal growth and yield from normal soils in which the predominant form of N is NO$_3^-$. 
Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy is based on the fact that:

Certain nuclei, such as $^1\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$, $^{31}\text{P}$, possess a permanent magnetic moment owing to nuclear spin. In an external magnetic field, there is a net orientation of nuclear spins in the direction of the field, at equilibrium.

NMR spectroscopy involves monitoring absorption of radio-frequency (rf) radiation by the spin population. The NMR experiment consists of placing the sample in a strong, homogeneous magnetic field which aligns the nuclear magnets, irradiation of the sample with an rf pulse of the appropriate frequency range to excite the nuclear spins (nuclear magnets aligned against the external magnetic field), detection of the excited nuclei as voltages induced in a tuned wire coil placed around the sample, addition of the signal following repeated exciting pulses (signal averaging), transformation of these signals into a conventional spectrum: a plot of intensity versus frequency. (More often, intensity is plotted against "chemical shift" measured in the dimensionless unit of ppm, defined as $(V_s-V_{\text{ref}}) \times 10^6/V_{\text{ref}}$, where $V_s$ and $V_{\text{ref}}$ are the absolute resonance frequencies of a particular sample peak and the reference peak, respectively) (Roberts, 1984).

What makes NMR most interesting and useful is that the precise frequency of the electromagnetic radiation at which the nuclei produce the NMR signal is a function of the chemical environment of the nuclei. For example, free phosphate groups in the vacuole and in the cytoplasm will give two different signals since the pH of these two compartments is not the same (Roberts et al., 1980; Shulman, 1983). ATP has three phosphate groups with different chemical environments; consequently, ATP produces three different NMR signals, one
for each phosphate group. Thus, the position of the peaks is indicative of the chemical environment of the nuclei. This property can be used to identify compounds or follow chemical reactions in living cells. Moreover, NMR spectroscopy can be used to estimate concentration of metabolites in the living cells since the intensity of the signals is proportional to the number of nuclei.
MATERIALS AND METHODS

Experiment 1

Corn seeds (*Zea mays* L.) of hybrid A632 x A619 were pregerminated in germination boxes and, after 3 days, each seedling was transplanted to a 1-liter pot containing nutrient solution. The pots were placed in a growth chamber [max temp: 30°C, min temp: 26°C, RH = 50%, daylength = 15 h (hours)]. The nutrient solution consisted of 0.5 mM KH$_2$PO$_4$, 0.5 mM MgSO$_4$, 0.7 mM CaSO$_4$, 1 mM KNO$_3$, and proper level of micronutrients (pH = 6.65). Air was constantly bubbled through the solution. The plants were grown for about 1 week before the treatments were applied. The treatments consisted of a factorial arrangement of 2 N levels (2 and 4 mM KNO$_3$) and 3 Cl levels (0, 1.5, and 3 mM CaCl$_2$) with 4 replications. All the other nutrients were maintained at the proper levels. Water was added regularly to replace that lost by evapotranspiration and N level in the nutrient solution and pH were followed daily. The experiment was harvested two weeks after application of the treatments. Root and shoot weights were determined as well as final pH and N uptake (estimated by disappearance from the solution). Nitrogen levels in solution were determined by the steam distillation method (Bremner and Keeney, 1965).
Experiment 2

Corn seeds (hybrid A632 x A619) were planted in 1-liter pots containing washed sand. The experiment was conducted in a greenhouse during February 1983. Temperatures fluctuated between 20 and 30°C and relative humidity fluctuated between 35 and 65%. After emergence, the plants were watered with nutrient solution (1 mM KH$_2$PO$_4$, 1 mM MgSO$_4$, 2 mM Ca(NO$_3$)$_2$, 2 mM (NH$_4$)$_2$SO$_4$, 1.4 mM CaSO$_4$, 46 μM H$_3$BO$_3$, 9 μM MnCl$_2$, 0.8 μM ZnSO$_4$, 0.3 μM NaMoO$_4$, 0.3 μM CuSO$_4$, 30 μM NaCl, 20 μM Fe chelate; pH = 6). The experiment consisted of a randomized complete block design with 3 treatments and 6 replications.

In the first treatment, the plants were watered with the basic nutrient solution plus 4.5 mM CaCl$_2$; in the second treatment, the plants were supplied with basic nutrient solution only; finally, in the third treatment, the plants received basic nutrient solution plus 3.5 mM Na$_2$SO$_4$. During the first few days after emergence, all the plants were watered with half strength nutrient solution. The plants were watered in excess to avoid accumulation of salts in the sand. The experiment was harvested 19 days after planting. Plant height, and root and shoot fresh and dry weights were measured. Two different procedures were used to estimate external pH. It was measured (1) in a 1:2.5 sand:CaCl$_2$ .01 M mixture, (2) using solution extracted from the pots by suction 24 h after the last irrigation with nutrient solution. Nitrate-N and
NH\textsubscript{4}-N levels remaining in the sand at the end of the experiment were analyzed using the steam distillation technique. Tissue samples were collected, ground, and their N percentage estimated using the micro-Kjeldahl technique.

Experiment 3

This experiment was conducted in a growth chamber. Seventy-five corn seeds (hybrid A632 x A619) were planted in each of 12 recipients containing 9 kg of sand. Water was added to the recipients until emergence; from that moment, the corn seedlings were watered daily with half strength nutrient solution (see materials and methods, Experiment 2). Relative humidity in the chamber fluctuated around 50%. Maximal and minimal temperatures were 31\degree C (during the day) and 18\degree C (at night), respectively. The plants were subjected to a 14-h day photoperiod with a photosynthetically active photon flux density (PPFD) of 400-460 \mu mol m\textsuperscript{-2}s\textsuperscript{-1}. Three different treatments with 4 replications were applied 8 days after planting (5 days after emergence). They consisted of the application of 25 meq of N per pot as Ca(NO\textsubscript{3})\textsubscript{2}, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, or a combination of both. Four hours later, 40 plants per pot were harvested. After the roots were separated from the shoots and seeds, they were washed once with water and 3 times with CaSO\textsubscript{4} 0.01 M. Then, they were dried with paper towels, weighed to determine fresh weight and frozen in liquid N\textsubscript{2}. 
The root content was extracted in 100 ml of NH$_4^+$ free water. The extracts were filtered and centrifuged at 4,000 x g for 30 minutes before their NO$_3^-$ and NH$_4^+$ concentrations were determined. The NO$_3^-$N concentration was measured by 3 different techniques: (1) steam distillation of the extract, (2) cadmium reduction method (using Nitra Ver V nitrate reagent for plant analysis, from Hach Chemical Company, Ames, Iowa), and (3) NO$_3^-$ electrode. The NH$_4^+$-N concentration was estimated by the steam distillation procedure.

Five ml of crude extract were taken from each sample and filtered through a 0.4 µm millipore filter. These filtrates were used for organic acid and amino acid analysis. Organic acid (malate) levels were determined using a C$_{18}$ microbonded column (Waters Associates) in a Beckman HPLC connected to a Cary 210 spectrophotometer. Absorbance was read at 214 nm. The carrier solution was 0.5% (NH$_4$)$_2$SO$_4$ taken to pH 2.8 with H$_3$PO$_4$. Part of the samples were sent to a laboratory for free amino acid analysis with a Durrum D-440 amino-acid analyzer.

Experiment 4

Corn seeds (hybrid A632 x A619) were planted in styrofoam cups (1 seed per cup). The cups contained 300 g of a 50:50 mixture of sand and Huntsville silt soil from the Hinds
farm (near Ames, Iowa). Initial analysis of the soil showed that it contained 104 kg ha\(^{-1}\) of P, 245 kg ha\(^{-1}\) of K, 0.4% of organic matter, and a pH of 7.35. The experiment was conducted in a growth chamber with day-night temperatures fluctuating between 28 and 15\(^{\circ}\)C, and relative humidity between 50 and 100%. The plants were subjected to a 14-h day photo-period with a PPFD of 400-460 \(\mu\)mol m\(^{-2}\)s\(^{-1}\).

The plants were watered with proper levels of nutrient solution for 9 days after emergence (see Experiment 2). Two days later, different treatments consisting of various concentrations of \(\text{NO}_3^-\) or \(\text{NH}_4^+\) were applied. The treatments were replicated twice. Plants were supplied with 30 ml of a solution of \((\text{NH}_4)_2\text{SO}_4\) or \(\text{Ca(NO}_3)_2\) ranging from 2 to 48 meq \(\text{L}^{-1}\). Five h later, the plants were harvested and shoot and root weights were determined. Nitrogen uptake was calculated as the difference between initial and final N in the pots; \(\text{NO}_3^-\)-N and \(\text{NH}_4^-\)-N in the soil were determined by the steam distillation procedure after extraction with 2 M KCl.

This experiment was preceded by calibration experiments in which denitrification, nitrification, as well as the amount of time required to detect uptake by the plants were estimated for the conditions of this system. In another calibration experiment, only half of the plants were supplied with nutrient solution and shoot and root fresh weights were followed for 11 days after emergence.
Experiment 5

A. Corn seedlings were grown as in the previous experiment. The experiment was a randomized complete block design with 8 treatments and 5 replications. The treatments were applied 11 days after emergence and they consisted of the application of 25 ml per pot of 8 different N solutions [(1) 8 meq L\(^{-1}\) Ca(NO\(_3\))\(_2\), (2) 16 meq L\(^{-1}\) Ca(NO\(_3\))\(_2\), (3) 32 meq L\(^{-1}\) Ca(NO\(_3\))\(_2\), (4) 8 meq L\(^{-1}\) Ca(NO\(_3\))\(_2\) plus 16 meq L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), (5) 16 meq L\(^{-1}\) Ca(NO\(_3\))\(_2\) plus 16 meq L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), (6) 32 meq L\(^{-1}\) Ca(NO\(_3\))\(_2\) plus 16 meq L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), (7) 16 meq L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\), and (8) 32 meq L\(^{-1}\) (NH\(_4\))\(_2\)SO\(_4\)]. Five h later, the seedlings were harvested, washed and dried with paper towels. Total fresh weight was determined and the second leaf was cut for nitrate reductase activity assay.

Nitrogen uptake was estimated by measuring the remaining N (NO\(_3\)-N + NH\(_4\)-N) in the soil (soil moisture level and total soil weight data were used in these calculations). Initial N level was estimated by taking soil samples before the application of the treatments. Ammonium-N and NO\(_3\)-N in the soil were measured using the steam distillation method after extraction with 2 M KCl. The technique presented by Blevins et al. (1978) was used for the nitrate reductase activity assay.

B. In a second experiment of the same type, a factorial arrangement of 2 sources of NO\(_3\)\(^-\) (KNO\(_3\) and Ca(NO\(_3\))\(_2\)) by two concentrations (10 and 16 meq L\(^{-1}\)) was replicated 4 times.
in a randomized complete block design. Twenty-five ml of the treatment solution were applied per pot 11 days after emergence. Five h later, the plants were harvested and fresh weight, nutrient uptake, and nitrate reductase activity were measured as in the previous experiment.

Experiment 6

This experiment was conducted in a greenhouse. Corn seeds (hybrid A632 x A619) were planted in pots on August 4, 1982. Each pot contained 9.1 kg of soil (Huntsville silt from the Hinds farm, Ames, Iowa). Leaching and watering were strictly controlled (any leachate was returned to the pots). The experiment consisted of 6 treatments in a randomized complete block design with 6 replications. A total of 1.8 g of N per pot as Ca(NO$_3$)$_2$ and/or (NH$_4$)$_2$SO$_4$ was applied in 7 split applications starting 30 days after planting. Proper amounts of K and P were added to the soil. The 6 treatments consisted of 6 NO$_3$-N/NH$_4$-N mixtures (100/0, 80/20, 60/40, 40/60, 20/80, and 0/100). The plants were harvested 63 days after planting. Soil pH, remaining N in the soil, N content in shoots and roots, and shoot and root dry weights were determined. Soil pH was determined on a 1:1 soil/water suspension. Total N in shoots and roots was determined by the micro-Kjeldahl method. The steam distillation procedure was also used to determine NH$_4$-N and NO$_3$-N levels in
soil (after extraction with 2 M KCl).

Experiment 7

A. The experiment was carried out in a greenhouse during June-July 1983. Air temperatures fluctuated between 20 and 38°C. Two corn seeds (hybrid A632 x A619) were planted in each of 52 pots containing 3.63 kg of soil from the Hinds farm, Ames, Iowa (Huntsville silt). Initial soil analysis showed that the soil contained 112 kg ha$^{-1}$ of P, 245 kg ha$^{-1}$ of K, 0.8% organic matter, 9 kg ha$^{-1}$ of NH$_4$ON, 15 kg ha$^{-1}$ of NO$_3$-N, and a pH of 6.65. A total of 1.02 g of N as Ca(NO$_3$)$_2$ and/or (NH$_4$)$_2$SO$_4$ was applied per pot in 8 split applications starting 11 days after planting. The experiment consisted of 6 treatments in a randomized complete block design with 6 replications. Five NO$_3$-N/NH$_4$-N mixtures (100/0, 75/25, 50/50, 25/75, and 0/100) constituted the different treatments. A total of 0.1 ml of nitrapyrin formation was applied per pot in 8 split applications with the N solution in order to inhibit nitrification. (There are 242 g of active ingredient per liter of formulation.) Watering was strictly controlled and any leachate was returned to the pots.

The experiment was harvested 35 days after planting. The variables measured were: plant height, total N (NO$_3$-N + NH$_4$-N) remaining in the soil; final NO$_3$-N/NH$_4$-N ratio in the soil, percentage of NO$_3$-N and NH$_4$-N used, soil pH, shoot
fresh weight and dry weight, N percentage in shoot, and the level of micro- and macronutrients in shoots. Soil pH was measured in a 1:1 soil/water mixture. Nitrogen measurements in soil and tissues were estimated by the steam distillation and micro-Kjeldahl techniques, respectively, while the levels of micro- and macronutrients in tissue were measured by inductively coupled plasma (ICP) atomic emission spectroscopy (AES) (Munter and Grande, 1980). Total sulfur was determined by dry combustion and measurement of evolved sulfur dioxide on a LECO sulfur determinator, model no. SC-132 by IR radiation.

B. Concomitantly, a second experiment of the same type was conducted using a randomized complete block design with 6 treatments and 6 replications. The treatments were:

(1) KNO₃, (2) Ca(NO₃)₂, (3) half (NH₄)₂SO₄ and half Ca(NO₃)₂, (4) same as 3 plus Cl₂Ca in a 1:1 Cl:N ratio, (5) (NH₄)₂SO₄, and (6) NH₄Cl. A total of 1.02 g of N per pot was applied in 8 split applications. Nitrapyrin was added to inhibit nitrification and leaching was controlled by returning any leachate to the pots. The plants were harvested 36 days after planting. Plant height, shoot fresh weight and dry weight, NO₃⁻-N + NH₄⁺-N remaining in the soil, final NO₃⁻-N/ NH₄⁺-N ratio in the soil, percentage of NO₃⁻ and NH₄⁺ used, soil pH, N percentage in leaves, and levels of micro- and macronutrients in tissue were measured as in part A.
Experiment 8

A. Corn plants (hybrid A632 x A619) were grown in a growth chamber where air temperatures fluctuated between 18°C at night and 30°C during the day and relative humidity fluctuated between 50 and 90%. The plants were subjected to a 14-h photoperiod with a PPFD of 400-460 μmol m⁻² s⁻¹. Each experimental unit consisted of 420 seeds planted in big pots containing 10 kg of sand (8.4% moisture). The seedlings were watered with nutrient solution (see Experiment 2). The experiment consisted of 3 treatments replicated 5 times. The treatments were applied 10 days after planting and they consisted of the application of 500 ml of 3 different solutions: 100 meq L⁻¹ Ca(NO₃)₂, 100 meq L⁻¹ (NH₄)₂SO₄, and 50 meq L⁻¹ Ca(NO₃)₂ plus 50 meq L⁻¹ (NH₄)₂SO₄. Five h after the application of the treatments, the plants were removed, washed, leaf samples taken, and root tips 2-3 mm long (around 1,500 per sample) were excised with a razor blade and each immediately placed in NMR tubes with a cold solution. The solution contained 50 mM glucose, 0.1 mM CaSO₄, and 10 meq L⁻¹ of NO₃⁻, NH₄⁺, or half and half according to the respective treatments. The tubes were kept in an ice-water mixture to inhibit any metabolic activity in the root tips. Due to the limitation of this step, the replications were conducted at different times. The glucose solution was frequently changed
to avoid anaerobiosis. Nitrogen uptake by the seedlings during the 5-h period was estimated by difference between initial and final N in the pots. The steam distillation unit was used in this step.

After cutting all the root tips, the tubes were taken to the NMR laboratory (Chemistry Department, ISU) and the $^{31}$P-NMR spectra were obtained immediately at 5°C with 1,600 scans at 0.41 seconds per scan using a Bruker WM-300 nuclear magnetic resonance spectrometer operated at 121 megahertz. Proton lock was used for homogeneity adjustment. Peaks were referred to 0.5 M methylene diphosphonic acid (adjusted to pH 8.9 with tris buffer) contained in a thin coaxial capillary tube placed in the NMR tube with the root tips.

B. Once the spectra were obtained, the root tips were placed in 25 ml of a 20 meq L$^{-1}$ N solution (Ca(NO$_3$)$_2$ or (NH$_4$)$_2$SO$_4$) containing also 50 mM glucose and 0.1 mM CaSO$_4$. Then, the root tips were bubbled with O$_2$ for 60 minutes at room temperature; after this, the root tips were placed in cold solution containing 20 meq L$^{-1}$ of Ca(NO$_3$)$_2$ or (NH$_4$)$_2$SO$_4$ according to the treatments and second spectra were obtained immediately at 5°C and 1,600 scans.

C. In order to study anaerobiosis, some samples were bubbled with N$_2$ for several minutes at room temperature; after this, the spectra were obtained at 5°C (1,600 scans). Then, the root tips were incubated in the NMR at 25°C.
Subsequent spectra were obtained at 25°C.

D. New batches of root tips were bubbled with O₂ for 15 minutes in a solution containing 50 mM glucose, 0.1 mM CaSO₄ and 20 meq L⁻¹ of N as Ca(NO₃)₂ or (NH₄)₂SO₄. Then, the root tips in their respective solutions were placed in the NMR spectrometer and ³¹P spectra were obtained every 5 minutes at 25°C in order to study the effect of NO₃⁻ and NH₄⁺ in the development of anaerobiosis. After almost 3 h of being in the NMR, the amount of NO₃⁻ reduced to NO₂⁻ was estimated by measuring the NO₂⁻ concentration in the root tip solution. Nitrite concentration was estimated by adding 1 ml of 1% sulfamilamide in 3 N HCl and 1 ml 0.02% of N-1 napthyl-ethylenediamine dihydrochloride in 95% ethanol to 2 ml of solution. Absorbance was read at 540 nm after proper color development. Initial NO₂⁻ concentration was also measured.

E. In order to estimate N uptake, new batches of root tips were placed in 15 ml of 2 different solutions: (a) 50 mM glucose, 0.1 mM CaSO₄ and 20 meq L⁻¹ Ca(NO₃)₂, and (b) 50 mM glucose, 0.1 mM CaSO₄ and 4 meq L⁻¹ Ca(NO₃)₂. The root tips were bubbled with O₂ to prevent anaerobiosis. Rate of N uptake was estimated by disappearance from the solution. A control without root tips was included to correct for evaporation of water. Root tip length, fresh and dry weight were determined 21 h after the initiation of the experiment.
Experiment 9

This experiment was conducted under irrigation in the field using corn (hybrid A632 x A619) during summer of 1982. The field plots were located on Huntsville silt (Hinds farm, Ames, Iowa). Nitrogen analysis of soil samples taken after anthesis for the control experimental units showed that the site contained 1.51 ppm NH$_4^-$-N and 3.19 ppm of NO$_3^-$-N in the upper 15 cm (soil moisture, 15%).

The field was fertilized with 49 kg ha$^{-1}$ of P and 200 kg ha$^{-1}$ of K in fall, and with 175 kg ha$^{-1}$ of N as urea in the early spring. The field also was treated with 3.06 kg ha$^{-1}$ of alachlor and 1.1 kg ha$^{-1}$ of metribuzin, and it was tilled in the conventional way. The final plant population was 55,000 plants ha$^{-1}$. Nitrogen was applied near flowering to study the disappearance of NO$_3^-$ and NH$_4^+$ from the soil.

The experiment was conducted utilizing a split-plot design. The subplot treatments consisted of application of 90 kg NH$_4^-$-N ha$^{-1}$ with four rates of nitrapyrin plus a check with no N application. The four rates of nitrapyrin were: 0, 741, 2244, and 6672 g ha$^{-1}$ of active ingredient and will be referred to as nitrapyrin 0, 1, 2, and 3, respectively.

The whole-plot treatments consisted of three different stages of application: at beginning of flowering (A), at two weeks after (B), and at three weeks after beginning of flowering (C). At the third stage of application, the treatment
with the higher rate of nitrapyrin was replaced by 90 kg of N as NO$_3$-N. The whole plots were single rows 9.14 m long, while the split plots were only 1 m long. The rows were 1 m apart. The solutions of N and nitrapyrin were applied at the proper time into the soil by making trenches approximately 12 cm deep at 25 cm from the row. The NO$_3$-N and NH$_4$-N in the soil was measured 2, 4, and 6 weeks after application of the treatments by taking 30-cm deep samples and analyzing them by the steam distillation method after extraction of the soil with 2 M KCl.

Experiment 10

This experiment was conducted under irrigation in the field using corn hybrid A632 x A619 during the summer of 1983. The field experimental plot was located at the Hinds farm. The corn was planted on May 9, 1983 in rows 75 cm apart. The field plots were located on Huntsville silt. Nitrogen analysis of soil samples taken before application of the treatments (end of May) showed that the site contained 3.99 ppm of NH$_4$-N and 6.75 ppm of NO$_3$-N in the upper 15 cm (soil moisture = 9.7%). The field was fertilized with 49 kg ha$^{-1}$ of P and 200 kg ha$^{-1}$ of K in the fall. Soil analysis of samples taken from the upper 15 cm in late spring indicated that the soil had 1.6% of organic matter, 127 kg ha$^{-1}$ of P, 220 kg ha$^{-1}$ of K, and a pH of 7.50. The field was
treated with 3.06 kg ha$^{-1}$ of alachlor and 1.1 kg ha$^{-1}$ of metribuzin and tilled in the conventional way. The final plant population was approximately 55,000 plants ha$^{-1}$. The experiment was conducted utilizing a split-plot design where the whole-plot treatments consisted of 2 rates of N (75 and 150 kg ha$^{-1}$), whereas the split-plot treatments consisted of two controls plus a factorial arrangement of 3 forms of N (urea plus nitrapyrin, urea, and KN0$_3$) by two forms of application. In the first form of application, all the N was applied at once on May 27, whereas in the second form of application, the N was applied in two split applications on May 27 and July 9. The two controls were: no N and 225 kg N ha$^{-1}$ in 3 split applications.

The treatments were replicated 4 times in blocks. The fertilizer was applied along the corn rows using a manual planter previously calibrated. Nitrapyrin was applied in solution on the urea at a rate of 3.2 kg ha$^{-1}$ and properly covered with soil. Approximately 600 mm of irrigation water were applied to the field from June 8 to August 17. Natural rainfall for June, July, and August was 179, 112, and 108 mm, respectively.

Soil samples 30 cm deep were taken 2, 4, and 6 weeks after May 27. Nitrate-N and NH$_4$-N were determined by the steam distillation method after extracting the soil with 2 M KCl. Three days after anthesis, leaves opposite and below the ear
were gathered for protein analysis. The leaves were cleaned, dried at 65°C and ground in a Wiley mill to pass through a 20-mesh stainless steel screen. The plots were machine harvested and grain yield per plot, weight of 100 seeds, and grain protein percentage were determined. A sample of grain was dried at 65°C and ground for total protein determination. Protein content for leaf and grain was determined by micro-Kjeldahl analysis.
RESULTS

Experiment 1

Some interacting effects of NO$_3^-$ and Cl in culture solution are summarized in Tables 1 and 2. Root fresh weight and shoot fresh weight did not differ significantly among treatments. The application of 1.5 or 3 mM CaCl$_2$ to the nutrient solution significantly decreased total NO$_3^-$-N uptake and NO$_3^-$-N uptake per unit root fresh weight. Moreover, NO$_3^-$-N uptake increased when the N concentration in the solution was increased from 2 to 4 meq L$^{-1}$. Final pH of the nutrient solution was affected by the N level (significant differences at $\alpha = 0.05$).

Experiment 2

The effects of Na and Cl on corn grown in sand culture with NO$_3^-$-N and NH$_4^-$-N is shown in Table 3. Fresh weight, dry weight, and height did not show any significant differences among the treatments. The pH of the sand as well as the pH of the solution extracted from the pots at the end of the experiment were higher with the Cl (CaCl$_2$) treatment. Both types of pH measurement showed a significant linear effect.

Nitrogen percentage in shoots was negatively correlated with shoot dry weight ($r = -0.96$, $\alpha = 0.0001$). The larger the plant the lower the N concentration was in tissue (Fig. 1).
Table 1. Effect of N and Cl concentration in the nutrient solution on root fresh weight, shoot fresh weight, N uptake, N uptake per unit of root fresh weight, and final pH of the sand; Experiment 1

<table>
<thead>
<tr>
<th>N (meq L⁻¹)</th>
<th>Cl</th>
<th>Root fresh weight (g)</th>
<th>Shoot fresh weight (g)</th>
<th>N uptake (mg N L⁻¹)</th>
<th>N uptake/root fresh wt. (mg N L⁻¹ g⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>3.24</td>
<td>4.46</td>
<td>10.88</td>
<td>3.36</td>
<td>6.81</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.49</td>
<td>4.98</td>
<td>7.89</td>
<td>2.26</td>
<td>6.72</td>
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<tr>
<td></td>
<td>6</td>
<td>3.83</td>
<td>5.13</td>
<td>8.76</td>
<td>2.29</td>
<td>6.74</td>
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<td>3.33</td>
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<td>14.42</td>
<td>4.33</td>
<td>6.90</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.37</td>
<td>4.36</td>
<td>9.90</td>
<td>2.94</td>
<td>6.92</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.63</td>
<td>4.56</td>
<td>9.99</td>
<td>2.75</td>
<td>6.90</td>
</tr>
</tbody>
</table>

SEM<sup>a</sup> 0.31 0.30 0.62 0.41 0.08

| 2          | 0-3-6 | 3.52                  | 4.86                   | 9.18                | 2.61                                   | 6.76|
| 4          | 0-3-6 | 3.44                  | 4.41                   | 11.44               | 3.33                                   | 6.91|

SEM 0.18 0.17 0.36 0.28 0.05

| 2,4        | 0     | 3.29                  | 4.37                   | 12.65               | 3.84                                   | 6.86|
|            | 3     | 3.43                  | 4.68                   | 8.90                | 2.59                                   | 6.82|
|            | 6     | 3.73                  | 4.86                   | 9.38                | 2.51                                   | 6.82|

SEM 0.22 0.21 0.44 0.29 0.06

<sup>a</sup>SEM = standard error of the mean.
Table 2. Statistical analysis for N uptake (1) and N uptake per unit of root fresh weight (2); Experiment 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS 1</th>
<th>MS 2</th>
<th>F 1</th>
<th>F 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blocks</td>
<td>3</td>
<td>15.67</td>
<td>0.22</td>
<td>10.31***</td>
<td>0.32</td>
</tr>
<tr>
<td>N level</td>
<td>1</td>
<td>30.76</td>
<td>3.26</td>
<td>20.24***</td>
<td>4.79*</td>
</tr>
<tr>
<td>Cl level</td>
<td>2</td>
<td>33.38</td>
<td>5.75</td>
<td>21.96****</td>
<td>8.46**</td>
</tr>
<tr>
<td>N*Cl</td>
<td>2</td>
<td>2.76</td>
<td>0.08</td>
<td>1.82</td>
<td>0.12</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>1.52</td>
<td>0.68</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

****,***,**,*Significant at α = 0.0001, 0.001, 0.01, and 0.05, respectively.

No significant treatment effect was found on N percentage in shoots; but when shoot dry weight was partialled out by covariance analysis, the Na treatment (\( \text{Na}_2\text{SO}_4 \)) showed a higher N percentage (with differences significant at \( \alpha = 0.05 \)). The Na treatment also showed a significantly higher N content in the shoot compared to the Cl treatment.

The \( \text{NO}_3^-\text{N/NH}_4^-\text{N} \) ratio remaining in the pots at the end of the experiment was highest for the Cl treatment (significant linear effect at \( \alpha = 0.05 \)). Finally, the \( \text{NO}_3^-\text{N} \) remaining in the pots was highest for the Cl treatment and lowest for the Na treatment with a significant linear effect at \( \alpha = 0.05 \) (Table 4).
Table 3. Effect of Cl and Na on the variables measured in Experiment 2

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatment</th>
<th>Cl⁻</th>
<th>Control</th>
<th>Na⁺</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root fresh weight (g)</td>
<td></td>
<td>5.60</td>
<td>5.52</td>
<td>5.85</td>
<td>0.28</td>
</tr>
<tr>
<td>Shoot fresh weight (g)</td>
<td></td>
<td>8.28</td>
<td>8.82</td>
<td>8.89</td>
<td>0.35</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td>48.37</td>
<td>48.50</td>
<td>46.88</td>
<td>1.46</td>
</tr>
<tr>
<td>pH in CaCl₂ 0.01 M</td>
<td></td>
<td>6.72</td>
<td>6.68</td>
<td>6.65</td>
<td>0.018</td>
</tr>
<tr>
<td>pH pure solution</td>
<td></td>
<td>6.46</td>
<td>6.32</td>
<td>6.30</td>
<td>0.015</td>
</tr>
<tr>
<td>N % in shoots</td>
<td></td>
<td>3.41</td>
<td>3.36</td>
<td>3.57</td>
<td>0.18</td>
</tr>
<tr>
<td>Total N in shoot (mg)</td>
<td></td>
<td>32.04</td>
<td>34.99</td>
<td>35.22</td>
<td>0.52</td>
</tr>
<tr>
<td>NO₃⁻-N/NH₄⁻-N ratio remaining</td>
<td></td>
<td>1.02</td>
<td>0.72</td>
<td>0.68</td>
<td>0.14</td>
</tr>
<tr>
<td>NO₃⁻-N remaining (mg N/100 g sand)</td>
<td></td>
<td>0.27</td>
<td>0.14</td>
<td>0.08</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Fig. 1. Relation of shoot dry weight to N percentage for corn seedlings (Experiment 2)
Table 4. Statistical analysis for the remaining NO$_3$-N in the pots (Experiment 2)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>14</td>
<td>0.0187</td>
<td>-</td>
</tr>
<tr>
<td>Blocks</td>
<td>4</td>
<td>0.0143</td>
<td>1.03</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.0468</td>
<td>3.37</td>
</tr>
<tr>
<td>Linear</td>
<td>1</td>
<td>0.0893</td>
<td>6.42*</td>
</tr>
<tr>
<td>LOF</td>
<td>1</td>
<td>0.0042</td>
<td>0.3</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.0139</td>
<td>-</td>
</tr>
</tbody>
</table>

*Significant at $\alpha = 0.05$.

Experiment 3

The concentrations of NO$_3$-N and NH$_4$-N in roots as affected by the form of N are shown in Fig. 2. The NO$_3$-N values are the average of the Kjeldahl and the cadmium reduction methods. Abnormal and useless values were obtained with the nitrate electrode. When NO$_3^-$ was applied as the N source, NO$_3$-N concentration in roots reached a maximum of 63.05 µg N per g of root fresh weight. This value decreased to 15.58 µg N per g of root fresh weight when the N was applied as NH$_4^+$. Contrarily, NH$_4$-N concentration increased from 20.80 to 68.76 µg N per g of root fresh weight for the same treatment comparison. Nitrate-N and NH$_4$-N concentration
Fig. 2. NO$_3$-N and NH$_4$-N concentration in roots as a function of the form of N. (Experiment 3) (1 represents 100% NO$_3$, 2 - 50/50, and 3 - 100% NH$_4$); the vertical bars indicate the standard error of the means (SEM).
in roots were intermediate when the N source was a combination of the two forms. The differences among the means presented in Fig. 2 were all highly significant ($\alpha = 0.001$).

Table 5 presents the free amino acid analysis of the extracts. The extracts derived from NH$_4^+$-fed plants had higher levels of aspartic acid plus asparagine, valine, methionine, and arginine, whereas those derived from NO$_3^-$-fed plants showed higher levels of serine, glutamic acid plus glutamine, proline, and alanine. However, these are the means of just two replications.

Figure 3 shows the results of the HPLC analysis. Peak 1 represents NO$_3^-$ and peak 2 malate. Glutamate and NO$_3^-$ standards showed peaks at exactly the same position. But, in the root extracts, peak 1 represents NO$_3^-$ because the glutamate concentration of the extracts (Table 5) was very low compared to the standards used and could not have produced those large signals. Moreover, the NO$_3^-$ concentration of the extracts (derived from the comparison between the samples and the standard) matched the results of the Kjeldahl and cadmium-reduction method analysis (calculation not shown). The extracts derived from NO$_3^-$-fed plants showed high NO$_3^-$ levels and absence of malate. Contrarily, those derived from NH$_4^+$-fed plants showed low NO$_3^-$ levels and presence of malate.
Table 5. Free amino acid content of corn roots as affected by the form of N supplied (Experiment 1)

<table>
<thead>
<tr>
<th>Extract</th>
<th>NO₃⁻</th>
<th>NH⁺⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM of AA per g root fresh weight</td>
<td></td>
</tr>
<tr>
<td>Aspartic + asparagine</td>
<td>651.9</td>
<td>728.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>355.7</td>
<td>344.1</td>
</tr>
<tr>
<td>Serine</td>
<td>897.9</td>
<td>750.0</td>
</tr>
<tr>
<td>Glutamic acid + glutamine</td>
<td>709.1</td>
<td>604.9</td>
</tr>
<tr>
<td>Proline</td>
<td>410.9</td>
<td>344.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>381.2</td>
<td>394.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>892.1</td>
<td>828.5</td>
</tr>
<tr>
<td>Valine</td>
<td>466.1</td>
<td>510.8</td>
</tr>
<tr>
<td>Methionine</td>
<td>94.1</td>
<td>130.9</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>291.7</td>
<td>306.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>655.7</td>
<td>650.8</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>244.2</td>
<td>248.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>275.1</td>
<td>263.6</td>
</tr>
<tr>
<td>Lysine</td>
<td>871.4</td>
<td>820.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>270.4</td>
<td>284.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>104.9</td>
<td>213.2</td>
</tr>
<tr>
<td>Total</td>
<td>7572.1</td>
<td>7424.8</td>
</tr>
</tbody>
</table>

Experiment 4

This experiment demonstrates that the efficiency of NO₃⁻-N and NH⁺⁺-N uptake decreases as their initial external levels increase. A preliminary result indicated that 5 hours
Fig. 3. HPLC spectra of corn root extracts (Experiment 3); peak 1 represents NO$_3^-$ and peak 2 - malate; other peaks were not identified (A = malate standard, B = NO$_3^-$ standard, C = extracts derived from NO$_3^-$-fed plants; D = extracts derived from NH$_4^+$-fed plants)
of treatment was an adequate time to detect N uptake since more than 25% of the initial soil N was depleted (Fig. 4). Moreover, the results of other calibration experiments showed no detectable denitrification or nitrification in the same period of time (data not shown). Figure 5 shows the progressive increase in total fresh weight of corn seedlings with and without N application. At 11 days after emergence, differences in fresh weight between N and non-N treatments became clear.

Figure 6 indicates that the regression of NO$_3^-$-N uptake and NH$_4^+$-N uptake on initial N (NO$_3^-$-N or NH$_4^+$-N, respectively) concentration was not linear and showed a tendency to a plateau. In other words, the efficiency of N utilization declined as the initial N levels increased. The linear and quadratic after linear effects were highly significant (Table 5). This indicates the existence of a mechanism for control of nutrient uptake.

Soil pH at the end of the experiment did not differ among the treatments (data not shown).

Experiment 5A and 5B

5A. This experiment investigates the interacting effects of NO$_3^-$ and NH$_4^+$ on nitrate reductase activity and N uptake.

Nitrate reductase activity (μmol NO$_3^-$ reduced to NO$_2^-$)
Fig. 4. Disappearance of N from the pots as a function of time (Experiment 4)
Fig. 5. Fresh weight accumulation of corn seedlings as a function of days after emergence and N supplied (Experiment 4)
Fig. 6. NO$_3$-N and NH$_4$-N uptake as a function of initial N concentration (NO$_3$-N or NH$_4$-N, respectively) (Experiment 4)
Table 6. Linear and quadratic effects for NO$_3$-N uptake and NO$_3$-N uptake per unit of root fresh weight as a function of initial NO$_3$-N concentration, and for NH$_4$-N uptake and NH$_4$-N uptake per unit of root fresh weight as a function of initial NH$_4$-N concentration (b$_1$ and b$_2$ are the regression coefficients for linear and quadratic effects, respectively, and α is the level of significance) (Experiment 4)

<table>
<thead>
<tr>
<th></th>
<th>Linear</th>
<th>Quadratic after linear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b$_1$</td>
<td>α</td>
</tr>
<tr>
<td>NO$_3$-N uptake</td>
<td>0.404</td>
<td>0.0001</td>
</tr>
<tr>
<td>NO$_3$-N uptake/root FW</td>
<td>0.165</td>
<td>0.0001</td>
</tr>
<tr>
<td>NH$_4$-N uptake</td>
<td>0.419</td>
<td>0.0001</td>
</tr>
<tr>
<td>NH$_4$-N uptake/root FW</td>
<td>0.153</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

per g of leaf fresh weight per h) was very low when NH$_4^+$ was the N source. When N concentration in the nutrient solution was increased from 8 to 32 meq NO$_3^-$ L$^{-1}$, nitrate reductase activity increased from 0.37 to 0.46 μmol of NO$_3^-$ reduced per g of leaf fresh weight per h (difference significant at α = 0.05; see Tables 7 and 8). Tables 7 and 8 show that when NH$_4^+$ was provided along with NO$_3^-$, nitrate reductase activity increased 10% compared to NO$_3^-$ alone, but this difference was not significant at α = 0.05. These results indicate that for the conditions of this experiment, NH$_4^+$ added to NO$_3^-$ did not reduce nitrate reductase activity in the leaves. Table 9
Table 7. Statistical analysis for the nitrate reductase activity data obtained in Experiment 5A

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>39</td>
<td>0.0079</td>
<td>-</td>
</tr>
<tr>
<td>Block</td>
<td>4</td>
<td>0.0101</td>
<td>2.06</td>
</tr>
<tr>
<td>Treatment</td>
<td>7</td>
<td>0.0184</td>
<td>3.76*</td>
</tr>
<tr>
<td>NH4-N vs rest</td>
<td>1</td>
<td>0.0437</td>
<td>8.91**</td>
</tr>
<tr>
<td>(trt 7 and 8 vs rest)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH4-N concentration</td>
<td>1</td>
<td>0.0048</td>
<td>0.98</td>
</tr>
<tr>
<td>(trt 7 vs 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO3-N vs NO3-N + NH4-N (A)</td>
<td>1</td>
<td>0.0101</td>
<td>2.06</td>
</tr>
<tr>
<td>(trt 1,2,3 vs 4,5,6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate concentration (B)</td>
<td>2</td>
<td>0.0244</td>
<td>4.98*</td>
</tr>
<tr>
<td>A*B</td>
<td>2</td>
<td>0.0108</td>
<td>2.20</td>
</tr>
<tr>
<td>Error</td>
<td>28</td>
<td>0.0049</td>
<td>-</td>
</tr>
</tbody>
</table>

**,*Significant at α = 0.01 and 0.05, respectively.
Table 8. Nitrate reductase activity\(^a\) of corn leaves as affected by the N concentration in the nutrient solution and by the addition of extra NH\(_4\); SEM (simple means) = 0.031 (Experiment 5A)

<table>
<thead>
<tr>
<th>NO(_3)-N concentration in solution (meq L(^{-1}))</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO(_3)-N</td>
<td>.338</td>
<td>.398</td>
<td>.416</td>
<td>.384</td>
<td>0.018</td>
</tr>
<tr>
<td>NO(_3)-N + 16 meq NH(_4)-N</td>
<td>.400</td>
<td>.360</td>
<td>.502</td>
<td>.421</td>
<td>0.018</td>
</tr>
<tr>
<td>Mean</td>
<td>.369</td>
<td>.379</td>
<td>.459</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.022</td>
<td>0.022</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^aNRA, \mu mol NO_3^- reduced/g leaf FW.h.\)

Table 9. Ammonium-N uptake as a function of initial NH\(_4\)-N concentration at different levels of initial NO\(_3\)-N concentration; SEM (simple means) = 0.32 (Experiment 5A)

<table>
<thead>
<tr>
<th>NH(_4)-N (mg N/pot)</th>
<th>NO(_3)-N (mg N/pot)</th>
<th>6.27</th>
<th>9.07</th>
<th>14.67</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>2.80</td>
<td>1.68</td>
<td>1.48</td>
<td>1.46</td>
<td>1.54</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>8.40</td>
<td>3.54</td>
<td>3.70</td>
<td>3.76</td>
<td>3.67</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>2.61</td>
<td>2.59</td>
<td>2.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
shows that, for the conditions of this experiment, NH$_4^-$-N uptake was not affected by the level of initial NO$_3^-$ concentration in the soil.

Nitrate-N uptake increased with the initial NO$_3^-$ concentration in soil (Table 10) with significant differences at $\alpha = 0.01$. High NH$_4^+$ reduced NO$_3^-$-N uptake, but this effect was not statistically significant at $\alpha = 0.05$.

Table 10. Nitrate-N uptake as a function of initial NO$_3^-$-N concentration at 2 levels of initial NH$_4^-$-N concentration; SEM (simple means) = 0.28 (Experiment 5A)

<table>
<thead>
<tr>
<th>NH$_4^-$-N (mg N/pot)</th>
<th>NO$_3^-$-N (mg N/pot)</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.80</td>
<td>2.34</td>
<td>3.60</td>
<td>4.14</td>
</tr>
<tr>
<td>8.40</td>
<td>2.28</td>
<td>2.74</td>
<td>3.96</td>
</tr>
<tr>
<td>Mean</td>
<td>2.31</td>
<td>3.17</td>
<td>4.05</td>
</tr>
<tr>
<td>SEM</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Treatments 3, 5, and 8 correspond to 32 meq L$^{-1}$ of NO$_3^-$-N, 32 meq L$^{-1}$ of a 50/50 NO$_3^-$-N/NH$_4^-$-N ratio, and 32 meq L$^{-1}$ of NH$_4^+$-N, respectively. When N was applied as a combination of NO$_3^-$ and NH$_4^+$, total N uptake was greater than with either source alone. This difference was significant at
\(a = 0.10\) (data not shown). The results of this experiment show that at 5 h after application of the treatments, \(\text{NH}_4^+\) added to \(\text{NO}_3^-\) did not reduce nitrate reductase activity in the leaves, and that the level of initial \(\text{NO}_3^-\) concentration did not affect total \(\text{NH}_4^-\)-N uptake. These results contrast with those obtained from long-term experiments by Schrader et al. (1972) and Warncke and Barber (1973). The reason for this discrepancy may be the duration of the experiments. Five hours may not be enough to show the effects of a regulatory feedback mechanism.

5B. A strong counter ion effect is demonstrated in this experiment. Tables 11 and 12 show the results of this experiment. Nitrate reductase activity was significantly higher with \(\text{KNO}_3\) than with \(\text{Ca(NO}_3)_2\) (\(\alpha = 0.01\)). Similarly, \(\text{NO}_3^-\)-N uptake (not shown) and \(\text{NO}_3^-\)-N uptake per g of fresh weight were significantly higher when \(\text{NO}_3^-\) was applied as \(\text{KNO}_3\) (\(\alpha = 0.01\)). Finally, Table 11 and Fig. 7 show a highly significant interaction between counter ion effect and concentration of \(\text{NO}_3^-\) supplied. The higher concentration of \(\text{NO}_3^-\) produced a higher uptake when K was the counter ion, but it did not produce an increase in uptake when Ca was the counter ion.
Table 11. Statistical analysis of the data obtained in Experiment 5B (1 = nitrate reductase activity, 2 = NO$_3$-N uptake per unit of total fresh weight

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>MS 1</th>
<th>MS 2</th>
<th>F 1</th>
<th>F 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>15</td>
<td>0.010</td>
<td>0.0083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block</td>
<td>3</td>
<td>0.005</td>
<td>0.0096</td>
<td>1.14</td>
<td>3.56</td>
</tr>
<tr>
<td>Treatments</td>
<td>3</td>
<td>0.034</td>
<td>0.0237</td>
<td>7.73**</td>
<td>8.78**</td>
</tr>
<tr>
<td>KNO$_3$ vs Ca(NO$_3$)$_2$ (A)</td>
<td>1</td>
<td>0.100</td>
<td>0.0262</td>
<td>22.73***</td>
<td>9.70**</td>
</tr>
<tr>
<td>10 meq vs 16 meq (B)</td>
<td>1</td>
<td>0.003</td>
<td>0.0126</td>
<td>0.68</td>
<td>4.67</td>
</tr>
<tr>
<td>A*B</td>
<td>1</td>
<td>0.0003</td>
<td>0.0324</td>
<td>0.07</td>
<td>12.00**</td>
</tr>
<tr>
<td>Error</td>
<td>9</td>
<td>0.0044</td>
<td>0.0027</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

***,**Significant at α = 0.001 and 0.01, respectively.

Experiment 6

The percentage of NH$_4$-N and NO$_3$-N uptake as a function of the supplied NO$_3$-N/NH$_4$-N ratio is shown in Fig. 8. The percentage of NO$_3$-N uptake (which is NO$_3$-N uptake divided by NO$_3$-N supplied to the pots) increased as NO$_3^-$ became less abundant in the NO$_3$-N/NH$_4$-N mixture (linear effect was significant at α = 0.0003). The percentage of NH$_4$-N uptake did not show a very clear trend.

Total N uptake and total N uptake per unit of root dry weight showed a significant quadratic effect after linear
Table 12. Nitrate reductase activity (μmol NO₃⁻ reduced/g leaf FW·h) as a function of NO₃-N concentration in the nutrient solution and type of counter ion present; SEM (simple means) = 0.033 (Experiment 5B)

<table>
<thead>
<tr>
<th>N meq L⁻¹</th>
<th>KNO₃</th>
<th>Ca(NO₃)₂</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.453</td>
<td>0.304</td>
<td>0.378</td>
<td>0.023</td>
</tr>
<tr>
<td>16</td>
<td>0.487</td>
<td>0.320</td>
<td>0.403</td>
<td>0.023</td>
</tr>
<tr>
<td>Mean</td>
<td>0.470</td>
<td>0.312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.023</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(α = 0.0055 and 0.0017, respectively) with maximal rates of uptake at a 40/60 NO₃⁻-N/NH₄⁺-N ratio (Fig. 9). Nitrogen percentage in shoots was significantly higher when the N was supplied as a balanced mixture of NO₃⁻ and NH₄⁺. Nitrogen percentage in roots showed a similar pattern but with the peak displaced toward a higher NO₃⁻-N/NH₄⁺-N ratio (data not shown).

No effect of the NO₃⁻-N/NH₄⁺-N ratio was observed in final soil pH, probably due to a good soil buffer capacity resulting from high levels of P applied.
Fig. 7. NO₃-N uptake as affected by initial NO₃-N concentration and counter ion present (Experiment 5B); the vertical bar indicates the SEM.
Fig. 8. Effect of the NO$_3$-N/NH$_4$-N ratio on the percentage of NO$_3$-N and NH$_4$-N uptake (Experiment 6); the vertical bars indicate the SEM.
Fig. 9. N uptake and N uptake per g of root dry weight as a function of the NO₃-N/NH₄-N ratio (Experiment 6); vertical bars = SEM
7A. Several effects of the NO$_3$-N/ NH$_4$-N ratio are studied in this experiment. Nitrate-N uptake, expressed as a percentage of total NO$_3$-N supplied, increased as the NO$_3$-N/NH$_4$-N ratio in the soil solution decreased. Contrarily, NH$_4$-N uptake, expressed as a percentage of total NH$_4$-N supplied, decreased as the NO$_3$-N/NH$_4$-N ratio decreased (Fig. 10). This indicates that corn plants tend to absorb proportionally more of the less abundant N form.

Soil pH decreased from 7.22 to 4.93 when the NO$_3$-N/NH$_4$-N ratio went from 100/0 to 0/100 (Fig. 11). This is evidence for the NO$_3^-$/OH$^-$ and NH$_4^+$/H$^+$ antiport mechanism. (Nitrification was not responsible for this pH change because it was inhibited with nitrapyrin.)

The regression of percentage NO$_3$-N uptake, percentage NH$_4$-N uptake, and pH on the NO$_3$-N/NH$_4$-N ratio all showed significant linear effect at $\alpha = 0.0001$. The analysis of the remaining soil N (NO$_3$-N + NH$_4$-N) indicated that total N uptake was maximum for a 50/50 NO$_3$-N/NH$_4$-N concentration (Fig. 12) (differences significant at $\alpha = 0.0001$). Figure 13 shows total N in shoots as a function of the NO$_3$-N/NH$_4$-N ratio.

The NO$_3$-N/NH$_4$-N ratio supplied to the 75/25 treatment was 3, but in the soil, this ratio increased to 8.01 at the end of the experiment. Contrarily, the initial ratio was
Fig. 10. Effect of the NO$_3$-N/NH$_4$-N ratio on the percentage of NO$_3$-N and NH$_4$-N uptake (Experiment 7A); vertical bars = SEM
Fig. 11. Soil pH as affected by the NO$_3$-N/NH$_4$-N ratio (Experiment 7A); vertical bar = SEM
Fig. 12. Total N uptake (calculated as depletion from the soil) as a function of the NO$_3$-N/NH$_4$-N ratio (Experiment 7A); vertical bar = SEM
Fig. 13. Effect of the NO₃-N/NH₄-N ratio on the N content in shoots of corn plants (Experiment 7A); vertical bar = SEM
0.33 for the 25/75 treatment but it declined to 0.10 at the end of the experiment. Again, this indicates that, when the NO$_3$-N/NH$_4$-N ratio is away from 1.0, corn plants favor uptake of the less abundant N form in an attempt to balance their nutrition.

The concentration of Ca in tissue decreased as the NO$_3$-N/NH$_4$-N ratio decreased mainly because NO$_3$-N was supplied as Ca(NO$_3$)$_2$. Potassium and Mg concentration in tissue also decreased as NH$_4^+$ became more abundant in the N solution, indicating the existence of a cation-anion balance effect (linear effects significant at $\alpha = 0.0001$) (Fig. 14 and 15). On the other hand, P concentration in tissue increased as NH$_4^+$ became more abundant, probably due to the same cation-anion balance effect (linear effect significant at $\alpha = 0.0001$) (Fig. 14). Sulfur concentration also increased, partly because NH$_4^+$ was supplied as (NH$_4$)$_2$SO$_4$, but the increase in S concentration had a significant linear effect ($\alpha = 0.0001$) and a significant quadratic effect after linear ($\alpha = 0.0013$) (Fig. 14). This quadratic effect in S concentration as NH$_4^+$ increased and NO$_3^-$ decreased in the N mixture is also interpreted as a cation-anion balance effect.

Figures 16, 17, and 18 show the effect of the treatments on the tissue concentration of micronutrients and Cd. Manganese concentration had a significant linear effect ($\alpha = 0.0001$) being maximum when all the N was applied as NH$_4$-N.
Fig. 14. Concentration of P, Ca, Mg, and S in shoots of corn plants as affected by the NO$_3$-N/NH$_4$-N ratio (Experiment 7A); vertical bars = SEM
Fig. 15. Concentration of K in shoots of corn plants as a function of the NO₃-N/NH₄-N ratio (Experiment 7A); vertical bar = SEM
Fig. 15. Effect of the NO₃-N/NH₄-N ratio on the concentration of Fe, Zn, Cu, and B in shoots of corn plants (Experiment 7A); vertical bars = SEM
Fig. 17. Effect of the NO₃-N/NH₄-N ratio on the concentration of Mn in shoots of corn plants (Experiment 7A); vertical bar = SEM
Fig. 18. Effect of the NO$_3$-N/NH$_4$-N ratio on the concentration of Cd in shoots of corn plants (Experiment 7A); vertical bar = SEM
This could be interpreted as a pH effect on the availability of Mn. The concentration of Cu in tissue was minimum for the 100% NH₄-N treatment. Copper, Zn, and B concentrations had maximum levels in tissue when the N was applied as a balance mixture of NO₃-N and NH₄-N. The concentration of Cd (heavy metal) had a significant linear effect (α = 0.0001) being maximum when all the N was supplied as NO₃⁻.

Finally, plant height and shoot fresh weight did not differ among the treatments (data not shown).

7B. The effect of readily versus nonreadily absorbed counter ions is analyzed in this experiment. Treatment 2 (Ca(NO₃)₂) had a significantly lower shoot dry weight than the rest of the treatments (17.41 vs 19.5 g). The rest of the values did not differ at the 5% level of significance (data not shown).

Table 13 shows the results of this experiment. The final soil pH responded to the form of N applied. The presence of Cl ions increased the soil pH significantly, probably due to a Cl⁻:OH⁻ antiport mechanism. When cation uptake exceeded anion uptake, the soil pH tended to decrease. Contrarily, when anion uptake was greater than cation uptake, the soil pH tended to increase. Total N content in shoots was greater when the N was supplied with a readily absorbed counter ion (K for NO₃⁻ and Cl for NH₄⁺). When the total N content in shoots for the KNO₃ and NH₄Cl treatments was
Table 13. Effect of different N forms on the variables measured in Experiment 7B

<table>
<thead>
<tr>
<th>Variable</th>
<th>KNO$_3$</th>
<th>Ca(NO$_3$)$_2$</th>
<th>50/50</th>
<th>50/50+Cl</th>
<th>(NH$_4$)$_2$SO$_4$</th>
<th>NH$_4$Cl</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO$_3^-$ uptake (mg NO$_3$-N/100 g dry soil)</td>
<td>22.92</td>
<td>22.49</td>
<td>13.15</td>
<td>10.40</td>
<td>-</td>
<td>-</td>
<td>0.56</td>
</tr>
<tr>
<td>NH$_4^+$ uptake (mg NH$_4$-N/100 g dry soil)</td>
<td>-</td>
<td>-</td>
<td>12.69</td>
<td>12.71</td>
<td>22.43</td>
<td>22.94</td>
<td>0.51</td>
</tr>
<tr>
<td>pH</td>
<td>7.12</td>
<td>7.21</td>
<td>5.92</td>
<td>6.26</td>
<td>4.91</td>
<td>5.47</td>
<td>0.08</td>
</tr>
<tr>
<td>Total N in shoots (g N/plant)</td>
<td>0.62</td>
<td>0.56</td>
<td>0.61</td>
<td>0.58</td>
<td>0.59</td>
<td>0.62</td>
<td>0.016</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>2103</td>
<td>2330</td>
<td>2479</td>
<td>2284</td>
<td>2954</td>
<td>2711</td>
<td>106</td>
</tr>
<tr>
<td>K (ppm)</td>
<td>53730</td>
<td>26804</td>
<td>24009</td>
<td>23951</td>
<td>20271</td>
<td>21490</td>
<td>790</td>
</tr>
<tr>
<td>Ca (ppm)</td>
<td>4713</td>
<td>7281</td>
<td>5067</td>
<td>7707</td>
<td>3752</td>
<td>6113</td>
<td>257</td>
</tr>
<tr>
<td>Mg (ppm)</td>
<td>2865</td>
<td>6362</td>
<td>4929</td>
<td>4776</td>
<td>3515</td>
<td>4592</td>
<td>172</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>50.9</td>
<td>54.0</td>
<td>66.3</td>
<td>76.7</td>
<td>53.2</td>
<td>61.6</td>
<td>2.32</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>4.00</td>
<td>4.70</td>
<td>6.37</td>
<td>5.32</td>
<td>2.93</td>
<td>4.77</td>
<td>0.34</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>65.3</td>
<td>78.8</td>
<td>76.6</td>
<td>67.7</td>
<td>68.3</td>
<td>70.4</td>
<td>5.21</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>39.2</td>
<td>47.0</td>
<td>64.7</td>
<td>70.7</td>
<td>98.0</td>
<td>129.2</td>
<td>9.54</td>
</tr>
<tr>
<td>Cd (ppm)</td>
<td>.33</td>
<td>.57</td>
<td>.47</td>
<td>.80</td>
<td>.19</td>
<td>.77</td>
<td>0.06</td>
</tr>
</tbody>
</table>
compared with the Ca(NO\textsubscript{3})\textsubscript{2} and (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} treatments, significant differences were found at $\alpha = 0.01$.

The level of P in tissue was not clearly affected by the different treatments. Treatment 1 had a very high level of K compared to the rest. This was expected because the source of N in this treatment was KNO\textsubscript{3}. Similarly, treatment 2, which represents Ca(NO\textsubscript{3})\textsubscript{2}, had high levels of Ca. The application of Cl to the 50/50 treatment and the replacement of (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} by NH\textsubscript{4}Cl both significantly increased the Ca level in tissue. Moreover, the NH\textsubscript{4}Cl treatment produced a 31% increase in Mg concentration compared to the (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} treatment. Also, the Mg concentration increased 122% when the KNO\textsubscript{3} treatment was replaced by Ca(NO\textsubscript{3})\textsubscript{2}. All these differences were highly significant.

The level of micronutrients in tissue also showed a response to the treatments. The presence of Cl ion increased the concentration of Zn and Cu in tissue. Moreover, all the micronutrient cations showed lower levels with KNO\textsubscript{3} than with Ca(NO\textsubscript{3})\textsubscript{2}. Finally, Cd concentration in tissue was higher for those treatments that favored anion uptake over cation uptake.

Experiment 8A - 8E

In this series of experiments, NMR is used to study the effect of the form of N on cytoplasmic and vacuolar pH
of excised corn root tips.

8A. Figure 19 shows the reference signal relative to that of 85% PO₄H₃. Figure 20 shows why root tips were necessary in these experiments. Root tips are used in order to obtain strong signals from the cytoplasm as well as from the vacuole. Cells in other parts of the plant are 90-95% vacuole; if these parts are used to obtain an NMR spectra, only vacuolar signals are clearly observed.

Phosphate groups in the vacuole showed peaks at around -15.50 ppm, whereas Pi groups in the cytoplasm showed peaks at -14.15 ppm. The conversion of ppm values to pH requires a standard curve (see Roberts et al., 1980). In this experiment, -14.11 ppm, -14.40 ppm, -15.02 ppm, and -15.55 ppm approximately correspond to pH values of 7.3, 7.0, 6.6, and 5.5, respectively. These conversion values can only be taken as an estimate for the present study.

The form of N did not affect the ppm value for the free Pi groups in the cytoplasm of the root tips. Consequently, the pH of the cytoplasm is not affected by the N form (see Fig. 21 and Table 14). Free Pi groups in root tip vacuole showed peaks at higher ppm values with NH₄⁺ than with NO₃⁻ (-15.48 vs -15.55); conversely, free Pi groups in the leaf vacuole showed peaks at higher ppm values with NO₃⁻ than with NH₄⁺ (-15.44 vs -15.67). This means that NO₃⁻ produced relatively lower vacuolar pH in root tips but higher vacuolar pH
Fig. 19. $^{31}$P NMR spectra of 85% PO$_4$H$_3$; the exact position of the peak is -16.426; the zero corresponds to the standard (Experiment 8A)
Fig. 20. $^{31}$P NMR spectra of corn root tips (A), whole corn roots (B), and corn leaves (C) (Experiment 8A); 1 = sugar phosphates, 2 = Pi in cytoplasm, 3 = Pi in vacuole, 4-5 = ATP
Fig. 21. Effect of the N form (A = NO$_3^-$, B = 50/50, C = NH$_4^+$) on the frequency of resonance of $^{31}$P in root tips (Experiment 8A); 1 = sugar phosphates, 2 = Pi in cytoplasm, 3 = Pi in vacuole
Table 14. Effect of the form of N on the frequency of resonance in root-tip vacuolar and cytoplasmic Pi and leaf vacuolar Pi (Experiment 8A)

<table>
<thead>
<tr>
<th></th>
<th>NO₃⁻ 50:50 ppm</th>
<th>NH₄⁺ 50:50 ppm</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root tip Cyt Pi</td>
<td>-14.18</td>
<td>-14.12</td>
<td>-14.16</td>
</tr>
<tr>
<td>Root tip Vac Pi</td>
<td>-15.55</td>
<td>-15.46</td>
<td>-15.48</td>
</tr>
<tr>
<td>Leaf Vac Pi</td>
<td>-15.44</td>
<td>-15.51</td>
<td>-15.57</td>
</tr>
<tr>
<td>SEM</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

in leaves when compared to NH₄⁺ (these differences were significant at α = 0.10).

Nitrogen uptake by the corn seedlings used to obtain the root tips was calculated. Figure 22 shows the results. Nitrogen uptake was a maximum from a 50:50 combination of NO₃⁻-N and NH₄⁺-N compared to either source alone (differences significant at α = 0.05).

8B and 8C. Figure 23 shows the development of anaerobiosis in corn root tips. Sugar phosphate peaks tended to disappear due to acceleration of glycolysis in the absence of O₂ (Pasteur effect). Moreover, the shift to the right in the cytoplasmic Pi signal indicates an acidification of the cytoplasm probably due to transient lactic fermentation (Roberts et al., 1984) and partly to CO₂ accumulation. (The precise ppm values at which the nuclei resonate is a function of pH
Fig. 22. Total N uptake as a function of N form for whole corn seedlings growing in sand culture (Experiment 8A)
Figure 23. Effect of anaerobiosis in the $^{31}$P spectra of corn root tips (Experiment 8C); A to C = increasing level of hypoxia; 1, sugar phosphates; 2, Pi in cytoplasm; 3, Pi in vacuole
with peaks moving to the right when the medium becomes more acid.)

In part A, the root tips could become somewhat anaerobic since 1 to 3 h occurred from the moment they were cut until they were placed in the NMR spectrometer. This could confound the N form effect. To check this, another experiment was carried out in which the root tips were bubbled with O₂ for 60 minutes in a 20 meq L⁻¹ of N (NO₃⁻ or NH₄⁺) solution (plus glucose and CaSO₄) immediately before obtaining the NMR spectra (part B of the experiment). Table 15 shows the results. Again, the nitrogen form did not affect the ppm value of the Pi groups (i.e., pH) in the cytoplasm of the root tips (Fig. 24).

Table 15. Effect of the form of N on the frequency of resonance of vacuolar and cytoplasmic Pi in corn root tips with excess O₂ (Experiment 8B)

<table>
<thead>
<tr>
<th></th>
<th>NO₃</th>
<th>NH₄</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyt Pi</td>
<td>-14.125</td>
<td>-14.101</td>
<td>0.029</td>
</tr>
<tr>
<td>Vac Pi</td>
<td>-15.678</td>
<td>-15.629</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.024</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 24. Effect of the N form on the frequency of resonance of $^{31}$P in root tips (Experiment 8B); the root tips were bubbled with O$_2$ for 60 minutes in a solution containing 50 meq L$^{-1}$ glucose, 0.1 mM CaSO$_4$ and 20 meq L$^{-1}$ of either Ca(NO$_3$)$_2$ (A) or (NH$_4$)$_2$SO$_4$ (B) right before obtaining the spectra; 1, sugar phosphates; 2, Pi in cytoplasm; 3, Pi in vacuole
8D. In anaerobiosis, the cytoplasmic Pi signal shifts to the right due to an acidification of this compartment. So, the shift in this signal could be considered as development of anaerobic conditions in the cell. The form of N affected the development of anaerobiosis in corn root tips as shown in Fig. 25A.

Nitrate (an electron acceptor) retarded the acidification of the cytoplasm compared to NH$_4^+$. Moreover, the cell died (as indicated by the destruction of the cell compartmentation) sooner when NH$_4^+$ was the N source (NH$_4^+$ cells died 2 hours after being placed in the NMR, whereas NO$_3^-$ cells were still alive at the end of the experiment).

Production of NO$_2^-$ by the corn root tips supplied with NO$_3^-$ was measured under anaerobic conditions. The value was 2.90 μmole of NO$_3^-$ reduced to NO$_2^-$ per g of dry weight per hour. A significant part of this NO$_2^-$ is extruded to the rhizosphere.

These results were communicated to Dr. J. Roberts at Stanford University and he cordially proposed to repeat this experiment with his modified NMR spectrometer (Roberts et al., 1982, 1984). He analyzed the effect of NO$_3^-$ on the development of cytoplasmic acidosis of corn root tips during hypoxia. Figure 25B shows the results. Nitrate clearly reduced the acidification of the cytoplasm after 9 h of perfusing the root cells with a 50 mM glucose/0.1 mM CaSO$_4$ solution saturated with N$_2$. The presence of NO$_3^-$ also decreased the amount
Fig. 25A. Shifts in cytoplasmic Pi signals of hypoxic corn root tips in a NO$_3^-$ (closed line) or NH$_4^+$ (dotted line) solution (Experiment 8D); time 0 represents initiation of development of anaerobiosis.
Fig. 25B. Effect of 25 mM Ca(NO$_3$)$_2$ on the frequency of resonance of $^{31}$P in corn root tips incubated for 9 h without O$_2$ (1 h spectra); 1' = control, perfused with N$_2$-saturated 50 mM glucose, 0.1 mM CaSO$_4$ solution; 2' = perfused with N$_2$-saturated 50 mM glucose, 0.1 mM CaSO$_4$, 50 meq L$^{-1}$ Ca(NO$_3$)$_2$ solution; 1 = sugar phosphates; 2 = Pi in cytoplasm; 3 = Pi in vacuole
of cytoplasmic acidosis during the first minutes of hypoxia.

After incubating the root tips for 21 h in a solution containing 20 meq L\(^{-1}\) NO\(_3\)\(^{-1}\), 50 mM glucose and 0.1 mM CaSO\(_4\), the roots grew to more than twice their original length (7 mm vs 3 mm). The rate of NO\(_3\) uptake was calculated for the root tips under these conditions and it was 0.48 mg NO\(_3\)-N (34 μmoles) per g of dry weight per h.

Experiment 9

The effect of a nitrification inhibitor is studied in this experiment. The natural log of the NO\(_3\)-N/NH\(_4\)-N ratio of soil samples collected at 2, 4, and 6 weeks after application of the treatments is presented in Fig. 25 and 27. Stages of application A and B were analyzed separately. Nitrapyrin effectively controlled nitrification, and this effect was also a function of nitrapyrin concentration.

Nitrapyrin significantly increased N (NO\(_3\)-N + NH\(_4\)-N) in the soil at all sampling times (see Fig. 28, stages A and B were pooled together and the higher level of nitrapyrin was not included) because it maintained the N in the band as NH\(_4\)\(^+\) which is less mobile and less subjected to leaching than NO\(_3\)\(^-\). The regression of N (NO\(_3\)-N + NH\(_4\)-N) in the soil on the rate of nitrapyrin had a highly significant linear effect (α = 0.0001). Moreover, N (NO\(_3\)-N + NH\(_4\)-N) in the band decreased linearly with time (significant at α = 0.0001) mainly
Fig. 26. Influence of nitrapyrin on the soil (band) NO$_3$-N/NH$_4$-N ratio (expressed as natural log) at 2, 4, and 6 weeks after application of 90 kg ha$^{-1}$ of NH$_4$-N sidedressing (Stage A, Experiment 9); the rates 0, 1, 2, and 3 of nitrapyrin represent 0, 741, 2244, and 6672 g ha$^{-1}$ of active ingredient, respectively; SEM for split means at the same whole mean level = 0.21; SEM for whole means at the same or different split mean level = 0.20
WEEKS AFTER APPLICATION

NAT LOG NO3-N/NH4-N RATIO

0 NITRAPYRIN 1 NITRAPYRIN 2 NITRAPYRIN 3 NITRAPYRIN
Fig. 27. Influence of nitrapyrin on the soil (band) NO$_3$-N/NH$_4$-N ratio (expressed as natural log) at 2, 4, and 6 weeks after application of 90 kg ha$^{-1}$ of NH$_4$-N sidedressing (Stage B, Experiment 9); the rates 0, 1, 2, and 3 of nitrapyrin represent 0, 741, 2244, and 6672 g ha$^{-1}$ of active ingredient, respectively; SEM for split means at the same whole mean level = 0.21; SEM for whole means at the same or different split mean level = 0.18
Figure 28. Effect of nitrapyrin and time after application on the soil (band) N (NO$_3$-N + NH$_4$-N) (Experiment 9); the rates 0, 1, and 2 represent 0, 741, and 2244 g ha$^{-1}$ of active ingredient, respectively; the control represents no N application; SEM for split means at the same whole mean level = 0.96; SEM for whole means at the same or different split mean level = 1.07
due to absorption by the plants and leaching after conversion of \( \text{NH}_4^+ \) to \( \text{NO}_3^- \).

The data for the third stage of application in which the third level of nitrapyrin was replaced by \( \text{NO}_3^- \) is presented in Figure 29. Total N application was the same for all the treatments. The figure shows that N in the band disappeared faster when \( \text{NO}_3^- \) was the N source. This demonstrates that \( \text{NO}_3^- \) is much more mobile in the soil and consequently subjected to leaching.

Experiment 10

The effect of the form of N on the efficiency of N fertilization is analyzed. The \( \text{NO}_3^-/\text{NH}_4^- \) ratio was followed for 6 weeks after the first application of the treatments. Figure 30 shows the natural log of the nitrogen ratio as a function of time after application. Nitrapyrin effectively inhibited nitrification. The ratio decreased with time for the \( \text{NO}_3^- \) treatment because \( \text{NO}_3^- \) is being leached or absorbed; on the other hand, the ratio increased for the \( \text{NH}_4^+ \) plus nitrapyrin and \( \text{NH}_4^+ \) without nitrapyrin treatments because \( \text{NH}_4^+ \) is slowly converted to \( \text{NO}_3^- \). These two opposite phenomena produced a highly significant time by N form interaction.

Figure 31 represents the disappearance of N (\( \text{NO}_3^- \) + \( \text{NH}_4^- \)) from the upper 30 cm of soil. The values at the second week after application are considered as 100%. Nitrogen
Fig. 29. $N (NO_3^-\text{N} + NH_4^-\text{N})$ in the soil (band) as affected by the rate of nitrpyrin, form of N, and time after application of 90 kg ha$^{-1}$ of N sidedressing (Experiment 9); rates 0, 1, and 2 represent 0, 741, and 2244 g ha$^{-1}$ of active ingredient, respectively; SEM for split means at the same whole mean level = 1.17; SEM for whole means at the same or different split mean level = 1.21
2 NITRAPYRIN  1 NITRAPYRIN  0 NITRAPYRIN  NITRATE

N IN SOIL (mg N/100g OF SOIL)

WEEKS AFTER APPLICATION
Fig. 30. Effect of nitrapyrin, form of N and time after application on the natural log of the NO₃-N/NH₄-N ratio in the soil (band) (Experiment 10); SEM for split mean at the same whole mean level = 0.24; SEM for whole means at the same or different split mean level = 0.30
NITRAPYRIN NO NITRATE
NITRAPYRIN
NAT LOG NO3-N/NH4-N RATIO
WEEKS AFTER APPLICATION

NAT LOG NO3-N/NH4-N RATIO

WEEKS AFTER APPLICATION
Fig. 31. Effect of nitrapyrin, form of N and time after application on the disappearance of N (NO$_3$-N + NH$_4$-N) from the soil (band) (Experiment 10); nitrogen in the soil is expressed as percentage of the N present at 2 weeks after application; SEM for split means at the same whole mean level = 7.02; SEM for whole means at the same or different split mean level = 6.93
disappeared faster when it was applied as NO$_3^-$ (differences significant at $\alpha = 0.10$).

Table 10 shows protein percentage of leaves sampled 3 days after anthesis, weight of 100 seeds, grain protein percentage, and grain yield as a function of N rate. All these variables except weight of 100 seeds increased with rate of N with a linear effect significant at $\alpha = 0.0001$.

Figures 32, 33, and 34 show the response of leaf protein percentage, grain protein percentage, and grain yield to the N form after pooling the data for the different N rates together and eliminating the controls. Splitting the N applications significantly increased leaf protein percentage at anthesis, grain protein percentage and grain yield. This practice should be recommended in those areas where soil characteristics and rainfall produce significant losses of N by leaching. The figures also show that applying the N as NH$_4^+$ (urea) plus nitrapyrin is another way of reducing the leaching effect. Without splitting the applications, urea plus nitrapyrin produced higher leaf protein percentage and grain yield than NO$_3^-$ (only the increase in grain yield was significant at $\alpha = 0.05$). Weight of 100 seeds was not affected by the treatments.
Table 16. Leaf protein percentage, weight of 100 seeds, grain protein percentage, and grain yield as affected by the rate of N in Experiment 10

<table>
<thead>
<tr>
<th>N kg/ha</th>
<th>Leaf protein (%)</th>
<th>Weight/100 seed (g)</th>
<th>Grain protein (%)</th>
<th>Grain yield (kg/plot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.03</td>
<td>24.72</td>
<td>7.69</td>
<td>5.55</td>
</tr>
<tr>
<td>75</td>
<td>17.38</td>
<td>26.34</td>
<td>9.06</td>
<td>10.26</td>
</tr>
<tr>
<td>125</td>
<td>18.21</td>
<td>26.13</td>
<td>9.75</td>
<td>10.81</td>
</tr>
<tr>
<td>225</td>
<td>21.33</td>
<td>26.09</td>
<td>11.50</td>
<td>13.05</td>
</tr>
<tr>
<td>SEM</td>
<td>0.31</td>
<td>0.23</td>
<td>0.19</td>
<td>0.26</td>
</tr>
<tr>
<td>(for 1 vs 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 32. Effect of the form of N (1 = urea plus nitrapyrin, 2 = urea without nitrapyrin, 3 = NO₃) on protein percentage of corn leaves (Experiment 10); the vertical bar represents the SEM.
LEAF PROTEIN PERCENTAGE

FORM OF NITROGEN
Fig. 33. Effect of the form of N (1 = urea plus nitrapyrin, 2 = urea without nitrapyrin, 3 = NO$_3$) on grain protein percentage in corn (Experiment 10); the vertical bar represents the SEM.
Fig. 34. Effect of the form of N (1 = urea plus nitrapyrin, 2 = urea without nitrapyrin, 3 = NO₃) on grain yield of corn (Experiment 10); the vertical bar represents the SEM.
GRAIN YIELD (kg PER PLOT)

SPLIT APPLICATION

NO SPLIT APPLICATION

FORM OF NITROGEN
DISCUSSION

The results of Experiment 6 and 7 (Fig. 8 and 10) indicate that corn plants favor absorption of the less abundant N form. Nitrate uptake, expressed as a percentage of total NO$_3^-$ applied, increased as the NO$_3^-$-N/NH$_4^+$-N ratio in the soil solution decreased. Contrarily, NH$_4^+$ uptake also expressed as a percentage of total NH$_4^+$ supplied, decreased as the ratio decreased. This was confirmed by the fact that during the experiments, the NO$_3^-$-N/NH$_4^+$-N ratio in the soil increased when NO$_3^-$ was the predominant form and decreased when NH$_4^+$ was the predominant form (nitrification was inhibited with nitrapyrin). The experiments also showed that total N uptake was greater when N was applied as a combination of NO$_3^-$ and NH$_4^+$ (Fig. 9 and 12). Similar results were found in Experiment 5. Moreover, in Experiment 8, total N uptake by corn seedlings growing in sand culture was also greater from a combination of NO$_3^-$ and NH$_4^+$ than from either source alone (Fig. 22).

Most of these experiments were conducted in soil, and their results agree with those conducted with nutrient solution by Warncke and Barber (1973) and Schrader et al. (1972). Warncke and Barber (1973) reported that the ratio of NO$_3^-$-N/NH$_4^+$-N absorbed by the corn seedlings moved toward 1.0 as compared to the ratio in the solution. Some further calculations were done with the data presented by Schrader et al.
(1972) and it was found that these data match the results presented in this work (data not shown).

A tendency toward a plateau was observed when NO\textsuperscript{3} \textsuperscript{-} uptake was plotted as a function of NO\textsuperscript{3} \textsuperscript{-} available in the soil (Fig. 6; Experiment 4). There was a significant quadratic effect after linear. Similar results were found for NH\textsubscript{4} \textsuperscript{+}. This indicates that the efficiency of NO\textsuperscript{3} \textsuperscript{-} or NH\textsubscript{4} \textsuperscript{+} uptake decreases as their external concentration increases.

It could be argued that saturation of the plasmalemma carrier capacity is responsible for all the previously discussed effects, but the problem seems to be more complicated than that. The results of Experiment 5B show that nitrate reductase activity in shoots, and nitrogen uptake, were significantly higher with KNO\textsubscript{3} than with Ca (NO\textsubscript{3})\textsubscript{2} as the N source (Fig. 7, Tables 11 and 12). Similar results were reported for nutrient solution experiments (Blevins et al., 1978; Smith, 1973). The data obtained in Experiment 3 (Fig. 2) clearly indicate that NO\textsuperscript{3} \textsuperscript{-} concentration in roots increases with the level of NO\textsuperscript{3} \textsuperscript{-} nutrition. This could be responsible for the decrease in efficiency of uptake at high external levels of NO\textsuperscript{3}. A slowly penetrating counter ion (Ca) can slow down the uptake of a rapidly penetrating ion (NO\textsuperscript{3}) (Lütge and Higinbotham, 1979). The absence of counter ions for NO\textsuperscript{3} \textsuperscript{-} translocation to the shoots would produce an accumulation of NO\textsuperscript{3} \textsuperscript{-} in the roots (Ben Zioni et al., 1971; Raven
and Smith, 1976). This higher NO\textsubscript{3} concentration would somehow decrease the uptake efficiency (Frost et al., 1978; Rufty et al., 1981; MacKown et al., 1981; Jackson et al., 1975).

Ammonium concentration in roots also increased with higher levels of NH\textsubscript{4} nutrition (Fig. 2) (Magalhaes and Wilcox, 1983b). Again, this higher concentration could be responsible for the decrease in efficiency of uptake at high external levels of NH\textsubscript{4}.

Cram (1973) and Glass (1975) proposed models in which ion uptake is controlled by feedback mechanisms exerted on the carriers directly or indirectly by the internal ion concentrations. Since NH\textsubscript{4} and NO\textsubscript{3} accumulated in corn roots as a function of external concentration, this is a possibility. However, Deane-Drummond and Glass (1983) found that NO\textsubscript{3} efflux was strongly correlated with internal NO\textsubscript{3} concentration in the cells, while NO\textsubscript{3} influx appeared to be independent of NO\textsubscript{3} concentration in the cells. The higher the NO\textsubscript{3} influx rate, the higher the internal concentration. This would enhance leaking and, consequently, diminish the uptake efficiency.

Tables 1, 2, and 3 show that the presence of Cl reduced NO\textsubscript{3} uptake and increased the external NO\textsubscript{3}-N/NH\textsubscript{4}-N ratio. Moreover, K favored NO\textsubscript{3} uptake (Fig. 7, Experiment 5) and total N content in shoots was greater when the N was supplied with a readily absorbed counter ion, i.e., K for NO\textsubscript{3} and Cl
for \( NH_4^+ \) (Table 13, Experiment 7B). The results of Experiment 7A clearly indicate that \( NH_4^+ \) nutrition reduced cation accumulation in tissue and increased inorganic anion content when compared to \( NO_3^- \) nutrition (Fig. 14 and 15). These results indicate the presence of a cation-anion balance effect. Magalhaes and Wilcox (1983a,b), Mattson (1966), Harada et al. (1968), Dijkshoorn and Van Wijk (1967), Blair et al. (1970), Kirkby and Hughes (1970), and Dibb and Welch (1976) found similar effects for different species under different conditions. With the exception of Mn (Fig. 17), all the micronutrient cations had minimum or low concentrations in tissue with all \( NH_4^+ \), which was the treatment with the most acid soil pH and the greatest availability of these micronutrients (Fig. 16). The maximal concentrations for the micronutrient cation was with the mixed N forms or with all \( NO_3^- \). The concentration of the B anion increased with \( NH_4^+ \) (except with 100% \( NH_4^-\)N). Then, the tissue concentration of the micronutrients, which are many-fold more dilute than the macronutrients, supports the cation-anion balance effect in corn nutrition. Soluble Cd concentration in the soil solution would increase if pH decreased. However, the concentration of Cd in tissue was maximal when all the N was applied as \( NO_3^- \) (Fig. 18). This evidence would also support the cation-anion balance effect. This effect is not restricted to N metabolism. The concentration of Mg in tissue increased with
high levels of Cl and decreased with high levels of K (both Cl and K are readily absorbed ions). Moreover, high levels of Cl significantly increased the concentration of Zn, Cu, and Cd in tissue (Table 13).

The results presented so far indicate that (1) when the NO$_3^-$-N/NH$_4^+$-N ratio in the soil is different from 1.0, the plants favor absorption of the less abundant N form; (2) the form of N absorbed influences the uptake of other ions; and (3) availability of other nutrients may influence the ratio of NO$_3^-$-N/NH$_4^+$-N absorbed. All this suggests that nutrient uptake by corn plant is strongly regulated toward a balanced cation-anion uptake. These effects are probably a function of the total N concentration and of the specific environment (Warren et al., 1980).

Many models in the literature deal with regulation of N uptake and cation-anion balance effect. Dejaegere et al. (1981) postulated for barley roots that there are just as many HCO$_3^-$ ions produced inside the cell as NH$_4^+$ ions absorbed and that all the HCO$_3^-$ is used when the NH$_4^+$ is assimilated in the roots. They also stated that NO$_3^-$ sustains its own uptake since the HCO$_3^-$ generated in the reduction of NO$_3^-$ is used for further NO$_3^-$ uptake. According to this, uptake of ions other than N would be independent of NO$_3^-$ or NH$_4^+$ uptake. But, in corn, NO$_3^-$ is mainly reduced in the shoots (Pate, 1973). Moreover, at high nutrient levels, NO$_3^-$ and NH$_4^+$ accumulate
in roots (Fig. 2). Then, the N form would influence the cation-anion balance of the plant. Smith (1973) suggested that low cation uptake (i.e., Ca(NO\textsubscript{3})\textsubscript{2}) reduces the activity of the cation/H\textsuperscript{+} exchange system and via feedback from cytoplasmic pH (low pH) reduces anion (NO\textsubscript{3}\textsuperscript{-}) uptake (and probably stimulates cation uptake). However, if roots are high in organic acids, anion (NO\textsubscript{3}\textsuperscript{-}) uptake would not be affected by low cation uptake because organic acid breakdown would produce HCO\textsubscript{3}\textsuperscript{-} to exchange for NO\textsubscript{3}\textsuperscript{-}. (The organic acid metabolism would act according to the pH stat mechanism.) This model would explain why the form of N influences the inorganic cation-anion content in the plant, why the availability of other nutrients affects the NO\textsubscript{3}\textsuperscript{-}-N/NH\textsubscript{4}\textsuperscript{+}-N ratio absorbed, and why the ratio of NO\textsubscript{3}\textsuperscript{-}-N/NH\textsubscript{4}\textsuperscript{+}-N absorbed moved toward 1.0 as compared to the ratio in the solution.

Figure 3 shows that organic acid content is lower with Ca(NO\textsubscript{3})\textsubscript{2} than with (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}. These data, together with those presented by Blevins et al. (1978) and Frost et al. (1978), where they showed a decrease in organic acid content of roots supplied with Ca(NO\textsubscript{3})\textsubscript{2}, would support Smith's model. However, Roberts et al. (1981, 1982) using NMR as a way to measure cytoplasmic pH found that intracellular pH was tightly regulated when corn root tips absorbed an unbalanced cation-anion ratio. The cytoplasmic pH of excised corn root tips did not change in the presence of 25 mM K\textsubscript{2}SO\textsubscript{4}, which induced
extrusion of 4 to 5 μeq H⁺ g⁻¹ h⁻¹. Moreover, the results of Experiments 8A and B indicate that the form of N (Ca(NO₃)₂, (NH₄)₂SO₄, or a combination of both) do not have any effect on the cytoplasmic pH of excised corn root tips (Fig. 21 and 24; Tables 14 and 15). The form of N clearly affects the uptake of other ions but this effect does not seem to be through changes in internal pH. Electropotential generated across the membranes (Cram, 1973) or another more direct effect at the site of the membranes are probably responsible for this phenomenon. This also could be partly responsible for the higher efficiency of NH₄⁺ uptake when NO₃⁻ predominates and also for the high efficiency of NO₃⁻ uptake when NH₄⁺ predominates.

When the uptake of anions and cations is unbalanced, the root must excrete OH⁻ or H⁺ to maintain electrical neutrality (Raven and Smith, 1976; Mengel et al., 1983). Many of the experiments presented here were conducted with plants growing in soil. In some of them, the pH of the soil was not affected by the treatments (Experiments 5 and 6), whereas, in others, it was (Experiment 7). However, a constant soil pH does not mean that the rhizospheric or apoplastic pH is not affected by the treatments. The pH changes in the rhizosphere zone are an important factor affecting the relative uptake of cations and anions (Riley and Barber, 1969) and the mineral nutrition of the plant in general (Clarkson and Hanson, 1980).
For the conditions of the present experiments, external pH could be partly responsible for the observed effects. It is considered that external pH is part of the mechanism that allows plants to balance their nutrition.

In general, plants do better with a combination of NO$_3^-$ and NH$_4^+$ than with either source alone (Schrader et al., 1972). Some of the reasons could be that with a combination of the two N forms (1) the plants have a balanced nutrition of all the other ions (this was already discussed); (2) the pH stress in the cytoplasm of the assimilating cells is reduced (Raven and Smith, 1976); and (3) energy is saved with some NH$_4^+$ available because this form of N is already reduced (however, high concentration of NH$_4^+$ is toxic to the plants). The results of Experiment 8 (A and B) show that the pH of the cytoplasm is not affected by the form of N (Fig. 21 and 24). Consequently, no evidence was found to conclude that cytoplasmic pH stress is the reason for a better growth or greater N uptake when a combination of the two forms is compared to either source alone.

Grain sink size in corn, which decreases considerably during flowering, is commonly a limiting factor for grain yield in temperate and subtropical regions. Grain sink size may be improved by increasing the assimilate supply to the ear during flowering (Tollenaar, 1977). By having some NH$_4^+$ available during flowering, corn plants could save energy
(because $NH_4^+$ is already reduced) which would be available for the developing ear. Consequently, sink capacity would increase. However, Andrade (1983) concluded that field-grown corn plants did not show any response in grain yield to different $NO_3^-$/$NH_4^-$N ratios during flowering.

Plants growing under field conditions are always subjected to a combination of $NO_3^-$ and $NH_4^+$ where $NO_3^-$ usually predominates. Nitrapyrin would affect the elemental composition of field-grown plants as well as the total amount of N absorbed because it alters the soil $NO_3^-$-N/$NH_4^-$N ratio. However, for this treatment to be effective, the whole soil mass must be treated with high rates of nitrapyrin. If $NH_4^+$ plus nitrapyrin are applied in a band, the effect of $NH_4^+$ uptake in that band is nullified by $NO_3^-$ uptake from the remaining soil mass. Moreover, the effects of the $NO_3^-$-N/$NH_4^-$N ratio are a function of the total amount of N supplied.

Under field conditions, the form of N has different implications; with $NH_4^+$ plus a nitrification inhibitor as the N source, leaching and denitrification can be reduced (Chancy and Kamprath, 1982). The results of Experiments 9 and 10 show that nitrapyrin effectively controls nitrification (Fig. 26, 27, and 30). Moreover, by keeping the N as $NH_4^+$, leaching and possibly denitrification were reduced (Fig. 28, 29, and 31). When leaching conditions prevail, the efficiency of N fertilizer can be improved by splitting the applications
or by using \( \text{NH}_4^+ \) plus nitrapyrin. Both practices increased leaf protein percentage at anthesis and grain yield (Fig. 32 and 34). When losses of N by leaching or by denitrification are small, no response to splitting the applications or to the use of nitrification inhibitors should be expected.

The N form has other important physiological effects. It was shown that the form of N did not affect the cytoplasmic pH of the assimilated cells under normal conditions. However, the form of N affected the cytoplasmic pH during development of anaerobic conditions. In anaerobiosis, the cytoplasmic Pi signal in the NMR spectra shifts to the right (pH drops) due to transient lactic fermentation (Fig. 23). This shift can be considered as development of anaerobic conditions in the cell. Nitrate retarded this acidification of the cytoplasm compared to \( \text{NH}_4^+ \) (Fig. 25A,B) and allowed the cells to survive for a longer period of time under hypoxia. Nitrate is an electron acceptor and could represent an alternative way to oxidize cytoplasmic and mitochondrial NADH (this would reduce lactic acid and ethanol formation). This reaction could be mediated by nitrate reductase or by some other system of electron transport. According to these results, the presence of \( \text{NO}_3^- \) is desirable for plant survival under anaerobic conditions. Part of the \( \text{NO}_3^- \) generated by this process is extruded to the rhizosphere following an electrochemical gradient. This could have important implications in denitrification due to the higher reactivity of \( \text{NO}_2^- \).
CONCLUSIONS

Nutrient uptake by corn plants is strongly regulated toward a balanced cation-anion uptake. The form of N absorbed influences the uptake of other ions, and availability of other nutrients may influence the ratio of NO$_3^-$-N/NH$_4^+$-N absorbed. This cation-anion balance effect could be partly responsible for the higher uptake efficiency of the less abundant N form. However, this effect could also be explained by the pump and leak mechanism of Deane-Drummond and Glass (1983).

As shown by the NMR experiments, cytoplasmic pH is not affected by the form of N and it does not seem to be involved in the cation-anion balance effect. Electropotential generated across the membranes or some other direct effect at the site of the membranes is probably responsible for this phenomenon. Organic acid synthesis or breakdown may directly respond to H$^+$ or OH$^-$ efflux during unbalanced cation-anion uptake, without changes in cytoplasmic pH (Roberts et al., 1981). Moreover, excess anion uptake, for example, would generate electropotential differences that would reduce anion uptake and stimulate cation uptake while electrochemical gradients would favor leaking of the anions absorbed in excess (pump and leak mechanism). Nitrogen assimilation into organic compounds in the roots would produce H$^+$ or OH$^-$ (for NH$_4^+$ and NO$_3^-$, respectively) that would be exchanged for further N uptake. The combination of these 3 processes would explain most of
the results obtained in this work. The relative significance of these 3 mechanisms would vary according to the form of N (NO$_3^-$ or NH$_4^+$), the type of counter ion present, the energetic status of the plant, etc. Apoplastic and rhizospheric pH could have significant effects on the relative uptake of cations and anions. External pH is considered as a part of the mechanism that allows plants to balance their nutrition electrochemically.

Under anaerobic conditions, nitrate, an electron acceptor, retarded the acidification of the cytoplasm compared to NH$_4^+$. This allowed the cells to survive for a longer period of time under hypoxia. Nitrite is generated under those conditions and part of it is extruded to the rhizosphere. This could have important implications in denitrification due to the higher reactivity of NO$_2^-$ in the soil.

Finally, when leaching conditions in the field prevail, the efficiency of N fertilizer can be improved by splitting the applications or by keeping the N in the NH$_4^+$ form.


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