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Effects of High Ammonium/Nitrate Ratios on Nitrification and Growth of Wheat in Hydroponics

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EFFECTS OF HIGH AMMONIUM/NITRATE RATIOS ON NITRIFICATION AND GROWTH OF WHEAT IN HYDROPONIC CULTURE

by

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ABSTRACT

Effects of High Ammonium/Nitrate Ratios on Nitrification and Growth of Wheat in Hydroponics

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Nitrogen is the only plant nutrient taken up as both a cation (NH_4^+) and anion (NO_3^-). Nitrate is considered the "safe" form of N and NH_4^+ is generally thought to be toxic, especially at high levels. High NH_4^+/NO_3^- ratios are thought to be toxic because they result in a rhizosphere pH low enough to damage root membranes, induced cation deficiencies, and build-up of NH_3 caused by delayed NH_4^+ assimilation. These factors can be minimized in hydroponic culture. The objective of these studies was to quantify the effects of high NH_4^+/NO_3^- ratios on nitrification and growth of wheat in hydroponics.

Two cultivars of wheat (*Triticum aestivum* L.) were grown to maturity with either 15% or 80% of the N supplied as NH_4^+. The effect of using Cl^- versus SO_4^{2-} as counter ions to NH_4^+ was also examined. Yield was not significantly affected by NH_4^+ ratio or counter ion. Seed protein was increased from 15 to 19% with high NH_4^+. Harvest index was reduced from 52 to 48% with 80% NH_4^+, but was unaffected by counter ion.

Rates of nitrification in hydroponic culture are not well quantified and could result in significant conversion of NH_4^+ to NO_3^- before plant uptake. An isotopic dilution study was conducted to quantify rates of nitrification in hydroponic culture. A $2 \times 2 \times 2$ factorial design was used to examine the effect of pH (5.8 or 7.0), inoculation with nitrifying bacteria, and the presence of plants. This study was done with wheat grown in vigorously-aerated, 2-L bottles. Each bottle contained 10 g of diatomaceous earth to provide surface area for microbial growth. Nitrate began to accumulate in 5 d in unplanted, inoculated bottles at pH 7.0; in 20 d at pH 5.8 with inoculation; but did not begin to accumulate in non-inoculated bottles (pH 5.8 or 7.0) until day 30. Nitrate never accumulated in any of the planted bottles, most likely because plants consumed the NO_3^- that was produced. Calculations from the isotopic dilution measurements indicated that the rate of nitrification averaged 58 μmol NO_3^- L^{-1} d^{-1} in the planted bottles, and averaged 270 μmol NO_3^- L^{-1} d^{-1} in unplanted bottles. Nitrification was likely reduced in the planted bottles because the reduced concentration of NH_4^+ limited nitrification.

To provide rapid, inexpensive measurement of nutrient concentration in hydroponic solution, five colorimetric tests were evaluated. Tests for NO_3^- and PO_4 were accurate and reliable, but the tests for SO_4^{2-}, SiO_2, and Fe need additional refinement.

(146 pages)

DEDICATION

To my Dad, who said my science fair ideas were too easy.

To my Mom, who set the example, if she can do it then so can I.

To my Husband, who always reminded me that I was doing this for fun.

ACKNOWLEDGMENTS

I would like to thank Dr. Bruce Bugbee for his support, advice, and open-door policy during this work. I also appreciate the help and suggestions from my committee members, Dr. Douglas Johnson, Dr. Rich Koenig, and especially Dr. Jeanette Norton.

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Very special thanks go to Peggy and Jared Evans for their assistance with wheat planting; you can be a scientist at any age. Thanks also to Rita and John Nelson for their help both in and out of the lab.

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Dawn J. Muhlestein

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Nitrogen is required at 2 to 3% of plant dry weight for optimal growth (Marschner, 1995). Nitrogen is the only nutrient absorbed by plants as either a cation (NH_4^+) or an anion (NO_3^-). Both forms are used as fertilizers; however, NH_4^+ fertilizer is usually less expensive than NO_3^- fertilizer. The NO_3^- form is usually considered the "safe" form of N, while NH_4^+ is considered toxic at high levels. High ratios of NH_4^+/NO_3^- may be toxic for three main reasons: acidification of the rhizosphere (Henry and Raper, 1989); induced Ca^{2+}, K^+, and Mg^{2+} deficiencies (Magalhaes and Wilcox, 1983; Cao and Tibbitts, 1993); and root carbohydrate deficiencies (Kafkafi, 1990; Lavoie et al., 1992). The effects of high NH_4^+ have been well studied in both field and hydroponic systems because NH_4^+may provide a more cost effective and energy efficient method of supplying nitrogen to plants. Management of N supply with high ratios of NH_4^+/NO_3^- also requires an understanding of root-zone N transformations, especially nitrification.

Plant Assimilation of N

Nitrate is readily mobile in plants and may be assimilated in roots or leaves. Nitrate can also be stored in vacuoles without danger to the plant. The NO_3^- form must be reduced to NH_4^+ before it can be assimilated, making NO_3^- assimilation energetically expensive (Marschner, 1995). In contrast, NH_4^+ must be immediately assimilated in the roots because it cannot be stored. Chaillou et al. (1991) found increased amino acid

concentrations in roots when N was supplied as NH_4^+ compared to NO_3^-. Because NH_4^+ is assimilated in the roots, a consistent supply of carbohydrates is required in the roots when the plant is supplied high amounts of NH_4^+ (Marschner, 1995). If carbohydrate supply to the roots is not sufficient for assimilation, excess NH_4^+ may accumulate in the roots where it can degrade to toxic NH_3 gas (Kafkafi, 1990; Lavoie et al., 1992). Carbohydrate levels are lower in NH_4^+-fed roots, and these levels decrease as root-zone temperatures increase (Marschner, 1995). Accordingly, plant growth is reduced and root growth is poor in plants supplied high NH_4^+ and high root-zone temperature (Kafkafi, 1990). Low light levels and other factors that affect photosynthesis could also decrease carbohydrates in roots at high NH_4^+ levels (Lavoie et al., 1992).

Effects of High NH_4^+ on Plant Growth

High NH_4^+ is associated with NH_4^+ toxicity. Ammonium toxicity includes any negative effect on plant growth associated with supplying N as NH_4^+. McEllhannon and Mills (1978) reported root damage to plants supplied 25% or more N as NH_4^+. Others reported decreased growth and yield with high NH_4^+ for a variety of species. High NH_4^+ ratios decreased yield and dry weights of all plant parts in cucumber (*Cucumis sativus* L.) (Alan, 1989), reduced plant dry weight and yield of sweet pepper (*Capsicum annuum* L.) (Marti and Mills, 1991b), reduced growth in potatoes (*Solanum tuberosum* L.) (Cao and Tibbitts, 1993), and reduced yield in wheat (Hooten, 1998).

In contrast to the negative effects of high NH_4^+ listed above, Camberato and Bock (1990) found that high ammonium increased the grain yield of wheat grown in soil. However, this increased yield was associated with increased tillering of the wheat plants,

not increased partitioning to the seeds. Silberbush and Lips (1991) also reported increased tillering with increased NH_4^+/NO_3^- ratio in sand culture.

High NH_4^+ also has been associated with increased plant N uptake. Leaf and stem N increases with NH_4^+ (Camberato and Bock, 1990). High NH_4^+ has also been shown to increase grain N content of wheat (Hooten, 1998; Camberato and Bock, 1990). Increased N content of grain is correlated with increased crude protein concentration, which indicates the potential for higher nutritional value when wheat is supplied high NH_4^+ (Marschner, 1995).

Mixed N – Low Levels of NH_4^+

Supplying both forms of nitrogen is commonly thought to result in the highest plant growth and yield. With mixed N the plant may more easily regulate intracellular pH and store N at lower energy costs (Marschner, 1995). The optimal ratio of NH_4^+/NO_3^- is generally considered to be between 30-50% of N supplied as NH_4^+. McElhannon and Mills (1978) found that maximum dry weight of all lima bean (*Phaseolus lunatus* L.) plant parts occurred when N was supplied as 75% or more NO_3^-, and leaf area decreased when 50% or more of N was supplied as NH_4^+. Sasseville and Mills (1979) reported that greatest seed dry weight of southernpeas (*Vigna unguiculata* L.) occurred when N was 50% or more NO_3^-. Many researchers reported similar positive results including increased wheat yield (Cox and Reisenauer, 1973; Sandoval-Villa et al., 1995), increased wheat plant growth (Gentry et al., 1989), increased shoot protein levels in sunflower (*Helianthus annuus* L.) (Weissman, 1964), and increased dry weights of all plant parts in lima bean (McElhannon and Mills, 1978). In contrast, Hooten (1998) found no

significant difference between NO_3^- only and low NH_4^+ treatments in biomass accumulation or seed yield for wheat grown in a recirculating hydroponic system.

Effect of NH_4^+ Uptake on Rhizosphere pH

As plants absorb NH_4^+, protons are extruded to maintain charge balance within the root. This extrusion of H^+ ions acidifies the rhizosphere. In contrast, when NO_3^- is absorbed, OH^-/HCO_3^- ions are released, which raises rhizosphere pH. The buffering capacity of soils causes these pH shifts to be localized in the soil close to plant roots. In hydroponic solutions, pH shifts may be significant throughout the system because the solution is not well buffered. Acidic pH associated with NH_4^+ uptake has been shown to reduce growth, N accumulation, and yield (Marcus-Wyner, 1983; Henry and Raper, 1989). Maintaining pH eliminates such problems (Maynard and Barker, 1969). Peet et al. (1985) found no significant difference in dry matter accumulation, partitioning between leaves and roots, or accumulation of N between tomato (*Lycopersicon esculentum* Mills) plants given 1.0 m*M* NO_3^- or 1.0 m*M* NH_4^+ when pH was controlled. In addition, Lea-Cox et al. (1999) confirmed that pH could be controlled by balancing the proportion of NO_3^-/NH_4^+ in solution.

Effect of NH_4^+ on Uptake of Other Nutrients

The form of N plays an important role in the cation-anion uptake relationship because 70% of cations and anions taken up by plants are NH_4^+ or NO_3^- (Marschner, 1995). The absorption of NH_4^+ inhibits uptake of other positively charged ions and favors the uptake of negatively charged ions. Plants supplied NH_4^+ as the source of N

have reduced amounts of K^+, Ca^{2+}, and Mg^{2+} compared to plants given NO_3^- (Magalhaes and Wilcox, 1983). Supplying N as NH_4^+ can also inhibit the uptake of NO_3^- (Youngdahl et al., 1982).

Ammonium Uptake Effects on Calcium

Calcium uptake is reduced when N is supplied as NH_4^+ compared to NO_3^-. Marti and Mills (1991a) reported reduced tissue Ca^{2+} when $NH_4^+$was part of the N supplied to bell pepper (*Capsicum annuum* L.) plants. Rideout and Raper (1994) also found decreased Ca^{2+} uptake in soybean (*Glycine max* L.) with increased NH_4^+. In cotton (*Gossypium hirsutum* L.) seedlings grown in soil, Adams (1966) found that supplemental $CaSO_4$ could reduce toxic effects of $(NH_4)_2HPO_4$. Adams suggested that toxic effects of NH_4^+ could be due to decreased Ca^{2+} uptake caused by calcium phosphate precipitation and NH_4^+ inhibition of Ca^{2+} uptake. Supplying additional Ca^{2+} alone did not completely correct the reduced absorption.

Supplying Ca^{2+} in conjunction with chloride may increase Ca^{2+} uptake. Koenig and Pan (1996) found that wheat grown in soil supplied with NH_4^+ had increased Ca^{2+} content when given supplemental Cl^-. In addition, plants fed NH_4^+ also had increased yield and N content with supplemental $CaCl_2$ compared to NH_4^+ alone (Fenn and Taylor, 1990; Koenig and Pan, 1996). The Cl^- ion may also increase the uptake of other cations such as Mg^{2+} and K^+ in addition to increased Ca^{2+} uptake (Jakobsen, 1992).

Additionally, $CaCl_2$ is much more soluble than $CaSO_4$. The maximum solubility of $CaSO_4$ is only 0.014 moles/L compared to 6.6 moles/L for CaCl (Weast, 1985). Because of this difference in solubility, supplying NH_4^+ with Cl^- may be better than SO_4^{2-} because of increased Ca^{2+} solubility.

Ammonium Uptake Effects on Nitrate

High NH_4^+ strongly suppresses uptake of NO_3^-, but high NO_3^- does not suppress NH_4^+ uptake (Marschner, 1995). Youngdahl et al. (1982) found that when supplied alone, plant carrier sites have similar affinity for NH_4^+ and NO_3^- (K_m), but NH_4^+ had a slightly higher maximum transport rate (V_{max}) in rice (*Oryza sativa* L.). Goyal and Huffaker (1986) also found higher V_{max} values for NH_4^+ in wheat, but they also reported higher K_m values for NH_4^+. If both forms of N are present, NH_4^+ uptake rates remain the same but NO_3^- uptake rates were severely inhibited (Youngdahl et al., 1982). Youngdahl et al. (1982) found that V_{max} of NO_3^- supplied with NH_4^+ dropped to approximately 1/3 the rate of only NO_3^-, but K_m dropped only slightly. The mechanism of inhibition is not known, but decreased NO_3^- uptake is expressed through effects on V_{max} of NO_3^-. Rapid NH_4^+ influx and decrease in trans-membrane potential may be responsible for decreased NO_3^- uptake (Marschner, 1995).

Ammonium Uptake Effects on Potassium

Because NH_4^+ inhibits the uptake of K^+, deficiencies in K^+ can occur when a high fraction of the N is supplied as NH_4^+. Scherer et al. (1987) reported that increased NH_4^+ reduced K^+ uptake by excised rice roots. However, supplying excess K^+ can ameliorate these effects (Ajayi et al., 1970).

Ammonium Uptake Effects on Magnesium

Magnesium uptake is also subject to cationic inhibition by root absorption of NH_4^+. Peet et al. (1985) found that Mg^{2+} uptake was substantially reduced for plants fed NH_4^+ compared to those fed NO_3^-. In addition, high levels of Ca^{2+} and K^+ may also

inhibit the uptake of Mg^{2+} (Marschner, 1995). When Ca^{2+} and K^{+} levels are increased to ameliorate deficiencies of those nutrients, Mg^{2+} may become deficient.

Cultivar Effects

Wheat cultivars can differ in their responses to nutrient stresses (Hooten, 1998). Veery-10 and USU-Line 10 are dwarf wheat cultivars, which have similar height and growth characteristics. However, Veery-10 appears to be more susceptible to Ca^{2+} deficiency, especially for the leaf tips (Bugbee et al., 1997). The Ca^{2+} sensitivity of Veery-10 may help determine NH_4^+ -induced Ca^{2+} deficiencies.

Microbial Conversion of NH_4^+

Microorganisms can convert NH_4^+ to NO_3^- through the two-step process of nitrification. Ammonium oxidizing bacteria such as those belonging to the *Nitrosomonas*, *Nitrosolobus*, and *Nitrosospira* genera oxidize NH_4^+ to NO_2^-. In the second step, NO_2^- is converted to NO_3^- by nitrite oxidizing bacteria such as *Nitrobacter* (Paul and Clark, 1996). Much of the NH_4^+ applied to the root-zone may be nitrified and enter the plant as NO_3^-. Norton and Firestone (1996) in a soil study estimated that 27% of ^{15}N supplied to plants as NH_4^+ was taken up as NO_3^- after being nitrified within a 24-h period.

Ammonium studies are often conducted in hydroponic solutions. Although no direct measurements of nitrification rates have been made *in situ*, nitrification was reported to be significant in 100% NH_4^+ nutrient solutions at pH 6.0 for aeroponic, sand culture, and hydroponic systems (Padgett and Leonard, 1993). Padgett and Leonard

(1993) used production of NO_3^- in solution, presence of NO_3^- in plants, and a bioassay as indicators of nitrification, and reported nitrification after only 5 to 8 d in a hydroponic system. However, the bioassay was conducted at pH 7 while the plants were grown at pH 6, so the relation of bioassay nitrification and in situ nitrification is unclear in the Padgett and Leonard studies (1993). However, nitrifying bacteria occur optimally within the pH range of 7.0 to 8.5 in liquid media (Allison and Prosser, 1993), and hydroponic solutions are typically controlled between pH 5 and 6.

Lang and Elliott (1991) found that the pH of soil-less potting media was a major factor controlling nitrification rate. They found no nitrifier activity below a pH of 5.6, but their assay was conducted at pH 7.0 so organisms operating at a lower pH may not have been detected. Surface-attached ammonia oxidizers can maintain activity at lower pH than suspended cells (Allison and Prosser, 1993). Root surfaces in hydroponic systems could provide the surface necessary for nitrification to occur at pH values lower than that found in hydroponic systems.

NH_4^+ and NO_3^- Availability in Soils

Negatively charged soil particles attract NH_4^+ ions and repel NO_3^- ions. This repulsion makes NO_3^- subject to leaching from soils. In contrast, NH_4^+ is not readily leached from soil, but can be lost through volatilization as NH_3 gas when the pH is above 7. In addition, NH_4^+ can be fixed in the interlayer of some clay minerals, which may make it unavailable for uptake (Havlin et al., 1999). The differences of mobility of NH_4^+ and NO_3^- in soil solution may determine N availability to roots.

Nitrogen in either form may also be made unavailable for plant uptake by microbial immobilization. In a greenhouse study with *Pinus ponderosa* seedlings grown in pots, Norton and Firestone (1996) found that 40% of NH_4^+ consumption and 30% of NO_3^- consumption was by microbial immobilization. Bristow et al. (1987) reported 37% of ^{15}N applied to a grass sward was immobilized into the microbial biomass; however, these numbers rapidly declined as microbial death released N back into the soil solution. Bristow et al. (1987) also noted large fluctuations in ^{15}N recovered from the microbial biomass, suggesting rapid cycling of mineral and immobilized N. Plants and microbes may be in direct competition with each other for N, and microbes can have higher uptake rates of NH_4^+ and NO_3^- compared to plants (Jackson et al., 1989).

NH_4^+ and NO_3^- Availability in Hydroponic Culture

Nutrient dynamics in hydroponic systems can be very different than soils. Nutrients arrive at root surfaces in soil by mass flow and diffusion. Nutrient levels in soil solution can be too low to supply the nutrient requirement by mass flow, and diffusive flux to the root occurs. In recirculating hydroponic systems, the flow rate is fast enough to supply all of the nutrients by mass flow. Microbial interactions in hydroponics are also limited, as equipment is cleaned to minimize microbes. Hydroponic solution is developed to make NH_4^+ and NO_3^- readily available for plant uptake and eliminate soil interactions.

SUMMARY AND CONCLUSIONS

High NH_4^+/NO_3^- ratios in hydroponic culture may result in many potential problems. High amounts of NH_4^+ can acidify the rhizosphere causing damage to roots. The uptake of NH_4^+ also inhibits uptake of other cations, especially Mg^{2+}, K^+, and Ca^{2+}. However, these problems can be alleviated, suggesting that NH_4^+ is not directly toxic. Nevertheless, Ca^{2+} uptake still needs to be increased in high NH_4^+ situations. Supplying NH_4^+ with the proper counterbalancing ions, such as Cl^-, may reduce Ca^{2+} uptake problems.

The problems associated with high NH_4^+ might be alleviated in hydroponics. In contrast, alleviating these problems in soils and media may be extremely difficult. Adding high amounts of NH_4^+ to soils with low pH, low nitrification, and low calcium supply is usually detrimental to plants. In such situations, maintaining elevated rhizosphere pH to alleviate the problems associated with high NH_4^+ would be difficult and expensive. Applying high NH_4^+ in soil-less media and the field may not be feasible in such situations unless nitrification can be enhanced or soil pH altered by liming.

NASA Advanced Life Support (ALS) systems used in space would have high levels of NH_4^+ in nutrient solutions as plant and human waste is recycled. The ability to grow plants under such conditions is essential to ALS systems. Studies in high NH_4^+ hydroponic culture are necessary to solve potential problems before attempting to grow plants with high NH_4^+ in space.

OBJECTIVES

The goal of this work was to evaluate the effect implications of high NH_4^+ in hydroponic growth systems. The objectives of the studies described in the following chapters were:

1. Examine the long-term effects of high NH_4^+/NO_3^- ratios on hydroponic wheat.
2. Quantify rates of nitrification in NH_4^+-based hydroponic solutions.
3. Evaluate methods of nutrient analysis for hydroponic solutions.

REFERENCES

Adams, F. 1966. Calcium deficiency and ammonium phosphate injury to cotton. Soil Sci. Soc. Proc. 30:485-488.

Ajayi, O., D.N. Maynard, and A.V. Barker. 1970. The effect of potassium on ammonium nutrition of tomato (*Lycopersicon esculentum* Mill). Agron. J. 62:818-821.

Alan, R. 1989. The effect of nitrogen nutrition on growth, chemical composition, and response of cucumbers (*Cucumis sativus* L.) to nitrogen forms in solution. J. Hortic. Sci. 64:467-474.

Allison, S.M., and J.I. Prosser. 1993. Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biol. Biochem. 25:935-941.

Bristow, A.W., J.C. Ryden, and D.C. Whitehead. 1987. The fate at several time intervals of ^{15}N-labelled ammonium nitrate applied to an established grass sward. J. Soil Sci. 38:245-254.

Bugbee, B., G. Koerner, R. Albrechtsen, W. Dewey, and S. Clawson. 1997. Registration of 'USU-Apogee' wheat. Crop Sci. 37:626.

Cao, W., and T.W. Tibbitts. 1993. Study of various NH_4^+/NO_3^- mixtures for enhancing growth of potatoes. J. Plant Nutr. 16:1691-1704.

Camberato, J.J., and B.R. Bock. 1990. Spring wheat responses to ammonium supply. I. Dry matter and nitrogen content. Agron. J. 82:463-467.

Chaillou, S., J.K. Vessey, J.F. Morot-Gaudry, C.D. Raper, L.T. Henry, and J.P. Boutin. 1991. Expression of characteristics of ammonium nutrition as affected by pH of the root medium. J. Exp. Bot. 42:189-196.

Cox, W.J., and H.M Reisenauer. 1973. Growth and ion uptake by wheat supplied nitrogen as nitrate, or ammonium, or both. Plant Soil 38:363-380.

Fenn, L.B., and R.M. Taylor. 1990. Calcium stimulation of ammonium absorption in radish. Agron. J. 82:81-84.

Gentry, L.E., X.T. Wang, and F.E. Below. 1989. Nutrient uptake by wheat seedling that differ in response to mixed nitrogen nutrition. J. Plant Nutr. 12:363-373.

Goyal, S.S., and R.C. Huffaker. 1986. The uptake of NO_3^-, NO_2^-, and NH_4^+ by intact wheat (*Triticum aestivum*) seedlings. Plant Physiol. 82:1051-1056.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 1999. Soil fertility and fertilizers: an introduction to nutrient management. Prentice Hall, Upper Saddle River, NJ.

Henry, L.T., and C.D. Raper. 1989. Effects of root-zone acidity on utilization of nitrate and ammonium in tobacco plants. J. Plant Nutr. 12:811-826.

Hooten, T.M. 1998. Ammonium and nitrate effects on growth, development, and nutrient uptake of hydroponic wheat. M.S. thesis. Utah State Univ., Logan.

Jackson, L.E., J.P. Schimel, and M.K. Firestone. 1989. Short-term partitioning of ammonium and nitrate between plants and microbes in an annual grassland. Soil Biol. Biochem. 21:409-415.

Jakobsen, S.T. 1992. Interaction between plant nutrients. II. Effect of chloride on activities of cations in soil solution and on nutrient uptake by plants. Scandinavian Soil Plant Sci. 42:213-217.

Kafkafi, U. 1990. Root temperature, concentration, and the ratio NO_3^-/NH_4^+ effect on plant development. J. Plant Nutr. 13:1291-1306.

Koenig, R.T., and W.L. Pan. 1996. Chloride enhancement of wheat responses to ammonium nutrition. Soil Sci. Soc. Am. J. 60:498-505.

Lang, H.J., and G.C. Elliott. 1991. Influence of ammonium:nitrate ratio and nitrogen concentration on nitrification activity in soilless potting media. J. Am. Soc. Hortic. 116: 642-645.

Lavoie, N., L.P. Vezina, and H.A. Margolis. 1992. Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. Tree Physiol. 11:171-183.

Lea-Cox, J.D., G.W. Stutte, W.L. Berry, and R.M. Wheeler. 1999. Nutrient dynamics and pH/charge balance relationships in hydroponic solutions. Acta Hortic. 481: 241-249.

Magalhaes, J.R., and G.E. Wilcox. 1983. Tomato growth and mineral composition as influenced by nitrogen form and light intensity. J. Plant Nutr. 6:847-862.

Marcus-Wyner, L. 1983. Influence of ambient acidity on the absorption of NO_3^- and NH_4^+ by tomato plants. J. Plant Nutr. 5:657-666.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, New York.

Marti, H.R., and H.A. Mills. 1991a. Calcium uptake and concentration in bell pepper plants as influenced by nitrogen form and stages of development. J. Plant Nutr. 14:177-1185.

Marti, H.R., and H.A. Mills. 1991b. Nutrient uptake and yield of sweet pepper as affected by stage of development and N form. J. Plant Nutr. 14:1177-1185.

Maynard, D.N., and A.V. Barker. 1969. Studies on tolerance of plants to ammonium nutrition. J. Am. Soc. Hortic. Sci. 94:235-239.

McElhannon, W.S., and H.A. Mills. 1978. Influence of percent NO_3^-/NH_4^+ on growth, N absorption, and assimilation by lima beans in solution culture. Agron. J. 70:1027-1032.

Norton, J.M., and M.K. Firestone. 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biol. Biochem. 28:351-362.

Padgett, P.E., and R.T. Leonard. 1993. Contamination of ammonium-based nutrient solutions by nitrifying organisms and the conversion of ammonium to nitrate. Plant Physiol. 101:141-146.

Paul, E.A., and F.E. Clark. 1996. Soil microbiology and biochemistry. 2nd ed. Academic Press, San Diego, CA.

Peet, M.M., C.D. Raper, L.C. Tolley, and W.P. Robarge. 1985. Tomato responses to ammonium and nitrate nutrition under controlled root-zone pH. J. Plant Nutr. 8:787-798.

Rideout, J.W., and C.D. Raper. 1994. Dry matter and nitrogen accumulation are not affected by superoptimal concentration of ammonium in flowing solution culture with pH control. J. Plant Nutr. 17:219-228.

Sandoval-Villa, M., G. Alcantar-Gonzalaz, and J.L. Tirado-Torres. 1995. Use of ammonium in nutrient solutions. J. Plant Nutr. 18:1449-1457.

Sasseville, D.N., and H.A. Mills. 1979. N form and concentration: effects on N absorption, growth, and total N accumulation with southernpeas. J. Am. Soc. Hortic. Sci. 104:586-591.

Scherer, H.W., J.E. Leggett, J.L. Sims, and P. Krasaesindhu. 1987. Interactions among ammonium, potassium, and calcium during their uptake by excised rice roots. J. Plant Nutr. 10:67-81.

Silberbush, M., and S.H. Lips. 1991. Potassium, nitrogen, ammonium/nitrate ratio, and sodium chloride effects on wheat growth. II. Tillering and grain yield. J. Plant Nutr. 14:765-773.

Weast, R.C. (ed). 1985. CRC handbook of chemistry and physics. CRC Press, Boca Raton, FL.

Weissman, G.S. 1964. Effect of ammonium and nitrate nutrition on protein level and exudate composition. Plant Physiol. 39:947-952.

Youngdahl, L.J., R. Pacheco, J.J. Street, and P.L.G. Vlek. 1982. The kinetics of ammonium and nitrate uptake by young rice plants. Plant and Soil 69:225-232.

CHAPTER 2

HIGH AMMONIUM EFFECTS ON YIELD

INTRODUCTION

Nitrogen is often the most limiting nutrient in plants. Nitrogen is the only nutrient absorbed as a cation and an anion, and can constitute 80% of the ions taken up by roots. NO_3^- is usually considered the "safe" form of N, while NH_4^+ is considered toxic at high levels. High ratios of NH_4^+/NO_3^- may be toxic for three main reasons: acidification of the rhizosphere (Henry and Raper, 1989); induced Ca^{2+}, K^+, and Mg^{2+} deficiencies (Magalhaes and Wilcox, 1983; Cao and Tibbitts, 1993); and root carbohydrate deficiencies (Kafkafi, 1990; Lavoie et al., 1992).

Effects of High NH_4^+ on Plant Growth

Nitrogen supplied with a high fraction as NH_4^+ is associated with NH_4^+ toxicity. McEllhannon and Mills (1978) reported root damage occurred in plants supplied 25% or more N as NH_4^+. High NH_4^+ ratios decreased yield and dry weights of all plant parts in cucumber (Alan, 1989), reduced plant dry weight and yield of sweet pepper (Marti and Mills, 1991), reduced growth in potatoes (Cao and Tibbitts, 1993), and reduced yield in wheat (Hooten, 1998). In contrast to the negative effects of high NH_4^+, Camberato and Bock (1990) found that high NH_4^+ increased the dry mass grain yield of wheat. However, this increased yield was associated with greater tillering of the wheat plants, not increased partitioning to the seeds.

High NH_4^+ has also been associated with increased plant N uptake. Leaf and stem N increased with increased NH_4^+ (Camberato and Bock, 1990). High NH_4^+ also increased grain N content of wheat (Camberato and Bock, 1990; Hooten, 1998). Increased N content of grain is correlated with increased crude protein concentration, which indicates the potential for higher nutritional value when supplied high NH_4^+.

Effect of NH_4^+ Uptake on Rhizosphere pH

As plants absorb NH_4^+, protons are extruded to maintain charge balance within the root. This extrusion of H^+ ions acidifies the rhizosphere. In contrast, as NO_3^- is absorbed, OH^-/HCO_3^- ions are released, which raises the pH. Acidic pH associated with NH_4^+ uptake reduced growth, N accumulation, and yield (Henry and Raper, 1989; Marcus-Wyner, 1983). Maintaining pH eliminated such problems (Maynard and Barker, 1969). Peet et al. (1985) found no significant difference in dry matter accumulation, partitioning between leaves and roots, or accumulation of N between tomato plants given 1.0 m*M* NO_3^- or 1.0 m*M* NH_4^+ when pH was controlled.

Effect of NH_4^+ on Uptake of other Nutrients

The form of N plays an important role in the cation-anion uptake relationship because 70% of cations and anions taken by plants are NH_4^+ or NO_3^- (Marschner, 1995). The absorption of NH_4^+ inhibits uptake of other positively charged ions and favors the uptake of negatively charged ions. Plants supplied NH_4^+ as the source of N had reduced amounts of K^+, Ca^{2+}, and Mg^{2+} compared to plants given NO_3^- (Magalhaes and Wilcox,

1983). Supplying N as NH_4^+ can also inhibit the uptake of NO_3^- (Youngdahl et al., 1982).

Cultivar Effects

Wheat cultivars can differ in their responses to nutrient deficiencies (Hooten, 1998). Veery-10 and USU- Line 10 are dwarf wheat cultivars, that have similar height and growth characteristics. However, Veery-10 appears to be more susceptible to Ca^{2+} deficiency, especially for the leaf tips (Bugbee et al., 1997).

OBJECTIVES AND HYPOTHESES

The objectives of this experiment were to study the long-term effects on wheat of high NH_4^+/NO_3^- ratios supplied with Cl^- or SO_4^{2-} counterbalancing ions. Specific objectives were to:

1. Quantify the effects of high NH_4^+/NO_3^- ratios on the wheat yield in hydroponic culture.

   Hypothesis 1: High NH_4^+ will reduce wheat yield.

2. Examine the effects of different counterbalancing ions (SO_4^{2-} and Cl^-) on growth, yield, and nutrient uptake of wheat.

   Hypothesis 2: Supplying NH_4^+ with Cl^- will improve yield and nutrient uptake, especially Ca^{2+} uptake.

3. Determine if Veery-10 and USU-Line 10 wheat cultivars respond similarly to the ammonium treatments.

Hypothesis 2: Veery-10 and USU-Line 10 will have similar growth, yield, and nutrient uptake.

MATERIALS AND METHODS

Two studies were conducted in a controlled environment in three independent, recirculating hydroponic systems with automated pH control, nutrient addition, and NO_3^- monitoring. Each system had four 25-L tubs, which were plumbed to a 180-L reservoir. The tubs were arranged in a randomized design (Figure 2-1).

The automated system is diagramed in Figure 2-2, and was previously described by Ritchie (1994), Smart and Bloom (1998), and Hooten (1998). The plant community was surrounded by reflective Mylar to prevent guard row effects. The plants were grown with elevated CO_2 (1200 ppm). The air temperature was maintained at 18°C until emergence, 23°C until anthesis, and 18°C until harvest. Solution temperature remained approximately 2°C higher than air temperature. Solution pH was maintained at 5.8 using a pH controller (Omega, Model PHCN-36) and automated addition of a base or acid.

A1 USU-Line 10	C2 Veery-10	B3 USU-Line 10	C4 Veery-10
B1 Veery-10	A2 Veery-10	C3 USU-Line 10	A4 USU-Line 10
C1 USU-Line 10	B2 USU-Line 10	A3 Veery-10	B4 Veery-10

Fig. 2-1. Randomization of treatments and cultivars for a trial. A,B, and C indicate the system to which a treatment is applied and 1-4 indicate replicate tubs. For example, A4 indicates system A replicate 4.

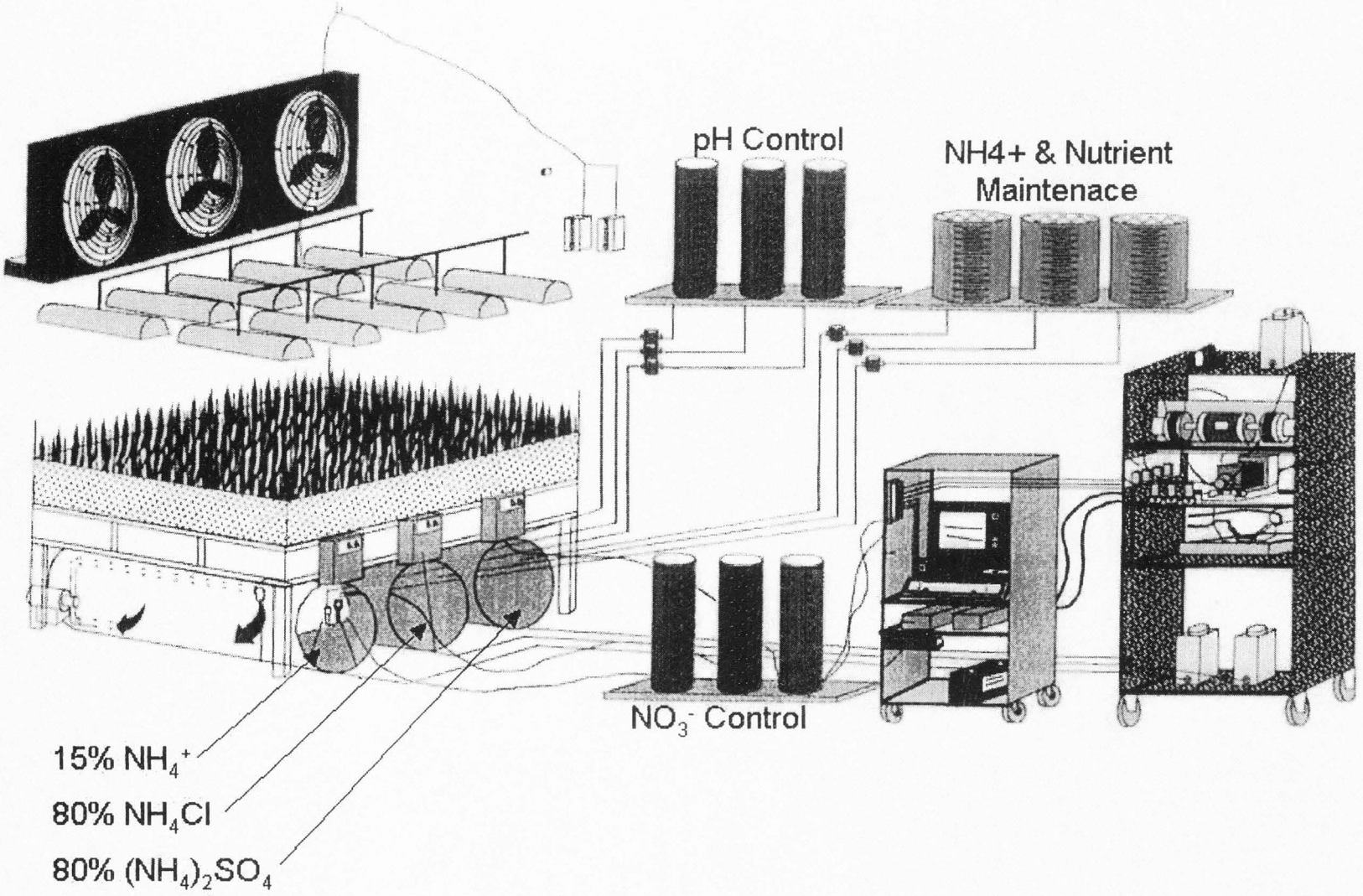

Figure 2-2. Automated nutrient maintainance system (adapted from Smart et al., 1998).

Nitrate concentration, pH, temperature, relative humidity, and CO_2 concentration were continuously monitored using a datalogger (Campbell Scientific, Model CR-10) and computer. All trials continued until physiological maturity.

Nutrient Solution

The nutrient solution used in both trials is shown in Table 2-1. Trials began as 100% NO_3^-, and NH_4^+ treatments began 6 days after emergence. The nutrient solution for each of the three systems was maintained at 280 L by automated addition of refill solution using a float switch. Automated control of pH occurred through the addition of 0.2 *M* HNO_3 to lower pH in low NH_4^+ treatments, or a mix of 0.025 *M* $Ca(OH)_2$ and 0.15 *M* KOH to raise pH in high NH_4^+ treatments.

Table 2-1. Nutrient solution.

		Starter solution		Refill solution	
Salt	Stock conc.	ml per 100 L	Final conc.	ml per 100 L	Final conc.
$Ca(NO_3)_2$	1 *M*	5	0.05 m*M*	100	1 m*M*
$CaSO_4$	Solid form	16.3 g	1.2 m*M*	0	0
KNO_3	2 *M*	0	0	200	4 m*M*
K_2SO_4	0.5 *M*	100	0.5 m*M*	0	0
KH_2PO_4	0.5 *M*	120	0.6 m*M*	120	0.6 m*M*
$MgSO_4$	0.25 *M*	200	0.5 m*M*	100	0.25 m*M*
K_2SiO_3	0.1 *M*	100	0.1 m*M*	100	0.1 m*M*
$FeCl_3$	50 m*M*	20	10 μ*M*	5	2.5 μ*M*
Fe-HEEDTA	100 m*M*	25	25 μ*M*	5	5 μ*M*
$MnCl_2$	60 m*M*	5	3 μ*M*	10	6 μ*M*
$ZnCl_2$	20 m*M*	20	4 μ*M*	20	4 μ*M*
H_3BO_3	20 m*M*	10	2 μ*M*	5	1 μ*M*
$CuCl_2$	20 m*M*	5	1.0 μ*M*	15	3 μ*M*
Na_2MoO_4	0.6 m*M*	15	0.09 μ*M*	5	0.03 μ*M*

Table 2-2. Description of treatments for yield trials.

Treatment Name	NH_4^+/NO_3^- Ratio (%)	NH_4^+ Form
Low NH_4^+	**15/75**	**$(NH_4)_2SO_4$ and NH_4Cl**
High NH_4Cl	**80/20**	**NH_4Cl**
High $(NH_4)_2SO_4$	**80/20**	**$(NH_4)_2SO_4$**

The NH_4^+/NO_3^- (%) treatments were: 15/85 with NH_4^+ supplied as $(NH_4)_2SO_4$ and NH_4Cl, 80/20 with NH_4^+ supplied as $(NH_4)_2SO_4$, and 80/20 with NH_4^+ supplied as NH_4Cl (Table 2-2).

Monitoring and Maintaining Nutrients in Solution

Nitrate in the three systems was monitored on an 18-min cycle using an ion selective electrode (Orion, Model 93-07) in a system similar to that described by Smart and Bloom (1993). Solution sampling was controlled by a series of solenoid valves, and each of the three systems was sampled for 6 min. A ratiomatic pump (Fluid Metering, Inc, Model QV-CKC) was used to pump the sample solution through the electrode block where the NO_3^- concentration was determined, and after the solution was returned to the solution tank. A 75-s lag time helped eliminate contamination among systems by purging solution in the tubing. If the NO_3^- concentration was below the 100 μM setpoint, a small amount of 200 mM NO_3^- solution was dispensed to bring NO_3^- concentration above the setpoint. The NO_3^- solution was composed of 50 mM $Ca(NO_3)_2$ and 100 mM KNO_3.

Ammonium was monitored using the Nesslerization colorimetric test and a colorimeter (LaMotte, Smart Colorimeter). Ammonium was added to the nutrient refill

solution. Small pulses of refill solution insured that the plants were exposed to a relatively constant concentration of ammonium. Other nutrients were also monitored throughout the trial using colorimetric tests.

Radiation

Ten high-pressure sodium (HPS) lamps supplied light. Plexiglass barriers located 15 cm below the lamps reduced the longwave radiation that would heat the plants. Day 0 began with two pairs of HPS lamps supplying a photosynthetic photon flux (PPF) of 450 μmol m^{-2} s^{-1}. On day 1, three pairs of HPS lamps increased the PPF to 650 μmol m^{-2} s^{-1}. Full light was supplied on day 6 with PPF of 1200 μmol m^{-2} s^{-1}. Plants had a 24-h photoperiod.

Plant Tissue Analysis

Total root, shoot, and seed mass was measured at harvest. Head number per m^2 was counted. Seeds per head and seed mass were calculated at harvest. Plant biomass and grain were analyzed for total N by combustion with a LECO CHN analyzer (Model CHN-1000) and NO_3^- by the chromotrophic acid method with a Lachat auto-analyzer. Plant biomass and grain were also analyzed for P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, and Mo by ICP-AES with a nitric acid peroxide digestion. Flag leaf samples were taken approximately every 14 d after anthesis throughout the trial and analyzed by ICP-AES, LECO-N, and Lachat for NO_3^-N. The Utah State University Plant Analysis Laboratory conducted the analyses. In addition, plant biomass and grain were analyzed for water-soluble Cl^- using a Hach colorimetric test kit.

Modeling Ca^{2+} Availability

Nutrient solution Ca^{2+} availability was modeled with the GEOCHEM-PC chemical speciation program (Parker et al., 1995). Solution was modeled for starting and ending nutrient concentrations.

Statistics

The data were analyzed two ways. Because the root zones of a treatment were all exposed to the same nutrient solution, a tub could be treated as a pseudo-replicate or as a true replicate blocked by trial. When evaluated as a pseudo-rep, the two tubs within a treatment were combined and each trial was a replicate. However, the tubs could also be treated as replicates blocked by trial, which allowed for analysis of missing data points. When analyzing the tubs as true replicates, general linear model (GLM) was used to account for missing data points. Because nutrient analysis was done on a sample representative of both tubs, ANOVA was used and trials were replicates in those cases. Statistical significance used here is from analysis with trials as blocks and tubs as replicates. Results of both methods are included in Appendix E.

RESULTS AND DISCUSSION

Total Biomass

Total dry biomass (Figure 2-3a) was not significantly affected by NH_4^+ treatment ($p=0.35$) or by cultivar ($p=0.87$). Trials 1 and 2 were significantly different ($p=0.03$), with Trial 1 averaging 4,921 g m^{-2} and Trial 2 averaging 4,425 g m^{-2}. These results agree

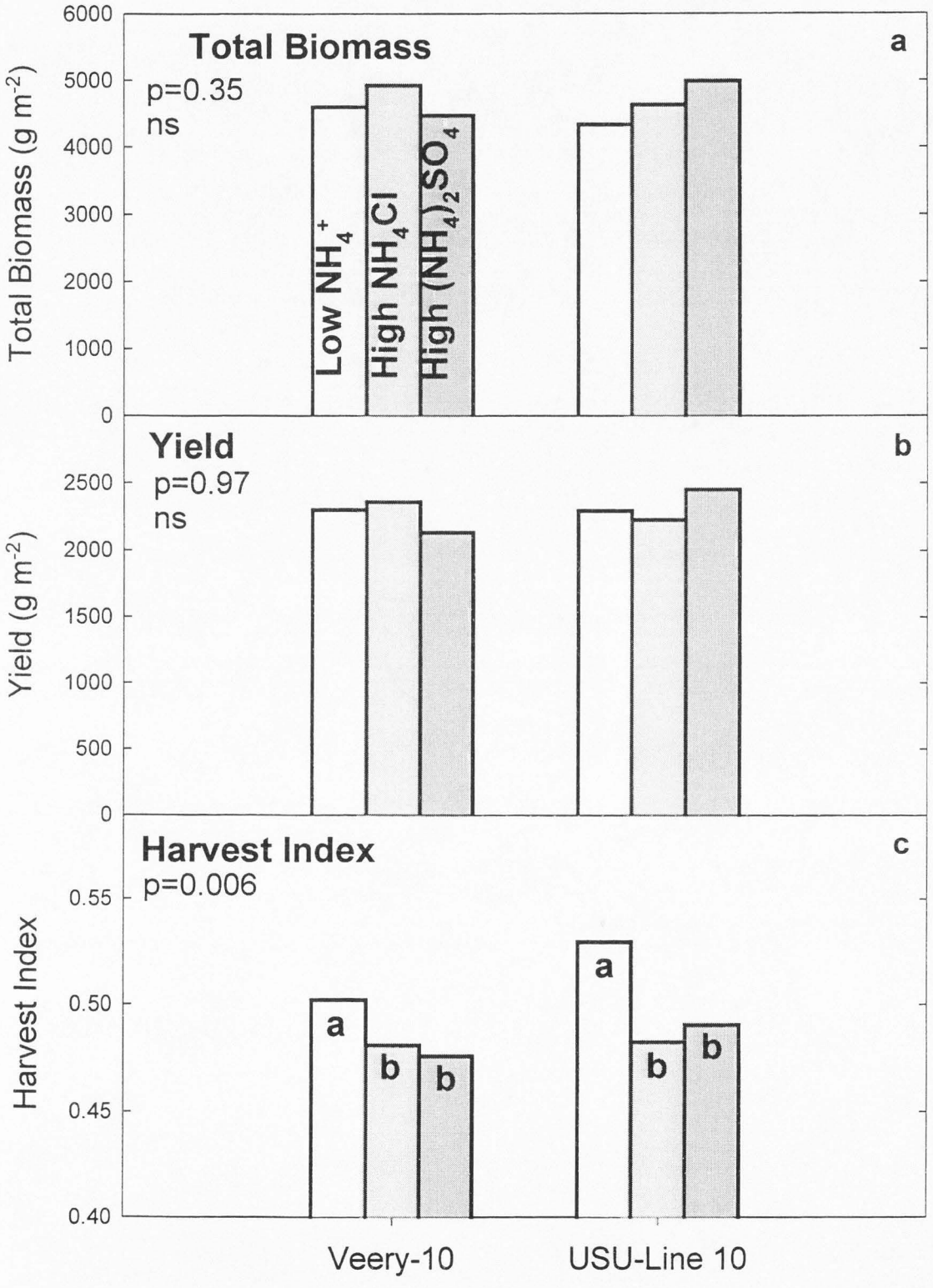

Fig. 2-3. The effect of NH_4^+ treatment on vegetative biomass, yield, and harvest index. Bars are the average of two replicate trials. The p-value shown is for differences among treatments.

with the previous work by Hooten (1998), Henry and Raper (1989), and Peet et al. (1985), who reported no significant difference in dry biomass accumulation between NO_3^--fed plants and NH_4^+-fed plants. Treatment comparisons are presented in Appendix A (Table A-1). The GLM and ANOVA results are shown in Appendix E (Tables E-1 and E-2).

Seed Yield

Seed yield did not differ between NH_4^+ treatments (Figure 2-3b, p=0.97). The low NH_4^+ treatment had an average seed yield of 2,297 g m^{-2}, the high NH_4Cl yielded 2,295 g m^{-2}, and the high $(NH_4)_2SO_4$ yielded 2,293 g m^{-2}. Cultivars also did not differ in yield (p=0.47). In contrast, Hooten (1998) reported a 20% reduction in seed yield for wheat grown in 80% NH_4^+. Air temperature averaged a few degrees warmer during seed set and fill in Hooten's (1998) studies. The cooler temperature in these studies during seed set and seed fill might have increased yield enough to minimize differences between treatments. This work suggests that wheat can be grown in high NH_4^+ with little effect on yield with the proper conditions. The GLM and ANOVA results are shown in Appendix E (Tables E-3 and E-4).

Harvest Index

Harvest index, the ratio of edible biomass to total biomass, was significantly reduced by high NH_4^+ (Figure 2-3c, p=0.006). The low NH_4^+ treatment had an average harvest index of 0.52, the high NH_4Cl treatment was 0.48, and the high $(NH_4)_2SO_4$ treamtent was 0.48. Harvest index was reduced by approximately 8% in the high NH_4^+

treatments in these studies, very different from the results of Hooten (1998), who reported a 21% reduction in harvest index in the high NH_4^+ treatment. Cultivar ($p=0.12$) and trial ($p=0.8$) main effects were not significant for harvest index. The GLM and ANOVA results are shown in Appendix E (Tables E-5 and E-6).

Yield Components

Heads per m^2

There were no significant differences in heads per m^2 among NH_4^+ treatments (Figure 2-4a, $p=0.43$). Heads per m^2 were also not significantly affected by trial ($p=0.56$) or cultivar ($p=0.67$). Camberato and Bock (1990) found that high NH_4^+ increased yield, which was associated with increased tillering of the wheat plants. Silberbush and Lips (1991) also reported increased tillering with increased NH_4^+/NO_3^- ratio. However, Hooten (1998) found no increase in wheat tillering with increasing NH_4^+. High NH_4^+ effects on tillering may be a function of plant density, which may explain the different results. A table comparing treatments is in Appendix A (Table A-2). The GLM and ANOVA are shown in Appendix E (Tables E-7 and E-8).

Seeds per Head

Seeds per head is indicative of pollination. The NH_4^+ treatment had no significant effect on seeds per head (Figure 2-4b, $p=0.09$). Average number of seeds per head was 19. Cultivars did not significantly differ in seeds per head ($p=0.24$). Average seed per head was 19.5 in Trial 1 and 17.8 in Trial 2, which were significantly different ($p=0.03$). Hooten (1998) reported a 26% decrease in seeds per head with high NH_4^+ compared to a NO_3^- only treatment. This reduction in seed set was the primary cause of yield reduction

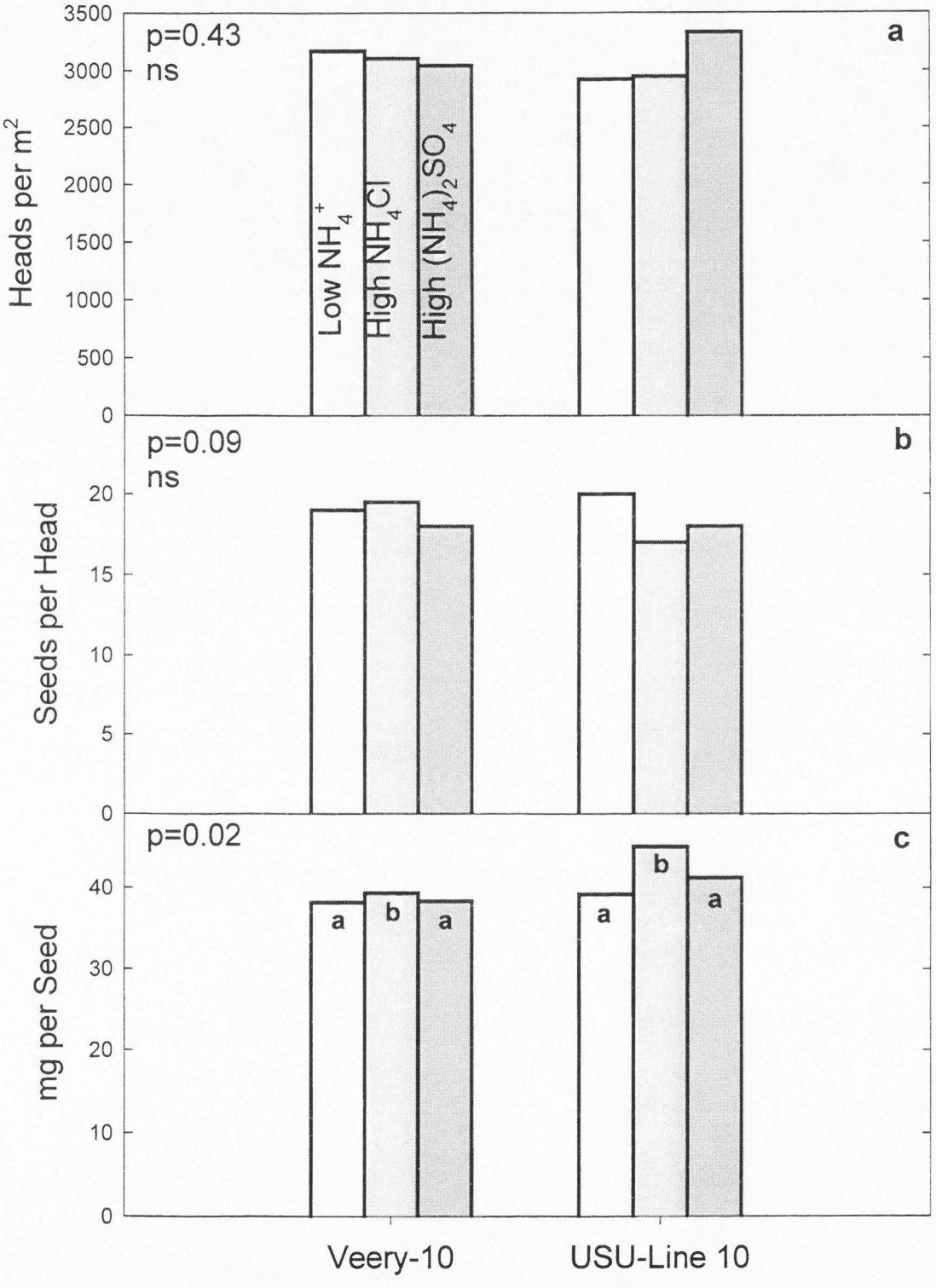

Fig. 2-4. NH_4^+ treatment had no significant effect on heads per m^2 or seeds per head. The high NH_4Cl treatment significantly increased seed mass. Each bar is the average of two replicate trials. The p-values are for treatment differences.

in Hooten's (1998) studies. The GLM and ANOVA are shown in Appendix E (Tables E-9 and E-10).

Seed Mass

Seed fill is characterized by seed mass (mg per seed). Although the increase in seed mass with high NH_4Cl compared to low NH_4^+ was statistically significant (Figure 2-4c, p=0.02), the increase was very small. Seed mass in the high NH_4Cl treatment averaged 41.7 mg, compared to 38.2 mg for low NH_4^+, and 39.0 mg for high $(NH_4)_2SO_4$. Koenig and Pan (1996) and Engel and Fixen (1994) also reported a similar increase of wheat kernel weight with Cl fertilization in soils. Cultivar also had a significant effect on seed mass (p=0.004) with Veery-10 averaging 38 mg and USU-Line 10 averaging 40.9 mg. The GLM and ANOVA are shown in Appendix E (Tables E-11 and E-12).

Sterile Heads

The NH_4^+ treatment had no statistically significant effect on % sterile heads, although high NH_4^+ did increase the % sterile heads (Figure 2-5, p=0.81). Hooten (1998) found that high NH_4^+ significantly increased sterile heads. Trial 1 and 2 did differ significantly (p= 0.01), probably due to a possible Pythium infection in the second trial. Trial 1 averaged 2.96% sterile heads compared to 7.06% for Trial 2. Cultivar had no significant effect on sterile heads (p=0.56). The GLM and ANOVA are shown in Appendix E (Tables E-13 and E-14).

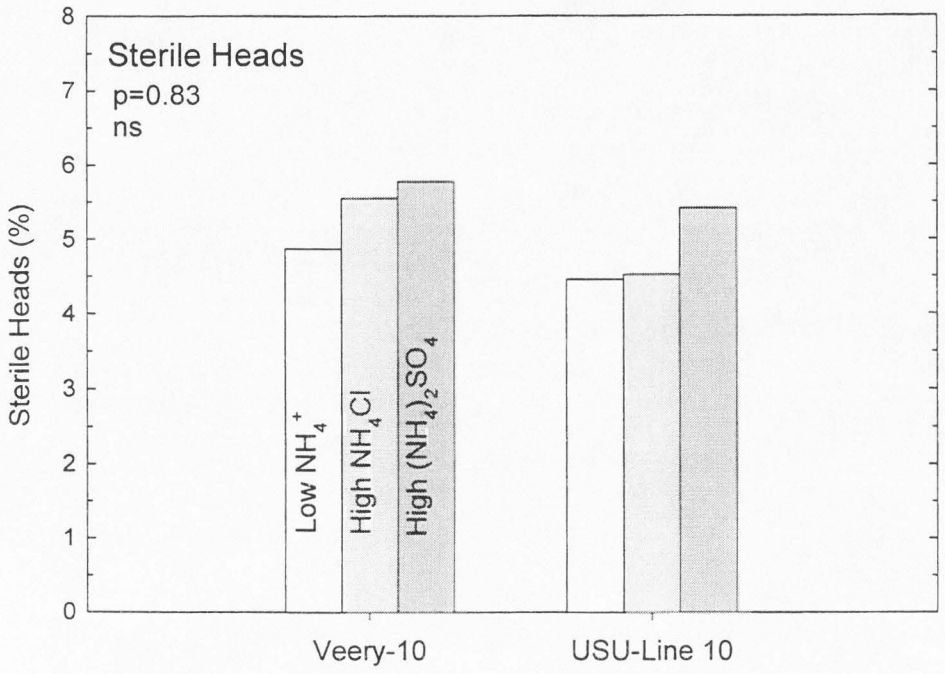

Fig. 2-5. NH_4^+ treatment effect on sterile heads. Percent sterile heads is the ratio of sterile heads /total heads. Each bar is the average of two replicate trials.

N in Plant Tissue

N in Seeds

High NH_4^+ treatments had significantly higher seed N content than the low NH_4^+ treatment (Figure 2-6a, p=0.001). Cultivar had no significant effect on N concentration in seeds. High NH_4^+ treatments averaged 33.5 g N kg^{-1}, compared to 24.7 g N kg^{-1} in low NH_4^+ seeds (see Appendix F, Tables F-5 and F-9). Higher N concentration is important because it correlates with % protein. Average protein concentration in wheat seeds is determined by multiplying %N by 5.83 (Marschner, 1995). High NH_4^+ treatments would have approximately 19.5% protein compared to 14.4% protein in the low NH_4^+ treatment.

Increased protein in seeds could be an important nutritional benefit of high NH_4^+-grown wheat. Additional tests to quantify protein content and determine types of protein present need to be completed to fully determine the potential nutritional benefits. The ANOVA is shown in Appendix E (Table E-15).

N in Vegetative Biomass

Differences between NH_4^+ treatments were not statistically significant (Figure 2-6b, p=0.11), although high NH_4Cl had higher N (17.2 g kg^{-1}) than low NH_4^+ (13.3 g kg^{-1}), and high $(NH_4)_2SO_4$ had the highest N concentration (19.5 g kg^{-1}). Cultivar also had no effect on N content in vegetative biomass. Plant tissue analysis is shown in Apendix F (Tables F-5 and F-9). The ANOVA is shown in Appendix E (Table E-16).

NO_3^- in Seeds

The NH_4^+ treatment had no significant effect on NO_3^- concentration in seeds (Figure 2-7a, p=0.89). Although the Veery-10 cultivar in the high NH_4Cl treatment had a much higher NO_3^--N content (0.09 g kg^{-1}) than the other treatment combinations, this difference was not statistically significant. The two wheat cultivars were not significantly different concerning NO_3^- content in seeds. The ANOVA is shown in Appendix E (Table E-17).

NO_3^- in Vegetative Biomass

The NH_4^+ treatment significantly affected NO_3^- in vegetative biomass (Figure 2-7b, p=0.03). High NH_4Cl significantly reduced NO_3^- content (2.1 g kg^{-1}) compared to

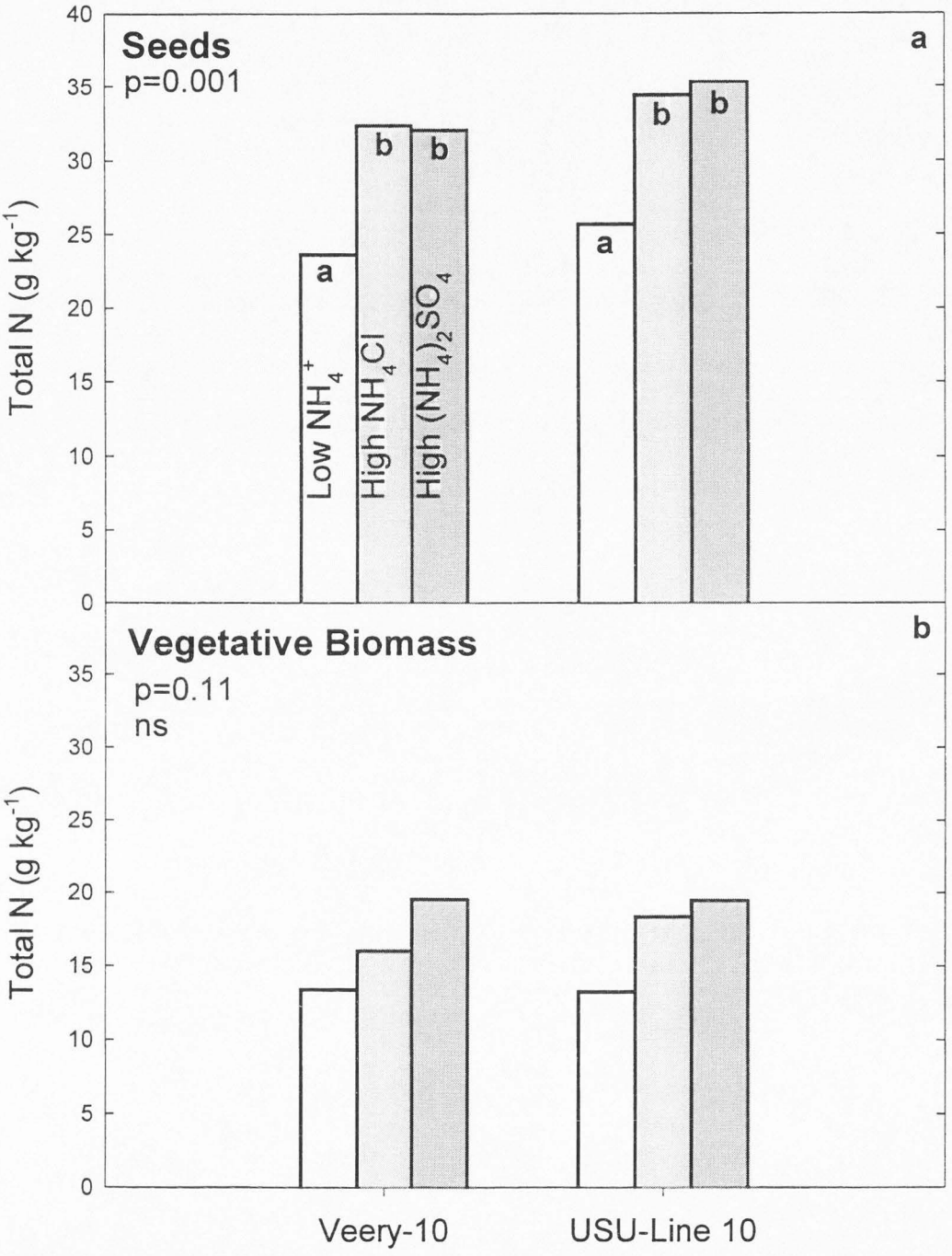

Fig. 2-6. The effect of NH_4^+ treatment on total N content of seeds and vegetative biomass. Total N was determined by combustion with a LECO CHN analyzer. Each bar is the average of two replicate trials. The p-values are for treatment differences.

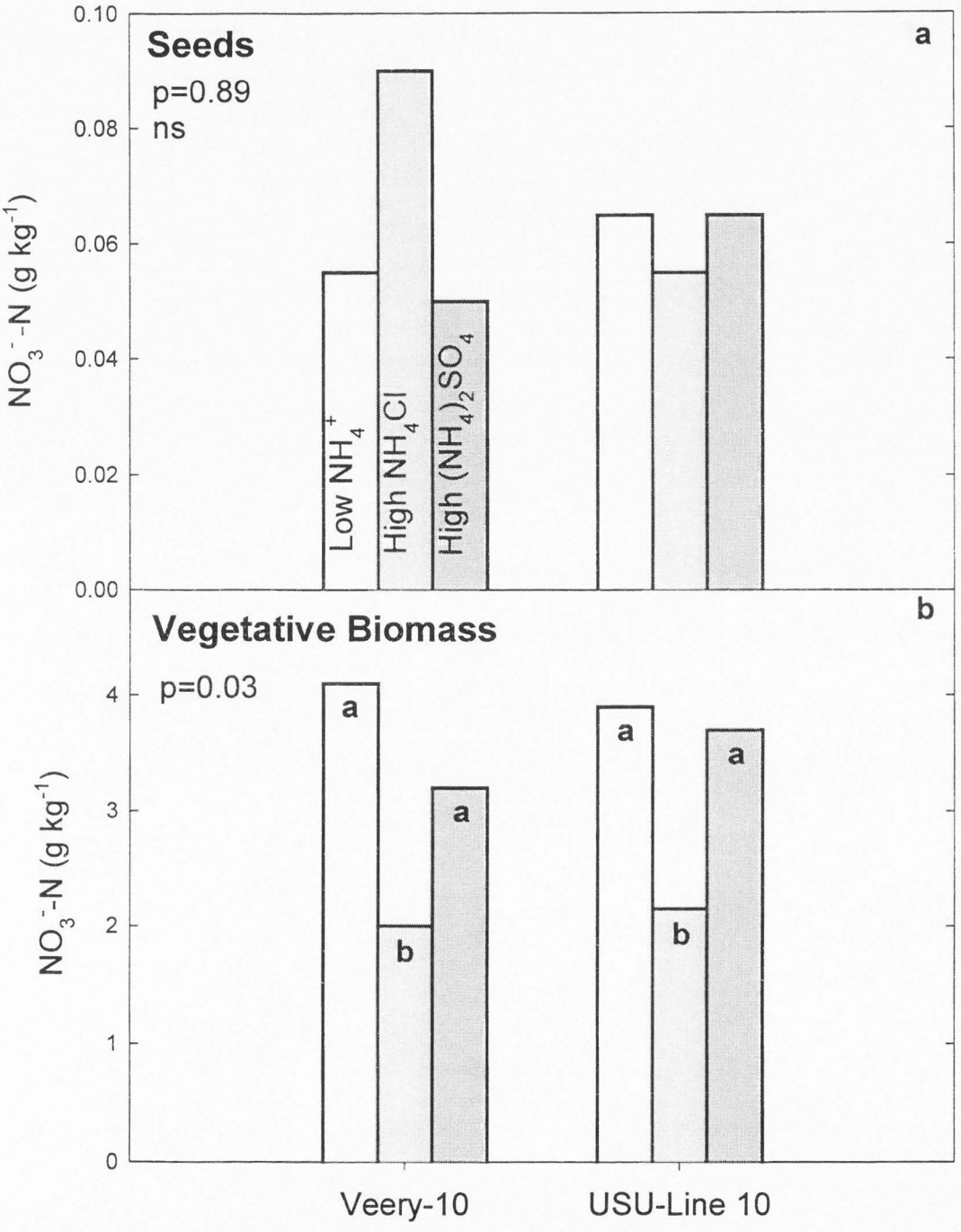

Fig. 2-7. The effect of NH_4^+ treatment on NO_3^- in seeds and vegetative biomass. NO_3^- concentration was determined by the chromotropic acid method using a Lachat autoanalyzer. Each bar is the average of two replicate trials. The p-values are for differences between treatments.

low NH_4^+ (4.0 g kg^{-1}) and high $(NH_4)_2SO_4$ (3.4 g kg^{-1}). The ANOVA is shown in Appendix E (Table E-18). Van der Boon et al. (1990) and Inal et al. (1995) found that high Cl^- reduced NO_3^- concentration in lettuce (*Latuca sativa* L.) and onion (*Allium cepa* L.) with NH_4^+ in the nutrient solution. Chloride may inhibit NO_3^- uptake, or replace NO_3^- as an osmoticum in the vacuole. Liu and Shelp (1995) suggested that Cl^- does not affect NO_3^- uptake, but instead reduces the ability to store NO_3^-. Because SO_4^{2-} uptake is relatively slow (Marschner, 1995), the plant apparently takes up more NO_3^- to supply charge balance for the NH_4^+ entering the plant.

Macro- and Micronutrients in Plant Tissue

Concentration of Macro- and Micronutrients in Flag Leaves

In general, cation concentrations were lower in high NH_4^+ treatments, and anion amounts were reduced in the low NH_4^+ treatment (Figures 2-8 and 2-9). However, there were a few exceptions. Figures 2-8 and 2-9 show concentrations of macro and micronutrients in flag leaves that were sampled every 2 wk following anthesis in both trials. Because cultivars were not significantly different, each point is the average of Veery-10 and USU- Line 10. Data are graphed without averaging cultivars in Appendix A (Figures A-1 through A-4). Plant nutrient analysis data are shown in Appendix F (Tables A-1 through A-9). Flag leaves were sampled because they are a good representation of plant nutrient status.

The NH_4^+ treatments did not significantly affect K^+ concentration in flag leaves, demonstrating that excess K^+ can alleviate deficiencies. As expected, Ca^{2+} concentration was lower in high NH_4^+ treatments. Counter ion appeared to have no effect on Ca^{2+}

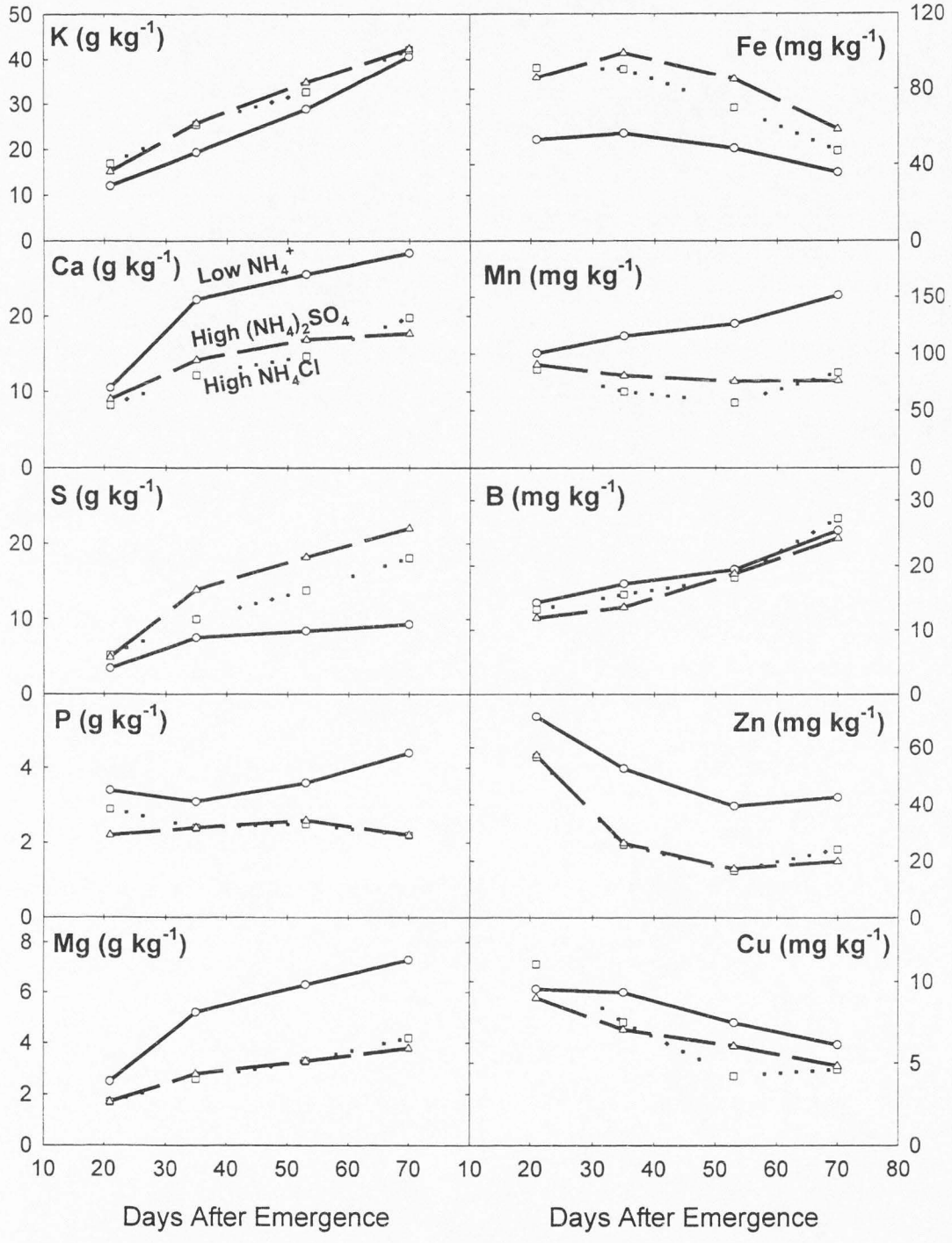

Fig. 2-8. The effect of NH_4^+ treatment on flag leaf content of 10 nutrients in Trial 1. Macronutrients are displayed in the left column as g kg^{-1}. Micronutrients are shown in the right column as mg kg^{-1}. Because there was no significant difference between cultivars, each point is the average of Veery-10 and USU-Line 10.

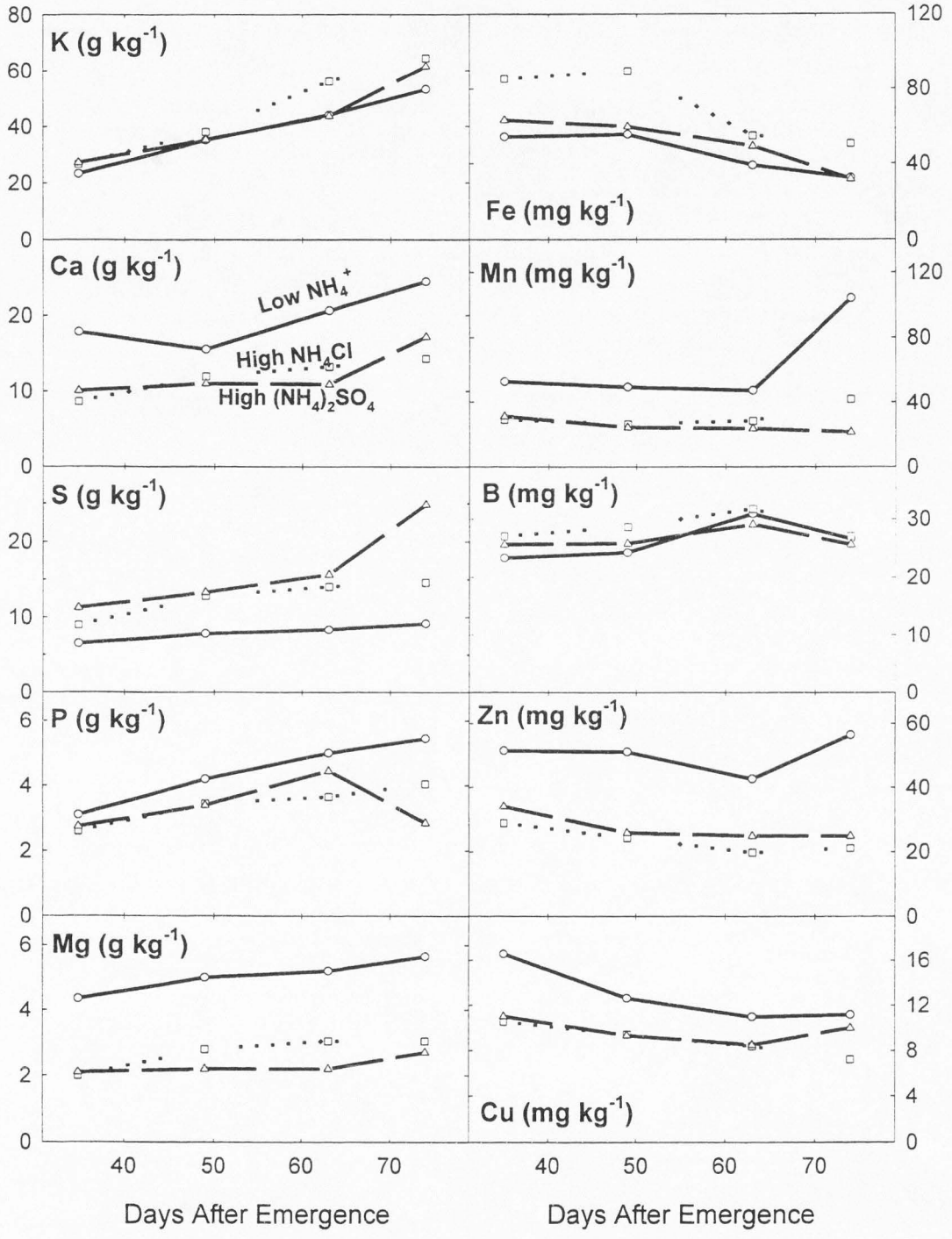

Fig. 2-9. The effect NH_4^+ treatment on flag leaf content of 10 nutrients in Trial 2. Macronutrients are displayed in the left column as g kg^{-1}. Micronutrients are shown the right column as mg kg^{-1}. Each point is the average of Veery-10 and USU-Line 10.

uptake in these studies. Unlike the Koenig and Pan (1996) study, these studies do not show increased Ca^{2+} uptake with excess Cl^-. Magnesium concentration was also reduced in high NH_4^+ treatments, and neither counter ion improved Mg^{2+} uptake. Uptake of Fe was only slightly improved in high NH_4^+ treatments. This is surprising because the high uptake of NH_4^+ should lower rhizosphere pH and improve Fe availability (Marschner, 1995). Indeed, Hooten (1998) found increased Fe with high NH_4^+. Concentrations of Mn and Zn were slightly reduced in the high NH_4^+ treatments. Concentration of Cu was only slightly reduced by high NH_4^+ in Trial 2. Reductions were expected in both trials, as NH_4^+ inhibits cation uptake. Uptake of S was highest in the high $(NH_4)_2SO_4$ treatment and lowest in the low NH_4^+ treatment. High NH_4^+ treatments were expected to have increased concentrations of S because it is taken up as an anion, and NH_4^+ uptake increases anion uptake. The high $(NH_4)_2SO_4$ treatment would be expected to have the most S because of the excess SO_4^{2-} in solution.

Calcium in Plant Tissue

Although high NH_4^+ reduced Ca in seeds, the difference was not statistically significant (Figure 2-10a, $p=0.08$). The NH_4Cl and $(NH_4)_2SO_4$ treatments did not differ in seed Ca levels. These levels may not be deficient, but higher Ca level in high NH_4^+ treatments is desirable in ALS systems for human health. The ANOVA is shown in Appendix E (Table E-19).

The reduction in Ca concentration in vegetative biomass of high NH_4^+ treatments was significant (Figure 2-10b, $p=0.004$). The ANOVA is shown in Appendix E (Table E-20). As for seeds, counter ion did not affect Ca in the biomass, indicating that Cl^- did

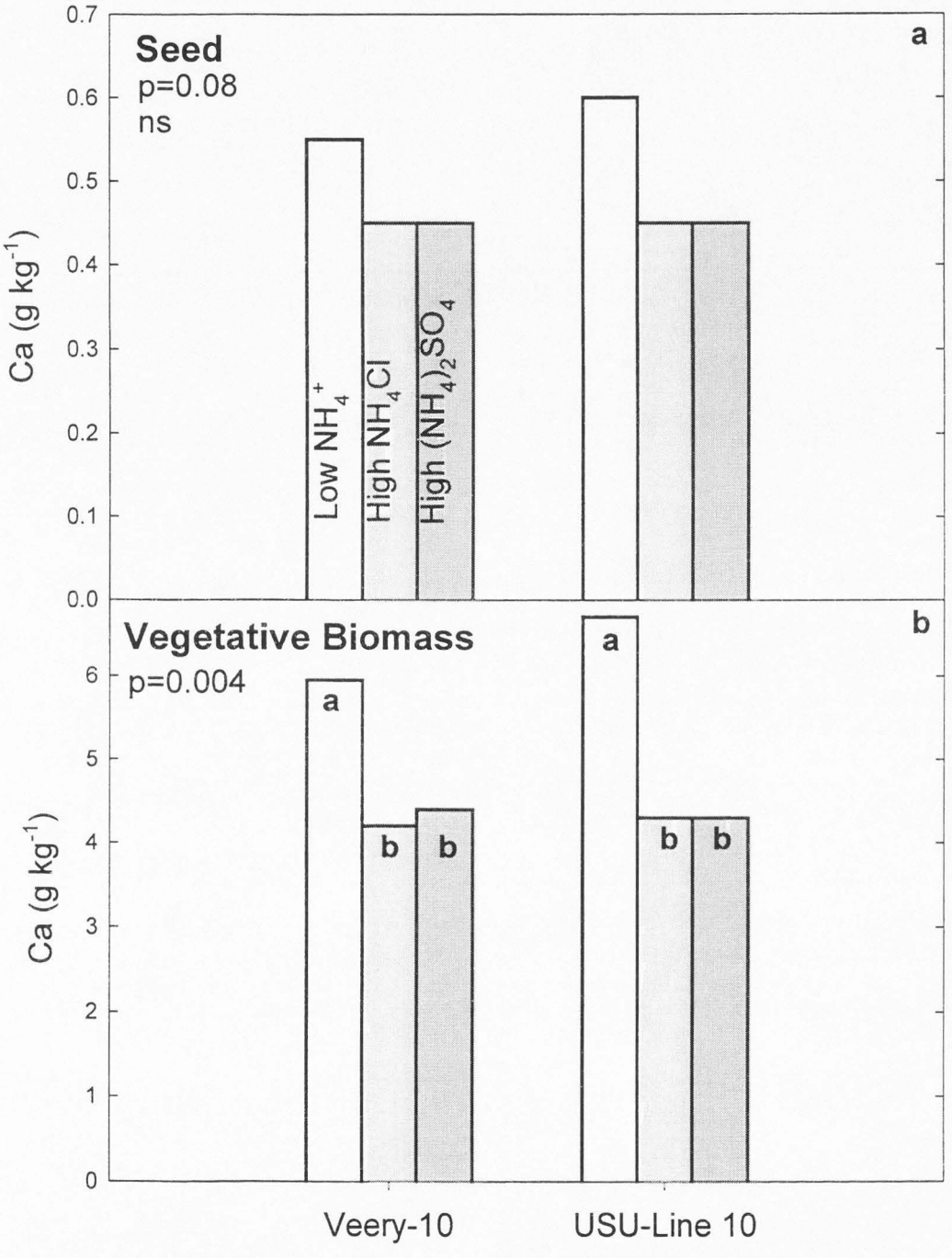

Fig. 2-10. The effect of NH_4^+ treatment on Ca in seeds and vegetative biomass. Each bar is the average of two replicate trials. The p-values are for treatment differences.

not help Ca uptake. However, these results may be due to SO_4^{2-} present in the NH_4Cl treatment and Cl^- present in the $(NH_4)_2SO_4$ treatment.

Treatment cross-contamination. During the study, there was treatment cross-contamination caused by the solution sampling system. System tests to quantify the contamination were run after the trials were completed. These tests indicated that the solution sampling lag time was too long, creating back contamination. Calculations showed that lag time is 14 s too long, causing approximately 214 mmoles to move from the high NH_4Cl treatment to the high $(NH_4)_2SO_4$ treatment during the entire trial (see Appendix D for description of the contamination tests). However, high NH_4Cl and high $(NH_4)_2SO_4$ treatments did maintain high concentrations of their respective counter ions in solution (Figure 2-11). The contamination of Cl^- in the high SO_4^{2-} treatment may have increased Ca uptake in that treatment; however, this contamination is small compared to the 5,200 mmoles of SO_4^{2-} added to the high $(NH_4)_2SO_4$ treatment. Contamination of other nutrients is not a concern because all three treatments contained equal amounts of those nutrients.

GEOCHEM-PC modeling of Ca^{2+} availability. Starting nutrient solutions for all three treatments were modeled with GEOCHEM-PC to estimate Ca^{2+} availability. Results showed that Ca^{2+} is equally available regardless of counter ion in starting solution (Table 2-3). To estimate Ca^{2+} availability at the end of the trial, SO_4^{2-} concentration was measured at 20 m*M* in solution. Solution Ca^{2+} was estimated at 10 m*M* based on calculated Ca^{2+} additions. Although the % of total Ca^{2+} was less available under these conditions, enough free Ca^{2+} remained for plant uptake (Table 2-3).

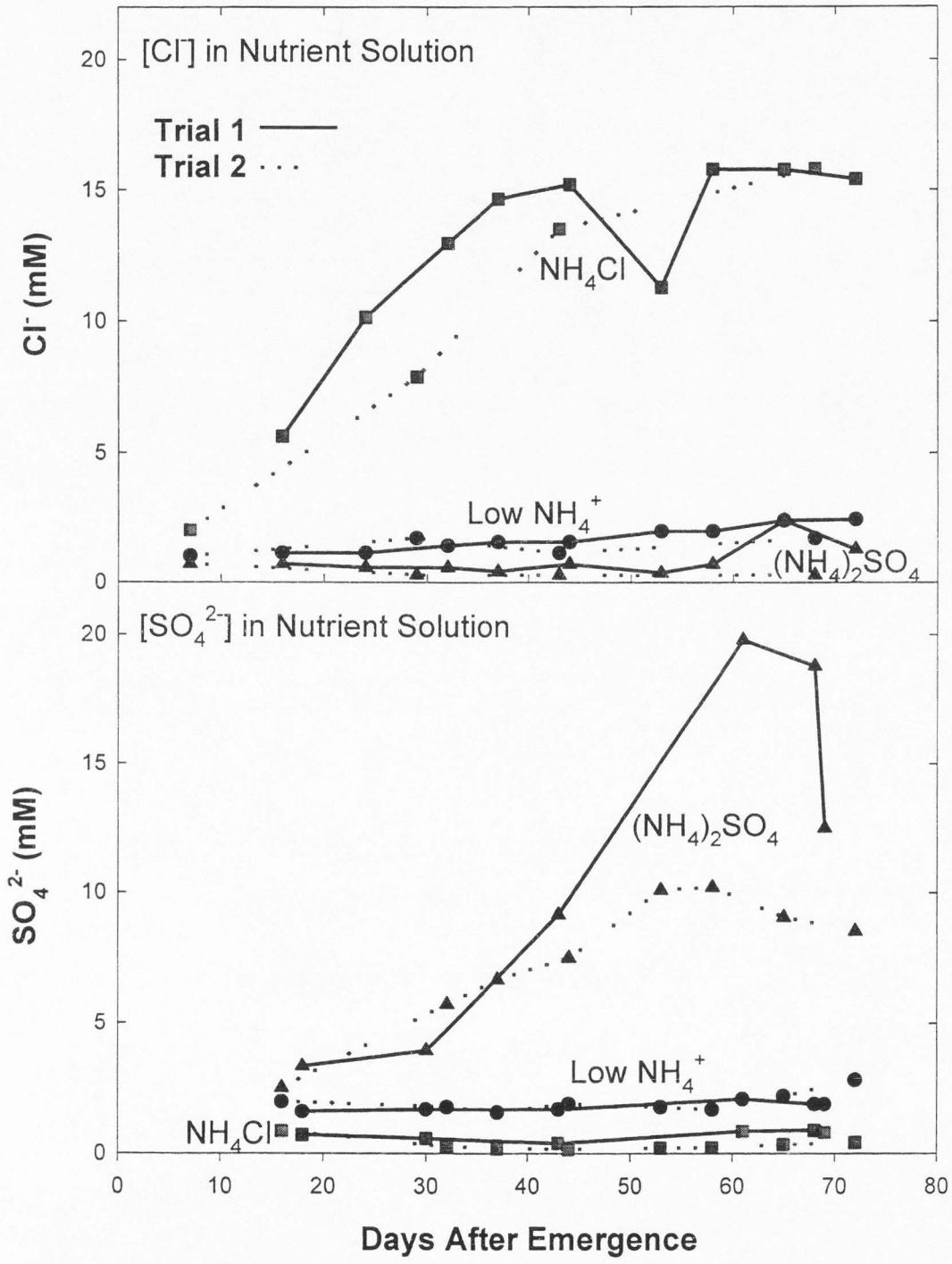

Fig. 2-11. Cl^- and SO_4^{2-} concentration in nutrient solution throughout Trials 1 and 2.

Table 2-3. Free concentration of Ca^{2+} in solution based on GECHEM-PC model.

Treatment	Total Ca^{2+} (mM)	Free Ca^{2+} (mM)
Low NH_4^+	1.19	0.94
High NH_4Cl	1.19	1.14
High $(NH_4)_2SO_4$ (Starting Soln)	1.19	1.03
High $(NH_4)_2SO_4$ (Ending Soln)	11.19	7.08

Sulfur in Vegetative Biomass

The NH_4^+ treatments had a significant effect on S in vegetative biomass (Figure 2-12a, $p<0.0001$). Low NH_4^+ had the least S (3.3 g kg^{-1}), high NH_4Cl was intermediate (4.3 g kg^{-1}), and $(NH_4)_2SO_4$ had the highest S concentration (6.7 g kg^{-1}). High $(NH_4)_2SO_4$ was expected to have the most S in tissue because SO_4^{2-} was the dominant counter ion in the nutrient solution. The high NH_4Cl treatment had higher SO_4^{2-} concentration than the low NH_4^+ treatment because of the higher NH_4^+ uptake in the NH_4Cl treatment. High NH_4^+ uptake requires large amounts of anions to balance the positive charge of NH_4^+. The ANOVA is shown in Appendix E (Table E-21).

USU-Line 10 and Veery-10 also had significantly different S concentrations ($p=0.03$). USU-Line 10 averaged 5.1 g kg^{-1} S and Veery-10 averaged 4.4 g kg^{-1} S. There was no interaction between cultivar and treatment ($p=0.41$).

Chloride in Vegetative Biomass

The high NH_4Cl treatment had significantly higher amounts of Cl in biomass compared to low NH_4^+ and high $(NH_4)_2SO_4$ (Figure 2-12b, $p=0.027$). It was surprising that $(NH_4)_2SO_4$ did not have more Cl than the low NH_4^+ treatment, similar to the high NH_4Cl having higher amounts of SO_4^{2-} in Figure 12a. Cultivar had no effect on Cl concentration ($p=0.84$). The ANOVA is shown in Appendix E (Table E-22).

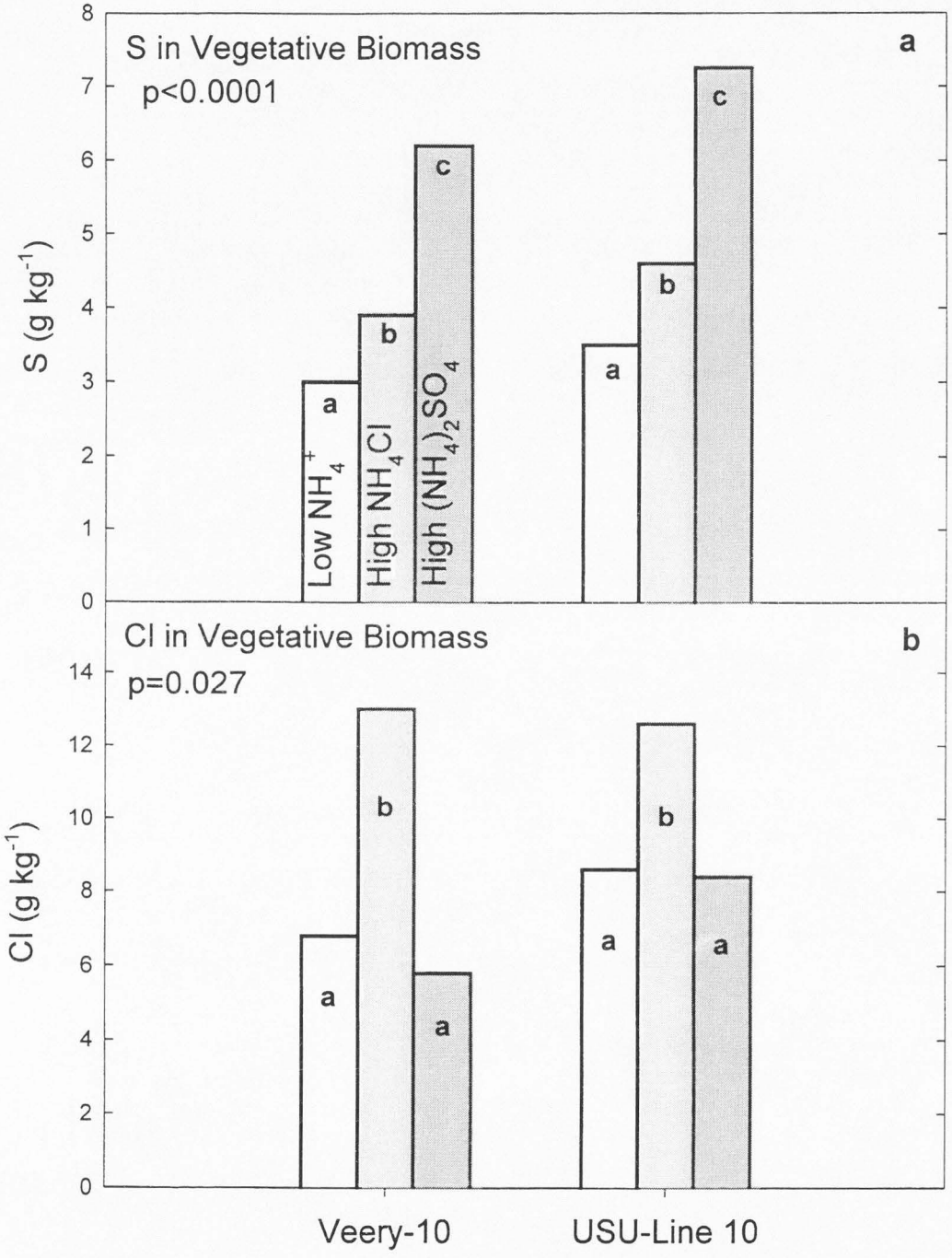

Fig. 2-12. The effect of NH_4^+ treatment on S and Cl in vegetative biomass. The p-value is for treatment differences. In the S graph, there is also a significant difference between cultivars (p=0.03). Each bar is the average of two replicate plots in each of two replicate trials.

Mass Balance

N Mass Balance

Mass recovery of N from both trials ranged from 73 to 106% (Table 2-4). Recovery of N typically averages 70% in solution culture (Bugbee, 1995). Trial 1 averaged 100% recovery, while Trial 2 averaged 80%. Calculations of plant tissue N are shown in Appendix A (Tables A-3 and A-4). The low NH_4^+ treatment had the highest N recovery in both trials. Reasons for abnormal N mass balance include NH_4^+ volatilization, denitrification, and treatment contamination. Loss of NH_4^+ through volatilization was a minor concern because the pH was maintained at 5.8, well below pH level that would cause significant loss of N as $NH_{3(gas)}$. The low NH_4^+ treatment was expected to have the lowest N recovery because of the high amounts of NO_3^- available for loss by denitrification but recoveries were similar or higher for NO_3^- treatments.

Table 2-4. Nitrogen mass recovery for Trials 1 and 2. All values, except recovery, are in mmoles.

Trial 1 Treatment	Total N added to system	Starting N	N left in system	N removed from system	N in plant material	Recovery (%)
Low NH_4^+	6,602	29	43	6,587	7,034	106
High NH_4Cl	9,661	29	112	9,578	9,589	100
High $(NH_4)_2SO_4$	13,047	29	633	12,443	11,764	94
Trial 2 Treatment						
Low NH_4^+	5,274	29	209	5,094	4,691	92
High NH_4Cl	8,577	29	46	8,560	6,209	73
High $(NH_4)_2SO_4$	8,194	29	332	7,891	5,883	75

Cl^- Mass Balance

Recovery of Cl^- for treatments varied between 31 to 50,000% (Table 2-5). Mass balance for the entire room (three systems combined) was ~65% for Trial 1 and ~55% for Trial 2. Recovery of Cl^- for the high $(NH_4)_2SO_4$ treatment differed enormously depending on whether a measured value or calculated value was used to estimate starting Cl^- concentration. Contamination of the high $(NH_4)_2SO_4$ treatment by HCl acid used to clean the system may account for the higher measured Cl^- value. Starting Cl^- concentration was the same in the other treatments for measured and calculated values. Calculated values are based on the nutrient solution recipe and volume of solution added to the system. If measured values are used for the high $(NH_4)_2SO_4$ treatment, Cl^- recovery is still much too high in both trials (591-1,800%).

Missing Cl^- was calculated to estimate contamination (Table 2-5). Missing or excess Cl^- for each treatment was calculated as the difference between Cl^- removed from the system and Cl^- in the plant material. Positive values in this column indicate missing Cl^-, while negative values indicate excess. In both trials, the high NH_4Cl treatment was missing more than 2,000 mmol of $Cl^-$and both high $(NH_4)_2SO_4$ and low NH_4^+ had large excesses of Cl^-. These data suggested treatment-to-treatment contamination caused by the solution sampling system. Systems tests to quantify contamination revealed that approximately 214 mmoles moved from the high NH_4Cl treatment to the high $(NH_4)_2SO_4$ treatment during a trial (see Appendix D for description of the contamination tests). However, this contamination does not completely account for the erroneous mass recoveries. Poor mass recovery may also be caused by measurement and sampling errors.

Table 2-5. Mass recovery of Cl^- for Trials 1 and 2. All values, except recovery, are mmoles. Missing Cl^- negative values are excess Cl^-, and positive values are missing Cl^-.

Trial 1 Treatment	Total Cl^- added to system	Starting Cl^-	Cl^- left in system	Cl^- removed from system	Cl^- in plant material	Missing Cl^-	Recovery (%)
Low NH_4^+	464	339	316	487	814	-327	167
High NH_4Cl	7,557	672	4,424	3,805	1,197	2,608	31
High $(NH_4)_2SO_4$	25	196* 37	78	143 -16	845	-702 -861	591* 5,281
Total	8,046	1,207* 1,048	4,818	4,435 4,276	2,856	1,579 1,420	64* 67
Trial 2							
Low NH_4^+	455	339	680	114	367	-253	322
High NH_4Cl	6,901	672	4,312	3,261	1,087	2,174	33
High $(NH_4)_2SO_4$	22	196* 37	198	20 -139	361	-341 -500	1,800* 50,000
Total	7,378	1,207* 1,048	5,190	3,395 3,236	1,815	1,580 479	53* 56

***Indicates use of measured value of starting Cl^-, all other values are calculated.**

Although the treatments were contaminated, the results from these studies are still valuable. The high $(NH_4)_2SO_4$ consistently had much more SO_4^{2-} than Cl^-, and vice versa for the high NH_4Cl treatment (Figure 2-11). However, additional studies must be conducted to determine if these counter ions do have different effects on Ca^{2+} uptake.

SO_4^{2-} Mass Balance

Recovery values of SO_4^{2-} ranged from 28 to 972% (Table 2-6). Total recovery for the entire room (three systems combined) was 73% for Trial 1 and 54% for Trial 2.

Table 2-6. Mass recovery of SO_4^{2-} for Trials 1 and 2. All values except recovery are in mmoles. Negative missing SO_4^{2-} values indicate excess SO_4^{2-}, and positive values indicate missing SO_4^{2-}.

Trial 1 Treatment	Total SO_4^{2-} added to system	Starting SO_4^{2-}	SO_4^{2-} left in system	SO_4^{2-} removed from system	SO_4^{2-} in plant material	Missing SO_4^{2-}	Recovery (%)
Low NH_4^+	356	476	524	308	431	-123	140
High NH_4Cl	94	280	224	150	541	-391	361
High $(NH_4)_2SO_4$	5,290	476	3,696	2,070	886	1,184	43
Total	5,740	1,232	4,444	2,528	1,858	670	73
Trial 2 Treatment							
Low NH_4^+	365	448	788	25	243	-218	972
High NH_4Cl	135	280	114	301	333	-32	111
High $(NH_4)_2SO_4$	3,344	616	2,392	1,568	448	1,120	28
Total	3,844	1,344	3,294	1,894	1,024	870	54

Because of the incorrect sampling lag time, approximately 176 mmoles of SO_4^{2-} moved from the high $(NH_4)_2SO_4$ treatment to low NH_4^+ treatment during the course of the experiment.

SUMMARY AND CONCLUSIONS

High NH_4^+ did not influence biomass production. Contrary to much of the literature, high NH_4^+ did not significantly reduce yield. Harvest index was reduced by

high NH_4^+. High NH_4^+ also increased N content in seeds. High N is correlated with increased protein content, which has important nutritional implications.

Veery-10 and USU-Line 10 did not respond differently to the NH_4^+ treatments. Counter ion had no significant effect on yield, harvest index, biomass, or Ca in plant tissue. Treatment contamination may account for the lack of difference between the high Cl^- and high SO_4^{2-} treatments.

These studies have significant implications for the NASA Advanced Life Support Program. Wheat can be grown under high NH_4^+ conditions with no significant effect on yield and increased grain protein, but it can be difficult. Calcium uptake is still much lower in high NH_4^+ treatments. Wheat grown in high NH_4^+ systems is apparently very sensitive to environmental conditions; a temperature variation of a few degrees can significantly alter yield results.

Future work needs to address the protein quality of the seeds and further clarify effects of counter ions. Future studies should also include other crop species. Because environmental conditions can be difficult to maintain in ALS systems, future studies must also evaluate the effects of varying light levels, photoperiods, and temperature regimes.

REFERENCES

Alan, R. 1989. The effect of nitrogen nutrition on growth, chemical composition, and response of cucumbers (*Cucumis sativus* L.) to nitrogen forms in solution. J. Hortic. Sci. 64:467-474.

Bugbee, B. 1995. Nutrient management in recirculating hydroponic culture. Hydroponic Soc. Am. Proc. 16:15-30.

Bugbee, B., G. Koerner, R. Albrechtsen, W. Dewey, and S. Clawson. 1997. Registration of 'USU-Apogee' wheat. Crop Sci. 37:626.

Camberato, J.J., and B.R. Bock. 1990. Spring wheat responses to ammonium supply. I. Dry matter and nitrogen content. Agron. J. 82:463-467.

Cao, W., and T.W. Tibbitts. 1993. Study of various NH_4^+/NO_3^- mixtures for enhancing growth of potatoes. J. Plant Nutr. 16:1691-1704.

Engel, R., and P. Fixen. 1994. Suppression of physiological leaf spot in winter wheat by chloride fertilization. Better Crops with Plant Food #323045/94211. Potash and Phosphate Institute, Norcross, GA.

Henry, L.T., and C.D. Raper. 1989. Effects of root-zone acidity on utilization of nitrate and ammonium in tobacco plants. J. Plant Nutr. 12:811-826.

Hooten, T.M. 1998. Ammonium and nitrate effects on growth, development, and nutrient uptake of hydroponic wheat. M.S. Thesis. Utah State Univ., Logan.

Inal, A., A. Günes, and M. Aktas. 1995. Effects of chloride and partial substitution of reduced forms of nitrogen for nitrate in nutrient solution on the nitrate, total nitrogen, and chloride contents of onion. J. Plant Nutr. 18:2219-2227.

Kafkafi, U. 1990. Root temperature, concentration, and the ratio NO_3^-/NH_4^+ effect on plant development. J. Plant Nutr. 13:1291-1306.

Koenig, R.T., and W.L. Pan. 1996. Chloride enhancement of wheat responses to ammonium nutrition. Soil Sci. Soc. Am. J. 60:498-505.

Lavoie, N., L.P. Vezina, and H.A. Margolis. 1992. Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. Tree Physiol. 11:171-183.

Liu, L., and B.J. Shelp. 1995. Impact of chloride on nitrate absorption and accumulation by broccoli (*Brassica oleracea* var. *italica*). Can. J. Plant Sci. 76:367-377.

Magalhaes, J.R., and G.E. Wilcox. 1983. Tomato growth and mineral composition as influenced by nitrogen form and light intensity. J. Plant Nutr. 6:847-862.

Marcus-Wyner, L. 1983. Influence of ambient acidity on the absorption of NO_3^- and NH_4^+ by tomato plants. J. Plant Nutr. 5:657-666.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, New York.

Marti, H.R., and H.A. Mills. 1991. Nutrient uptake and yield of sweet pepper as affected by stage of development and N form. J. Plant Nutr. 14:1177-1185.

Maynard, D.N., and A.V. Barker. 1969. Studies on tolerance of plants to ammonium nutrition. J. Am. Soc. Hortic. Sci. 94:235-239.

McElhannon, W.S., and H.A. Mills. 1978. Influence of percent NO_3^-/NH_4^+ on growth, N absorption, and assimilation by lima beans in solution culture. Agron. J. 70:1027-1032.

Parker, D.R., W.A. Norvell, and R.L. Chaney. 1995. GECHEM-PC: A chemical speciation program for IBM an compatible personal computers. SSSA Special Publication 42:253-269. SSSA, Madison, WI.

Peet, M.M., C.D. Raper, L.C. Tolley, and W.P. Robarge. 1985. Tomato responses to ammonium and nitrate nutrition under controlled root-zone pH. J. Plant Nutr. 8:787-798.

Ritchie, K.B. 1994. Influences of nitrogen supply and elevated CO_2 on nitrogen consumption, nitrogen loss, tissue nitrogen concentration, and yield of hydroponic wheat. M.S. Thesis. Utah State Univ., Logan.

Silberbush, M., and S.H. Lips. 1991. Potassium, nitrogen, ammonium/nitrate ratio, and sodium chloride effects on wheat growth. II. Tillering and grain yield. J. Plant Nutr. 14:765-773.

Smart, D.R., and A.J. Bloom. 1993. Relationships between the kinetics of NH_4^+ and NO_3^- absorption and growth in the cultivated tomato (*Lycopersicon esculentum* Mill. cv. T-5). Plant Cell Environ. 16:259-267.

Smart, D.R., and A.J. Bloom. 1998. Investigations of ion absorption during NH_4^+ exposure I. Relationship between H^+ efflux and NO_3^- absorption. J. Exp. Bot. 49:95-100.

Smart, D.R., K. Ritchie, A.J. Bloom, and B.B. Bugbee. 1998. Nitrogen balance for wheat canopies (*Triticum aestivum* cv. Veery 10) grown under elevated and ambient CO_2 concentrations. Plant Cell Environ. 21:753-763.

Van der Boon, J., J.W. Steenhuizen, and E.G. Steingröver. 1990. Growth and nitrate concentration of lettuce as affected by total nitrogen and chloride concentration, NH_4/NO_3 ratio and temperature of the recirculating nutrient solution. J. Hortic. Sci. 65:309-321.

Youngdahl, L.J., R. Pacheco, J.J. Street, and P.L.G. Vlek. 1982. The kinetics of ammonium and nitrate uptake by young rice plants. Plant and Soil 69:225-232.

CHAPTER 3

NITRIFICATION IN HYDROPONIC CULTURE

INTRODUCTION

Microorganisms can convert NH_4^+ to NO_3^- through the two-step process of nitrification. Ammonium-oxidizing bacteria such as those belonging to the *Nitrosomonas* and *Nitrosospira* genera oxidize NH_4^+ to NO_2^-. In the second step, NO_2^- is converted to NO_3^- by nitrite-oxidizing bacteria such as *Nitrobacter* (Paul and Clark, 1996). Much of the NH_4^+ applied to the root-zone may be nitrified and enter the plant as NO_3^-. Norton and Firestone (1996), in a soil study, estimated that 27% of ^{15}N supplied to plants as NH_4^+ was taken up as NO_3^- after being nitrified.

Ammonium studies are often conducted in hydroponic solutions. Significant nitrification has been reported in ammonium-based nutrient solutions for aeroponic, sand culture, and hydroponic systems (Padgett and Leonard, 1993). Plants were grown in aeroponic, sand, or hydroponic culture with 100% NH_4^+-based nutrient solutions. Padgett and Leonard (1993) used accumulation of NO_3^- in solution, presence of NO_3^- in plants, and a bioassay as indicators of nitrification, and reported nitrification after only 5 to 8 d in a hydroponic system. However, the bioassay in this study could only confirm the presence of nitrifying bacteria; it could not quantify nitrification rates in their NH_4^+ systems. Padgett and Leonard (1993) cultured solution samples from the hydroponic systems in conditions favorable for nitrification. Because the NH_4^+ systems did not have optimal nitrifying conditions, the assay could not be an accurate representation of nitrification rates in the actual plant growth systems.

Nitrifying bacteria function optimally within the pH range of 7.0 to 8.5 in liquid media (Allison and Prosser, 1993), and hydroponic solutions are typically controlled between pH 5 and 6. Lang and Elliott (1991) found that the pH of soil-less potting media was a major factor controlling nitrification rate. Lang and Elliott (1991) found no nitrifier activity below pH 5.6, but their assay was conducted at pH 7.0 so organisms operating at a lower pH may not have been detected. Surface-attached ammonia oxidizers can maintain activity at lower pH than suspended cells (Allison and Prosser, 1993). Root surfaces in hydroponic systems could provide the surface necessary for nitrification to occur at lower pH values found in hydroponic systems.

OBJECTIVES AND HYPOTHESES

The overall objective of this study was to measure nitrification rates in hydroponic solution. Specific objectives were to:

1. Determine if nitrification occurs at significant rates in non-inoculated standard hydroponic culture at pH 5.8.

    Hypothesis 1: Nitrification will not be significant in non-inoculated hydroponic culture at pH 5.8.

2. Determine if nitrification can be enhanced in hydroponics by elevating pH and/or inoculation with nitrifying bacteria.

    Hypothesis 2: Inoculation and elevated pH will increase nitrification in hydroponic solution.

MATERIALS AND METHODS

Preliminary studies (Appendix C) indicated that small amounts of nitrification could occur in high NH_4^+ hydroponic studies. In this study, plants were grown in a large walk-in growth room. Single plants of *Triticum aestivum* (cv. USU-Apogee) were grown in 2-L Nalgene bottles filled with hydroponic solution. Aeration supply was pulled through a simple filter (glass wool sandwiched by two foam plugs) by a double-head pump (Thomas Industries Inc.) to filter out airborne dust and contaminants. After emergence, seedlings were transplanted to the 2-L bottles. Isolite was added to each of the bottles (10 g dry weight) to increase surface area for microbial growth.

Treatments

The experimental design was a 2 × 2 × 2 factorial with four replications. Sixteen bottles were maintained at pH 7.0, and 16 bottles were maintained at pH 5.8. Three days after transplanting, half of the bottles for each pH were inoculated with 3% (by volume) of each culture of *Nitrosomonas europea* and *Nitrobacter winogradskyi*, and half were not inoculated. Half of the bottles were planted, and half were left unplanted. Treatments were arranged in a randomized complete block design.

Nitrosomonas europea bacteria were propagated in American Type Culture Collection (ATCC) medium 929 (ATCC, 2000a). *Nitrobacter winogradskyi* bacteria were propagated in ATCC medium 480 (ATCC, 2000b). The cultures were grown for 4 wk and then transferred to 2-L at a 1:50 dilution and grown for another 2 wk to make a total of 6 L of inoculum, and 60 mL of each culture was added to each 2-L bottle.

Culture samples were plated on dilute nutrient agar and incubated for 3 wk to insure that there was no heterotrophic contamination in the inoculum.

Environmental Conditions

Plants were transplanted on day 0 and given a PPF of 450 μmol m^{-2} s^{-1} supplied by two pairs of high pressure sodium (HPS) lamps. On day 1, a third pair of HPS lamps was added to supply a PPF of 650 μmol m^{-2} s^{-1}. Air temperature was maintained at 22° C until anthesis when the temperature was lowered to 18° C. Plants were grown with elevated CO_2 (1,200 μmol mol^{-1}).

Nutrient Solution and pH Control

Plants were grown in hydroponic solution (Table 3-1). Solution volume was maintained by daily addition of refill nutrient solution. Nitrogen was supplied as NH_4^+. MES buffer in pH 5.8 bottles and MOPS buffer in pH 7.0 bottles helped maintain pH, which was checked and adjusted with 1 *M* KOH or HNO_3 on a daily basis. Preliminary studies determined appropriate Fe-chelate for pH 7 solution (Appendix B). Ammonium was monitored using a colorimetric test and a Lamotte colorimeter. Solution NO_3^- concentration was monitored throughout the study to measure net production of NO_3^- with an ion selective electrode, colorimeter, and Lachat autoanalyzer.

Analysis of Nitrification and Nitrate Consumption

An $^{15}NO_3^-$ isotopic dilution technique was used to measure nitrate production and consumption rates in the bottles during a 24-h time period (Figure 3-1). Isotopic dilution

Table 3-1. Nutrient solution composition.

Salt	Stock conc.	Starter solution mL added per 100 L	Starter solution Final conc.	Refill solution mL added per 100 L	Refill solution Final conc.
	M		m*M*		m*M*
$(NH_4)_2SO_4$	1	50	0.5	100	1
$CaSO_4$	Solid form	20.66 g	1.2	17.22 g	1
K_2SO_4	0.5	100	0.5	400	4
KH_2PO_4	0.5	120	0.6	120	0.6
$MgSO_4$	0.25	200	0.5	100	0.25
K_2SiO_3	0.1	100	0.1	100	0.1
	m*M*		μ*M*		μ*M*
$FeCl_3$	50	20	10	5	2.5
Fe-HEEDTA	100	25	25	5	5
$MnCl_2$	60	5	3	10	6
$ZnCl_2$	20	20	4	20	4
H_3BO_3	20	10	2	5	1
$CuCl_2$	20	5	1	15	3
Na_2MoO_4	0.6	15	0.09	5	0.03

consists of adding isotope to the product pool of the transformation (Stark, 2000). In this study, the product pool was the NO_3^- in the bottle, and the transformation of interest was nitrification. Applying the isotope to the product pool prevents stimulation of the production rate. The gross production rate is estimated from the rate at which $^{15}NO_3^-$ is diluted by influx of $^{14}NO_3^-$ through nitrification (Stark, 2000). Gross consumption rate is calculated based on the rate of $^{15}NO_3^-$ disappearance from the pool (Stark, 2000). Calculations of rates work under the assumption that consumption does not discriminate between $^{14}NO_3^-$ and $^{15}NO_3^-$ and that rates are constant throughout the 24-h incubation period.

At t_0, 1 m*M* of KNO_3 enriched with 35% $^{15}NO_3^-$ was supplied to all of the bottles. Solution samples were taken 20 min after isotope addition and again after 24 h. Samples

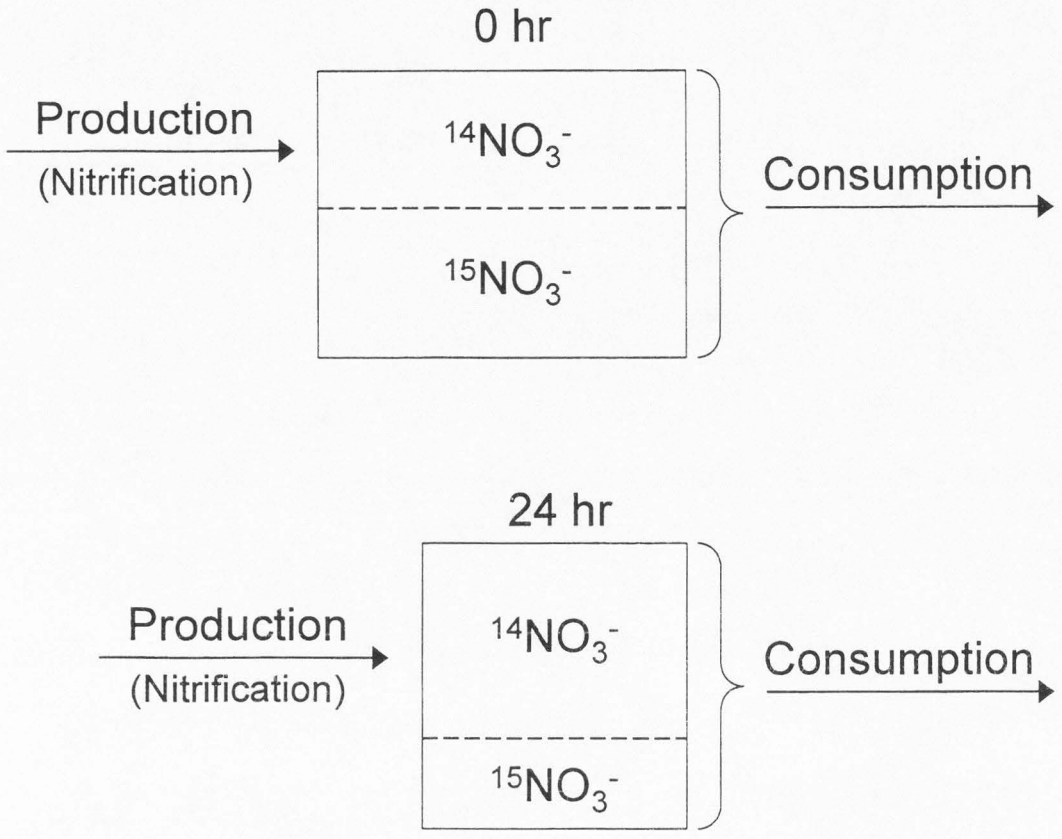

Fig. 3-1. Illustration of isotopic dilution technique. Solution samples are taken from the NO_3^- pool and are analyzed for total NO_3^- and $^{15}NO_3^-$, from which production and consumption rates are calculated (adapted from Stark, 2000).

were then analyzed on a Lachat autoanalyzer for total NO_3^-. Solutions were diffused and analyzed by mass spectrometry to determine $^{15}NO_3^-$ enrichment.

The diffusion technique described by Stark and Hart (1996) was used to concentrate inorganic N before ^{15}N analysis by mass spectrometry. A 3-ml sample of solution was placed in specimen cups containing 17 ml of 2 *M* KCl. MgO was added to each cup to raise the pH of the solution. The cups were left open for 1 wk to volatilize NH_4^+. After 1 wk, DDI water was added to bring the total volume in the cups back to 20 ml. Devarda's Alloy was added to convert all NO_3^- to $NH_{3(gas)}$. NH_3 was trapped on

acidified filter paper disks sealed between two strips of Teflon tape. The specimen cups were sealed with the trap and solution inside for 1 wk. Traps were then removed and analyzed for ^{15}N by continuous flow direct combustion-mass spectrometry at the University of California at Davis. Ratios of $^{15}NO_3^-$ to $^{14}NO_3^-$ at t_0 and t_{24} were used to calculate NO_3^- consumption and production rates according to the equations of Stark (2000).

RESULTS AND DISCUSSION

NO_3^- Accumulation

Accumulation of NO_3^- measured in bottles showed a large difference between planted and unplanted bottles. Planted bottles showed no NO_3^- accumulation throughout the study (Figure 3-2). Graphs of raw data are located in Appendix A (Figure A-5). Based on calculations of potential NO_3^- uptake, plants could easily consume any NO_3^- produced by nitrifiers.

Unplanted bottles had significant accumulation of NO_3^-, regardless of inoculation (Figure 3-3). However, inoculated bottles began accumulating NO_3^- earlier than non-inoculated bottles. Graphs of the raw data are located in Appendix A (Figure A-6). Net NO_3^- accumulation was not affected by treatment, but the time to achieve that rate was. Inoculated bottles at pH 5.8 took approximately 20 d to accumulate NO_3^-; in contrast, inoculated bottles with a pH of 7 took only 10 d, and non-inoculated bottles took almost 30 d. Before beginning the experiment, the bottles were cleaned with bleach and then HCl acid. This cleaning procedure may have been effective enough to delay

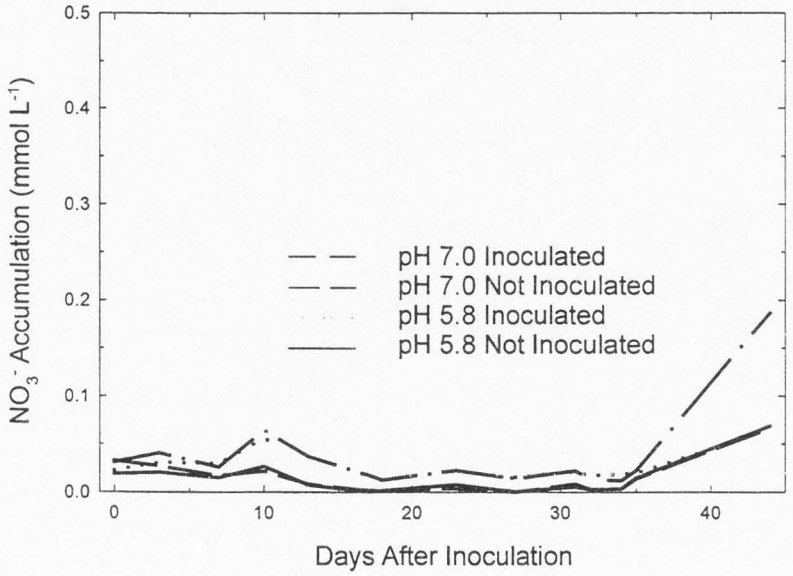

Fig. 3-2. Net NO_3^- accumulation in planted bottles. Each line is the average of four replicate bottles. NO_3^- is measured by an ion-selective electrode. The y-axis scale is 1/10 of the y-axis in Figure 3-3.

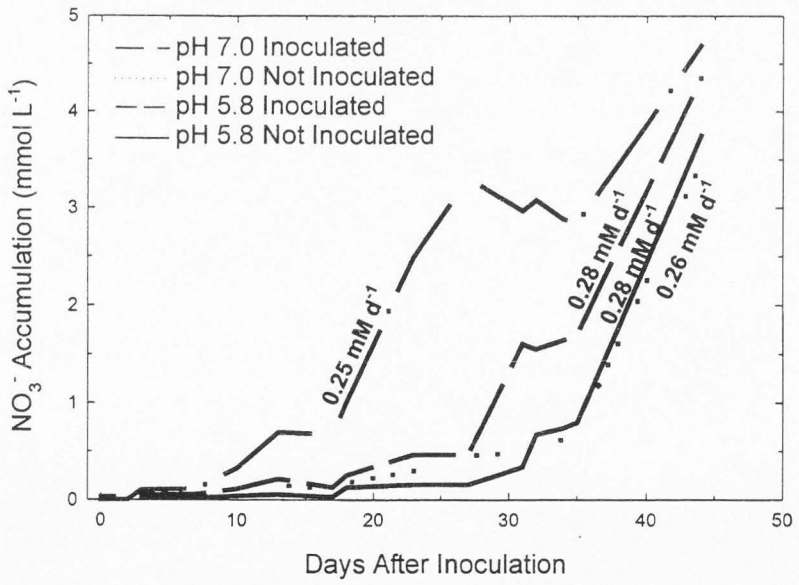

Fig. 3-3. Net NO_3^- accumulation in unplanted bottles. Each line is the average of four replicate bottles. NO_3^- was measured with an ion-selective electrode.

accumulation of NO_3^- in non-inoculated bottles. If contamination between bottles could be minimized, the lag time for NO_3^- accumulation in non-inoculated bottles could be even longer. This lag time contradicts studies by Padgett and Leonard (1993) who found significant nitrification after only 5 to 8 d in non-inoculated hydroponic systems.

Inoculated bottles maintained at pH 7 had reduced NO_3^- accumulation between day 25 and 35. The reduction could be due to instrument error, but this is not likely because these results were verified by measurements made with the cadmium reduction method using a Lachat Autoanalyzer (Figure A-7). The decrease in the rate of NO_3^- accumulation could be a change in either consumption or production of NO_3^-. Factors that would decrease production include decreased pH and product inhibition. Nitrifying bacteria can be very sensitive to pH. The optimum range falls between 6.6 and 8.0, and nitrification may be reduced below pH 6. Fluctuations in pH regime of the pH 7 bottles may account for reduced NO_3^- accumulation, but the pH was never measured below 6. As NO_3^- accumulates, it can inhibit nitrification; however, NO_3^- production later continued to increase when NO_3^- remained at the same concentration. Conditions increasing denitrification would increase NO_3^- consumption. These conditions include transient anaerobic conditions and changes in the composition of the denitrifier population. Because extreme reductions in pH and inhibition by NO_3^- did not occur, the transient factors increasing denitrification are the best explanation for the reduced NO_3^- accumulation.

Isotopic Dilution

Twenty minutes after adding the isotope, mass recovery of $^{15}NO_3^-$ averaged above 90% (Table A-5). Results of the isotopic dilution showed that nitrification rates were very low in planted bottles compared to unplanted bottles ($p<0.0001$, Table 3-2). The GLM is shown in Appendix E (Table E-23). Planted bottles had much lower rates of NO_3^- production, which could be caused by lower O_2 concentrations and competition between plants and nitrifiers for NH_4^+. Problems with N concentrations in planted bottles throughout the study may have further limited the growth of nitrifiers in planted bottles. Levels of N became very low in planted bottles due to a miscalculation in the nutrient solution recipe. A more detailed description of the problem is contained in Appendix D.

Production of NO_3^- was not affected by pH ($p=0.78$); however, there was a significant interaction between planting and inoculation ($p=0.034$). Inoculation increased NO_3^- production in unplanted bottles, but had no effect in planted bottles. Rate of NO_3^- production in non-inoculated bottles is most likely due to contamination by daily maintenance during the 50-d experiment. As solution levels and pH were maintained in

Table 3-2. Effect of inoculation on NO_3^- production. All values are the average of four replicates ± 1 standard deviation. Numbers followed by the same letter are not significantly different.

Treatments		pH 7.0 NO_3^- Production ($\mu mol\ L^{-1}\ d^{-1}$)	pH 5.8 NO_3^- Production ($\mu mol\ L^{-1}\ d^{-1}$)
Planted	Inoculated	29 ± 36 a	91 ± 74 a
	Not inoculated	65 ± 58 a	48 ± 7 a
Unplanted	Inoculated	300 ± 130 b	380 ± 130 b
	Not inoculated	240 ± 120 b	160 ± 79 b

Table 3-3. Comparison of net NO_3^- production calculated by isotopic dilution and NO_3^- accumulation in unplanted bottles. Each rate is the average of four replicates. NO_3^- accumulation rates are based on the steepest slope of the lines in Figure 3-3.

	pH 7.0 isotope ($\mu mol\ L^{-1}\ d^{-1}$)	pH 7.0 NO_3^- accum. ($\mu mol\ L^{-1}\ d^{-1}$)	pH 5.8 isotope ($\mu mol\ L^{-1}\ d^{-1}$)	pH 5.8 NO_3^- accum. ($\mu mol\ L^{-1}\ d^{-1}$)
Inoculated	**262**	**250**	**220**	**280**
Not inoculated	**240**	**260**	**160**	**280**

the bottles, solution carryover could easily contaminate non-inoculated bottles. The net rate of NO_3^- production in unplanted bottles calculated by isotopic dilution (gross production – consumption) correlated with all but one of the rates calculated by NO_3^- accumulation before adding isotope (Table 3-3).

Rate of NO_3^- consumption was significantly higher in planted bottles ($p=0.0002$, Table 3-4). Measured rates of consumption include all avenues of NO_3^- loss. Although plant uptake likely accounts for most of the consumption, microbial assimilation and denitrification can also contribute to loss of NO_3^-. The observed high levels of NO_3^- consumption in some of the unplanted bottles were unexpected because conditions in

Table 3-4. Effect of inoculation on NO_3^- consumption. All values are the average of four replicates ± 1 standard deviation. Numbers followed by the same letter are not significantly different.

Treatments		pH 7.0 NO_3^- consumption ($\mu mol\ L^{-1}\ d^{-1}$)	pH 5.8 NO_3^- consumption ($\mu mol\ L^{-1}\ d^{-1}$)
Planted	**Inoculated**	**400 ± 110 a**	**130 ± 120 a**
	Not inoculated	**330 ± 170 a**	**270 ± 96 a**
Unplanted	**Inoculated**	**38 ± 98 b**	**160 ± 200 b**
	Not inoculated	**-41 ± 110 b**	**-48 ± 110 b**

these bottles were maintained to minimize NO_3^- consumption. Because there were no plants in the bottles, consumption must be due to be microbial activity or some other physical/chemical processes. The most likely explanation for high rates of NO_3^- consumption is that the unplanted bottles somehow became anaerobic or had anaerobic microsites. Inoculation and pH had no significant effect on NO_3^- consumption. The GLM is shown in Appendix E (Table E-24).

SUMMARY AND CONCLUSIONS

Based on data from ^{15}N dilution and NO_3^- accumulation, inoculation was effective in unplanted bottles, and rate of NO_3^- production was not affected by pH. Inoculation may have been effective early in the study in planted bottles, but because of N limitations in those bottles, nitrifier population activity may have been reduced to ineffective levels. These results contrast with those of Padgett and Leonard (1993), who suggested that nitrification is a significant problem in high-NH_4^+ systems. Rates of nitrification may eventually account for significant rates of NO_3^- production, but the lag time may be long. If nitrification in planted systems can be improved, inoculation of nitrifiers may be very useful in ALS systems.

Results from this study are based on inoculation with pure cultures. Inoculating with a mixed culture, such as that used for wastewater treatment, may improve nitrification in hydroponic systems. Future studies should account for the N problems faced in this experiment. Aeration levels should also be increased in planted bottles to ensure that anaerobic conditions are not the cause of NO_3^- loss. Isotopic dilution should also be conducted early in the life cycle before cultural conditions are difficult to

maintain in the solution. Using larger solution volumes to increase nutrient and pH buffering capacity may also make maintenance easier.

REFERENCES

Allison, S.M., and J.I. Prosser. 1993. Ammonia oxidation at low pH by attached populations of nitrifying bacteria. Soil Biol. Biochem. 25:935-941.

ATCC. 2000a. Media formulations [Online]. Available at http://phage.atcc.org/cgi-bin/searchengin...cg?view=media,1200386&text=ATCC+%26+929 (verified 4 Dec. 2000).

ATCC. 2000b. Media formulations [Online]. Available at http://phage.atcc.org/cgi-bin/searchengin...cg?view=media,1057564&text=ATCC+%26+480 (verified 4 Dec. 2000).

Lang, H.J., and G.C. Elliott. 1991. Influence of ammonium:nitrate ratio and nitrogen concentration on nitrification activity in soilless potting media. J. Am. Soc. Hortic. 116: 642-645.

Norton, J.M., and M.K. Firestone. 1996. N dynamics in the rhizosphere of *Pinus ponderosa* seedlings. Soil Biol. Biochem. 28:351-362.

Padgett, P.E., and R.T. Leonard. 1993. Contamination of ammonium-based nutrient solutions by nitrifying organisms and the conversion of ammonium to nitrate. Plant Physiol. 101:141-146.

Paul, E.A., and F.E. Clark. 1996. Soil microbiology and biochemistry. 2nd ed. Academic Press, San Diego, CA.

Stark, J.M. 2000. Nutrient transformations. p. 215-234. *In:* O.E. Sala, R.B. Jackson, H.A. Mooney, and R. Howarth (eds). Methods in Ecosystem Science. Springer-Verlag, New York.

Stark, J.M., and S.C. Hart. 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci. Soc. J. 60:1846-1855.

CHAPTER 4

NUTRIENT ANALYSIS

INTRODUCTION

Monitoring the concentration of nutrients in solution is helpful in assessing plant health and quantifying nutrient use. Real-time measurements of nutrient concentration are essential to managing a recirculating root-zone solution. Methods used to analyze nutrient concentration should be accurate, easy, quick, and inexpensive.

OBJECTIVES AND HYPOTHESES

The main objective of this study is to evaluate several methods of nutrient analysis for hydroponic solution. Specifically, the objectives were to:

1. Quantify the accuracy of the Lamotte Smart Colorimeter for NO_3^-, PO_4, SO_4^{2-}, SiO_2, and Fe.

   Hypothesis: The colorimeter will consistently measure the above nutrients accurately.

2. Quantify the accuracy of the Hach Cl titrimetric test kit.

   Hypothesis: The Hach test kit will accurately measure Cl.

COLORIMETER

A colorimeter is a simplified spectrophotometer. The LaMotte Smart Colorimeter evaluated here has several colored filters to facilitate analysis at six different wavelengths (420 nm, 460 nm, 510 nm, 530 nm, 570 nm, and 605 nm). The colorimeter comes pre-programmed with 42 precalibrated tests for LaMotte reagent systems. The colorimeter

costs $1,000 to $2,000 depending on the number of test kits purchased. Colorimetric tests not included can be programmed. The colorimeter is useful for small numbers of samples and when rapid analysis is required, but exact procedures must be followed to get good results. The test range for many of the kits is too low for hydroponic solutions, and dilutions can contribute to errors. Most colorimetric procedures are subject to matrix and inter-element interferences (Jones et al., 1991). Although the precalibration provided with the colorimeter may speed up analysis, a calibration curve made with similar matrix as the samples may improve results. The following sections describe the evaluation of the test kits. Solutions were made with known quantities of the nutrient to be tested and compared to measured values.

Nitrate

The cadmium reduction method is used with the colorimeter. Powdered cadmium is used to reduce NO_3^- to NO_2^-. Reduced NO_2^- and NO_2^-, originally present in the sample, are determined by diazotization of NO_2^- and sulfanilamide followed by coupling with N-(1 naphthyl)-ethylenediamine dihydrochloride to form a pink-purple dye, which is measured colorimetrically. This method is described in the *Standard Methods for the Examination of Water and Wastewater* (Clesceri et al., 1998), but the cadmium reduction occurs in a glass column packed with cadmium-coated copper granules, and the sample is analyzed in a spectrophotometer at 543 nm.

If nitrite is present in the sample, NO_3^- concentration is artificially inflated because the NO_3^- is reduced and measured as NO_2^-. Strong oxidizers and reducers also interfere. High concentrations of iron and copper in samples may cause low results.

This test is fairly complicated and time consuming (~15 min per sample). Several, small volumes must be accurately measured at several points during the test. Due to the low range (0-3 ppm NO_3^--N, 0-0.214 m*M* NO_3^-), samples usually have to be diluted. Because of the many volume measurements, there are many opportunities for mistakes. However, with care this test method can be accurate. When samples do not have to be diluted, it is much easier to get accurate results (Table 4-1).

The Lachat auto-analyzer can also be used to test for NO_3^- in solution samples. The Lachat also uses the cadmium reduction method, with the potential for the same interferences as the colorimeter. But, as described in *Standard Methods for the Examination of Water and Wastewater* (Clesceri et al., 1998), the reduction of NO_3^- takes place in a column packed with copper-cadmium granules. A standard curve is made of six NO_3-N concentrations processed through the Lachat. This method is used by the USU Analytical Laboratory. The method can be difficult to use because standards and reagents have to be made accurately before each use and the instrumentation can cost $15,000-$50,000 (Jones et al., 1991). However, the automation is excellent for running many samples at one time.

Table 4-1. Tests of NO_3^- colorimeter analysis method. Measured NO_3^- is the average of the replicate tests ± one standard deviation.

Actual [NO_3^-] (m*M*)	Number of replicates	Average measured [NO_3^-] (m*M*)	Range of % error
6	6	9.68 ± 3.87	26 to130
3	12	3.15 ± 0.27	2 to 53
0.1	1	0.107	7
0.098	1	0.098	0
0.02	1	0.022	10

A NO_3^- selective electrode develops a potential across a thin, porous membrane that holds a water-immiscible liquid ion exchanger in place. The electrode responds to NO_3^- activity rather than concentration so background ionic strength must be held constant by adding excess SO_4^{2-} to samples and standards. Interferences include high amounts of Cl^-, HCO_3^-, NO_2^-, CN^-, S^{2-}, Br^-, I^-, ClO_3^-, and ClO_4^-. Electrostatic "noise" from other electronic equipment, such as pumps and controllers, can also interfere with the signal from the electrode. Care should be taken to avoid electrostatic interferences from such equipment. The NO_3^- electrode costs $550, and replacement membranes are $60. It is most useful in situations requiring continuous monitoring, or when immediate analysis is necessary.

Phosphate

Phosphate is determined with the vanadomolybdophosphoric acid method, which has a range of 0 to 0.84 m*M* PO_4 (0 to 80 ppm). In acid conditions, orthophosphate reacts with ammonium vanadomolybdate to form vanadomolybdophosphoric acid, which is yellow in color. Negative interferences are caused by the presence of arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, and thiocyanate. This test is relatively simple and takes approximately 5 min to complete. Because concentrations in nutrient solutions are relatively low, dilutions are usually unnecessary and good results are easy to obtain (Table 4-2).

Sulfate

The colorimeter test uses the barium chloride method. In an acid medium with

Table 4-2. Results from tests of the phosphate colorimetric method. Measured PO_4 is the average of the replicate tests ± one standard deviation.

Actual [PO_4] (m*M*)	Number of replicates	Average measured [PO_4] (m*M*)	Range of % error
0.5	27	0.51 ± 0.11	0 to 12

barium chloride, a barium sulfate suspension is formed. The precipitate is white, and suspension absorbance is proportional to sulfate present. The test has a range of 0 to 1.04 m*M* SO_4^{2-} (0 to 100 ppm). Suspended particles and silica concentrations above 500 mg/L will interfere.

The test is relatively simple. Only a small amount of barium chloride powder has to be added to 10 mL of sample. The test requires about 5 min to complete. Because the test range is so large, dilutions are rarely required. However, it has been difficult to obtain accurate results with this method (Table 4-3).

Silica

Silica forms a complex with ammonium molybdate to form a yellow color. The test range is 0 to 1.25 m*M* SiO_2 (0 to 75 ppm). Interferences are caused by high concentrations of iron and sulfide. Because of the large test range, dilutions are usually not necessary. However, this test is fairly complicated and requires three reagents. Good

Table 4-3. Evaluation of sulfate test kit. Measured SO_4^{2-} is the average of the replicate tests ± one standard deviation.

Actual [SO_4^{2-}] (m*M*)	Number of replicates	Average measured [SO_4^{2-}] (m*M*)	Range of % error
0.5	9	0.77 ± 0.15	10 to 98
0.25	21	0.29 ± 0.09	0 to 63

Table 4-4. Evaluation of silica test kit. Measured SiO_2 is the average of the replicate tests ± one standard deviation.

Actual [SiO_2] (m*M*)	Number of replicates	Average measured [SiO_2] (m*M*)	Range of % error
0.1	21	0.22 ± 0.13	-30 to 400

results have been difficult to obtain with this method (Table 4-4), perhaps because normal nutrient solution concentrations are very low.

Iron

In the iron-bipyridyl test, ferric iron is reduced to ferrous iron, which then forms a colored complex with bipyridyl. The test range is 0 to 0.09 m*M* Fe (0 to 5 ppm). Interferences include strong oxidizers, copper, and cobalt in excess of 5 ppm. This test is relatively simple and requires about 5 min to complete. Accurate results have been difficult to obtain in nutrient solutions (Table 4-5). Much of the iron in a nutrient solution is chelated, which may account for inaccurate results. In addition, iron concentrations in nutrient solutions are very low.

HACH CHLORIDE TEST KIT

The Hach Cl^- is a titrimetric method for quantifying Cl^- concentration. The Hach chloride test kit uses K_2CrO_3 as the indicator and $AgNO_3$ as the titrant. At the endpoint

Table 4-5. Evaluation of iron test kit. Measured Fe is the average of the replicate tests ± one standard deviation.

Actual [Fe] (μ*M*)	Number of replicates	Average measured [Fe] (μ*M*)	Range of % error
7.5	15	8.3 ± 2.4	-60 to 60

the color of the solution changes from yellow to red-brown. The kit has a low-range method (0-100 mg $Cl^- L^{-1}$) and a high-range method (100-400 mg $Cl^- L^{-1}$). This method is very effective for testing both nutrient solution and plant tissue samples. The kit was evaluated on several aqueous extractions of plant tissue with known Cl^- concentrations (Table 4-6).

Table 4-6. Evaluation of Hach Cl^- test kit use with plant tissue extractions. The measured concentration is the average of two replicates ± one standard deviation.

Known concentration (%)	Average measured concentration (%)	% Error
0.2	0.2 ± 0	0
0.66	0.7 ± 0.018	6
0.93	1.0 ± 0.035	7
1.49	1.6 ± 0	7

SUMMARY AND CONCLUSIONS

The LaMotte Smart Colorimeter is very useful in situations requiring rapid turn-around with few samples. The colorimeter is also very affordable compared to electrodes and auto-analyzers. However, many of the colorimeter methods require considerable experience and are difficult to obtain accurate results. At this time, only the NO_3^- and PO_4 tests consistently produce accurate results with the colorimeter. Perhaps with more time and practice the other test kits could be more reliable. However, methods requiring that much practice may not be useful for researchers and growers.

REFERENCES

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton (eds). 1998. Standard methods for the examination of water and wastewater treatment. 20th ed. American Public Health Association, Washington, DC.

Jones, J.B., B. Wolf, and H.A. Mills. 1991. Plant analysis handbook: a practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing, Athens, GA.

CHAPTER 5

SUMMARY AND CONCLUSIONS

There was no significant effect of high NH_4^+ on wheat biomass production. Contrary to much of the literature, high NH_4^+ did not significantly reduce yield, but harvest index was slightly reduced. High NH_4^+ also increased N content in seeds. High N is correlated with increased protein content, which has important nutritional implications.

Counter ion (Cl^- vs. SO_4^{2-}) had no significant on yield, harvest index, biomass, or Ca in plant tissue. Cross contamination of the nutrient solutions may account for some of the lack of difference between the high Cl^- and high SO_4^{2-} treatments.

Based on data from ^{15}N dilution and NO_3^- accumulation, inoculation was effective in unplanted bottles. Inoculation may have been effective early in the study in planted bottles, but because of N limitations in those bottles nitrifier populations may have been reduced to ineffective levels. If nitrification in planted systems can be improved to match unplanted hydroponic systems, it has the potential to provide significant amounts of NO_3^- in high-NH_4^+ systems.

All of the nutrient analysis methods evaluated here are applicable in different situations. The Lachat auto-analyzer is most appropriate for large numbers of samples run at the same time. The NO_3^- electrode is best suited for continuous sampling of solution. The LaMotte Smart Colorimeter is very useful for testing NO_3^- and PO_4 in situations requiring rapid turn-around with few samples, but is not yet effective with Fe, Si, or SO_4^{2-}.

These studies have significant implications for the NASA Advanced Life Support Program. This research demonstrates that wheat can be grown in high NH_4^+ conditions

with no significant effect on yield and increased grain protein. However, growing wheat under such conditions can require intensive management, and Ca uptake is still inhibited. Nitrification in hydroponic systems has the potential to provide enough NO_3^- to alleviate problems associated with growing plants in 100% NH_4^+. If nitrification could be established in hydroponic plant systems, no NH_4^+ would have to be converted to NO_3^- when waste material is recycled in ALS. Inoculating plant systems would also provide functional redundancy to the nitrification currently done in microbial bioreactors that recycle plant and human waste in ALS systems.

These studies established an effective method to study long-term effects of high NH_4^+/NO_3^- ratios on crops. Future work for ALS should quantify the effects of high NH_4^+ on other ALS candidate crops such as lettuce, soybean, and rice. Research should also evaluate the effects of 100% NH_4^+ on growth and yield. To alleviate the management difficulties with high NH_4^+, further research with nitrifier inoculation in hydroponic culture is recommended.

APPENDICES

APPENDIX A. SUPPLEMENTAL FIGURES AND TABLES

Table A-1. The effect of NH_4^+ treatment on vegetative biomass, yield, and harvest index. All data are means of two trials ± one standard deviation.

	Veery-10	USU-Line 10
Total Biomass (kg m^{-2})		
Low NH_4^+	**4.59 ± 0.29**	**4.33 ± 0.55**
High NH_4Cl	**4.92 ± 0.25**	**4.67 ± 0.18**
High $(NH_4)_2SO_4$	**4.47 ± 0.91**	**4.99 ± 0.71**
Yield (kg m^{-2})		
Low NH_4^+	**2.30 ± 0.01**	**2.29 ± 0.28**
High NH_4Cl	**2.36 ± 0.13**	**2.23 ± 0.02**
High $(NH_4)_2SO_4$	**2.13 ± 0.52**	**2.45 ± 0.38**
Harvest Index (%)		
Low NH_4^+	**50.2 ± 3**	**53.0 ± 0.3**
High NH_4Cl	**48.0 ± 0.1**	**47.9 ± 1.2**
High $(NH_4)_2SO_4$	**47.6 ± 1.8**	**49.0 ± 0.5**

Table A-2. The effect of NH_4^+ treatment on heads per m^2, seeds per head, and seed mass. All data are means of two trials ± one standard deviation.

	Veery-10	USU-Line 10
Heads per m^2		
Low NH_4^+	3171 ± 305	2922 ± 3.5
High NH_4Cl	3105 ± 350	2942 ± 19.4
High $(NH_4)_2SO_4$	3043 ± 263	3330 ± 124
Seeds per Head		
Low NH_4^+	19 ± 1.4	20 ± 1.41
High NH_4Cl	20 ± 2.1	17 ± 0
High $(NH_4)_2SO_4$	18 ± 1.4	18 ± 0
Seed Mass (mg)		
Low NH_4^+	38.2 ± 1.1	39.2 ± 2.8
High NH_4Cl	39.2 ± 1.8	45.1 ± 0.4
High $(NH_4)_2SO_4$	38.3 ± 2.2	41.2 ± 4.7

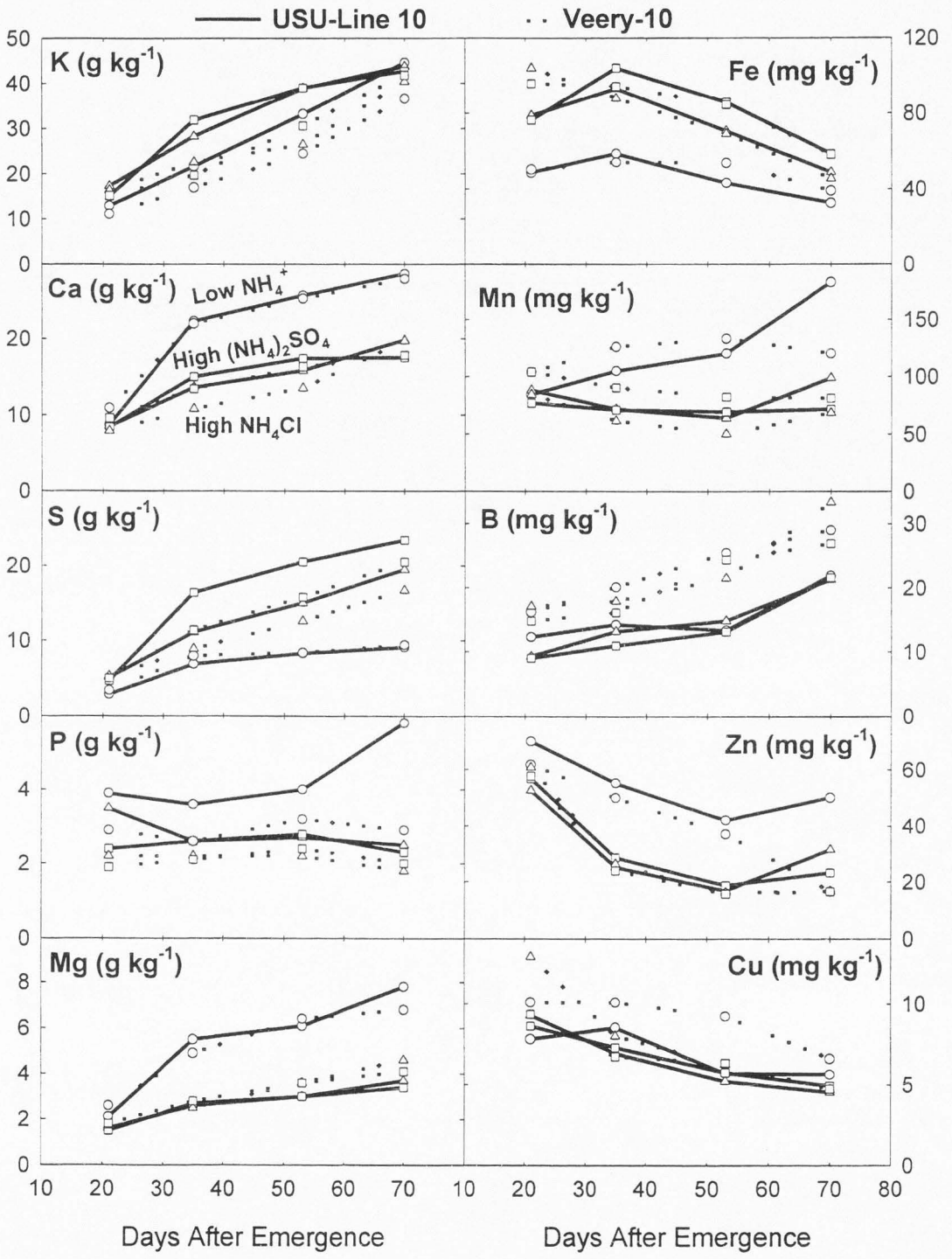

Fig. A-1. The effect of NH_4^+ treatment on flag leaf content of 10 nutrients in Trial 1. Macro-nutrients are displayed in the left column as g kg^{-1}. Micro-nutrients are shown in the right column as mg kg^{-1}. o represents the low NH_4^+ treatment, Δ represents the high NH_4Cl treatment, and represents the high $(NH_4)_2SO_4$ treatment.

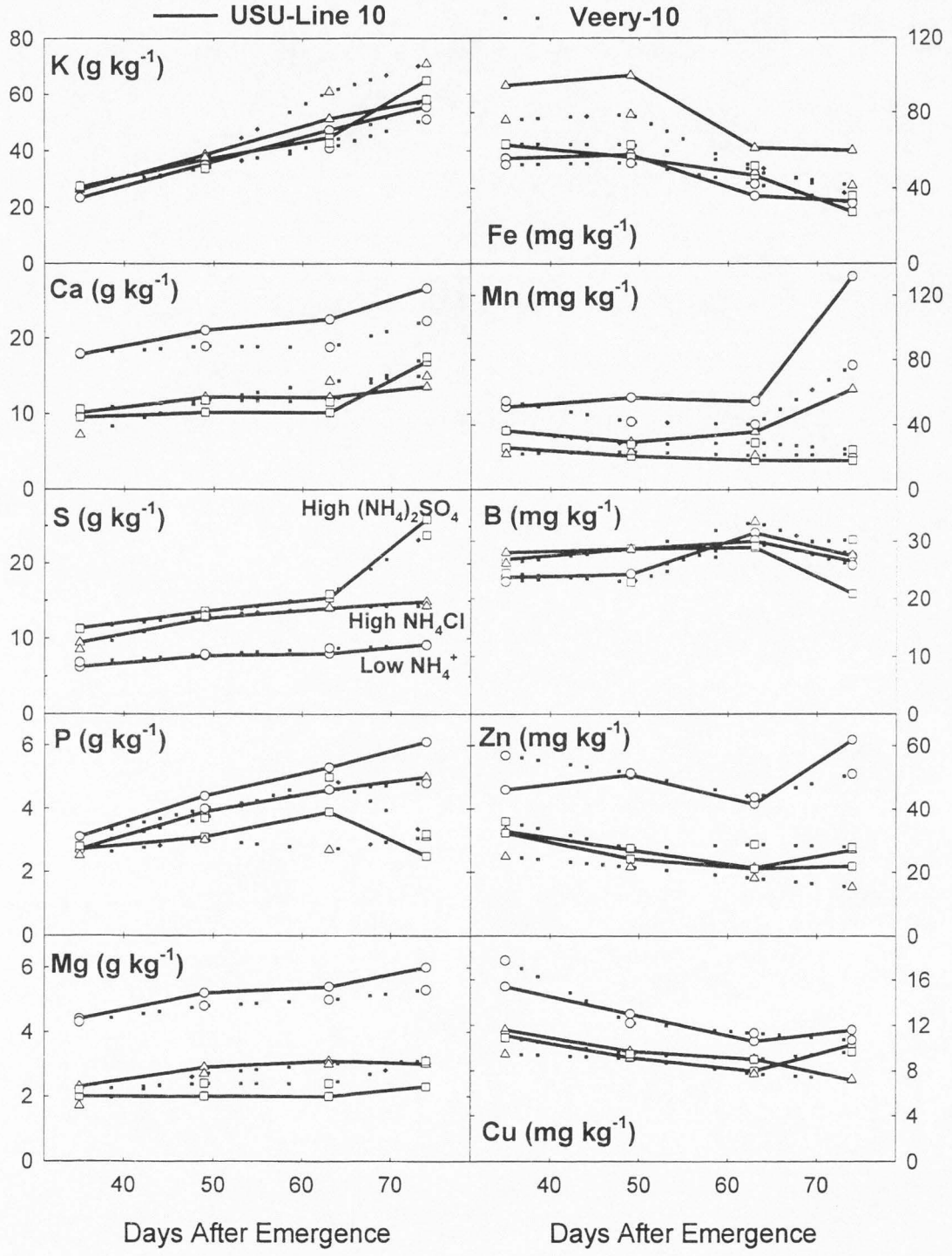

Fig. A-2. The effect of NH_4^+ treatment on flag leaf content of 10 nutrients in Trial 2. Macro-nutrients are displayed in the left column as g kg^{-1}. Micro-nutrients are shown in the right column as mg kg^{-1}. o represents the low NH_4^+ treatment, Δ represents the high NH_4Cl treatment, and represents the high $(NH_4)_2SO_4$ treatment.

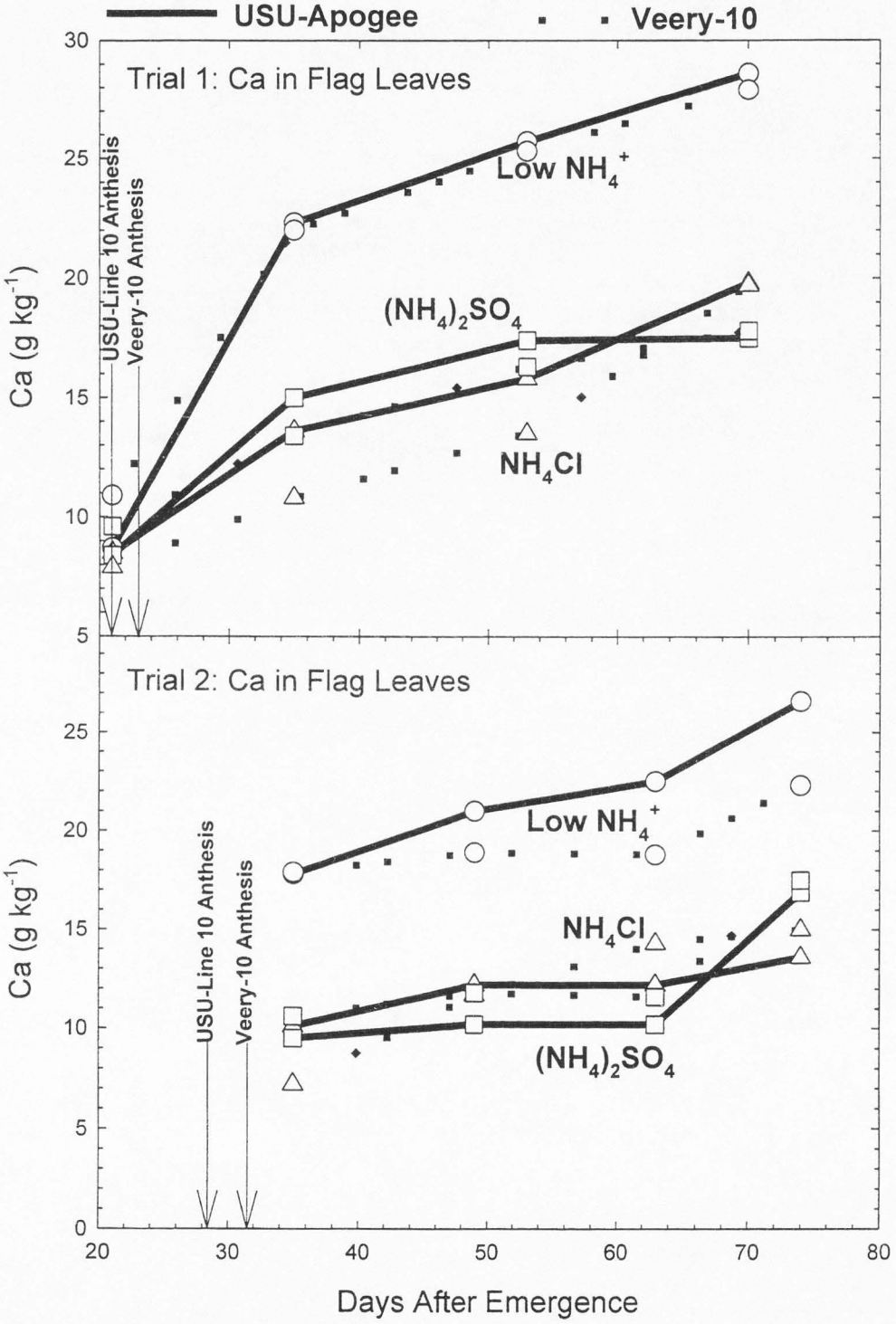

Fig. A-3. The effect of NH_4^+ treatment on flag leaf Ca content in Trials 1 and 2. ○ represents the low NH_4^+ treatment, Δ represents the high NH_4Cl treatment, and represents the high $(NH_4)_2SO_4$ treatment.

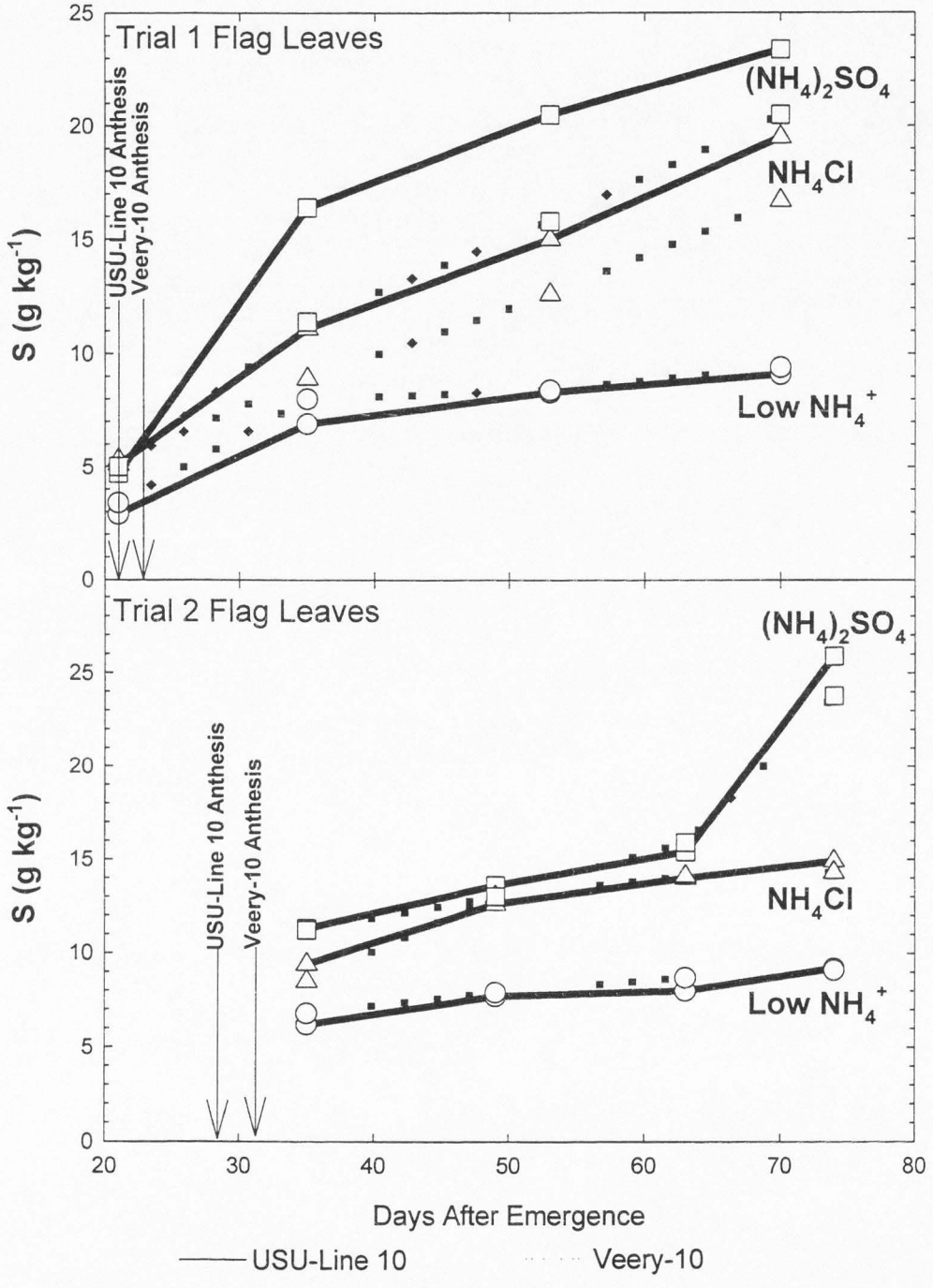

Fig. A-4. The effect of NH_4^+ treatment on flag leaf S content. o represents the low NH_4^+ treatment, Δ represents the high NH_4Cl treatment, and represents the high $(NH_4)_2SO_4$ treatment.

Table A-3. Calculation of total N in plant tissue for Trial 1.

Total N in Plant Tissue for Trial 1								
Treatment	Cultivar	Veg. Biomass (g)	N in Biomass (%)	Biomass N (mmoles)	Seed Mass (g)	N in Seed (%)	Seed N (mmoles)	Total N in Tissue (mmoles)
Low NH_4^+	USU-Line 10	1887	1.44	1940	996	2.74	1949	3890
Low NH_4^+	Veery-10	1756	1.36	1706	923	2.18	1437	3144
Total		**3643**		**3646**	**1919**		**3386**	**7034**
High NH_4Cl	USU-Line 10	1652	2.07	2443	790	3.66	2065	4509
High NH_4Cl	Veery-10	2040	1.93	2812	982	3.23	2266	5080
Total		**3692**		**5255**	**1772**		**4331**	**9589**
High $(NH_4)_2SO_4$	USU-Line 10	2198	2.35	3690	1088	3.64	2829	6521
High $(NH_4)_2SO_4$	Veery-10	2044	2.05	2993	1000	3.15	2250	5243
Total		**4242**		**6683**	**2088**		**5079**	**11764**

Table A-4. Calculation of total N in plant tissue for Trial 2.

Total N in Plant Tissue for Trial 2								
Treatment	Cultivar	Veg. Biomass (g)	N in Biomass (%)	Biomass N (mmoles)	Seed Mass (g)	N in Seed (%)	Seed N (mmoles)	Total N in Tissue (mmoles)
Low NH_4^+	USU-Line 10	738	1.21	638	839	2.40	1438	2076
Low NH_4^+	Veery-10	1003	1.32	946	915	2.55	1667	2615
Total		**1741**		**1584**	**1754**		**3105**	**4691**
High NH_4Cl	USU-Line 10	1003	1.60	1146	897	3.22	2063	3211
High NH_4Cl	Veery-10	992	1.27	900	906	3.24	2097	2998
Total		**1995**		**2046**	**1803**		**4160**	**6209**
High $(NH_4)_2SO_4$	USU-Line 10	919	1.54	1011	873	3.42	2133	3145
High $(NH_4)_2SO_4$	Veery-10	821	1.86	1091	706	3.26	1644	2738
Total		**1740**		**2102**	**1579**		**3777**	**5883**

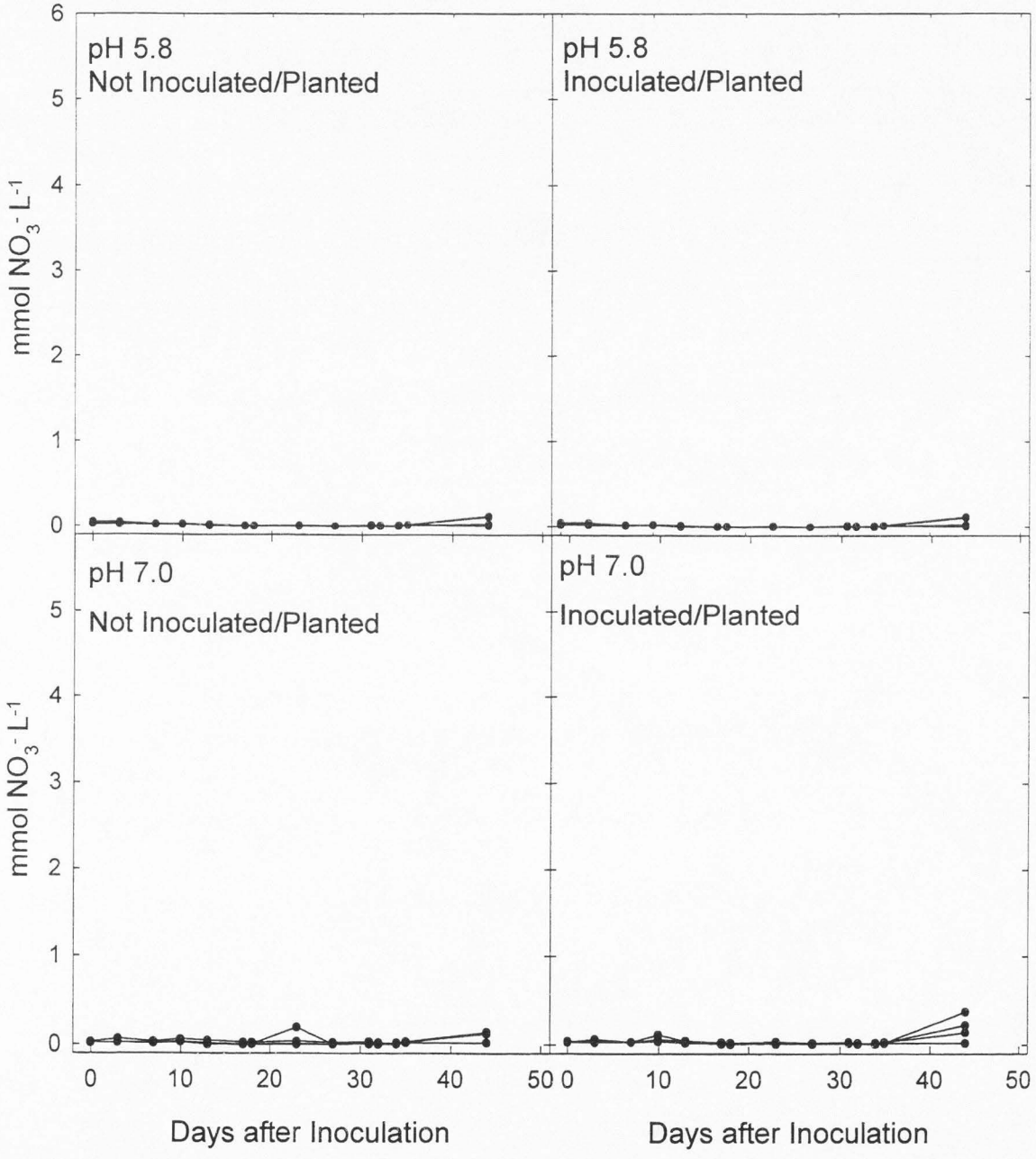

Fig. A-5. Nitrate accumulation in planted bottles before isotopic dilution. Each line is from one replicate bottle. Nitrate was measured with an ion-selective electrode.

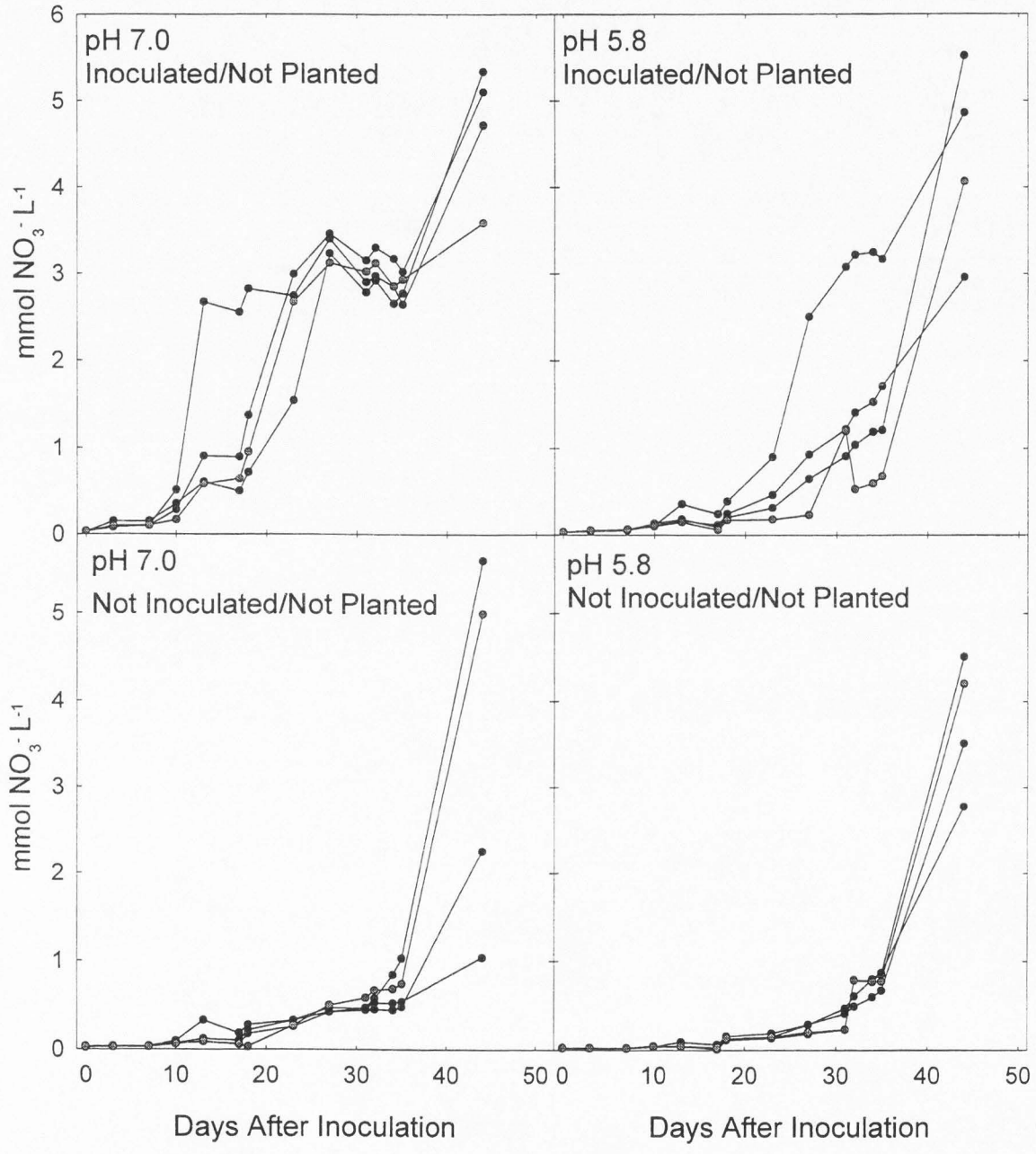

Fig. A-6. Nitrate accumulation in unplanted bottles before isotopic dilution. Each line is from one replicate. Nitrate was measured with an ion-selective.

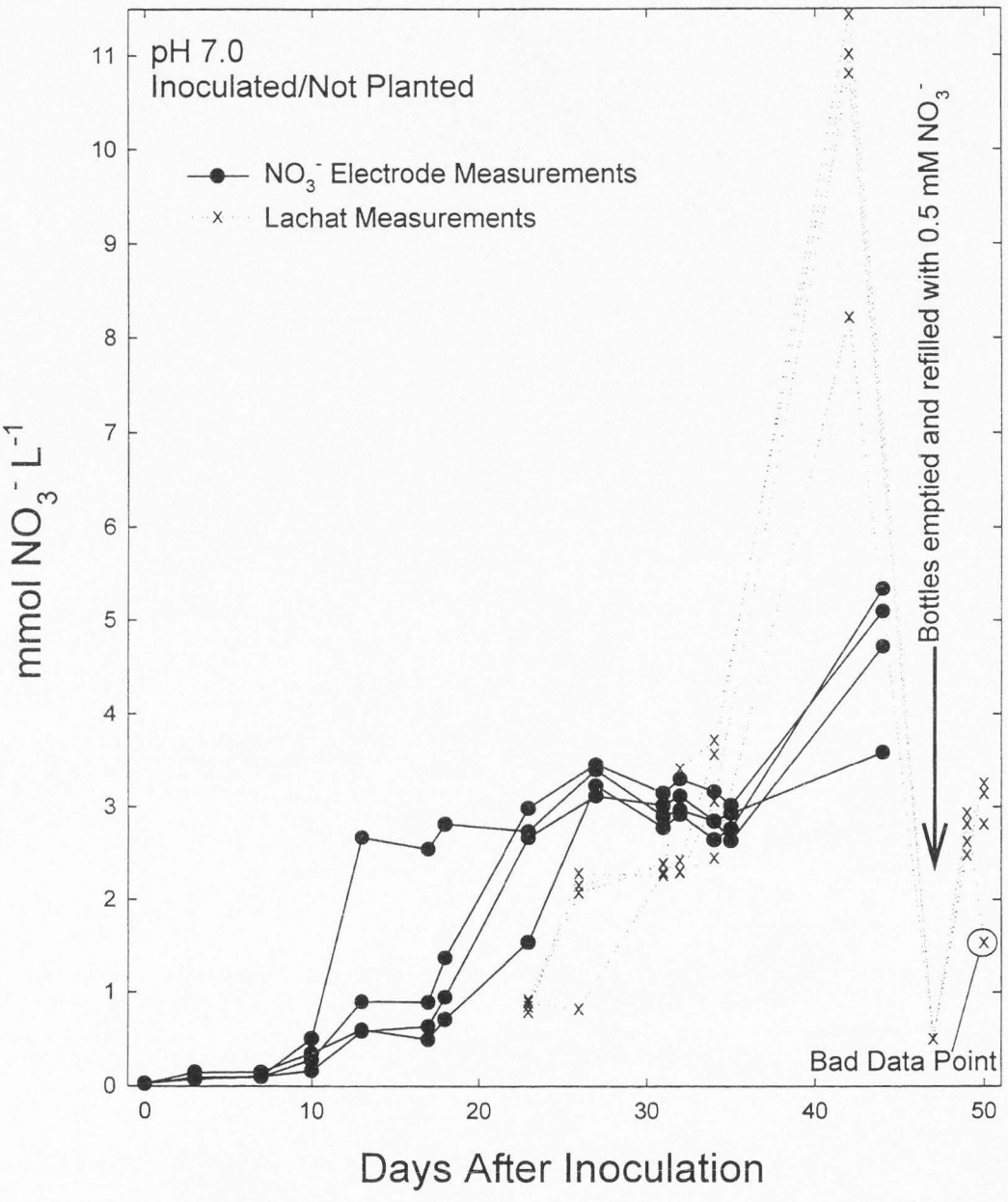

Fig. A-7. NO_3^- accumulation in pH 7, unplanted, and inoculated bottles.

Table A-5. Recovery of $^{15}NO_3^-$ 20 minutes after adding isotope to bottles.

			Recovery at t_0 (%)				Average Recovery (%)
pH 5.8	Planted	Inoculated	85	82	92	103	91
		Non-Inoculated	97	93	96	98	96
	Unplanted	Inoculated	95	111	97	96	100
		Non-Inoculated	93	90	94	94	93
pH 7.0	Planted	Inoculated	84	52	95	92	81
		Non-Inoculated	81	98	85	89	88
	Unplanted	Inoculated	93	97	**	101	97
		Non-Inoculated	92	91	95	99	94
					Total Average Recovery		92

** Indicates missing data.

APPENDIX B. CHELATE STUDIES

INTRODUCTION

Iron is required in plants at 50-100 mg kg^{-1} of plant dry weight (Marschner, 1995). Iron serves an important role in photosynthesis as a constituent of many enzymes in Photosystem I and II (Marschner, 1995). Although iron is abundant in soils, its availability is limited. In well-aerated systems, iron solubility depends on ferric oxides and the following reaction: $Fe(OH)_3 + 3H^+ \Leftrightarrow Fe^{3+} + H_2O$ (Kochian, 1991). Plants use two basic strategies to obtain iron from deficient systems. Dicotyledons and non-graminaceous plants are known as Strategy I plants, which increase release of protons and reducants into the rhizosphere in response to iron deficiency (Marschner, 1995). Graminaceous monocots, Strategy II plants, release phytosiderophores into the rhizosphere to increase iron uptake (Marschner, 1995).

Chelates help maintain iron in a form available for plant uptake. Metals bound in chelate rings essentially lose cation characteristics, which makes them less available for participation in chemical reactions (Wallace, 1989). Vaious chelates have different stabilities and bind metals at different strengths (See Table B-1).

Table B-1. Log of equilibrium constants of some metal chelates. The higher the value, the greater the stability (Wallace, 1989).

Metal Ion	EDTA	HEEDTA	DTPA	EDDHA
Fe^{3+}	25.1	19.8	28.6	33+
Ca^{2+}	10.7	8.1	10.9	7.2
Zn^{2+}	16.4	14.5	18.8	9.3
Cu^{2+}	18.7	17.4	21.5	>15
Mn^{2+}	13.9	10.7	15.6	?

Choosing the proper chelate is not based solely on the stability constant, but also on plant species and pH. For instance, grasses have difficulty removing iron from chelates with high stability so HEEDTA is often used (Wallace, 1989). Dicotyledons and

nongraminaceous plants appear to be able to remove iron from strong chelates by reducing the iron to Fe^{2+} and then absorbing it (Kochian, 1991). Evidence suggests that the dicot Fe^{3+} reduction is done by a plasma-membrane bound Fe(III) reduction system (Kochian, 1991). Different chelates are also effective at different pH ranges.

OBJECTIVES

The goal of these studies was to quantify the effects of different Fe-chelates on plant growth in hydroponic solution. Specifically, I was interested in evaluating the effect of EDDHA, EDTA, DTPA, and HEEDTA on chlorophyll content, biomass accumulation, and Fe concentration of soybean and wheat.

MATERIALS AND METHODS

Two experiments were conducted to evaluate the effect of different chelates in hydroponic culture. The first experiment was done in 12, 30-L tubs with a plant canopy. The second experiment was done with single plants in 2-L Nalgene bottles. Both experiments used Fe-HEEDTA, Fe-EDDHA, and Fe-EDTA; and the first experiment also used Fe-DTPA. These experiments were done to determine the best chelate for growing plants in high pH regimes.

Plant Community Study

Wheat plants were planted at a density of 500 plants m^{-2} in 12, 30-L tubs in a growth chamber. Ten high pressure sodium (HPS) lamps supplied a photosynthetic photon flux (PPF) of 1,100 $\mu mol\ m^{-2}\ s^{-1}$. The plants were grown with a 24-hr

photoperiod at 22° C. Plants were grown at pH 5.8, 6.5, or 7.0 with EDDHA, EDTA, HEEDTA, or DTPA (See Table B-2). There were two replicate treatments of HEEDTA at each pH and one replicate of all other treatments. All plants were grown with Fe-HEEDTA until 11 d after emergence, when solutions were replaced to begin chelate treatments.

Table B-2. Treatment arrangement for plant community chelate experiment.

EDDHA 7.0	**HEEDTA 5.8**	**EDTA 6.5**	**HEEDTA 5.8**
HEEDTA 6.5	**DTPA 6.5**	**HEEDTA 7.0**	**EDTA 7.0**
EDDHA 6.5	**DTPA 7.0**	**HEEDTA 7.0**	**HEEDTA 6.5**

Flag leaves were sampled for ICP analysis throughout the life cycle. Plants were harvested 22 d after emergence, and dry mass of roots and shoots were measured. Shoot tissue was also analyzed by ICP. A chlorophyll meter (Minolta, SPAD-502) was used to quantify plant color.

Single Bottle Study

Soybean (*Glycine max* (L.) Merr. cv. Hoyt) and wheat (*Triticum aestivum* (L.) cv. USU-Apogee) were grown in 2-L Nalgene bottles with hydroponic solution. Plants were germinated in Isolite, transferred to the 2-L bottles after emergence, and grown in a greenhouse with supplemental HPS lamps. The photoperiod was 16-h, and PPF was 650 $\mu mol\ m^{-2}\ s^{-1}$. Temperature in the greenhouse was maintained at 23° C. The nutrient solution composition is listed in Table B-3. Solution pH was stabilized with MES buffer

for pH 5.8, MOPs buffer for pH 7.0, and maintained with manual additions of HNO_3 or KOH as needed.

Table B-3. Nutrient solution composition for chelate studies.

		STARTER		VEGETATIVE GROWTH	
Salt	**Stock Conc**	**ml/100 L**	**Final Conc**	**ml/100 L**	**Final Conc**
$Ca(NO)_3$	1 M	100	1 mM	100	1 mM
KNO_3	2 M	50	1 mM	250	5 mM
KH_2PO_4	0.5 M	100	0.5 mM	250	1.25 mM
$MgSO_4$	0.25 M	200	0.5 mM	600	1.5 mM
K_2SiO_3	0.1 M	100	0.1 mM	100	0.1 mM
K_2SO_4	0.5 M	0	0 mM	100	0.5 mM
$FeCl_3$	50 mM	10	5 μM	3	1.5 μM
Fe-Chelate	100 mM	40	40 μM	10	10 μM
$MnCl_2$	60 mM	10	6 μM	15	9 μM
$ZnCl_2$	20 mM	30	6 μM	20	4 μM
H_3BO_3	20 mM	300	60 μM	300	60 μM
$CuCl_2$	20 mM	20	4 μM	20	4 μM
Na_2MoO_4	1 mM	10	0.1 μM	10	0.1 μM

Three chelates and three pH treatments were applied to nine wheat and nine soybean plants. Three soybean and wheat plants were treated with Fe-EDDHA, Fe-HEEDTA, or Fe-EDTA. The nutrient solution in three bottles of each treatment were then maintained at pH 5.8 (standard) or pH 7.0, with the third bottle beginning at pH 5.8 and elevated to pH 7.0 on Day 10 (Table B-4).

Table B-4. Single-bottle treatment scheme for chelate study.

	pH 5.8	**pH 5.8→7.0**	**pH 7.0**
EDDHA	Soybean & Wheat	Soybean & Wheat	Soybean & Wheat
HEEDTA	Soybean & Wheat	Soybean & Wheat	Soybean & Wheat
EDTA	Soybean & Wheat	Soybean & Wheat	Soybean & Wheat

A chlorophyll meter (Minolta, SPAD-502) was used to indirectly measure chlorophyll by quantifying plant color. At the end of the study, total biomass dry weight was measured. Geochem, a chemical equilibrium model, was also run to predict availability of iron in the different treatments.

RESULTS AND DISCUSSION

Plant Community Study

The highest Fe concentration occurred in wheat plants grown at pH 5.8 with HEEDTA (Figure B-1a). At pH 6.5, plants grown with EDDHA or DTPA had the highest Fe content. At pH 7.0, DTPA produced the highest Fe concentration. HEEDTA plants had reduced dry mass at pH 7.0 (Figure B-1b).

Single-Bottle Study

Chelate and pH had little effect on soybean chlorophyll meter readings, although HEEDTA had the highest chlorophyll meter reading in all pH treatments (see Figure B-2a). HEEDTA produced much higher wheat chlorophyll readings in all pH ranges (See Figure B-2b). Because HEEDTA is not an effective chelate at high pH, it is surprising that chlorophyll was so high in the pH 7.0, HEEDTA treatment. Previous studies with wheat in this lab indicated that HEEDTA was ineffective at pH 7.0 because plants became extremely chlorotic.

During the study, soybean chlorophyll readings changed very little in all treatments (Figure B-3). In contrast, chlorophyll readings of wheat treated with EDDHA at pH 7.0 decreased as the study progressed (Figure B-3a). Chlorophyll readings of

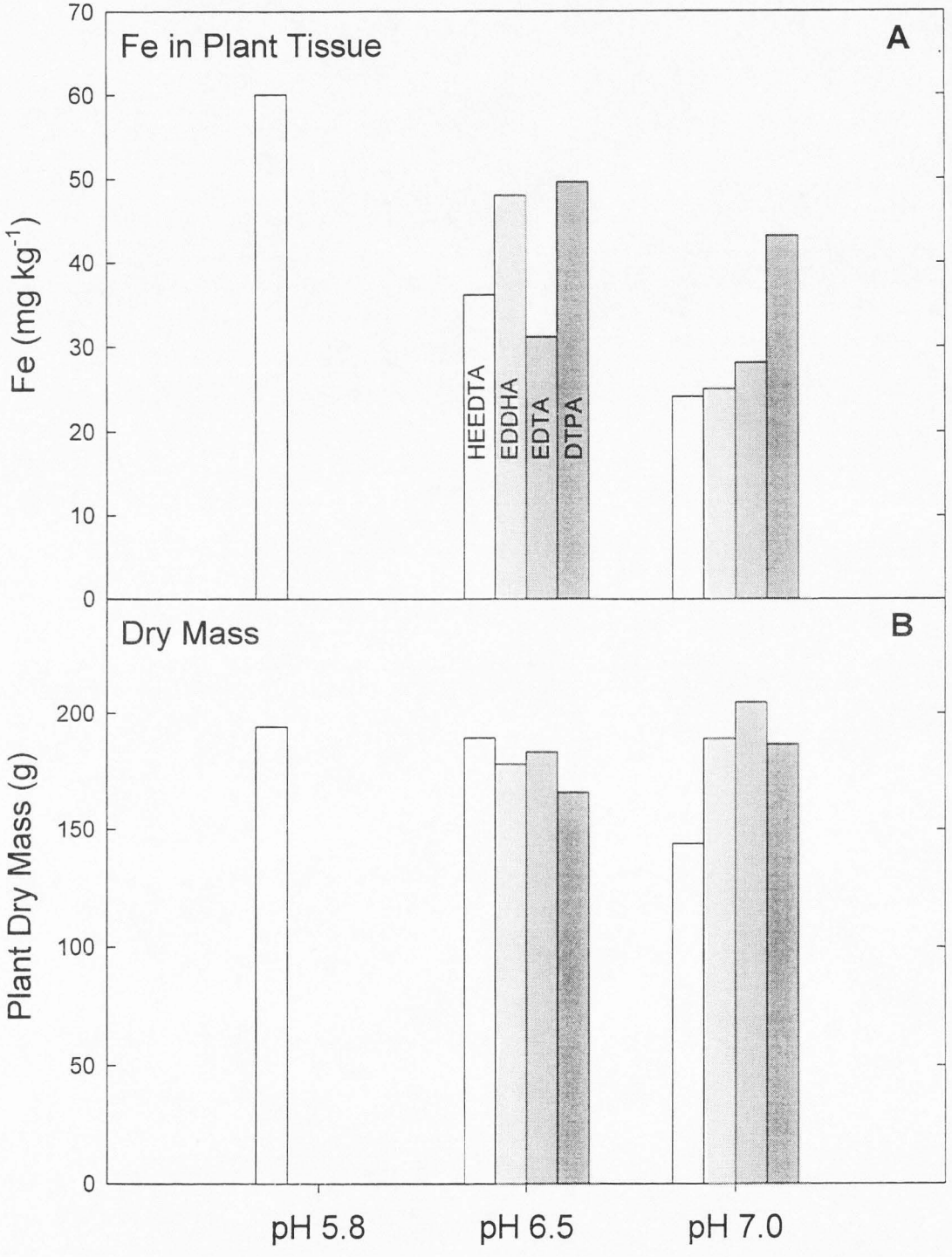

Fig. B-1. Iron content and plant dry mass in wheat plant community chelate study.

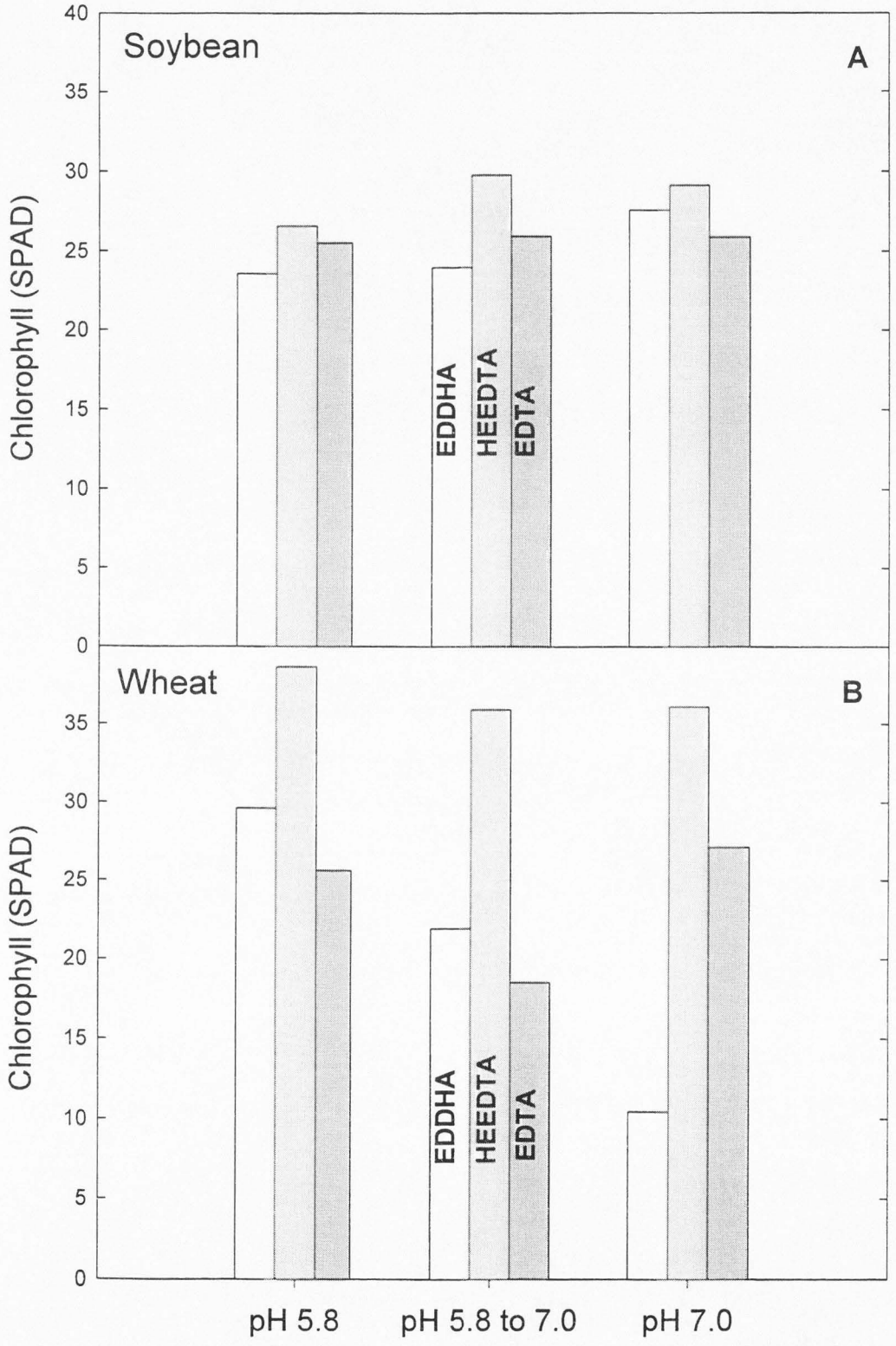

Fig. B-2. Chlorophyll meter readings (SPAD) averaged over time in single-bottle chelate study.

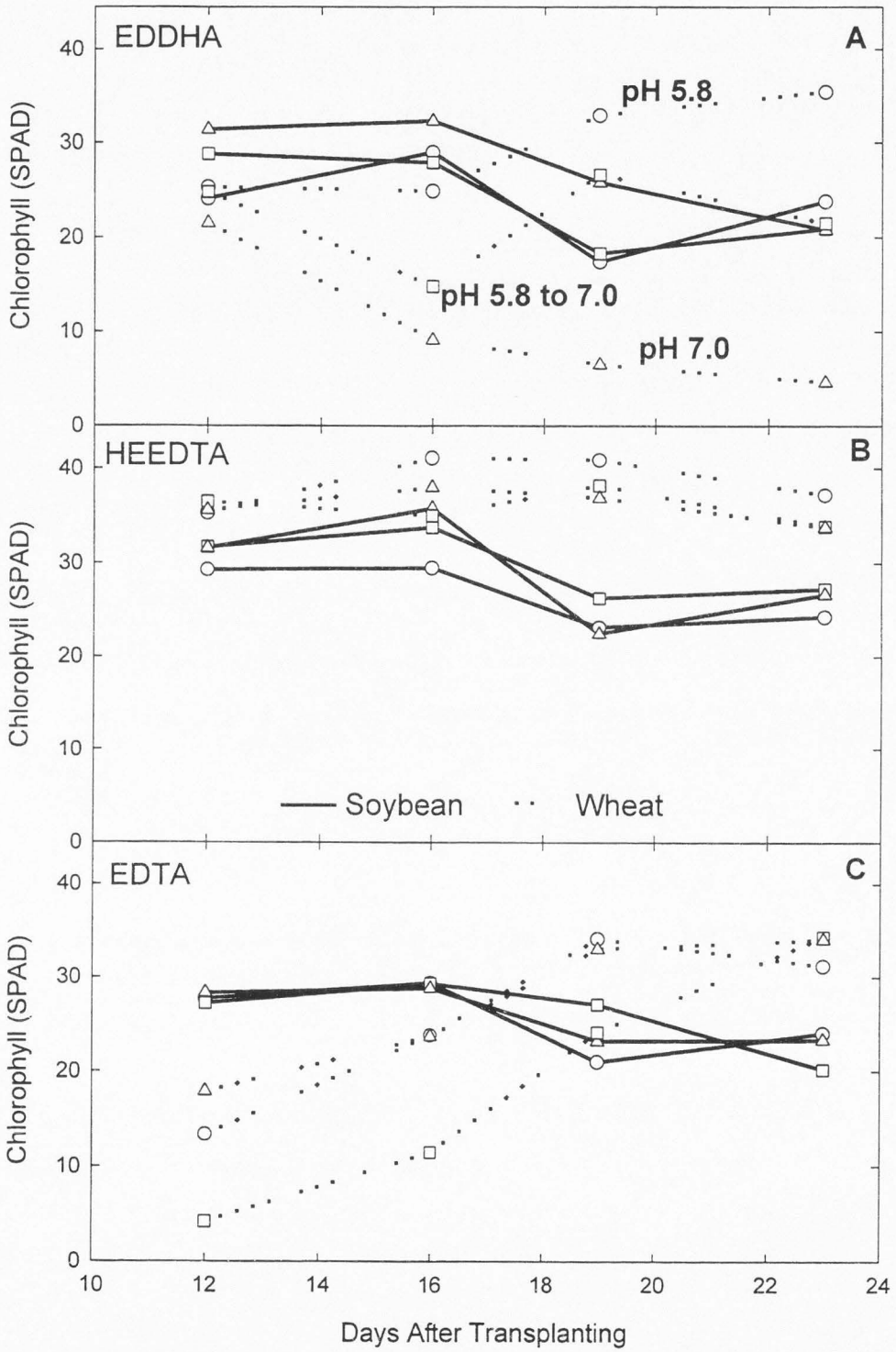

Fig. B-3. Chlorophyll meter readings in relation to time in the single-bottle chelate study. o represents plants at pH 5.8, Δ represents plants at pH 7.0, and represents plants started at pH 5.8 and shifted to pH 7.0

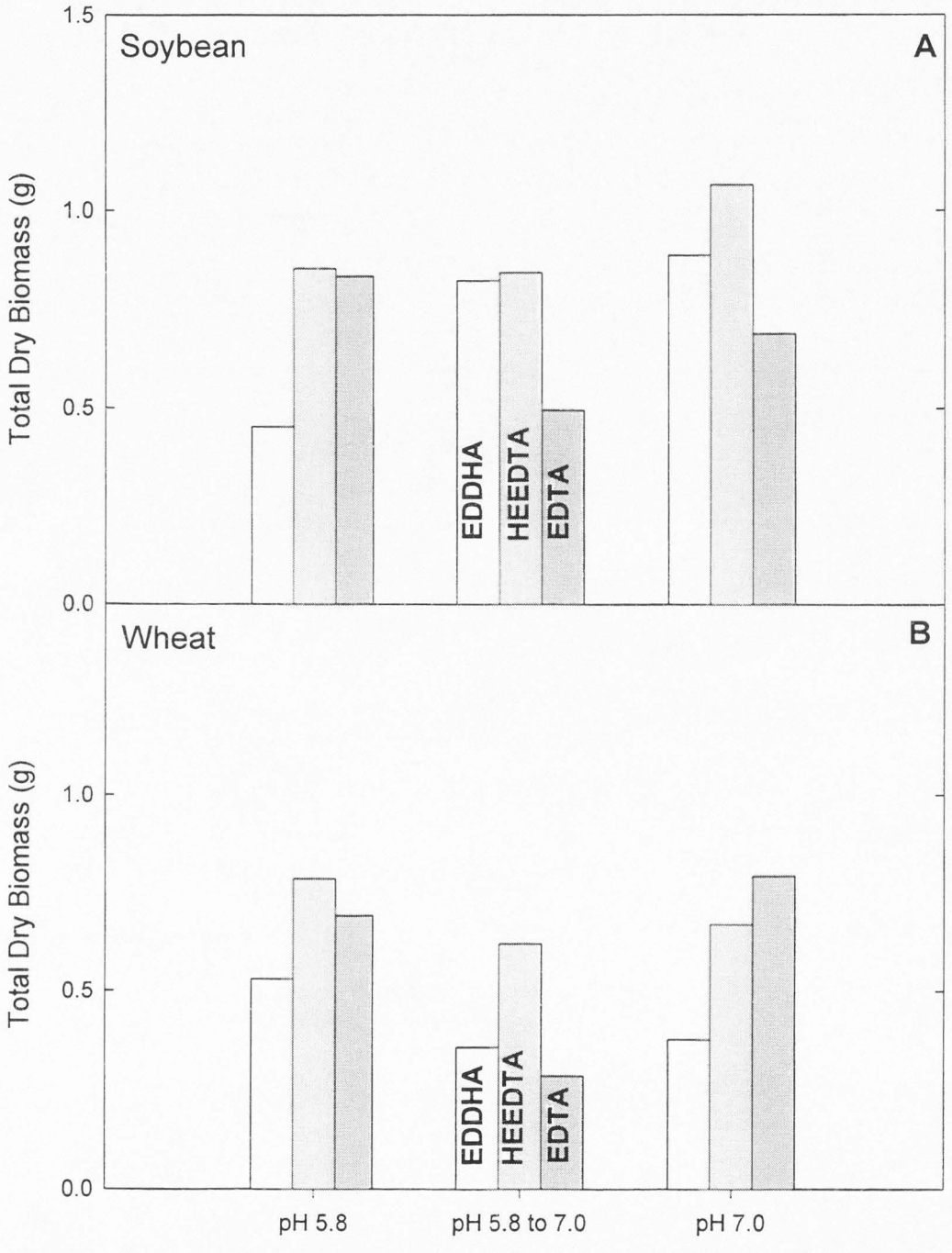

Fig. B-4. The effect of chelate and pH on total biomass of soybean and wheat plants in the single-bottle chelate study.

wheat treated with HEEDTA in all pH treatments remained constant (Figure B-3b). Chlorophyll increased with time in wheat treated with EDTA in all pH treatments (Figure C-3c). Increasing chlorophyll indicated that the plants were better able to acquire iron from EDTA as time progressed.

Soybean biomass was always highest with HEEDTA chelate, although the EDTA produced similar biomass at pH 5.8 and EDDHA had similar biomass at the other pHs (Figure B-4a). Wheat biomass was highest with HEEDTA at pH 5.8 and pH 5.8 to 7.0 (Figure B-4b). Wheat also had high biomass with EDTA at pH 5.8 and pH 7.0 (Figure B-4b).

Without replication, it is impossible to determine if these trends are significant. Preliminary studies with wheat canopies indicated that HEEDTA was ineffective at pH 7.0. Wheat grown at pH 7.0 with HEEDTA was more chlorotic and smaller in stature than wheat grown at the same pH in EDDHA or EDTA. However, the preliminary study was conducted in a high light environment with a 24-h photoperiod. The rapid growth rate from the high daily PPF may have induced Fe deficiency that was not present in the current study.

Results from the GEOCHEM-PC model are presented in Table B-5. The –log free concentration indicates the availability of the free ion in solution, the higher the number the less available the ion. Chelate had little effect on free concentration, but pH 7.0 reduced free concentration. Reduced availability at higher pH was probably due to increased Fe^{3+} bound in iron hydroxides. Less of the Fe was complexed with EDTA and HEEDTA at pH 7.0, which is probably due to reduced effectiveness of those chelates at higher pH. The % Fe complexed with HEEDTA is much lower than Fe complexed with

Table B-5. Iron free concentration and percent complexed with chelate from the GEOCHEM-PC model.

Chelate	-log Free Concentration		% Fe Complexed with Chelate	
	pH 5.8	pH 7.0	pH 5.8	pH 7.0
HEEDTA	14.720	17.184	62.1	49.2
EDTA	14.722	17.184	67.8	51.8
EDDHA	14.728	17.184	81.5	80.4

EDDHA. Because EDDHA has a much higher stability value with iron than HEEDTA, it has much more complexed Fe.

Visual differences between plants grown with different chelates were small. Soybean chlorosis could only be seen in the pH 5.8/EDDHA treatment, and the chlorosis consisted of a few light-colored mottles. Wheat had chlorosis in the pH 7.0/EDDHA and pH 5.8 to 7.0/EDTA treatments.

SUMMARY AND CONCLUSIONS

According to the results from the single bottle study, HEEDTA would be the most effective chelate for wheat at all pH levels evaluated in this study; however, in the community study, EDDHA was a better chelate. The combination of low stability values and low % Fe complexed makes HEEDTA a good choice for wheat. In contrast, EDDHA has high stability values and high complexed Fe, making Fe difficult to remove from the chelate. Either HEEDTA or EDDHA would be effective chelates for soybean. Soybean grew very well with HEEDTA at all pH regimes. As a dicot, soybean can also effectively remove iron from EDDHA.

When choosing a chelate, complexes formed between the chelate and other metals must also be considered. Chelates also bind Zn, Cu, and Mn making those nutrients less

available for plant uptake. Much research with synthetic chelates remains to be done. Plant tissue concentrations of Fe, Zn, Cu, and Mn would be extremely useful in determining the most effective chelate and could be determined by ICP. Further replication of this study would also help characterize plant responses to different chelates. In addition, there are also several synthetic chelates (such as DTPA and HBED) and chelate alternatives (such as organic acids) that should be studied.

REFERENCES

Kochian, L.V. 1991. Mechanisms of micronutrient uptake and translocation in plants. *In:* Mortvedt, J.J., F.R. Cox, L.M. Sheman, and R.M. Welch (eds). Micronutrients in Agriculture, 2nd Ed. SSSA. Madison, WI.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, New York.

Wallace, A. 1989. Regulation of micronutrients and use of chelating agents in solution culture. HAS Proceedings 10th Annual Conference.

APPENDIX C. PRELIMINARY NITRIFICATION STUDIES

INTRODUCTION

Padgett and Leonard (1993) suggested that nitrification can cause the transformation of significant amounts of NH_4^+ to NO_3^- in hydroponic, sand, and aeroponic culture. This means that plants in high-NH_4^+ systems may be taking up transformed NO_3^- rather than NH_4^+. Here we report the results of two preliminary studies conducted to quantify rates of nitrification in hydroponic systems.

OBJECTIVES

The goal of this study was to quantify nitrification rates in hydroponic systems. Specific objectives were to:

1. Determine if nitrification occurs at significant rates in non-inoculated standard hydroponic culture at pH 5.8.
2. Determine if nitrification can be enhanced in hydroponics by elevating pH and/or inoculation with nitrifying bacteria.

MATERIALS AND METHODS

Both studies were conducted in a controlled growth room. Both experiments had treatments maintained at pH 5.8 or pH 7.0 and were inoculated with nitrifiers or not inoculated.

Plant Community Study

USU-Apogee wheat was grown in 12 independent 30-L tubs. Tubs were filled with nutrient solution and aerated. Eleven days after emergence, half of the tubs were

maintained at pH 5.8 and the other half at pH 7. Also on Day 11, half of the tubs at each pH were inoculated with *Nitrobacter winogradskyi* and *Nitrosomonas europea* (10% by volume). Nitrifier activity was estimated by nitrification potential on root samples.

Nitrification potentials were completed for root samples taken before inoculation on Day 11 and at the end of the experiment on Day 25. The method used for nitrification potentials followed that described by Hart et al. (1994). Root samples were placed in 250-mL Erlenmeyer flasks containing phosphate buffer and $(NH_4)_2SO_4$, to which $NaClO_4$ was added to prevent the conversion of NO_2^- to NO_3^-. The flasks were placed on a shaker to maintain aeration. The solution was sampled four times during a 24-h period. Samples were analyzed for NO_2^- with a Lachat autoanalyzer to estimate potential nitrification rates.

Single-Bottle Study

USU-Apogee was grown in 16, 2-L bottles. Half of the bottles were maintained at pH 5.8 and half at pH 7. Half of the bottles in each pH regime were inoculated with 33% inoculum of *Nitrobacter wynogradskyi* and *Nitrosomonas europea*. One unplanted bottle of each treatment served as a control. Nutrient solution contained only NH_4^+ as the N source. Solution NO_3^- concentration was monitored throughout the experiment to estimate nitrification. Wheat was harvested 38 days after transplanting to bottles. Unplanted, control bottles were maintained for another 40 days.

RESULTS AND DISCUSSION

Plant Community Study

Nitrification potentials before inoculation revealed consumption of NO_3^- rather than production (Figures C-1a and C-2a). After inoculation, no nitrification was observed during nitrification (Figures C-1b,c and C-2b,c). Because the nitrification potential does not measure gross production or consumption, this test was unable to provide definitive evidence of nitrification (or the lack of nitrification).

The mass of root samples used in the nitrification potential evaluation may have been too large. The large mass of roots could have supplied large amounts of carbon for heterotrophic microbes capable of denitrification. If large amounts of N were denitrified or heterotrophic organisms were competing with the nitrifiers, the nitrification potential would not be able to provide an accurate estimate of nitrification.

Single-Bottle Study

Plant and root dry mass were decreased in bottles that were not inoculated in both pH regimes (Figure C-3). Because plants were supplied N as 100% NH_4^+, the increased dry mass in inoculated bottles could be indirect evidence of nitrification. Nitrification would have supplied some of the N as NO_3^- and provided anions to counter-balance NH_4^+ uptake.

Unplanted, control bottles were maintained after plants were harvested. Working under the assumption that there would be no significant consumption in unplanted bottles, NO_3^- accumulation was measured to estimate nitrification rates. Solution in these

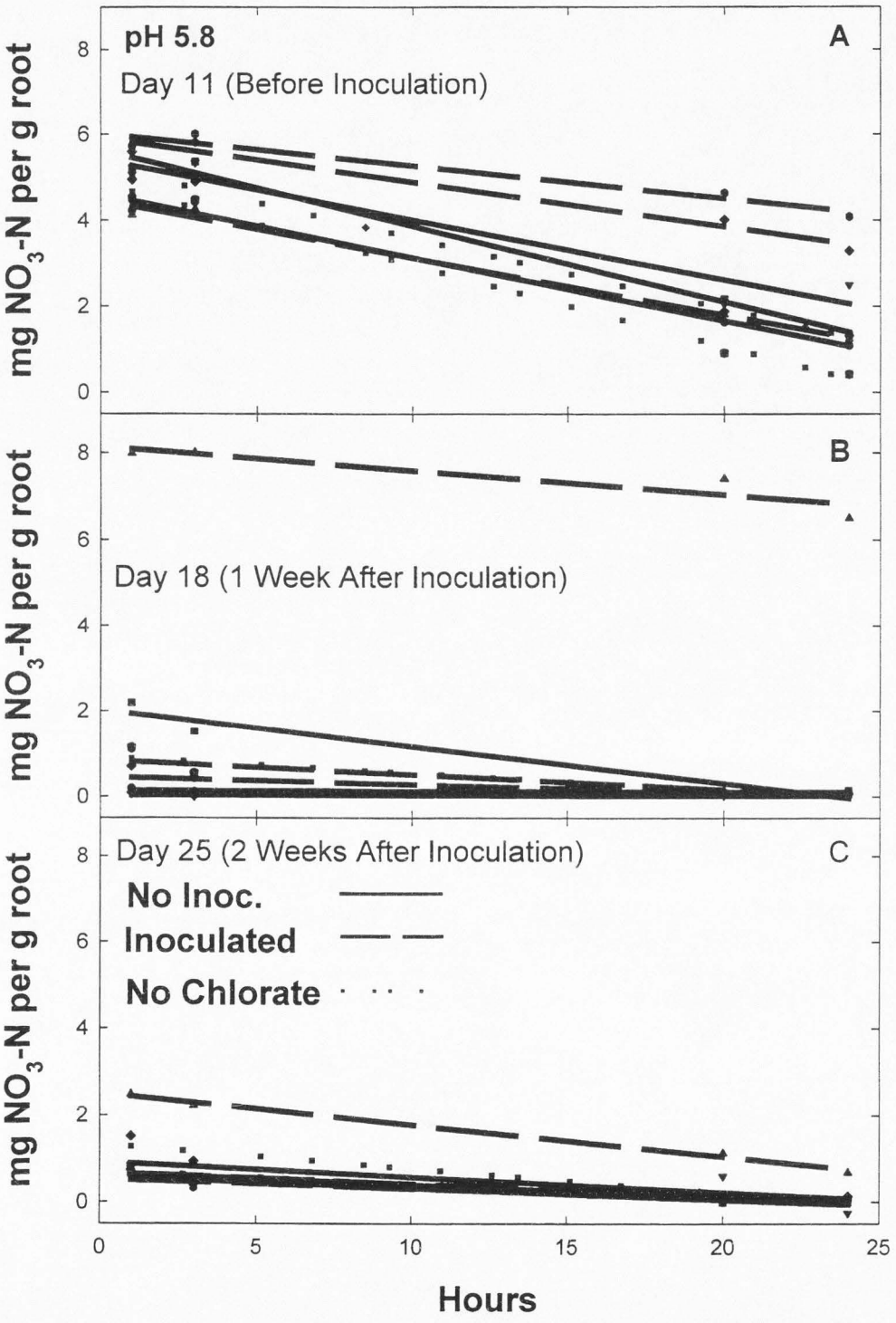

Fig. C-1. Potential nitrification in pH 5.8 treatments. Each line represents data from one tub.

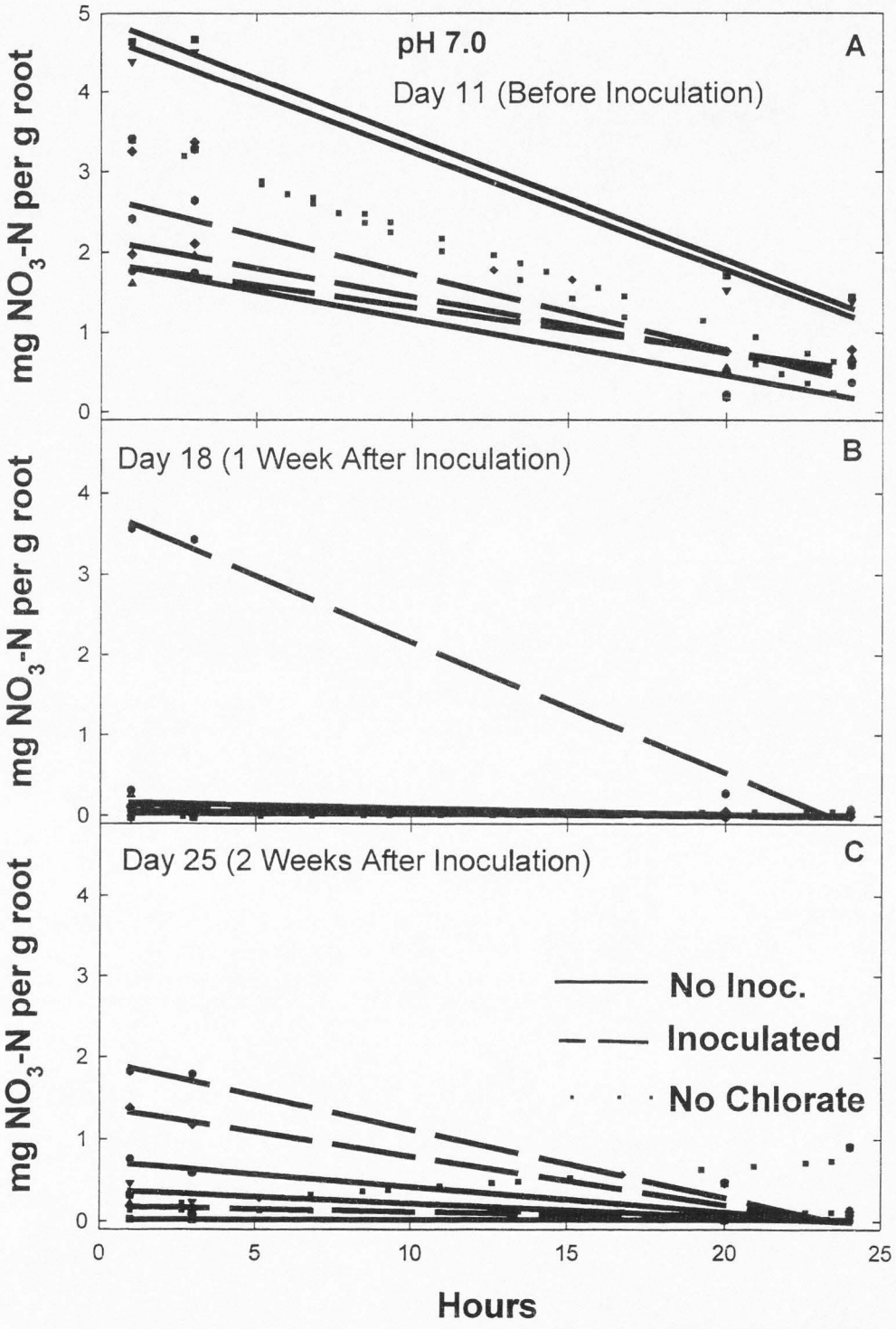

Fig. C-2. Potential nitrification in pH 7.0 treatments. Each line represents data from one tub.

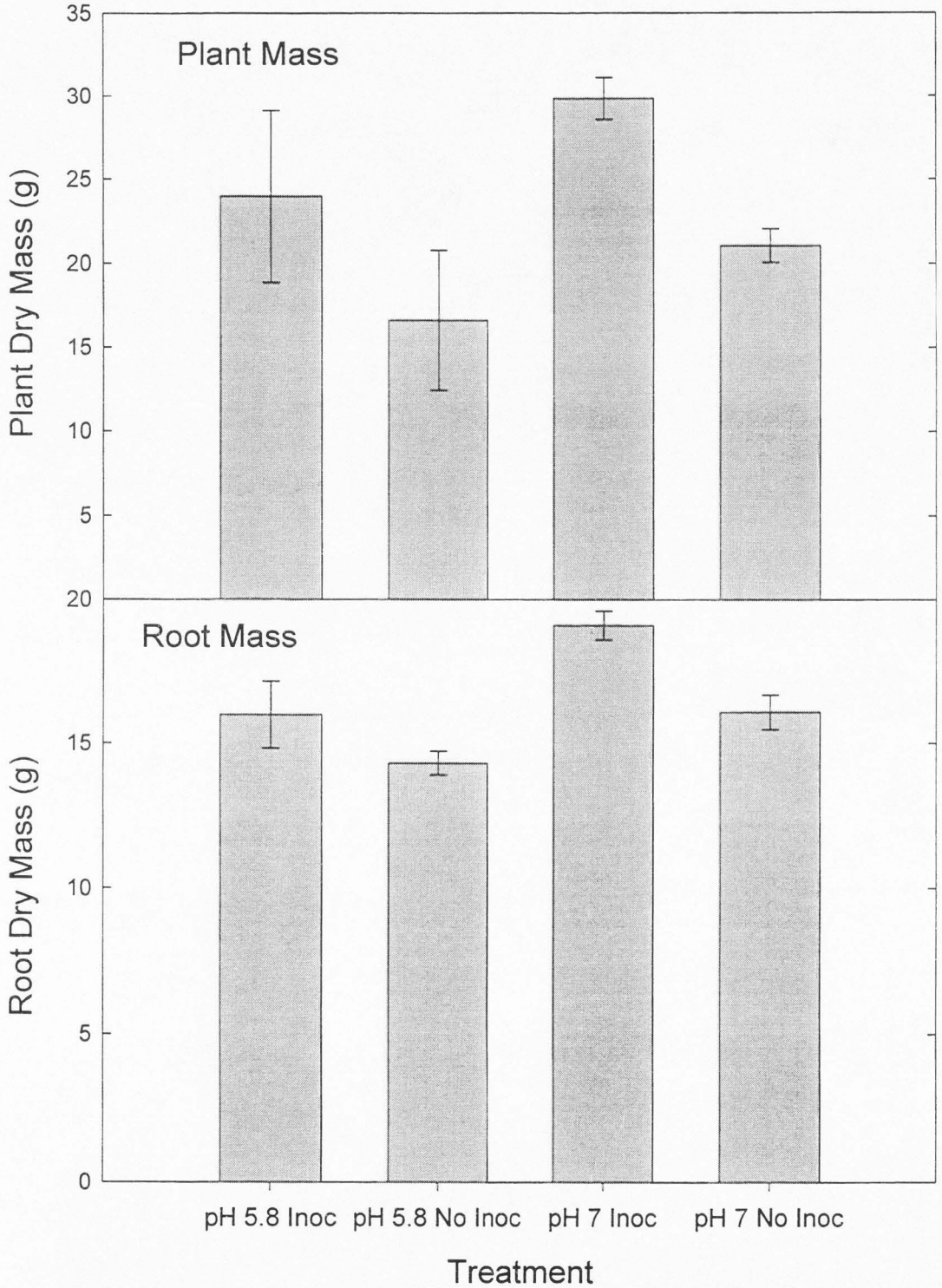

Fig. C-3. Dry plant and root mass of plants harvested 38 days after transplanting. Each bar is the average of four replicates and error bars are ± one standard deviation.

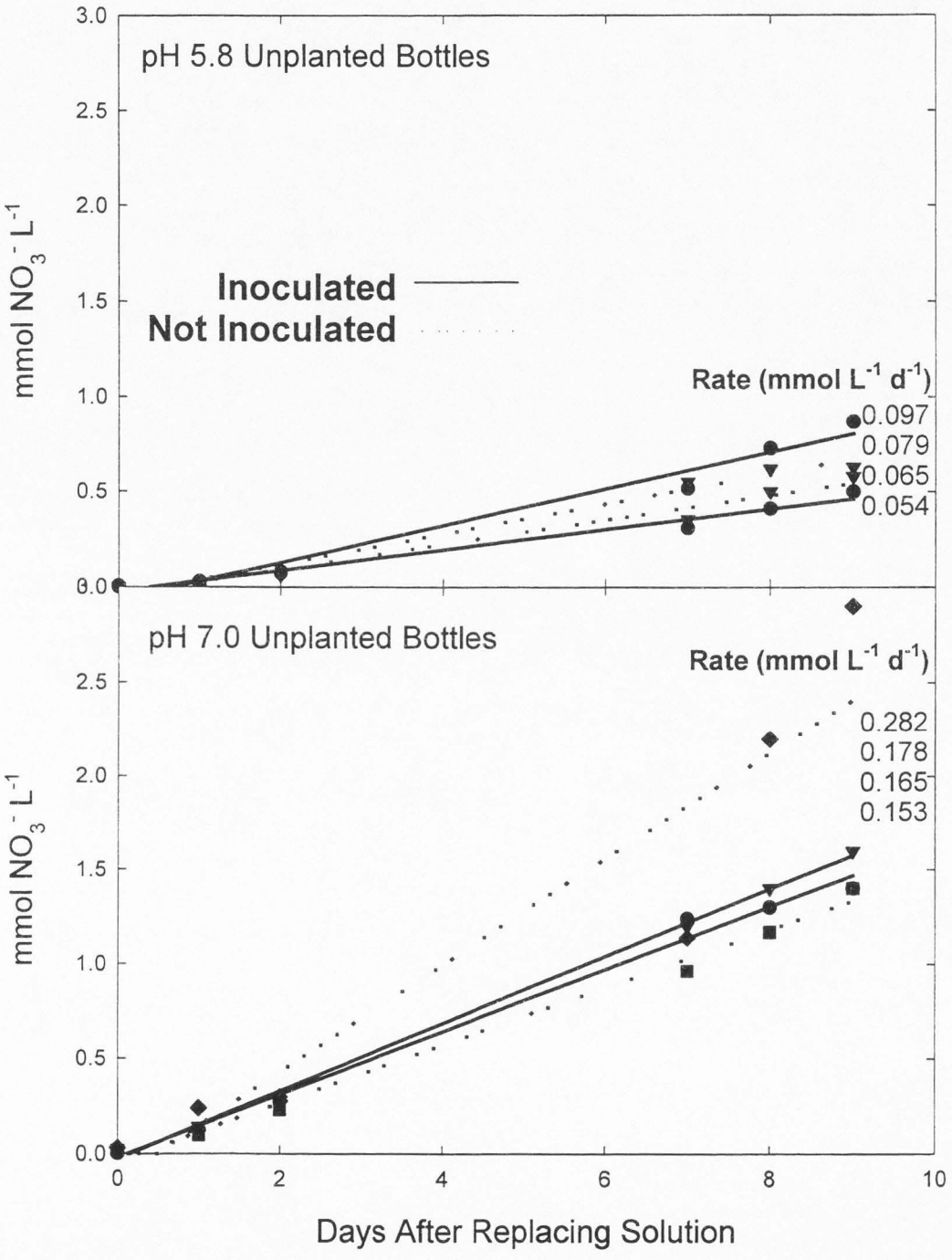

Fig. C-4. Nitrate accumulation in unplanted bottles.

bottles was periodically replaced to prevent inhibition of nitrification by NO_3^- accumulation. Non-inoculated and inoculated bottles had similar NO_3^- production rates (Figure C-4), probably due to contamination during the long study. Rates of NO_3^- accumulation were reduced at pH 5.8 compared to pH 7.0 (Figure C-4). Nitrification rates should be higher at pH 7 because it is in the optimum pH range.

Measurement of NO_3^- accumulation only allows the calculation of net NO_3^- production, that is, NO_3^- produced in excess of consumption. Gross production and consumption cannot be estimated from the measurements. In addition, plants can take up any NO_3^- produced by nitrifiers so rates of production cannot be quantified by measuring NO_3^- accumulation in the presence of plants.

SUMMARY AND CONCLUSIONS

In these experiments there was no way to eliminate NO_3^- consumption, so measurements of NO_3^- concentration was really a measurement of net NO_3^- production. To accurately quantify rates of NO_3^- production and consumption at the same time, an isotope method was required. These studies led to the isotopic dilution experiment described in Chapter 3. These two studies did provide circumstantial evidence of nitrification in hydroponic systems, but the results could not definitively quantify nitrification.

REFERENCES

Hart, S.C., J.M. Stark, E.A. Davidson, and M.K. Firestone. 1994. Nitrogen mineralization, immobilization, and nitrification. In: Methods of Soil Analysis Part 2-Microbiological and Biochemical Properties. R.W. Weaver, J.S. Angle, and P.S. Bottomley (eds). SSSA, Madison, WI.

Padgett, P.E. and R.T. Leonard. 1993. Contamination of ammonium-based nutrient solutions by nitrifying organisms and the conversion of ammonium to nitrate. Plant Physiol. 101:141-146.

APPENDIX D. EXTENUATING CIRCUMSTANCES

CONTAMINATION TESTS

Materials and Methods

Each of the three recirculating systems in the White Room were filled with 100 L of solution. The first system contained 5 m*M* Cl^- (as $CaCl_2$), the second DI water, and the third 5 m*M* SO_4^{2-} (as K_2SO_4). Solution samples from all three tanks were tested for Cl^- and SO_4^{2-} before the solution was recirculated, after starting recirculation, 1 h after the sampling system was started, and 23 h after the sampling system was started. Solution was also collected directly from the return tube during the 75-s lag time.

Results

Based on the data (Tables D-1 through D-5), the lag time is too long (back contamination). Calculations showed that the lag time was 11 to 14 s too long. Using the 14-s estimate, ~214 mmoles Cl^- would move from the high Cl treatment and ~176 mmoles SO_4 from the high SO_4 treatment during the yield trials. Those losses account

Table D-1. Contamination test before turning on recirculation pumps.

Treatment	Cl m*M* (ppm)	SO_4^{2-} m*M* (ppm)
Cl	5.6 (200)	0.07 (7)
DI	0.4 (15)	0.06 (6)
SO_4^{2-}	0.4 (15)	3.1 (300)

Table D-2. Contamination test after turning on recirculation pumps.

Treatment	Cl m*M* (ppm)	SO_4^{2-} m*M* (ppm)
Cl	5.6 (200)	0.06 (6)
DI	1.7 (60)	0.08 (8)
SO_4^{2-}	1.5 (55)	3.6 (350)

Table D-3. Contamination test 1 hour after turning on sampling system.

Treatment	Cl m*M* (ppm)	SO_4^{2-} m*M* (ppm)
Cl	5.6 (200)	0.07 (7)
DI	1.7 (60)	0.07 (7)
SO_4^{2-}	1.5 (55)	3.02 (290)

Table D-4. Contamination test 23 hours after turning on sampling system.

Treatment	Cl m*M* (ppm)	SO_4^{2-} m*M* (ppm)
Cl	5.6 (200)	0.07 (7)
DI	1.7 (60)	0.08 (8)
SO_4^{2-}	1.7 (60)	3.02 (290)

Table D-5. Contamination of solution sample from lag time in sampling system return lines.

Treatment	Cl m*M* (ppm)	SO_4^{2-} m*M* (ppm)
Cl	5.6 (200)	0.07 (7)
DI	1.7 (60)	0.54 (52)
SO_4^{2-}	2.25 (80)	2.71 (260)

for 30-100% of the missing Cl or SO_4 in the yield trials, but does not account for all of the mass balance error.

POSSIBLE N DEFICIENCY IN NITRIFICATION ^{15}N STUDY

A few weeks after plants were transferred to the 2-L bottles, leaves became chlorotic. In addition, any spike of NO_3^- was consumed within 24 h. We expected to see some NO_3^- remain in the bottles after 24 h because of the presence of NH_4^+ in the root zone. The plants should have taken up the NH_4^+ and left some NO_3^-.

Initially the plants were given an 8-h dark period because we thought the chlorosis was due to Ca deficiency caused by the high NH_4^+. However, the dark period had no effect, and the plants actually became more chlorotic.

On Day 17 all of the bottles were checked for NH_4^+ and NO_3^- content with a colorimeter and electrode. All of the planted bottles and a few of the unplanted bottles were extremely low in N. The plants were all N-stressed. The nutrient solution composition was developed as a NO_3^- recipe, and NH_4^+ was just substituted for the NO_3^-. Unfortunately, much of the N in the NO_3^- recipe actually comes from pH control in the form of HNO_3. When the NH_4^+ was substituted for the NO_3^-, the N from pH control was unaccounted for, which lead to N deficiency in the plants.

Upon discovery of the error, all unplanted bottles were given a 2m*M* spike of NH_4^+. Planted bottles were spiked with 4m*M* NH_4^+ and 1m*M* NO_3^-. Bottles were given excess N until the NO_3^- and NH_4^+ began to accumulate in the bottles, meaning the plants had recovered.

APPENDIX E. STATISTICAL ANALYSES

Table E-1. GLM for total biomass.

The GLM Procedure

Dependent Variable: Biomass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2807611.427	401087.347	1.56	0.2215
Error	15	3853590.270	256906.018		
Corrected Total	22	6661201.697			

R-Square	Coeff Var	Root MSE	Bio Mean
0.421487	10.87179	506.8590	4662.148

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	516349.266	258174.633	1.00	0.3894
cv	1	1072.319	1072.319	0.00	0.9493
trial	1	1506732.000	1506732.000	5.86	0.0286
trt*cv	2	729112.750	364556.375	1.42	0.2726
cv*trial	1	54345.092	54345.092	0.21	0.6522

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	582376.302	291188.151	1.13	0.3480
cv	1	6747.413	6747.413	0.03	0.8734
trial	1	1464770.767	1464770.767	5.70	0.0305
trt*cv	2	716347.024	358173.512	1.39	0.2784
cv*trial	1	54345.092	54345.092	0.21	0.6522

Table E-2. ANOVA for total biomass.

The ANOVA Procedure

Dependent Variable: Biomass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	660218.680	132043.736	0.44	0.8102
Error	6	1819766.870	303294.478		
Corrected Total	11	2479985.550			

R-Square	Coeff Var	Root MSE	Bio Mean
0.266219	11.82961	550.7218	4655.450

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	227528.1650	113764.0825	0.38	0.7023
cv	1	514.8300	514.8300	0.00	0.9685
trt*cv	2	432175.6850	216087.8425	0.71	0.5277

Table E-3. GLM for seed yield.

The GLM Procedure

Dependent Variable: seed yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	839684.759	119954.966	1.80	0.1613
Error	15	1001395.393	66759.693		
Corrected Total	22	1841080.152			

R-Square	Coeff Var	Root MSE	seed Mean
0.456083	11.24152	258.3790	2298.435

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	663.3799	331.6899	0.00	0.9950
cv	1	28932.6395	28932.6395	0.43	0.5203
trial	1	639975.6586	639975.6586	9.59	0.0074
trt*cv	2	169054.3125	84527.1562	1.27	0.3104
cv*trial	1	1058.7685	1058.7685	0.02	0.9015

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	4448.6641	2224.3320	0.03	0.9673
cv	1	36693.8617	36693.8617	0.55	0.4699
trial	1	606703.8127	606703.8127	9.09	0.0087
trt*cv	2	167321.8307	83660.9154	1.25	0.3138
cv*trial	1	1058.7685	1058.7685	0.02	0.9015

Table E-4. ANOVA for seed yield.

The ANOVA Procedure

Dependent Variable: seed yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	119772.2375	23954.4475	0.28	0.9067
Error	6	509030.5250	84838.4208		
Corrected Total	11	628802.7625			

R-Square	Coeff Var	Root MSE	seed Mean
0.190477	12.69221	291.2704	2294.875

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	28.1150	14.0575	0.00	0.9998
cv	1	10782.0075	10782.0075	0.13	0.7337
trt*cv	2	108962.1150	54481.0575	0.64	0.5588

Table E-5. GLM for harvest index.

The GLM Procedure

Dependent Variable: HI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.01020672	0.00145810	3.61	0.0176
Error	15	0.00605850	0.00040390		
Corrected Total	22	0.01626522			

R-Square	Coeff Var	Root MSE	HI Mean
0.627518	4.073650	0.020097	0.493348

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	0.00614213	0.00307106	7.60	0.0052
cv	1	0.00118286	0.00118286	2.93	0.1076
trial	1	0.00169611	0.00169611	4.20	0.0584
trt*cv	2	0.00056304	0.00028152	0.70	0.5135
cv*trial	1	0.00062259	0.00062259	1.54	0.2335

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	0.00600680	0.00300340	7.44	0.0057
cv	1	0.00110682	0.00110682	2.74	0.1186
trial	1	0.00144565	0.00144565	3.58	0.0780
trt*cv	2	0.00064373	0.00032186	0.80	0.4689
cv*trial	1	0.00062259	0.00062259	1.54	0.2335

Table E-6. ANOVA for harvest index.

The ANOVA Procedure

Dependent Variable: HI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.00408742	0.00081748	2.88	0.1152
Error	6	0.00170350	0.00028392		
Corrected Total	11	0.00579092			

R-Square	Coeff Var	Root MSE	HI Mean
0.705832	3.417237	0.016850	0.493083

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.00310067	0.00155033	5.46	0.0446
cv	1	0.00072075	0.00072075	2.54	0.1622
trt*cv	2	0.00026600	0.00013300	0.47	0.6471

Table E-7. GLM for heads per m^2.

The GLM Procedure

Dependent Variable: heads

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	899305.707	99922.856	1.37	0.2920
Error	13	945581.250	72737.019		
Corrected Total	22	1844886.957			

R-Square	Coeff Var	Root MSE	heads Mean
0.487458	8.722568	269.6980	3091.957

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	111043.2065	55521.6033	0.76	0.4859
cv	1	7354.3750	7354.3750	0.10	0.7556
trial	1	17992.9934	17992.9934	0.25	0.6272
trt*cv	2	330466.2345	165233.1173	2.27	0.1425
trt*trial	2	355133.8971	177566.9485	2.44	0.1259
cv*trial	1	77315.0000	77315.0000	1.06	0.3213

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	130464.3229	65232.1615	0.90	0.4317
cv	1	14000.0000	14000.0000	0.19	0.6681
trial	1	25515.0000	25515.0000	0.35	0.5638
trt*cv	2	335853.9062	167926.9531	2.31	0.1387
trt*trial	2	337471.8750	168735.9375	2.32	0.1375
cv*trial	1	77315.0000	77315.0000	1.06	0.3213

Table E-8. ANOVA for heads per m^2.

The ANOVA Procedure

Dependent Variable: heads

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	230604.0000	46120.8000	0.92	0.5266
Error	6	300847.0000	50141.1667		
Corrected Total	11	531451.0000			

R-Square	Coeff Var	Root MSE	heads Mean
0.433914	7.254892	223.9222	3086.500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	61526.0000	30763.0000	0.61	0.5722
cv	1	4961.3333	4961.3333	0.10	0.7637
trt*cv	2	164116.6667	82058.3333	1.64	0.2709

Table E-9. GLM for seeds per head.

The GLM Procedure

Dependent Variable: seedhead

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	43.96933230	4.88548137	2.49	0.0659
Error	13	25.50892857	1.96222527		
Corrected Total	22	69.47826087			

R-Square	Coeff Var	Root MSE	seedhd Mean
0.632850	7.527634	1.400795	18.60870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	10.88897516	5.44448758	2.77	0.0992
cv	1	2.50803571	2.50803571	1.28	0.2787
trial	1	14.28958333	14.28958333	7.28	0.0182
trt*cv	2	9.79166667	4.89583333	2.50	0.1210
cv*trial	1	6.37500000	6.37500000	3.25	0.0947
trt*trial	2	0.11607143	0.05803571	0.03	0.9709

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	11.28273810	5.64136905	2.87	0.0925
cv	1	2.90496032	2.90496032	1.48	0.2453
trial	1	11.71607143	11.71607143	5.97	0.0296
trt*cv	2	10.56398810	5.28199405	2.69	0.1052
cv*trial	1	6.21607143	6.21607143	3.17	0.0985
trt*trial	2	0.11607143	0.05803571	0.03	0.9709

Table E-10. ANOVA for seeds per head.

The ANOVA Procedure

Dependent Variable: seedhead

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	13.60416667	2.72083333	1.61	0.2874
Error	6	10.12500000	1.68750000		
Corrected Total	11	23.72916667			

R-Square	Coeff Var	Root MSE	seedhd Mean
0.573310	7.006048	1.299038	18.54167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	5.79166667	2.89583333	1.72	0.2574
cv	1	1.68750000	1.68750000	1.00	0.3559
trt*cv	2	6.12500000	3.06250000	1.81	0.2419

Table E-11. GLM for seed mass (mg).

The GLM Procedure

Dependent Variable: seedmass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	179.7838859	19.9759873	4.36	0.0085
Error	13	59.6143750	4.5857212		
Corrected Total	22	239.3982609			

R-Square	Coeff Var	Root MSE	mass Mean
0.750982	5.354738	2.141430	39.99130

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	35.89236801	17.94618401	3.91	0.0467
cv	1	55.31358036	55.31358036	12.06	0.0041
trial	1	48.73781689	48.73781689	10.63	0.0062
trt*cv	2	26.69773091	13.34886545	2.91	0.0902
cv*trial	1	2.66103554	2.66103554	0.58	0.4598
trt*trial	2	10.48135417	5.24067708	1.14	0.3490

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	50.08854167	25.04427083	5.46	0.0190
cv	1	63.00243056	63.00243056	13.74	0.0026
trial	1	51.10243056	51.10243056	11.14	0.0053
trt*cv	2	24.40354167	12.20177083	2.66	0.1075
cv*trial	1	1.93287500	1.93287500	0.42	0.5275
trt*trial	2	10.48135417	5.24067708	1.14	0.3490

Table E-12. ANOVA for seed mass (mg per seed).

The ANOVA Procedure

Dependent Variable: seedmass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	66.5441667	13.3088333	2.03	0.2076
Error	6	39.4250000	6.5708333		
Corrected Total	11	105.9691667			

R-Square	Coeff Var	Root MSE	mass Mean
0.627958	6.375205	2.563364	40.20833

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	24.93166667	12.46583333	1.90	0.2299
cv	1	30.40083333	30.40083333	4.63	0.0750
trt*cv	2	11.21166667	5.60583333	0.85	0.4720

Table E-13. GLM for % sterile heads.

The GLM Procedure

Dependent Variable: sterile

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	108.5786712	12.0642968	1.14	0.4004
Error	13	137.1265723	10.5481979		
Corrected Total	22	245.7052435			

R-Square	Coeff Var	Root MSE	sterile Mean
0.441906	63.64977	3.247799	5.102609

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	2	3.97060598	1.98530299	0.19	0.8307
cv	1	2.24913938	2.24913938	0.21	0.6519
trial	1	98.57745054	98.57745054	9.35	0.0092
trt*cv	2	2.01153876	1.00576938	0.10	0.9097
cv*trial	1	0.76396820	0.76396820	0.07	0.7921
trt*trial	2	1.00596830	0.50298415	0.05	0.9536

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	2	4.46568497	2.23284249	0.21	0.8120
cv	1	3.78240573	3.78240573	0.36	0.5596
trial	1	96.22203018	96.22203018	9.12	0.0098
trt*cv	2	1.47538497	0.73769249	0.07	0.9328
cv*trial	1	0.91773018	0.91773018	0.09	0.7727
trt*trial	2	1.00596830	0.50298415	0.05	0.9536

Table E-14. ANOVA for % sterile heads.

The ANOVA Procedure

The ANOVA Procedure

Dependent Variable: sterile

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	3.12734167	0.62546833	0.06	0.9960
Error	6	60.19715000	10.03285833		
Corrected Total	11	63.32449167			

R-Square	Coeff Var	Root MSE	sterile Mean
0.049386	62.11738	3.167469	5.099167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	1.77126667	0.88563333	0.09	0.9167
cv	1	1.08600833	1.08600833	0.11	0.7533
trt*cv	2	0.27006667	0.13503333	0.01	0.9867

Table E-15. ANOVA for % N in seeds.

The ANOVA Procedure

Dependent Variable: Nseeds

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2.28007500	0.45601500	10.80	0.0058
Error	6	0.25335000	0.04222500		
Corrected Total	11	2.53342500			

R-Square	Coeff Var	Root MSE	totN Mean
0.899997	6.720760	0.205487	3.057500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	2.09040000	1.04520000	24.75	0.0013
cv	1	0.18007500	0.18007500	4.26	0.0845
trt*cv	2	0.00960000	0.00480000	0.11	0.8944

Table E-16. ANOVA for % N in vegetative biomass.

The ANOVA Procedure

Dependent Variable: Nbiomass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.83366667	0.16673333	1.43	0.3360
Error	6	0.70160000	0.11693333		
Corrected Total	11	1.53526667			

R-Square	Coeff Var	Root MSE	totN Mean
0.543011	20.51731	0.341955	1.666667

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.77811667	0.38905833	3.33	0.1066
cv	1	0.01470000	0.01470000	0.13	0.7350

Table E-17. ANOVA for NO_3^- in seeds.

The ANOVA Procedure

Dependent Variable: no3seed

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.00002067	0.00000413	0.19	0.9553
Error	6	0.00013000	0.00002167		
Corrected Total	11	0.00015067			

R-Square	Coeff Var	Root MSE	no3seed Mean
0.137168	73.49600	0.004655	0.006333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.00000517	0.00000258	0.12	0.8897
cv	1	0.00000033	0.00000033	0.02	0.9053
trt*cv	2	0.00001517	0.00000758	0.35	0.7182

Table E-18. ANOVA for NO_3^- in vegetative biomass.

The ANOVA Procedure

Dependent Variable: no3bio

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.08177500	0.01635500	2.78	0.1224
Error	6	0.03525000	0.00587500		
Corrected Total	11	0.11702500			

R-Square	Coeff Var	Root MSE	no3bio Mean
0.698782	24.14128	0.076649	0.317500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.07865000	0.03932500	6.69	0.0296
cv	1	0.00067500	0.00067500	0.11	0.7462
trt*cv	2	0.00245000	0.00122500	0.21	0.8174

Table E-19. ANOVA for % Ca in seeds.

The ANOVA Procedure

Dependent Variable: □ased

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.00048542	0.00009708	2.03	0.2075
Error	6	0.00028750	0.00004792		
Corrected Total	11	0.00077292			

R-Square	Coeff Var	Root MSE	ca Mean
0.628032	13.96071	0.006922	0.049583

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.00037917	0.00018958	3.96	0.0802
cv	1	0.00005208	0.00005208	1.09	0.3373
trt*cv	2	0.00005417	0.00002708	0.57	0.5958

Table E-20. ANOVA for % Ca in vegetative biomass.

The ANOVA Procedure

Dependent Variable: cabiomass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.21241667	0.04248333	6.85	0.0182
Error	6	0.03722500	0.00620417		
Corrected Total	11	0.24964167			

R-Square	Coeff Var	Root MSE	ca Mean
0.850886	17.15424	0.078767	0.459167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.20707917	0.10353958	16.69	0.0035
cv	1	0.00120000	0.00120000	0.19	0.6755
trt*cv	2	0.00413750	0.00206875	0.33	0.7289

Table E-21. ANOVA for % S in vegetative biomass.

The ANOVA Procedure

Dependent Variable: SO4biomass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.26094167	0.05218833	29.68	0.0004
Error	6	0.01055000	0.00175833		
Corrected Total	11	0.27149167			

R-Square	Coeff Var	Root MSE	SO4bio Mean
0.961141	8.812431	0.041932	0.475833

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	0.24331667	0.12165833	69.19	<.0001
cv	1	0.01400833	0.01400833	7.97	0.0303
trt*cv	2	0.00361667	0.00180833	1.03	0.4130

Table E-22. ANOVA for % Cl in vegetative biomass.

The ANOVA Procedure

Dependent Variable: Clbiomass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.74854167	0.34970833	2.86	0.1166
Error	6	0.73375000	0.12229167		
Corrected Total	11	2.48229167			

R-Square	Coeff Var	Root MSE	Clbio Mean
0.704406	33.17334	0.349702	1.054167

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	2	1.71166667	0.85583333	7.00	0.0270
cv	1	0.00520833	0.00520833	0.04	0.8433
trt*cv	2	0.03166667	0.01583333	0.13	0.8809

Table E-23. GLM for gross NO_3^- production rates.

The GLM Procedure

Dependent Variable: production

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	459350.5910	45935.0591	6.94	0.0001
Error	20	132338.8929	6616.9446		
Corrected Total	30	591689.4839			

R-Square	Coeff Var	Root MSE	prod Mean
0.776337	51.76930	81.34460	157.1290

Source	DF	Type I SS	Mean Square	F Value	Pr > F
block	3	61027.6803	20342.5601	3.07	0.0511
pH	1	355.3499	355.3499	0.05	0.8191
plant	1	302558.9104	302558.9104	45.72	<.0001
pH*plant	1	5334.8633	5334.8633	0.81	0.3799
inoc	1	29665.9352	29665.9352	4.48	0.0470
pH*inoc	1	23361.5600	23361.5600	3.53	0.0749
plant*inoc	1	33539.7984	33539.7984	5.07	0.0358
pH*plant*inoc	1	3506.4935	3506.4935	0.53	0.4751

Source	DF	Type III SS	Mean Square	F Value	Pr > F
block	3	36747.0238	12249.0079	1.85	0.1705
pH	1	510.7890	510.7890	0.08	0.7840
plant	1	323439.0219	323439.0219	48.88	<.0001
pH*plant	1	2391.4310	2391.4310	0.36	0.5545
inoc	1	35626.5219	35626.5219	5.38	0.0310
pH*inoc	1	26630.4765	26630.4765	4.02	0.0586
plant*inoc	1	34462.6071	34462.6071	5.21	0.0336
pH*plant*inoc	1	3506.4935	3506.4935	0.53	0.4751

Table E-24. GLM for gross NO_3^- consumption rates.

The GLM Procedure

Dependent Variable: prod

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	804826.698	80482.670	3.55	0.0076
Error	20	453296.786	22664.839		
Corrected Total	30	1258123.484			

R-Square	Coeff Var	Root MSE	prod Mean
0.639704	97.04725	150.5485	155.1290

Source	DF	Type I SS	Mean Square	F Value	Pr > F
block	3	55962.6267	18654.2089	0.82	0.4965
pH	1	23477.3571	23477.3571	1.04	0.3209
plant	1	483353.6683	483353.6683	21.33	0.0002
pH*plant	1	90180.5817	90180.5817	3.98	0.0599
inoc	1	24729.3802	24729.3802	1.09	0.3087
pH*inoc	1	5163.1850	5163.1850	0.23	0.6383
plant*inoc	1	57375.8893	57375.8893	2.53	0.1273
pH*plant*inoc	1	64584.0097	64584.0097	2.85	0.1069

Source	DF	Type III SS	Mean Square	F Value	Pr>F
block	3	60898.9643	20299.6548	0.90	0.4606
pH	1	15231.3336	15231.3336	0.67	0.4220
plant	1	483840.6234	483840.6234	21.35	0.0002
pH*plant	1	84735.8791	84735.8791	3.74	0.0674
inoc	1	25169.3791	25169.3791	1.11	0.3045
pH*inoc	1	4979.4643	4979.4643	0.22	0.6443
plant*inoc	1	51862.5609	51862.5609	2.29	0.1460
pH*plant*inoc	1	64584.0097	64584.0097	2.85	0.1069

APPENDIX F. PLANT NUTRIENT ANALYSES

Table F-1. ICP analysis of flag leaves from day 21 of Trial 1.

USU #	Ident.	Al	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg		%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg		
9176	T1-A1+4-Apogee	2.47	10.8	0.87	<	<	<	7.82	48.7	1.12	0.21	84.6	3.57	<	<	0.34	<	0.29	<	5.59	70.0
9177	T1-B2+3-Apogee	<	9.0	0.84	<	<	<	8.63	76.4	1.51	0.15	77.0	3.35	<	<	0.24	<	0.47	<	3.65	56.8
9178	T1- C1+3-Apogee	<	9.3	0.85	<	<	<	9.29	78.8	1.73	0.16	88.1	3.76	<	<	0.35	<	0.51	<	5.27	52.6
9179	T1-A2+3-Veery	3.75	16.2	1.09	<	<	<	10.1	50.3	1.11	0.26	104	3.45	10.1	<	0.29	<	0.34	<	5.98	61.7
9180	T1-B1+4-Veery	<	14.8	0.96	<	<	<	9.36	95.8	1.52	0.18	104	2.90	11.7	<	0.19	<	0.50	<	4.48	57.7
9181	T1-C2+4-Veery	<	17.1	0.79	<	<	<	12.9	104	1.64	0.17	84.5	2.87	<	<	0.22	<	0.53	<	4.95	60.8
9182	T1-Greenhouse-Apogee	<	10.7	0.33	<	<	<	4.12	47.3	1.39	0.29	206	7.57	<	<	0.32	<	0.22	<	15.0	18.8
9183	T1-Greenhouse-Veery	5.41	19.9	0.28	<	<	<	4.36	55.0	1.30	0.24	159	5.40	21.8	<	0.30	<	0.22	<	13.2	23.9
Reporting Limits		1	1	0.001	1	1	1	2.5	2.5	0.01	0.001	1	2.5	10	5	0.001	2.5	0.002	25	1.5	2.5

A = Low NH_4^+
B = High $(NH_4)_2SO_4$
C = High NH_4Cl
Apogee = USU-Line 10
Veery = Veery-10

Table F-2. ICP analysis of flag leaves from days 35 and 53 of Trial 1.

USU #	Ident	Al	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg			%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg		
899	1A	21.2	14.25	<	2.23	<	1.40	<	8.55	58.7	2.17	0.55	105	<	14.4	<	0.36	<	0.69	<	11.0	55.2
900	2A	10.0	20.13	<	2.20	<	1.41	<	10.1	54.1	1.70	0.49	126	<	11.0	<	0.26	<	0.80	<	10.7	50.0
901	3A	6.4	10.99	<	1.50	<	1.05	<	7.31	104.1	3.19	0.27	71.1	<	12.4	<	0.26	<	1.64	<	6.86	28.6
902	4A	6.8	16.13	<	1.34	<	<	<	6.75	94.3	1.97	0.28	90.3	<	9.09	<	0.21	<	1.14	<	7.28	24.0
903	5A	7.7	13.17	2.69	1.36	<	<	<	6.96	93.2	2.83	0.26	71.8	<	13.0	<	0.26	<	1.11	<	8.38	25.2
904	6A	7.7	17.91	2.38	1.08	<	<	<	8.02	88.0	2.26	0.25	61.6	<	18.8	<	0.22	<	0.89	<	7.67	26.1
905	1B	7.4	13.37	<	2.57	<	1.58	<	5.69	43.1	3.32	0.61	120	<	12.9	<	0.40	<	0.83	<	12.4	42.0
906	2B	7.9	25.46	<	2.53	<	1.60	<	9.22	53.8	2.45	0.64	133	<	14.8	<	0.32	<	0.84	<	11.7	37.0
907	3B	11.4	13.14	<	1.74	<	1.11	<	5.74	85.8	3.90	0.30	69.4	<	12.2	<	0.28	<	2.05	<	8.14	18.7
908	4B	6.3	24.40	<	1.63	<	<	<	6.34	84.6	3.06	0.36	82.2	<	19.0	<	0.24	<	1.58	<	9.79	15.7
909	5B	7.6	14.83	2.82	1.58	<	<	<	5.21	70.7	3.89	0.30	63.9	<	16.1	<	0.27	<	1.50	<	10.1	16.9
910	6B	8.3	21.50	2.91	1.35	<	<	<	6.25	69.2	2.65	0.36	50.0	<	12.1	<	0.22	<	1.26	<	10.6	16.2
Reporting Limits		3	5	2	0.001	1	1	1	2.5	2.5	0.005	0.001	1	2.5	10	7.5	0.001	5	0.001	25	2	2

A = Day 35
B = Day 53
1 & 2 = Low NH_4^+
3 & 4 = High $(NH_4)_2SO_4$
5 & 6 = High NH_4Cl
Evens = Veery-10 Odds = USU-Line 10

Table F-3. ICP analysis of seeds and biomass from harvest of Trial 1.

USU #	Ident	Al	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg			%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg		
9295	T1 Har AA1 B10	<	9.24	<	0.61	<	<	<	5.91	23.5	3.99	0.15	83.9	3.0	18.6	<	0.35	<	0.34	<	3.34	65.9
9296	T1 Har AA4 B10	<	7.30	<	0.66	<	<	<	6.28	25.1	4.56	0.15	65.8	3.1	22.4	<	0.34	<	0.36	<	3.31	71.5
9297	T1 Har AB2 B10	<	6.49	<	0.42	<	<	<	4.69	45.3	3.80	0.12	63.5	<	10.6	<	0.25	<	0.72	<	2.48	42.3
9298	T1 Har AB3 B10	<	6.80	<	0.42	<	<	<	4.28	39.9	4.31	0.10	47.6	<	13.0	<	0.18	<	0.69	<	2.42	32.0
9299	T1 Har AC1 B10	<	9.55	<	0.42	<	<	<	3.89	31.2	3.20	0.11	59.3	<	13.3	<	0.23	<	0.46	<	3.22	35.7
9300	T1 Har AC3 B10	<	8.27	<	0.34	<	<	<	5.21	32.4	3.01	0.10	59.4	<	14.0	<	0.23	<	0.46	<	2.93	31.9
9301	T1 Har VA2 B10	<	11.6	<	0.60	<	<	<	7.08	25.3	3.26	0.13	48.9	<	22.1	<	0.20	3.07	0.31	<	3.46	36.4
9302	T1 Har VA3 B10	<	10.2	<	0.52	<	<	<	7.45	30.4	3.08	0.12	42.3	<	19.0	<	0.17	<	0.29	<	2.93	30.6
9303	T1 Har VB1 B10	<	11.9	<	0.48	<	<	<	7.22	40.5	3.53	0.11	49.4	<	17.4	<	0.20	3.67	0.58	<	3.05	28.6
9304	T1 Har VB4 B10	<	11.7	<	0.43	<	<	<	5.66	126	3.08	0.10	45.5	<	17.3	<	0.19	<	0.52	<	2.86	22.6
9305	T1 Har VC2 B10	3.41	10.4	<	0.42	<	<	<	6.80	37.1	3.04	0.09	40.9	<	15.2	<	0.14	4.68	0.39	<	3.63	26.2
9306	T1 Har VC4 B10	2.88	10.2	<	0.41	<	<	<	5.14	247	3.25	0.10	39.6	<	15.6	<	0.17	<	0.39	<	3.49	22.9
9307	T1 AA1 Seeds	3.72	<	<	0.07	<	<	<	9.11	42.4	0.62	0.14	49.6	<	12.6	<	0.45	<	0.16	<	1.42	57.1
9308	T1 AA4 Seeds	6.13	<	<	0.06	<	<	<	8.32	34.6	0.62	0.12	37.3	<	<	<	0.44	<	0.16	<	<	44.5
9309	T1 AB2 Seeds	<	<	<	0.06	<	<	<	6.42	37.0	0.50	0.13	49.4	<	<	<	0.42	<	0.20	<	0.44	52.3
9310	T1 AB3 Seeds	<	<	<	0.05	<	<	<	6.11	31.4	0.57	0.12	37.4	<	<	<	0.38	<	0.19	<	0.37	39.3
9311	T1 AC1 Seeds	4.39	<	<	0.05	<	<	<	6.08	32.3	0.48	0.12	37.6	<	17.3	<	0.38	<	0.18	<	<	40.5
9312	T1 AC3 Seeds	<	<	<	0.05	<	<	<	5.97	34.3	0.51	0.12	44.5	<	<	<	0.38	<	0.19	<	<	46.7
9313	T1 VA2 Seeds	<	<	<	0.06	<	<	<	8.76	35.7	0.52	0.12	36.6	<	<	<	0.40	<	0.15	<	<	49.7
9314	T1 VA3 Seeds	<	5.20	<	0.05	<	<	<	8.73	35.3	0.50	0.12	39.0	<	11.2	<	0.39	<	0.15	<	<	50.9
9315	T1 VB1 Seeds	<	<	<	0.05	<	<	<	4.85	41.7	0.54	0.11	38.8	<	<	<	0.38	<	0.18	<	<	38.1
9316	T1 VB4 Seeds	<	<	<	0.05	<	<	<	5.08	42.8	0.55	0.12	39.4	<	11.1	<	0.39	<	0.18	<	<	40.1
9317	T1 VC2 Seeds	<	5.60	<	0.05	<	<	<	5.33	38.6	0.52	0.11	39.0	<	10.4	<	0.36	<	0.17	<	<	39.1
9318	T1 VC4 Seeds	<	<	<	0.05	<	<	<	5.47	37.8	0.54	0.12	40.1	<	<	<	0.37	<	0.17	<	<	40.3
Reporting Limits		2.5	5	2	0.003	1	1	1	2.5	2.5	0.01	0.003	1	3	10	5	0.001	3	0.001	25	2	2.5

First A = USU-Line 10
V = Veery-10
A = Low NH_4^+
B = High $(NH_4)_2SO_4$
C = High NH_4Cl
B10 = Biomass
Seeds = Seeds

Table F-4. ICP analysis of flag leaves from harvest of Trial 1.

USU #	Ident.	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg				%	mg/kg					%			mg/kg			%	mg/kg	%	mg/kg		
4596	1 AV	10.7	<	29.0	<	2.79	<	<	<	6.61	39.0	3.66	0.68	120	<	16.9	<	0.29	<	0.94	<	11.0	35.0
4597	1 BV	5.37	<	26.9	<	1.78	<	<	<	4.75	58.2	4.18	0.41	81.2	<	15.1	<	0.20	<	2.05	<	10.3	16.6
4598	1 CV	5.56	<	33.3	4.11	1.97	<	<	<	4.72	45.4	4.04	0.46	68.4	<	12.8	<	0.18	<	1.67	<	13.5	16.7
4599	1 AA	9.51	<	21.9	<	2.86	<	<	<	5.64	32.5	4.46	0.78	183	<	21.5	<	0.58	<	0.91	<	12.4	50.1
4600	1 BA	7.54	<	21.6	<	1.75	<	<	<	4.92	58.6	4.27	0.34	71.6	<	11.3	<	0.23	<	2.34	<	8.0	23.2
4601	1 CA	9.31	<	21.3	3.49	1.98	<	<	<	4.54	48.8	4.38	0.37	98.9	<	18.3	<	0.25	<	1.95	<	12.1	31.5
Reporting Limits		5	10	5	2	0.01	2	2	2	3	3	0.01	0.01	1	3	10	8	0.01	3	0.01	25	2	3

A = Low NH_4^+
B = High $(NH_4)_2SO_4$
C = High NH_4Cl
Second A = USU-Line 10
V = Veery-10

Table F-5. NO_3^- and LECO-N analysis of biomass and seeds from harvest of Trial 1.

First A = USU-Line 10
V = Veery-10
A = Low NH_4^+
B = High $(NH_4)_2SO_4$
C = High NH_4Cl
Bio = Biomass Seeds = Seeds

USU #	Ident	NO3-N --mg/kg--	Leco-N ---%---
9295	T1-Har-AA1-Bio	3350	1.35
9296	T1-Har-AA4-Bio	4060	1.53
9297	T1-Har-AB2-Bio	3035	2.62
9298	T1-Har-AB3-Bio	3615	2.07
9299	T1-Har-AC1-Bio	1985	1.81
9300	T1-Har-AC3-Bio	1770	2.32
9301	T1-Har-VA2-Bio	3615	1.42
9302	T1-Har-VA3-Bio	3225	1.30
9303	T1-Har-VB1-Bio	3850	2.13
9304	T1-Har-VB4-Bio	2615	1.96
9305	T1-Har-VC2-Bio	2125	1.87
9306	T1-Har-VC4-Bio	2365	1.98
9307	T1-AA1-Seeds	32	2.74
9308	T1-AA4-Seeds	49	2.73
9309	T1-AB2-Seeds	17	3.68
9310	T1-AB3-Seeds	61	3.60
9311	T1-AC1-Seeds	15	3.54
9312	T1-AC3-Seeds	36	3.77
9313	T1-VA2-Seeds	19	2.74
9314	T1-VA3-Seeds	14	2.62
9315	T1-VB1-Seeds	41	3.05
9316	T1-VB4-Seeds	34	3.24
9317	T1-VC2-Seeds	31	3.31
9318	T1-VC4-Seeds	41	3.14

Table F-6. ICP analysis of flag leaves from day 35 of Trial 2.

USU #	Ident.	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg				%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg		
7570	34A1	5.85	<	28.0	<	1.01	<	<	<	11.6	94.3	2.61	0.23	36.3	3.96	<	<	0.27	<	0.94	<	4.17	32.9
7571	35B1	8.11	<	23.7	<	1.78	<	<	<	15.4	56.0	2.32	0.44	51.0	7.61	<	<	0.31	<	0.62	<	5.27	46.0
7572	35C1	5.42	<	26.8	<	0.95	<	<	<	11.1	62.9	2.70	0.20	26.0	5.29	<	<	0.27	<	1.13	<	3.38	32.3
7573	35A2	7.82	<	26.1	<	0.72	<	<	<	9.44	76.1	2.72	0.17	22.0	3.05	<	<	0.25	<	0.85	<	2.97	24.9
7574	35B2	9.01	<	22.9	<	1.79	<	<	<	17.7	52.5	2.33	0.43	54.6	8.45	<	<	0.31	<	0.68	<	5.29	56.8
7575	35C2	7.52	<	24.4	<	1.06	<	<	<	10.9	63.4	2.74	0.22	36.4	5.21	<	<	0.28	<	1.12	<	3.94	36.0
Reporting Limits		5	10	5	3	0.01	2	2	2	3	3	0.01	0.01	2	3	10	8	0.01	3	0.01	25	2	3

A = High NH_4Cl
B = Low NH_4^+
C = High $(NH_4)_2SO_4$
1 = USU-Line 10
2 = Veery-10

Table F-7. ICP analysis of flag leaves from day 49 of Trial 2.

USU #	Ident	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Sr	Zn
		mg/kg				%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg			
7577	49AA	12.0	<	28.7	<	1.22	<	<	<	9.70	100	3.86	0.29	29.4	4.35	21.3	<	0.39	<	1.26	<	155	5.03	27.0
7578	49BA	7.52	<	24.2	3.35	2.10	<	<	<	13.0	57.9	3.53	0.52	56.9	8.42	12.4	<	0.44	<	0.77	<	116	6.50	50.8
7579	49CA	10.6	<	28.7	<	1.02	<	<	<	9.14	56.6	3.69	0.20	20.7	5.83	11.1	<	0.31	<	1.36	<	67.8	3.88	24.2
7580	49AV	9.45	<	28.6	3.43	1.17	<	<	<	9.09	79.0	3.76	0.27	23.4	4.96	11.6	<	0.30	<	1.30	<	174	5.32	21.6
7581	49BV	6.56	<	24.3	<	1.89	<	<	<	12.2	53.4	3.49	0.48	41.9	8.76	<	<	0.40	<	0.79	<	203	5.90	51.3
7582	49CV	7.59	<	22.9	<	1.18	<	<	<	9.48	63.0	3.38	0.24	28.0	5.37	<	<	0.37	<	1.30	<	169	4.61	27.6
7582 dup		6.92	<	23.0	<	1.10	<	<	<	10.4	57.3	3.42	0.25	27.9	5.34	<	<	0.36	<	1.29	<	135	4.69	27.7
Reporting Limits		5	10	5	3	0.01	2	2	2	3	3	0.01	0.01	2	3	10	5	0.01	3	0.01	25	3	2	3

A = High NH_4Cl
B = Low NH_4^+
C = High $(NH_4)_2SO_4$
Second A = USU-Line 10
V = Veery-10

Table F-8. ICP analysis of flag leaves from days 63 and 74 (harvest) of Trial 2.

USU #	Ident	Al	As	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Si	Sr	Zn
		mg/kg				%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg			
7658	63AA	6.92	<	30.0	3.42	1.22	<	<	<	9.03	61.3	5.13	0.31	35.7	4.86	17.9	<	0.46	<	1.40	<	216	5.87	21.4
7659	63BA	9.09	<	31.5	3.64	2.25	<	<	<	10.6	36.0	4.73	0.54	54.4	8.31	23.2	<	0.53	<	0.80	<	194	7.05	41.6
7660	63CA	6.94	<	28.9	<	1.02	<	<	<	7.95	46.8	4.46	0.20	18.1	6.16	16.9	<	0.39	<	1.54	<	215	4.00	21.1
7661	63AV	12.4	<	33.4	3.87	1.43	<	<	<	7.70	48.6	6.10	0.30	21.1	4.52	27.8	<	0.27	<	1.41	<	123	7.33	18.1
7662	63BV	6.69	<	30.2	2.78	1.88	<	<	<	11.3	42.2	4.09	0.50	40.2	6.37	15.4	<	0.46	<	0.87	<	68	6.36	43.7
7663	63CV	8.51	<	29.2	<	1.16	<	<	<	8.99	51.9	4.28	0.24	29.0	6.90	11.8	<	0.50	<	1.59	<	151	4.90	28.8
7664	HAA	8.26	<	26.5	3.83	1.36	<	<	<	7.15	60.0	5.78	0.30	62.0	4.75	19.6	<	0.50	<	1.49	<	145	7.26	27.0
7665	HBA	9.29	<	27.4	4.29	2.66	<	<	<	11.6	33.0	5.54	0.60	131.8	6.96	30.8	<	0.61	<	0.92	<	172	8.41	61.8
7666	HCA	10.5	<	20.9	2.59	1.69	<	<	<	10.3	27.3	6.50	0.23	18.0	6.28	51.5	<	0.25	<	2.59	<	184	6.74	21.9
7667	HAV	9.45	<	27.8	3.66	1.50	<	<	<	7.24	41.4	7.09	0.31	21.9	4.83	30.6	<	0.31	<	1.43	<	150	7.18	15.3
7668	HBV	7.51	<	25.8	3.34	2.23	<	<	<	10.7	32.0	5.10	0.53	76.8	6.15	26.3	<	0.48	<	0.91	<	142	7.24	51.0
7669	HCV	7.42	<	30.3	2.97	1.75	<	<	<	9.68	36.1	5.80	0.31	24.8	8.31	30.7	<	0.32	<	2.38	<	161	7.10	28.0
dup 7669	HCV	7.74	<	30.2	2.95	1.75	<	<	<	9.90	36.1	5.75	0.31	24.9	6.29	29.8	<	0.32	<	2.37	<	242	7.05	28.1
Reporting Limits		5	10	5	3	0.01	2	2	2	3	3	0.01	0.01	2	3	10	8	0.01	4	0.01	25	3	2	3

A = High NH_4Cl
B = Low NH_4^+
C = High $(NH_4)_2SO_4$
Second A = USU-Line 10
V = Veery-10

Table F-9. ICP, NO_3^-, and LECO-N analysis of biomass and seeds from harvest of Trial 2.

USU #	Ident	Al	B	Ba	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	Pb	S	Se	Sr	Zn
		mg/kg			%	mg/kg					%		mg/kg				%	mg/kg	%	mg/kg		
4579	AVS	<	6.97	<	0.04	<	<	<	7.15	39.6	0.58	0.12	40.5	<	17.8	<	0.45	9.08	0.17	<	<	44.8
4580	BVS	3.58	<	<	0.05	<	<	<	9.26	29.9	0.58	0.11	40.2	<	<	<	0.42	12.5	0.14	<	<	47.6
4581	CVS	4.29	5.06	<	0.04	<	<	<	8.44	28.8	0.54	0.11	32.5	<	14.0	<	0.41	14.3	0.18	<	<	45.6
4582	AAS	<	<	<	0.04	<	<	<	8.87	34.8	0.56	0.13	52.7	<	<	<	0.44	28.3	0.16	<	<	58.1
4583	BAS	<	<	<	0.06	<	<	<	9.97	28.4	0.64	0.12	42.4	<	12.1	<	0.46	21.4	0.15	<	<	51.5
4584	CAS	<	<	<	0.04	<	<	<	7.69	29.6	0.57	0.11	31.3	<	14.3	<	0.41	9.54	0.18	<	<	42.5
4585	AVB	4.39	6.13	<	0.42	<	<	<	7.31	26.0	4.36	0.07	29.6	<	22.2	<	0.19	<	0.39	<	2.42	26.3
4586	BVB	8.47	7.36	<	0.63	<	<	<	8.61	25.5	4.03	0.11	37.8	3.53	26.6	<	0.25	<	0.34	<	2.71	38.6
4587	CVB	6.17	9.32	<	0.42	<	<	<	8.44	29.1	3.43	0.07	24.0	<	31.7	<	0.24	<	0.68	<	1.97	24.9
4588	AAB	7.45	8.29	<	0.48	<	<	<	8.49	35.6	3.96	0.10	64.0	3.71	21.1	<	0.32	<	0.46	<	2.55	45.7
4589	BAB	5.81	6.37	<	0.70	<	<	<	9.02	174	4.13	0.10	46.3	3.57	26.5	<	0.26	<	0.34	<	2.56	50.2
4590	CAB	4.67	6.33	<	0.44	<	<	<	7.64	25.5	4.18	0.06	17.1	2.87	65.6	<	0.17	3.21	0.75	<	2.18	20.7
dup 4590		4.02	8.24	<	0.42	<	<	<	7.79	22.4	4.11	0.06	16.0	2.59	28.3	<	0.17	3.61	0.73	<	2.03	19.1
Reporting Limit		3	5	2	0.001	1	1	1	2.5	2.5	0.01	0.001	1	2.5	10	5	0.002	2.5	0.001	25	1.5	2.5

USU #	Ident	LECO-N	NO3-N
		%	mg/kg
4579	AVS	3.24	144
4580	BVS	2.55	90
4581	CVS	3.26	83
4582	AAS	3.22	78
4583	BAS	2.40	89
4584	CAS	3.42	88
4585	AVB	1.27	2135
4586	BVB	1.32	4850
4587	CVB	1.86	4240
4588	AAB	1.60	2405
4589	BAB	1.21	4090
4590	CAB	1.54	3685

A = High NH_4Cl
B = Low NH_4^+
C = High $(NH_4)_2SO_4$
Second A = USU-Line 10
V = Veery-10
S = Seeds B = Biomass