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Is Nitrate Necessary to Biological Life Support?

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ABSTRACT

Urea is 85% of the recycled nitrogen in a life support system. Urea is quickly converted to NH_4^+ but nitrification to NO_3^- is difficult. Supplying NH_4^+ directly to plants eliminates the need for a nitrifying bioreactor. Most plant physiology textbooks indicate that NH_4^+ is toxic to plants, but we now know that this may not be true if pH is rigorously controlled. However, the long-term effects of high NH_4^+/NO_3^- uptake ratios are poorly understood. In four studies, two cultivars of wheat were grown to maturity with NH_4^+/NO_3^- ratios from 0 to 0.85 in recirculating hydroponic solution. In the third and fourth studies, NH_4^+ was supplied as $(NH_4)_2SO_4$, NH_4Cl, or both. Contrary to conventional wisdom, there was no beneficial effect of supplying 25% of the N as NH_4^+ compared to a nitrate control. The high NH_4^+ treatment (85% NH_4^+) reduced seed yield by 20% in the first two studies, but yield was not reduced in the third and fourth studies. Increasing calcium and potassium supply in the nutrient solution appears to be critical to ameliorating the detrimental effects of NH_4^+. Seed protein concentration was increased from 17 to 22% at the highest NH_4^+ level. These studies indicate that it may be possible to eliminate the need to recycle N as NO_3^- in regenerative life support systems.

INTRODUCTION

Nitrogen is the only essential element that can be absorbed as both a cation (NH_4^+) and an anion (NO_3^-). Plants absorb NH_4^+ much faster than NO_3^-, and the form of N can have a significant effect on the uptake of other nutrients by competitive inhibition. The form of N absorbed also affects the pH of the rhizosphere. Absorption of NH_4^+ results in an efflux of H^+ and the rhizosphere pH can shift downward as much as two units (from pH 7 to 5) in a short time. In contrast, absorption of NO_3^- results in an efflux of OH^-, increasing rhizosphere pH [1]. Lea-Cox *et al* [2] recently confirmed that balancing the proportion of NO3/NH_4^+ in solution can be used to control pH.

A mixture of about 30/70% NH_4^+/ NO_3^- is commonly believed to increase yield compared to NO_3^- only [1,3], but increasing the NH_4^+ fraction above 50% of the total N is believed to reduce yield [4]. However, several short-term studies indicate that the detrimental effects of high levels of NH_4^+ can be ameliorated if root-zone pH is controlled between pH 5 and 6 [5,6,7]. The long-term effects of high NH_4^+ on yield have been studied in soil [8], but have not been studied in solution culture with pH control.

Increased NH_4^+ uptake typically decreases the uptake of other cations, especially K^+, Ca^{2+}, and Mg^{2+}. Cox and Reisenauer [12] found that K, Ca, and Mg decreased in wheat shoots and roots as NH_4^+ concentration in solution culture increased. Magalhaes and Wilcox [13] reported that NH_4^+ uptake decreased the uptake of K^+, Ca^{2+}, and Mg^{2+} in tomato plants. However, studies in both solution and soil culture have shown that NH_4^+ toxicity symptoms can be minimized in tomato plants by increasing K^+ in the root zone [14,15]. Roots actively absorb K+, but Ca^{2+} is passively absorbed from solution. It is not known if higher levels of Ca^{2+} in solution culture can ameliorate NH_4^+-induced Ca^{2+} deficiency. Ammonium may directly decrease Mg^{2+} uptake by cationic inhibition. In addition, magnesium deficiencies have been induced in crop plants by high applications of K^+ and Ca^{2+} fertilizer [1]. The use of high levels of K^+ and/or Ca^{2+} to overcome NH_4^+-induced deficiencies can thus cause Mg deficiency.

The principle of electroneutrality requires that ammonium must be supplied with a charge counter-balancing anion. In hydroponic solutions, the only logical options are SO_4^{2-} or Cl^-, because they are highly soluble, and non-phytotoxic. The choice of anion may affect growth, yield, and nutrient uptake. Koenig and Pan [8] found that the addition of supplemental Cl^- in high NH_4^+ soil increased grain yield and also appeared to increase Ca^{2+} uptake.

A thorough understanding of N nutrition of hydroponically grown crops is important to the development of NASA's Bioregenerative Life-support System (BLS). A

BLS is a self-contained biological, physical, and chemical system that recycles food, air, and water between humans and plants for long term missions in space. In a typical BLS, about 85% of the nitrogen available for plant growth is from urea [9]. Urea is quickly converted to NH_4^+ and CO_2 by bacterial urease [9], but further nitrification to NO_3^- in a bioreactor is difficult [11]. If the potentially detrimental effects of NH_4^+ on plant growth can be ameliorated, the nitrifying bioreactor can be eliminated and NH_4^+ can be recycled directly to the plant root-zone.

Our objective was to study the long-term effects of high NH_4^+ ratios and counterbalancing ions on growth, yield, and nutrient uptake of two cultivars of wheat.

MATERIALS AND METHODS

Plants were grown to harvest in a 3x4 m walk-in growth room containing three recirculating hydroponic systems. Each system had four randomly located 25-liter tubs, which were plumbed to a 180-liter reservoir. This system has been described in detail by Smart *et al* [16]. Two replicate tubs of each cultivar of dwarf wheat (cv. USU-Apogee and cv. Veery-10) were grown for each treatment.

Nutrient solution pH was maintained at 5.8 by automated addition of acid or base. Plants were grown with a 24-h photoperiod from ten, 1000-W high pressure sodium lamps that provided a PPF of 1300 µmol m^{-2} s^{-1}. Air temperature was 23°C until anthesis and was decreased to 17°C after anthesis. Solution temperature was 1.5°C above air temperature. Adjustments were made to air temperature to control rate of development. NO_3^- concentration, pH, temperature, and relative humidity were monitored with a datalogger (Campbell Scientific Inc., model CR10T) and the data was continuously graphed on a computer. The CO_2 was maintained at 1200 µmol mol^{-1} for all four trials. The CO_2 was supplied from compressed gas cylinders, filtered with magnesium perchlorate (Air Repair) and monitored by an infrared gas analyzer.

Nitrate was monitored with an ion selective electrode (Orion model 93-07). When the NO_3^- concentration was depleted to the set point of 100µM, the data logger opened a solenoid, which increased NO_3^- by 10µM. The nitrate was monitored and adjusted every 18-min. in each system. Ammonium concentration was monitored colorimetrically using the Nesslerization method using a colorimeter (LaMotte, SMART Colorimeter) at least twice a week.

Four trials were conducted with NO_3^-/NH_4^+ ratios ranging from 0 to 85%. The three N-treatments were started as 100% NO_3^- in all three trials and the NH_4^+ treatments were initiated on day seven. The first two trials had one 100% NO_3^- treatment, one 25% NH_4^+ treatment, and one 85% NH_4^+ treatment. The third and fourth trial consisted of two 80% NH_4^+ treatments with one treatment supplied as NH_4Cl and the other as $(NH_4)_2SO_4$, the third treatment had N supplied as 15% NH_4^+ supplied as both NH_4Cl and $(NH_4)_2SO_4$.

Total root and shoot dry biomass were measured at harvest. Head numbers per m^2 were counted at harvest. Seed mass was weighed and yield expressed as g m^{-2}. Grain and plant biomass were analyzed for total N by combustion with a LECO CHN analyzer (Model CHN-1000). Plant biomass and grain were analyzed for 11 essential elements (P, K, Ca, Mg, S, Fe, Mn, Zn, B, Cu, and Mo) by ICP-AES at the Utah State University Plant Analysis Laboratory.

RESULTS AND DISCUSSION

Ammonium fraction had no significant effect on total dry biomass for any of the trials (Figure 1A). Seed yield was not different between the NO_3^- only treatment and the 25% NH_4^+ treatment.

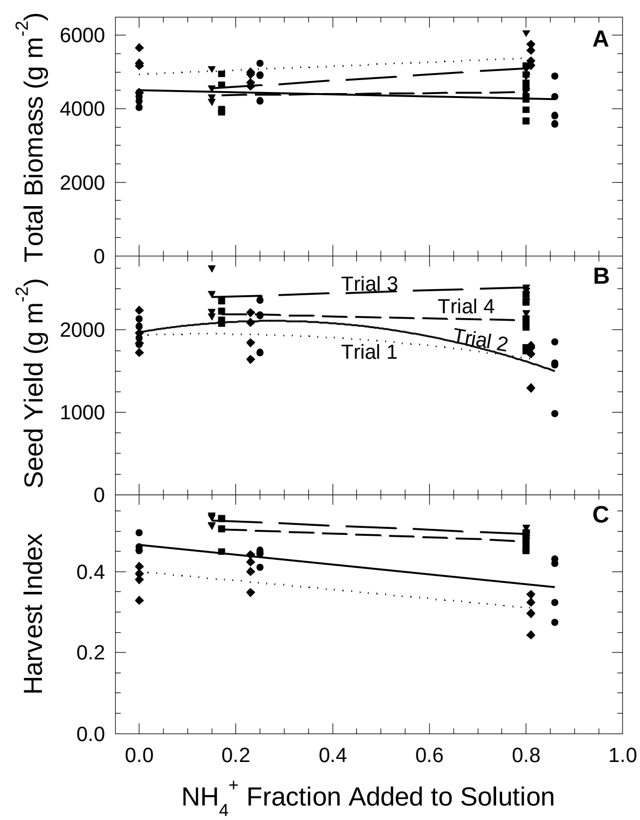

Figure 1. The effect of ammonium fraction on total biomass, seed yield, and harvest index of all four trials.

In the first two trials seed yield was reduced by 22% in the high NH_4^+ treatment compared to the low NH_4^+ treatment ($P \leq 0.01$). However, high NH_4^+ did not have a significant effect on yield in the third and fourth trials (Figure 1B).

Differences between trials may be due to slightly cooler temperatures during seed-fill and longer time period for seed-fill during the third and fourth trials.

Harvest index was reduced by 21% ($P \leq 0.001$) with high NH_4^+ compared to NO_3^- only and low NH_4^+ treatments in Trials 1 and 2 (Figure 1C). Harvest index is the ratio of seed yield to total dry biomass so this reduction is not surprising. Differences of harvest index were not statistically significant for Trials 3 and 4.

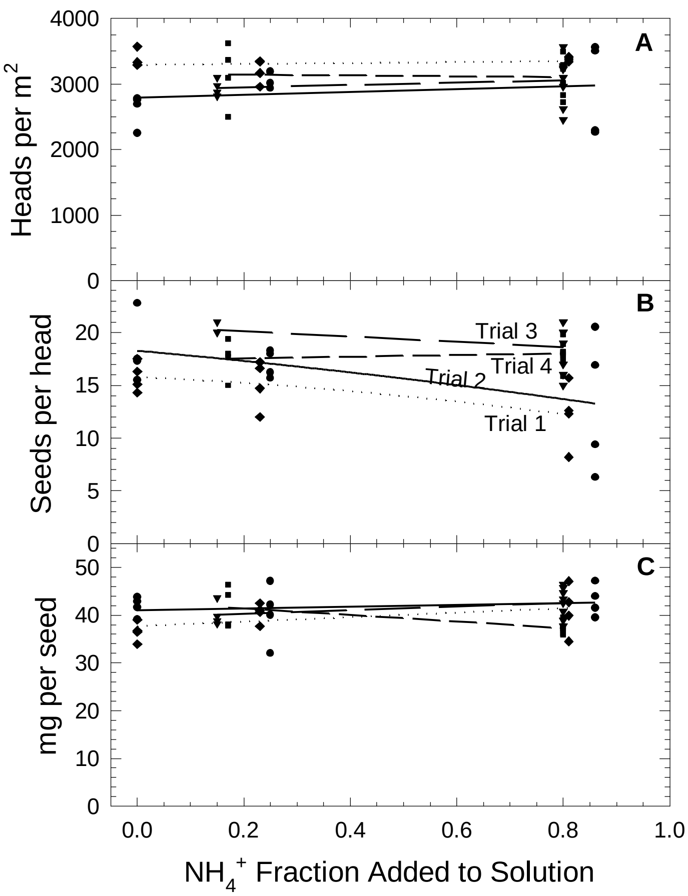

Figure 2. The effect of ammonium fraction on heads per m^2, seeds per head, and mass per seed. Heads per m^2 includes both fertile and sterile heads.

There was no difference between the sulfate and chloride counterbalancing anions in any of the trials.

Total number of heads m^{-2} is indicative of tillering. Ammonium fraction had no effect on heads m^{-2} (Figure 2A). Seeds per head is an indicator of seed set. High NH_4^+ fraction decreased seeds per head in three trials, but was only statistically significant ($P \leq 0.01$) in the first two trials (Figure 2B). The decrease in seeds per head with increasing NH_4^+ suggests that more carbon was partitioned to some other plant part. Seed mass is representative of grain fill. Ammonium fraction had no significant effect on seed mass in any of the trials (Figure 2C).

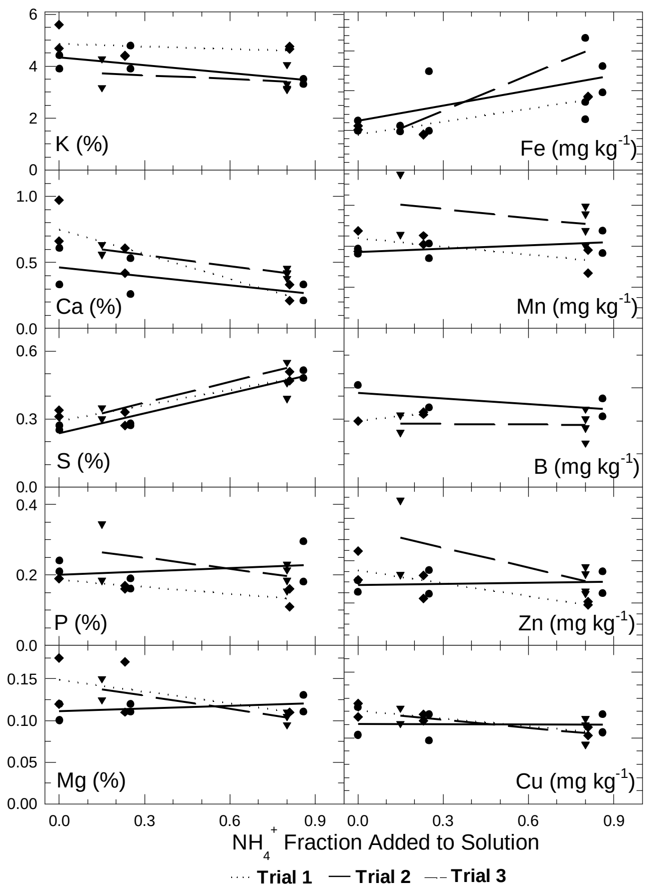

Figure 3. The effect of ammonium fraction on the concentration of ten essential elements in the biomass at harvest. Macronutrients are in the left column, micronutrients in the right column. Data for Trial 4 are not yet available.

Nutrients were all sufficient with the possible exception of Ca^{2+}. Calcium decreased with increased NH_4^+ fraction. In contrast, the concentration of iron and sulfur increased with high NH_4^+ fraction and K^+ remained constant (Figure 3).

The concentration of nitrogen in the seeds increased significantly ($P \leq 0.001$) with increased NH_4^+ fraction (Figure 4). There was no significant difference between N in seeds for the NO_3^- only and low NH_4^+ treatments in the first two trials. Average protein concentration is obtained by multiplying %N by 5.83 [1]. The average protein concentration of the three trials was 22% for the high NH_4^+ treatments, 17% for the low NH_4^+ treatments, and 17% for the NO_3^- only treatments. The increase in protein content is significant considering that the protein concentration in bread wheat is 12 to 15%.

Increasing NH_4^+ fraction resulted in increased N in biomass ($P \leq 0.001$) for three trials (Figure 4). Previous studies have found no significant difference in whole plant N between plants given NH_4^+ or NO_3^- [6,7]. However, the previous studies were short term and did not go to harvest. The longer treatment periods of our studies may allow the plants to accumulate more N.

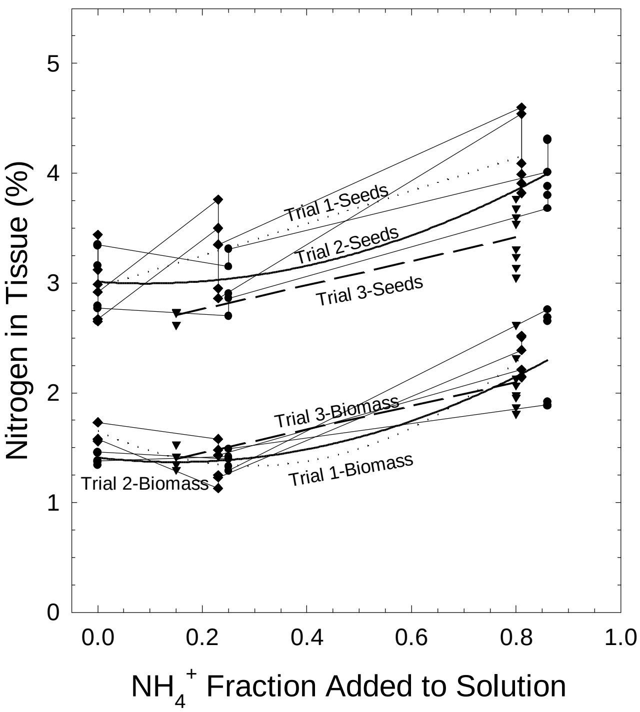

Figure 4. The effect of ammonium fraction added to the nutrient solution on total nitrogen in seeds and total nitrogen in biomass. Data for Trial 4 is not yet available.

CONCLUSIONS

Almost all of the nitrogen in a regenerative system can be returned to the plant root-zone as ammonium, but there may be a small reduction in yield.

Either chloride or sulfate can be used as the counterbalancing anion.

High ammonium increases seed protein nitrogen, which should enhance flour quality and improve nutrition. Future studies will examine the effects of high ammonium on lettuce and rice.

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