Effects of Ammonium Nitrogen on Lettuce Grown on Pumice in a Closed Hydroponic System

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Abstract. Two successive lettuce crops were grown in spring 2005 in a completely closed hydroponic system. The ratio of ammonium to total nitrogen (N_r) in the fresh nutrient solution (FNS) introduced into the closed system to compensate for plant uptake was 0, 0.1, 0.2 and 0.3 on a molar basis. In all N_r treatments, the concentrations of total N, K, Ca, Mg, P, and micronutrients in the FNS were identical, but that of SO_4^{2-} increased as N_r increased, to compensate electrochemically for the enhanced NH_4^+ and decreased NO_3^- supply. The highest fresh and dry weights per plant were attained with the highest ammonium supply ($N_r = 0.3$) but, even when no NH_4^+ was included in the FNS as an N source, the plants were healthy without apparent nutritional disorders. The ammonium concentration in the drainage solution dropped to nearly zero in all treatments some days after the initiation of recycling, which implies a preferential uptake of NH_4 -N over NO_3 -N. The root zone pH, as indicated by the values measured in the drainage solution, decreased slightly as N_r increased, and ranged from 6.5 to 8.0 in all treatments. The leaf K, Ca, Mg, and Fe concentrations were not influenced, whereas those of P, Mn, Zn, and Cu were enhanced by the increasing NH_4^+ supply. The increased ammonium supply did not enhance the utilization of N in plant metabolism, although it reduced the nitrate concentration of the internal leaves in the early spring experiment. The leaf micro-nutrient concentrations were clearly more than critical levels even when NO_3^- was the sole N source for lettuce, whereas the P concentration approached the lowest critical level when N_r was 0 or 0.1. The stimulation of lettuce growth as N_r was increased to 0.3 may be a consequence of enhanced P uptake resulting from better control of pH in the root zone.

Nitrogen is the only nutrient that can be supplied to plants via fertigation in both anionic (NO_3^-) and cationic (NH_4^+) form, whereas the uptake rates of both N forms are influenced by their external concentrations (Forde and Clarkson, 1999). However, the manipulation of NH_4 -N/ NO_3 -N in the nutrient solution supplied to the plants, while maintaining the same total-N concentration, may considerably modify the total cation-to-anion uptake ratio. Changes in the total cation-to-anion uptake ratio have a profound impact on the pH in the root zone (Imas et al., 1997; Lea-Cox et al., 1996). Indeed, the imbalance of total cation over anion uptake in the rhizosphere originating from enhanced NH_4^+ uptake is electrochemically compensated for by the release of protons (H^+), which results in a lowering of the medium pH

(Bolan et al., 1991; Imas et al., 1997). Similarly, the excess of anion over cation uptake by the plants resulting from the supply of NO_3^- as the sole or dominant N form is compensated for by H^+ influx or equivalent anion extrusion (Barber, 1984; Imas et al., 1997; Magalhaes and Wilcox, 1983), which increases the pH of the external solution. The pH of the external medium may influence the uptake of P and micronutrients, thereby inducing serious nutritional disturbances (Adams, 2002; Imas et al., 1997; Islam et al., 1980; Savvas et al., 2003). Furthermore, the form of N supplied may influence the uptake of other macronutrients resulting from ion antagonism (Marschner, 1995). The N form also influences plant metabolism, resulting from differences in the intracellular assimilation paths (Gerendás et al., 1997; Raab and Terry, 1994) and the accumulation of nitrate (Blom-Zandstra, 1989).

As a rule, the use of ammonium as the sole or principal N source results in impaired growth and yield reductions (Chaillou et al., 1986; Chance et al., 1999; Claussen and Lenz, 1999; Guo et al., 2002; Raab and Terry, 1994). This is ascribed to the high toxicity of ammonia at an intracellular level (Barker and Mills, 1980; Givan, 1979; Raab and Terry,

1994). The current recommendation for soil-less culture is that NH_4 -N should not exceed 25% of the total-N supply (Sonneveld, 2002). However, plant species differ in their response to the NH_4 -N/total-N supply ratio and root zone pH (Akl et al., 2003; Beevers and Hageman, 1983; Claussen and Lenz, 1999; Islam et al., 1980). The changes in nutrient concentrations and pH in the root zone of soilless-grown plants may be rapid as a result of the restricted volume of the rooting medium and a lack of buffering capacity. Moreover, the impact of a particular NH_4 -N/total-N supply ratio may be quite different in closed hydroponic systems than in open ones (Savvas and Gizas, 2002). Hence, to prevent nutrient disturbances and to optimize growth in crops grown in closed hydroponic systems, specific knowledge is required for each particular plant species concerning its response to the ratio of NH_4 -N to total-N supply via the nutrient solution.

High NO_3^- concentrations in edible plant parts constitute a potential threat for human health and, therefore, many countries have set maximum permissible values by legislation (Blom Zandstra, 1989; Van der Boon et al., 1990). Many plants tend to accumulate nitrate in their leaves, and lettuce is one of these (Gent, 2003). To restrict the accumulation of NO_3^- in lettuce, a partial substitution of NH_4^+ for NO_3^- has been tested in crops grown in recirculating nutrient solution (Ikeda and Osawa, 1981; Van der Boon et al., 1990) and in pots filled with an organic substrate (Scaife et al., 1986). The previously mentioned investigations focused mainly on plant growth and tissue nitrate levels. The current study was designed to investigate the responses of lettuce to NH_4 -N/total-N when the plants are grown on an inert substrate (pumice) under Mediterranean climatic conditions and the drainage solution is completely recycled. To understand better the mechanisms underlying the effects of NH_4^+ and NO_3^- on lettuce growth, our study focuses not only on plant growth and tissue nitrate concentrations, but also on the nutrient status in the root zone and the uptake of nutrients.

Materials and Methods

Two successive experiments with lettuce (*Lactuca sativa* L., cv. Vardac) were carried out in a glasshouse located in Arta (lat. 39°7'N, long. 20°56'E), Greece. The experiments were conducted in 12 independent hydroponic installations enabling complete recycling of the drainage solution, which corresponded to 12 experimental units. Each experimental unit comprised two channels, 5 m in length and 0.25 m in width. The channels were filled with pumice (particle size range, 0–5 mm) to a depth of 8 cm. Each channel accommodated 40 plants and, hence, the volume of substrate per plant was 2.5 L. All channels were covered with black–white polyethylene sheets to avoid water evaporation. Nutrient solution was automatically applied via a drip irrigation system at a rate

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of 0.12 L per plant per irrigation event, at intervals depending on solar radiation intensity, which was monitored using a pyranometer. The solar integral at which an irrigation event was triggered was regularly adjusted to values maintaining the drainage percentage within a range of 35% to 50%. This schedule resulted in three to eight irrigation applications per day to each experimental unit, depending on the prevailing weather conditions. The nutrient solution supplied to the crop, henceforth termed *irrigation solution*, was prepared by blending the entire amount of drainage solution occurring after each irrigation event with fresh nutrient solution (FNS) and adjusting the pH to 5.6 using nitric acid. A fully automated installation was used to blend FNS with drainage solution up to a constant preset volume, adjust pH, and supply the resultant irrigation solution to the crop. This process was performed separately for each of the 12 experimental units in a successive sequence.

Four different treatments of FNS were obtained by injecting nitrogen at four $\text{NH}_4\text{-N}$ -to-total-N ratios (N_r) corresponding to 0.0, 0.1, 0.2, and 0.3 on a molar basis. The total-N concentration was 11.7 mM in the FNS of all four treatments, which were arranged in a randomized complete block design with three blocks. The nutrient concentrations in all treatments of FNS were as follows: 9.2 mM K, 2.0 mM Ca, 1.0 mM Mg, 1.3 mM P, 40 μM Fe, 5 μM Mn, 4 μM Zn, 0.75 μM Cu, 30 μM B, and 0.5 μM Mo. To attain identical K, Ca, Mg, total-N, and P concentrations in all treatments, the changes in the $\text{NH}_4\text{-N}$ -to-total-N ratio were balanced by varying the sulfate concentration from 0.8 to 4.2 mM and the electrical conductivity of the FNS (EC) from 1.78 to 2.14 $\text{dS}\cdot\text{m}^{-1}$. The mean EC values in the drainage solution ranged from 1.84 to 2.32 $\text{dS}\cdot\text{m}^{-1}$ and from 1.92 to 2.44 $\text{dS}\cdot\text{m}^{-1}$ in the early and late spring experiments, respectively. According to Shannon and Grieve (1999), differences in EC within this range in the root zone are not expected to influence the growth of lettuce significantly, and hence any significant differences should be ascribed to the N_r treatments.

In the first experiment, planting took place on 21 Feb. at the stage of three true leaves and harvesting on 5 Apr. 2005, whereas in the second experiment (which repeated the first) the plants were planted on 21 Apr. and harvested on 25 May 2005. In the early spring experiment, the inside air temperature ranged from 14 to 18 °C during the night and from 15 to 27 °C during the day, whereas the maximum and minimum daily solar radiation inside the greenhouse was 156 $\text{Wh}\cdot\text{m}^{-2}$ and 3398 $\text{Wh}\cdot\text{m}^{-2}$ (average, 1707 $\text{Wh}\cdot\text{m}^{-2}$). In the late spring experiment, the air temperature inside the greenhouse fluctuated from 16 to 22 °C (night) and from 17 to 29 °C (day), whereas the maximum and minimum daily solar radiation was 743 $\text{Wh}\cdot\text{m}^{-2}$ and 3788 $\text{Wh}\cdot\text{m}^{-2}$ (average, 2425 $\text{Wh}\cdot\text{m}^{-2}$). The mean root temperature was 16.5 °C and 20.7 °C in the early and the late spring experiments respectively. In both experiments, the plants

were harvested at the stage of commercial maturity (complete head formation) following the common growing practice for the particular cultivar.

Immediately after planting, the plants of each treatment were supplied with the FNS corresponding to each treatment. Recycling of the drainage solution was initiated 3 d after planting by automatically mixing FNS with drainage solution as described earlier, and this process was continued until the end of the experiment. The pH of the drainage solution, which was considered to be an indicator for the pH status in the root environment, was measured and recorded automatically in an Access® database whenever irrigation solution was prepared. At regular time intervals, three samples of drainage solution were selected from each treatment and used to determine the NH_4^+ concentration at 640 nm by the phenate method and the NO_3^- concentration at 540 nm after reduction to nitrite by cadmium and formation of a diazo complex (Eaton et al., 1995). To monitor the nutrient status in the root zone, the concentrations of all metallic nutrients (K, Ca, Mg, Mn, Zn, Fe, Cu) and P in the drainage solution were also measured at two sampling dates. P was determined colorimetrically as phosphomolybdate blue complex at 880 nm. The metallic cations were measured by atomic emission spectrophotometry (Perkin Elmer, AAnalyst 100), whereas NH_4^+ , P, and NO_3^- were determined using a continuous flow analyzer, type San++ of SKALAR (Breda, The Netherlands).

At crop termination, all the plants from each experimental unit were harvested, and their weight was recorded individually. The plants collected from each channel were used to calculate a mean fresh weight per plant and thus for each treatment a mean of six measured values was computed. Subsequently, two plants randomly selected from each channel were divided into roots and shoot, weighed, and oven-dried at 65 °C to constant weight. The roots were separated from the substrate by placing them on a sieve (mesh size, 6.35 mm), and washing them with tap water at low pressure to remove adhering pumice grains. After drying, the shoot samples were powdered using a ball mill, passed through a 40 mesh sieve, subjected to dry ashing in a muffle furnace at 550 °C for 5 h, and used to extract K, Ca, Mg, Fe, Mn, Zn, Cu, and P by means of 1 N HCl. The concentrations of these nutrients in the water extracts were measured as described earlier for the drainage solution samples. Furthermore, four additional plants were randomly selected from each treatment, separated into young inner leaves (50 g of the youngest leaves per sample) and outer leaves (50 g of the oldest leaves per sample) and used for the determination of their nitrate concentration, which was measured colorimetrically at 410 nm by the nitration of salicylic acid as described by Cataldo et al. (1975). In addition, the powdered shoot samples were used to determine the concentration of organically bound N after Kjeldhal digestion (Mills and Jones, 1996).

Fig. 1. Fresh and dry weight of lettuce as influenced by $\text{NH}_4\text{/total-N}$ (N_r) in the fresh nutrient solution introduced into a closed hydroponic system to compensate for plant uptake. Vertical bars indicate $\pm$ SEM. Values of the same curve marked with different letters are significantly different at $P \leq 0.05$.

All data were subjected to single-factor two-way analysis of variance. When a significant F test was obtained, all possible comparisons between the four treatment means were carried out by using Duncan's multiple range test ($P=0.05$). The PlotIT3.2® work package was used for both statistical analysis and plotting graphs.

Results

Plant growth. The supply of 10% ($N_r = 0.1$) or 20% ($N_r = 0.2$) of total N in the form of ammonium to lettuce plants grown in a completely closed hydroponic system had no effect on the fresh and dry weight of the plant shoot at the stage of commercial maturity in comparison with nitrate alone (Fig. 1). However, the further increase of N_r to 0.3 significantly increased both the fresh and dry shoot weight of lettuce in the early spring experiment, whereas in the late spring only the fresh

weight was significantly enhanced. The increase of the $\text{NH}_4\text{-N}$ -to-total-N supply ratio to 0.3 also enhanced root fresh weight in the early spring experiment compared with the supply of 0, 0.1, and 0.2 of total N in the form of NH_4^+ , although root dry weight was not recorded in that experiment. In the late spring experiment, the results regarding the root fresh weight were not confirmed, whereas the highest root dry weight was measured when N_r was 0.2.

Nutrient solutions. The concentration of ammonium in the drainage solution, which indicates the NH_4^+ level in the root zone, ranged from 0 to 1.1 mM on day 15 after planting in the early spring experiment, in proportion to the ratios at which it was supplied (Fig. 2). However, on day 23 after planting and subsequently, the NH_4^+ concentrations in the drainage solution dropped to nearly zero in all treatments involving the delivery of a part of the total-N in the form of

NH_4^+ , irrespective of the level of N_r . Depletion of NH_4^+ in the root zone of lettuce, as indicated by the concentrations measured in the drainage solution, was also observed in the late spring experiment, despite a constant NH_4^+ delivery to the plants via the irrigation solution in three of the four treatments, which was proportional to N_r . The concentration of NO_3^- in the drainage solution was consistently reduced in both growing seasons as N_r increased (Fig. 3). The concentrations of metallic nutrients and P in the drainage solution did not differ significantly between the treatments (data not shown).

The pH in the drainage solution decreased in both experiments as N_r increased (Fig. 4). However, the pH rose above 6.5 on day 14 (early spring crop) and day 8 (late spring crop) after planting even at the two higher N_r treatments and remained above that level throughout the cultivation. The average pH values calculated from the entire growth period were significantly lower as N_r increased, with a consistent mean separation between the four treatments in the early spring experiment and a separation of the two lower N_r levels from the two upper ones in the late spring experiment (Fig. 4).

Plant tissue. In both experiments, the increased supply of ammonium had no effect on the concentrations of macronutrient cations in the leaves of lettuce (Fig. 5). However, the leaf P concentration was enhanced as N_r rose to levels above 0 and 0.1 in the early and the late spring experiment respectively. With respect to the micronutrient cations, the increase of NH_4^+ supply did not affect the leaf Fe concentration in either of the two experiments or the leaf Cu content in the early spring crop, but the levels of Zn and Mn in the leaves were enhanced in both experiments by the increase in N_r (Fig. 6). In particular, the leaf Cu concentration in the late spring experiment was enhanced by the supply of 10% of N in the form of NH_4^+ ($N_r = 0.1$) in comparison with NO_3^- as the sole N supply, but the further increase of N_r to 0.2 and 0.3 did not additionally raise the leaf Cu level. The leaf Zn content was elevated in both experiments as N_r rose from 0 to 0.1, but to increase the leaf Zn concentration further, an N_r level of 0.3 was required in the early spring crop, whereas in late spring no additional increase of Zn was observed as N_r rose to 0.2 or 0.3. In both experiments, the leaf Mn concentration was not influenced by the increase of N_r from 0 to 0.2, but the further elevation of N_r to 0.3 significantly enhanced the leaf Mn levels.

The increase of $\text{NH}_4\text{-N}$ /total-N from 0 to 0.3 in the FNS that was introduced into the closed system to replace the amounts of nutrients and water taken up by the plants reduced the concentration of nitrate in the inner young leaves of lettuce in the early spring, but not in the late spring (Table 1). However, increasing the NH_4^+ supply to plants had no effect on the NO_3^- concentration of the outer leaves of lettuce in either experiment. The organically bound N in the leaves declined significantly in the early spring

Fig. 2. Influence of $\text{NH}_4\text{/total-N}$ (N_r) in the fresh nutrient solution introduced into a closed hydroponic system to compensate for nutrient and water uptake by lettuce on the NH_4 concentration 1) in drainage solution (solid lines) and 2) in irrigation solution supplied to the crop (dotted line). Vertical bars indicate $\pm$ SEM.

Fig. 3. Influence of $\text{NH}_4/\text{total-N}$ (N_r) in the fresh nutrient solution introduced into a closed hydroponic system to compensate for nutrient and water uptake by lettuce on the NO_3 concentration in the drainage solution. Vertical bars indicate $\pm$ SEM.

Fig. 4. Influence of $\text{NH}_4/\text{total-N}$ (N_r) in the fresh nutrient solution introduced into a closed hydroponic system to compensate for nutrient and water uptake by lettuce on drainage solution pH. Points represent average values of 3-d intervals, whereas mean values correspond to the entire growing period. Different letters adjacent to mean pH values in each experiment indicate significant differences at $P \leq 0.05$.

experiment as N_r increased, but in the late spring experiment no significant differences between treatments could be established.

Discussion

Many investigators have reported a stimulatory effect of $\text{NH}_4\text{-N}$ on plant growth and development when ammonium is not the major source of N (Gerendás and Sattelmacher, 1990; Gerendás et al., 1997; Savvas et al., 2003; Sonneveld, 2002). However, most plants exhibit growth reduction and severe toxicity symptoms if ammonium is the dominant or sole form of supplied nitrogen (Gerendás et al., 1997; Guo et al., 2002; Raab and Terry, 1994). Even if ammonium is not the predominant source of N, the growth of many plant species may be restricted when the $\text{NH}_4\text{-N}/\text{total-N}$ ratio (N_r) exceeds a threshold (Bar-Tal et al., 2001; Sonneveld, 2002). Plant species vary in their response to a particular N_r (Beevers and Hageman, 1983; Claussen and Lenz, 1999; Stensvand and Gislerød, 1992). Furthermore, this response relates to the rooting medium (Magalhaes and Wilcox, 1984), the root temperature (Frota and Tucker, 1972; Ganmore-Neumann and Kafkafi, 1980), and the root zone pH (Claussen and Lenz, 1999; Marschner, 1995).

The rooting medium (soil, soilless substrate, pure nutrient solution) influences the response of plants to the $\text{NH}_4\text{-N}/\text{total-N}$ supply ratio due mainly to its chemical properties, specifically the cation exchange capacity, which may alter NH_4^+ availability in the root zone (Magalhaes and Wilcox, 1984) and the rhizosphere pH (Chaignon et al., 2002). However, in our experiments, the growth medium was the same in all treatments. High temperatures in the root zone are generally associated with a higher susceptibility of plants to increasing NH_4^+ supply, presumably because of enhanced consumption of carbohydrates by root respiration, which may decrease the availability of carbon skeletons for ammonia detoxification (Gerendás et al., 1997; Kafkafi, 1990). The root temperature was similar in all the treatments of each experiment in the current study, but was generally higher in late spring than in early spring. The different temperature conditions between the two trials may account for the weaker responses of lettuce to NH_4^+ in the late spring experiment in comparison with those observed in early spring. With respect to the impact of the root zone pH on $\text{NH}_4\text{-N}$ nutrition, a level of about 6 seems to be optimal for growth in ammonium-fed plants (Wiesler, 1997). Lower pH levels restrict the conversion of NH_4^+ to organic N compounds in the root, thereby increasing the transport of free ammonium to the shoot with detrimental consequences for plant metabolism (Barker et al., 1966). An alkaline root zone pH is also harmful to NH_4^+ -fed plants because of the enhanced conversion of NH_4^+ to free ammonia, which is highly toxic to root cells (Marschner, 1995).

Although the impact of NH_4^+ on plant growth depends strongly on the root zone

Fig. 5. Concentrations of K, Ca, Mg, and P in the leaves of lettuce as influenced by NH_4^+ /total-N (N_r) in the fresh nutrient solution introduced into a closed hydroponic system to compensate for nutrient and water uptake by the plants. Vertical bars indicate $\pm$ SEM. Values of the same curve marked with different letters are significantly different at $P \leq 0.05$.

Fig. 6. Concentrations of micronutrient cations in the leaves of lettuce as influenced by NH_4^+ /total-N (N_r) in the fresh nutrient solution introduced into a closed hydroponic system to compensate for nutrient and water uptake by the plants. Vertical bars indicate $\pm$ SEM. Values of the same curve marked with different letters are significantly different at $P \leq 0.05$.

pH, the latter is also influenced by the NH_4^+ -N/total-N supply ratio as outlined earlier. Hence, in some experiments, to exclude secondary NH_4^+ effects on the plants because of their impact on the root zone pH, the latter was consistently adjusted to the same level in all treatments (e.g., Claassen and Lenz, 1999; Guo et al., 2002). Because our experiments were aimed at investigating the impact of the NH_4^+ supply in closed hydroponic systems under commercial growing conditions, the pH was adjusted only in the irrigation solution delivered to the plants. As a result, differences in the anion-to-cation uptake ratio brought about by the level of supplied NH_4^+ caused differences in the root zone pH. Hence, growth enhancement at the highest N_r level was probably the result of a more favorable root zone pH. Possible mechanisms underlying pH-related growth improvement are discussed later. Nevertheless, even the inclusion of 30% of total-N in the form of NH_4^+ was not sufficient to decrease the pH in the root zone of lettuce to levels between 5 and 6, which are considered optimal for most hydroponically grown plants (Adams, 2002; Sonneveld, 2002). The establishment of relatively high pH levels in all treatments and in both experiments was obviously a result of NH_4^+ depletion in the root zone.

According to Sonneveld (2002), the supply of 10% to 15% of total-N in the form of NH_4^+ is optimal for most crops grown hydroponically on inert substrates, whereas an N_r higher than 0.15 to 0.20 is associated with an increased risk of ammonium toxicity. In our experiments, even the supply of 30% of total-N in the form of NH_4^+ was not harmful, but rather beneficial, to lettuce. However, in both trials, the ammonium concentration in the root zone was extremely low after the initial 2 to 3 weeks of growth, despite the much higher concentrations in the irrigation solution. Conversion of ammonium to ammonia is negligible at pH values < 7.5 (De Rijck and Schrevens, 1997), and thus depletion of NH_4^+ resulting from gaseous losses of ammonia is not likely. Indeed, depletion of NH_4^+ in the drainage solution occurred even when N_r was 0.3, although the pH in the root zone was consistently less than 7.5, particularly in the late spring experiment. Nitrification of NH_4^+ may have occurred to some extent, but this was presumably not the main reason for the depletion of ammonium from the root zone. Indeed, the NO_3^- concentration in the drainage solution decreased with increasing NH_4^+ supply (Fig. 3), obviously reflecting the reduced NO_3^- levels in the FNS, which were necessary to maintain the same total-N concentration in all treatments. However, a complete microbial conversion of NH_4^+ into NO_3^- would have resulted in similar nitrate concentrations in the drainage solution of all treatments, given that the total-N supply was identical in all treatments and the nitrogen concentrations in the plant tissues were similar or even lower at high N_r . Depletion of NH_4^+ in the drainage solution resulting from adsorption by pumice is also unlikely, because this substrate has a very

Table 1. Concentrations of nitrate and organically bound N in the leaves of lettuce grown in a closed hydroponic system as influenced by $\text{NH}_4/\text{total-N}$ in the fresh nutrient solution introduced into the system to compensate for nutrient and water uptake by the plants.^z

$\text{NH}_4/\text{total-N}$ (mol basis)	Early spring expt			Late spring expt		
	NO_3 ($\text{mg}\cdot\text{kg}^{-1}$ fresh wt)		Organic-N ($\text{mg}\cdot\text{g}^{-1}$ dry wt)	NO_3 ($\text{mg}\cdot\text{kg}^{-1}$ fresh wt)		Organic N ($\text{mg}\cdot\text{g}^{-1}$ dry wt.)
	Internal leaves	External leaves		Internal leaves	External leaves	
0.0	1621 a	5348 a	50.72 a	1601 a	4028 a	44.16 a
0.1	1611 ab	5448 a	47.22 b	1237 a	4774 a	43.41 a
0.2	1471 b	5185 a	47.11 b	1531 a	4697 a	43.68 a
0.3	1296 c	4926 a	45.71 b	1448 a	4651 a	41.15 a

^zValues are means of four measurements. Mean separation in columns is by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $P \leq 0.05$.

low cation exchange capacity (Gizas et al., 1999; Raviv et al., 2002). Thus, it seems that lettuce belongs to that group of species that is capable of absorbing NH_4^+ more rapidly than NO_3^- from a mixed $\text{NO}_3^-/\text{NH}_4^+$ source (Forde and Clarkson, 1999). Depletion of NH_4^+ in the drainage solution was also observed in gerbera grown in a closed hydroponic system (Savvas and Gizas, 2002). When a plant species with a preference for NH_4^+ absorption is grown in closed hydroponic systems, N_r should be increased to higher levels than those suggested for systems with free drainage to prevent NH_4^+ depletion in the root zone, which might result in excessively high pH levels in the rhizosphere.

Many investigators concerned with the effects of NH_4^+ on plants have reported reduced uptake of major cations (K, Ca, Mg) with increasing external NH_4^+ concentrations (e.g., Chance et al., 1999; Pill and Lambeth, 1977; Wiesler, 1997). However, under the conditions of our experiment, soon after initiation of drainage solution recycling there was hardly any NH_4^+ in the root zone to influence the uptake of K, Ca, or Mg antagonistically. As a consequence, the concentrations of the latter in the leaves of lettuce were not significantly reduced by the enhanced ammonium supply. In contrast, the concentrations of P, Mn, and Zn in the leaves of lettuce increased as N_r rose. It is well-known that these nutrients become less soluble as the pH increases, which is particularly marked as pH increases to levels more than 6.0 (Adams, 2002; Marschner, 1995). Hence, the effect of the increased NH_4^+ supply on P, Mn, and Zn, as well as on Cu in the late spring experiment may be ascribed to the impact of NH_4^+ on the rhizosphere pH. Despite the high rhizosphere pH, the leaf concentrations of Mn, Zn, and Cu were in all treatments far above the critical levels for deficiency (Marschner, 1995; Mills and Jones, 1996), which rules out growth stimulation at the highest N_r level resulting from a more abundant supply of micronutrients. However, when N_r was 0 or 0.1, the leaf P concentration was only slightly above $3 \text{ mg}\cdot\text{g}^{-1}$ dry weight, which is considered to be the critical level for P deficiency (Marschner, 1995), whereas in the treatment with the highest $\text{NH}_4\text{-N}/\text{total-N}$ supply ratio the leaf P was significantly higher ($4.1 \text{ mg}\cdot\text{g}^{-1}$ and $4.4 \text{ mg}\cdot\text{g}^{-1}$ dry weight in the early and late spring trials respectively). Hence, the stimulatory effect of NH_4^+ on lettuce growth was presumably associated with a more efficient P supply,

resulting from a more favorable pH in the root zone, although energy savings (Raab and Terry, 1994) and alterations in the phytohormone balance (Smiciklas and Below, 1992; Wiesler, 1997) may have also been involved. On the other hand, because the increase of N_r by maintaining the same total-N level did not enhance the concentration of organic N in the shoot of lettuce, the stimulation of growth at the highest N_r level was not the result of improved N nutrition.

Restriction of leaf NO_3^- in lettuce with increasing $\text{NH}_4\text{-N}/\text{total-N}$ has also been observed by Van der Boon et al. (1990). Gent (2003) reported increased NO_3^- levels in the leaves of lettuce grown in a closed hydroponic system as the external NO_3^- concentration was elevated. However, in our study, a reduction of NO_3^- concentration as N_r increased was observed only in the inner (younger) leaves and only in the early spring. Given that nitrate is transported via the transpiration stream to the leaves and accumulates in the vacuoles (Pate, 1980), it seems that the storage capacity of the latter in the old leaves was saturated as a result of prolonged transpiration, thereby minimizing the impact of different external NO_3^- concentrations on NO_3^- deposition. The different response of lettuce to N_r with respect to NO_3^- accumulation in the leaves observed in the early and late spring experiments seems to originate from seasonal differences, particularly solar radiation, which has a strong impact on the nitrate reductase activity (Marschner, 1995), and temperature, which accelerates both nitrification and plant metabolism. Van der Boon et al. (1990) also found a more profound effect of N_r on the leaf NO_3^- concentration of lettuce in the winter than in the summer.

In conclusion, lettuce is not susceptible to micronutrient deficiencies if $\text{NO}_3\text{-N}$ is the only N source in hydroponically grown crops, although under such conditions the pH in the root zone may reach levels more than 7. Lettuce preferentially takes up $\text{NH}_4\text{-N}$ over $\text{NO}_3\text{-N}$, and this may lower the nitrate concentrations in the internal leaves, depending on the growth season. However, the rapid uptake of $\text{NH}_4\text{-N}$ by lettuce does not enhance the level of organically bound N in the shoot when the $\text{NH}_4\text{-N}/\text{total-N}$ supply ratio increases but the total-N supply remains constant. The supply of at least 30% of total-N in the form of NH_4SO_4 by maintaining the concentrations of total N and all other nutrients except SO_4^{2-} at the standard suggested

levels seems to stimulate lettuce growth in pumice when the drainage solution is recycled. The stimulation of lettuce growth by NH_4^+ may be associated with enhanced phosphorus uptake resulting from better control of pH in the root zone.

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