Effects of Different $\text{NH}_4\text{:NO}_3$ Ratios on Growth and Nutrition Uptake in *Iris germanica* ‘Immortality’

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Abstract. The form of nitrogen (N) in fertilizer can influence plant growth, nutrient uptake, and physiological processes in the plant. However, few studies have been conducted on the effects of N form on tall bearded (TB) iris (*Iris germanica* L.). In this study, five $\text{NH}_4\text{:NO}_3$ ratios (0:100, 25:75, 50:50, 75:25, and 100:0) were applied to investigate the response of TB iris to different N form ratios. $\text{NH}_4\text{:NO}_3$ ratios in fertilizer did not affect the leaf, root, and rhizome dry weight, or total plant dry weight. Plant height and SPAD reading were affected by $\text{NH}_4\text{:NO}_3$ ratios in some months, but not over the whole growing season. Neither spring nor fall flowering was influenced by $\text{NH}_4\text{:NO}_3$ ratios. Across the whole growing season, leachate pH was increased by higher $\text{NH}_4\text{:NO}_3$ ratios. At the end of the growing season, concentrations of phosphorous (P), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) in leaf; calcium (Ca), magnesium (Mg), Mn, boron (B) in root; and N, P, Mg, Fe, Mn, and Zn in rhizome tissues were affected by $\text{NH}_4\text{:NO}_3$ ratios. Greater $\text{NH}_4\text{:NO}_3$ ratios increased the uptake of Fe, Mn, and Zn. The net uptake of N was unaffected by $\text{NH}_4\text{:NO}_3$ ratios, which indicates TB iris may not have a preference for either ammonium or nitrate N.

Nitrogen is an important macronutrient needed by plants and often required in the highest amount of all the mineral elements. Normally, N is supplied to plants in form of nitrate (NO_3^-), ammonium (NH_4^+), or urea [$\text{CO}(\text{NH}_2)_2$]. Both the rate and the form of N fertilizer can influence plant growth and must be managed appropriately to maximize plant growth and development (Bar-Yosef et al., 2009; Bernstein et al., 2005; Niu et al., 2011).

Usually a large proportion of urea from fertilizer would be converted to ammonium in soil or medium and then absorbed by plants. In other words, urea may be considered to be the same as ammonium during the uptake process. Thus, the two major N forms taken up by plants are NO_3^- and NH_4^+ . Many

fertilizers provide nitrogen in one or both of these forms. The optimal $\text{NH}_4\text{:NO}_3$ ratio depends on many factors, such as plant species, age of the plant, application timing, climate, and location (Marschner, 2012).

The responses of plant growth to N forms or $\text{NH}_4\text{:NO}_3$ ratio vary with species (Bar-Yosef et al., 2009; Bernstein et al., 2005; Hewins and Hyatt, 2010; Mendoza-Villarreal et al., 2015; Niu et al., 2011). Usually, plants adapted to acid soils prefer NH_4^+ , whereas plants adapted to high pH soils prefer NO_3^- (Marschner, 2012). Solutions with 67:33 $\text{NH}_4\text{:NO}_3$ ratio produced greater biomass than other ratios in mesquite (*Prosopis velutina*) (Hahne and Schuch, 2006). Seventy-five percent of NO_3^- in total N is preferable for improving growth and flowering in hybrid phalaenopsis orchid (*Phalaenopsis*) (Wang, 2008). Rohozinski et al. (1986) found supplying ammonium to apple trees (*Malus pumila*) increased floral initiation. In some plants, N form has no significant effects on plant growth, for instance, the dry weight of shoot and root, and root-to-shoot ratio in Texas mountain laurel (*Sophora secundiflora*) were unaffected by $\text{NH}_4\text{:NO}_3$ ratio (Niu et al., 2011).

Chlorophyll content can be affected by $\text{NH}_4\text{:NO}_3$ ratios. In endive (*Cichorium endivia*), chlorophyll content increased with increasing $\text{NH}_4\text{:NO}_3$ ratios due to its tolerance to ammonium nutrition (Bonasia et al., 2008). In apple (*Malus domestica*), sole ammonium nutrition led to the lowest chlorophyll content (Sotiropoulos et al., 2005). This negative

effect of high ammonium ratio on chlorophyll content could be caused by low pH in the medium reducing the enzyme activity and cell growth (Mashayekhi-Nezamabadi, 2000) or ammonium accumulation increasing leaf sensitivity to ethylene which enhanced chlorophyll loss (Hsu et al., 2003). But, in some plants, $\text{NH}_4\text{:NO}_3$ ratio had no effect on chlorophyll content or SPAD reading, such as in garlic mustard (*Alliaria petiolata*) (Hewins and Hyatt, 2010).

The process of up taking NH_4^+ or NO_3^- has a strong impact on the uptake of other cations and anions and rhizosphere pH. When roots take up NO_3^- , which has a negative charge, and NH_4^+ , which has a positive charge, they typically release an identically charged molecule to maintain a balanced pH inside the plant cells. For example, the assimilation process of one molecule of NH_4^+ produces one proton which will be excreted into the external rhizosphere, reducing rhizosphere pH (Marschner, 2012). Since NO_3^- has a negative charge, the process of NO_3^- uptake is associated with an uptake of protons from the rhizosphere that leads to increasing pH (Hinsinger et al., 2003).

High levels of NH_4^+ can also inhibit the uptake of cations such as calcium and magnesium from the substrate and thus induce a deficiency of those elements in the crop (Adams, 1966; Siddiqi et al., 2002). This decreased uptake of essential cations could cause more problems for plant growth and metabolism. For instance, calcium deficiency led to “toppling” disorder in tulip (*Tulipa*) (Nelson and Niedziela, 1998). However, ammonium applications can also benefit uptake of nutrients. For instance, in calcareous soils, ammonium application reduces the incidence of iron (Fe) deficiency (Mills and Jones, 1997). In addition, ammonium-fed plants accumulate more phosphate and sulfate due to acidification of the rhizosphere, whereas nitrate depresses the uptake of those essential anions (Marschner, 2012). Thus, most of the time, supplying proper $\text{NH}_4\text{:NO}_3$ ratio results in the highest growth rates and plant yields (Kafkafi, 1990; Santamaría and Elia, 1997).

The objective of this study was to investigate the effects of $\text{NH}_4\text{:NO}_3$ ratios on plant growth, flowering, and uptake of nutrients in ‘Immortality’ TB iris.

Materials and Methods

This study was conducted under natural conditions in Starkville, MS (lat. 33°27' N, long. 88°47' W). Starkville has a humid temperate climate with long summers and short, mild winters. The monthly average air temperature in Starkville is 17, 21, 25, 26, 26, 23, 17, 11, and 6 °C in April, May, June, July, August, September, October, November, and December, respectively. The monthly average relative humidity in Starkville is 66%, 69%, 70%, 73%, 73%, 73%, 70%, 70%, and 74% in April, May, June, July, August, September, October, November, and December, respectively.

Rhizomes (average caliper = 4.7 cm and length = 5.8 cm) of ‘Immortality’ TB iris

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(Schreiner's Iris Gardens, Salem, OR) were potted in Aug. 2012 into 3.78-L (23 cm diameter; 16 cm height) round plastic pots (one rhizome per pot) containing a commercial substrate with no starter fertilizer (Fafard growing mix 2; Sun Gro Horticulture, Agawam, MA). From 28 Aug. to 28 Sept. 2012, plants were supplied twice per week with 400 mL of modified Hoagland's solution (Hoagland and Aron, 1950) containing 10 mM N from NH_4NO_3 to provide basic nutrients for fall growth.

On 5 Apr. 2013, before the start of the NH_4NO_3 ratio treatments, five plants were harvested for background dry weight and nutrient composition. The experiment was a completely randomized design with five treatments and 16 replications in each treatment. Five treatments of NH_4NO_3 at 0:100, 25:75, 50:50, 75:25, and 100:0, which had the same concentrations of N (12 mM), K^+ (10 mM), and PO_4^{3-} (5 mM), were used. The nutrient solutions were prepared by adding analytical grade chemicals KNO_3 , NH_4NO_3 , $\text{Ca}(\text{NO}_3)_2$, CaCl_2 , $(\text{NH}_4)_2\text{SO}_4$, Na_2SO_4 , K_2SO_4 , KH_2PO_4 , KCl , and MgSO_4 to tap water with the composition shown in Table 1. Other micronutrients, including Fe (0.1 mM), Mn (0.01 mM), Zn (10^{-3} mM), Cu (10^{-3} mM), and B (0.05 mM), were also added to all nutrient solutions. Plants were supplied with 400 mL solution containing one of five NH_4NO_3 ratio twice per week from 8 Apr. 2013 to 17 Sept. 2013.

Throughout the experiment, plant height, leaf SPAD readings (SPAD-502; Minolta Camera Co., Japan, one of the first two fully expanded leaves was selected to measure SPAD reading), and pH and electrical conductivity (EC) in the leachate (using the pour-through extraction method) were measured monthly. Plants were watered before collecting the leachate. After the container has drained for 30 min, 100 mL water was poured on the surface of substrate to extract ≈ 50 mL leachate.

At the end of the growing season, four plants from each treatment were randomly selected and destructively harvested on 5 Dec. 2013. Each plant was divided into leaves, roots, and rhizomes. All samples were oven-dried at 60 °C until constant weight and dry weights were recorded by tissue type. All

samples were ground to pass a 40-mesh sieve using a Wiley Mill (Thomas Scientific, Swedesboro, NJ) for tissue nutrient analyses. Net uptake of nutrients in 2013 was estimated by subtracting the nutrient content in the plant on 5 Apr. 2013 from nutrient content on 5 Dec. 2013. The N content of each structure (leaf, root, and rhizome) was calculated by multiplying the dry mass by its N concentration. Nitrogen allocation to leaves, roots, and rhizomes was calculated by dividing N content in each tissue by the total plant N.

Data were analyzed by using the five NH_4NO_3 ratio treatments as a one-factor study. Continuous response data were analyzed using linear models with GLM procedure of SAS 9.3 (SAS Institute, Cary, NC) and count data were analyzed using generalized linear mixed models (Poisson distribution and log link function) with the GLIMMIX procedure of SAS. Data collected over time were analyzed by month. Means were compared using Tukey's honestly significant difference test.

Results and Discussions

Plant height and SPAD reading. From April to August, plant height was unaffected by N forms, except in June in which plant height with 100:0 NH_4NO_3 ratio was significantly shorter than the others (Fig. 1A). The effects of N form on plant height may vary among different species; for example, in pepper (*Capsicum annuum*), decreasing NH_4NO_3 ratio led to shorter and more compacted plants (Bar-Tal et al., 2001), whereas in tomato (*Lycopersicon esculentum*), NH_4NO_3 ratio had no effect on plant height (Sandoval-Villa et al., 2001).

It is well known that SPAD readings are highly linearly related to chlorophyll content (Wang et al., 2005). Other research also indicated SPAD readings may be used to indicate N status in plant leaves (Ghosh et al., 2013; Islam et al., 2009). In June, SPAD readings of plants receiving 75:25 NH_4NO_3 were greater than other treatments (Fig. 1B). In August, SPAD readings of plants receiving 75:25, 50:50, and 25:75 NH_4NO_3 were greater than those receiving sole ammonium or nitrate form fertilizer. In November, SPAD readings of plants fertigated

with 100:0, 75:25, and 50:50 NH_4NO_3 were greater than those with 25:75 and 0:100 NH_4NO_3 ratios. During other months, there was no significant difference on SPAD reading of plants receiving different N form.

From April to July, plant leaf SPAD readings showed a declining trend irrespective of NH_4NO_3 ratios. In a previous study with 'Immortality' iris, chlorophyll content decreased during high temperatures in summer and increased after August (Pei, 2006). This might explain the declining trend in leaf SPAD readings in our study. The declining trend of chlorophyll content caused by high temperatures also been noticed with other plants. For example, in creeping bent grass (*Agrostis stolonifera*), chlorophyll content decreased when soil temperature was high (Liu and Huang, 2004).

In this study, SPAD readings were higher in those treatments with both ammonium and nitrate. In tomato, high NH_4^+ concentration in fertilizer decreased SPAD reading which indicates as a result of the NH_4^+ toxicity effect chlorophyll molecular were degraded (Sandoval-Villa et al., 1999). While in head endive, plants were darker with NH_4^+ -fed treatment (Santamaria and Elia, 1997). Greater NH_4NO_3 ratios enhanced Fe uptake, which may be correlated with higher chlorophyll concentration (Roosta, 2014). NH_4NO_3 ratio may influence leaf chlorophyll content or SPAD reading differently depending on plant species. The results of SPAD readings in this study indicated fertilizer with both ammonium and nitrate may benefit the growth of TB iris.

Table 1. Chemical and nutrient composition of fertigation solution of five NH_4NO_3 ratios at a constant total nitrogen (N) concentration of 12 mM.

NH_4NO_3 ratios	Chemical composition (mM)									
	KNO_3	NH_4NO_3	$\text{Ca}(\text{NO}_3)_2$	CaCl_2	$(\text{NH}_4)_2\text{SO}_4$	Na_2SO_4	K_2SO_4	KH_2PO_4	KCl	MgSO_4
0:100	5	0	3.5	0	0	2.5	0	5	0	1
25:75	0	3	3	0	0	0	2.5	5	0	1
50:50	0	6	0	3	0	0	2.5	5	0	1
75:25	0	3	0	3	3	0	0	5	5	1
100:0	0	0	0	3	6	0	0	5	5	1

Nutrient composition (mM)									
	N	NH_4^+	NO_3^-	PO_4^{3-}	K^+	Ca^{2+}	Mg^{2+}	Na^+	SO_4^{2-}
0:100	12	0	12	5	10	3.5	1	5	2.5
25:75	12	3	9	5	10	3	1	0	2.5
50:50	12	6	6	5	10	3	1	0	2.5
75:25	12	9	3	5	10	3	1	0	3
100:0	12	12	0	5	10	3	1	0	6

Fig. 1. (A) Plant height and (B) leaf SPAD reading of container-grown 'Immortality' tall bearded iris. Plants were treated with different NH_4NO_3 ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and data were collected weekly in 2013. ns, *Nonsignificant or significant at $P \leq 0.05$, respectively.

Flowering. Neither spring nor fall flowering (including number of inflorescences per plant and inflorescence stem length) was influenced by N form (data not shown). In butter cup (*Ranunculus asiaticus*), when percentage of ammonium increased from 10% to 30%, number of flowers was affected (Bernstein et al., 2005). When NH₄:NO₃ ratios were greater than 60:40, cut rose (*Rosa*) yield declined due to calcium and potassium deficiency in leaves induced by ammonium in a closed hydroponic system (Bar-Yosef et al., 2009). Flower production of gerbera (*Gerbera jamesonii*) was highest at the substrate NH₄:NO₃ ratios 33:67 in one experiment and 25:75 or 50:50 in another experiment (Guba, 1994).

Dry weight. The dry weight of leaves, roots, rhizomes, and total plant, and shoot-to-root ratio (sum of leaf and rhizome dry weight divided by root dry weight) were unaffected by the NH₄:NO₃ ratios (data not shown). The impact of NH₄:NO₃ ratios on the accumulation of biomass varied among plant species. In both Texas mountain laurel and garlic mustard, N form had no significant effects on biomass of both leaf and root (Hewins and Hyatt, 2010; Niu et al., 2011). In prairie gentian (*Eustoma grandiflorum*), the dry weight of leaf, stem, and shoot increased linearly with increasing NH₄:NO₃ ratios (Mendoza-Villarreal et al., 2015). In addition, if available N form was not the one preferred by plants, then it may cause N-deficiency

symptoms, such as lower dry biomass and larger root-to-shoot ratio (Garbin and Dillenburg, 2008). Thus, no changes of dry weight using different NH₄:NO₃ ratios indicate TB iris may not have a preference for either ammonium or nitrate N form.

Leachate EC and pH. In general, leachate EC was higher in substrate treated with higher NH₄⁺ ratio (Table 2). A possible explanation could be higher NH₄⁺ ratio led to lower pH which increased solubilization of salt elements from fertilizer.

Throughout the growing season, pH of the leachate ranged from 6.2 to 7.5 and decreased with higher NH₄⁺ ratios (Table 2). According to growing practice, the suitable pH for growing TB iris is 6.8 (slightly acidic) (Morris, 2011). The pH in treatments with 25:75 and 50:50 NH₄:NO₃ ratios are closer to this suggested pH. In this study, the lowest pH is 6.2 with 100:0 NH₄:NO₃ ratio treatment, whereas, in 'Safari Sunset' cone-bush (*Leucadendron*), rhizosphere pH decreased below pH 5.0 at high NH₄⁺ application (Silber et al., 2001).

The changes of pH caused by NH₄:NO₃ ratio in fertilizer may also affect the uptake of other cations or anions. In rose, low pH with greater percentage of NH₄⁺ in solution caused Ca and K deficiency in leaves. High pH with a greater percentage of nitrate led to Ca and Mn precipitation and reduced the availability of these nutrients (Bar-Yosef et al., 2009).

Tissue nutrient concentrations. In this study, N concentration in leaves and roots was unaffected by NH₄:NO₃ ratios (Table 3), whereas another rhizomatous plant siam tulip (*Curcuma alismatifolia*), N concentration in leaves was highest in plants receiving nitrate as the N source (Inkham et al., 2011). NH₄:NO₃ ratios in fertilizer significantly affected concentration of P, Fe, Mn, Zn, and Cu in leaves; concentration of Ca, Mg, Mn, and B in roots; and concentration of N, P, Mg, Fe, Mn, and Zn in rhizomes.

In turnip (*Brassica rapa*) leaves, the highest P and K concentration was in 75:25 NH₄:NO₃ ratio treatment, the highest B, Cu, Fe, Mn, and Zn concentration was in 50:50 NH₄:NO₃ ratio treatment (Simonne et al., 1993). Whereas, in this study, the highest Cu, Fe, Mn, and Zn concentrations in leaves were observed with the 100:0 NH₄:NO₃ ratio. There was a decreasing trend in Ca and Mg concentration in leaves, roots, and rhizomes with higher NH₄:NO₃ ratios, which is consistent with results of turnip (Simonne et al., 1993). Due to the antagonism between NH₄⁺ and Ca²⁺ in the process of uptake, greater NH₄⁺ ratio in fertilizer can cause a decrease in Ca concentration in plant tissues (Siddiqi et al., 2002). Thus, mixed NH₄:NO₃ nutrition is recommended to balance the uptake of cation-anion and minimize pH changes in the root environment (Roosta, 2014). In addition, ammonium takes less energy to be assimilated into amino acids than nitrate (Escobar et al., 2006),

Table 2. Leachate pH and electrical conductivity (EC) of container-grown 'Immortality' tall bearded iris. Plants were treated with different NH₄:NO₃ ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

NH ₄ :NO ₃ ratios	May		June		July		August		September		October		November	
	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC
0:100	7.31 a ²	2.1 b	7.47 a	3.6 b	7.29 a	6.9 bc	7.22 a	3.2 b	7.36 a	6.7 ab	7.81 a	4.4 a	7.50 a	3.9 a
25:75	7.22 ab	2.7 b	7.42 a	3.5 b	6.94 ab	10.9 a	7.01 b	2.9 b	7.18 ab	4.6 b	7.34 b	3.9 a	7.18 ab	3.9 a
50:50	7.08 c	3.1 b	7.18 b	4.3 ab	6.80 b	4.0 c	7.15 ab	3.1 b	6.85 bc	5.5 b	7.15 bc	4.0 a	7.17 ab	4.2 a
75:25	6.96 c	3.2 b	6.95 c	4.3 ab	6.25 c	8.3 ab	6.67 b	3.1 b	6.34 d	7.7 a	6.92 cd	4.9 a	6.79 bc	5.7 a
100:0	6.56 d	5.7 a	6.67 d	5.3 a	6.19 c	8.4 ab	6.24 c	5.0 a	6.51 cd	7.0 ab	6.67 d	4.9 a	6.37 c	4.3 a

²Means within a column followed by different letters denotes significant differences between N treatments (Tukey's honestly significant difference, $P \leq 0.05$).

Table 3. Concentration of nutrients in leaves, roots, and rhizomes of container-grown 'Immortality' tall bearded iris. Plants were treated with different NH₄:NO₃ ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

NH ₄ :NO ₃ ratios	N ² (%)	C (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
Leaf											
0:100	2.4 a ²	45.9 a	0.54 a	4.7 a	1.5 a	0.48 a	39.0 c	8.5 c	22.0 bc	3.5 c	30.5 a
25:75	2.1 a	46.0 a	0.53 a	4.2 a	1.5 a	0.47 a	36.8 c	11.8 c	20.3 c	6.8 bc	36.8 a
50:50	2.1 a	46.7 a	0.51 a	4.1 a	1.4 a	0.44 a	36.5 c	10.8 c	22.3 bc	8.5 ab	37.5 a
75:25	2.5 a	47.2 a	0.38 b	4.2 a	1.3 a	0.38 a	74.0 b	30.8 b	29.0 b	11.0 b	32.0 a
100:0	2.5 a	47.3 a	0.42 b	4.2 a	1.3 a	0.38 a	112.8 a	49.5 a	40.8 a	12.0 a	34.3 a
Root											
0:100	0.87 a	49.5 a	0.24 a	3.0 a	0.45 a	0.17 a	71.5 a	15.8 c	14.5 a	5.0 a	13.5 ab
25:75	0.92 a	49.1 a	0.34 a	3.3 a	0.43 a	0.18 a	77.8 a	19.0 c	18.8 a	9.3 a	14.0 a
50:50	0.87 a	48.6 a	0.32 a	3.9 a	0.35 b	0.18 a	62.5 a	17.3 c	15.3 a	6.8 a	13.8 ab
75:25	0.88 a	49.5 a	0.27 a	3.5 a	0.32 b	0.15 b	78.8 a	25.0 b	18.5 a	6.8 a	12.5 bc
100:0	0.79 a	48.5 a	0.29 a	4.0 a	0.32 b	0.15 b	65.8 a	30.8 a	21.3 a	7.5 a	12.0 c
Rhizome											
0:100	2.7 ab	48.2 a	0.46 a	1.6 a	0.56 a	0.29 a	46.3 b	6.3 d	14.5 c	7.8 a	9.8 a
25:75	2.5 bc	48.4 a	0.38 bc	1.5 a	0.56 a	0.28 a	45.3 b	6.5 cd	20.0 bc	10.3 a	9.8 a
50:50	2.0 c	47.9 a	0.33 c	1.4 a	0.52 a	0.21 b	49.8 b	8.0 b	14.3 c	8.3 a	9.3 a
75:25	3.1 a	48.9 a	0.40 ab	1.5 a	0.48 a	0.23 b	62.8 a	14.0 b	28.3 ab	11.0 a	10.0 a
100:0	3.0 ab	48.4 a	0.41 ab	1.6 a	0.45 a	0.23 b	65.0 a	16.0 a	37.0 a	13.3 a	9.5 a

²N = nitrogen; P = phosphorous; K = potassium; Ca = calcium; Mg = magnesium; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper; B = boron.

²Means within a column followed by different letters denotes significant differences between N treatments (Tukey's honestly significant difference, $P \leq 0.05$).

Table 4. Net nutrient uptake of container-grown 'Immortality' tall bearded iris. Plants were treated with different $\text{NH}_4\text{:NO}_3$ ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

$\text{NH}_4\text{:NO}_3$ ratios	N ^z (g)	P (g)	K (g)	Ca (g)	Mg (g)	Fe (mg)	Mn (mg)	Zn (mg)	Cu (mg)	B (mg)
0:100	1.31 a ^y	0.23 a	0.57 a	0.18 a	0.11 a	33.71 c	5.56 b	10.33 b	0.07 a	0.24 a
25:75	1.00 a	0.18 a	0.49 a	0.16 a	0.07 a	30.54 c	6.16 b	11.75 b	0.17 a	0.30 a
50:50	0.90 a	0.17 a	0.49 a	0.17 a	0.05 a	34.75 bc	6.85 b	10.58 b	0.14 a	0.30 a
75:25	1.55 a	0.21 a	0.80 a	0.23 a	0.07 a	51.65 a	14.68 a	19.88 a	0.19 a	0.24 a
100:0	1.24 a	0.18 a	0.68 a	0.13 a	0.04 a	48.88 ab	16.63 a	22.13 a	0.23 a	0.26 a

^zN = nitrogen; C = carbon; P = phosphorous; K = potassium; Ca = calcium; Mg = magnesium; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper; B = boron.

^yMeans within a column followed by different letters denotes significant differences between N treatments (Tukey's honestly significant difference, $P \leq 0.05$).

therefore using mixed $\text{NH}_4\text{/NO}_3$ nutrition has energy-saving effects.

Uptake of nutrients. The net uptake of N was unaffected by $\text{NH}_4\text{:NO}_3$ ratios (Table 4). This result suggests TB iris may not have a preference for either ammonium or nitrate N form. Tall bearded iris could have an efficient series of uptake pathways as well as a metabolism, which allow TB iris take up both high concentrations of ammonium or nitrate. For instance, in *Arabidopsis thaliana*, the high- and low-affinity transporter systems for nitrate regulate nitrate uptake in plants with the large variations of nitrate availability (Orsel et al., 2002).

No antagonism between NH_4^+ and Ca^{2+} , Mg^{2+} , and K^+ was indicated by the net uptake of the elements. In this study, S and Cl concentration in fertigation solution varied among the different $\text{NH}_4\text{:NO}_3$ ratios. Sulfur is essential for converting N into biomass production and insufficient S availability may decrease the uptake of N from the soil (Ceccotti, 1995); however, there was no difference in uptake of N with varied S concentration in this study. The lowest N:S ratio in this study was greater than 7:1, the optimum N:S ratio reported by Janzen and Bettany (1984), indicating the availability of S was sufficient.

Even though this study had 0 mm Cl concentration in 0:100 and 25:75 $\text{NH}_4\text{:NO}_3$ treatments, no Cl-deficiency symptom, such as wilted leaves or curling young leaves, was observed. For some plants, growth was unaffected by withholding Cl supply (Johnson et al., 1957) and the input of Cl from irrigation water and rain may satisfy a plant's need (Marschner, 2012).

The uptake of Fe, Mn, and Zn was significantly increased by higher $\text{NH}_4\text{:NO}_3$ ratios. Higher $\text{NH}_4\text{:NO}_3$ ratios induced low pH in the rhizosphere, which increased Fe^{2+} , Mn^{2+} , and Zn^{2+} availability and uptake (Marschner, 2012). Another study with container-grown 'Immortality' TB iris demonstrated the following spring flowering was affected by the amount of storage nitrogen from the previous year (Zhao et al., 2016). Thus, greater amounts of Fe, Zn, and Cu uptake in the previous year might influence growth and flowering the following season. The higher uptake of nutrients by plants was reflected in the higher concentrations of these elements in plant tissues.

Nitrogen allocation to leaves, roots, and rhizomes. Nitrogen allocation was not statistically different between treatments. Allocation of N was greatest to rhizomes (75% to

84%), followed by leaves (10% to 18%) and roots (7% to 8%) in Dec. 2013. This result is consistent with previous research that the rhizome is a major N-storage organ in winter (Zhao et al., 2016). Storing N in rhizome could maintain the viability of plants through winter and increase the residence time of N in plants.

In summary, $\text{NH}_4\text{:NO}_3$ ratios affected substrate leachate EC and pH in the whole growing season, SPAD and plant height only in some months. The uptake of Fe, Mn, and Zn was affected by $\text{NH}_4\text{:NO}_3$ ratio, which could be related to the changes in pH in the rhizosphere. $\text{NH}_4\text{:NO}_3$ ratios did not influence flowering, dry weight accumulation, or net uptake of N and some other nutrients, which could be indicators of N form preference of plants. Thus, 'Immortality' TB iris may not have a preference for either ammonium or nitrate N form.

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