




# Mitigating water stress by increasing $\text{NO}_3^-:\text{NH}_4^+$ ratio in young *Eucalyptus urophylla* plants

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Received: 4 February 2021 / Accepted: 12 July 2021 / Published online: 30 July 2021  
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## Abstract

**Key message** Mixed N fertilization with  $\text{NO}_3^-:\text{NH}_4^+$  ratio of 0.50:0.50 mitigates negative water stress effects on growth in young *Eucalyptus urophylla* plants.

**Abstract** Plant tolerance to water deficit can be influenced by several factors, including the available ionic forms of fertilization. The goal of this study was to assess the effects of nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) ratios in N fertilization on growth-related morphophysiological and biochemical traits, with the aim of mitigating water stress in young *Eucalyptus urophylla* plants. A greenhouse experiment was arranged in a completely randomized design and factorial scheme  $5 \times 2$ , with different  $\text{NO}_3^-:\text{NH}_4^+$  ratios (0.0:1.0  $\times$  0.25:0.75  $\times$  0.50:0.50  $\times$  0.75:0.25  $\times$  1.0:0.0) and water regimes based on irrigation at 90 and 30% of pot capacity. The results showed that water deficit inhibited plant growth, resulting in lower plant height, and smaller stem diameter, total leaf area, and leaf, stem and root dry mass. Decreases in stomatal conductance, net photosynthesis, and the content of reducing sugar and starch are involved in growth inhibition. Increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio can mitigate negative water deficit effects on leaf water potential, stomatal conductance, and photosynthesis. Furthermore, leaf nitrate reductase activity improves under mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization, compared with  $\text{NO}_3^-$  or  $\text{NH}_4^+$  fertilization alone. Despite this, variations in N sources proved to be ineffective in preventing growth inhibition under water deficit. However, the  $\text{NO}_3^-:\text{NH}_4^+$  ratio of 0.50:0.50 provided the best performance of morphophysiological traits, regardless of the water regime. This was particularly relevant at irrigation levels under 30% since fertilization with an  $\text{NO}_3^-$  ratio equal to  $\text{NH}_4^+$  can mitigate water stress effects on plant growth, despite not preventing damage to morphophysiological traits.

**Keywords** Woody plants · Nitrogen · Mineral nutrition · Water deficit

## Introduction

The increasing demand for wood in Brazil has led to the expansion of *Eucalyptus* cultivation throughout the country. However, in many regions low and/or unstable rainfall distribution can limit the establishment of new forest stands. Water deficit negatively affects seedling growth, and can threaten their survival in the field. Water deficit-tolerant

genotypes are usually recommended for cultivation in these regions, but this strategy alone may not be sufficient to prevent the harmful physiological effects of low soil water availability, especially in the early growth stages.

Plant nutritional status is one of the determining factors for growth under water deficit conditions, and nitrogen is the most essential nutrient. Inorganic nitrogen is absorbed as  $\text{NO}_3^-$  and  $\text{NH}_4^+$  ionic forms (Hawkesford et al. 2012) although it is now acknowledged that organic N forms (e.g., amino acids) are also used by plants (Warren 2006, 2009). Nitrate absorption and assimilation both demand energy, either for the influx from the apoplast to the cytosol across the plasma membrane or for the subsequent reduction to nitrite ( $\text{NO}_2^-$ ) and  $\text{NH}_4^+$ , mediated by nitrate reductase (NR) and nitrite reductase (NiR), respectively (Taiz et al. 2017). In contrast, ammonium absorption requires less energy and

Communicated by R. Guy.

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can occur passively (Li et al. 2013), although it can become toxic when it accumulates in plant tissues.

Previous studies have reported that  $\text{NO}_3^-$  and  $\text{NH}_4^+$  have different effects on the performance of some growth-related morphophysiological and biochemical traits that improve tolerance to water deficit, such as root dry mass (Holzschuh et al. 2011), water uptake (Gao et al. 2010; Faustino et al. 2015), photosynthesis rate (Cao et al. 2018), osmoregulator content such as reducing sugar and proline, and antioxidant activity (Zhang et al. 2011b; Fernández-Crespo et al. 2012). The beneficial effects of mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization have been attributed to such factors as the maintenance of intracellular pH stability, lower energy demand for N assimilation, regulation in the uptake of other cations, rational use of carbon skeletons, and mitigation of  $\text{NH}_4^+$  toxicity (Li et al. 2013; Hachiya and Sakakibara 2016).

Previous studies have shown that the highest growth rate is reached in *Eucalyptus urophylla* seedlings under hydroponic medium with mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization with a 0.75:0.25 ratio (Guimarães et al. 2014). Mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization improves the tolerance of water deficit in corn (Zhang et al. 2011a) and rice (Cao et al. 2018; Wang et al. 2018) found that mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization in *Camellia oleifera* seedlings, regardless of water regime, increased leaf N, chlorophyll, soluble sugar and protein content, and promoted higher enzymatic activity.

Mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization can also improve the absorption efficiency of other nutrients (Holzschuh et al. 2011; Wang et al. 2018), thereby mitigating the negative effects of water deficit (Ashraf et al. 2011; Waraich et al. 2011). Moreover, it can promote auxin accumulation in the roots (Fu et al. 2020; Meier et al. 2020), favoring expansion and deepening (Hachiya and Sakakibara 2016; Liu et al. 2019). The increase in root/shoot ratio improves the water absorption capacity, as observed in *Populus deltoides* (Woolfolk and Friend 2003) and *Oryza sativa* L. (Holzschuh et al. 2011) under mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization. Despite the positive effects on some species, there is still a knowledge gap regarding the ideal  $\text{NO}_3^-:\text{NH}_4^+$  ratio to provide more tolerance to water stress in young *Eucalyptus* plants.

We hypothesized that a high  $\text{NO}_3^-:\text{NH}_4^+$  ratio could reduce water deficits in *Eucalyptus* seedlings through changes in stomatal conductance, improvements in photosynthesis rate and osmoregulation, higher root/shoot ratio and increased water and nutrient absorption. Thus, the goal of this study was to assess the effects of different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in N fertilizer on growth-related morphophysiological and biochemical traits, with the aim to mitigate water stress in young *Eucalyptus urophylla* plants.

## Materials and methods

### Site description and experimental design

A greenhouse experiment was carried out at State University of Southwest Bahia, in Vitória da Conquista, Bahia, Brazil (14°53'08"-S, 40°48'02"-W, 881 m asl), from October to December 2019. The local climate is *Cwb* type (dry-winter subtropical highland climate), according to the Köppen-Geiger classification. During the experimental period, the average temperature and relative humidity inside the greenhouse were  $24 \pm 2$  °C and  $61.5\% \pm 5\%$ , respectively.

The experiment was arranged in a completely randomized design and factorial scheme  $5 \times 2$ , four replicates (one plant per pot), with five different  $\text{NO}_3^-:\text{NH}_4^+$  ratios (0.0:1.0  $\times$  0.25:0.75  $\times$  0.50:0.50  $\times$  0.75:0.25  $\times$  1.0:0.0) and two water regimes based on irrigation at 90 and 30% of pot capacity.

### Plant material and growing conditions

The seedlings of *Eucalyptus urophylla* clone AEC 144 used for this study were produced in small tubes (54 cm<sup>3</sup>) containing the following substrates: bovine manure (40%), vermiculite (40%) and coconut husk powder (20%) (Oliveira Júnior et al. 2011). Seedlings 120-days-old, with 6–8 fully-expanded leaves on average, and approximately 20 cm in height were planted in 15 dm<sup>3</sup> pots using sand previously washed with deionized water as a substrate which was fertilized in two stages, using a nutrient solution proposed by Hoagland and Arnon (1952) (Table 1).

**Table 1** Chemical composition of the nutrient solution supplied per plant for different  $\text{NO}_3^-:\text{NH}_4^+$  ratios

Chemicals	$\text{NO}_3^-:\text{NH}_4^+$ ratios				
	0.0:1.0	0.25:0.75	0.50:0.50	0.75:0.25	1.0:0.0
Quantity supplied (mmol)					
$\text{KH}_2\text{PO}_4$	1	1	1	1	1
$\text{NH}_4\text{Cl}$	15	11.25	7.5	3.5	–
KCl	5	2.2	–	7.6	–
$\text{CaCl}_2$	5	5	3.75	–	–
$\text{MgSO}_4$	2	2	2	2	2
$\text{KNO}_3$	–	3.75	5	1.2	5
$\text{Ca}(\text{NO}_3)_2$	–	–	2	5	5
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.089	0.089	0.089	0.089	0.089
Quantity supplied ( $\mu\text{mol}$ )					
$\text{H}_3\text{BO}_3$	46.25	46.25	46.25	46.25	46.25
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	9.15	9.15	9.15	9.15	9.15
$\text{ZnCl}_2$	0.74	0.74	0.74	0.74	0.74
$\text{CuCl}_2$	0.29	0.29	0.29	0.29	0.29
$\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$	0.11	0.11	0.11	0.11	0.11

The first fertilization was carried out immediately after the seedlings were planted in the pots, and the ionic strength of the nutrient solution was gradually increased (30%, 60 and 100%) every 3 days, to aid in seedling adaptation to the new substrate. Twenty-one days after planting, the second fertilization was conducted using only the nutrient solution at its maximum ionic strength. For both fertilizations, the volume of nutrient solution supplied was sufficient to maintain the substrate moisture at 90% of the pot capacity, which was measured by the gravimetric method. The electrical conductivity of the solution was kept below  $1.5 \text{ mS cm}^{-1}$ , and the pH was  $5.5 \pm 0.2$ , using HCl 0.1 M solution. Water regimes were established 45 days after planting. Fifteen days after water regimes started, we carried out measurements of morphology, plant water status, gas exchange, and biochemical and metabolic traits.

### Plant water status

Plant water status was assessed at predawn in fully expanded leaves ( $n=4$ ) from the middle part of the canopy. A pressure chamber (Model 1000, PMS, Albany, USA) was used for leaf water potential measurements, according to Scholander et al. (1965). Relative water content (RWC) measurements were based on fresh (FM), turgid (TM), and dry mass (DM) from 10 leaf disc samples, and data were used in this equation (Weatherley 1950):

$$\text{RWC} = \frac{(\text{FM} - \text{DMM})}{(\text{TMM} - \text{DM})} \times 100.$$

### Gas exchange

Net photosynthesis ( $A$ ), transpiration ( $E$ ) and stomatal conductance ( $g_s$ ) were measured from 8:00 to 10:00 in fully expanded leaves ( $n=4$ ) from the middle part of the canopy, using an infrared gas analyzer (IRGA LCPro, ADC, UK). Measurements were performed under a photon irradiance of  $900 \text{ mmol m}^{-2} \text{ s}^{-1}$  and a  $\text{CO}_2$  air concentration of  $375 \text{ mmol mol}^{-1}$ . The intrinsic water use efficiency (iWUE) was calculated as the ratio between  $A$  and  $g_s$  ( $A/g_s$ ).

### Biochemical and metabolic traits

Fully expanded leaf samples ( $n=4$ ) were oven-dried at  $70^\circ \text{C}$  for 2–3 days prior to analysis of reducing sugar, proline and starch content. Reducing sugar was extracted from 200 mg of dry leaves after immersion in 15 mL of  $\text{KH}_2\text{PO}_4$  0.1 M buffer solution,  $3 \times$  centrifuged at 2500g for 45 min. The supernatant was taken as the extract, and the reducing sugar content was determined using a spectrophotometer (Miller 1959). Starch was extracted from 250 mg of

dry leaves previously subjected to fat removal by hexane, followed by immersion in 5 mL of  $\text{H}_2\text{SO}_4$  0.5 M at  $100^\circ \text{C}$  for 1 h. Starch content was determined using a spectrophotometer, according to Normative Instruction N<sup>o</sup> 20 (Brasil 1999). Proline was extracted from 200 mg of fresh leaves, after immersion in 6 mL of 3% ( $w/v$ ) sulfosalicylic acid,  $3 \times$  centrifuged at 7500 rpm for 10 min. The supernatant was taken as the extract, and proline content was determined in a spectrophotometer (Bates et al. 1973).

Leaf nitrate reductase activity *in vivo* was assessed as described by Guimarães et al. (2014). The enzymatic assay was based on a 500 mg sample of fresh fragmented leaves ( $n=4$ ), after immersion in 5 mL of  $\text{KH}_2\text{PO}_4$  0.1 M buffer, 3% ( $v/v$ ) *n*-propanol, and  $\text{KNO}_3$  (0.1 M) at pH 7.5, and a water bath at  $30^\circ \text{C}$  in the dark. After 1 h, a 1 mL aliquot from the assay was added to a medium consisting of 1 mL of 1% ( $w/v$ ) sulfanilamide in HCl (1.5 M), 1 mL of 0.02% ( $w/v$ ) *n*-1-naphthylethylenediamine di-HCl, and 1 mL of deionized water. Nitrite content was determined using a spectrophotometer.

### Morphophysiological traits

Measurements of plant height and stem diameter (measured at the root collar) were measured using a graduated ruler and digital caliper, respectively. For total leaf area, a leaf area meter (LICOR, LI-3100) was used. The dry mass of leaves, stem and roots was obtained after drying in an oven at  $70 \pm 5^\circ \text{C}$ , followed by weighing on a scale.

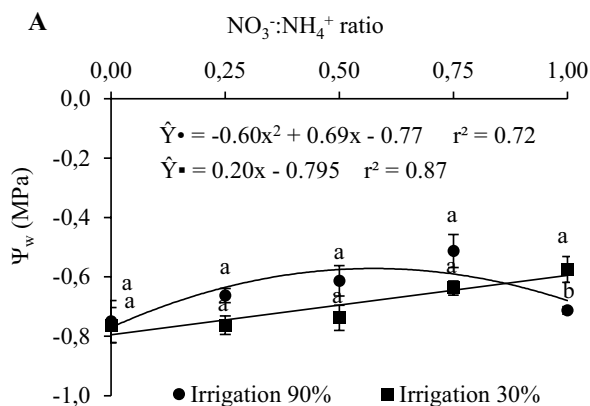
### Statistical analysis

The data were subjected to an analysis of variance. Mean values from the water regimes were compared using Tukey's test. Regression models were adjusted for data from the  $\text{NO}_3^-:\text{NH}_4^+$  ratios. When traits were not adjusted to the regression models, data from  $\text{NO}_3^-:\text{NH}_4^+$  ratios were compared using Tukey's test, using the SISVAR statistical program (Ferreira 2011).

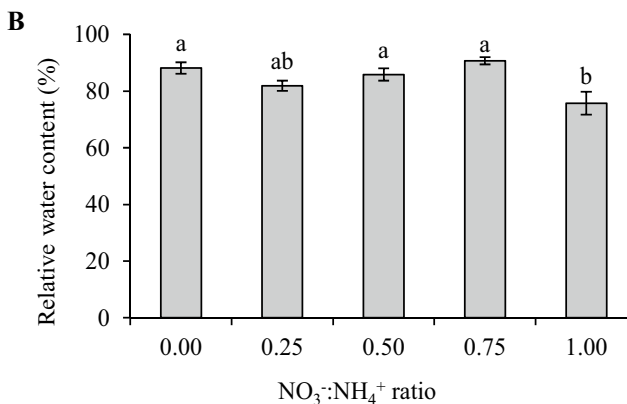
## Results

### Plant water relations

The results only showed a significant interaction ( $p < 0.005$ ) between  $\text{NO}_3^-:\text{NH}_4^+$  ratios and water regimes in relation to leaf water potential ( $\Psi_w$ ). As an isolated factor, the  $\text{NO}_3^-:\text{NH}_4^+$  ratios influenced both  $\Psi_w$  and relative water content ( $p < 0.001$ ). Regarding  $\Psi_w$ , there was a quadratic effect on plants under 90% irrigation in response to increasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio (Fig. 1). There was an increase in  $\Psi_w$ , followed by a decrease from an estimated 0.58:0.42 ratio.



**Fig. 1** **A** Leaf water potential ( $\Psi_w$ ) in young *Eucalyptus urophylla* plants, clone AEC 144, at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios and irrigations 90% (black circle) and 30% (black square). Same lowercase letters for each  $\text{NO}_3^-:\text{NH}_4^+$  ratio indicate that  $\Psi_w$  does not differ between water



regimes by Tukey's test ( $p < 0.01$ ). **B** Relative water content in young *Eucalyptus urophylla* plants, clone AEC 144, at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios. Same lowercase letters indicate that the data does not differ by Tukey's test ( $p < 0.01$ )

However, under 30% irrigation, increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio caused a linear increase. The water regime effect was significant only at the 1.0:0.0 ratio, where  $\Psi_w$  was higher under 30% irrigation (Fig. 1A). Regarding relative water content, there was a significant decrease at 1.0:0.0, irrespective of water regime, although this data was similar to that observed for the 0.25:0.75 ratio (Fig. 1B).

### Gas exchange

The results showed a significant interaction ( $p < 0.001$ ) between the  $\text{NO}_3^-:\text{NH}_4^+$  ratios and water regimes for all gas exchange traits. The increase in  $\text{NO}_3^-:\text{NH}_4^+$  ratio under 30% irrigation caused linear increases in  $A$ ,  $E$  and  $g_s$ , and a linear decrease in  $iWUE$ . Conversely, under 90% irrigation, these traits were not influenced by the different  $\text{NO}_3^-:\text{NH}_4^+$  ratios. Results from  $A$ ,  $E$ , and  $g_s$  were higher under 90% irrigation than under 30% irrigation, for the majority of the  $\text{NO}_3^-:\text{NH}_4^+$  ratios, except at 1.0:0.0, where there was no difference between water regimes. The performance of  $iWUE$ , in turn, was consistently higher under 30% irrigation, irrespective of the  $\text{NO}_3^-:\text{NH}_4^+$  ratio (Fig. 2).

### Biochemical and metabolic traits

The results showed a significant interaction between  $\text{NO}_3^-:\text{NH}_4^+$  ratios and water regimes for reducing sugar ( $p < 0.001$ ), starch ( $p < 0.005$ ), and proline ( $p < 0.005$ ) contents, as well as nitrate reductase (NR) activity ( $p < 0.001$ ).

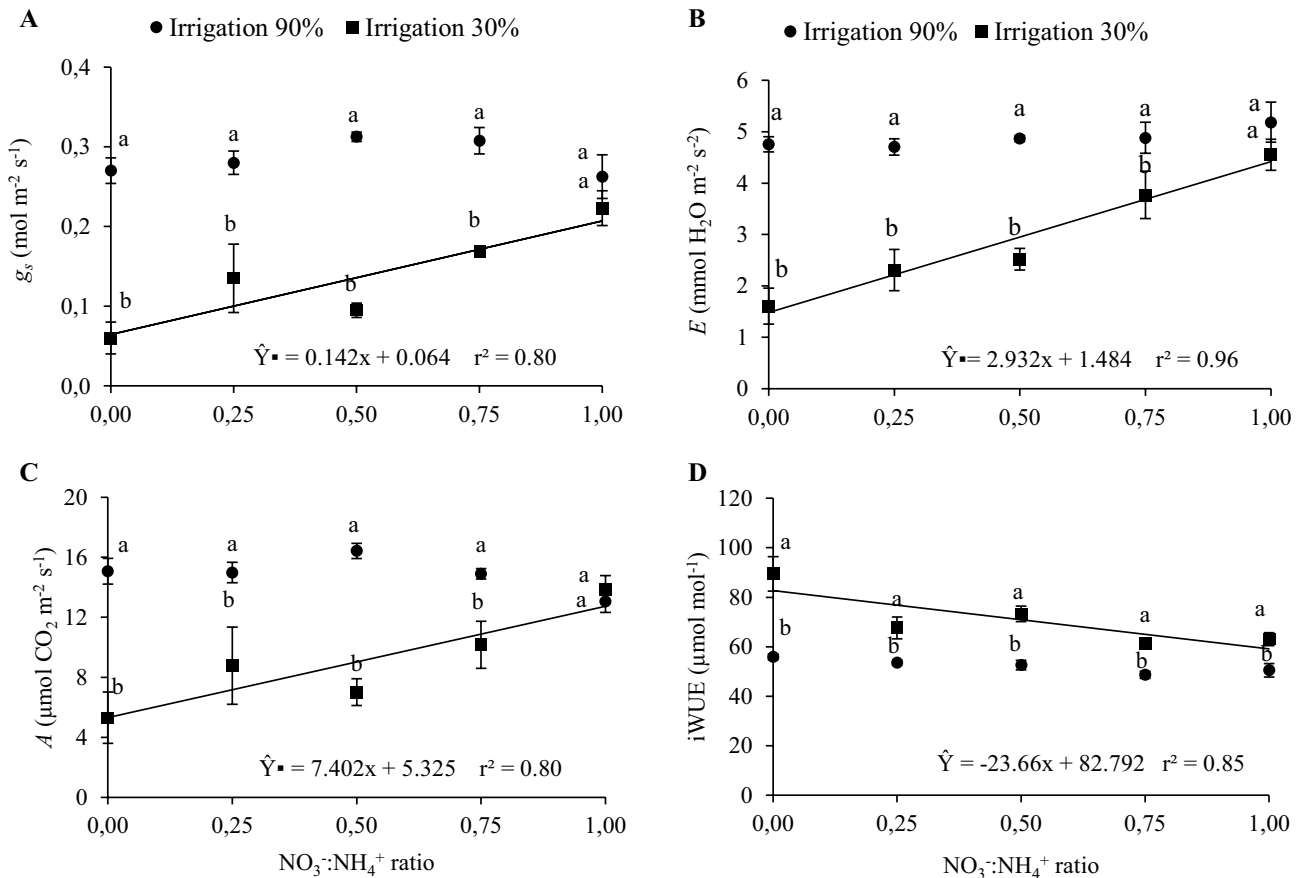
Regarding reducing sugar, there was no difference between the water regimes when the  $\text{NO}_3^-:\text{NH}_4^+$  ratio was 0.0:1.0. However, the other ratios showed a lower reducing sugar under 30% irrigation than under 90%. With 30% irrigation, reducing sugar decreased linearly in response

to an increased  $\text{NO}_3^-:\text{NH}_4^+$  ratio (Fig. 3A). Starch content, in turn, was lower under irrigation 30% than under 90%, regardless of the  $\text{NO}_3^-:\text{NH}_4^+$  ratio. The increased  $\text{NO}_3^-:\text{NH}_4^+$  ratio caused a slight quadratic effect at 30%, with starch content decreasing up to an estimated 0.61:0.39 ratio (Fig. 3B). Proline content increased under 30% irrigation at most of the  $\text{NO}_3^-:\text{NH}_4^+$  ratios, except at 0.75:0.25, where it did not differ between water regimes. This increase was more pronounced in fertilization with only one ionic N form than with mixed  $\text{NO}_3^-:\text{NH}_4^+$  fertilization (Fig. 3C).

Leaf nitrate reductase activity was not influenced by water regimes, fertilization with  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , or mixed N fertilization at 0.75:0.25. Enzymatic activity at 0.25:0.75 and 0.50:0.50 was higher under 30% irrigation. At 90%, RN activity showed a slight decline, in response to an increased  $\text{NO}_3^-:\text{NH}_4^+$  ratio, whereas with 30% irrigation, the increase in  $\text{NO}_3^-:\text{NH}_4^+$  ratio caused a quadratic effect, with enzymatic activity increasing to an estimated 0.46:0.54 ratio (Fig. 3D).

### Morphophysiological traits

The results showed no significant interaction between  $\text{NO}_3^-:\text{NH}_4^+$  ratios and water regimes in relation to plant height, stem diameter, total leaf area, and leaf, stem and root dry mass. Nevertheless, both  $\text{NO}_3^-:\text{NH}_4^+$  ratios and water regimes showed significant differences for all traits ( $p < 0.001$ ). In general, increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio had positive effects on growth-related morphophysiological traits, which reached the best performance at 0.50:0.50, despite some similarities with higher  $\text{NO}_3^-:\text{NH}_4^+$  ratios, depending on the variable (Table 2). As for water regimes, 30% irrigation negatively affected all morphophysiological



**Fig. 2** **A** Stomatal conductance ( $g_s$ ), **B** transpiration ( $E$ ), **C** net photosynthesis ( $A$ ), and **D** intrinsic water use efficiency (iWUE) in young *Eucalyptus urophylla* plants, clone AEC 144, at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios and irrigations 90% (black circle) and 30% (black square). Same lowercase letters for each  $\text{NO}_3^-:\text{NH}_4^+$  ratio indicate that data do not differ between water regimes by Tukey's test ( $p < 0.01$ )

traits, especially leaf and stem dry mass, which decreased 28.96 and 32.30%, respectively (Table 3).

Fifteen days after starting treatments with mixed N fertilization, typical symptoms of  $\text{NH}_4^+$  toxicity were observed in plants at 0.0:1.0 and 0.25:0.75. The first symptoms appeared at stage B (Silva et al. 2020) of leaf development, when a purple to reddish color became noticeable at leaf edges, followed by upper edge curling (Fig. 4A). These symptoms evolved to necrosis at stage C of leaf development (Fig. 4B).

## Discussion

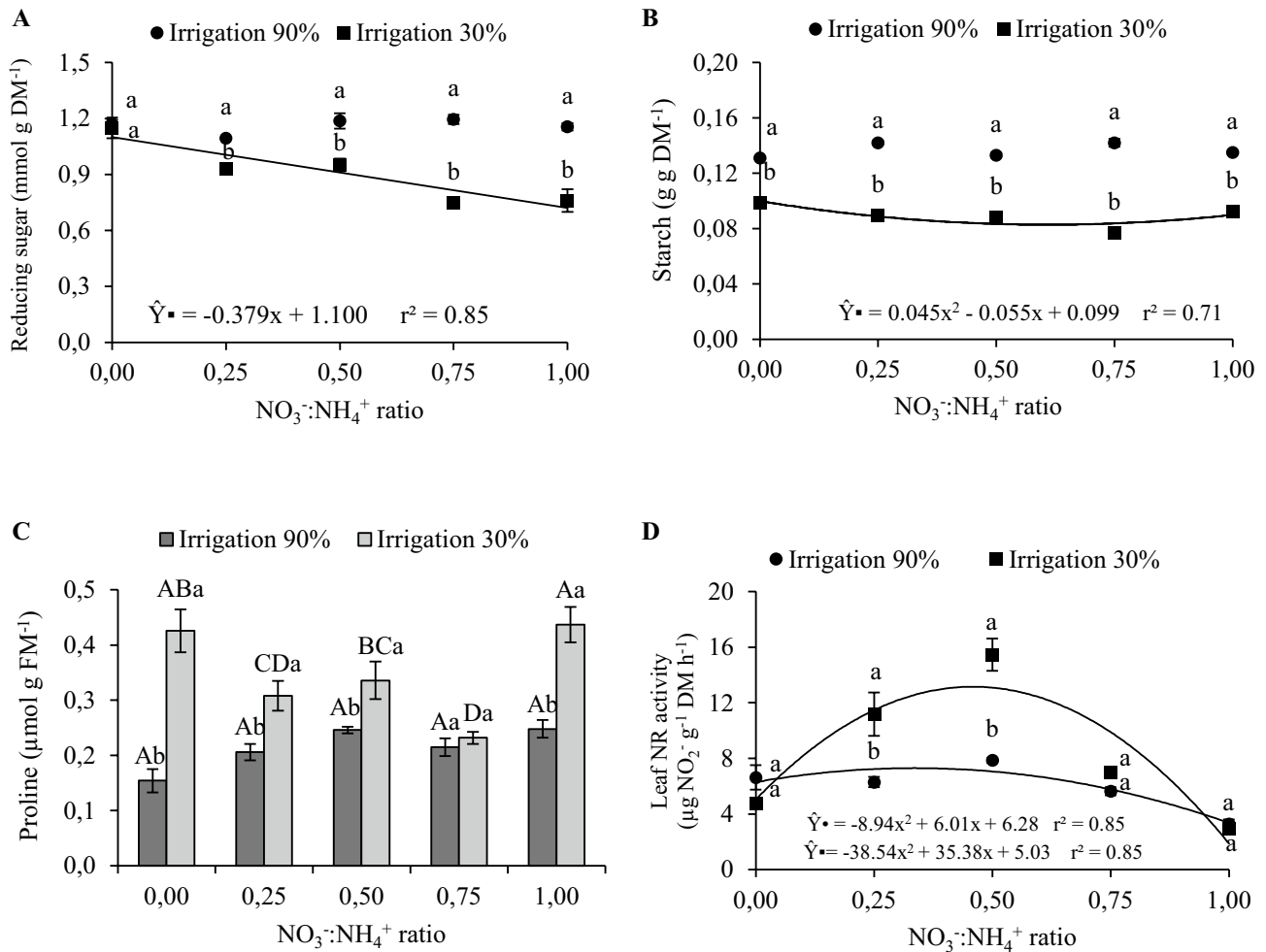
The results showed that increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio under water deficit was not effective in preventing significant decreases in growth-related morphophysiological traits such as plant height, stem diameter, total leaf area, and leaf, stem and root dry mass (Table 3). Regarding dry mass, these effects were more pronounced on leaf and stem, which reduced in size by 28.96 and 32.30%, respectively, when compared to well-watered plants. Due to the smaller

ratios and irrigations 90% (black circle) and 30% (black square). Same lowercase letters for each  $\text{NO}_3^-:\text{NH}_4^+$  ratio indicate that data do not differ between water regimes by Tukey's test ( $p < 0.01$ )

decrease in root dry mass (18.83%), there was likely a higher source-to-sink assimilate partitioning for this organ, which favors water uptake, thus providing higher tolerance to water deficit. The best morphophysiological traits were achieved at  $\text{NO}_3^-:\text{NH}_4^+$  ratios of 0.50:0.50, irrespective of water regime (Table 2). This effect is especially interesting for plants under 30% irrigation since it helps to mitigate the effects of water stress on plant growth.

For gas exchange,  $g_s$  was one of the most important factors, showing a strong correlation with  $E$  and  $A$ . Under 30% irrigation,  $g_s$ ,  $E$ , and  $A$  values were lower at low  $\text{NO}_3^-:\text{NH}_4^+$  ratios (Fig. 2). These results agree with those of Cramer and Lewis (1993) in wheat, and Lopes et al. (2004) in barley, who determined that  $g_s$  and  $A$  values were significantly lower in  $\text{NH}_4^+$ - than in  $\text{NO}_3^-$ -fertilized plants.

Water deficit usually promotes partial stomatal closure, reducing  $g_s$ ,  $E$ , and  $A$  (Sharma et al. 2020). In our study, the results revealed that increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio attenuated the water deficit effect on gas exchange affected by water potential (Fig. 2). The increase in  $A$ , in response to increased  $\text{NO}_3^-$  supply, partly relates to the concomitant



**Fig. 3** Leaf contents of **A** reducing sugar, **B** starch and **C** proline, and **D** nitrate reductase activity in young *Eucalyptus urophylla* plants, clone AEC 144, at different NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> ratios and irrigations 90%

(black circle) and 30% (black square). Lower case letters compare water regimes in each NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> ratio, while capital letters compare NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> ratios in each water regime, by Tukey's test ( $p < 0.01$ )

**Table 2** Effects of NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup> ratio on plant height (H), stem diameter (D), total leaf area (TLA), and leaf, stem and root dry mass (LDM, SDM and RDM) in young *Eucalyptus urophylla* plants, clone AEC 144, irrespective of water regimes

Traits	NO <sub>3</sub> <sup>-</sup> :NH <sub>4</sub> <sup>+</sup> ratio				
	0.0:1.0	0.25:0.75	0.50:0.50	0.75:0.25	1.0:0.0
H (cm)	41.50 ± 0.68 c	41.94 ± 1.76 bc	48.18 ± 1.54 a	44.12 ± 1.08 bc	45.31 ± 0.52 ab
D (cm)	6.18 ± 0.37 bc	5.83 ± 0.23 c	7.05 ± 0.24 a	6.73 ± 0.26 ab	6.63 ± 0.17 ab
TLA (cm <sup>2</sup> )	1320.49 ± 88.75 b	1565.54 ± 137.45 b	1942.70 ± 106.70 a	1599.48 ± 98.36 ab	1628.26 ± 89.42 ab
LDM (g)	7.52 ± 0.69 b	8.33 ± 0.96 b	10.54 ± 0.92 a	10.48 ± 0.65 a	10.52 ± 0.54 a
SDM (g)	5.24 ± 0.74 bc	4.52 ± 0.40 c	7.15 ± 0.59 a	6.35 ± 0.73 ab	6.61 ± 0.41 ab
RDM (g)	5.96 ± 0.47 c	7.09 ± 0.90 bc	8.74 ± 0.67 a	8.52 ± 0.58 ab	8.34 ± 0.29 ab

Lower-case letters compare data on the same line using Tukey's test ( $p < 0.05$ )

decrease in NH<sub>4</sub><sup>+</sup> supply, thus mitigating the negative effects of this cation on gas exchange. A high NH<sub>4</sub><sup>+</sup> content may also be involved in stomatal closure (Foyer et al. 2003), as  $g_s$  and  $A$  are decreased. These deleterious effects of high

NH<sub>4</sub><sup>+</sup> content on gas exchange corroborate other studies on strawberry, sugar cane, and fir, even under well-watered conditions (Rothstein and Cregg 2005; Tabatabaei et al. 2006; Pissolato et al. 2019). In contrast, in our study NO<sub>3</sub><sup>-</sup>:NH<sub>4</sub><sup>+</sup>

**Table 3** Effects of water regimes on plant height ( $H$ ), stem diameter ( $D$ ), total leaf area (TLA), and leaf, stem and root dry mass (LDM, SDM and RDM) in young *Eucalyptus urophylla* plants, clone AEC 144, irrespective of  $\text{NO}_3^-:\text{NH}_4^+$  ratios

Traits	Water regimes	
	Irrigation 90 %	Irrigation 30 %
$H$ (cm)	46.15 ± 0.85 a	42.27 ± 0.76 b
$D$ (cm)	6.97 ± 0.17 a	6.01 ± 0.13 b
TLA (cm <sup>2</sup> )	1741.32 ± 83.92 a	1481.27 ± 59.57 b
LDM (g)	11.74 ± 0.46 a	8.34 ± 0.42 b
SDM (g)	7.12 ± 0.30 a	4.82 ± 0.27 b
RDM (g)	8.76 ± 0.41 a	7.11 ± 0.48 b

Lower-case letters compare data on the same line using Tukey's test ( $p < 0.05$ )

ratios did not cause significant changes in gas exchange under 90% irrigation, and this result verifies that of Guimarães et al. (2014), also using *Eucalyptus urophylla*.

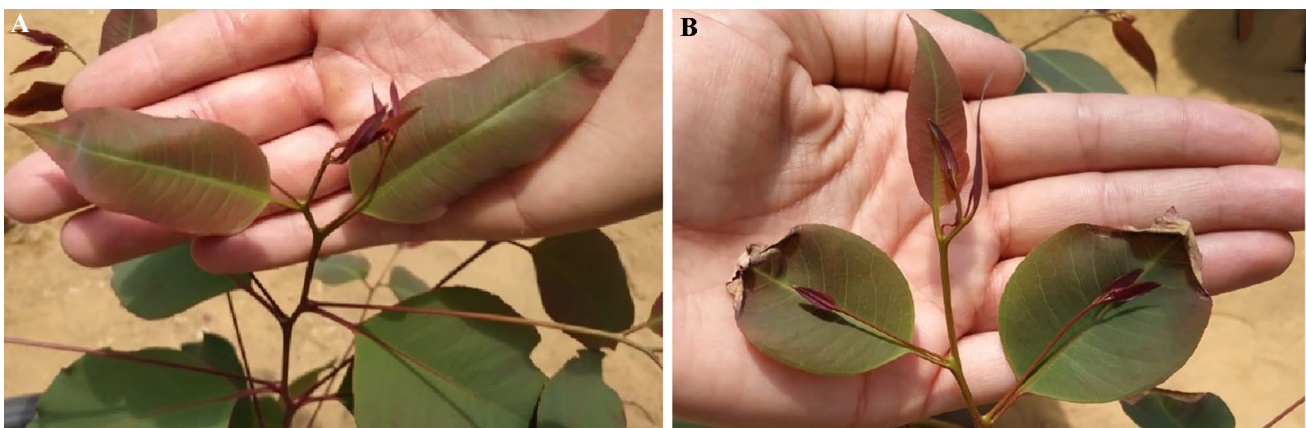
The reasons for  $\text{NH}_4^+$ -supplemented plants inducing decreased  $g_s$  remains unclear, but a more restricted osmotic adjustment is assumed to be involved. Rhizosphere acidification due to  $\text{NH}_4^+$  assimilation restricts cation absorption compared to fertilization mainly with  $\text{NO}_3^-$  sources (Basra and Goyal 2002). Decreased  $g_s$  values may reflect a low  $\text{NO}_3^-$  supply, which is not only a relevant osmolyte (McIntyre 1997; Lopes and Araus 2006), but also an essential anion for cation translocation through the xylem (Guo et al. 2003; Wang et al. 2012). According to Marschner (2012), plants mainly-fertilized with  $\text{NH}_4^+$  reveal less Ca, Mg, and K content than those with  $\text{NO}_3^-$  as the main agent. In this regard, it should be noted that a lower K content may restrict stomatal function (Laporte et al. 2002). In our study, some slight signs of root senescence were observed in plants that

were mainly  $\text{NH}_4^+$ -fertilized, which may cause decreased  $g_s$  (Basra and Goyal 2002; Britto and Kronzucker 2013).

$\text{NO}_3^-:\text{NH}_4^+$  ratios less than 0.50:0.50 caused a decrease in root dry mass (Table 2), which may be related to the limiting effect of high  $\text{NH}_4^+$  content on water uptake, leading to a hardening water stress. Therefore, it is assumed that under 30% irrigation a decrease in  $\text{NH}_4^+$  supply due to an increase in the  $\text{NO}_3^-:\text{NH}_4^+$  ratio must have contributed to improved water uptake, thus increasing leaf  $\Psi_w$ . Moreover, the increase in  $\text{NO}_3^-:\text{NH}_4^+$  ratio up to 0.75:0.25 may have contributed to maintaining leaf relative water content, in whole or in part, despite the increase in  $E$  (Fig. 1).

Increasing  $\Psi_w$  concomitant with increased  $\text{NO}_3^-:\text{NH}_4^+$  ratio under 30% irrigation corroborates Faustino et al. (2015) with *Pinus taeda*. According to those authors, increasing  $\text{NO}_3^-$  supply induces changes in root dry mass and hydraulic conductance, providing more tolerance to water deficit. However, the increase in  $\Psi_w$  may also relate to a decreased  $\text{NH}_4^+$  supply, as evidenced in studies on other species under water stress, in which  $\text{NH}_4^+$  fertilization complicates water uptake making it further even more difficult, thus reducing  $\Psi_w$  (Pill and Lambeth 1977; Wu et al. 2017). Conversely, under 90% irrigation,  $\text{NO}_3^-:\text{NH}_4^+$  ratios higher than an estimated 0.58:0.42 caused a decrease in  $\Psi_w$ , which reached the lowest value at 1.0:0.0.

The decrease in reducing sugar and starch content, as observed in plants under 30% irrigation (Fig. 3), may be another consequence of stomatal closure under water deficit, which impedes  $\text{CO}_2$  absorption, negatively affecting carbohydrate synthesis (Hartmann et al. 2020). Under 30% irrigation, increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio decreased the reducing sugar content (Fig. 3A) concomitantly with an increase in  $A$ . This suggests a short-term conversion from reducing sugar to sucrose, and subsequent source-to-sink assimilate partitioning, corroborated by a similar positive effect on plant



**Fig. 4** Symptoms of  $\text{NH}_4^+$  toxicity at stages B [5A] and C [5B] of leaf development in young *Eucalyptus urophylla* plants, clone AEC 144, fertilized by  $\text{NO}_3^-:\text{NH}_4^+$  at 0.0:1.0 and 0.25:0.75 ratios

height, stem diameter, and leaf, stem, and root dry mass (Table 2).

With 30% irrigation, increasing the  $\text{NO}_3^-:\text{NH}_4^+$  ratio up to an estimated 0.61:0.39 caused a slight decline in starch content (Fig. 3B). As the  $\text{NO}_3^-:\text{NH}_4^+$  ratio increases, so does the demand for  $\text{NO}_3^-$  reduction, which is energetically expensive (Nunes-Nesi et al. 2010) and is avoided when  $\text{NH}_4^+$  is the main N source (MacNeill et al. 2017). In tobacco (*Nicotiana tabacum* L.) and soybean (*Glycine max* L.) leaves with increasing  $\text{NO}_3^-$  supply, the starch content decreases as the carbon is reallocated to metabolites of the Krebs cycle, organic acids, and amino acids (Veau et al. 1992; Scheible et al. 1997). The increasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio in *Arabidopsis* results in a decline in starch content and an increase in metabolites associated with tricarboxylic acids (Hachiya et al. 2012; Sato and Yanagisawa 2014).

Proline content was higher in water-stressed plants, particularly in those fertilized solely with  $\text{NO}_3^-$ - or  $\text{NH}_4^+$ -over those receiving mixed N (Fig. 3C). The increase in leaf proline content usually occurs both in water deficit conditions (Hossain and Fujita 2010) and fertilization with only  $\text{NH}_4^+$  fertilization (Fernández-Crespo et al. 2012; Kováčik and Klejdus 2014; Ravazzolo et al. 2020). In our study, it suggested that either  $\text{NO}_3^-$  or  $\text{NH}_4^+$  root accumulation may be involved in a pronounced increase in proline content. In *Arabidopsis thaliana* L. mutants under water stress, increased proline content is related to  $\text{NO}_3^-$  accumulation in the roots (Chen et al. 2012). In *Triticum durum* L. (var. Amilcar), however, the increase in proline content was seen as a response to  $\text{NH}_4^+$  toxicity (Torralbo et al. 2019).

Different  $\text{NO}_3^-:\text{NH}_4^+$  ratios influenced leaf NR activity, but only in plants under 30% irrigation. This finding disagrees with other studies that suggest water deficit as an inhibitory factor of NR activity (Zahoor et al. 2017; Huang et al. 2018). Leaf NR activity increased up to an estimated 0.46:0.54 ratio with 30% irrigation. This response may be due to both the positive effect of increased  $\text{NO}_3^-$  supply on enzymatic kinetics (Sauro 2011) and to an attenuation of the inhibitory effect of  $\text{NH}_4^+$  on NR activity (Botella et al. 1993). However, this result was reversed under higher  $\text{NO}_3^-:\text{NH}_4^+$  ratios, suggesting that a high  $\text{NO}_3^-$  content may inhibit leaf NR activity (Fig. 3D). Similar findings were reported by Wang et al. (2018) and Zhang et al. (2019).

Increasing  $\text{NO}_3^-:\text{NH}_4^+$  from the 0.5:0.5 ratio attenuated leaf  $\text{NH}_4^+$  toxicity occurred with the 0.0:1.0 and 0.25:0.75 ratios (Fig. 4). Symptoms of  $\text{NH}_4^+$  toxicity also occur in other species (Guo et al. 2007; Helali et al. 2010; Wang et al. 2018), and can lead to a disruption in hormonal homeostasis (Walch-Liu et al. 2000), reduced photosynthesis (Pissolato et al. 2019), oxidative stress (Wang et al. 2010), acidification of cellular organelles, and photophosphorylation inhibition (Bittsánszky et al. 2015).

The results also showed that mixed N fertilization favored NR activity more than only  $\text{NO}_3^-$  or  $\text{NH}_4^+$  supply. Under water stress, NR activity maintenance is necessary because this enzyme acts in the synthesis of nitric oxide (Pissolato et al. 2020), which plays an important role as in mitigating water stress, as observed in other species (Cai et al. 2015; Pissolato et al. 2020).

The positive effect of increasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio on morphophysiological traits under water deficit can be attributed, to some extent, to a close relationship among  $\text{NO}_3^-$  ratio, NR activity, and A. Carbon skeletons derived from  $\text{CO}_2$  assimilation are required for amino acid synthesis, after  $\text{NO}_3^-$ - $\text{NH}_4^+$  reduction. In this process, NR acts as a key enzyme that provides metabolic regulation in  $\text{NO}_3^-$  reduction to ensure that  $\text{NH}_4^+$  production does not exceed the availability of carbon skeletons for amino acid synthesis (Heldt and Piechulla 2011).

## Conclusions

The growth of young *Eucalyptus urophylla* plants is negatively affected by water deficit, resulting in lower plant height, less stem diameter, total leaf area, and dry mass of leaves, stems, and roots. Reduction in turgor pressure, associated with decreases in stomatal conductance, net photosynthesis, and reducing sugar and starch contents are the main physiological and biochemical changes that lead to growth inhibition.

Mixed N fertilization with increased  $\text{NO}_3^-:\text{NH}_4^+$  ratio can mitigate negative water deficit effects on leaf water potential, stomatal conductance, and photosynthesis. Furthermore, leaf nitrate reductase activity improved with mixed  $\text{NO}_3^-:\text{NH}_4^+$ , compared with only fertilizing with  $\text{NO}_3^-$  or  $\text{NH}_4^+$ . Despite this, variations in N sources proved to be ineffective in preventing growth inhibition in young plants under water deficit.

The best performance of growth-related morphophysiological traits was achieved at a  $\text{NO}_3^-:\text{NH}_4^+$  ratio of 0.50:0.50, regardless of the water regime. These data can be seen as particularly relevant for plants under 30% irrigation, since fertilization with an  $\text{NO}_3^-$  ratio equal to  $\text{NH}_4^+$ , despite not preventing damage to morphophysiological traits, can mitigate water stress effects on plant growth.

**Author contributions statement** Conceptualization: PC and PS. Greenhouse plant cultivation and data collection: PS, RB, MB, MS, SM, and NM; laboratory chemical analyses: PS, RB, MB, LS, MS, and MA; data analyses and interpretation: PS, PC, and LS; original writing and English language



translation: PC and PS. All authors have provided consent for the publication of the final version of the manuscript.

## Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest.

## References

- Ashraf M, Akram NA, Al-Qurainy F, Foolad MR (2011) Drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients. In: Lorenz AJ, Chao S, Asoro FG, Heffner EL, Hayashi T, Iwata H, Smith KP, Sorrells ME, Jannink JL (eds) *Advances in agronomy*. Academic Press, London, pp 249–296. <https://doi.org/10.1016/B978-0-12-387689-8.00002-3>
- Basra AS, Goyal SS (2002) Mechanisms of improved nitrogen-use efficiency in cereals. In: Kang MS (ed) *Quantitative genetics, genomics and plant breeding*. CAB International, Louisiana State University, Baton Rouge, pp 269–288
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil*. <https://doi.org/10.1007/BF00018060>
- Bitsánszky A, Pilinszky K, Gyulai G, Komives T (2015) Overcoming ammonium toxicity. *Plant Sci*. <https://doi.org/10.1016/j.plantsci.2014.12.005>
- Botella MA, Cruz C, Martins-Loução MA, Cerdá A (1993) Nitrate reductase activity in wheat seedlings as affected by  $\text{NO}_3^-/\text{NH}_4^+$  ratio and salinity. *J Plant Physiol*. [https://doi.org/10.1016/S0176-1617\(11\)80394-9](https://doi.org/10.1016/S0176-1617(11)80394-9)
- Brasil (1999) Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Instrução Normativa nº 20, de 21 de jul. de 1999. Oficializa os métodos analíticos físico-químicos, para controle de produtos cárneos e seus ingredientes – sal e salmoura. Diário oficial da União. Brasília, DF, 27 jul. 1999
- Britto DT, Kronzucker HJ (2013) Ecological significance and complexity of N-source preference in plants. *Ann Bot*. <https://doi.org/10.1093/aob/mct157>
- Cai W, Liu W, Wang WS, Fu ZW, Han TT, Lu YT (2015) Overexpression of rat neurons nitric oxide synthase in rice enhances drought and salt tolerance. *PLoS One*. <https://doi.org/10.1371/journal.pone.0131599>
- Cao X, Zhu C, Zhong C, Hussain S, Zhu L, Wu L, Jin Q (2018) Mixed-nitrogen nutrition-mediated enhancement of drought tolerance of rice seedlings associated with photosynthesis, hormone balance and carbohydrate partitioning. *Plant Growth Regul*. <https://doi.org/10.1007/s10725-017-0352-6>
- Chen CZ, Lv XF, Li JY, Yi HY, Gong JM (2012) *Arabidopsis* NRT1.5 is another essential component in the regulation of nitrate reallocation and stress tolerance. *Plant Physiol*. <https://doi.org/10.1104/pp.112.199257>
- Cramer MD, Lewis OAM (1993) The influence of nitrate and ammonium nutrition on the growth of wheat (*Triticum aestivum*) and maize (*Zea mays*) plant. *Ann Bot*. <https://doi.org/10.1006/anbo.1993.1119>
- Faustino LI, Moretti AP, Graciano C (2015) Fertilization with urea, ammonium and nitrate produce different effects on growth, hydraulic traits and drought tolerance in *Pinus taeda* seedlings. *Tree Physiol*. <https://doi.org/10.1093/treephys/tpv068>
- Fernández-Crespo E, Camañes G, García-Agustín P (2012) Ammonium enhances resistance to salinity stress in citrus plants. *J Plant Physiol*. <https://doi.org/10.1016/j.jplph.2012.04.011>
- Ferreira DF (2011) Sisvar: um sistema computacional de análise estatística. *Ciênc Agrotec*. <https://doi.org/10.1590/s1413-7054011000600001>
- Foyer CH, Parry M, Noctor G (2003) Markers and signals associated with nitrogen assimilation in higher plants. *J Exp Bot*. <https://doi.org/10.1093/jxb/erg053>
- Fu Y-F, Zhang Z-W, Yang X-Y, Wang C-Q, Lan T, Tang X-Y, Chen G-D, Zeng J, Yuan S (2020) Nitrate reductase is a key enzyme responsible for nitrogen-regulated auxin accumulation in *Arabidopsis* roots. *Biochem Biophys Res Commun*. <https://doi.org/10.1016/j.bbrc.2020.08.057>
- Gao YX, Li Y, Yang XX, Li HJ, Shen QR, Guo SW (2010) Ammonium nutrition increases water absorption in rice seedlings (*Oryza sativa* L.) under water stress. *Plant Soil*. <https://doi.org/10.1007/s11104-009-0245-1>
- Guimarães MMC, Cairo PAR, Neves OSC (2014) Crescimento de *Eucalyptus urophylla* em meio hidropônico com diferentes proporções de nitrato e amônio. *Floresta Ambient*. <https://doi.org/10.4322/ffloram.2014.011>
- Guo FQ, Young J, Crawford NM (2003) The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in *Arabidopsis*. *Plant Cell*. <https://doi.org/10.1105/tpc.006312>
- Guo S, Zhou Y, Shen Q, Zhang F (2007) Effect of ammonium and nitrate nutrition on some physiological processes in higher plants—growth, photosynthesis, photorespiration, and water relations. *Plant Biol*. <https://doi.org/10.1055/s-2006-924541>
- Hachiya T, Sakakibara H (2016) Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *J Exp Bot*. <https://doi.org/10.1093/jxb/erw449>
- Hachiya T, Watanabe CK, Fujimoto M, Ishikawa T, Takahara K, Kawai-Yamada M, Uchimiya H, Uesono Y, Terashima I, Noguchi K (2012) Nitrate addition alleviates ammonium toxicity without lessening ammonium accumulation, organic acid depletion and inorganic cation depletion in *Arabidopsis thaliana* shoots. *Plant Cell Physiol*. <https://doi.org/10.1093/pcp/pcs012>
- Hartmann H, Bahn M, Carbone M, Richardson AD (2020) Plant carbon allocation in a changing world - challenges and progress: introduction to a Virtual Issue on carbon allocation. *New Phytol*. <https://doi.org/10.1111/nph.16757>
- Hawkesford W, Horst M, Kichey T, Lambers H, Schjoerrin J, Möller IS, White P (2012) Functions of macronutrients. In: Marschner P (org.) *Marschner's mineral nutrition of higher plants*. Academic Press, London, pp 135–189. <https://doi.org/10.1016/B978-0-12-384905-2.00006-6>
- Helali SM, Nebli H, Kaddour R, Mahmoudi H, Lachaâl M, Ouerghi Z (2010) Influence of nitrate-ammonium ratio on growth and nutrition of *Arabidopsis thaliana*. *Plant Soil*. <https://doi.org/10.1007/s11104-010-0445-8>
- Heldt H-W, Piechulla B (2011) *Plant biochemistry*. Academic Press, London
- Hoagland DR, Arnon DI (1952) The water culture method for growing plants without soil. The College of Agriculture, University of California, Berkeley
- Holzschuh MJ, Bohnen H, Anghinoni I, Pizzolato TM, Carmona FDC, Carlos FS (2011) Absorção de nutrientes e crescimento do arroz com suprimento combinado de amônio e nitrato. *Rev Bras Ciênc Solo*. <https://doi.org/10.1590/S0100-06832011000400030>
- Hossain MA, Fujita M (2010) Evidence for a role of exogenous glycinebetaine and proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under

- salt stress. *Physiol Mol Biol Plants*. <https://doi.org/10.1007/s12298-010-0003-0>
- Huang L, Li M, Zhou K, Sun T, Hu L, Li C, Ma F (2018) Uptake and metabolism of ammonium and nitrate in response to drought stress in *Malus prunifolia*. *Plant Physiol Bioch*. <https://doi.org/10.1016/j.plaphy.2018.03.031>
- Kováčik J, Klejdus B (2014) Induction of phenolic metabolites and physiological changes in chamomile plants in relation to nitrogen nutrition. *Food Chem*. <https://doi.org/10.1016/j.foodchem.2013.07.074>
- Laporte MM, Shen B, Tarczynski MC (2002) Engineering for drought avoidance expression of maize NADP-malic enzyme in tobacco results in altered stomatal function. *J Exp Bot*. <https://doi.org/10.1093/jexbot/53.369.699>
- Li SX, Wang ZH, Stewart BA (2013) Responses of crop plants to ammonium and nitrate N. *Adv Agron*. <https://doi.org/10.1016/B978-0-12-405942-9.00005-0>
- Liu B, Wu J, Yang S, Schiefelbein J, Gan Y (2019) Nitrate regulation of lateral root and root hair development in plants. *J Exp Bot*. <https://doi.org/10.1093/jxb/erz536>
- Lopes MS, Araus JL (2006) Nitrogen source and water regime effects on durum wheat photosynthesis and stable carbon and nitrogen isotope composition. *Physiol Plant*. <https://doi.org/10.1111/j.1399-3054.2006.00595.x>
- MacNeill GJ, Mehrpouyan S, Minow MAA, Patterson JA, Tetlow IJ, Emes MJ (2017) Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. *J Exp Bot*. <https://doi.org/10.1093/jxb/erx291>
- Marschner P (2012) *Marschner's mineral nutrition of higher plants*, 3rd edn. Academic Press, London
- Mcintyre GI (1997) The role of nitrate in the osmotic and nutritional control of plant development. *Aust J Plant Physiol*. <https://doi.org/10.1071/PP96064>
- Meier M, Liu Y, Lay-Pruitt KS, Takahashi H, Von Wirén N (2020) Auxin-mediated root branching is determined by the form of available nitrogen. *Nat Plants*. <https://doi.org/10.1038/s41477-020-00756-2>
- Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem*. <https://doi.org/10.1021/ac60147a030>
- Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. *Mol Plant*. <https://doi.org/10.1093/mp/ssq049>
- Oliveira Júnior OA, Cairo PAR, Novaes AB (2011) Características morfológicas associadas à qualidade de mudas de *Eucalyptus urophylla* produzidas em diferentes substratos. *Rev Árvore*. <https://doi.org/10.1590/S0100-67622011000700003>
- Pill WG, Lambeth VN (1977) Effects of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  nutrition with and without pH adjustment on tomato growth, ion composition, and water relation. *J Am Soc Hortic Sci* 102:78–81
- Pissolato MD, Silveira NM, Machado EC, Zambrosi FCB, Sodek L, Ribeiro RV (2019) Photosynthesis and biomass accumulation in young sugarcane plants grown under increasing ammonium supply in nutrient solution. *Theor Exp Plant Physiol*. <https://doi.org/10.1007/s40626-019-00154-w>
- Pissolato MD, Silveira NM, Prataviera PJC, Machado EC, Seabra AB, Pelegrino MT, Sodek L, Ribeiro RV (2020) Enhanced nitric oxide synthesis through nitrate supply improves drought tolerance of sugarcane plants. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2020.00970>
- Ravazzolo L, Trevisan S, Forestan C, Varotto S, Sut S, Dall'acqua S, Malagoli M, Quaggiotti S (2020) Nitrate and ammonium affected the overall maize response to nitrogen availability by triggering specific and common transcriptional signatures in roots. *Int J Mol Sci*. <https://doi.org/10.3390/ijms21020686>
- Rothstein DE, Cregg BM (2005) Effects of nitrogen form on nutrient uptake and physiology of Fraser fir (*Abies fraseri*). *For Ecol Manag*. <https://doi.org/10.1016/j.foreco.2005.08.043>
- Sato S, Yanagisawa S (2014) Characterization of metabolic states of *Arabidopsis thaliana* under diverse carbon and nitrogen nutrient conditions via targeted metabolomic analysis. *Plant Cell Physiol*. <https://doi.org/10.1093/pcp/pct192>
- Sauro HM (2011) *Enzyme kinetics for systems biology*. Future Skill Software and Ambrosius Publishing, Lexington
- Scheible WR, Gonzalez-Fontes A, Lauerer M, Muller-Rober B, Caboche M, Stitt M (1997) Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell*. <https://doi.org/10.1105/tpc.9.5.783>
- Scholander PF, Bradsterret ED, Hemmingsen EA, Hammel HT (1965) Sap pressure in vascular plants. *Science*. <https://doi.org/10.1126/science.148.3668.339>
- Sharma A, Kumar V, Shahzad B, Ramakrishnan M, Sidhu GPS, Bali AS, Handa N, Kapoor D, Yadav P, Khanna K, Bakshi B, Rehman A, Kohli SK, Khan EA, Parihar RD, Yuan H, Thukral AK, Bhardwaj R, Zheng B (2020) Photosynthetic response of plants under different abiotic stresses: a review. *J Plant Growth Regul*. <https://doi.org/10.1007/s00344-019-10018-x>
- Silva JRJ, Cairo PAR, Bomfim RAA, Barbosa MP, Souza MO, Leite TC (2020) Morphological and physiological changes during leaf ontogeny in genotypes of *Eucalyptus* young plants. *Trees*. <https://doi.org/10.1007/s00468-020-01955-2>
- Tabatabaei SJ, Fatemi LS, Fallahi E (2006) Effect of ammonium:nitrate ratio on yield, calcium concentration, and photosynthesis rate in strawberry. *J Plant Nutr*. <https://doi.org/10.1080/01904160600767575>
- Taiz L, Zeiger E, Moller IM, Murphy A (2017) *Fisiologia e desenvolvimento vegetal*, 6th edn. Artmed, Porto Alegre
- Torralbo F, González-Moro MB, Baroja-Fernández E, Aranjuelo I, González-Murua C (2019) Differential regulation of stomatal conductance as a strategy to cope with ammonium fertilizer under ambient versus elevated  $\text{CO}_2$ . *Front Plant Sci*. <https://doi.org/10.3389/fpls.2019.00597>
- Veau EJI de, Robinson JM, Warmbrodt RD, Kremer DF (1992) Photosynthetic metabolism in the source leaves of  $\text{N}_2$ -fixing soybean plants. *Plant Physiol*. <https://doi.org/10.1104/pp.99.3.1105>
- Walch-Liu P, Neumann G, Bangerth F, Engels C (2000) Rapid effects of nitrogen form on leaf morphogenesis in plants. *J Exp Bot*. <https://doi.org/10.1093/jexbot/51.343.227>
- Wang C, Zhang SH, Wang PF, Li W, Lu J (2010) Effects of ammonium on the antioxidative response in *Hydrilla verticillata* (L.f.) royle plants. *Ecotox Environ Safe*. <https://doi.org/10.1016/j.ecoenv.2009.08.012>
- Wang R, Chen L, Chen J, Chen Y, Zhang Z, Wang X, Peng Y, Peng S, Li A, Wei X (2018) Different nitrate and ammonium ratios affect growth and physiological characteristics of *Camellia oleifera* Abel seedlings. *Forests*. <https://doi.org/10.3390/f9120784>
- Wang Y-Y, Hsu P-K, Tsay Y-F (2012) Uptake, allocation and signaling of nitrate. *Trends Plant Sci*. <https://doi.org/10.1016/j.tplants.2012.04.006>
- Waraich EA, Ahmad R, Ashraf MY (2011) Role of mineral nutrition in alleviation of drought stress in plants. *Aust J Crop Sci* 5:764–777
- Warren CR (2006) Potential organic and inorganic N uptake by six *Eucalyptus* species. *Funct Plant Biol*. <https://doi.org/10.1071/fp06045>
- Warren CR (2009) Uptake of inorganic and amino acid nitrogen from soil by *Eucalyptus regnans* and *Eucalyptus pauciflora* seedlings. *Tree Physiol*. <https://doi.org/10.1093/treephys/tpn037>
- Weatherley PE (1950) Studies in the water relations of the cotton plant: the field measurements of water deficits in leaves. *New Phytol*. <https://doi.org/10.1111/j.1469-8137.1950.tb05146.x>

- Woolfolk WTM, Friend AL (2003) Growth response of cottonwood roots to varied  $\text{NH}_4^+:\text{NO}_3^-$  ratios in enriched patches. *Tree Physiol.* <https://doi.org/10.1093/treephys/23.6.427>
- Wu H, Chen X, Zhang Y, Wu F (2017) Effects of different  $\text{NH}_4^+/\text{NO}_3^-$  ratio and water condition on physiological characteristics of rice seedlings. *Agric Sci Technol* 18:1908–1911
- Zahoor R, Zhao W, Abid M, Dong H, Zhou Z (2017) Potassium application regulates nitrogen metabolism and osmotic adjustment in cotton (*Gossypium hirsutum* L.) functional leaf under drought stress. *J Plant Physiol.* <https://doi.org/10.1016/j.jplph.2017.05.001>
- Zhang L, Gao M, Li S, Li S, Liang Z (2011a) Growth, water status and photosynthesis in two maize (*Zea mays* L.) cultivars as affected by supplied nitrogen form and drought stress. *Pak J Bot* 43:1995–2001
- Zhang L, Wang K, Zhang X, Lu L, Li Y, Gao M, Wang C, Hu J, Liang Z (2011b) Role of nitrate nutrition in alleviation of the adverse effects of drought stress on maize cultivars: biomass production and antioxidative capacity. *Pak J Bot* 43:2869–2874
- Zhang J, Lv J, Dawuda MM, Xie J, Yu J, Li J, Xiaodan Z, Tang C, Wang C, Gan Y (2019) Appropriate ammonium-nitrate ratio improves nutrient accumulation and fruit quality in pepper (*Cap-sicum annuum* L.). *Agronomy* 10:10. <https://doi.org/10.3390/agronomy9110683>

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