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Nutritional management and physiological responses of *Atriplex nummularia* Lindl. on the improvement of phytoextraction in salt-affected soil

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ABSTRACT

Soil salinity is a significant abiotic stress and poses risks to environmental sustainability. Thus, the improvement of the time for recovering the salt-affect soil is crucial for the phytoextraction process using halophytes plants, especially regarding on nutritional management. We evaluated the responses of *Atriplex nummularia* Lindl. to nitrogen (N) and phosphorus (P) under different salinity levels. The treatments comprised doses of N (N1 = 80 kg ha^{-1}) and P (P1 = 60 kg ha^{-1}): (1) without N and P (N0P0) (control); (2) with N and without P (N1P0); (3) without N and with P (N0P1); and (4) with N and P (N1P1) and five levels of electrical conductivity from irrigation water: 0.08, 1.7, 4.8, 8.6, and 12.5 dS m^{-1}. The. We evaluated dry biomass of leaves, stems, and roots 93 days after transplantation. We also assessed the leaf and osmotic water potential, the osmotic adjustment, and the nutrient contents (N, P, Na, and K). N application increased 22.3, 17.8, and 32.8% the leaf biomass, stem biomass, and osmotic adjustment, respectively; and consequently, boosts Na extraction in 27.8%. Thus, the time of the phytoextraction process can be improved with N fertilizer at a rate of 80 kg ha^{-1}.

NOVELTY STATEMENT

Very few studies have investigated the nutrient dynamics responses in *Atriplex* species in salt-affected soils; thus, this study represents a novelty. We tested the management of nitrogen (N) and phosphate (P) fertilizers to increase crop yield and optimize the phytoextraction process in salt-affected soils. We believe our results contribute to the improvement of the knowledge of this relevant topic, mainly in terms of the recovery of areas degraded by salinity. There is a paucity of studies associating salinity and nutritional management of soils worldwide.

KEYWORDS
Abiotic stress; leaf nitrogen; leaf phosphorus; osmotic adjustment; water relations

Introduction

Soil salinity is a significant abiotic stress and poses risks to environmental sustainability (Gheyi *et al.* 2022). Salinity affects roughly 833 million ha worldwide (FAO 2021). Salt accumulation in the rhizosphere hinders crop yield, due to damage to plant growth, nutritional balance changes, and toxicity caused by the excess of ions absorbed by plants (Munns *et al.* 2020a, 2020b).

Excess of salts in the soil leads to phosphorus (P) deficiency (Dey *et al.* 2021), which affects plant metabolism and nitrogen (N) uptake, reducing nitrate and ammonium assimilation (Bouras *et al.* 2021). Inhibition of water absorption, influenced by the osmotic potential, causes nutritional deficiency to plants in saline soils (Munns *et al.* 2020b). Moreover, high ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}, and Cl^-/NO_3^- in the soil cause toxicity and nutritional imbalance in salt-affected soils (Taufiq *et al.* 2018).

Halophytes are plant species capable of surviving in salinity-affected soils, as they tolerate concentrations >200 mM NaCl in the soil solution (Nikalje *et al.* 2019; Levinsh 2020). *Atriplex nummularia* Lindl. is a halophyte with high tolerance to salinity (Ramos *et al.* 2004; Souza *et al.* 2012). There are many reports in the literature on the physiology of *Atriplex* species (Lins *et al.* 2018; Paulino *et al.* 2020; Monteiro *et al.* 2024; Pasalar *et al.* 2024); nevertheless, few studies have investigated the nutrient dynamics in saline soils as well as the response of *Atriplex* species to nitrogen (N) and phosphate (P) fertilization to increase crop yield and optimize the phytoextraction of salts (Cunha *et al.* 2017; Zouari *et al.* 2023).

The recovery time of salt-affected soils can be reduced with the use of mineral and organic fertilizers in the phytoextraction process (Karimi *et al.* 2021; Eissa *et al.* 2022). Very few studies have investigated the nutrient dynamics responses in *Atriplex* species in salt-affected soils. Cunha

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et al. (2017) tested the efficiency of nitrogen and phosphorus fertilization in *A. nummularia* Lindl. in the highly saline soil and it was observed the increasing of Na extraction with the N application. The nitrogen fertilization seems improve the performance of different *Atriplex* species (*A. nummularia*, *A. lentiformis*, and *A. canescens*) with increasing of biomass. However, it is important to highlight the lack of results regarding on nutritional management and physiological responses in different electrical conductivity of irrigation water (Cunha *et al.* 2017; Karimi *et al.* 2021; Eissa *et al.* 2022; Pasalar *et al.* 2024).

The present study aims to provide a better understanding of plant growth, biomass production, water relations, and nutrient extraction capacity of *A. nummularia* Lindl. in terms of N and P availability in the soil under different salinity levels of the irrigation water to optimize the phytoextraction process of salt in the recovery of salt-affected soils.

Material and methods

Soil sampling and initial characterization

The cultivation substrate for the experiment was Fluvic Neosol from the Nossa Senhora do Rosário settlement in the municipality of Pesqueira, Pernambuco State, Brazil (8°23′46.1″ S 36°51′34.8″ W), located in the Agreste region of the state (Figure 1).

The soil was sampled at a layer 0–30 cm deep. The soil was air-dried, crushed, and sieved through a 4-mm mesh to preserve the microaggregates and field representation. The physical and chemical analyses were carried out on samples of air-dried fine earth.

For the soil physical characterization were determined the particle size composition (sand, silt, and clay), water-dispersed clay (WDC), dispersion degree (DD), flocculation degree (FD), bulk density (BD), particle density (PD), total porosity (TP) and soil moisture at field capacity according to Teixeira *et al.* (2017) (Table 1).

For chemical characterization, a saturation paste was prepared in the soil solution and the electrical conductivity (EC) of the saturation extract was measured (Richards 1954). For the exchangeable fraction of the soil, the pH was measured in water (1:2.5) and in KCl solution, the potential acidity (H + Al) was extracted with 1.0 mol L^{-1} of calcium acetate at pH 7.0 and determined volumetrically with NaOH solution (Teixeira *et al.* 2017). The exchangeable cations Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with KCl 1.0 mol L^{-1} and determined using atomic absorption spectrophotometry, while Al^{3+} was quantified by titration with NaOH 0.025 mol L^{-1}.

K^+, Na^+, and available P were extracted with the Mehlich-1 solution (HCl 0.05 mol L^{-1} and H_2SO_4 0.0125 mol L^{-1}). K^+ and Na^+ were measured by flame photometry and P was quantified by colorimetry. Total organic carbon (TOC) was quantified through the organic matter (OM) oxidation by potassium dichromate, according to the Yeomans and Bremmer (1988) method adapted from Pribyl (2010) (Table 2). The results were used to calculate the sum of bases (SB), effective cation exchange capacity (CEC), total CEC, percentage of saturation per base, and percentage of exchangeable Na.

Seedlings of *A. nummularia* Lindl

The seedlings were propagated *via* cuttings from a single matrix to reduce genetic variability between clones (Figure 2A). Seedlings at four months of age were transplanted into pots filled with 5 kg of soil (Figure 2B). The experiment was conducted in a greenhouse for 93 days (Figure 2C).

Treatments

The treatments consisted of applying doses of N (N1 = 80 kg ha^{-1}) and P (P1 = 60 kg ha^{-1}) as follows: (1) without addition of N and P (N0P0) (control treatment), (2) with addition of N and without P addition (N1P0), (3) without N addition and with P addition (N0P1), and with addition of N and P (N1P1) in combination with irrigation using five EC levels in the irrigation water (0.08, 1.7, 4.8, 8.6, and 12.5 dS m^{-1}) forming a 4 × 5 double factorial with four replications totaling 80 experimental units in a randomized block design. Ammonium sulfate and triple superphosphate were the sources for fertilization. The N and P doses were based on the results found by Cunha *et al.* (2017). The soil moisture was kept at 70% of the field capacity and the irrigation was carried out daily by weighing the pots.

Irrigation water

Irrigation water was prepared using salts NaCl, $CaCl_2.2H_2O$, and $MgSO_4.7H_2O$ at the proportion 7:2:1, respectively (Table 3) to simulate the proportions of ions predominant in the water sources used for irrigation in the Brazilian Northeast (Costa and Medeiros 2017).

Assessments of plants

Leaf water potential (Ψw)

The leaf water potential (Ψw) was measured at 80 days after transplantation (DAT) at predawn (Ψpdw) in apical branches using the Scholander Pressure chamber (model 1515D Pressure Chamber Instrument, PMS Instrument Company, Albany, OR, USA).

Osmotic potential (Ψo)

The leaf osmotic potential (Ψo) was determined in the sap of the same leaves used for water potential. The sap was obtained by macerating the leaves with liquid N and centrifuging at 10,000 g for 10 min at 4 °C until the plant residues were decanted and a translucent supernatant was obtained. A 10 µL aliquot was used to determine osmolality using a Vapor Pressure Osmometer (Wescor Vapro® Model 5600). The Ψo was estimated using the van't Hoff equation (Equation 1) (Souza *et al.* 2012).

$$\Psi o(\text{MPa}) = -RTC \tag{1}$$

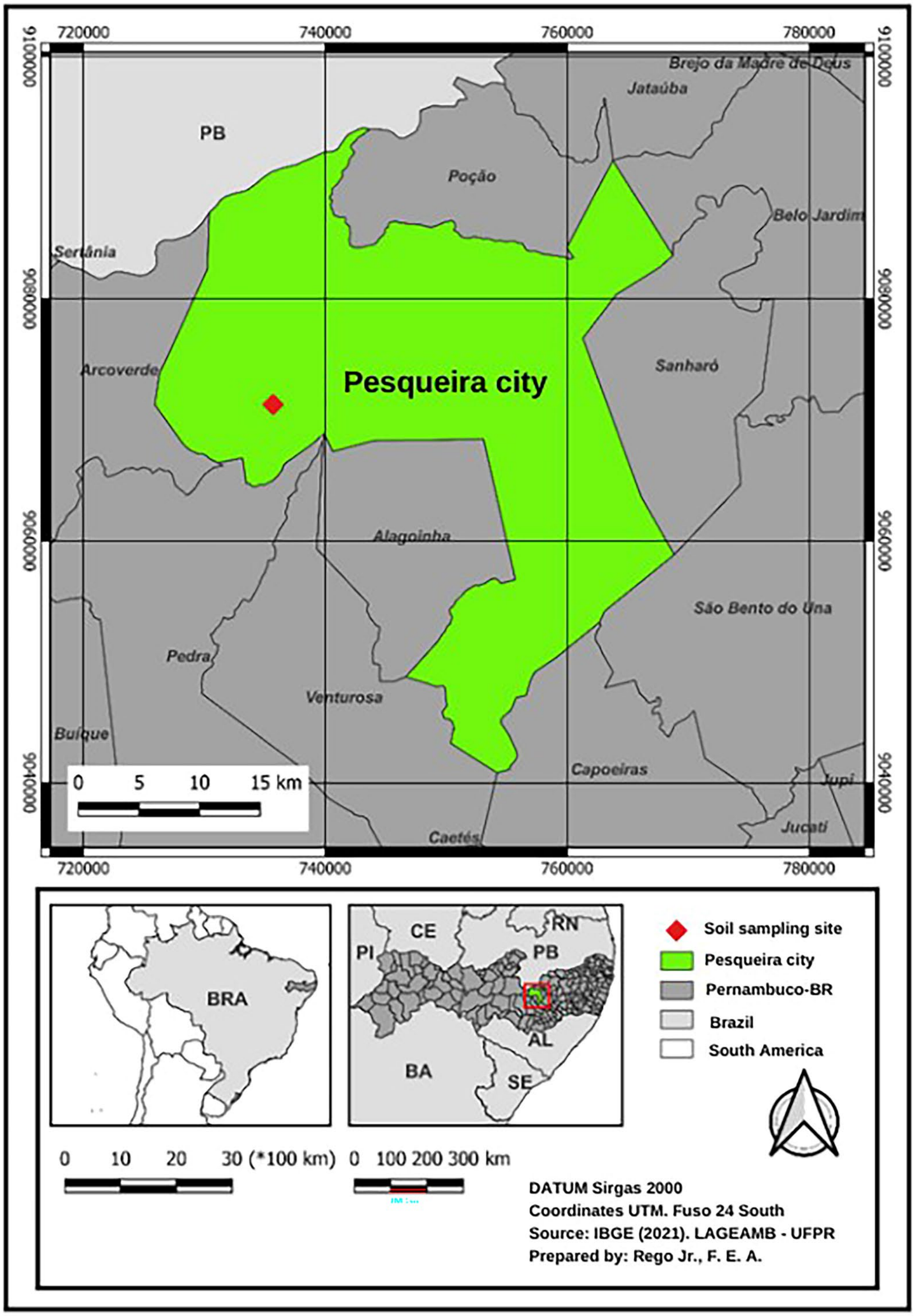

Figure 1. Geographic location of the municipality of Pesqueira, Pernambuco State, Brazil.

Where: R is the universal gas constant ($8.314.10^{-6}$ MPa m^3 mol^{-1} K^{-1}), T is the absolute temperature (K), and C is the solute concentration expressed in mol kg^{-1} (Souza *et al.* 2012).

Osmotic adjustment

The osmotic adjustment (OA) was determined at 80 DAT in leaves from the middle third of the plant. The leaves were saturated with deionized water for 24 h in the dark at 4 °C. After reaching turgor, water excess in the leaf was removed using a paper towel and the leaf was subsequently macerated in liquid N. The sap was then extracted, filtered, and centrifuged at 10,000 g for 10 min at 4 °C. Finally, the supernatant

Table 1. Initial physical characterization of the Fluvic Neosol used in the experimental units.

Total sand	Silt	Clay	WDC	BD	PD	DD	FD	TP	Θcc
--------------	$g.kg^{-1}$	--------------		--- $g.cm^{-3}$ ---		---------	%	---------	cm^3 cm^{-3}
200	560	240	100	1.34	2.60	42	58	0.49	0.46

DD: ADC/Clay; FD: (1 − DD); DD: dispersion degree; FD: flocculation degree; TP: total porosity.; Θcc: volumetric soil moisture at field capacity.

was collected. A 10 µL aliquot was used to determine osmolality using a Vapor Pressure Osmometer (Wescor Vapro® Model 5600). The Ψo was estimated using the van't Hoff equation 1. The OA was obtained from equation 2 (Blum and Jordan 1985).

$$OA_{total} = \Psi ne^{100} - \Psi e^{100} \quad (2)$$

Table 2. Initial chemical characterization of the Fluvic Neosol used in the experimental units.

Variables	Value
	Saturation Extract
EC (dS m^{-1})	1.51
	Exchange complex
pH (1:2.5)	5.76
Na^+ ($cmol_c$ dm^{-3})	0.57
K^+ ($cmol_c$ dm^{-3})	0.40
Ca^{2+} ($cmol_c$ dm^{-3})	11.68
Mg^{2+} ($cmol_c$ dm^{-3})	3.90
P (mg kg^{-1})	120.51
SB ($cmol_c$ dm^{-3})	16.54
Al^{3+} ($cmol_c$ dm^{-3})	0.00
$H^+ + Al^{3+}$ ($cmol_c$ dm^{-3})	3.07
Effective CEC ($cmol_c$ dm^{-3})	16.54
T (%)	19.61
V (%)	84.33
ESP (%)	2.89
TOC (g kg^{-1})	18.17

EC: electrical conductivity; SB: sum of bases; $H^+ + Al^{3+}$: potential acidity; effective CEC: effective cation exchange capacity; T: total cation exchange capacity; V: saturation percentage per base; ESP: exchangeable sodium percentage; TOC: total organic carbon.

Where: OA_{total} is the total osmotic adjustment, Ψne^{100} is the osmotic potential of plants standard treatment (control group) at full turgor, and Ψe^{100} is the osmotic potential of plants subjected to saline stress at full turgor.

Mineral composition of plant tissues

Plant shoots were collected at the end of the experiment (93 DAT). The plants were cut close to the soil surface and separated into leaves and stems. On the other hand, the roots collected were cleaned under running water until complete removal of the soil and then dried with paper towels. The plant materials (leaves, stems, and roots) were dried in a forced air circulation oven at 65 °C, until constant weight to determine the dry mass weight.

The dry mass of leaves, stems, and roots were ground in a Willey mill, and sulfuric digestion was carried out (Silva 2009). The N concentration was determined using the Kjeldahl method (Silva 2009), where the plant extract was transferred to the Kjeldahl semi-micro distiller. The distilled sample was titrated with sulfuric acid solution to determine total N. The extraction of sodium (Na), potassium (K), and phosphorus (P) was carried out with HCl 1 mol L^{-1} according to the methodology described in Teixeira *et al.* (2017). Subsequently, the Na and K concentration were determined by flame emission photometry (Digimed, model DM-64-4E), while the P concentration was determined by photocolorimetry (BEL Photonics, model 1105). The Na, K, P, and N were evaluated considering the concentration and content. The units from concentration and content are g kg^{-1} and

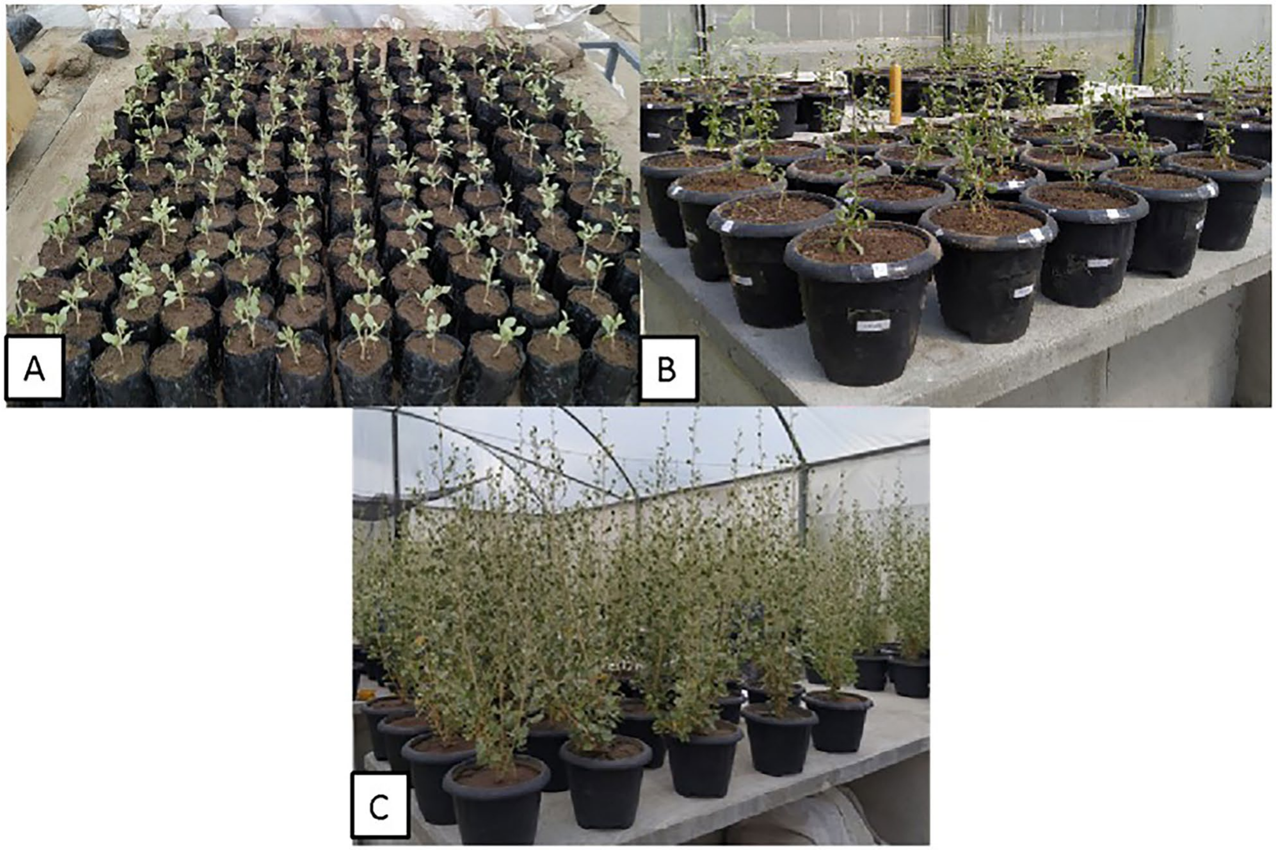

Figure 2. Development of seedlings (A), acclimatization phase of transplanted seedlings (B), and experimental units at 93 days after transplantation (DAT) (C).

g plant^{-1}, respectively. The expression content was used only to Na because the potential phytoextraction in *A. nummularia* Lindl.

Statistical analyses

The experiment was designed in randomized block in a double factorial with 4 types of fertilization and 5 levels of electrical conductivity with 4 replicates. After the tests of homogeneity of variances and normality, the results of the soil and plant analyses were subjected to descriptive statistics using measurements of central tendency and dispersion. In addition, the ANOVA, Scott-Knott test ($p < 0.05$), and adjustments of regression equations were made.

Results and discussion

Water potential and osmotic potential of leaves

The water potential (Ψw) and the osmotic potential (Ψos) of leaves of *A. nummularia* Lindl. plants showed a linear behavior inversely proportional to the EC increase of irrigation water (Figure 3).

The most negative values of Ψw were observed in plants irrigated with higher salinity water (12.5 dS m^{-1}), obtaining −3.84 MPa, while in less saline treatments (0.08 and 1.7 dS m^{-1}), the lowest values were −2.57 and −2.46 MPa, respectively (Figure 3A).

Table 3. Salt concentration (g L^{-1}) to obtain the electrical conductivity values of the irrigation water used in the experiment.

EC dS m^{-1}	Salt concentration (g L^{-1}) NaCl	 $CaCl_2.2H_2O$	 $MgSO_4.7H_2O$	Total g L^{-1}
0.08	–	–	–	–
1.7	0.5936	0.1696	0.0848	0.848
4.8	1.834	0.524	0.262	2.62
8.6	3.64	1.04	0.520	5.20
12.5	5.418	1.548	0.774	7.74

EC: electrical conductivity.

The effect of salinity on Ψos of *A. nummularia* Lindl. at 80 DAT was represented by accumulated reductions of 33.50, 9.93, 47.01, and 87.25% under 1.7, 4.8, 8.6, and 12.5 dS m^{-1}, respectively (Figure 3B).

The reduction of Ψw of *A. nummularia* Lindl. plants in response to salinity is an adaptive strategy mediated by the accumulation of high concentrations of inorganic ions and/or organic solutes (Munns *et al.* 2020a), which contributes to water absorption regulation and ensures plant growth in soils with salt excess (Munns *et al.* 2020b).

There was a decrease in Ψw and Ψos as salt concentration increased; however, the potential values of the plants evaluated were similar, regardless of the N and P treatments. This shows that plants maintained the water status, confirming the capacity of *A. nummularia* Lindl. to thrive under saline stress conditions.

Plant survival under saline conditions concerns their capacity to maintain cell turgor at low osmotic potential and keep low levels of cytosolic Na^+ with high K^+/Na^+ ratios. These resources ensure the biochemical processes of the OA (Paulino *et al.* 2020).

In response to both water stress (30% of field capacity) and saline stress (100 mM NaCl), *A. canescens* exhibits a reduction in its Ψos of 76% compared to the control treatment, reaching values below −2 MPa (Guo *et al.* 2020). Similarly, other *Atriplex* species, including *A. halimus* and *A. atacamensis*, have been observed to exhibit similar responses to saline stress (0.5% NaCl) and water stress (PEG) with Ψos values lower than −2.5 MPa (Orrego *et al.* 2020). The reduction in their potentials contributes to maintaining the water status of the crop under conditions of salinity and/or water deficit. This is often evidenced by the maintenance of relative water content (Guo *et al.* 2020; Orrego *et al.* 2020; Paulino *et al.* 2020)."

Osmotic adjustment (OA)

The interaction between saline levels and nutritional management was significant ($p < 0.05$) to the OA of *A. nummularia* Lindl. plants (Figure 4).

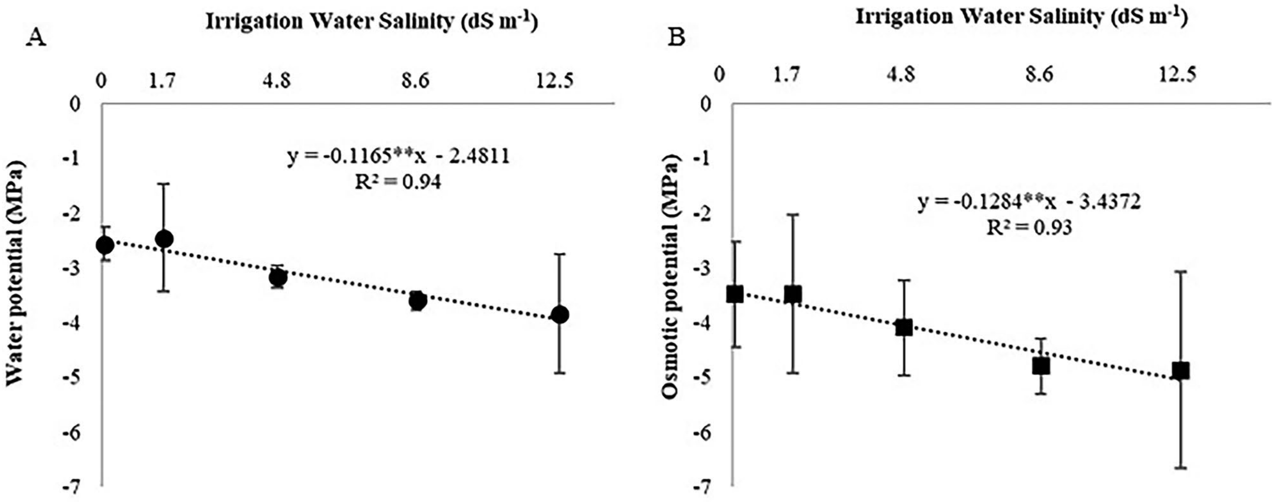

Figure 3. Leaf water potential (A) and leaf osmotic potential (B) of *A. nummularia* Lindl. under different salinity levels of irrigation water at 80 DAT. **Significant at $p \leq 0.01$ by *F* test.

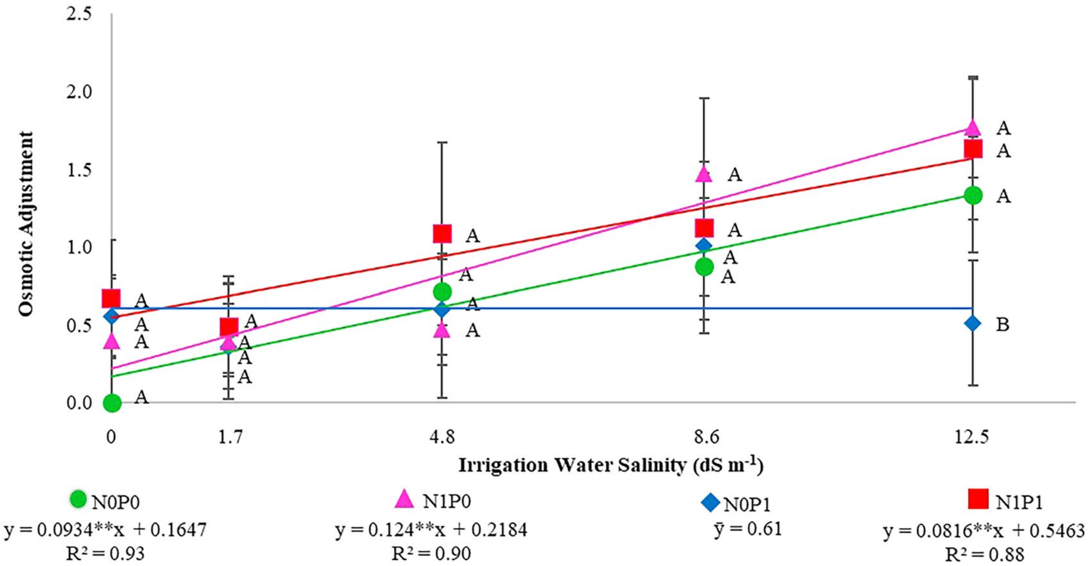

Figure 4. Osmotic adjustment of *A. nummularia* Lindl. under different salinity levels of irrigation water and nitrogen and phosphorus fertilization management at 80 DAT. Means followed by equal letters do not differ from each other using the Scott-Knott test ($p < 0.05$). N0P1 did not obtain significant regression analysis ($p > 0.05$). **Significant at $p \leq 0.01$ by *F* test.

For N and P management (N0P0, N1P0, N1P1), a linear increase is observed as the salinity levels of irrigation water increase. For N0P1, no significant effect was observed by the regression analysis, with an average OA value of 0.61.

Salinity affects the OA values of *A. nummularia* Lindl. subjected to N and P fertilization. Plants irrigated with saline water showed an increase in the OA values as the salt concentration increased, except for the N0P1 management.

In both *Suaeda salsa*, a halophytic plant known for thriving in low nitrogen concentration areas, and in *Atriplex* species, nitrogen supply has been demonstrated to enhance nutrient extraction, particularly under saline conditions (Cunha *et al.* 2017; Wang *et al.* 2022; Pasalar *et al.* 2024). The expansion of the root system, with an emphasis on elongating the roots closer to the surface and increasing their depth, can contribute to ionic balance and nutrient absorption. This facilitates the uptake of halophyte ions (Na^+ and K^+), consequently contributing to OA in crops (Cunha *et al.* 2017; Wang *et al.* 2022).

An excess of P in the soil can result in a reduction in K^+ absorption due to an antagonistic effect between these elements. This effect is exacerbated under water stress and, similarly, under saline stress, the contribution of potassium to OA may decline due to an increased contribution of sodium. For instance, in *A. canescens* plants, the contribution of K^+ to OA decreased from 54 to 32%, and in *Karelinia caspia*, it dropped from 44 to 7%, when compared to control plants and those under saline stress (Guo *et al.* 2020; Li *et al.* 2022).

The contribution of inorganic solutes (Na^+ and K^+) to OA is contingent upon the inherent characteristics of the studied species. In species that exclude Na^+, K^+ assumes a more significant role in OA, whereas in species that exclude K^+, Na^+ assumes a more prominent position (Levinsh *et al.* 2021). Consequently, when contemplating the implications of P fertilization, it is essential to consider the species and the inorganic solute that is most relevant for OA.

The OA in response to salt stress is a physiological adaptation of plants subjected to salt-affected environments (Nikalje *et al.* 2019; Munns *et al.* 2020b). The OA efficiency requires the use of Na^+ and Cl^- in the vacuoles and K^+, leading to a decrease in the toxic concentration of ions in the cytoplasm (Nikalje *et al.* 2019).

The role of Na^+ in the OA is often discussed in halophytes. In the present study, findings of Na concentration in plant tissues are related to Na contribution to plant adaptation to salt-induced stress conditions. It is proposed that both K^+ and Na^+ play a role in the OA of plants in response to high soil salinity and that Na^+ ions contribute more efficiently than K^+ ions in this response (Ramos *et al.* 2004). Levinsh (2020) also observed evidence of a high relative contribution of saline ions when plants were subjected to substrates with concentrations between 0.88 and 11.9 g kg^{-1} of NaCl (3.8 and 39.8 dS m^{-1}) from the soil saturation extract to *Atriplex glabriuscula*.

In halophytic plants, sodium is extensively utilized in the osmotic adjustment process. This occurs due to several factors, including the high mobility of the element within the plant, the capacity of compartmentalization, the partial substitution of potassium, and the lower energy expenditure compared to the production of compatible organic solutes (Munns *et al.* 2020a; Levinsh *et al.* 2021; Wang *et al.* 2022). These factors become evident due to hyperaccumulation of Na^+ in the aerial part of the plant, as observed by Levinsh (2020) in *A. glabriuscula* plants under salinity. The Na^+ concentrations in these plants exceeded 300% compared to control plants.

In plants of the *A. canescens* species cultivated under conditions of high salinity, the proportion of Na^+ in the OA plants can reach ~20% (Guo *et al.* 2020). When the energy expenditure associated with the transport and allocation of sodium by the plant is compared to the expenditure involved in the production of compatible organic solutes, it becomes evident that the process is more energetically efficient when it occurs through the transport and accumulation of Na^+ and Cl^- ions (Munns *et al.* 2020a).

Biomass of A. nummularia Lindl

The leaf dry mass (LDM) (Figures 5A,C) and stem dry mass (SDM) (Figures 5B,D) were significantly affected by salinity levels and nutritional management ($p < 0.05$). However, there was no significant effect on the root dry mass (RDM) ($p > 0.05$).

The results of the coefficient of determination of $R^2 = 0.73$ and 0.96 indicate that the LDM and SDM of *A. nummularia* Lindl. assumed a quadratic behavior with an increase in the salinity of irrigation water, respectively. The maximum estimated values of LDM and SDM were 19.62 and 37.47 g per plant for the EC values of 4.48 and 2.80 dS m^{-1}, respectively (Figures 5A,B).

N doses increased LDM by 22.32% and SDM by 17.76%, compared to treatments without N doses (Figures 5C,D). LDM values indicate the capacity of *A. nummularia* Lindl. to tolerate salinity levels (Munns *et al.* 2020a). SDM was more sensitive to increased salinity levels in irrigation water, as SDM values were reduced at lower EC levels in irrigation water.

The application of nitrogen fertilization has been demonstrated to increase the production of various halophytes, even in the presence of abiotic stress. This phenomenon is attributed to a number of factors, including ionic homeostasis, the maintenance of the photosynthetic apparatus, and the induction of antioxidant activity (Karimi *et al.* 2021; Eissa *et al.* 2022; Wang *et al.* 2022).

In plants of *A. nummularia* L. cultivated in saline soil (52 dS m^{-1}) and subjected to nitrogen fertilization at a rate of 80 mg dm^{-3}, an observed dry leaf mass production of ~11.2 g was recorded, in comparison to 3.2 g in the control group plants (Cunha *et al.* 2017). In our study, the N1P0 treatment yielded a value of 20.5 g, while the control group produced 17.0 g. The superior results observed in this study

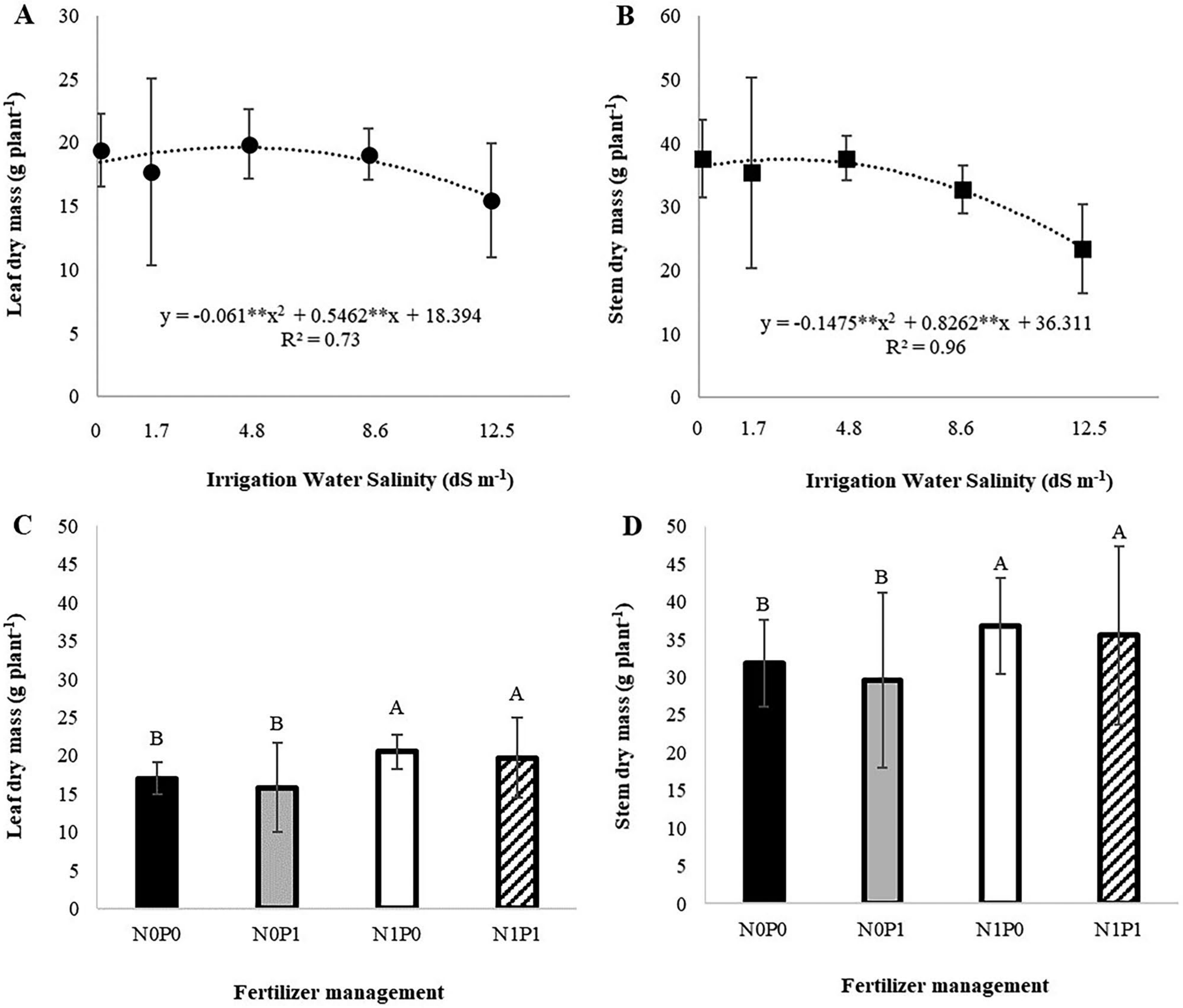

Figure 5. Dry mass of leaves (A) and stem (B) of *A. nummularia* Lindl. under different salinity levels of irrigation water and dry mass of leaves (C) and stem (D) under different fertilizer management at 93 DAT. N0P0 = no fertilizer addition; N0P1 = dose of 60 kg ha^{-1} of phosphorus; N1P0 = dose of 80 kg ha^{-1} of nitrogen; N1P1 = doses of 80 and 60 kg ha^{-1} of nitrogen and phosphorus, respectively. Means followed by equal letters do not differ from each other using the Scott-Knott test ($p < 0.05$). **Significant at $p \leq 0.01$ by *F* test.

may be attributed to the duration of the experiment and the salinity level imposed on the plants.

In addition to salinity, nitrogen utilization demonstrated encouraging results in the phytoremediation of soils contaminated by heavy metals. Eissa *et al.* (2022) have observed a 40% increase in root dry mass and a 20% increase in aboveground biomass in *A. lentiformis* (Torr.) S. Wats (Eissa *et al.* 2022).

Phosphorous (P) plays a crucial role in plant development. However, due to the risk of antagonism and cross-interaction with other essential elements in the same signaling pathways (Wang *et al.* 2021), P supplementation may not result in significant improvements in halophyte species, even in environments with low availability of this element (Doncato and Costa 2023). This phenomenon was also observed in the growth variables of the plant *A. nummularia* L., with the following order of response: N1P0 > N1P1 > N0P0 > N0P1.

Na, K, N, and P in plant tissue

The Na concentration in leaf, stem, and roots were significantly affected ($p < 0.05$) by the change in salinity levels in irrigation water (Figure 6). Conversely, fertilizer application did not show a significant response ($p > 0.05$). The quadratic pattern was observed in regression models to leaf, stem, and roots.

The highest Na concentration was observed in the leaves, followed by the stem and the roots. The maximum estimated Na contents for each plant part were 31.80 (leaves), 14.01 (stem), and 12.80 (roots) mg g^{-1} for EC values of 9.16, 10.00, and 9.93 dS m^{-1}, respectively. This means that, for EC levels between 9 and 10 dS m^{-1}, the Na content in the leaves were 2.27 times higher than in the stem and 2.48 times higher than in the roots. High Na contents in the leaves of *A. nummularia* Lindl. are common, as the species allocates more than 70% of Na allocation to leaf tissues. However, these values may vary depending on soil moisture and nutrients (Souza *et al.* 2012; Zouari *et al.* 2023).

Atriplex nummularia Lindl. has adaptive mechanisms, such as regulation and compartmentalization of ions in specialized structures and synthesis of organic compounds in the cytoplasm, which protect cellular components from dehydration (Lins *et al.* 2018).

The Na content (g $plant^{-1}$) in the plant was affected significantly ($p < 0.05$) by salinity levels as well as by fertilizer application; however, there was no interaction between the factors ($p > 0.05$).

The Na content in the leaves of *A. nummularia* Lindl. plants irrigated with different salt concentrations provided a quadratic response pattern, with a coefficient of determination $R^2 = 0.86$ and a maximum leaf Na content of 0.60 g per plant for EC of 8.05 dS m^{-1} (Figure 7A).

The N contents in the treatments that received N doses were significantly higher (27.8%) than in the other treatments without N addition, as shown by the Scott-Knott test ($p < 0.05$) (Figure 7B).

The K concentration in the leaves was significantly affected ($p < 0.05$) by salinity levels in irrigation water and showed a quadratic behavior $R^2 = 0.89$ when salinity was higher in irrigation water (Figure 8). Melo *et al.* (2016) found similar K concentration to leaves (40 g kg^{-1}) in *A. nummularia* Lindl. irrigated with saline water of 10 dS m^{-1}.

Atriplex nummularia Lindl. showed a preference to absorb Na^+ over K^+. Na^+ accumulation in leaves is consistent with the capacity of this species to compartmentalize Na^+ in the vacuoles and use Na^+ rather than K^+ for osmotic adjustment (Levinsh 2020). The results showed that the increase in Na^+ concentration in *A. nummularia* Lindl. leaves were concomitant with a lower absorption of K^+, which remained stable with increasing salt concentrations.

Studies have shown that plants grown in saline environments undergo ionic imbalance and nutrient deficiency, similar to our results (Ashraf and Harris 2004). Furthermore, high Na^+ concentrations can inhibit K^+ uptake due to physicochemical similarity, which promotes ionic competition for binding sites in membrane transporters.

The findings in the present study highlight the capacity of *A. nummularia* Lindl. to replace K^+ for Na^+ in nonspecific functions, such as osmotic adjustment, accumulating Na^+ in the vacuoles (Paulino *et al.* 2020). Thus, K^+ is

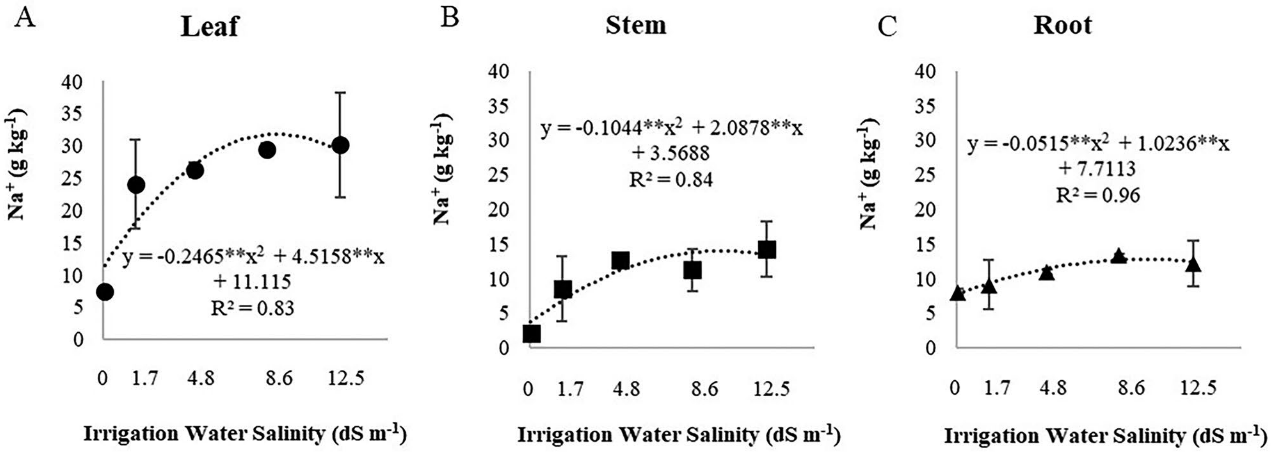

Figure 6. Sodium (Na^+) concentration in tissues of leaves (A), stem (B), and root (C) of *A. nummularia* Lindl. under different salinity levels of irrigation water at 93 DAT. **Significant at $p \leq 0.01$ by *F* test.

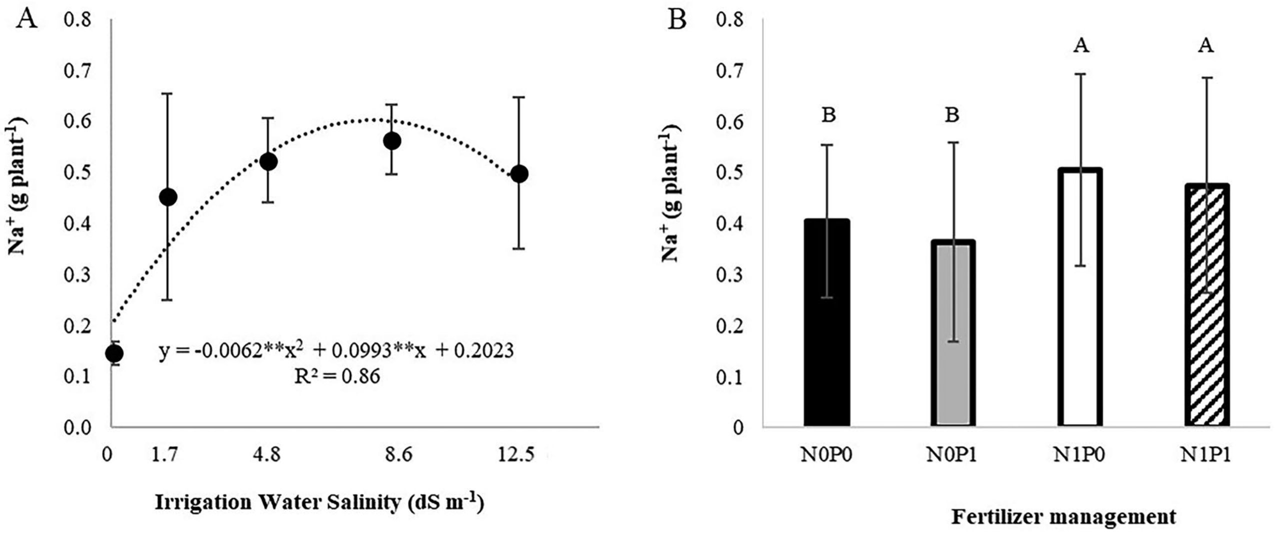

Figure 7. Sodium (Na^+) content in leaf tissue under different salinity levels of irrigation water (A) and under different doses of fertilizers (B) of *A. nummularia* Lindl. at 80 DAT. N0P0 = no fertilizer addition; N0P1 = dose of 60 kg ha^{-1} of phosphorus; N1P0 = dose of 80 kg ha^{-1} of nitrogen; N1P1 = doses of 80 and 60 kg ha^{-1} of nitrogen and phosphorus, respectively. Means followed by equal letters do not differ from each other using the Scott-Knott test ($p < 0.05$). **Significant at $p \leq 0.01$ by *F* test.

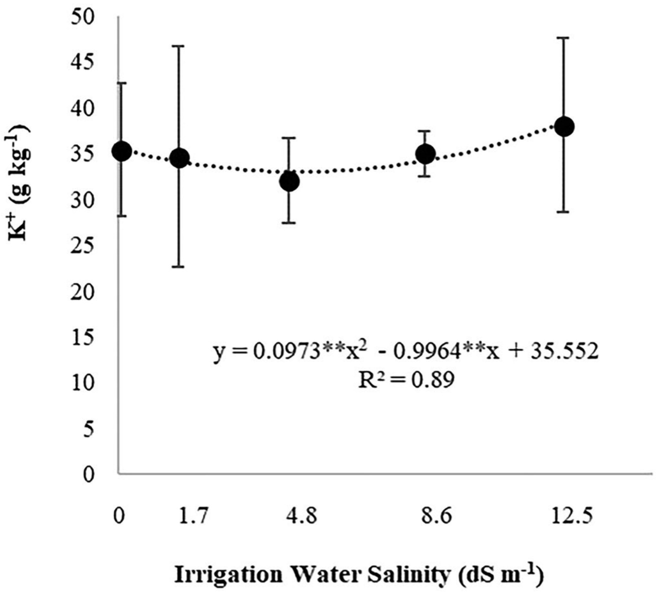

Figure 8. Potassium (K^+) concentration in the leaf tissue of *A. nummularia* Lindl. under different salinity levels of irrigation water at 93 DAT. **Significant at $p \leq 0.01$ by *F* test.

preserved for vital functions of plants, although K^+ also plays an important role in cell expansion, particularly for young leaves. Furthermore, some mechanisms for Na^+ uptake may not represent a cost to maintain membrane potential (Munns *et al.* 2020a).

Salinity levels significantly ($p < 0.05$) affected N concentration in the leaves and roots as well as P concentration in the leaves and stems of *A. nummularia* Lindl. plants. A quadratic pattern was observed for the N concentration in plant leaves due to increased salinity levels in irrigation water (Figure 9A). A linear increase is observed for the roots, as the salinity levels of irrigation water increase (Figure 9B), while the stem did not show a significant trend for the N concentration, with an average of 6.4 g kg^{-1}. The P concentrations in leaves (Figure 9C) and stems (Figure 9D) decreased linearly with increasing water salinity. However, there was no significant effect on the roots with an average of 1.5 g kg^{-1}.

The P and N concentrations in the leaves are in accordance with the average values reported in the literature for *Atriplex* (El-Shatnawi and Abdullah 2003; Eissa and Ahmed 2016; Pasalar *et al.* 2024). In our study, P supplementation did not promote a significant increase in biomass production, in Na extraction, and in the OA, possibly due to the high P content in the soil (Table 2).

It has been observed that phosphorus-based organic and/or chemical fertilizers tend to enhance sodium extraction by plants of the *Atriplex* species. This phenomenon has been observed in species, such as *A. canascens*, *A. leucoclada*, and *A. lelentiformis*, which exhibit ~10% higher Na^+ and K^+ extractions compared to fertilizers with lower P concentrations (Pasalar *et al.* 2024). However, it is essential to note that soils where these fertilizers are commonly applied often have low P levels. For instance, in the previously mentioned study, the soil had only 7 mg kg^{-1} of P.

Halophyte plants typically develop in environments with low concentrations of P. As a result, they have adapted mechanisms for such conditions (Dey *et al.* 2021). Additionally, excess P is typically stored in the plant vacuoles (Wang *et al.* 2021). However, in the case of halophyte plants, vacuoles are commonly associated with storing Na^+ and Cl^- ions, with the aim of preventing the toxicity of these ions in the cytoplasm (Flowers and Colmer 2015; Nikalje *et al.* 2018; Guo *et al.* 2020).

Such statements would justify the linear reductions in P concentrations in plant tissues due to increased salinity levels in irrigation water. These findings corroborate the results observed by Alghamdi *et al.* (2023), who noted an increase

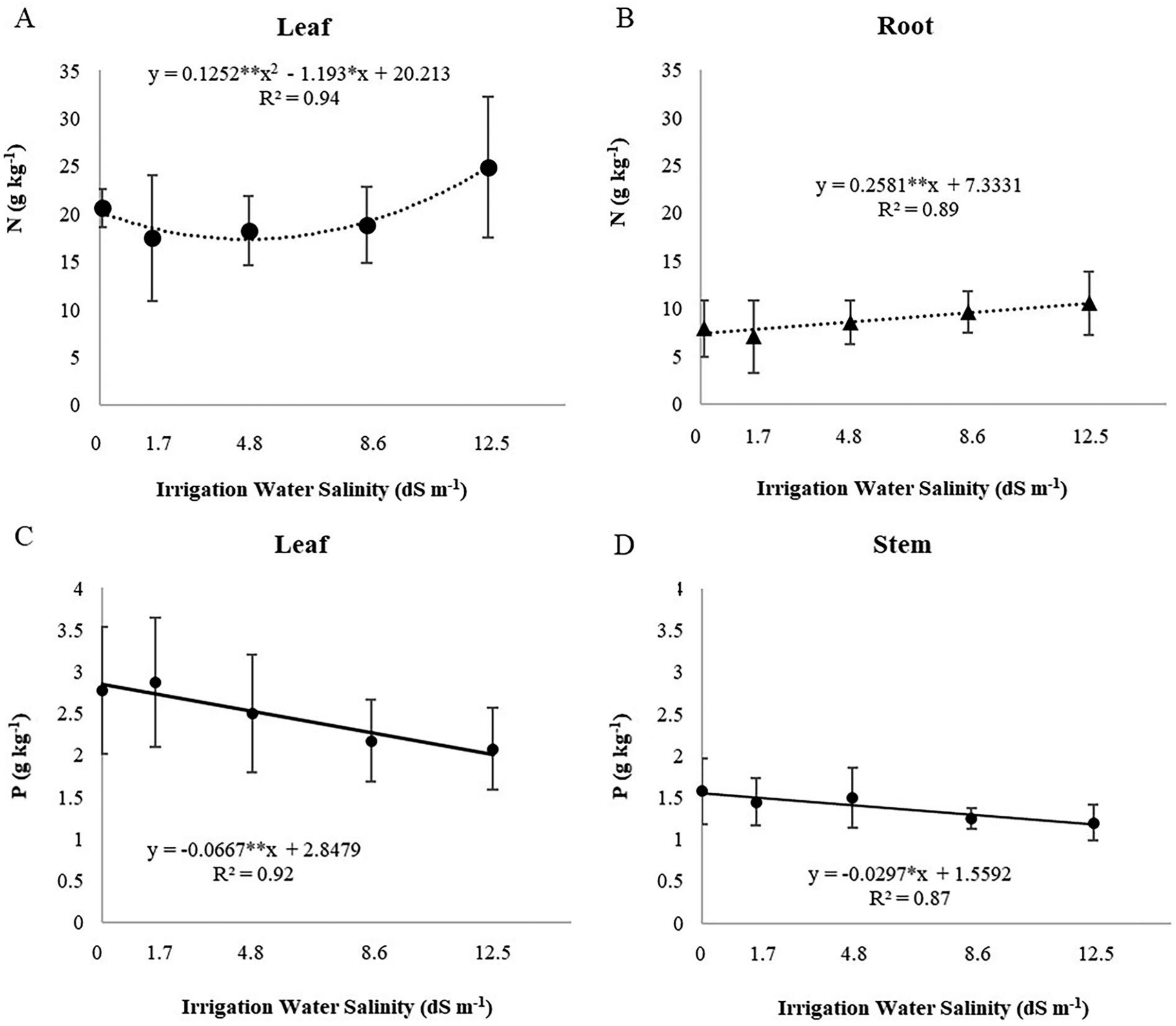

Figure 9. Nitrogen concentration in leaf tissue (A) and roots (B) and phosphorus concentration in leaf tissue (C) and stem (D) of *A. nummularia* Lindl. under different salinity levels in irrigation water at 93 DAT. **, *Significant at $p \leq 0.01$ and $p < 0.05$, respectively by *F* test.

in P concentration and a reduction in Na^+ in *A. nummularia* L. through Na^+ reduction in the soil and greater nutrient availability.

In the present study, the soil already exhibited elevated concentrations of phosphorous (120 mg kg^{-1}), and the addition of fertilizer did not result in enhanced crop performance. Previous studies have also failed to identify a beneficial effect of P fertilization on the growth of *A. nummularia* L. (Cunha *et al.* 2017) and *A. canescens* (Karimi *et al.* 2021). Additionally, a reduction in the beneficial effects of nitrogen application on sodium extraction was observed when P fertilization was employed.

Conclusions

1. N application under salinity increases plant biomass, favoring Na extraction and the osmotic adjustment, attenuating the salinity effects on *A. nummularia* Lindl.
2. It is suggested to cultivate *A. nummularia* Lindl. irrigated with water of up to 4.8 dS m^{-1} with N fertilizers at a rate of 80 kg ha^{-1}.
3. P supplementation for *A. nummularia* Lindl. cultivation is not recommended considering the P contents in the soil studied.
4. According with our findings, we suggest for future research to test the effects of micronutrients on plant growth and physiological traits in different types of soils. Also, it is important to study the better ratio of nutrients in plant tissue for improve the fertilizer recommendation for *A. nummularia* Lindl.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Francisco E. de A. Rêgo Júnior, Lucas Y. C. Leal, Martha K. S. S. Paulino, and Monaliza A. dos Santos. The first draft of the manuscript was written by Francisco E. de A. Rêgo Júnior and Edivan R. de Souza and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

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