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CROP PRODUCTION

Leaf gas exchange and mineral nutrition of *Clitoria Ternatea* intercropped with forage cactus under supplemental brackish water irrigation

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ABSTRACT. Supplemental irrigation using moderately saline water and intercropping are effective strategies for mitigating the adverse effects of water stress on leaf physiology, particularly during dry periods. This study aimed to assess the impact of supplemental irrigation with brackish water (3.0 dS m^{-1}) on leaf gas exchange and mineral concentrations in butterfly pea (*Clitoria ternatea* L.) when intercropped with forage cactus (*Opuntia stricta*). Conducted under a semi-arid climate in 2022 and 2023, the experimental design was a randomized block in split plots, with four replicates. The main plots consisted of two water scenarios: no irrigation and supplemental irrigation. The subplots comprised three cropping systems: butterfly pea in monoculture (B) (1.0 x 0.1 m), forage cactus (2.0 x 0.1 m) intercropped with a line of butterfly pea (P+1B), and forage cactus (3.0 x 0.1 m) intercropped with two lines of butterfly pea (P+2B). Supplemental irrigation was applied to the butterfly pea from February to June during dry spells and fully during the onset of the dry season (July and August). This irrigation approach alleviated water stress impacts on stomatal conductance, net photosynthesis rate, transpiration rate, and internal CO_2 concentration, and enhanced the levels of K^+, Ca^{2+}, Cl^-, and Na^+. Additionally, the P+2B system, when supplemented, led to increased accumulation of N and P. Intercropping improved leaf gas exchange and reduced Cl^- concentration in *C. ternatea*, thus benefiting its agronomic performance.

Keywords: intercropping; salinity; photosynthesis; mineral elements; semi-arid.

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Introduction

The Brazilian semi-arid region, characterized by irregular rainfall and frequent droughts, relies heavily on groundwater sources for productive activities (Amaral et al., 2021). However, these sources often have high salt concentrations, leading to soil salinization and agricultural damage (Dias et al., 2021). In Ceará, wells in the crystalline basement offer low flow rates, making full irrigation with brackish water unfeasible; however, supplemental irrigation has proven viable (Cavalcante et al., 2021; Lessa et al., 2023). During dry periods, this method can mitigate the adverse effects of water deficits on plant growth and enhance forage production. Furthermore, it can improve the CO_2 assimilation rate, crucial for boosting crop yields (Cavalcante et al., 2022).

Water availability is a critical factor for the success of forage plant production, affecting growth, biomass, and nutritional value (Testa et al., 2011). Adapted crops like forage cactus and butterfly pea are poised to significantly impact future livestock feeding in tropical semi-arid regions under abiotic stress (Mudgal et al., 2018). Intercropping, a globally adopted sustainable practice, positively affects crop productivity and water and nutrient use efficiency (Fan et al., 2020). It enhances the root zone environment for better nutrient and water uptake (Gouda et al., 2018) and improves the canopy environment, increasing photosynthetic rates and crop productivity (Chi et al., 2023).

Exploring intercropping systems that incorporate salt-tolerant plants with low water demands can advance biosaline agriculture and forage production in salt-impacted, water-scarce areas. Among such plants are forage cactus (*Opuntia* sp. and *Nopalea* sp.) and butterfly pea (*Clitoria ternatea* L.) (Barros et al., 2004; Fonseca et al., 2019), which are critical in providing water and energy to ruminants and other animals, especially during dry seasons (Dubeux Júnior et al., 2021). However, relying solely on forage cactus may not satisfy the nutritional demands of animals, necessitating its combination with higher fiber and protein sources like grasses and legumes (Santos et al., 2023). *Clitoria ternatea*, native to tropical Asia and belonging to the Fabaceae family, is known as butterfly bean, butterfly pea, or cunhã (Oguis et al., 2019). It is widely cultivated in Brazil, particularly in warm climates (Gonçalves et al., 2024), and enriches forage nutritional value and nitrogen fixation in systems (Silva et al., 2023).

Limited research, such as Araújo et al. (2021), indicates that microclimatic conditions in intercropping systems with maize and cowpea can lessen the impact of salinity on cowpea productivity. However, data on photosynthetic parameters and mineral nutrition of *C. ternatea* intercropped with forage cactus under supplemental brackish water irrigation are scarce as well. We hypothesize that intercropped systems with supplemental brackish water exhibit superior physiological performance and enhanced mineral absorption by *C. ternatea* compared to rainfed cultivation. Thus, the study aimed to evaluate the effects of supplemental irrigation with brackish water and various production systems on leaf gas exchange and mineral element concentration in the butterfly pea crop.

Material and methods

The study was conducted from February to August 2022 and 2023, in Riacho das Pedras, located in the municipality of General Sampaio, Ceará State, Brazil (4º03'10" S; 39º27'16" W, elevation 105 m). This region features a warm tropical, mild semi-arid climate, with the rainy season extending from January to April and average temperatures ranging from 26 to 28°C (Instituto de Pesquisas Econômicas do Ceará [IPECE], 2017).

Meteorological data collected during the experimental period are presented in Figure 1 (A and B), sourced from the Meteorological Station at the Vale do Curu Experimental Farm of the Federal University of Ceará (UFC), located in Pentecoste, Ceará Statge, Brazil (03º47'34" S; 39º16'13" W, elevation 45 m). Both General Sampaio and Pentecoste are situated within the Vale do Curu microregion of Ceará State, Brazil.

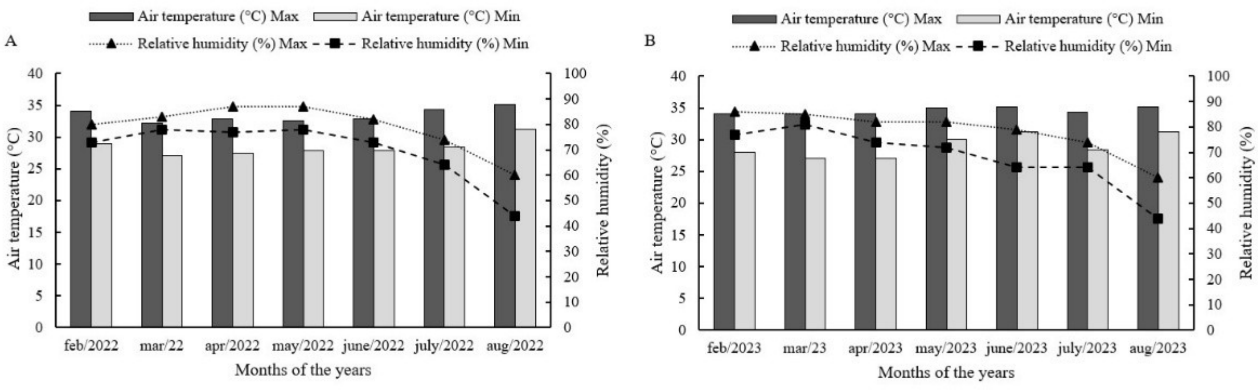

Figure 1. Air temperature and relative humidity from February to August in 2022 (A) and in 2023 (B).

The experiment used a randomized block design in a split-plot arrangement, with four replications. The main plots were defined by two water regimes: non-irrigated and with supplemental irrigation. The subplots consisted of three different production systems that combined butterfly pea and forage cactus: butterfly pea in monoculture (B), forage cactus intercropped with a line of butterfly pea (P+1B), and forage cactus intercropped with two lines of butterfly pea (P+2B).

Crops were established post the onset of the rainy season, in February 2022 and 2023 for butterfly peas, and in February 2022 for forage cactus, covering an area of 0.2 ha. The propagation material for the forage cactus was cladodes from the cultivar 'Orelha de Elefante Mexicana'. The planting followed spacings of 2 x 0.1 m for the P+1B system and 3 x 0.1 m for the P+2B system. Butterfly pea was propagated from seeds sourced from the Seeds Laboratory at the Federal University of Ceará. Before sowing, the butterfly pea seeds were mechanically scarified using sandpaper and soaked in warm water at 80°C for 12 hours to break seed dormancy. Planting of the butterfly pea was done at a spacing of 1 x 0.1 m. Thinning occurred one week after sowing, maintaining 10 plants per linear meter.

Table 1 shows the chemical properties and texture of the soil before the beginning of the experiment. To adjust the soil pH to a suitable level, liming was performed in January 2022 using dolomitic limestone at a rate of 2.82 t ha^{-1}.

Table 1. Chemical and granulometric characteristics of the soil collected in December 2021.

Depth	K^+	Ca^{2+}	Mg^{2+}	SB	Na^+	H+Al	CEC	P	pH	ECe	ESP
cm				$cmol_c\ dm^{-3}$				mg kg^{-1}	H_2O	dS m^{-1}	%
0-20	0.22	0.51	0.27	1.0	0.19	0.8	1.8	3.1	5.67	0.29	10.0
Depth	Sand			Silt			Clay			Textural class	
cm				(%)							
0-20	85.37			8.5			6.13			Sandy loam	

Potential of hydrogen (pH), Electrical conductivity of saturation extract (ECe), Sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Hydrogen + Aluminum (H+Al), Phosphorus (P), Sum of bases (SB), Cation exchange capacity (CEC), Exchangeable sodium percentage (ESP).

Phosphate fertilization for butterfly peas occurred 30 days after sowing, using simple superphosphate at a rate of 50 kg ha^{-1} of P_2O_5 in a single application, following the guidelines of Martins et al. (2012). Potassium fertilization was divided into three applications at 30, 60, and 90 days after sowing (DAS), using potassium chloride (KCl) at a dose of 60 kg ha^{-1} of K_2O as per the recommendations by Salgado et al. (2010). No nitrogen fertilization was applied to the butterfly pea, in line with the recommendations of Salgado et al. (2010), considering the species' proficiency in biological nitrogen fixation.

For forage cacti, only organic fertilizer was used, specifically cattle manure at 30 tons per hectare annually, as recommended by Santos et al. (2006). The manure was applied adjacent to the plant rows at the onset of the rainy seasons in 2022 and 2023.

Supplemental irrigation utilized a drip system, with one irrigation line per plant row. This system employed flexible polyethylene drip tapes, featuring emitters with a flow rate of 1.7 L h^{-1}, emitter spacing at 0.20 m, a service pressure of 101.32 kPa, and a distribution uniformity coefficient of 90%. Irrigation depth was tailored to the specific water volume available at the site and the water needs of each species, dictating the supplementary water volume for each plant. The irrigation rate was calculated from the volume applied per plant relative to the covered area. Butterfly peas received supplemental irrigation from February to August in 2022 and 2023 during dry spells, while forage cactus was irrigated only in the dry season, from July to December. The supplemental water was sourced from a blend of water from a deep well (70 m deep) and an Amazon well.

The water from the deep well exhibited the following chemical properties: a pH of 8.4, electrical conductivity (EC) ranging from 4.0 to 5.0 dS m^{-1}, and ion concentrations of 35.7 $mmol_c\ L^{-1}$ for Na^+, 0.33 mmolc L^{-1} for K^+, 14.2 $mmol_c\ L^{-1}$ for Ca^{2+}, 14.4 $mmol_c\ L^{-1}$ for Mg^{2+}, and 42.87 $mmol_c\ L^{-1}$ for Cl^-, with a sodium adsorption ratio (SAR) of 9.44 $(mmol_c\ L^{-1})^{0.5}$. The Amazon well water had a pH of 8.19, EC ranging from 2.0 to 3.0 dS m^{-1}, and ion concentrations of 12.7 $mmol_c\ L^{-1}$ for Na^+, 0.1 $mmol_c\ L^{-1}$ for K^+, 9.6 $mmol_c\ L^{-1}$ for Ca^{2+}, 9.8 $mmol_c\ L^{-1}$ for Mg^{2+}, and 18.9 $mmol_c\ L^{-1}$ for Cl^-, with a SAR of 4.10 $(mmol_c\ L^{-1})^{0.5}$. Both water sources were classified as C4S1 according to Richards (1954), indicating low sodicity risks and high salinity risks.

Supplemental irrigation involved a blend of these two water sources, maintaining an average EC of 3.0 dS m^{-1}. Each irrigation event delivered 0.9 liters to each butterfly pea plant. In 2022, thirteen supplemental irrigation events were conducted: four before the first cut and nine between the first and second cuts. In 2023, twelve events were implemented: three before the first cut and nine thereafter. The total water depth applied to the butterfly pea during its first growth cycle was 121.8 mm, with 37.5 mm before the first cut and 84.7 mm between the first and second cuts. In the second growth cycle, the total applied water depth was 112.8 mm, comprising 28.1 mm until the first cut and 84.7 mm up to the second cut.

Figure 2 depicts the precipitation events, supplemental irrigation events, and the timing of samplings for leaf gas exchange and plant material analyses.

In the first growth cycle, butterfly pea plants were measured for leaf gas exchange at 82 and 180 days after sowing (DAS); in the second cycle, these measurements occurred at 120 DAS. Moreover, stomatal conductance (gs), net photosynthesis rate (A), transpiration (E), and internal CO_2 concentration (Ci) were measured on butterfly pea mature leaves using an infrared gas analyzer (LCi System, ADC, Hoddesdon, UK) in an open system with an airflow rate of 300 mL min^{-1}. Measurements were performed between 10:00 AM and 12:00 PM, employing an artificial light source providing approximately 1,200 µmol m^{-2} s^{-1}.

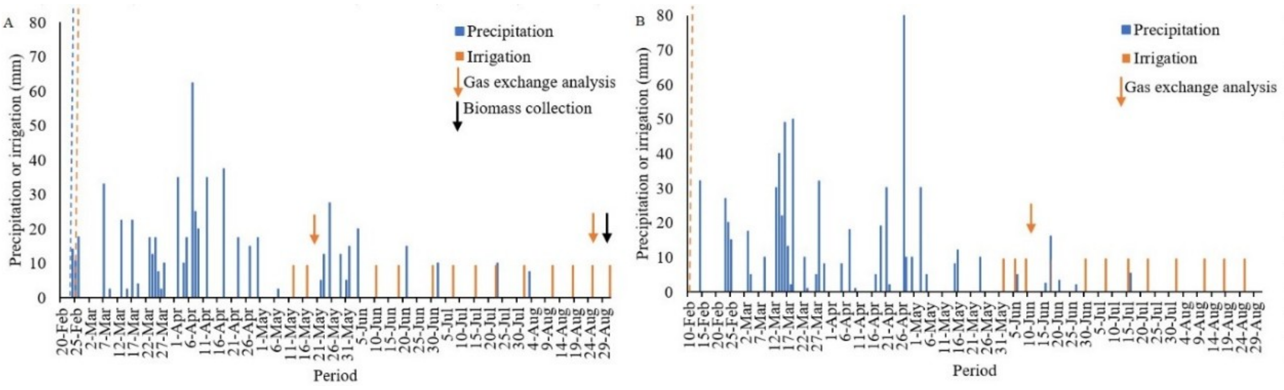

Figure 2. Water supply via precipitation and supplemental irrigation events for butterfly pea during the first (A) and second (B) growth cycles in General Sampaio, Ceará State, Brazil. Dashed gray lines indicate the onset of forage cactus cultivation, while dashed orange lines refer to the onset of butterfly pea cultivation. Orange arrows indicate when leaf gas exchange analysis was performed, while black arrows indicate when *Clitoria ternatea* L samples were collected for mineral content analysis.

To analyze nitrogen, phosphorus, potassium, magnesium, calcium, sodium, and chlorine contents, butterfly pea shoot samples were collected at the end of the first cycle in 2022. The collected plant material was rinsed with distilled water and dried in a forced air circulation oven at 65°C until a constant weight was achieved. Subsequently, the dried samples were weighed, finely ground using a Willey mill, stored in labeled containers, and sent for chemical analysis.

Nitrogen content was determined using wet digestion with H_2SO_4, followed by vapor dragging and water distillation in a semi-micro-Kjeldahl apparatus, with NH_4^+ quantified through titration using sulfuric acid (Meneghetti, 2018). For the levels of P, K^+, Mg^{2+}, Ca^{2+}, and Na^+, the samples underwent incineration in an electric muffle furnace at temperatures between 450 and 550°C. The resultant ash was then dissolved in a 1 mol L^{-1} HCl solution for extraction. Na^+ and K^+ concentrations were measured using flame photometry, P was assessed using molybdenum blue spectrophotometry, and Ca^{2+} and Mg^{2+} were determined through atomic absorption spectrophotometry (Meneghetti, 2018). Chloride (Cl^-) was measured using an aqueous extract and titrated with silver nitrate ($AgNO_3$) in the presence of potassium chromate as an indicator (Malavolta et al., 1997).

Data were first subjected to a normality test (Shapiro-Wilk test) at a 5% probability level. Subsequently, an analysis of variance (ANOVA) was performed, and the means were compared using the Tukey test at a 0.05 probability level. Statistical analyses were conducted using the ASSISTAT software version 7.7 beta (Silva & Azevedo, 2016).

Results

According to Table 2, before the first cut in both 2022 and 2023, the stomatal conductance of butterfly peas was significantly influenced by different water scenarios and production systems, as well as by their interaction ($p < 0.05$). The net photosynthesis rate was impacted by the interaction of these factors ($p < 0.01$) only before the first cut in 2023, while in 2022, the rate was solely affected by the production systems factor before the first cut. There were also isolated effects observed for internal CO_2 concentration ($p < 0.05$) and transpiration rate ($p < 0.01$).

In 2022, before the first cut, butterfly pea plants in the rainfed water scenario exhibited higher stomatal conductance in the monoculture production system, measuring 0.82 mol m^{-2} s^{-1}. However, under supplemental irrigation during dry spells, the intercropping systems P+1B and P+2B recorded higher conductance values of 0.95 and 1.17 mol m^{-2} s^{-1}, respectively (Figure 3A). In 2023, before the first cut, supplemental irrigation with brackish water resulted in increases in stomatal conductance by 57.7, 73.1, and 73.1% in the B, P+1B, and P+2B production systems, respectively, compared to the non-supplemented scenario, with the highest conductance observed in the P+2B system at 0.93 mol m^{-2} s^{-1} (Figure 3B).

In terms of photosynthesis, before the first cut in 2022, the rate in the P+2B system was statistically higher than that in the monoculture, showing a 15.26% increase in the intercropped system compared to the monoculture (Table 2). Similarly, in 2023, the net photosynthesis rates before the first cut in the intercropped systems supplemented with brackish water were higher, registering 24.16 and 25.97 µmol m^{-2} s^{-1} for P+1B and P+2B, respectively. For the monoculture, the photosynthesis rates remained similar across treatments with and without brackish water supplementation, averaging 19.09 µmol m^{-2} s^{-1} (Figure 3C).

Table 2. Summary of variance analysis for leaf gas exchange of the butterfly pea intercropped with forage cactus under supplemental irrigation with brackish water before the first cut.

Variation source	Mean square							
	Before the first cut							
	2022	2023	2022	2023	2022	2023	2022	2023
	gs		A		E		Ci	
Block	0.025ns	0.01ns	27.46ns	15.39ns	0.075ns	9.21*	1424.05ns	1796.11ns
Water scenario (W)	0.33*	1.45**	6.66ns	294.14**	6.39**	42.88**	2917.65ns	8362.66*
Residue 1	0.01	0.017	18.43	3.49	0.078	0.43	662.29	806.11
Production system (P)	0.08*	0.1**	42.33*	5.61ns	0.003**	0.001**	1621.83*	2741.54ns
Interaction (W x P)	0.11*	0.074*	2.80ns	104.32*	0.569ns	0.12ns	863.97ns	2325.79ns
Residue 2	0.01	0.0073	9.19	15.42	0.24	1.23	287.87	1537.77
CV (%) (W)	12.33	28.5	16.07	9.65	5.09	11.0	10.11	13.19
CV (%) (P)	15.29	18.44	11.35	20.29	8.92	18.53	6.67	18.21
Treatment	mol H_2O $m^{-2}s^{-1}$		μmol CO_2 $m^{-2}s^{-1}$		mmol H_2O $m^{-2}s^{-1}$		μmol $m^{-2}s^{-1}$	
Without supplementation	0.73 b	0.21 b	26.18 a	15.85 b	4.99 b	4.66 b	243.50 a	196.66 b
With supplementation	0.97 a	0.71 a	27.24 a	22.85 a	6.02 a	7.34 a	265.55 a	234.00 a
(B)	0.80 ab	0.37 b	24.10 b	19.09 a	5.49 a	5.99 a	241.66 b	194.12 a
(P+1B)	0.78 b	0.42 b	27.59 ab	18.67 a	5.53 a	6.01 a	252.25 ab	223.62 a
(P+2B)	0.97 a	0.59 a	28.44 a	20.29 a	5.51 a	6.00 a	269.76 a	228.25 a

Coefficient of variation (CV), Stomatal conductance (gs), Photosynthetic rate (A), Transpiration (E), Internal CO_2 concentration (Ci), Non-significant (ns), Significant at 1% probability (**), Significant at 5% probability (*).

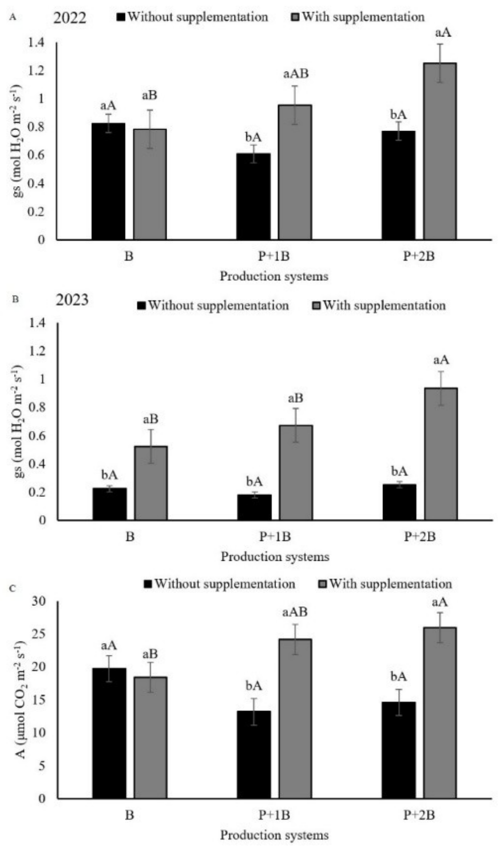

Figure 3. Stomatal conductance (A and B) and net photosynthetic rate (C) of butterfly pea plants intercropped with forage cactus under supplemental brackish water irrigation before the first cut in 2022 and 2023. For interaction (W x P), averages followed by lowercase letters on bars correspond to production systems, while uppercase letters correspond to water scenarios. Bars with the same letters do not differ significantly according to the Tukey test at a 5% significance level. Vertical bars represent standard errors (n = 4).

In 2022, before the first cut, the absence of supplemental irrigation resulted in a reduced transpiration rate for butterfly peas due to water stress during dry spells (Table 2). Before the first cut in 2023, plants not supplemented with brackish water exhibited a 36.5% reduction in transpiration, due to partial stomatal closure (Table 2). No significant differences were observed in transpiration rates before the first cut in 2022 and 2023 across the evaluated production systems (Table 2), indicating that intercropping did not negatively affect leaf transpiration.

The internal CO_2 (Ci) concentration was elevated in the P+1B and P+2B systems, showing increases of 4.2% and 10.5% respectively, compared to the monoculture system before the first cut in 2022 (Table 3). The higher Ci in the P+2B system was justified by its greater stomatal conductance relative to other systems. In 2023, before the first cut, the internal CO_2 concentration in butterfly pea plants supplemented with brackish water was 16.0% higher than in the non-supplemented scenario, indicating that using lower-quality water during dry spells can benefit the functioning of the crop's photosynthetic apparatus (Table 3).

Both stomatal conductance and photosynthetic rate were significantly affected by water scenarios ($p < 0.01$) and production systems ($p < 0.05$) before the second cut in 2022. Additionally, production systems also influenced transpiration ($p < 0.01$) and internal CO_2 concentration ($p < 0.05$) (Table 3).

Table 3. Summary of analysis of variance for leaf gas exchange in butterfly pea combined with forage cactus under supplementary irrigation with brackish water before the second cut.

Variation source	Before the second cut			
	gs	A	E	Ci
Block	0.0003^{ns}	1.65^{ns}	1.34^{ns}	251.07^{ns}
Water scenario (W)	0.04**	268.13**	71.19**	5987.88*
Residue 1	0.0001	1.13	0.28	186.05
Production system (P)	0.0024*	13.05*	1.45^{ns}	2420.61^{ns}
Interaction (W x P)	0.0009^{ns}	7.44^{ns}	0.40^{ns}	880.89^{ns}
Residue 2	0.0005	2.47	0.59	700.16
CV (%) (W)	14.23	13.19	13.44	7.84
CV (%) (P)	28.16	19.54	19.32	15.21
Treatment	mol H_2O m^{-2} s^{-1}	μmol CO_2 m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	μmol m^{-2} s^{-1}
Without supplementation	0.03 b	4.74 b	2.26 b	158.13 b
With supplementation	0.13 a	11.40 a	5.70 a	189.72 a
(B)	0.07 b	7.47 ab	3.58 a	154.55 a
(P+1B)	0.10 a	9.52 a	4.43 a	179.06 a
(P+2B)	0.07 ab	7.18 b	3.94 a	188.18 a

Coefficient of variation (CV), Stomatal conductance (gs), Photosynthetic rate (A), Transpiration (E), Internal CO_2 concentration (Ci), Non-significant (ns), Significant at 1% probability (**), Significant at 5% probability (*).

Supplemental irrigation with brackish water resulted in a 76.9% increase in the stomatal conductance of butterfly peas before the second cut (Table 3). However, there was an 86.6% reduction in conductance values before the second cut compared to those observed before the first cut in 2022 (Table 3). This decrease could be attributed to more stressful environmental conditions brought on by the onset of the dry season and increased salt accumulation in the soil.

In the regrowth phase before the second cut, stomatal conductance in butterfly pea leaves was higher in the intercropped systems, with the P+1B system registering the highest value at 0.10 mol m^{-2} s^{-1} (Table 3). Supplemental irrigation with brackish water also enhanced the net photosynthetic rate before the second cut (Table 3), mirroring the pattern seen in stomatal conductance. The highest photosynthetic rate was observed in the P+1B system, though it was 65.5% lower than the rate before the first cut (Tables 2 and 3). Both the transpiration rate and internal CO_2 concentration before the second cut were higher in treatments that received supplemental irrigation with brackish water (Table 3).

The concentrations of nitrogen, phosphorus, magnesium, and sodium were significantly affected by the interaction between water scenarios and production systems ($p < 0.05$). Water scenarios also influenced the concentrations of calcium and potassium ($p < 0.05$) (Table 4).

Supplemental irrigation with brackish water led to an increase in nitrogen content in the butterfly pea exclusively within the P+2B system, achieving 37.5 g kg^{-1}, which was 15.5 and 14.2% higher than in the monoculture and P+1B systems, respectively, when supplemented (Figure 4A). Similarly, phosphorus levels were notably higher with brackish water irrigation only in the P+2B system, showing increases of 51.6% and 30.0% compared to the monoculture and P+1B systems, respectively, when supplemented (Figure 4B).

Supplemental irrigation with brackish water resulted in a 21.6% increase in the potassium content of the butterfly pea crop compared to rainfed farming (Table 4). Conversely, the highest magnesium contents were observed in the rainfed scenario across all evaluated production systems, with increases of 20.9%, 20.5%, and 19.4% for systems B, P+1B, and P+2B, respectively, when compared to their supplemented counterparts (Figure 4C). However, brackish water supplementation enhanced the absorption of calcium by the crop, showing a 14.28% increase in calcium content compared to the non-supplemented scenario (Table 4).

Table 4. Mineral element contents of butterfly pea in systems intercropped with cactus and under supplementation with brackish water.

Variation source	Mean square						
	N	P	K^+	Mg^{2+}	Ca^{2+}	Na^+	Cl^-
Block	20.25^{ns}	0.0012^{ns}	5.36^{ns}	0.10^{ns}	2.70^{ns}	0.0028*	0.006^{ns}
Water scenario (W)	0.47^{ns}	0.0014^{ns}	459.37*	127.72**	9.86*	5.95**	0.66*
Residue 1	8.81	0.0008	33.31	0.40	0.95	0.06	0.034
Production systems (P)	19.91*	0.0057**	89.67*	3.82^{ns}	16.56^{ns}	0.70**	0.31**
Interaction (W x P)	21.67*	0.004*	38.49^{ns}	0.013*	9.37^{ns}	0.48*	0.002^{ns}
Residue 2	4.18	0.00076	21.68	2.43	5.70	0.081	0.013
CV (%) (W)	8.82	23.39	15.98	3.12	11.57	16.17	21.94
CV (%) (P)	6.08	22.67	12.89	7.64	28.24	18.23	19.56
Treatment	g kg^{-1}						
Without supplementation	33.53 a	0.11 a	31.75 b	22.72 a	7.81 b	1.06 b	0.41 b
With supplementation	33.81 a	0.12 a	40.50 a	18.11 b	9.10 a	2.06 a	0.75 a
(B)	32.69 b	0.09 b	39.96 a	20.17 a	9.09 a	1.90 a	0.81 a
(P+1B)	32.83 ab	0.12 ab	33.84 a	19.88 a	9.60 a	1.41 b	0.46 b
(P+2B)	35.49 a	0.14 a	34.56 a	21.19 a	6.67 a	1.37 b	0.46 b

Coefficient of variation (CV), Nitrogen (N), Phosphorus (P), Potassium (K^+), Magnesium (Mg^{2+}), Calcium (Ca^{2+}), Sodium (Na^+), Chlorine (Cl^-), Non-significant (ns), Significant at 1% probability (**), Significant at 5% probability (*).

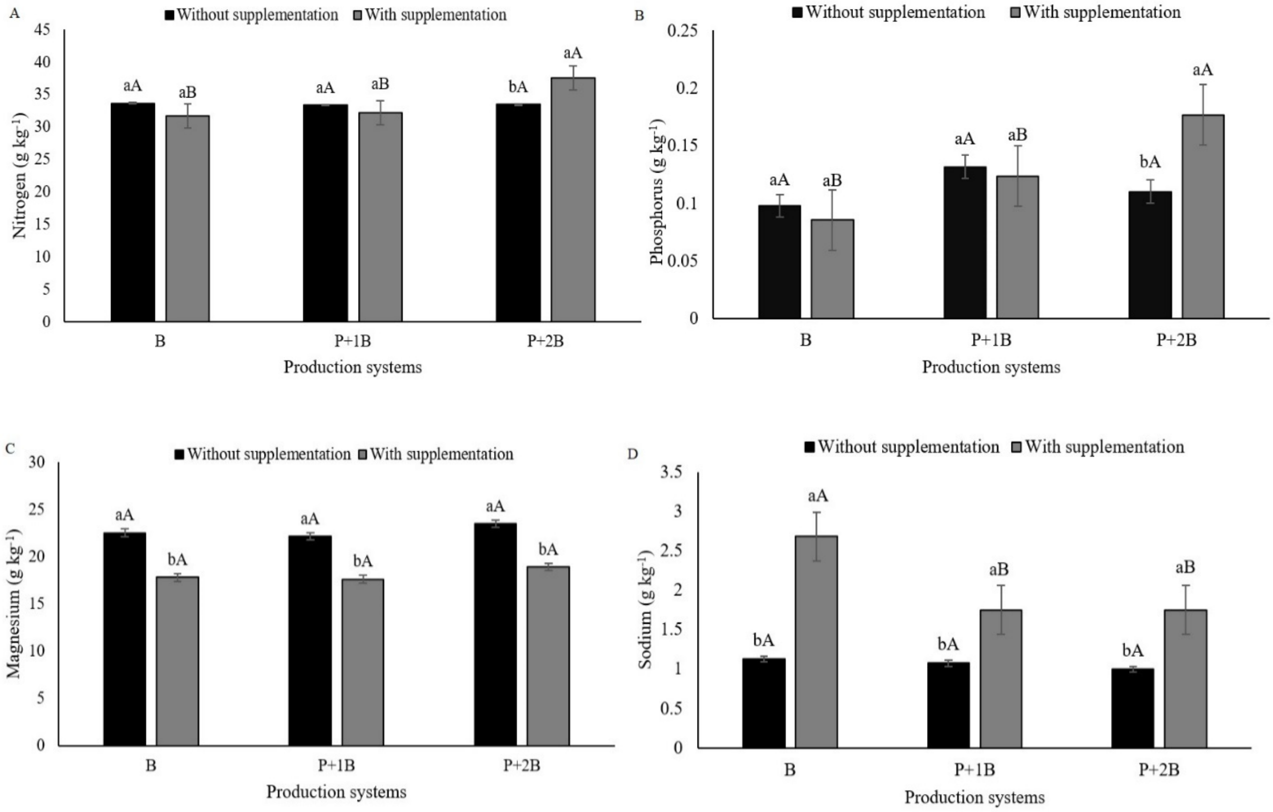

Figure 4. Shoot concentrations of nitrogen (A), phosphorus (B), magnesium (C), and sodium (D) in butterfly peas under monoculture and intercropped with forage cactus, irrigated with brackish water. Averages followed by lowercase letters in the bars correspond to production systems, while uppercase letters correspond to water scenarios. Bars with the same letters do not differ significantly according to the Tukey test at a 5% significance level. Vertical bars represent the standard error (n = 4).

Butterfly pea plants in the intercropped systems (P+1B and P+2B) exhibited lower sodium contents (1.75 g kg^{-1} for both) compared to the monoculture system when supplemented with brackish water, indicating reductions of 34.7% (Figure 4D). Although there was no reduction in sodium levels with supplementation, the observed level was low. Additionally, supplemental irrigation with brackish water led to a 45.3% increase in chlorine content in butterfly pea plants compared to rainfed conditions (Table 4). This increase is due to the presence of Cl^- in the irrigation water. Regarding chlorine content relative to production systems, a similar trend to sodium content was noted, with reductions of 43.2% observed in the intercropped systems P+1B and P+2B compared to monoculture (Table 4).

Discussion

Supplemental irrigation with brackish water during the dry spells led to increased stomatal conductance in butterfly peas within the intercropped systems before the first cut in 2022 and across all systems evaluated in 2023 (Figure 3A and B). Similarly, enhancements in the photosynthetic rate were noted in the intercropped systems when supplemented before the first cut (Figure 3C). These results indicate that in the forage cactus-butterfly pea intercropping systems, the detrimental effects of water and salt stress on stomatal conductance and net photosynthetic rate were mitigated by supplemental irrigation with brackish water. Conversely, water stress reduced the stomatal conductance (gs) of butterfly pea plants across all production systems, as it restricts access to essential resources for photosynthesis through stomatal closure and diminished internal water transport (Taiz et al., 2021).

The observed increases in conductance and photosynthesis in butterfly pea leaves intercropped with forage cactus can be attributed to water complementarity within the cultivation systems, particularly with the application of supplemental irrigation. The forage cactus-butterfly pea intercropping facilitated spatial complementarity of the crops, which, according to Montezano and Peil (2006), enhances the utilization of light, water, and nutrients within the productive ecosystem. In such diverse planting systems, complex interactions among plants, soil, and microbiota occurred (Cappelli et al., 2022), contributing to reduced soil water evaporation.

In 2022, the stomatal conductance (gs) in the scenario without supplemental irrigation was higher compared to the same scenario in 2023. The observed reduction in 2023 can be attributed to more severe dry spells occurring before the measurement of leaf gas exchange that year (Figure 1), resulting in lower gs values due to increased water stress and more adverse atmospheric conditions.

Supplemental brackish water irrigation during dry spells led to increased transpiration rates and internal CO_2 concentration in *C. ternatea* L. plants (Table 3). Water deficits during these periods significantly reduced gs, E, and Ci, proving to be more detrimental than the salt stress induced by using brackish water for supplemental irrigation. This finding suggests the potential for increasing full irrigation events with brackish water in butterfly pea crops, depending on the water salinity level (Lessa et al., 2023). The decrease in transpiration and internal CO_2 concentration may be linked to stomatal closure; lower stomatal conductance increases resistance to CO_2 diffusion and reduces transpiration (Guimarães et al., 2022). Cavalcante et al. (2022), in their study of supplemental irrigation with brackish water in maize under tropical conditions, observed transpiration reductions caused by water stress in normal, drought, and severe drought scenarios without supplemental irrigation.

Intercropping with forage cactus was beneficial for the photosynthetic rate and internal CO_2 concentration of butterfly pea plants (Tables 3 and 4), particularly in the P+1B system. The spatial configuration of the P+1B system resulted in less shading and/or interspecific competition, creating favorable conditions for enhanced gs, Ci, and A in butterfly pea leaves. According to Gouda et al. (2018) and Chi et al. (2023), improvements in the root zone environment from intercropping can enhance nutrient and water absorption, while enhancements in the canopy environment can boost photosynthetic rates. Jo et al. (2022) reported that net photosynthesis in intercropped soybean leaves increased by 21.9% compared to soybeans grown in monoculture. They attributed this increase to the higher chlorophyll content observed in intercropped systems.

The lower gas exchange values (gs, A, E, and Ci) observed in butterfly pea plants before the second cut can be attributed to the plants being from the regrowth following the first cut, compounded by adverse atmospheric conditions such as high temperatures and low relative humidity, which are characteristic of the dry season in the Brazilian semi-arid region. It is worth noting that 85 days after the first cut of the butterfly peas, there is a significant decline in both dry matter production and crude protein concentration (Alencar & Guss, 2016).

In the P+2B system supplemented with brackish water, the butterfly pea plants showed no signs of saline stress affecting N and P absorption (Figure 4A and B). This resilience can be attributed to the beneficial effects of supplemental irrigation on the symbiotic root system, enhancing N and P uptake. It is crucial to mention that the symbiotic process is facilitated by an enzymatic complex known as nitrogenase, which directly involves elements such as Ca, Fe, Mo, Mg, Co, Ni, and P (Prado, 2020). Therefore, the increased water availability near the root zone enhanced the absorption of these essential and beneficial nutrients, primarily through mass flow, leading to an increase in biological N fixation.

The enhanced N and P concentration in the intercropped butterfly pea (P+2B) when supplemented may be linked to increased root growth and greater soil moisture retention. Xie et al. (2022) observed that in an intercropped system of peanuts and cotton, the highest accumulations of nitrogen and phosphorus occurred both in vegetative tissues and reproductive organs, suggesting a synergistic effect in intercropping that benefits nutrient uptake.

Supplemental brackish water irrigation during dry spells increased K, Ca, and Cl^- contents in butterfly pea plants, suggesting that irrigation-added salts can contribute to plant nutrition and development to a certain extent (Table 4). Notably, Ca and Cl^- were present at high concentrations in irrigation water, potentially enhancing the availability and absorption of these elements. Ashraf el al. (2017) noted that increasing the leaf K^+/Na^+ ratio and Ca^{2+} concentrations is crucial for enhancing plant tolerance to salt stress.

Conversely, supplementation with brackish water at 3.0 dS m^{-1} negatively affected Mg absorption across all production systems (Figure 4C). This reduction is attributed to ionic competition with soluble salts present in irrigation water. Notably, high concentrations of Ca^{2+} and especially K^+ in the soil can inhibit Mg absorption due to ionic competition, potentially leading to Mg deficiency in plants (Prado, 2020).

Lower Na uptake was observed in butterfly pea plants within intercropped systems (P+1B and P+2B) with forage cactus when supplemented with brackish water, compared to those in monoculture (Figure 4D). Forage cactus may have extracted a significant amount of Na from the soil solution, thereby reducing its availability near butterfly pea roots and subsequently decreasing Na^+ absorption by this crop. Chehab et al. (2020), assessing the impact of supplemental irrigation with brackish water (2.0 dS m^{-1}) and the use of organic amendments on nutrient content in olive trees intercropped with broad beans in a semiarid region, noted an increase in leaf Na content in intercropped olive plants, a finding that aligns with ours. Nonetheless, Na^+ levels in butterfly peas remained low and did not reach potentially toxic levels, as assessed at the end of the rainy season.

Intercropping also led to reduced Cl^- concentrations in shoots of butterfly pea (Table 5), due to enhanced Cl^- uptake by the forage cactus. Similarly, Hu et al. (2020) discovered that cauliflower intercropped with *Paspalum vaginatum* experienced a significant reduction in Cl^- in its rhizosphere, with a 58.8% decrease compared to monoculture controls.

Conclusion

Supplemental brackish water irrigation during dry spells alleviated the impacts of water deficit on butterfly pea plants by enhancing stomatal conductance, net photosynthesis rate, transpiration rate, and internal CO_2 concentration, while increasing potassium, calcium, chlorine, and sodium levels. When supplemented, the P+2B intercropping system provided a notable increase in nitrogen and phosphorus assimilations by butterfly pea plants. Additionally, intercropping led to improvements in net photosynthetic rate and internal CO_2 concentration, along with chlorine content reductions in *Clitoria ternatea* L. plants, thereby enhancing its agronomic performance.

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