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RESEARCH ARTICLE

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Growth and nutrients content of *Atriplex canescens* across a soil electric conductivity gradient

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Abstract

Atriplex canescens canopy cover, biomass production, and nutrient content were evaluated after four years of livestock grazing exclusion and mechanical shrub removal, except *A. canescens* in an arid rangeland with a slightly saline soil and with a long history of heavy livestock grazing in the southern Chihuahuan Desert, Mexico (24° N). Twenty 3 × 3 m paddocks were established in terrains with three levels of electric conductivity (EC): <1.4 dS/m (n=7), 1.4–1.6 dS/m (n=7) and >1.6 dS/m. *Atriplex canescens* canopy cover was higher (49.5%; $p<0.01$) on paddocks with soil EC >1.6 dS/m than paddocks with soil EC <1.4 and 1.4–1.6 dS/m (32.1 and 22.9%, respectively). Above-ground biomass did not differ between paddocks with soils with EC of <1.4 and 1.4–1.6 dS/m (1309 ± 535 and 1372 ± 180 kg DM/ha), but biomass increased 2.7 times ($p<0.01$) when soil EC was greater than 1.6 dS/m. The soil EC had no effect on the levels of crude protein (range 13.6 to 14.3%), neutral detergent fiber (range 56.5 to 57.7%) and ash (range 14.5 to 16.4%). In vitro dry matter digestibility (IVDMD) of the foliage of *A. canescens* was not affected by of soil EC level (range 60.4 to 62.2%). It was concluded that in an arid rangeland with slight saline soil, the increase in salinity favors canopy cover and biomass production of *A. canescens* without altering nutrient content and IVDMD of this fodder shrub.

Additional keywords: aerial cover; biomass production; nutrient content; rangeland; soil salinity.

Abbreviations used: DM (dry matter); EC (electric conductivity); IVDMD (in vitro dry matter digestibility).

Authors' contributions: Design of the experiment, data analysis and manuscript preparation: MM, JRA. Field work: JEG UMC, LAR.

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Introduction

The *Atriplex* genus has several plant species that grow in ecosystems with high salinity, scarce humidity and high temperature (Ramos *et al.*, 2004). These shrubs have been used for erosion control and rangeland rehabilitation in salt-affected and degraded areas (Rani *et al.*, 2013; de Souza *et al.*, 2014). These shrubs are also utilized by farmers in dry ecosystems as maintenance feed for livestock during the drought feed gap (Norman *et al.*, 2004; Pearce *et al.*, 2010) or for landscaping purposes (Panta *et al.*, 2014; Ventura *et al.*, 2015).

Competition among plants in arid environments favors species that are able to become established in harsh environments and are able to reproduce and expand in the ecosystem. Plant species with higher tolerance to salinity, drought, and high ambient temperature are more successful to reproduce in low-resource environments (de Souza *et al.*, 2012). *Atriplex* shrubs have adaptations enabling them to tolerate the adverse effect of salts internally or excrete salt from cells and tissues (Flowers & Colmer, 2008). Therefore, they have an advantage over forage species unable to deal with salt in the soil and are therefore good competitors in arid zones with saline soils and can provide a supplementary feed source for livestock and wildlife under arid and semi-arid conditions (Aganga *et al.*, 2003; Norman *et al.*, 2010).

Many species of *Atriplex* are good forage resource for livestock because of their adequate crude protein content and high dry matter digestibility (Pinos-Rodríguez *et al.*, 2007; Mellado *et al.*, 2012); however, animals must combine the ingestion of *A. canescens* with more nutritious understory species because total dietary intake is limited by the salt content of *A. canescens* as animals ingest large amounts of salty shrubs (Masters *et al.*, 2005).

Atriplex canescens occurs in many different arid ecosystems of the Chihuahuan desert and represents a significant source of forage for small ruminants, cattle, and wildlife (El Shaer, 2010; Mellado *et al.*, 2012). In these ecosystems, the salt content of soils can mitigate the negative effects of water stress. For instance, plants in drying soils tend to thrive better in saline than in nonsaline soils, because salt-stressed plants grow not as much as those growing in nonsaline soils, therefore, they drain soil moisture slower than nonsalt-stressed plants.

Currently, little information is available on the effect of salt concentration of soil on the biomass production and nutrient content of *A. canescens* under field conditions. This knowledge is important to understand its ecological adaptations and provide valuable guidance on the exploitation of rangelands with mild concentrations of subsoil salt. The present study addresses the following questions: (1) what effect does salinity have on biomass production of *A. canescens*, (2) does nutrients of this shrub change in response to salinity? Our working hypotheses were: increasing salinity levels would have a positive effect on biomass production; the magnitude of the soil salinity affects the nutrient content of this woody shrub. Our objective was to quantify the canopy cover and biomass production of *A. canescens* on a moderately productive clay loam site. An additional objective was to assess the effect of degree of salinity of soil on the nutrient content of *A. canescens*.

Material and methods

Study site

The study area (162 ha) was located in the Noria de Guadalupe research Station located 14 km south from Concepción del Oro (Mexico) at 24° 20' N and 101° 23' W at an elevation of 1,800 m. Mean annual precipitation at the study site is 317 mm with about 60% falling during the June-October summer growing season. Summer rainfall occurs as convective, intense thunderstorms highly localized and of short duration. Most of the remaining rain falls during the December-April winter rainy season. The mean annual temperature is 12.2° C. The soil is yermosol slightly alkaline (pH=7.6), very poor in organic matter (2.2 ± 0.4%; mean and SD) and nitrogen (53 ± 9.1 kg/ha) and with slight salinity (1.2-2.7 dS/m). Soil depths at the study site range from 66 to 125 cm. Over the study area, the mean percentage of sand, silt, and clay was 44.1, 34.9 and 21.0, respectively.

The vegetation is dominated by creosotebush (*Larrea tridentata* (DC) Coville) along with tarbush (*Flourensia cernua* DC.), fourwing saltbush (*Atriplex canescens* (Pursh) Nutt.) and lechuguilla (*Agave lechuguilla* Torr). Important suffrutescents include mariola (*Parthenium incanum* H.B.K.) and desert zinnia (*Zinnia acerosa* DC). The main perennial grasses are *Bouteloua karwinskii* (E. Fourn) Griffiths.), creeping muhly (*Muhlenbergia repens* (J. Presl) Hitchc.) and fluffgrass (*Erioneuron pulchellum* (Kunth) Tateoka). The most abundant forbs are velvety Nerisyrenia (*Nerisyrenia camporum* (A. Gray) Greene) and copper globemallow (*Sphaeralcea angustifolia* (Cav.) G. Don).

This site has been managed for decades to supported communal grazing of horses, cattle, sheep and goats with a stocking rate far above the grazing capacity recommended for this ecosystem. At the study area, all shrubs except *A. canescens* were removed, severing them below the ground level cutting the roots as much as possible with a pickax and manually removed in 25 ha. The root balls of shrubs were pulled out by using a spade shovel and a mattock. Then the site was fenced with six strands of barbed wire to exclude the grazing livestock.

Four years after the enclosure was established, 25 samples of soil were randomly taken from the top 30 cm, within this terrain in order to identify zones of low (<1.4), moderate (1.4-1.6) and moderately high (>1.6) levels of electric conductivity (EC). EC of soil (samples previously air-dried and powdered), was determined with an electrical conductivity meter, model 19101–00 (Cole-Parmer Instrument Co., Vernon Hills, IL, USA). For this determination, 2 g of soil was suspended in 10 mL double distilled water and gently stirred to obtain a homogeneous suspension.

Then, twenty 3 × 3 m paddocks were established in areas predominantly occupied by *A. canescens* with low (n=7), moderate (n=7) and moderately high (n=6) electric conductivity values. The 9-m^2 plot size was selected because it was large enough to include a representative sample of the plant community. Three soil samples were again collected on bare soil from each paddock and place in plastic bags.

Soil sampling and analyses

Soil was sampled in June 2015, with samples collected with a flat-bladed shovel from the upper 30 cm. Five samples from the same depth, sampling site and paddock were mixed together and were air-dried at 35°C and crushed to pass a 2 mm sieve. The pH was measured with a glass electrode in water (pH H_2O)

and 1M KCI (pH KCI) with a 1:1 soil: solution ratio. Samples were analyzed for total N by microKjeldhal and autoanalyzer, and total P by reduction with amino-naphtol sulphonic acid and autoanalyzer (Bray & Kurtz, 1945).

Aerial cover and biomass determination

Paddock sampling of *A. canescens* canopy coverage involved a visual estimation of this variable from the top of a 150 cm flexible ladder. *A. canescens* canopy cover represented the percentage of land surface area occupied by *A. canescens* canopies within the 9 m^2 square plots as viewed from above. All plants of *A. canescens* were hand-cut at 2 cm above ground by using large pruning shears; the entire crown of a shrub was sampled if the base of the plant fell within the plot. Individual plants were cut into small manageable pieces and placed in large cotton sacs bags.

The plant material was dried at 60°C to a constant weight and fractions weighed. The leaves (without petioles), twigs, branches, stems and seeds were separated by hand. Values obtained from each paddock were transformed to kg/ha. Numbers of plants with a height greater than 1 m were counted in paddocks prior to whole plant harvesting. Samples for biomass estimation and chemical analyses were taken by clipping all plants of *A. canescens* within the 20 quadrats on a single sampling date.

Dried leaves samples were ground to pass a 1-mm screen using a Wiley mill (Thomas Model 4 Wiley® Mill, Swedesboro, NJ, USA), and analyzed for ash (method 942.05), nitrogen (method 954.01) and ether extract (method 920.39), according to AOAC (1997) official methods. Neutral detergent fiber (NDF) was assessed by the procedure of Van Soest *et al.* (1991) excluding alpha amylase but with sodium sulfite and reported excluding the residual ash. Acid detergent fiber (ADF) was analyzed according to AOAC (1997; method 973.18) and reported excluding the residual ash.

The mineral content was determined by dry-ashing the foliage samples at 550°C in a furnace, and dissolving the ash in 10% HCI, and filtered. Sodium (Na) and potassium (K) were determined by flame photometer while an atomic absorption spectrometer was used to determine Ca, Mg, Zn, and Cu (Perkin-Elmer, Waltham, MA, USA; AOAC, 1997). Phosphorus was determined by the ascorbic acid method (John, 1970). The in vitro dry matter digestibility (IVDMD) was determined by incubation of *A. canescens* leaves samples in filter bags in a Daisy II incubator (ANKOM Technology Corp., Macedon, NY, USA) with rumen inoculum from a rumen-fistulated Holstein steer and buffer in a 1:4 ratio for 48 h at 39°C under anaerobic conditions.

Statistical analysis

The paddocks served as blocks to examine soil EC level differences. Canopy coverage data were analyzed as a completely random design with each experimental unit being the canopy coverage of the seven quadrats per level of EC of soil. A generalized linear model logistic regression analysis was used (SAS Inst. Cary, NC, USA) because percentage or proportion data usually fit a binomial distribution. The LSMEANS/diff statement was used to compare the means.

Dry matter production of total biomass, leaves and seeds per ha was analyzed as a completely randomized block design using the MIXED procedure of SAS (SAS Inst.). Mean separation among levels of EC of the soil was completed using the PDIFF option of the LS MEANS statement (SAS Inst.). A non-linear regression was used to test the relationships between the level of EC of the soil and biomass production of *A. canescens* (CurveExpert PRO 2.6.3).

Results

Despite fairly similar number of plants per paddock ($p>0.05$) for sites with low, moderate and moderately high soil EC readings, *A. canescens* canopy cover was about two times higher ($p<0.01$) in the paddocks with the highest soil EC readings compared to sites with lower EC of soil (Table 1). No statistical difference was detected in canopy cover between paddocks with low and moderate EC of soil.

Data of the 20 paddocks showed an average of 1401 *A. canescens* plants greater than 1 m of height/ha, with no significant difference between sites with low, moderate and moderately high readings for soil EC. *A. canescens* in paddocks with soil EC greater than 1.6 mS/m displayed significant increases in biomass (DM), when compared to paddocks with soils with EC<1.4 or 1.4 to 1.6 mS/m (Fig. 1). Likewise, DM of leaves of *A. canescens* was significantly higher in paddocks with values of soil EC greater than 1.6 mS/m.

A. canescens biomass production showed a significant positive correlation with values of soil EC (Fig. 2). Ash content of foliage of *A. canescens* increased with increasing soil EC but these differences were not statistically significant. Crude protein of *A. canescens* foliage ranged from 12.4 to 15.7%, with no differences between values obtained from plants growing in soils with low, moderate or moderately high soil EC readings

Table 1. *Atriplex canescens* canopy cover estimates, biomass production and number of plants/ha in an arid environment with soils with three different electric conductivity readings for the 0 to 30 cm layer. Values are means ± standard deviation.

Item	<1.4 dS/m	1.4-1.6 dS/m	>1.6 dS/m
Aerial cover (%)	32.1 ± 15.6^{a}	22.9 ± 9.7^{a}	49.5 ± 18.9^{b}
Biomass (kg/ha)	1309 ± 535^{a}	1372 ± 565^{a}	3916 ± 1294^{b}
Leaves (kg/ha)	672 ± 272^{a}	737 ± 300^{a}	2303 ± 1146^{b}
Seeds (kg/ha)	100 ± 54^{a}	250 ± 161^{ab}	431 ± 365^{b}
Number of plants/ha	1112 ± 818	1468 ± 825	1624 ± 954

^{a,b}Means within a row followed by different superscript are significantly different at $p<0.05$.

(Table 2). NDF and FDF levels in *A. canescens* foliage averaged 57.3 and 31.4%, respectively, with no differences between *A. canescens* growing in sites with soils varying in EC values. No significant effects were observed for soil EC readings regarding percentages of IVDMD of *A. canescens* foliage. Levels of soil salinity did not modify the macro and microelements content of *A. canescens*'s foliage (Table 2).

Discussion

A notable feature of the present study was that the aerial cover of *A. canescens* was stimulated by increased values of EC of soil. Changes in aerial cover can be influenced by two processes: recruitment of new *A. canescens* plants or coalescing of expanding *A. canescens* patches. In the experimental area, the number of plants of *A. canescens* was very similar among paddocks of different EC of soil which suggests that a greater development and expansion and possibly an increased number of stems per plant of *A. canescens* occurred with the highest EC of soil, which lead to a considerable coalescing of this shrub. A greater *A. canescens* canopy cover affects both vertical and horizontal water fluxes, therefore, the greater expansion of *A. canescens* in slightly saline soils makes it a particularly prized fodder shrub to improve soil and water conservation.

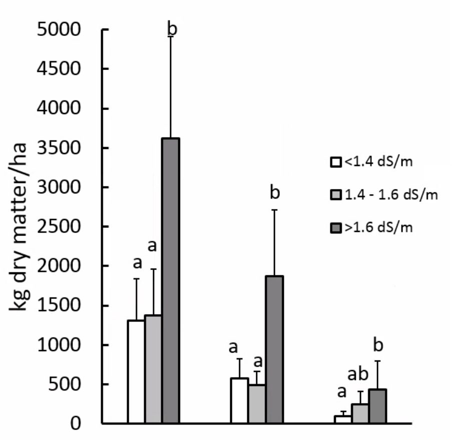

Figure 1. Mean aboveground live biomass, leaves and seeds production per hectare from paddocks with *Atriplex canescens* excluded from grazing and where all other shrubs, except *A. canescens*, had been eliminated from this terrain four years ago. Within parts of the plant, means with different letters differ statistically ($p<0.05$).

As soil EC increased, the likelihood of greater biomass was enhanced. This is in line with results of Gomez-Silveira *et al.* (2009) and Pan *et al.* (2016) where the growth of *Atriplex* species seedlings was enhanced by moderate salinity and unaffected by high salinity. This positive association has also been reported for other *Atriplex* species (Redondo-Gómez *et al.*, 2007; Bouchenak *et al.*, 2012; Tsutsumi *et al.*, 2015). The mechanisms promoting the growth improvement at moderate salinity levels are not fully understood. In halophyte plants, a moderate increase of soil salinity leads to a transient increase of stomata number, maximum opening area and size, which is an adjustment to a particular demand of CO_2 (Shabala *et al.*, 2012; Koyro *et al.*, 2013). Carboxylation capacity does not seem to be adversely affected by salinity in *Atriplex portulacoides*, but it is stimulated by moderate salt concentrations which contribute to increased growth rates.

Additionally, a moderate increased salt content of soil seems to enhance photosynthetic capacity and

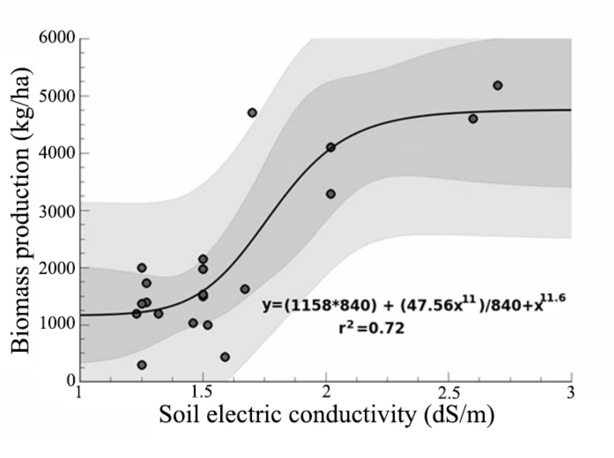

Figure 2. Association between soil electric conductivity and biomass production of *Atriplex canescens* in a semiarid rangeland of northern Mexico.

Table 2. Nutrient content of *Atriplex canescens* growing in soils with three different electric conductivity readings in an arid environment. Values are means ± standard deviation.

Item	<1.4 dS/m [2]	1.4-1.6 dS/m	>1.6 dS/m
Ash	14.5 ± 2.0	15.7 ± 1.8	16.4 ± 2.0
Protein (% DM)	13.6 ± 0.8	13.7 ± 0.9	14.3 ± 1.1
Ether extract (% DM)	1.2 ± 0.1	1.2 ± 0.2	1.1 ± 0.2
NDF (% DM)	57.7 ± 3.7	56.5 ± 5.0	57.7 ± 3.6
ADF (% DM)	30.6 ± 1.9	31.3 ± 2.1	32.2 ± 1.9
Hemicellulose (% DM)	27.1 ± 2.1	25.2 ± 3.4	25.5 ± 3.3
Lignin (% DM)	4.9 ± 1.3	4.7 ± 1.2	5.0 ± 1.2
Cellulose (% DM)	24.3 ± 1.8	24.7 ± 1.8	26.6 ± 2.4
Sodium (% DM)	5.0 ± 1.1	5.6 ± 1.5	5.8 ± 1.9
Potassium (% DM)	2.8 ± 0.9	3.2 ± 0.6	2.8 ± 0.4
Magnesium (% DM)	1.1 ± 0.2	1.2 ± 0.3	1.3 ± 0.2
Calcium (% DM)	0.47 ± 0.08	0.55 ± 0.09	0.57 ± 0.11
Phosphorus (% DM)	0.53 ± 0.11	0.54 ± 0.12	0.47 ± 0.08
Copper (mg/kg MS)	4.1 ± 1.6	4.1 ± 1.4	4.1 ± 1.1
Zinc (mg/kg MS)	92.8 ± 14.0	104 ± 13.9	97.8 ± 11.5
Digestibility MS (%)	62.2 ± 2.8	60.4 ± 4.3	61.8 ± 2.3
Net energy for lactation (Mcal/kg)[1]	1.56 ± 0.05	1.54 ± 0.06	1.51 ± 0.05

[1] EM_L=2.392–0.0273*ADF. [2] Electric conductivity readings did not significantly affect (p<0.05) any of the variables in this table.

consequently the growth of *A. canescens*. Pan *et al.* (2016) suggest that Na^+ promote the C_4 photosynthetic process of *A. canescens* plants and consequently improve the water use efficiency under high levels of soil salinity. Another mechanism for xero-halophytes to increase growth rate is the development of a greater photosynthetic area, allowing its fastest growth to occur at various soil salinity levels (Redondo-Gómez *et al.*, 2006). Additionally, moderate soil salinity led to an increase in the water use efficiency in halophytes (Koyro, 2006; Redondo-Gómez *et al.*, 2007; Hussin *et al.*, 2013).

Nutritional status of *A. canescens* relative to readings of soil EC did not show any significant change. Total soluble carbohydrates and total soluble protein in leaves of *A. nummularia* are increased as water salinity increased (Hussin *et al.*, 2013). The divergence between the aforementioned study and the present trial derives from the fact the former experiment was carried out in hydroponic greenhouse system and the later was conducted in the field with much narrower salinity levels. Results of the present study suggest that photosynthesis, biochemical and photochemical capacities of the leaves of *A. canescens* in slightly saline soils were not impaired.

A. canescens met the protein and energy needs of livestock in different physiological stages and is considered adequate as forage at any stage of development (Pinos-Rodríguez *et al.*, 2007; Mellado *et al.*, 2012). Likewise, the foliage of *A. canescens* presented adequate digestible energy (based on IVDMD), and concentrations of all mineral elements for grazing farm animals and wild ruminants (Suttle, 2010), although adequate water at low salinity levels would also be necessary since *A. canescens* contains high quantities of sodium and potassium. Copper supplementation would be advisable for animals foraging this rangeland with predominance of *A. canescens*, as this element fails to meet the Cu needs of grazing ruminants and could cause copper deficiencies in livestock and wildlife that do not have access to copper supplements or other forages high in copper concentration.

The IVDMD range of *A. canescens* at all sites fell within the range (34.3 to 80%; different digestibility techniques) for DM digestibility noted for different *Atriplex* species (van der Baan *et al.*, 2004; Pinos-Rodríguez *et al.*, 2007; Askar *et al.*, 2016). The NDF content of leaves of *A. canescens* was higher that values found by other researchers (Ben Salem *et al.*, 2004; Otal *et al.*, 2010). As NDF is closely associated with feed intake, these data suggests that the moderate NDF values of the leaves of *A. canescens* in this trial would allow adequate intakes by small stock and cattle.

A. canescens would be a good choice to consider in revegetating rangelands because it offers substantial quantities of forage and nutritional qualities generally adequate to meet requirements of livestock and wildlife, although high ingestion of this plant material with elevated salt content requires increased water intake and could depress feed intake.

In the present study the ash content of leaves of *A. canescens* ranged from 14.5 to 16.4, values lower than those reported for other *Atriplex* species, where ashes exceed 20% of the DM (Khan *et al.*, 2000; Abu-Zanat *et al.*, 2003; Meneses *et al.*, 2012). The mineral composition of *A. canescens* appeared adequate to meet the requirements of small ruminants, cattle and wild herbivores grazing such types of forage (Suttle, 2010).

Leaf sodium content increased with increasing soil EC values, but this difference was not statistically significant. This is contrary to other studies where sodium concentration in the foliage of *Atriplex* increases significantly with increasing soil NaCl concentrations (Benzarti *et al.*, 2014). However, most studies where the association between soil salinity and sodium content of *Atriplex* plants have been carried out in pots (inert material) with the use of complete nutrient solution added with different salinities. Likewise, Na^+ and Cl^- in leaves did not decrease K^+. These results indicate that *A. canescens* in the site of the present study was just slightly challenged with salinity which did not result in salt-induced nutritional imbalances.

Cations (Ca, K, and Mg) concentrations did not decrease with an increase of soil salinity and levels of these minerals were adequate and constant throughout the EC values in the soil, which does not agree with results found for other halophytes (Ayala & O'Leary, 1995; Khan *et al.*, 2000; Belkheiri & Mulas, 2013). However, most studies where increased levels of NaCl have induced decreases in Ca, K and Mg in halophytes have been carried out in plant growth chambers and results under controlled conditions normally does not translate back to field situations (Poorter *et al.*, 2016).

In summary, results of the present study indicate that, under field conditions, *A. canescens* is strongly stimulated by soil electric conductivity values >1.6 dS/m, which suggests that, in drying soils, the accumulation of moderate levels of salt would alleviate rather than exacerbate the stress load on this fodder shrub. Additionally, these results show that nutrient contents of foliage of *Atriplex canescens* are not adversely affected by slight salinity stress in an arid climate.

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