



Influence of *Atriplex canescens* (Pursh) Nutt. on soil properties and nutrient content of dominant plant species in a semi-arid region

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Article Info	Abstract
Article type: Research Article	The use of halophyte species is a suitable application for reclamation of saline soils. <i>Atriplex canescens</i> is considered an adaptable and productive plant species in Iran. The enclosure applied after <i>Atriplex</i> cultivation in arid and semiarid regions. Various soil and vegetation properties were investigated to study the effects of <i>A. canescens</i> plantation and rangeland enclosure on soil and vegetation characteristics. The results indicated that soil organic matter (SOM), EC, pH, and bulk density (SBD) values were significantly higher than those of other areas. The highest values of electrical conductivity (EC) and SOM were reported as 11.82 dS m⁻¹ and 0.51 % in the sub-canopy treatment. The maximum and the minimum values of soil pH were reported as 9.92 and 7.11 in control and sub-canopy treatments, respectively. Compared with control, various soil nutrient contents (Cu, Fe, Mg, Mn, Na, and Zn) were also remarkably higher in sub-canopy treatment. The highest quantitative amount of Na was reported as 185.93 mg kg⁻¹ in the sub-canopy soil and the lowest (173.4 mg kg⁻¹) was seen in the control region. As observed in the Tall Wheatgrass, prevalent weed associated to the shrubs, similar trends were seen in Proline concentration, catalase (CAT), and peroxidase (POD) enzyme activity and the maximum contents were seen in sub-canopy treatments. The changing trend of total chlorophyll content was unlike the changing trend of Proline, Cat, and POD. However, the highest total chlorophyll concentration was reported in the control region treatment (8.32 mg g⁻¹ FW), and the lowest concentration was reported in the sub-canopy treatment. In general, <i>A. canescens</i> had positive effects on the soil and vegetation characteristics of the study area.
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Introduction

The large extent of arid and semi-arid areas in Iran (90% of the country's total area) (Bazrafshan et al., 2020) makes it necessary to pay attention to the reclamation of these rangelands by alternative species that are more compatible with drought. Among abiotic stresses, salinity and drought have a negative impact on the establishment and production of plants (Waseem et al., 2023), especially in arid and semi-arid regions (Sepehry et al., 2012; Akhzari, 2015; Haghighi et al., 2018).

The use of halophytic species for reclamation of saline regions not only have economic benefits, but also is considered ecologically beneficial. The halophyte species of the Chenopodiaceae family have higher amounts of crude protein and ash than grasses and legumes (Davis, 1979). Nemati (1997) reported that *A. canescens* has the highest survival capacity of seedlings in saline and arid regions and was introduced as the most adaptable and productive *Atriplex* species in Iran. *Atriplex* plantation is one of the usual methods of rangeland improvement in arid and semiarid areas. As a non-native species, *A. canescens* has been used for arid and semi-arid rangeland reclamations in Iran. Today, a wide area of dry rangeland in Iran has been cultivated by this type of plant and every year its cultivation area is increased.

Various soil physical (SBD) (Ghazavi and Vali, 2013); chemical (EC and pH) (Hanta et al., 2005; Ahmadi et al., 2018); and nutritional properties (Jafari et al., 2003; Khalkhali et al., 2005) are affected after *Atriplex* establishment. Compared with the control area, the percentage of soil lime (CaCO_3) (Abbasi Khalaki et al., 2015); soil nutrient content (Arévalo et al., 2020), and sodium adsorption ratio (SAR) (Mohebbi et al., 2016) at sub-canopy soil of *A. canescens* have increased significantly. Although *A. canescens* increases soil surface nutrient content, it may have negative effects on the soil by increasing sodium content and EC (Hanta et al., 2005; Ahmadi et al., 2018).

Abiotic stresses significantly affect the concentration of secondary metabolites (Nicolas-Espinosa et al., 2023). Secondary metabolites are responsible for the

protection of plants against environmental stress (Nicolas-Espinosa et al., 2023). These compounds protect plants against destructive factors such as pests, diseases, harmful soil conditions, and environmental stress and create conditions for the plant to continue its life.

There is a strong relationship between plant adaptation to abiotic stress and the activity of antioxidant enzymes (Mishra et al., 2023). These stresses cause the production of compounds with reactive oxygen species (ROS) that damage protein, lipids, carbohydrates, and nucleic acids (Voloshina et al., 2023). Plants use enzymatic (such as catalase and superoxide dismutase) and non-enzymatic (such as phenolic compounds and carotenoids) defense systems to clean and detoxify ROS compounds from the cell surface.

Proline is one of the amino acids that accumulate in plants in response to abiotic stress, especially salt stress, and acts as an osmolyte or molecular chaperone to maintain the structure of plant proteins from damage caused by stress (Koç and Karayığit, 2023). Proline increases plants tolerance to stress through mechanisms such as osmotic regulation, protection of enzymes and proteins, stabilization of intracellular structures such as membranes, and antioxidant role (Ashraf and Foolad, 2007). In addition, changes in chlorophyll concentration have been reported previously in many plant species as a result of their exposure to abiotic stress during growth (Kiran and Baysal Furtana, 2023; Nikzad et al., 2023; Urmi et al., 2023).

The cultivation of *Atriplex canescens* may exert both positive and negative impacts on soil properties and native plant species. Enhancing the physico-chemical characteristics of the soil can contribute to the overall improvement of ecosystem conditions in semi-arid regions. Additionally, the production of osmolytes and enzymes in native plants reflects their adaptability to the presence of non-native species. *Atriplex* plantations can induce various ecological changes, which may either benefit or harm the soil and native vegetation in these environments. Following

the cultivation of *A. canescens*, the area was enclosed to prevent disturbances. Therefore, the objective of this study was to investigate the effects of *A. canescens* plantation on soil physico-chemical properties, as well as on osmolyte and enzyme production in native plant species.

Materials and methods

The study area is Khan Abad Village, Kaboudarahang, a city in Hamadan Province (N 35° 10' 00", E 48° 44' 20"). *A. canescens* was planted in this area in 2016. The average rainfall of the area is 330 mm per year and the climate is cold semi-arid based on Amberger climate classification.

Soil and vegetation sampling

Soil and vegetation sampling was conducted using a systematic random method along 100-meter transects across three treatments: sub-canopy, between the shrubs, and a control area. Soil samples were collected from the surface layer at a depth of 0–20 cm. The dominant plant species across all three treatments was tall wheatgrass (*Agropyron elongatum*). Therefore, to evaluate the impact of *Atriplex canescens* plantation on proline concentration, chlorophyll content, and enzyme activity, samples were specifically taken from this species.

Three 100-meter transects were established in each of the sub-canopy, between-shrubs, and control regions. The control area was located adjacent to the cultivated site and shared similar topographical, climatic, and edaphic conditions. Along each transect, 20 soil and vegetation samples were collected in mid-May 2022. Vegetation was sampled using 1×1 meter quadrats, and 200-gram soil samples were collected using an auger from the 0–20 cm soil depth.

Quantitative analysis of soil physicochemical characteristics

Soil pH was measured in a 1:1 water-to-soil suspension and its EC was measured in saturated extract (Gupta, 2000). SBD was measured by standard Core Sampler

(Schipper and Sparling, 2000). Fe, Cu, Mn, and Zn were extracted from soil by Lindsay and Norvell's (1978) method and then were measured by an atomic absorption device. After neutralization with acid and titration with soda, the soil calcium carbonate equivalent was represented by Richard and Donald's (1996) method. Quantitative analyses of Mg and Na content in soil were carried out by atomic absorption spectrophotometer.

Preparation of enzyme extract from plant samples

The plant tissue of *A. elongatum* (0.5 g) was crushed by 100 mM phosphate buffer (pH=7.0) containing 1% polyvinyl pyrrolidone and 1 mM EDTA on homogenized ice. Then, it was centrifuged at 15000 g for 10 minutes and the plant enzyme extract, was kept at 25° C to measure the enzyme activity (Talaat et al., 2023).

Assay of catalase (CAT) enzyme activity

Catalase (CAT) enzyme activity was measured spectrophotometrically by monitoring the decomposition of hydrogen peroxide at a wavelength of 240 nm for 30 seconds, using a modified method based on Aebi (1984), with a UV-visible spectrophotometer. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 15 mM hydrogen peroxide, and 50 µL of the prepared plant extract. CAT activity was calculated based on the amount of hydrogen peroxide decomposed, expressed in nanomoles per minute.

Peroxidase (POD) enzyme activity

POD enzyme activity was measured with Kar and Mishra's (1976) method. Based on the mentioned method, the test mixture, which included 125 µM of phosphate buffer (with pH = 6.8), 50 µM of pyrogallol, and 50 µM of hydrogen peroxide was prepared. One mL of plant enzyme extract was added to the prepared solution. The prepared test mixture was kept at 25° C for 5 minutes for reaction. After this period, 0.5 mL of sulfuric acid with a volume ratio of 5% was added to stop the experiment. The amount of formed

purpurogallin was determined by the absorbance at 420 nm using a UV-visible spectrometer.

Measuring the amount of Proline and total chlorophyll content

Proline content was extracted and measured using the method of Bates et al. (1973). For this purpose, 0.2 g of fresh plant leaves was homogenized in 4 mL of 3% sulfosalicylic acid. Then, 2 mL of the extract was mixed with 2 mL of acid ninhydrin solution and 2 mL of glacial acetic acid. The mixture was incubated in a hot water bath for one hour. After incubation, 4 mL of toluene was added, and the mixture was vortexed for 30 seconds. Following a 20-minute rest period, the upper colored phase was separated and its absorbance was measured at 520 nm using a spectrophotometer. Proline concentration was determined using a standard calibration curve. Total chlorophyll content in the leaves was determined following the method described by Arnon (1949).

Data analysis

Statistical analysis of the data was carried out using SPSS version 22. First, the normality of the data was checked using the Kolmogorov–Smirnov test, and the homogeneity of the variances was checked using the Lyon test. The T-test was used to investigate the effect of *A. canescens* plantation on soil and vegetation.

Results

Effect of *A. canescens* cultivation on soil physicochemical characteristics

The results of the analysis of variance (ANOVA) of data from the physicochemical characteristics of soil showed no significant

difference in the quantity of calcium carbonate equivalent (Table 1). However, ANOVA results showed remarkable differences ($P \leq 0.05$) for pH and SBD traits in three studied treatments (control region, inter-rows, and sub-canopy soil). SBD ranged from 2.87 to 1.12 g cm⁻³ in control and inter-rows regions, respectively. The quantity of organic matter and EC were significantly higher ($P \leq 0.05$) in sub-canopy soil than those of inter-rows and control region (Table 1). So the highest values of EC and SOM were reported as 11.82 dS m⁻¹ and 0.51 % in the sub-canopy treatment (Table 1). The highest and the lowest values of soil pH were reported as 9.92 and 7.11 in control and sub-canopy treatments, respectively.

Effects of *A. canescens* plantation on soil nutrition content

The ANOVA results showed a remarkable effect of *A. canescens* plantation on the concentration of nutrients in the soil (Table 2). In general, the trend of all the investigated nutrients in this research showed significant differences ($P \leq 0.05$) between the concentration of nutrients in the control and in the soil of inter-rows and sub-canopy regions. Comparison of average data obtained from Cu, Fe, Mg, Mn, and Zn showed that the change of these nutrients in the control region, inter-rows and sub-canopy soil has a similar pattern. Thus, the highest and the lowest concentrations of mentioned nutrients (Cu, Fe, Mg, Mn, and Zn) are seen in the sub-canopy soil and the control region, respectively. Also, ANOVA results showed significant differences between Na content in various treatments and the highest quantitative amount of Na was reported as 185.93 mg kg⁻¹ in the sub-canopy soil and the lowest (173.4 mg kg⁻¹) was seen in the control region (Table 2).

Table 1. The effect of *A. canescens* plantation on physicochemical properties of soil

Soil characteristics	Treatments		
	Control region	Inter-rows	sub-canopy soil
Organic matter (%)	0.42± 0.06 b	0.45± 0.03 b	0.51± 0.02 a
Calcium carbonate equivalent (%)	11.83± 1.32 a	12.67± 0.91 a	13.92± 0.65 a
EC (dS m ⁻¹)	8.43± 0.63 b	10.13± 0.25 a	11.82± 0.34 a
pH	9.92± 0.24 a	7.54± 0.52 b	7.11± 0.41 b
Bulk density (g cm ⁻³)	2.87± 0.01 a	1.12± 0.32 b	1.53± 0.13 b

Dissimilar letters in each row indicate significance at the 5% probability level.

Table 2. Effects of *A. canescens* cultivation on soil nutrition content

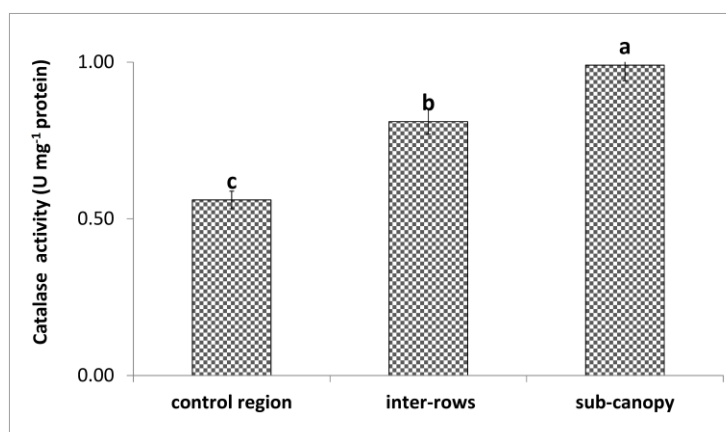
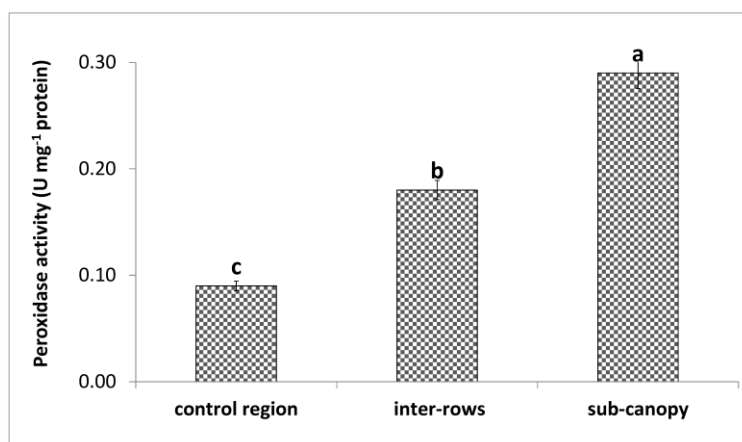
Soil nutrition content	Treatments		
	Control region	Inter-rows	sub-canopy soil
Cu (mg kg ⁻¹)	3.12± 0.04 b	3.65± 0.02 ab	4.82± 0.01 a
Fe (mg kg ⁻¹)	41.2± 3.72 b	50.1± 2.85 a	53.3± 3.03 a
Mg (mg kg ⁻¹)	6.32± 0.41 b	9.65± 0.34 a	11.92± 0.52 a
Mn (mg kg ⁻¹)	7.82± 0.31 b	8.11± 0.57 ab	11.92± 0.37 a
Na (mg kg ⁻¹)	173.4± 4.54 b	182.6± 4.89 a	185.93± 3.74 a
Zn (mg kg ⁻¹)	4.84± 0.09 a	5.63± 0.12 ab	6.01± 0.08 a

Dissimilar letters in each row indicate significance at the 5% probability level.

Effect of *A. canescens* plantation on CAT and POD enzymes activity in *A. elongatum* as a dominant plant in the study area

CAT enzyme activity increased significantly ($P \leq 0.05$) in sub-canopy treatment (Figure 1). The highest quantitate value of CAT enzyme activity was observed in the sub-canopy treatment as 0.99 U mg⁻¹ protein. Also, the lowest amount of CAT activity was seen in

the control treatment and was equal to 0.56 U mg⁻¹ protein. Similar to CAT enzyme activity compared to the control region and inter-rows treatments, POD enzyme activity in *A. elongatum* significantly increased in the sub-canopy treatment. Thus, the highest amount of POD activity was reported in the sub-canopy treatment as 0.29 U mg⁻¹ protein and the lowest in the control region treatment as 0.09 U mg⁻¹ protein (Figure 2).

**Figure 1.** Effect of *A. canescens* plantation on CAT enzyme activity in *A. elongatum***Figure 2.** Effect of *A. canescens* cultivation on POD enzymes activity in Tall Wheatgrass

Effect of *A. canescens* plantation on Proline and total chlorophyll content in *A. elongatum* as a dominant plant in the study area

The results from this research showed a significant ($P \leq 0.05$) effect of *A. canescens* cultivation on the amount of Proline in plants grown in the control region, inter-rows, and sub-canopy soil. The results of comparing the average Proline concentration in *A. elongatum* showed the lowest amount of Proline in plants grown in control soil ($1.82 \mu\text{mol g}^{-1} \text{FW}$) and the highest amount (3.42

$\mu\text{mol g}^{-1} \text{FW}$) in plants grown in the sub-canopy treatment (Figure 3).

The trend of total chlorophyll concentration was unlike that of Proline. The ANOVA results indicated that there was a significant difference ($P \leq 0.05$) between the chlorophyll concentration in different treatments (control region, inter-rows, and sub-canopy). In general, the highest total chlorophyll concentration was reported in the control region treatment and was equal to $8.32 \text{ mg g}^{-1} \text{FW}$, and the lowest concentration was reported in the sub-canopy treatment as $5.11 \text{ mg g}^{-1} \text{FW}$ (Figure 4).

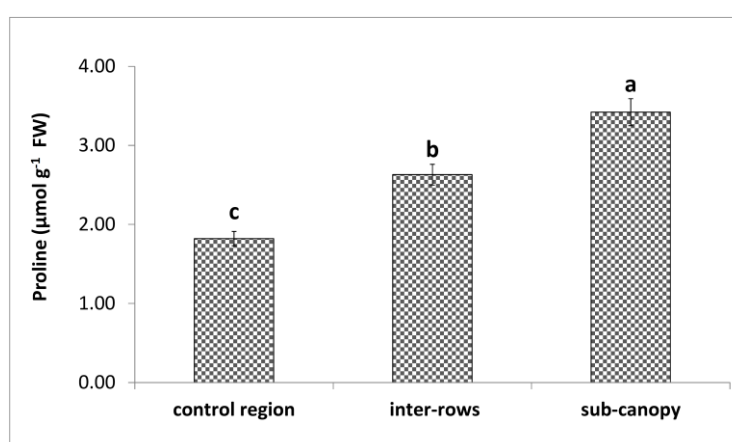


Figure 3. Effect of *A. canescens* plantation on the amount of Proline content in plants grown in the study area

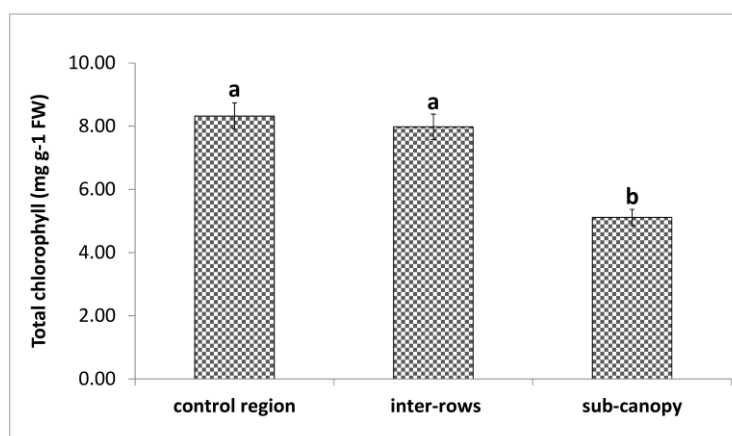


Figure 4. Effect of *A. canescens* cultivation on total chlorophyll concentration of *A. elongatum*

Discussion

Effect of *A. canescens* cultivation on physicochemical properties and nutrition content of soil

Various drought episodes and improper livestock grazing have degraded native rangeland vegetation in the study area. To

rehabilitate these areas, shrub species—particularly *Atriplex*—are commonly used. *Atriplex* is highly resistant to prevalent environmental stresses in Iran and is often established in arid and saline rangelands. However, as a non-native plant species, *Atriplex* may exert both positive and

negative effects on soil properties and native plant communities.

Compared to the control region, the electrical conductivity (EC) of soils in the *Atriplex*-cultivated areas was significantly higher, indicating increased salinity. Following the plantation of *A. canescens*, the area was fenced to create an enclosure, and livestock grazing was prohibited. Consequently, leaf litter deposition and rainfall-induced leaching of salts from the plant—originally absorbed from deeper soil layers—contributed to surface soil salinization. Numerous studies have reported similar findings, showing significant increases in EC values after *A. canescens* cultivation (Hanta et al., 2005; Ahmadi et al., 2018). These results are consistent with those of West and Ibrahim (1983), Hanta et al. (2005), and Khalkhali et al. (2005). Supporting these findings, Alharby et al. (2014) reported that halophytic plants such as *Atriplex* species absorb sodium and chloride ions to maintain osmotic balance and ensure optimal growth. However, these ions are often excreted into the rhizosphere, thereby elevating soil salinity.

Atriplex cultivation also led to a decrease in soil pH. This decline in pH beneath the *Atriplex* canopy is attributed to increased vegetation cover and elevated soil organic matter (SOM). The decomposition of SOM generates humic acids, which in turn lower soil pH. In contrast, livestock excreta in the control area possess alkaline properties (Brummerloh & Kuka, 2023), contributing to the higher soil pH observed there. Nonetheless, Rahimizadeh et al. (2010) reported similar pH reductions in *Atriplex*-cultivated areas.

Soil bulk density (SBD) was significantly lower in the cultivated area compared to the control site. This difference is likely due to the absence of livestock grazing in the enclosure, which prevented soil compaction. In contrast, the control region was subjected to trampling from grazing animals. Xiao et al. (2024) also reported a significant increase

in SBD in grazed rangelands, supporting the present findings.

The calcium carbonate equivalent (CCE) did not differ significantly between the studied areas, consistent with the findings of Boudjabi and Chenchouni (2022). However, *Atriplex* cultivation increased SOM and soil nutrient concentrations beneath the shrub canopy (Hanta et al., 2005), enhancing soil fertility (Jafari et al., 2003; Khalkhali et al., 2005). The observed nutrient enrichment was likely due to leaf litter deposition from *A. canescens*. West and Ibrahim (1983) also noted that *Atriplex* plantations improved SOM content, microbial activity, and mineral availability in soils.

Livestock grazing typically reduces vegetation cover and limits organic residue input into the soil. As a result, grazing acts as a disturbance that suppresses decomposer microbial activity, slows SOM decomposition, and ultimately reduces rangeland soil productivity (Reeder et al., 2004; Hui & Jackson, 2005). Other studies (Javadi et al., 2005; Azarnivand et al., 2009; Golluscio et al., 2009; Liu et al., 2012) have confirmed that grazing leads to the removal of aboveground plant biomass and causes soil compaction through trampling. Additionally, trampling crushes surface plant litter, increasing soil temperature and accelerating decomposition rates. Therefore, under certain conditions, grazing can also contribute to elevated nutrient concentrations in surface soils.

Effect of *A. canescens* plantation on CAT and POD activity, Proline, and total chlorophyll contents in *A. elongatum* as a dominant plant in the studied area

Abiotic stresses cause changes in the concentration of biochemical indicators such as Proline, CAT, POD, and chlorophyll by affecting various physiological, biochemical and hormonal processes. These changes may increase the resistance of plants to environmental stresses and are considered an indicator to evaluate the adaptation of plants in abiotic stressful regions. Various

mechanisms are activated in the plant to reduce the toxic oxidative effects caused by abiotic stresses. POD and CAT production are the two main systems for enzymatic defense against peroxidative damage by ROS compounds. Compared to the control and inter-rows treatment regions, CAT and POD enzyme activity in *A. elongatum* increased significantly in the sub-canopy treatment. Soil salinity at the sub-canopy treatments was significantly higher than that of all other treatments. In arid and semi-arid regions, destructive impacts of drought stress are escalated in saline soils. Therefore, the plant under the bushes faced more stressful conditions which caused CAT and POD enzyme activity to increase. In agreement with this research, increasing activity of CAT and POD under environmental stress has been reported in previous research (Mishra et al., 2023; Voloshina et al., 2023).

The results from this research showed a significant effect of *A. canescens* cultivation on the amount of Proline in plants grown in the control region, inter-rows, and sub-canopy soil. In agreement with this research, Koç and Karayığit (2023) reported Proline accumulates in plant tissues in response to abiotic stresses. Proline acts as an osmolyte or molecular chaperone (Koç and Karayığit, 2023) for osmotic regulation, protection of enzymes and proteins, stabilization of intracellular structures such as membranes, and antioxidant role (Ashraf and Foolad, 2007).

In general, the highest total chlorophyll concentration was reported in the control region treatment, and the lowest concentration was reported in the sub-canopy treatment. One of the reasons for the decrease in the concentration and decomposition of chlorophyll under stress is due to the increase in the activity of the chlorophyllase enzyme. It seems that in the present study, the activity of the chlorophyllase enzyme increased with the increase in salinity levels, and as a result, it led to the decomposition of chlorophyll and a decrease in its concentration. A similar report by El-Dakak et al. (2023) stated a

remarkable decrease in chlorophyll concentration under abiotic stress.

Conclusions

Livestock grazing, as one of the most significant ecological disturbances, can have detrimental impacts on the soil and vegetation of semi-arid rangelands. Identifying suitable plant species for restoring degraded rangelands is crucial for maintaining ecosystem sustainability. The selection of plants for rangeland restoration must be carried out with great care, as introducing species with undesirable traits may cause environmental harm and disrupt the biological balance of these ecosystems. Changes in soil physicochemical properties vary under different climatic and edaphic conditions, leading to diverse outcomes. Such changes may improve soil conditions and promote restoration; however, the cultivation of non-native species can sometimes produce adverse environmental effects.

According to the results of this study, *Atriplex canescens* significantly increased soil organic carbon and nutrient content in the sub-canopy areas compared to surrounding regions. Additionally, soil bulk density (SBD) decreased significantly in the inter-row areas relative to the control sites. However, the presence of *A. canescens* also elevated electrical conductivity (EC) and soluble sodium in surface soils, potentially causing notable surface soil degradation.

Overall, the cultivation of *A. canescens* demonstrated a generally positive effect on the soil quality in the study area. Moreover, *Atriplex* cultivation enhanced the activities of catalase (CAT) and peroxidase (POD) enzymes in *Agropyron elongatum*, the dominant native plant species in the region. Proline concentration in *A. elongatum* also increased, while total chlorophyll content decreased. Therefore, it can be concluded that *A. canescens* cultivation has a positive influence on the native plant species in the studied rangelands.

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