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Effects of *Bromus kopetdaghensis* Drobov Pit-Seeding on Soil and Vegetation Characteristics in a Semi-Arid Rangeland

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ABSTRACT

Aims: Pit-seeding is widely recognized as a primary method for rangeland improvement in hilly and sloping areas. This research aimed to investigate the impact of pit-seeding on soil and vegetation properties in the semi-arid rangelands of Hamadan Province.
Materials & Methods: Systematic random sampling was conducted in May 2022. Ten 100-meter transects were established in the pit-seeding area, and an equal number of samples were collected from an adjacent uncultivated control area.
Findings: ANOVA analysis revealed no significant differences in soil bulk density (SBD), soil moisture content (SMC), or pH values between pit-seeding and control regions. However, the electrical conductivity (EC) of soil in the control region was 5.9 $ds.m^{-1}$, which was significantly higher than 4.3 $ds.m^{-1}$ in the pit-seeding area. Statistical comparisons ($p<0.05$) of soluble sugar levels showed that plants in the pit-seeding area had significantly higher sugar content than those in the control region. Additionally, the highest numerical values for bioindicators (richness, evenness, and diversity) were observed in the pit-seeding area, while the lowest values were recorded in the control region. Proline, catalase (CAT), and peroxidase (POD) levels were significantly higher in the control region, likely due to livestock grazing. Soil nutrient levels, including calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), were also notably greater in the control region.
Conclusion: Pit-seeding improved vegetation quality and strengthened ecological bioindicators in semi-arid rangelands, fostering healthier plant communities. This method effectively reduced soil electrical conductivity, creating more favorable conditions for vegetation growth. Moreover, pit-seeding maintained higher soil nutrient levels, underscoring the importance of sustainable grazing practices in preserving ecosystem balance.

Keywords: Grazing, Proline; Catalase; Peroxidase; Soil Nutrient.

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Introduction

Rangelands serve as critical ecosystems that support livestock-dependent communities, provide essential ecological services, and contribute to biodiversity conservation. Livestock grazing and forage utilization are among the primary activities in these landscapes, significantly influencing their structure and function [1, 2]. While grazing is a fundamental component of rangeland management, its effects vary based on environmental conditions. In arid regions, where soil fertility is typically low, livestock grazing encourages the growth of resistant species. Conversely, in humid areas with higher soil fertility, grazing increases plant tolerance and enhances species diversity [3].
Despite the benefits of controlled grazing, unsustainable grazing practices pose a significant threat to rangeland integrity, resulting in soil degradation, vegetation loss, and biodiversity decline. Excessive livestock pressure often results in the depletion of soil nutrients, reduction in organic matter, and disturbance of the natural regeneration cycle. Understanding the ecological consequences of grazing, as well as viable restoration solutions is essential for ensuring the long-term sustainability of semi-arid rangelands [4].
Unregulated grazing has been identified as a key driver of rangeland degradation, particularly in arid and semi-arid ecosystems [5]. Overgrazing exacerbates soil erosion, reduces vegetation cover, and accelerates the processes of desertification. These disturbances significantly impair rangeland productivity by limiting nutrient cycling and weakening ecosystem resilience [4]. Heavy grazing is also associated with a decline in soil fertility caused by the loss of organic matter and reduced plant biomass [6]. Research indicates that nutrient availability in enclosed rangelands is substantially higher than in heavily grazed areas, illustrating the detrimental effects of excessive livestock pressure [7].

Beyond soil degradation, intense grazing pressure harms biodiversity [8]. Rangeland biodiversity plays a crucial role in environmental stability, enhancing ecosystem resilience against climate fluctuations and human disturbances [9]. Species richness and evenness are fundamental indicators of biodiversity, directly influencing the sustainability of rangelands [10]. As grazing intensity increases, shifts in species composition occur, resulting in the dominance of less palatable species and a decline in overall ecosystem function.

Numerous studies have investigated the detrimental effects of livestock grazing on soil fertility, vegetation structure, and rangeland stability. Increased grazing intensity has been shown to reduce nitrogen levels, particularly in deep soil layers compared to surface layers [11, 12]. Additionally, grazing depletes phosphorus stocks, as plants absorb phosphorus from the soil and release it upon decomposition [13]. Over time, potassium depletion also occurs due to the removal of forage biomass, further exacerbating soil degradation [14]. These disruptions to soil nutrient dynamics hinder plant regeneration, decrease biomass production, and reduce rangeland productivity, reinforcing the need for sustainable restoration techniques.

The physical properties of soil are also adversely affected by heavy grazing. Research has shown that excessive grazing reduces soil porosity, resulting in increased soil compaction and bulk density [15]. Soil bulk density changes immediately after grazing and trampling, limiting water infiltration and affecting root development [16, 17]. These structural changes further restrict the growth potential of desirable plant species, reinforcing the cycle of degradation.

Among various restoration strategies, pit-

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seeding has been identified as an effective technique for rehabilitating degraded rangelands, particularly in mountainous and sloping landscapes. This method involves creating small depressions in the soil and planting seeds within them, which enhances moisture retention, seed germination rates, and plant establishment. One promising species used in pit-seeding is *Bromus kopetdaghensis*, a drought-tolerant species within the Poaceae family, valued for its forage production, soil stabilization properties, and ecological benefits [18, 19, 20]. Given its ability to protect soil, increase nutrient availability, and improve vegetation structure, *Br. kopetdaghensis* is considered an ideal candidate for rangeland rehabilitation efforts.

This study aims to evaluate the effectiveness of pit-seeding as a restoration strategy, specifically focusing on its impact on soil physicochemical properties and plant physiological responses in the semi-arid rangelands of Hamadan Province. By comparing pit-seeded areas with adjacent uncultivated control sites, this research seeks to determine the method's potential to enhance soil fertility, improve vegetation quality, and restore ecosystem functionality. Furthermore, the study aims to examine how pit-seeding interacts with broader rangeland management practices, including livestock grazing intensity and adaptive ecological restoration techniques.

Given the increasing pressures of climate change, land degradation, and unsustainable grazing practices, evidence-based restoration approaches are essential for mitigating the environmental impacts of rangeland disturbances. Understanding the mechanisms by which pit-seeding influences soil fertility, nutrient availability, and plant diversity will provide valuable insights into the development of sustainable rangeland management strategies. The implementation of scientifically backed restoration techniques, such as pit-seeding, is critical for ensuring long-term productivity, enhancing ecosystem resilience, and preserving biodiversity in semi-arid rangelands.

Materials & Methods

Experimental Design and Data Gathering

The present research was conducted in a natural rangeland of Ghatar Aghaj Village, located in the western parts of Iran, near Kaboudarahang City, Hamadan Province (N 35°34'49"; E 48°27'24"). The climate of the region is semi-arid, with an elevation ranging from 1936 to 2016 m above sea level and an average annual precipitation of 299.6 mm. Soil and vegetation sampling in this research was done by systematic randomized method along 100-meter transects using a soil sampling cylinder and standard plots (for vegetation sampling) in May 2022.

In this way, ten 100-meter transects were established in the pit-seeding area, then 10 plots were determined along each transect, and 500 gr of soil was taken from the depth of 0-30 cm in each plot. Similarly, the same number of samples was also taken in the adjacent area that was not cultivated and was considered as a control area. In each plot, plant species were registered and classified based on vegetative form, lifespan and durability, canopy percentage, density, abundance, and production. Based on the gathered data, Shannon-Wiener (Eq. 1) [21] and Simpson (Eq. 2) [22] indices were used to evaluate plant species diversity [23]. Species richness values were calculated using Margalef (Eq. 3) [24] and Menhinick (Eq. 4) [25] indices and the evenness of plant species was assessed using the Simpson index (Eq. 5) [10, 23]. The equations of bioindicators used in this research are shown in Table 1.

Soil Analysis

Bulk density, electrical conductivity (EC), and pH of soil were measured using

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Table 2) The t-test results of soil pH, EC, SBD, and SMC values in pit-seeding and control regions.

Treatments	pH	EC ($ds.m^{-1}$)	SBD ($g.cm^{-3}$)	SMC (%)
Control Region (Grazed area)	8.3± 0.11 [a]	5.9± 0.08 [a]	1.27± 0.03 [a]	13.53± 1.12 [a]
Pit-seeding Region (Exclosure area)	7.9± 0.13 [a]	4.3± 0.07 [b]	1.33± 0.01 [a]	15.11± 0.83 [a]

Different letters in the same column represent a significant difference at the 5% level.

Table 3) The t-test results of soil Ca, Mg, Cu, Fe, Mn, and Zn contents in pit-seeding and control regions.

Treatments	Ca	Mg	Cu	Fe	Mn	Zn
Control Region (Grazed area)	5.62±0.03 [a]	13.2±0.04 [a]	5.32±0.02 [a]	62±0.19 [a]	13.5±0.12 [a]	8.36±0.12 [a]
Pit-seeding Region (Exclosure area)	3.86±0.01 [b]	9.3±0.02 [b]	3.11±0.01 [b]	53±0.11 [b]	10.3±0.07 [b]	6.49±0.05 [b]

Different letters in the same column represent a significant difference at the 5% level.

standard methods. In this research, soil pH and electrical conductivity (EC) values were measured using pH and EC meter in saturated paste extract. SBD was determined by the Foth method [26]. The content of Fe, Zn, Cu, and Mn in soil samples was measured by the acid digestion method [27], and the titration method was used for Ca and Mg content determination [28].

Vegetation Analysis

The catalase (CAT, EC 1.11.1.6) and peroxidase (POD, EC 1.11.1.7) activities were assessed following the procedure described by Chance and Maehly [29]. Briefly, 1 mL of H2O2 (40 mM) and 1.9 mL of 50 mM buffer (1mL) were added into 100 µL of the enzyme extract for the determination of CAT activity. The variation in absorbance was measured every 20 s for 3 min at 240 nm by using a spectrophotometer (Specord 205-Analytik Jena). For POD activity, 1 mL of 20 mM guaiacol, 900 µL of 40 mM H2O2, and 1 mL of 50 mM phosphate buffer were added into 100 µL of the enzyme extract. The change in POD activity was recorded every 20 s for 3 min at 470 nm. One unit of CAT and POD activities was considered as 0.01 absorbance changes per minute. The quantity of proline content was determined based on the modified method of Bates et al. [30] and expressed as $\mu mol.g^{-1}.FW^{-1}$.

To measure the concentration of soluble sugars, 0.1 g of dry vegetation powder was added to 5 ml of 2.5 normal hydrochloric acid and placed in a hot water bath at 100 °C for 3 hours. In the next step, the samples were neutralized with sodium carbonate. Then, the volume of the samples was increased to 100 ml with distilled water, and the samples were centrifuged at 3500 g for 10 minutes. In the next step, 0.5 ml was taken from the upper part of the samples and made up to 1 ml with distilled water. After that, 4 ml of anthrone solution was added to the samples, and they were placed in a boiling water bath for 8 minutes. Finally, the absorbance was measured at a wavelength of 630 nm using a spectrophotometer [31].

Statistical Analysis

Before the statistical comparison of the collected data, the normality of the soil and vegetation data was evaluated using the Kolmogorov-Smirnov normality test performed in the SPSS (version 24) software. Diversity, richness, and evenness indices were calculated for selected sites using PAST (version 5) software. For the statistical

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comparison of the data obtained in the two pit-seeding and control areas, an unpaired t-test was used, and the Duncan test was performed in SPSS software to compare the means ($p < 0.05$).

Findings

The comparison of the average soil pH data in the two areas did not show significant differences. In general, the investigated soil pH was alkaline, with a value greater than 7. However, in the control area, the pH level was slightly higher than that of the pit-seeding region (Table 2). Moreover, compared with the pit-seeding region, the EC result of the control region showed a significant difference and was recorded at 5.9 days.m^{-1} (Table 2).

The t-test analysis revealed no significant difference between SBD values in pit-seeding and control regions, but all measured values were greater than one in both study regions. On the other hand, the soil moisture content of pit-seeding and control did not show a significant difference ($p<0.05$). However, the soil moisture content in the pit-seeding area was slightly higher than in the control area (Table 2). The overall changes in soil nutrients followed the same pattern, and the contents of Ca, Mg, Cu, Fe, Mn, and Zn were significantly higher in the control region (Table 3).

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The results of the t-test obtained from the calculation of diversity, richness, and evenness indices are shown in Table 4. The numerical values of species diversity and richness in all calculated indicators have the significantly highest amounts in the pit-seeding region (exclosure area) ($p < 0.05$). There is no significant difference between the numerical values of the evenness index (Simpson) in the exclosure area and the control region (Table 4).

Table 4) The results of analysis of the t-test comparing species diversity, richness, and evenness indices in the two study areas.

Bioindicator	Pit-seeding Region (Exclosure area)	Control Region (Grazed area)
Shannon-Wiener (Diversity)	1.54±0.09 [a]	1.33±0.13 [b]
Simpson (Diversity)	0.87±0.04 [a]	0.69±0.06 [b]
Margalef (Richness)	2.89±0.41 [a]	1.52±0.56 [b]
Menhinick (Richness)	1.94±0.03 [a]	1.52±0.11 [b]
Simpson (Evenness)	0.18±0.01 [a]	0.15±0.02 [a]

The statistical comparison ($p < 0.05$) of soluble sugar amounts in the two regions indicated that the plants in the pit-seeding area have significantly higher soluble sugar levels compared to those in the control area (Figure 1).

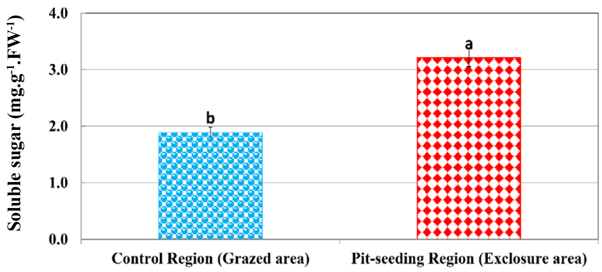

Figure 1) The results of the test comparing plant's soluble sugar in the two study areas.
Different letters represent a significant difference at the 5% level.

The t-test results showed that the changes in proline (Figure 2), CAT (Figure 3), and POD (Figure 4) in the two investigated regions have a similar trend. The amount of proline, CAT, and POD in the control region, which was mainly exposed to livestock grazing, was significantly more than that of the pit-seeding area (Figures 2, 3, and 4). Based on the results, a significant difference was found in proline content in pit-seeding (exclosure area) and grazed regions. The maximum amount of proline was found at 4 μmol.g^{-1}.FW^{-1} and the minimum recorded as 2.1 μmol.g^{-1}.FW^{-1} (Figure 2).

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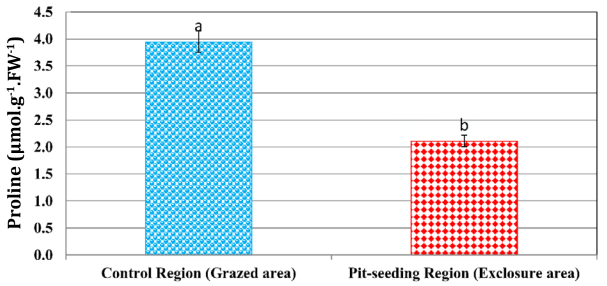

Figure 2) The results of the t-test comparing plant's proline content in pit-seeding (exclosure area) and grazed regions.
Different letters represent a significant difference at the 5% level.

The CAT and POD significantly increased in the grazed region, and the numerical values were recorded at 1.67 and 0.39 Units.mg^{-1}, respectively (Figures 3 and 4).

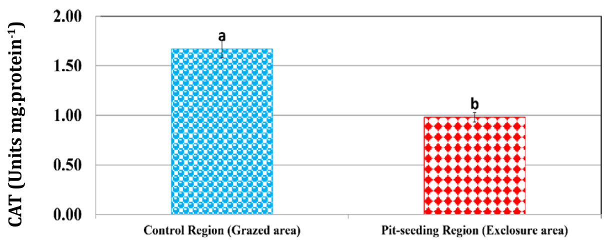

Figure 3) The t-test results of CAT content in pit-seeding and control regions.
Different letters represent a significant difference at the 5% level.

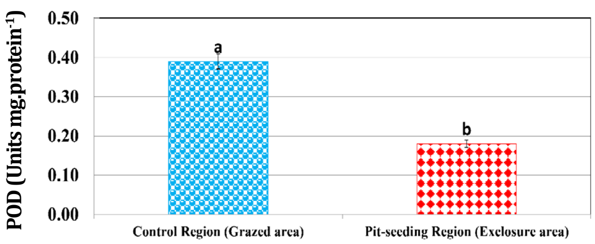

Figure 4) The t-test results of POD content in pit-seeding and control regions.
Different letters represent a significant difference at the 5% level.

Discussion

The results of the present research indicated that there were no significant differences between soil pH in pit-seeding and control regions. These results are in agreement with the findings of Liebig et al. [32] and Zhang et al. [33]. In contrast, Yadav et al. [34] reported that organic acids and inorganic acids, such as carbonic acid, increase in the soil due to the increase in organic matter. It is expected that in the exclosure regions, soil alkalinity will also increase compared to the grazed rangelands. Although carbonic acid is a weak acid, the constant production of that in the soil causes lime leaching from these soils and pH decrement [35]. On the other hand, Ghobadi and Akhzari [36] reported that changes in soil chemical parameters in different climates and edaphic conditions have a dissimilar trend. Therefore, these changes may have a distinct trend in other regions.

Improper use, excessive use of rangelands, and destruction of vegetation all increase soil dryness, which causes an increase in evaporation [37] and, as a result, the tendency for more soil salinity [38]. The results of this study showed a significant difference in soil electrical conductivity (EC) between pit-seeding and control regions. In agreement with the results of current research, Teague and Kreuter [38] also reported that the electrical conductivity of the pit-seeding area was lower than that of the grazed area due to the increase in vegetation and the decrease in evaporation and transpiration in the exclosure regions. The electrical conductivity increase in the soil of grazed rangeland may be caused by the reduction of soil fertility and the rise in cation exchange capacity.

The data average comparison of the SBD in the two studied regions did not show any significant differences. SBD is one of the factors that changes with grazing and trampling due to soil compaction [17]. Vegetation and soil porosity reduction in grazed rangelands caused by livestock trampling [15] increase SBD in arid and semi-arid rangelands. As a result of livestock trampling, the soil aggregates are splashed and turned into finer particles, which are located in the pores of the soil and increase the SBD [39]. Moreover, Heavy livestock grazing can lead to soil compaction, reducing soil porosity [40].

Comparing the effect of *Br. kopetdaghensis* pit-seeding and livestock grazing on the soil water content revealed no significant differences. These results are in contrast with those of Ghobadi and Akhzari [36], who reported that the SMC has decreased significantly in the grazed region. Inconsistent results were also reported by Wang et al. [41], who stated that the amount of SMC in grazed regions is significantly higher than those of pit-seeding rangelands. A possible reason for this disagreement may be related to livestock grazing. Animal light grazing did not lead to significant soil compaction in grazed areas.

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The Simpson and Shannon-Wiener diversity indices yielded the same results in the studied areas. Simpson's index changes between zero and 1, and a numerical value close to 1 indicates the highest species diversity [42]. According to the results, the Simpson index was estimated to be 0.87 in the pit-seeding region (exclosure area) and 0.69 in the control region (grazed area), indicating a generally low diversity of plant species in the study area. The Shannon-Wiener index typically ranges from 1.5 to 3.5, and in exceptional cases, this value may be less than 1.5 or greater than 3.5 [43]. In this study, the value of this index was found to be 1.54 in the pit-seeding region and 1.33 in the Grazed area, which generally indicates low diversity of vegetation cover.

The comparison of the numerical values of the richness indices (Margalef and Menhinick) in the two investigated regions showed a significant difference ($p<0.05$). The highest numerical value of these indicators was obtained in the pit-seeding region, and the lowest value was obtained in the control region. Therefore, the application of pit-seeding has improved the condition of the vegetation. This is following previous findings reported by Gillen et al. [44], who stated that livestock grazing can affect the structure of plant communities in rangeland ecosystems. Hickman et al. [45] have also noted that livestock grazing can increase annual plant growth and species richness, but it also leads to soil disturbance, which in turn causes ecosystem instability.

Based on t-test results ($p<0.05$), the amount of soluble sugar in the plants of the pit-seeding area was significantly higher than that of the control area. The content of soluble sugars may be a helpful method in selecting salt and drought-resistant species. Soluble sugars represent the most important source of energy in the diet of livestock; therefore, increasing their amount in the plant means increasing the yield of the plant. In the pit-seeding region (exclosure area), nitrogen-containing compounds are a suitable selective absorber for ammonium cations and reduce the leaching of nitrogen from the root environment [46].

Based on the results of this research, the changes in proline, CAT, and POD in the two investigated regions have a similar trend. The amount of proline, CAT, and POD in the control region, which was exposed to livestock grazing, was significantly higher than that in the pit-seeding area. Livestock grazing as abiotic stress leads to the formation of reactive oxygen species (ROS) in plant cells. Different plants have various mechanisms to reduce the harmful effects of ROS. Oxygen free radicals in the plant cell are removed by some antioxidant enzymes such as CAT and POD [47]. The results of this research indicated that CAT and POD activity increased remarkably in the grazed region (Figure 6). These results were in agreement with the reports of Shafi et al. [48], who stated that antioxidant enzymes increased under

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stressful conditions. Proline affects the solubility of various proteins and enzymes in plants and prevents them from changing their nature. The production of this protein increases significantly under biotic and abiotic stresses.

The statistical comparison ($p<0.05$) of nutrient data revealed that the contents of Ca, Mg, Cu, Fe, Mn, and Zn were significantly higher in the control region. Any environmental factor that causes movement and increases the accessibility of nutrients improves the absorption of these elements by plants [49]. Therefore, the reason for the increase in soil nitrogen content in the exclosure area compared to the grazed area can be attributed to the addition of litter due to the decomposition of plants.

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Authors' Contributions: S Talebi was responsible for the field sampling and laboratory analyses, while **D Akhzari** developed the experimental design, performed the data analysis, and prepared the manuscript. Both authors have read and approved the final version of the manuscript.

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