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ASSESSMENT OF SOIL SEED BANK CHARACTERISTICS IN A MOUNTAIN GRASSLAND OF IRAN

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Abstract

Soil seed bank is an important reservoir for restoration of degraded rangeland ecosystems. The study was conducted to assess the ability of soil seed bank to restore the aboveground vegetation in a mountain grassland, north-west of Iran. We studied characteristics of the soil seed bank and similarity between seed bank and ground flora at two soil depths of 0-5 and 5-10 cm. In order to predict persistent seeds, the relationship between seed weight and variance of seed dimensions were calculated. The results showed that Poaceae in ground flora and soil seed bank was the dominant family. Cryptophytes and hemicryptophytes had higher abundance in both depths. The seed bank composition did not differ significantly ($p > 0.5$) between the two burial depths, however, the total seed bank density was significantly ($p < 0.01$) higher in the 0-5cm depth (134 seedlings) relative to 5-10 cm (81 seedlings). Species composition of the seed bank and aboveground flora was similar (Sorensen's similarity index = 76%). The relationship between seed mass and variance of seed dimension showed that seed size and shape were not related to persistence of the soil seed bank of the selected species. Our results suggest that the soil seed bank can play an important role in the rehabilitation of aboveground vegetation in the mountain grasslands of Iran, if they are threatened.

Keywords: mountain grassland, restore, seed bank, seed density

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1. Introduction

Iranian rangelands with an area of 84.8 million hectares cover 50 percent of Iran's lands (Squires et al., 2017). Only 9.3 million ha of Iranian rangelands (10.3%) are in good conditions, while the share of average rangelands is 41.4 percent and the rest (48.3%) is allocated to poor rangelands (Farahpour and Marshall, 2001). The quantity and quality of Iran's rangelands are decreasing due to different factors especially increase in population and demand for food, overgrazing and drought. Increasing degradation of rangelands vegetation is causing the extinction of many plant species. In this regard, studies related to soil seed bank and evaluation of the potential of this

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bank in restoring vegetation of rangelands is doubly important.

Plant community composition and distribution are mainly influenced by seed and seedling environment (Briceño et al., 2015). The soil seed bank is an important seed storage system for passive restoration of deteriorating herbaceous species diversity, cover, and production in degraded ecosystems (Vieira and Overbeck, 2020; Yehnjong et al., 2017). The seeds buried in the soil seed bank after germination come out of the composition of the seed bank and affect the composition of the vegetation by establishment in aboveground vegetation. Awareness of seed bank composition is essential in designing rangeland conservation and restoration programs, and policy making in arid ecosystems (Ma et al., 2010). One of the applications of soil seed bank identifications is to recognize the regenerative power of the native plant composition of each habitat. Soil seed bank studies determine guidelines for supporting species whose seed density is low in the soil and needs support to increase the population (Fourie, 2008). The soil seed bank is considered as a genetic reserve of plant species.

Seed bank density varies with soil depth depending on the seed characteristics (e.g. size, shape, and weight), predation, soil structure and disturbances. The presence of seeds in the lower depths of the soil can be attributed to their durability (Auld et al., 2000). Also, the ability of seeds to penetrate deep into the soil depends on the morphological seed characteristics (e.g. size and shape), seed predation (Wen-Ming et al., 2004), soil features such as humidity and texture (Özaslan Parlak et al., 2011), trampling (Rusterholz et al., 2011) and physiological needs of the seed as well as the activity of living organisms (Bertiller and Ares, 2011; Leck, 2012). Of course, it is believed that the smaller seeds have higher probability to be buried in the deep layers of the soil (Luzuriaga et al., 2005).

Another important issue in seed banks is seed persistence in the soil (Peco et al., 2003), which strongly affects the structure of plant community. Based on the longevity of seeds in the seed banks, there are two groups: transient and persistent. In a transient seed bank, none of the seeds produced in a given year remain viable in the habitat for more than one year, whereas seeds in the persistent seed bank are one or more years old (Walck et al., 2005). The persistence of seeds in the soil depends largely on environmental conditions and seed related physical and physiological properties. Seed bank persistence is crucial for seeds to disperse over time and spreads the risk of recruitment failure in a variable and changing environment. Seed dimensions have been proposed as good indicators for predicting seed persistence (Baskin and Baskin, 2014; Mndela et al., 2020). Thompson et al. (1993) used the variance of seed length, width, and depth for measuring sphericity of a range of British herbaceous species. They recommended that the mechanisms underlying these relationships between seed dimensions and persistence were because of burial process and rates of predation. Seeds of elongated and flat-seeded species germinate more rapidly than those of round-seeded species, because elongated and flat seeds are more likely to have higher post-dispersal seed predation than round seeds. However, empirical data supporting this prediction are lacking. Transient seed bank species had significantly heavier seeds and higher variance of seed shape than persistent species in a field study (Cerabolini et al., 2003). Therefore, in general, it can be said that transient seeds have a more important role in the restoration of vegetation in degraded rangelands.

Considering the extent and diversity of Iran's flora, intensity of rangeland destruction and the importance of soil seed bank in the restoration of aboveground vegetation, the role of soil seed bank studies in Iranian rangelands is highlighted. However, only little data are available on the mountainous rangelands of Iran. No research has been done on the persistence of seed in mountainous rangelands of Iran. The unique rangelands of these climates spread over mountainous areas throughout Iran at altitudes above 2500 meters.

The main purpose of this research is to assess the ability of soil seed bank to restore the aboveground vegetation in Iranian grasslands. The study intended to answer the following questions:

(i) What is the composition and density of seeds buried in the soil?

(ii) How much similarities exist between soil seed bank and aboveground vegetation?

(iii) Are there any differences between shallower (0-5 cm) and deeper (5-10 cm) soil layers on seed bank composition and density?

(iv) What is the relationship between seed dimensions and seed weights in related to persistent seed banks?

2. Material and methods

2.1. Description of study site

The study was done in Arshad-Chaman rangeland, in the Azerbaijani part of the Iran-o-Touranian vegetation zone with a mountainous climate located 60 km south of Tabriz, East-Azerbaijan province, north-west of Iran (Fig. 1). The area is 365 ha and located near Sahand Mountain (37° 43´N and 46° 19´E). Elevation at the study site ranges from 2700 to 3420 m.a.s.l. This area is a part of Lake Urmia Watershed basin and is one of the nomad’s mountain ranges of East-Azerbaijan province. The area has an average annual rainfall of 202.7 mm, the maximum temperature of 16.8°C, the minimum temperature of 7.8°C and, the average temperature of 12°C. Humidity is high in December to May, with the length of the wet season being six months. The fluctuations of humidity in the humid months vary from 11.9 mm to 49.2 mm. The soil characteristics are shown in Table 1. There is no livestock grazing in the study area and it has been under exclosure for 15 years, but a section of the rangeland is under rotational grazing system.

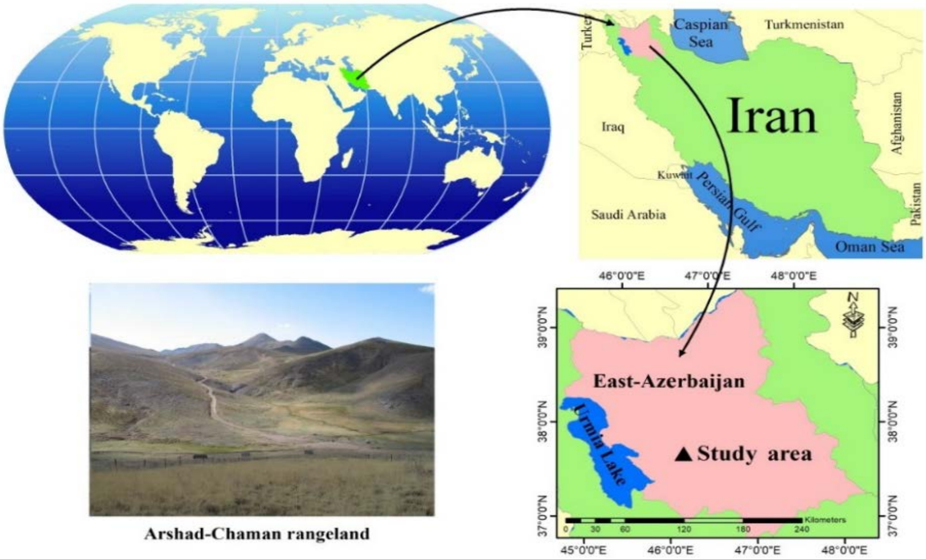

Fig. 1. The location of study area in East-Azerbaijan province of Iran

Table 1. The soil characteristics of Arshad-Chaman grassland in East-Azerbaijan province of Iran

Soil texture	*PH*	*EC (ds/m)*	*CaCO₃ (%)*	*Organic carbon (%)*	*Total nitrogen (%)*	*Phosphorous (ppm)*	*Potassium (ppm)*
Sandy loam	6.12	0.894	0.918	1.424	0.144	4.354	290.48

Livestock are usually present in the area under grazing from early June to early September (3 months). In some years, the entrance of livestock may be delayed until mid-June due to the cold weather.

2.2. Soil and plant sampling

Soil samples for seed bank analysis were collected at the beginning of September before the germination of seeds in the field. Twenty square plots (1-m^2) were systematically placed along four transects. In each plot, five soil cores were collected by a metal pipe (3 cm in diameter and 5 cm deep) from burial depths of 0-5 cm and 5-10 cm, giving a total of 200 samples (2 depths × 5 cores × 20 plots). In total, a volume of 7065 cm^3 soil was collected from two depths. The 0-5 and 5-10 cm depths were selected because most of the seeds are found at 0-5 cm depth and some at a depth of 5-10 cm. After manual removal of gravel and roots, the soil samples of each depth were bulked, transported to the laboratory, and air-dried. The percent herbaceous cover was estimated at a species level as the proportional ground covered by plants per 1-m^2 quadrats in June during peak production.

To measure seed dimensions of all species observed in the seed bank, seed samples were collected in the grassland. To test the relationship between seed size, shape and persistence, the variance of seed dimensions was used. A minimum of 10 and a maximum of 100 seeds per species were weighed, and the dry weight of the seeds was recorded. Variance in seed dimensions was obtained by transforming the average length, depth, and width of 15 seeds for each species, so that length was unity and calculating the variance of the transformed values. This gives a value for seed shape such that spherical seeds have a variance of 0 and elongated or flattened seeds have variances of up to 0.33 (Moles et al., 2000; Thompson et al., 1993).

The method used for determining seed density in this study was the germination method (Roberts, 1981). The germination method gives an estimate of seed density of viable seeds only in a known volume of soil. The soil samples were kept at 3-4ºC for 2-3 days (Stark et al., 2008). Then, the samples were spread at 1-cm depth on sterilized sand in plastic pots in a greenhouse with a temperature between 10 and 15ºC. Three plastic pots filled with only sterilized sand were maintained throughout the study as controls. The pots were watered manually to field capacity every day and emerging seedlings were identified, recorded, and removed daily from the pots. Seedlings that could not be identified were replanted in a new pot for later identification. After six months, when no new seedlings germinated for three weeks, the identification of the seedlings was completed (Chu et al., 2019). In order to estimate the number of seeds per square meter (100 cm^2) of soil, we planted this volume of soil in rectangular trays with thickness of one centimeter.

2.3. Statistical analysis

To show the species composition of the soil seed bank, relative frequency and relative density of emerged seedlings of soil seed bank were calculated. For standing vegetation, relative frequency and relative coverage were calculated. These can be presented in the form of the following formula (Myers and Bazely, 2003), Eqs. (1-6):

$$Frequency = \frac{No.\ of\ plots\ or\ pots\ in\ which\ the\ species\ or\ seeding\ is\ present}{Total\ no.\ of\ pots\ or\ plots\ sampled} \quad (1)$$

$$Relative\ frequency\ (\%) = \frac{Frequency\ of\ the\ species\ or\ seedling}{Frequency\ of\ all\ the\ species\ or\ seedling} \times 100 \quad (2)$$

$$Density = \frac{No.of\ individual\ of\ the\ seedling}{Total\ no.of\ pots\ sampled} \quad (3)$$

$$Relative\ density\ (\%) = \frac{Density\ of\ an\ individual\ seedling}{Sum\ of\ density\ of\ all\ the\ seedlings} \times 100 \quad (4)$$

$$Coverage = \frac{Total\ coverage\ percentage\ of\ the\ species}{Total\ no.\ of\ plots\ sampled} \quad (5)$$

$$Relative\ coverage\ (\%) = \frac{Coverage\ percentage\ of\ the\ species}{Sum\ of\ coverage\ percentage\ of\ all\ the\ species} \times 100 \quad (6)$$

The Shapiro-Wilks test was used to determine if the data were normally distributed. A popular non-parametric test to compare outcomes between two related groups that are not normally distributed and the samples are small is the Wilcoxon signed ranks test. Therefore, the difference in the total number of individuals of seedlings and species composition of soil seed bank between two depths was tested using a non-parametric Wilcoxon signed ranks test. Species were classified into different functional groups according to their life form (Raunkiaer, 1934*).* Also, the percentage of annual and perennial species was calculated. Species similarity between the aboveground vegetation and the soil seed bank was calculated using the Sørenson similarity index (Magurran, 2004) (Eq. 7).

$$Q = \frac{2c}{a+b} \quad (7)$$

where: *a* is the number of species in the seed bank; *b* the number of species in established vegetation; *c* the number of species existing in both lists.

The same process was used for species similarity between the 0-5 cm and 5-10 cm depths in soil seed bank, where (a) and (b) are the numbers of species in the depths of 0-5 cm and 5-10 cm of the soil seed bank, respectively, and (c) is the number of species shared in both depths. To visualize the floristic composition of the seedlings between the two depths, we performed a nonmetric multidimensional scaling (nMDS) based on seed abundance. NMDS is a technique used to simplify multivariate data into a few important axes to facilitate recognition and interpretation of patterns and differences among groups. NMDS makes no assumptions about the data (Faith et al., 1987) and is considered among suitable methods for graphical representation of floristic ordination (Clarke, 1993). NMDS uses the rank order of similarity relationships between samples. The accurate of NMDS reflected by the stress value. NMDS with a stress value lower than 0.3 represents all relationship accurately. The samples that did not provide any germination were excluded from the analysis.

Additionally, we used the non-parametric analysis of similarities (ANOSIM) approach to partition dissimilarity between the two depths and used 9999 random permutations to test the significance of the partition (Clarke, 1993). ANOSIM provides a way to test statistically whether there is a significant difference between two groups of sampling units. This test checks to see if the similarities between the group and within the group are the same. ANOSIM uses a ranked dissimilarity matrix as input instead of raw data and gives the R-value that shows the strength of the factors (depth) on the separation of samples. R-value is supposed to vary between zero and 1. R-value close to 1 indicates a high separation between levels of factors, while R-value close to zero indicates no separation between levels of factors.

The similarity percentages analysis (SIMPER) was used to identify the species that contributed most to differences between two depths of seed bank. The SIMPER states the contribution of individual species to the overall Bray-Curtis dissimilarity (Clarke, 1993). All statistical analyses were run using PAST v.4 (Hammer et al., 2001), Excel, and SPSS software.

3. Results and discussion

3.1. Standing vegetation and the soil seed bank composition

In the aboveground vegetation, we recorded 21 species, belonging to 11 families. Poaceae, Asteraceae and Fabaceae were the most dominant families. Regarding the percentage cover, *Festuca ovina*, *Astracantha aurea*, and *Bromus tomentellus* had the highest density in the aboveground vegetation (Fig. 2).

In the soil seed bank, we recorded 11 species, belonging to 6 families with Poaceae, Fabaceae and Asteraceae being the most dominant families at both depths. Also, *Veronica orientalis* and *B. tomentellus* were the most dominant herbaceous species at both depths. The frequency of these species collectively, was 55 and 40% at 0-5 and 5-10 cm depths, respectively (Table 2). Since Poaceae, Fabaceae and Asteraceae families have the largest frequency in Iran's flora and make up about 40% of Iranian plant species (Asadi, 2019; Ghahremaninejad and Nejad Falatoury, 2016), their significant frequency in aboveground vegetation and soil seed bank was expected.

Table 2. Species composition, life form, relative density, relative frequency and relative coverage percentage of the standing vegetation and seedlings of soil seed bank in study area

Species	*Family*	*Raunkiaer*	*Life form*	*Standing vegetation based on cover*		*0-5 cm soil seed bank based on seedlings*		*5-10 cm soil seed bank based on seedlings*	
				CP%	*FR%*	*DE%*	*FR%*	*DE%*	*FR%*
Alopecurus textilis Boiss.	Poaceae	He	PG	5.2	7.4				
Astracantha aurea (Willd.) Podlech	Fabaceae	Ch	Bu	15.7	11.7	4.5	3.7	15.2	14.8
Astragalus pinetorum Boiss.	Fabaceae	He	PF	3.6	3.5	4.5	3	5.7	6.2
Bromus tomentellus Boiss.	Poaceae	Cr	PG	9.3	11.3	13.6	14.9	19	23.5
Campanula stevenii M. Bieb.	Campanulaceae	He	PF	0.3	1.7				
Chenopodium foliosum Asch.	Amaranthaceae	Th	AF	0.6	0.9				
Cirsium haussknechtii Boiss.	Asteraceae	He	PF	0.5	0.9				
Eremogone dianthoides (Sm.) Ikonn.	Caryophyllaceae	He	PF	6.8	9.5	3	1.5	1.9	1.2
Festuca ovina L.	Poaceae	Cr	PG	26.9	12.6	10.6	9	9.5	11.1
Festuca rubra L.	Poaceae	Cr	PG	14.5	6.9	9.1	6	5.7	4.9
Gagea confusa A. Terracc.	Liliaceae	Cr	PF	0.2	0.9	4.5	2.2		
Onobrychis cornuta (L.) Desv.	Fabaceae	Ch	Bu	1.4	2.6	4.5	6	5.7	6.2
Papaver orientale L.	Papaveraceae	Th	AF	0.2	0.4				
Poa araratica Trautv.	Poaceae	Cr	PG	0.4	1.3	10.6	5.2	11.4	7.4
Polygonum cognatum Meisn.	Polygonaceae	He	PF	0.2	0.9			3.8	3.7
Stachys lavandulifolia Vahl	Lamiaceae	Ch	PF	0.2	0.4				
Tanacetum aureum (Lam.) Greuter & al.	Asteraceae	He	PF	3.6	4.3	13.6	9		
Taraxacum azerbaijanicum Soest	Asteraceae	He	PF	1.8	6.1				
Thymus kotschyanus Boiss. & Hohen.	Lamiaceae	Ch	Bu	4.9	6.9				
Tragopogon marginatus Boiss. & Buhse	Asteraceae	He	PF	0.3	1.7			3.8	2.5
Veronica orientalis Mill.	Plantaginaceae	He	PF	3.4	8.2	21.2	39.6	19	18.5

Note: CP%= Relative coverage percentage, DE% = Relative density%, FR% = Relative frequency%, He = Hemicryptophyte, Ch = Chamaephyte, Cr = Cryptophyte, Th = Therophyte, PG = Perennial grass, Bu = Bush, PF = Perennial forb, AF = Annual forb

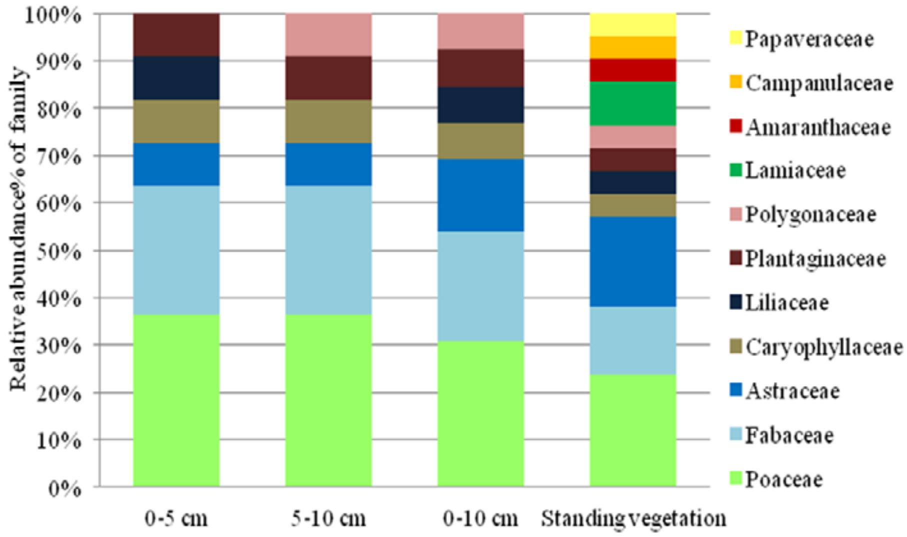

Fig. 2. Family of species in soil seed bank seedling and standing vegetation of study area

Long life and easy distribution of seeds are other important reasons for abundance of these families in the study area. In some other rangelands of Iran such as mountain grassland of north Alborz (Nazari et al., 2014) and Chaharbagh rangelands of Golestan province, northern Iran (Salarian et al., 2016), the significant share of these three families in the composition of the soil seed bank has been mentioned. The remarkable abundance of Poaceae in the vegetation of the earth has caused that this family has a significant share in the composition of aboveground vegetation and soil seed bank as mentioned in many other regions (e.g. Ghebrehiwot et al., 2012; Miao et al., 2016; Price et al., 2010).

3.2. The life form of the soil seed bank and aboveground vegetation

Perennial plant species, forbs and grasses in particular made up the majority of species types in both soil seed bank (85%) and standing vegetation (76%). Guan et al. (2019) obtained the same results in coastal wetland ecosystems in eastern China, while Yang et al. (2018) showed that the capacity of perennial vegetation in soil seed banks is lower than that of annual vegetation. The frequency of bush form increased with burial depths, whereas perennial forbs declined with depth. The main reason for the contribution of most perennial species to aboveground vegetation is the exclosure of the area. Livestock grazing increases the abundance of annual species and reduces the share of perennial species. The high share of perennial species in aboveground vegetation caused the share of these species in the soil seed bank to increase. In addition, repeated droughts in Iran, including the study area in the years before the study, have been effective in reducing the abundance of annual species.

Hemicryptophytes and cryptophytes were the most highly represented life form in the soil seed bank and standing vegetation. These two life forms represented over 70% of the species recorded in standing vegetation and more than 80% in the soil seed bank. In these two groups of plants, the regenerating buds are either placed inside the soil (cryptophytes) or under snow inside the litters (hemicryptophytes) that are characteristic of mountainous ecosystems. The mountainous situation of Arshad-Chaman (elevation range between 2700 to 3420 m.a.s.l with cold climate and existence of snow) has caused the dominancy of these two life forms in this area. Plant species belonging to these two life forms play an important role in soil consolidation and support of other species due to having an extensive root system.

3.3. Sphericity index, volume, weight, and dimensions of seeds

In this study, we measured the dimensions and weight of 13 seeds that germinated from the soil seed bank in the study area. Seeds have a weight ranging from 0.0002 g for *Eremogone dianthoides* and *Poa araratica* to 0.008 g for *O. cornuta*. The sphericity index varied from a minimum of 19.16 for *Tragopogon marginatus* to a maximum of 76.72 for *Veronica orientalis*. Seed volume ranged from 5.55 to 346.65 mm^3. This is a wide range of seed volume indicating that a wide range of seed shapes was present in the soil seed bank (Table 3). After calculating the variance of seed dimensions, this variable was plotted against seed weight (Fig. 3).

Although, the results of some research on several floras (e.g. Cerabolini et al., 2003; Thompson et al., 1993) have shown that the persistence of a seed in a soil seed bank largely depends on the size and shape of the seed, but this relationship was not observed in this study. Thompson et al. (1993) stated that compact seeds which weigh less than 3 mg are all persistent in the soil and the suggested underlying mechanism is predation. Small seeds experience less predation and are more likely to become buried, which itself offers significant protection from predation by vertebrates. In this study, the variance and seed weight of six species were less than 0.14 and 3 mg, respectively; indicating that all seeds out of this range are short lived.

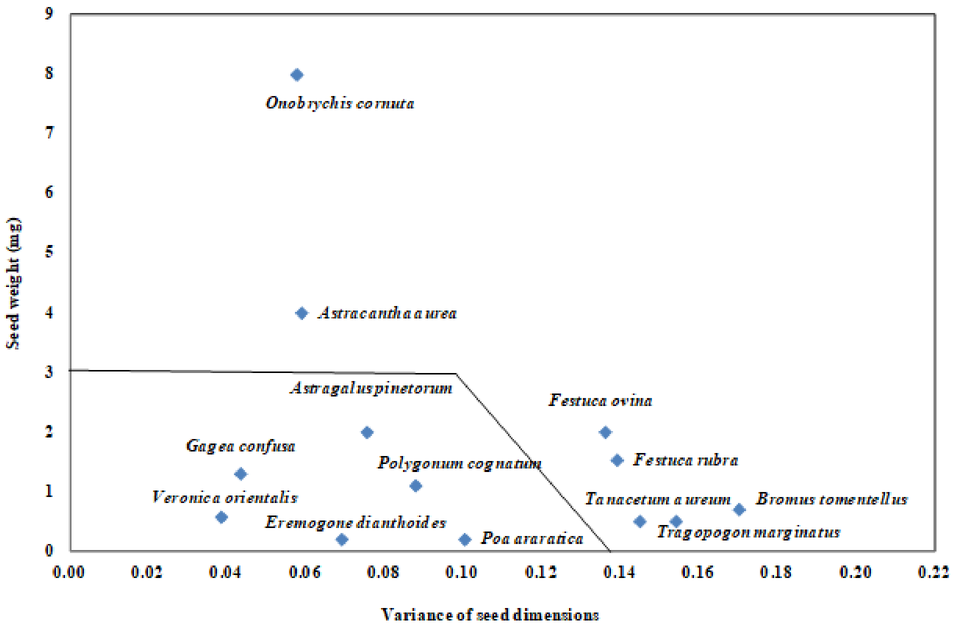

Fig. 3. Relationship between seed mass and variance of seed dimension. The solid line shows the boundary found by Thompson et al., (1993) that species in this region have persistence seeds. All species with at least two seeds in the 5-10 cm layer are assumed to have a persistent seed bank

Table 3. The properties measured in the soil seed bank of the study area

Species of soil seed bank	*Average Length (mm)*	*Average width (mm)*	*Average thickness (mm)*	*Average Weight (g)*	*Weight for 1000 seeds (g)*
Astracantha aurea	3.177	2.164	1.206	0.004	4
Astragalus pinetorum	3.271	2.481	1.016	0.002	2
Bromus tomentellus	11.128	1.622	0.92	0.0007	0.7
Eremogone dianthoides	1.719	1.378	0.56	0.0002	0.2
Festuca ovina	4.651	0.947	0.715	0.002	2
Festuca rubra	7.11	0.84	0.713	0.0015	1.53
Gagea confusa	6.61	4.17	3.004	0.0013	1.3
Onobrychis cornuta	4.04	2.6	1.541	0.0080	8
Poa araratica	2.941	1.052	0.669	0.0002	0.2
Polygonum cognatum	2.765	1.121	0.708	0.0011	1.1
Tanacetum aureum	4.565	0.888	0.669	0.0005	0.5
Tragopogon marginatus	13.95	1.245	1.103	0.0005	0.5
Veronica orientalis	1.963	1.721	1.014	0.0006	0.57

In our research, there are some species with persistent seeds that have large and/or elongated or flattened seeds. *Bromus tomentellus* and *O. cornuta* are the best examples of the fact that the first species have high value of seed variance and the second species have a seed weight of 8 mg. On the other hand, at a depth of 5-10 cm in the soil, there were no seeds of the *G. confusa*, while this species is in the range of persistent species. Overall, seed size and shape do not appear to be related to persistence in the soil in our research. This result, combined with similar evidence from Australia (Leishman and Westoby, 1998), New Zealand (Moles et al., 2000), Iran (Thompson et al., 2001) and Spain (Peco et al., 2003) shows that using diaspore morphology to predict the ability of a species' seeds to persist in the soil is not appropriate in all floras.

Due to the different results of the relationship between seed shape and size and its persistence, it is emphasized that seed persistence in the soil is not wholly determined by seed size and shape. Germination requirements, dormancy mechanisms, and resistance to pathogens also contribute to persistence (Moles et al., 2005). Different results can be due to the differences in the evolutionary history of seed dispersal, contrasts in vegetation structure, occurrence of disturbances in habitats, relative influence of seed predation and differences in rate of burial.

3.4. Seedlings density of the soil seed bank

We recorded 215 seedlings from the total soil seed bank (134 seedlings in depth of 0-5 cm and 81 seedlings in the depth of 5-10 cm) in a volume of 7065 cm^3 soil collected from two depths. As a result, it was estimated that 304 seedlings/m^2 in the depth of 0-10 cm. The seedlings density was higher at 0-5 cm and there was a significant difference between the two depths of the soil seed bank (Wilcoxon signed ranks test = -2.453, p = 0.014). This coincides with the general idea that the maximum seed density lies close to the surface (Muvengwi and Ndagurwa, 2015; Sandrine et al., 2006). We attribute the decline in seed density with soil depth to very little vertical dispersion of seed by soil organisms such as ants that are contributed in moving seeds from the surface depth to lower depths. Low density of soil seed bank seedlings can be related to unfavorable climatic conditions in the studied rangeland and seed dormancy characteristics.

3.5. The similarity between the soil seed bank and aboveground vegetation

A total of 13 and 21 species were recorded in the seed bank and vegetation, respectively. All species found in the soil seed bank were also found in aboveground vegetation. In other word, the seed bank mirrored the aboveground vegetation composition (Sorensen's index = 76%). Therefore, it can be said that the species composition of aboveground vegetation and soil seed bank were closely related. In total, the diversity of aboveground vegetation species was much greater than the diversity of soil seed bank species. The reason why a number of species were present only in aboveground vegetation is that these plants are either unable to germinate in the greenhouse due to the provision of suitable conditions such as the loss of seed dormancy or the need for special conditions or they may have low-longevity and vitality seed bank.

3.6. Similarity of the seed bank composition between the burial depths

The number of species common to both depths (0-5 cm and 5-10 cm) was nine, leading the Sørenson similarity index between two depths to be 82%. The species richness (the number of species types) of seed bank did not differ significantly between two depths (Wilcoxon signed ranks test = -0.713, p = 0.476). The results of the nMDS test based on the Bray-Curtis similarity matrix showed that species composition of the soil seed bank in two depths is similar. The value of a stress factor was 0.2. This statistic shows how species are distributed at two depths of 0-5 cm and 5-10 cm (Fig. 4). The one-way ANOSIM based on Bray-Curtis similarity index showed that there is a statistically significant difference (p = 0.04) in the abundance of seedlings between two depths of the soil seed bank. The R-value was 0.09 that suggests an even distribution of high and low ranks between two depths. In other words, there is a small separation between seedling compositions of two depths in the soil seed bank of the study area (Fig. 5).

The R value gives the strength of the depth factor on the seedling of the soil seed, so the results showed that there was a little separation between levels of depth factor (0-5 cm versus 5-10 cm seedlings).

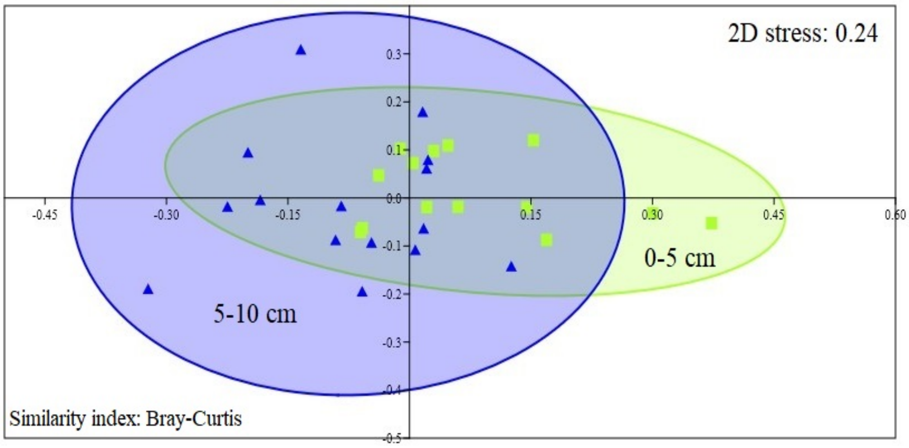

Fig. 4. Two-dimensional space obtained from the nMDS analysis that shows the high similarity (shown by some overlap of data) between two depths (0-5 cm (squares) and 5-10 cm (triangles)) of soil seed bank in the study area

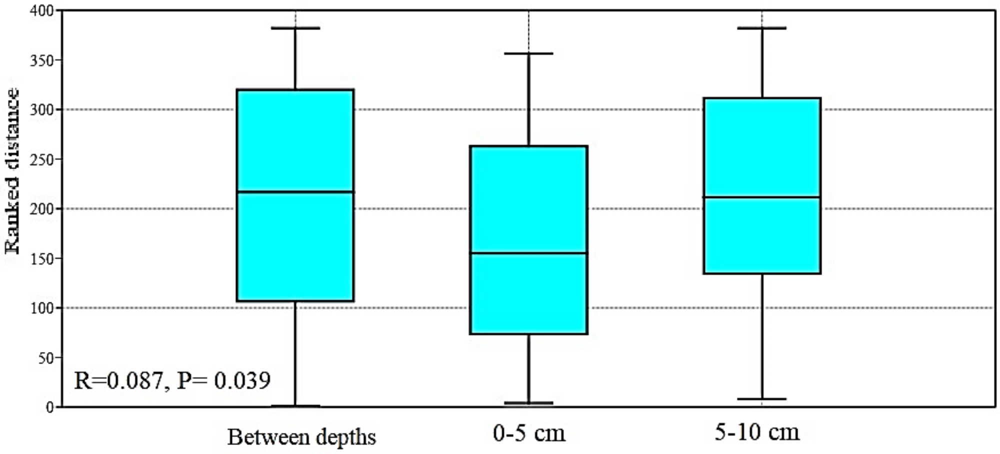

Fig. 5. Box whisker plot of ANOSIM analysis showing the changes in the abundance of seedling of the two depths of soil. Black horizontal bar in the box indicates median; bottom of the box indicates 25th percentile; and top of the box indicates 75th percentile

Table 4. SIMPER analysis (analysis of similarities) showing the percentage of contribution of individual of each species in dissimilarity between two depths of soil seed bank

Species	*Average dissimilarity*	*Contribution (%)*	*Cumulative contribution (%)*	*Mean abundance (Depth of 0-5 cm)*	*Mean abundance (Depth of 5-10 cm)*
Veronica orientalis	17	25	25	3.8	1.0
Bromus tomentellus	11	15	40	1.4	1.3
Astracantha aurea	7	10	50	0.4	0.8
Festuca ovina	7	9	59	0.9	0.6
Tanacetum aureum	6	9	69	0.9	0.0
Onobrychis cornuta	5	7	76	0.6	0.3
Festuca rubra	5	7	83	0.6	0.3
Poa araratica	4	6	88	0.5	0.4
Astragalus pinetorum	3	4	92	0.3	0.3
Polygonum cognatum	2	2	95	0.0	0.2
Gagea confusa	1	2	97	0.2	0.0
Tragopogon marginatus	1	2	99	0.0	0.1
Eremogone dianthoides	1	1	100	0.1	0.1

The SIMPER analysis (similarity percentage analysis) as a post hoc test for ANOSIM determined that *V. orientalis* with about 25% Contribution in dissimilarity, most express the between-depth effect. As mentioned before, the seeds of this species have lower weight and were observed more abundance at a depth of 0-5 cm in the soil. *Bromus tomentellus* and *A. aurea* contributed about 15%, and 10%, respectively to the total dissimilarity between two depths of the soil. As the ANOSIM results showed, there was no big effect for depth factor in terms of seedling composition, so these species did not show much contribution in the dissimilarity between the two depths. *Bromus tomentellus* had almost the same mean abundance at two depths and *A. aurea* had a higher frequency at a depth of 5-10 cm due to heavier seeds (Table 4).

4. Conclusions

In this study we assessed characteristics of soil seed bank (seed composition of two depths of 0-5 cm and 5-10 cm, dimensions, weight, and its similarity with standing vegetation) of a mountain rangeland in Iran. The results showed that species composition of the seed bank and aboveground flora was similar and there was high value of Sorenson's index between them. There was no difference between shallower (0-5 cm) and deeper (5-10 cm) soil layers on seed bank based on species composition, however the total number of seedlings was significantly higher in the 0-5cm depth. Because of the most species in the 5-10 cm layer belonged to persistent seed bank, it can be said that the soil seed bank can play an important role in restoring the aboveground vegetation of mountainous grasslands of the study area, where other rehabilitation activities are expensive due to unfavorable weather and geomorphological conditions.

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