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Fire effects on soil seed banks under different woody plant species in Mazandaran province, Iran

Misagh Ghasempour [a], Reza Erfanzadeh [a,*], Péter Török [b,c]

[a] Rangeland Management Department, Faculty of Natural Resources, Tarbiat Modares University, Tehran, Iran
[b] ELKH-DE Functional and Restoration Ecology Research Group, Debrecen, Hungary
[c] Polish Academy of Sciences, Botanical Garden - Center for Biological Diversity Conservation in Powsin, Warszawa, Poland

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ABSTRACT

Soil seed banks play a major role in the post-fire regeneration of semi-arid mountain grasslands. Plant species present before fire can determine the soil seed bank (SSB) characteristics in fire-prone ecosystems. However, it remains unclear how the density and species composition of the SSB under the canopy cover of specific shrub species may be affected by fire. Therefore, we designed a study to test the effects of prescribed burning on the viable SSBs under the canopies of two shrub species: *Berberis integerrima* (with open canopies) and *Onobrychis cornuta* (with dense canopies). We selected 20 study sites that included separate patches of the two shrub species and nearby herbaceous patches as control. Soil sampling was carried out pre- and post-burning of shrub canopies and the control patch. Soil samples were collected at two depths: 0–5 cm and 5–10 cm, and transported to the greenhouse for germination experiments. The results showed that canopy fire of shrubs decreased SSB density and species richness. However, the effects of shrub burning on SSB were species-specific and these reductions were more pronounced for canopy burning of *O. cornuta* than for *B. integerrima*. Total SSB densities decreased by 61% for *B. integerrima* and 71% for *O. cornuta* after canopy fire at soil depth of 0–5 cm. At soil depth of 5–10 cm, total SSB density decreased by 45% under *O. cornuta* after canopy fire, while canopy fire of *B. integerrima* did not affect SSB density. Herbaceous patch burning had no statistically significant effects on SSB density at soil depths of 0–5 cm and 5–10 cm. The comparison of quantitative and qualitative similarity indices between pre- and post-fire species composition of shrub canopy showed that the statistically significant effect of *B. integerrima* burning on SSB composition was less pronounced compared with *O. cornuta*. This study indicated that seeds stored within the soil under certain shrub species are at a high risk of mortality during fire. Thus, successful post-fire recovery does not rely on the seed bank reservoir under these shrubs.

1. Introduction

The soil seed bank (SSB) is a collection of viable seeds stored in the soil of a specific area. The SSB may be supplied by the surrounding vegetation, as well as by seeds that have been transported from elsewhere by wind or animals (Zou et al., 2021). The SSB is a successional archive that reflects the management history and composition of vegetation in the past (Thompson et al., 1997). The study of viable plant seeds in the soil is an important principle in vegetation ecology and macroecology, due to their key role in vegetation dynamics. For example, SSBs determine the potential responses of plant communities to disturbances, and can contribute to the overall plant community resilience (Erfanzadeh et al., 2021).

Fire is one major type of ecological disturbance that is linked to SSBs in both forest and grassland ecosystems (Fernandes et al., 2021). Given the importance of SSBs in the recovery and restoration of vegetation in the degraded areas after fire, the effects of fire on SSB have been extensively studied in different grasslands (e.g., Snyman, 2005 in semi-arid grasslands; de Oliveira et al., 2019 in neotropical grasslands; Cuello et al., 2020 in temperate grasslands), where it can affect both above- and below ground habitat conditions. For example, fire may affect grassland functioning by the reduction or elimination of aboveground green biomass and litter (Snyman, 2004). Fire events are responsible for maintaining structure and diversity of plant communities (Bond and Keeley, 2005), opening gaps within the vegetation, and creating new colonization sites for species establishment (Fidelis et al., 2012).

* Corresponding author at: Rangeland Management Department, Faculty of Natural Resources, Tarbiat Modares University, Tehran, Iran.
E-mail address: Rezaerfanzadeh@modares.ac.ir (R. Erfanzadeh).

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Conditions in the burnt gaps may differ from the rest of the habitat, with higher daily temperature fluctuations in the upper soil layers (Daibes et al., 2017). Nutrient availability may be enhanced following fire by the mobilization of organic phosphorus and potassium, which may directly influence seed recruitment and plant communities.

Alongside effects on aboveground vegetation, several studies have shown that fire alters the frequency and composition of SSBs (Kiss et al., 2018). Seed dormancy contributes to species persistence, where species with viable seeds in the soil can bridge unfavourable conditions for establishment. Thus, SSBs support the long-term persistence of species, helping avoid extinction. In fire-prone ecosystems, conditions for seedling establishment are particularly favourable just after fire. Consequently, many plants create SSBs that are released from dormancy only after a fire event and after being exposed to fire-related factors, such as heat (Luna, 2020).

The vast majority of studies in SSB-fire relationships have dealt with Mediterranean ecosystems, where fire is considered an essential structuring force of plant communities (e.g., Céspedes et al., 2012; Manela et al., 2019; Luna, 2020; Magaña Ugarte et al., 2021). In addition, SSB-fire relationships have been studied in forest ecosystems and shrublands, where prescribed burning is increasingly included in the management of these type of ecosystems (Konsam et al., 2020; Lipoma et al., 2018). The extent to which fires may affect soil seed banks in other ecosystems, such as semi-arid grasslands with shorter or less intense evolutionary history of fire, is much less known (Jaureguiberry and Díaz, 2015).

In Iran, fire has been a frequent event in mountainous grasslands for decades. For example, 67 large fires were registered during the 1957–1997 period in a single protected area, Golestan National Park in Alborz (Safaian et al., 2005). According to the reports of the Deputy Agriculture Ministry, most of the grassland fires were caused unintentionally by accidental human error and/or by careless visitors. Dried-out vegetation provides more fuel and can easily ignite, leading to rapid fire spread, even in winter (Bashmaghi et al., 2017). Post-fire grassland restoration is a priority for regional nature managers. This conservation goal requires understanding of post-fire vegetation recovery.

Berberis integerrima and *Onobrychis cornuta* are native shrub species covering large areas in the mountainous grasslands in the Iran-Turan region (Heshmati, 2007). Both shrubs play an important role as a SSB reservoir, increasing SSB density and richness through seed trapping (Erfanzadeh et al., 2014; Erfanzadeh et al., 2020). Accidental and unintentional fires have been reported in the recent years in areas where these species occur (Erfanzadeh et al., 2014). Both shrub species have a high potential to increase the severity of fire in the grasslands through fuel load (Niknam et al., 2018). Since the canopy structure is different between the two species, with a low stature and dense compact form in *O. cornuta,* and an open canopy with thorny tall branches in *B. integerrima*, it can be supposed that the severity and intensity of canopy fire may vary between the two species, leading to differing implications for the associated SSBs.

The objectives of this study were to assess the effect of fire on germination and emergence of the SSBs located under cover of different shrub species. To investigate this, soils were collected under the canopy of different shrub species and in herbaceous control patches, before and after burning in late winter, and were germinated in a greenhouse. In particular we wanted to test the following hypotheses: i) The density and species richness of SSBs beneath the canopy of *O. cornuta* and *B. integrrima* will decrease after fire, while these changes will be less pronounced in SSBs under the herbaceous patch; ii) The seed banks of the upper soil layers are more affected by the fire than those of the lower soil layers, in both shrub and herbaceous control plots.

2. Materials and Methods

2.1. Study area

The study was conducted in mountainous grasslands of Baladeh Watershed in Mazandaran province, Iran (36° 16′30″N - 36° 18′19″N; 51° 49′30″E -51° 51′17″E). The elevation is between 2750 and 2985 m a.s.l. with an average annual rainfall of ca. 390 mm and average annual temperature 5.5 °C. Two characteristic fire-prone shrubs with different features and landforms were selected for the study: 1) *Berberis integerrima* L. (Berberidaceae), is an upright deciduous shrub with an average of 1.8 m tall. The canopy structure is open with thorny branches. In the study site, this species is damaged by pests (*Malacosoma neustria* L.),has not produced newly developed twigs in the recent years (Fig. 1). This species provides a large amount of fuel for fire in the area. 2) *Onobrychis cornuta* (L.) Desv. (Fabaceae), is a perennial, deciduous, branched spiny shrub, forming cushions, that reaches up to 60 cm in height in this area. The canopy structure is low stature with a dense compact form. This species is easily burned, due to its compact form, small leaves and dry thin branches. We also selected herbaceous control patches (plots) close to the selected plots of shrubs. The mean area of each herbaceous control patch was 0.25 to 1 m^2, similar to the surface area of the neighbouring individual shrub. These controlpatches were dominated by perennial grasses, such as *Festuca ovina* and *Bromus tomentellus*. Enough dry vegetation for fire fuel was present in the herbaceous control patches, i.e. 1.5 kg to 2 kg per m^2. Since the experimental fires were conducted at the end of winter, herbaceous species were dry and were burnt easily.

2.2. Site selection and shrub burning

We selected five sampling areas, in which four sampling sites were located in each area (in total 20 sites). In each site, the two species of shrub and the herbaceous control patch were present relatively close to each other (Fig. 2). The distance between the sampling areas was at least 300 m, with at least 50 m distance between two sampling sites nested in each area. Soil sampling was performed at the end of winter, following the natural cold stratification of SSBs. Under each individual shrub canopy and herbaceous control patch, 10 soil cores were collected at random to a depth of 10 cm, with a 5 cm diameter auger. The soil samples were then divided into two vertical segments of 0–5 cm and 5–10 cm depth (Auld and Denham, 2006). The soil cores from each depth were pooled per each individual shrub and for each control patch, totalling six samples for each site and 120 samples for all sites. We used a portable picnic gas stove for burning shrub canopies and herbaceous control patches (Fig. 2). Although the two shrub species are of conservation interest in the region, both shrub species are neither endangered nor officially protected. All necessary permits for burning were obtained from the corresponding authorities (General Department of Natural Resources and Watershed Management of Nowshahr, Mazandaran Province). The second soil sampling was done during the few days following canopy burning (post-burning), after the soil became cold. All of the soil samples were transported to the greenhouse for seed germination experiments (Niknam et al., 2018). Soil temperatures during burning were not recorded, as the thermocouples or other apparatus were not available.

2.3. Greenhouse experiments

Each soil sample was distributed evenly in trays (25 cm × 35 cm) over a mix of potting soil and sand that had been sterilized at 150 °C for 24 h. A total of 240 trays were used (20 (sites) × 2 (pre- and post-burning) × 3 (patch types) × 2 (depths)) which were set in a greenhouse during the germination period. The sterilized potting soil layer was 3 cm and the field-collected soil layer was ca. 1.2 cm in the trays. The germination trays were labelled and distributed randomly on benches with natural light and temperature conditions (varied between 15 °C and 26 °C) and irrigated every second day when it was necessary (Hadinezhad et al., 2021). In addition, 24 control trays containing sterilized material only were randomly placed between the sample trays to detect airborne seed contamination. Germinated seedlings were identified, counted and then removed from the trays in every two weeks.

Fig. 1. The *Berberis integerrima* community in the mountainous grasslands of Baladeh Watershed, Iran. The shrub is without green-fresh twigs and leaves in the growth season due to the pest (*Malacosoma neustria* L.); a prone-fire species in all seasons (the photo was taken in the spring). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

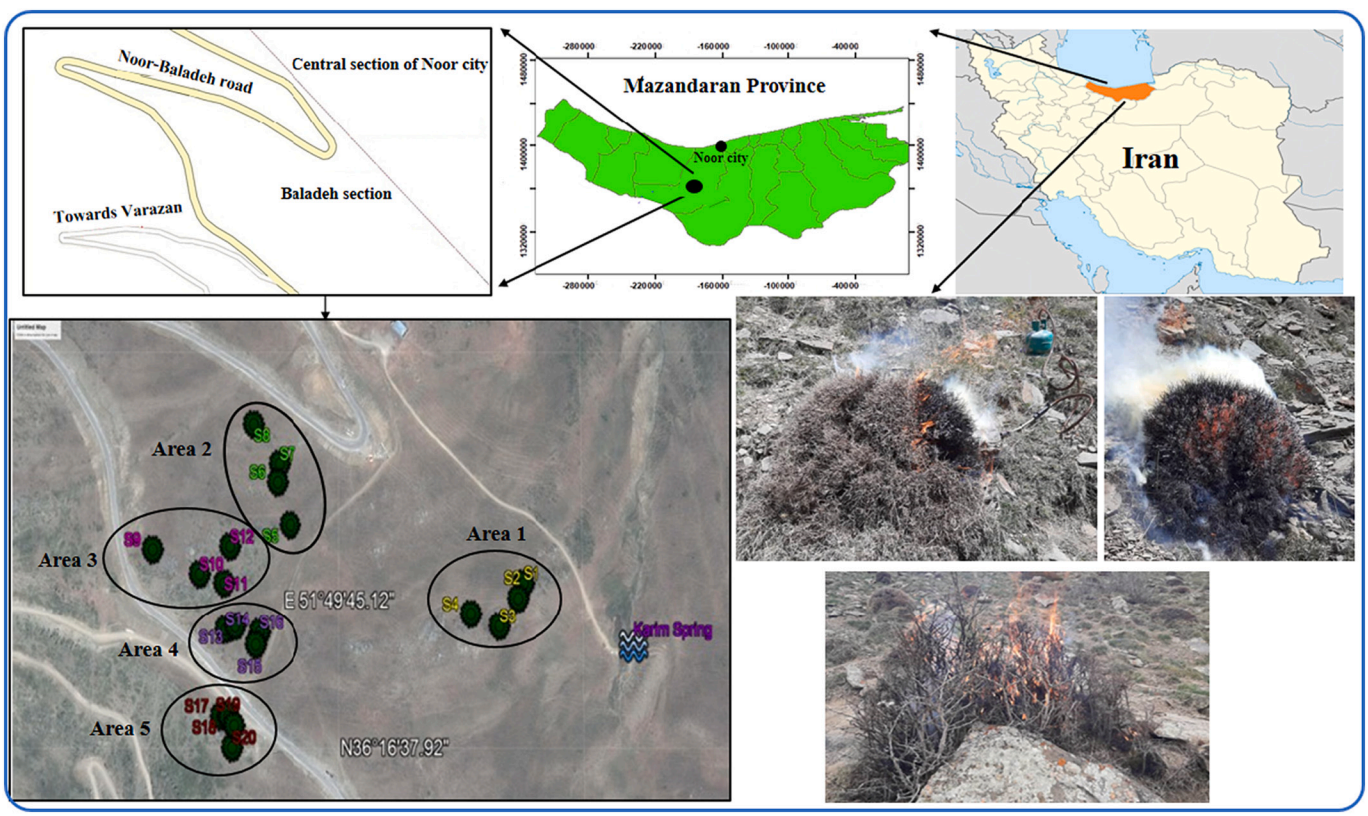

Fig. 2. Geographical location of the study area and five main sites with twenty sites in which two shrub species (*Berberis integerrima* and *Onobrychis cornuta*) were found closed to each other in each site.

The seedlings were identified to species level. Approximately 10% of plants were identified to functional groups, i.e. forb, grass and woody plants. Plant species were identified using publications on the flora of Iran (Ghahraman, 1986-2014; Rechinger, 1964). After a period of five months, when no further seedlings emerged, the trays were left to dry for two weeks. This allowed the samples to be crumbled to expose deeper buried seeds to the light. After the dry period, samples were re-irrigated, and the germination lasted for another month.

2.4. Data analyses

Total SSB density and the abundance of each functional type (annuals, perennials, forbs, grasses and shrubs) of SSB were calculated per square meter and then log-transformed (lg(x + 1)) to improve normality. In addition, total SSB richness was calculated per soil sample (number of species observed under each patch at each soil depth). Two-way repeated measure ANOVA models with least significant difference (LSD) tests were used to compare total SSB density, total SSB species richness and total abundance functional groups (annuals, perennials, forbs, grasses and shrubs) between shrubs (and herbaceous patch) and pre- and post-fire with respect to the sampling depths (0–5 cm and 5–10 cm). Total SSB density, total SSB richness and total SSB density of each functional group were introduced in the two-way repeated measure ANOVA model as dependent variables. Patch type and sampling depth

(two-way) were introduced as fixed factors. In addition, in case of statistically significant fire-patch interactions on SSB variables, a paired *t*-test was used to compare SSB characteristics pre- and post-fire in each depth and in each shrub and control patch, separately. All statistical analyses were done using SPSS ver. 17.0 (SPSS Inc., USA; www.spss.com).

We also carried out a non-metric dimensional scaling (NMDS) to visually analyse the distribution of SSB composition in the space delimited by the patches pre- and post-fire (six groups). Since the fire effects on SSB density and composition were mostly prominent in the soil depth of 0–5 cm, we used the data of this depth for further analysis in NMDS. The quantitative data (seedling densities) were used for NMDS analyses. The NMDS analyses were done using the 'vegan' package (Oksanen et al., 2019) in R version 3.6.1 (R Core Team, 2015).

In addition, changes in floristic composition of SSBs between "pre" and "post" burning were analyzed through the qualitative Sørensen and the quantitative Czekanowsky similarity indices (Kent and Coker, 1994) in all three plot types. One-way ANOVA was used to compare similarity indices among three different patches for each depth separately.

3. Results

3.1. Soil seed bank composition

In total 5795 seedlings emerged from 61 species in the pre-fire soil sample, and 4114 seedlings emerged from 60 species in the post-fire soil sample (pooled data, see Appendix 1 and 2). The most abundant species in both pre- and post-fire seed banks was *Sisymbrium loeselii,* with 751 (pre-fire) and 615 (post-fire) seedlings. At a soil depth of 0–5 cm pre-fire, the total number of species found under was 52, while 57 species were found under and 48 species under the control patch. In the post-fire soil sample at the same soil depth, the total number of species found were 53 under *B. integerrima*, 42 under *O. cornuta* and 47 under the control patch. At a soil depth of 5–10 cm pre-fire, 47 species were found under *B. integerrima*, 55 species under *O. cornuta,* and 25 under the control patch. At the same soil depth post-fire, 51 species were found under *B. integerrima*, 35 species under *O. cornuta* and 29 species were found under the control patch.

The studied patch types were separated along the second axis of NMDS which was visible in the ordination diagram (Fig. 3). The NMDS results showed no distinct groups for the two sampling dates (pre- and post-fire sampling date) of the SSB for any of the shrub or control patches. In accordance with this, the values for similarity between the pre- and post- fire SSB were relatively high in all three patches and at both soil depths. The mean values of Sørensen similarity indices at a soil depth of 0–5 cm were 0.45, 0.66 and 0.71 and at a soil depth of 5–10 cm were 0.61, 0.70 and 0.63 in *O. cornuta*, *B. integerrima* and the herbaceous control patches, respectively (Fig. 4A). The lowest Sørensen similarity between pre- and post-fire SSB was observed in *O. cornuta,* at both soil depths (statistically significant at 0–5 cm and non-significant at 5–10 cm depths, Table 1). The mean values of the Czekanowski similarity indices at a soil depth of 0–5 cm were 0.31 for *O. cornuta*, 0.37 for *B. integerrima*

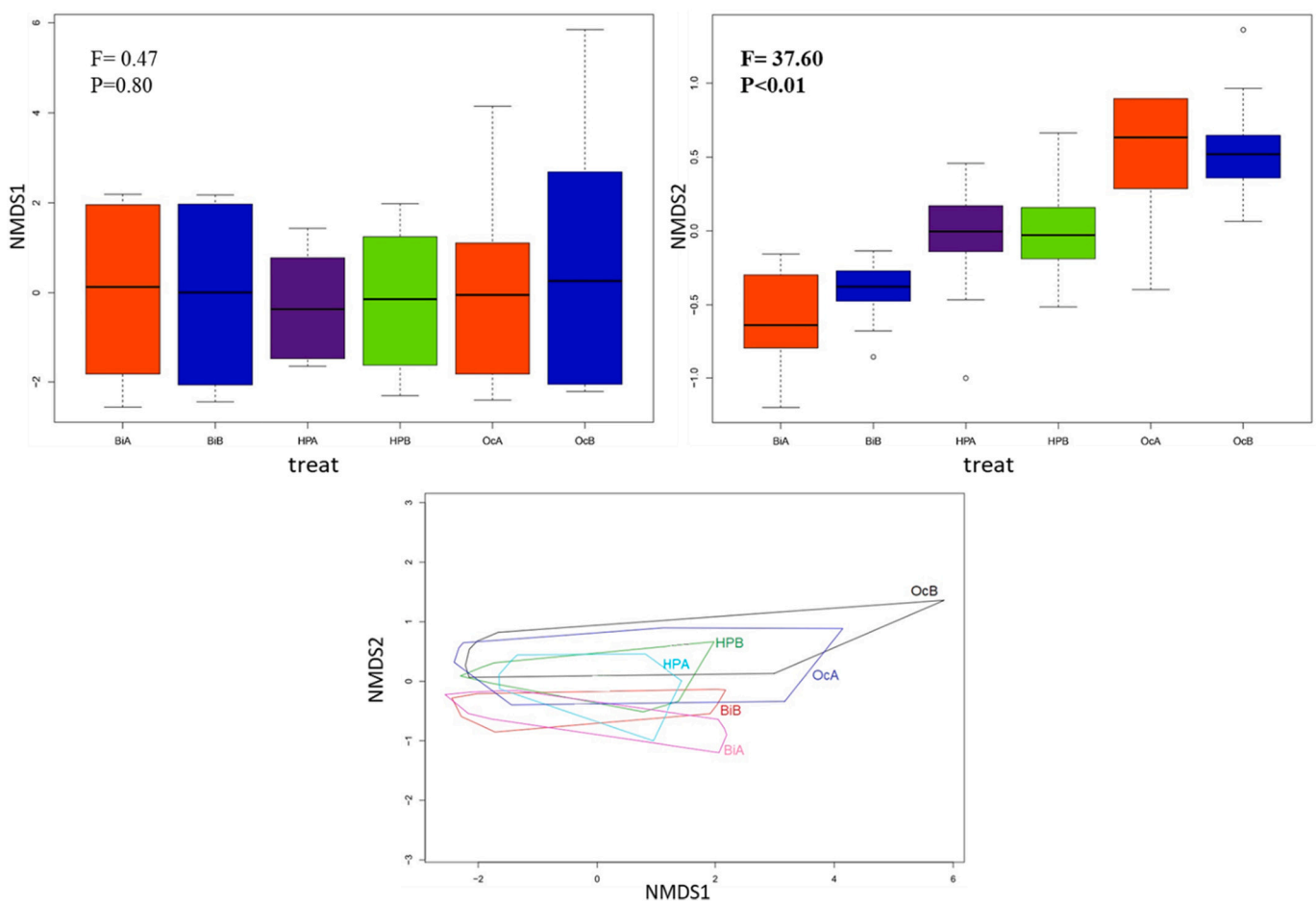

Fig. 3. Non-metric multidimensional scaling (NMDS) of soil seed bank (SSB) composition (0–5 cm) under two shrub species (*Berberis integerrima* and *Onobrychis cornuta*) and herbaceous patch in pre- and post-fire (R2 = 0.99 for Non-metric fit, R2 = 0.98 for Linear fit and Stress = 0.006) (BiB=*B. integerrima* before burning, BiA = *B. integerrima* after burning, OcB = *O. cornuta* before burning, OcA = *O. cornuta* after burning, HPB = herbaceous patch before burning and HPA = herbaceous patch after burning).

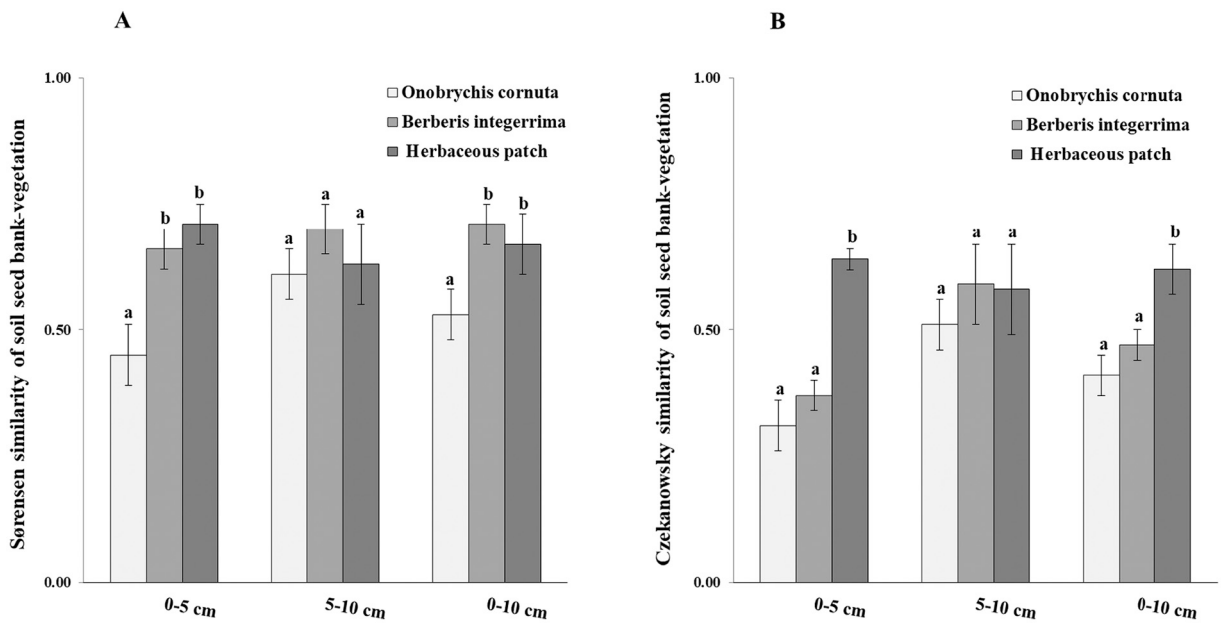

Fig. 4. Mean values±SE of Sørensen (A) and Czekanowsky (B) similarities of SSB composition between pre- and post-fire under three vegetation types (*Onobrychis cornuta*, *Berberis integerrima* and herbaceous control patch) at different soil depths.

Table 1
The results of one-way ANOVA, comparing Sørensen and Czekanowski SSB similarities between pre- and post-fire in three vegetation plot types (*Onobrichys cornuta*, *Berberis integerrima* and herbaceous patch) in two different soil layers (0–5 cm and 5–10 cm), separately. Bold digits show significant results at $P < 0.05$.

		Sørensen index					Czekanowski index			
		df	Sum of squares	Mean of squares	F value	*P* value	Sum of squares	Mean of squares	F value	P value
Depth 0–5 cm	Between Groups	2	0.73	0.36	7.64	**<0.01**	1.29	0.65	17.04	**<0.01**
	Within Groups	57	2.74	0.05			2.16	0.04		
	Total	59	3.47				3.48			
Depth 5–10 cm	Between Groups	2	0.30	0.15	1.96	0.15	0.08	0.04	0.39	0.69
	Within Groups	57	4.41	0.08			5.76	0.10		
	Total	59	4.71				5.84			
Depth 0–10 cm	Between Groups	2	0.76	0.39	5.90	**<0.01**	0.86	0.43	5.70	**<0.01**
	Within Groups	117	7.52	0.06			8.88	0.08		
	Total	119	8.28				9.75			

and 0.64 for the control patches. At a soil depth of 5–10 cm the mean values of the Czekanowski similarity indices were 0.51 for *O. cornuta*, 0.59 for *B. integerrima* and 0.58 for the control patches (Fig. 4B). The lowest statistically significant Czekanowski similarity between pre- and post-fire SSB were observed in *O. cornuta* and *B. integerrima* at a soil depth of 0–5 cm.

3.2. Total soil seed bank density and richness

The results of the two-way repeated measure ANOVA showed that fire, patch, soil depth and their interactions had significant effects on total SSB density and richness (Table 2). Significant effects of interaction between patch and fire (patch × fire) on total SSB density and species richness in both soil depths were observed (Table 2). Paired *t*-test results showed that fire decreased total SSB density under *B. integerrima* at a soil depth of 0–5 cm ($t = 12.5$, $P < 0.01$) and had no significant effect on total SSB density at 5–10 cm depth. Fire decreased total SSB density beneath *O. cornuta* at both soil depths (0–5 cm: $t = 9.70$, $P < 0.01$ and 5–10 cm: $t = 3.79$, $p < 0.01$). Total SSB density in herbaceous control patches showed no significant changes between pre- and post-fire at both soil depths (Fig. 5A and Fig. 5B). Canopy fire had no significant effects on SSB richness beneath *B. integerrima* ($t = 0.68$, $P = 0.54$) and the control patch ($t = -1.16$, $P = 0.26$) at 0–5 cm depth. At the 5–10 cm depth, fire had significant positive effects on SSB species richness in the control and

Table 2
Repeated measures results of total soil seed bank density and species richness through data analyses of data of two soil depths and each depth separately.

		Density			Richness		
	source	df	F-value	*p*-value	df	F-value	p-value
Total	Fire	1	12.49	0.01	1	0.52	0.47
	Patch type	2	4.45	0.05	2	1.79	0.17
	Soil depth	1	30.53	0.01	1	18.80	0.01
	Fire× Patch	2	27.45	0.01	2	27.78	0.01
	Fire× Depth	1	34.49	0.01	1	0.82	0.36
	Patch× Depth	2	3.99	0.05	2	3.6	0.05
	Fire× Patch× Depth	2	8.29	0.01	2	7.54	0.01
0–5 cm	Fire	1	40.19	0.01	1	0.04	0.84
	Shrub	2	4.07	0.05	2	1.49	0.23
	Fire× Shrub	1	34.97	0.01	2	5.25	0.01
5–10 cm	Fire	1	0.06	0.81	1	1.24	0.27
	Shrub	2	4.42	0.05	2	5.43	0.01
	Fire× Shrub	2	8.72	0.01	2	26.30	0.01

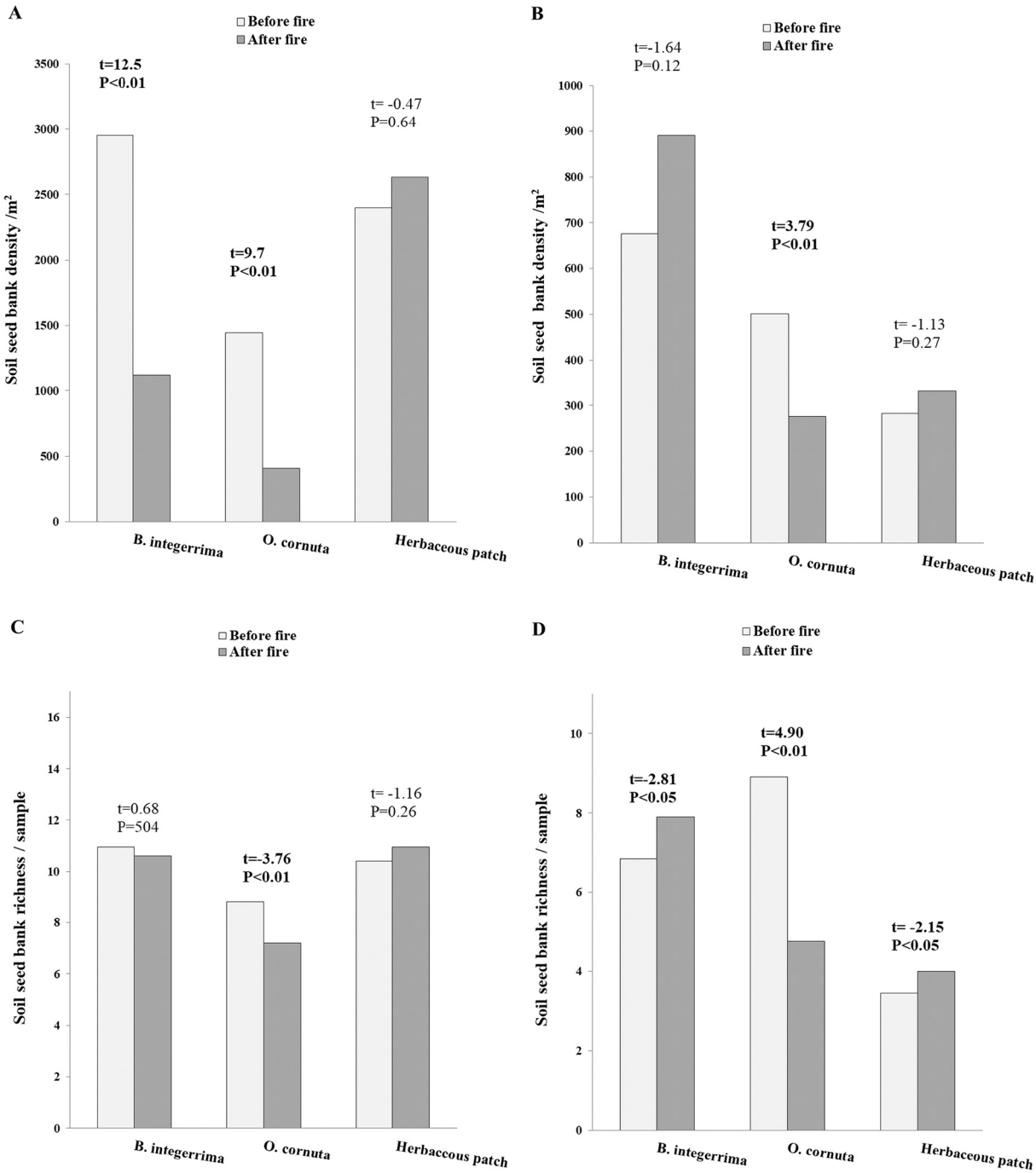

Fig. 5. Mean values ±SE of total soil seed bank density and richness under three patches (*Berberis integerrima*, *Onobrychis cornuta* and herbaceous patch) at 0–5 cm (A and C, respectively) and 5–10 cm (B and D, respectively). The paired t-test was used to compare soil seed bank density and richness between pre- and post-fire of patches (bold digits show significant results).

under *B. integerrima* and significant negative effects on SSB richness under *O. cornuta* (Fig. 5C and Fig. 5D).

3.3. Functional groups in soil seed bank

Repeated measures analysis showed that fire, vegetation type, soil depth and the interaction among them had significant effects on functional groups of SSB in most cases. Significant effects of interaction were observed between patch and fire (patch × fire) on annuals ($F = 8.30$, $P < 0.01$), perennials ($F = 14.27$, $P < 0.01$) and forbs ($F = 35.61$, $P < 0.01$). At the 5–10 cm soil depth, the effects of patch × fire on perennials ($F = 12.45$, $P < 0.01$), forbs ($F = 7.08$, $P < 0.01$) and shrubs ($F = 5.26$, $P < 0.01$) were significant.

Canopy fire of *B. integerrima* decreased the SSB density for perennials, forbs and grasses at the 0–5 cm soil depth. In contrast, no significant effects were found at the 5–10 cm depth (Table 3). Canopy fire of *O. cornuta* showed significant negative effects on all functional groups of the SSB, with the exception of shrubs, at the 0–5 cm soil depth. At the 5–10 cm soil depth, canopy fire of *O. cornuta* had significant negative effects on all functional groups of SSB, with the exception of annuals (Table 3). Burning of the herbaceous control patches decreased the SSB of grasses (marginally significant, $P < 0.1$), but had no significant effects

Table 3
The results of the paired t-test to compare functional groups of soil seed bank of different vegetation plot types between pre- and post-fire.

Functional groups	Patch	Depth	Burning	mean	SD	df	t-value	p-value
Annuals	*B. integerrima*	0–5	pre	2.73	0.39	19	1.75	0.096
			post	2.61	0.26			
		5–10	pre	2.53	0.45	19	−1.71	0.105
			post	2.66	0.46			
	Herbaceous patch	0–5	pre	3.16	0.64	19	−0.65	0.52
			post	3.21	0.61			
		5–10	pre	2.29	0.45	19	0.07	0.939
			post	2.28	0.42			
	O. cornuta	0–5	pre	2.83	0.57	19	6.26	0.000
			post	2.51	0.58			
		5–10	pre	2.37	0.36	19	1.63	0.121
			post	2.25	0.31			
Perennials	*B. integerrima*	0–5	pre	3.29	0.33	19	8.75	0.000
			post	2.82	0.39			
		5–10	pre	2.64	0.43	19	−0.64	0.532
			post	2.69	0.45			
	Herbaceous patch	0–5	pre	2.99	0.42	19	0.55	0.591
			post	2.95	0.48			
		5–10	pre	2.30	0.43	19	−1.91	0.071
			post	2.39	0.32			
	O. cornuta	0–5	pre	2.93	0.73	19	8.37	0.000
			post	2.50	0.80			
		5–10	pre	2.58	0.49	19	5.29	0.000
			post	2.29	0.48			
Forbs	*B. integerrima*	0–5	pre	3.39	0.29	19	12.15	0.000
			post	3.00	0.26			
		5–10	pre	2.83	0.39	19	−1.89	0.074
			post	2.96	0.41			
	Herbaceous patch	0–5	pre	3.31	0.49	19	−0.74	0.470
			post	3.36	0.51			
		5–10	pre	2.48	0.42	19	−0.19	0.851
			post	2.49	0.42			
	O. cornuta	0–5	pre	3.12	0.57	19	10.99	0.000
			post	2.70	0.71			
		5–10	pre	2.69	0.45	19	3.073	0.006
			post	2.50	0.37			
Grasses	*B. integerrima*	0–5	pre	2.27	0.48	19	2.37	0.029
			post	2.12	0.45			
		5–10	pre	2.16	0.44	19	0.83	0.421
			post	2.10	0.39			
	Herbaceous patch	0–5	pre	2.68	0.66	19	2.042	0.055
			post	2.51	0.67			
		5–10	pre	1.99	0.39	19	−0.49	0.630
			post	2.03	0.32			
	O. cornuta	0–5	pre	2.46	0.76	19	3.92	0.001
			post	2.26	0.58			
		5–10	pre	2.077	0.36	19	3.18	0.005
			post	1.91	0.30			
Shrubs	*B. integerrima*	0–5	pre	1.77	0.18	19	−0.85	0.404
			post	1.83	0.25			
		5–10	pre	1.72	0.07	19	−1.00	0.331
			post	1.74	0.09			
	Herbaceous patch	0–5	pre	174	0.15	19	0.51	0.612
			post	1.72	0.07			
		5–10	pre	1.77	0.21	19	0.14	0.893
			post	1.76	0.15			
	O. cornuta	0–5	pre	1.79	0.28	19	0.77	0.450
			post	1.75	0.15			
		5–10	pre	1.82	0.19	19	2.67	0.015
			post	1.72	0.067			

on the SSB of other functional groups at the 0–5 cm soil depth. At a soil depth of 5–10 cm, burning of herbaceous control patches had no significant effects on the SSB of any functional groups (Table 3).

4. Discussion

Our study demonstrated that the effect of plant canopy fire on the underlying SSB was highly dependent on the vegetation type. For both vegetation plots characterized by shrub species, the number of seeds in the soil under the shrub canopies after burning was depleted relative to the pre-fire seed bank levels. No such effect was detected for the density of the SSBs in the herbaceous control patches. In addition, canopy burning of *O. cornuta* decreased SSB species richness, while significant changes were not observed between pre- and post-fire SSB species richness in *B. integerrima* and herbaceous patches. These results confirmed our first hypothesis.

One likely explanation for this phenomenon is that the fuel load influences the survival of viable seeds in the SSB after fire. Auld and Denham (2006) demonstrated that in burnt sites, few seeds will be available for a period after a fire. The authors found that fuel accumulation and consumption of greater quantities of fuel on the soil surface affected the number of species in the post-fire SSB. Soil temperatures

during fires vary among different microsites depending on fuel availability, with lower temperatures in open microsites than in those with higher fuel accumulation (Carrington, 2010). The highest amounts of litter are often found under shrubs, compared with herbaceous patches or bare soil (Erfanzadeh et al., 2021). Shrubs may have higher litter content beneath their canopies due to wind-blown litter trapping (Yan et al., 2016), facilitative effects on herbaceous biomass (Erfanzadeh et al., 2021) and individual shrub growth during previous years. Consequently, higher amounts of fuel under shrub canopies may lead to lethally high temperatures for seeds during fire (Tangney et al., 2020). Thus, successful post-fire recovery may not rely solely on the SSB buried under shrub canopies.

Nevertheless, very hot fires can be useful for seed germination in some cases. For instance, Dairel and Fidelis (2020) found that in some species, dormancy was broken when seeds were exposed to high temperature and therefore high temperatures produced by fire enhanced their germination. In our study, we also found that seedling emergence increased in some species, e.g. *Erodium cicutarium*, after shrub canopy burning. Interestingly, the seedlings of some species such as *Cirsium vulgare* appeared after shrub fire, while they were absent before shrub burning. It appears that the seeds of these species are tolerant to the fire temperature and/or fire dependent for germination. Heat-stimulated germination is known for many species, including those from the Fabaceae family (e.g., Ruprecht et al., 2013). Risberg (2015) reported that some species of Geraniaceae in Sweden are fire-dependent and have a heat-triggered germination. Other species that showed a positive reaction to canopy shrub burning could be due to the thickness of the seed coat, in which the heat destroyed the skin and facilitated germination (Risberg, 2015).

The decrease in SSB density following fire was more pronounced in the top 5 cm of soil than at a depth of 5 to 10 cm for most plant functional groups in our study. These results supported our second hypothesis that the detrimental effects of fire on seed survival lessen with increasing soil depth. Both the magnitude and duration of heat shock associated with fire have been shown to reduce with soil depth elsewhere (Auld and Denham, 2006), thus influencing seed survival. Previous studies have reported that during the passage of a fire, the greatest depletion of the SSB occurred in the upper soil layer, where maximum temperatures occur (e.g. Tangney et al., 2020). Williams et al. (2004) reported that seeds buried in deeper soil layers should be less affected by fire, because temperature sharply decreases with soil depth, although significant effects of fire-related factors on seed germination in deep soil layers have been reported in some case studies, such as Read et al. (2000). Our results showed that the number of seedlings under *B. integerrima* decreased after the fire only at the upper soil layer, while this reduction occurred for both soil depths (0–5 cm and 5–10 cm) under *O. cornuta*. The differing canopy structures of these two shrub species could be the reason for this variation. Schwilk (2003) argues that canopy architecture is a more important factor than fuel load of plants with regards to fire intensity. Indeed, *B. integerrima* has an open canopy that may trap and produce less litter compared with the compact and dense canopy of *O. cornuta* (Erfanzadeh et al., 2020), which may produce a hotter fire. In addition, the presence of the pest in our study areas inhibited the production of new woody growth in *B. integerrima*, restricting canopy development and therefore fuel load. During the burning of *B. integerrima,* only small and thin stems could be burned, while thick stems remained largely intact. In contrast, the dense canopy of *O. cornuta* burned successfully and produced a very hot ash with long-lasting heat that could be felt when we returned to the field for post-fire soil sampling. As a result, the intensity and durability of the high temperatures in the case of *O. cornuta* patches may be consistently higher than *B. integerrima* canopies, therefore reaching the SSB in the deeper soil layers and reducing overall SSB density.

Burning of herbaceous control patches had no significant effects on the number of viable seeds in the SSBs. Although we did not record the temperatures, it was observed that in open grassland plots, fire was usually fast and likely produced lower temperatures compared with areas of shrub. Therefore, seeds may be able to survive the occurrence of fire in these patches, as long as they are not directly damaged by the flames. Ruprecht et al. (2013) found that fires in grasslands have a minor influence on the soil seed bank. We expected that the weak heat shock produced by burning in herbaceous control plots could be a factor promoting seed germination of species in the greenhouse, as found in previous studies (Céspedes et al., 2012; Luna et al., 2019), but our results showed non-significant effects of fire on SSBs under control plots. In grassland ecosystems, seeds normally tolerate heat-shocks associated with low intensity fires, but are negatively affected by higher heat doses (Fernandes et al., 2021). Fire could therefore play a key role in the germination process and seedling establishment of many grassland species in open areas (Fernandes et al., 2021). Moreover, high spatial diversity of seedling germination among the SSBs of shrub and herbaceous vegetation types suggest the importance of spatial heterogeneity of fire temperature. Tangney et al. (2018) reported on spatio-temporal heterogeneity in soil temperatures following fire. The authors state that soil temperatures near the surface reached 250 °C in some patches, and $\geq$ 100 °C for durations up to >2.5 h. Consequently, spatial diversity in soil temperatures during fires among different vegetation types leads to a heterogeneity in the distribution of safe sites for seeds, and in the likelihood of post-fire germination of species, according to their different response strategies to heat (Tangney et al., 2020). High post-fire survival rates of seeds would enable seedling recruitment and ensure the maintenance of the SSB under herbaceous areas (see also Daibes et al., 2018; Dairel and Fidelis, 2020).

Although the results of NMDS showed little difference of SSB composition between pre- and post-fire, similarity indices indicated that fire altered SSB composition to a greater extent under *O. cornuta* compared with the other two vegetation types. Both the qualitative Sørensen and quantitative Czekanowsky similarity indices decreased from pre- to post-fire levels for *O. cornuta* (at 0–5 cm), while only the quantitative similarity index (at 0–5 cm) decreased following burning of *B. integerrima.* This result indicates that *O. cornuta* canopy fire affects both number of seeds (density) and the type of species in the SSB, while canopy fire of *B. integerrima* may affect only the seed density.

Significant effects of canopy burning of the two shrub species on total seedling germinations of the shrub functional group were not observed, while surprisingly, number of germinated seedlings of both shrubs (*B. integerrima* and *O. cornuta*) was enhanced under their own canopies after burning. This indicated that probably the dormancy of the seeds of these shrub species was ceased by fire-related factors, such as heat, and should be considered in the future studies on SSBs. Cold stratification (naturally or artificially) is a well-known treatment for samples in SSB studies. We suggest that the researchers consider hot stratification as a treatment for seed dormancy breaking in their studies. In addition, the seeds buried under shrubs are expected to be an important component in the survival of their own populations after fire.

5. Conclusions

This study demonstrated that fire affects the capacity for shrubs to maintain SSBs. Shrub burning damages viable seed banks buried in soil beneath their canopies. In unburnt grasslands, seeds buried in the soil under the canopy of woody species can play an important role in the recovery of aboveground vegetation after disturbances, while in burnt areas, these seeds deplete their roles in plant recovery. Grassland managers should consider that fire is able to damage shrubby grasslands to a greater extent than grasslands with no woody cover. However, this type of negative effect of shrub canopy fires on the density of SSBs may be species-specific. Different shrubs with different canopy architectures may support fires of varying temperatures and intensities, in turn affecting seed germination. These processes should be considered by land managers for optimal mountain grassland restoration.

CRediT authorship contribution statement

Misagh Ghasempour: Conceptualization, Methodology, Software, Data curation. **Reza Erfanzadeh:** Visualization, Investigation, Data curation, Formal analysis, Methodology, Supervision. Writing-Original draft preparation. **Péter Török:** Methodology, Validation, Writing-Reviewing and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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