

## Role of Plant Patches in Preserving Flora from the Soil Seed Bank in an Overgrazed High-mountain Habitat in Northern Iran

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### ABSTRACT

Seed bank is a central topic for plant community restoration. We determined the potential and regeneration capacity of soil seed banks of woody plant patches in conservation of the vegetation in an alpine habitat, since vegetation has completely disappeared in some sites by intensive grazing in the habitat. The study was done in mountainous area of Alborz in Iran. A total of 20 individual shrubby patches were selected and two quadrats were established in and out of each patch. Soil samples were then collected from each quadrat in spring, 2011. Above-ground vegetation was estimated in each quadrat in the growing season. The soil seed bank was determined by Seedling Emergence method in the greenhouse. The results showed that the soil seed bank density inside the shrub patches was much higher than outside the patches. This differentiation was more pronounced for forbs. However, seed density of the between-patches was strongly correlated to seed density of the within-patches, indicating the so-called patch effect. Similarity between the soil seed bank and aboveground vegetation was lower inside the patches than outside. This study revealed that the limited woody patches that have remained in the study area could play an important role in conservation of herbaceous and palatable species by their positive effects on the aboveground vegetation and the soil seed bank.

**Keywords:** Alpine grassland, Nurse plant. Palatable species, Patch effect, Seed dispersal.

### INTRODUCTION

In Alborz mountain-chain communities, northern Iran, vegetation cover occurs as relatively small individual shrubby patches embedded in a short and intensively grazed grassland matrix, as well as in other mountain regions of the world (Callaway *et al.*, 2002; Escudero *et al.*, 2004). This spatial and temporal pattern has also been described in highly stressful habitats such as arid and semi-arid environments (Maestre and Cortina, 2005; Caballero *et al.*, 2008) and alpine habitats (Ma *et al.*, 2010). Although

the effect of these patches on soil seed density is widely known in arid and semi-arid ecosystems, we still lack information on the effect of the patches on seed composition and density in alpine ecosystems, which have harsh environment, i.e. low temperature and short growth period.

Assessing differences in seed density and composition of patches and between-patches (open area) can clarify the relationship between patterns and processes of the soil seed bank. In fact, knowledge of the particular location of different seed types may explain the spatial pattern of plant

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recruitment at the different microhabitat (Marone *et al.*, 2000; Marone *et al.*, 2004).

Moreover, shrubs that constitute a patch act as nurse plant for herbaceous species. The nurse plants are those that facilitate the growth and development of other plant species beneath their canopy (Ren *et al.*, 2008). As a matter of fact, the nurse plants play an important role in conservation and recovery of degraded sites (Padilla and Pugnaire, 2006). In recent years, the function of nurse plant has been investigated in different habitats around the world, including Mediterranean mountain and alpine habitats (Castro *et al.*, 2004; Cavieres *et al.*, 2006). In most of these studies, the effects of nurse plants on aboveground vegetation were investigated, while their role in belowground flora (soil seed bank) was hardly mentioned, particularly in alpine habitats. Generally, the species richness and density of aboveground vegetation under nurse plants is higher than that in the open sites (Cavieres *et al.*, 2006). Therefore, we hypothesize that seed-trapping and higher recruitment success under the nurse plant could increase seed input to the soil and enhance the density and richness of seeds buried in the soil, thereby increasing the similarity between soil seed bank and aboveground vegetation.

Intensive grazing by sheep and goat has a long history in alpine grasslands in the Alborz mountain rangelands in Iran, where unvegetated gaps have been formed by long-term intensive grazing. Therefore the restoration of degraded sites is a concern for rangeland managers in these areas. Consequently, from a restoration point of view, our results could be interpreted as an evaluation of the potential role of seeds buried in the soil of plant patches in restoration or conservation of vegetation.

## MATERIALS AND METHODS

### Study Area

The study was done in Alborz mountain rangeland in Vaz watershed, Iran (51° 55'

15"E to 52°12' 15"E and 36° 12' 30" to 36° 30' 15" N), with an elevation of 3,000 m asl. The average annual temperature is 15.5°C from 0.8°C in January to 30.2°C in July. The average annual precipitation is ca. 500 mm. The soil type is a sub-alpine meadow soil with sandy and loamy sand texture and mean depth of 50 cm. The community of herbaceous species were dominant in the aboveground vegetation of the study area with a ca. 65% mean total cover including *Festuca ovina* L., *Brachypodium pinnatum* (L.) P. Beauv., *Asperula odorata* L., *Plantago major* L. (Erfanzadeh *et al.*, 2013). The vegetation has completely disappeared in some sites by intensive grazing (ca. five animals per hectare), emerging bare soil gaps. Sheep are the predominant grazers in the region, with a few goats during April to September each year. They are penned in at night and let out to graze during the day.

The sampling area is characterized by a succession of wide hills with gentle slopes. Cushion plants (*Onobrychis cornuta* (L.) Desv. subsp. *cornuta* and *Acantholimon erinaceum* Boiss.) are individually distributed in the entire area. However, *Berberis integerrima* Bung. usually aggregate into patches, where we compared soil seed characteristics with open areas (grassland). Unlike other patchy habitats around the world, in our study, the patches were very sparsely and individually distributed in the area, with maximum two patches per hectare (Figure 1).

### Sampling Methods

Soil seed bank sampling was carried out after natural cold seed stratification and snow melting occurred in early spring, 2011. A total of 20 individual shrubby patches were selected ranging 10 to 30 m<sup>2</sup> in surface area. In each patch, a 1×1 m plot (hereafter called within-patch) and its pair in the bare soil close to the patch (hereafter called between-patch) were established. In some cases in which the area of the patches were large, we established at least two plots inside



**Figure 1.** A shrubby patch (*Berberis integerrima*) with cushion plant species around it.

and two plots outside the patches. In each plot, ten random soil cores were collected to a depth of 10 cm using a 5 cm diameter auger, (Erfanzadeh *et al.*, 2013). Soil samples were taken at two depths (0-5 and 5-10 cm) and the samples were then pooled per soil layer for each plot. The volume of each sample exceeded 0.8 liter of soil, necessary to determine the species composition of the seed bank, as suggested by Hutchings (1986).

The resulting soil samples were spread in 25×35 cm plastic trays in a greenhouse in a sterile substrate of peat and sand (1:1). The trays were randomly placed on shelves with a natural light regime and were kept moist by regular tap water spraying. Air temperature varied between 14 and 25°C. About five additional trays were filled with the sterile mixture to detect possible seed contamination. Emerged seedlings were identified and removed as soon as possible. After 6 months, the trays were left to dry for 2 weeks and the soil in the trays was then crumbed to expose deeper buried seeds to the light. After watering the samples for another 4 weeks, no new seedlings emerged.

Nevertheless, the residual soil was checked for the remaining seeds by viewing small random samples taken from the trays under microscope and probing seeds with a needle in order to distinguish between firm and empty seeds. Since the number of seeds that remained in the investigated soil samples was negligible, we did not need to correct germinating data for remaining viable seeds (see also Erfanzadeh *et al.*, 2010).

The aboveground vegetation composition was measured in all plots located within and between-patches during the growing season 2011. Plant species nomenclature follows Rechinger (1964).

### Data Analysis

The number of total germinable seeds of all species, seeds of forbs, seeds of grasses, and seeds of shrubs were recalculated to square meter for each soil layer. Correlation between the species composition of aboveground vegetation and the soil seed bank was separately assessed by Sørensen's



qualitative similarity index (Kent and Coker, 1994) for each soil depth.

To compare the composition and abundance of species in the vegetation and the seed bank both within and between-patches, a multivariate ordination was conducted using Detrended Correspondence Analysis (DCA) (Hill and Gauch, 1980). DCA was used to examine the variation in plant species composition, and was applied to the data of species frequency. Graphical plots of data ordinations were constructed using CANODRAW 4.5 (Smilauer, 1992). All analyses were performed using the CANOCO 4.5 program, following the default options for DCA (condensed data, detrended by segments and ...). For seed bank data, species frequencies were calculated as the number of seedlings of one species divided by the total number of seedlings in the seed bank for each plot. The data set finally consisted of one statistical matrix: the "sample-species" matrix Y with species frequency data for both seed bank and vegetation data set, simultaneously.

Seed density was transformed to  $\sqrt{x + 0.5}$  to meet normal distribution (Busso and Bonvissuto, 2009). To examine the patch effect on seed density, this variable was compared among the within- and between-patches using paired Student's t test and correlated using Pearson's r (Aguilar and Sala, 1994).

Total seed density was classified into forbs, grasses, and shrubs. Seed density of each functional group was compared among the within-patch and between-patch using paired Student's t test.

## RESULTS

### Aboveground Vegetation and Seed Bank Composition

The aboveground vegetation in the within-patch comprised of 23 species, of which 4 species occurred both in the aboveground vegetation and in the seed bank and 19

species occurred only in aboveground vegetation. In the vegetation of the between-patches, 19 species were recorded, of which 6 species occurred both in the aboveground vegetation and the seed bank and 13 species solely in the aboveground vegetation (Table 1). In total, 80 species were recorded in the study area (both in the vegetation and the seed bank).

Palatable species such as *Sanguisorba minor*, *Lathyrus pratensis*, *Lotus corniculatus* and *Dactylis glomerata* were not observed in the between-patches neither in the seed bank nor in the aboveground vegetation, while they occurred in the within-patches.

### Correlation Between Soil Seed Bank and Aboveground Vegetation

The mean Sørensen index per plot in 0-5, 5-10 and 0-10 cm depths significantly differed between the within- and the between-patches. In all depths, the highest similarity between seed bank and aboveground vegetation was in the between-patch (Figure 2).

Detrended correspondence analysis (DCA) of the soil seed bank and vegetation plots identified groupings of species composition (Figure 3). The distance between the within-patch vegetation and within-patch seed bank groups was more than the distance between the between-patch vegetation and the between-patch groups.

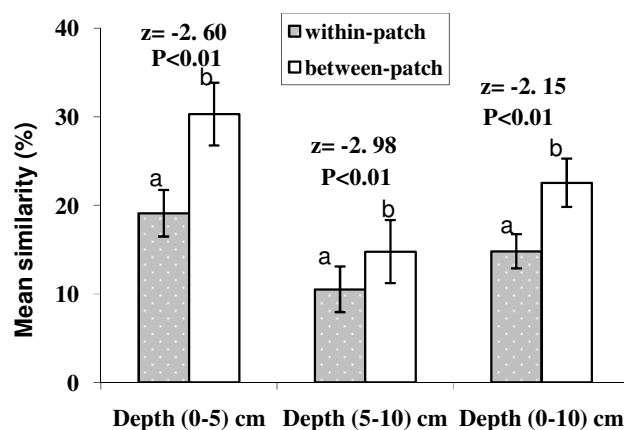
### Seed Bank Density

The mean number of seeds per plot of forbs differed significantly between the within-patch and the between-patch, while it was not significantly different for grasses and shrubs between the within-patch and the between-patch (Figure 4).

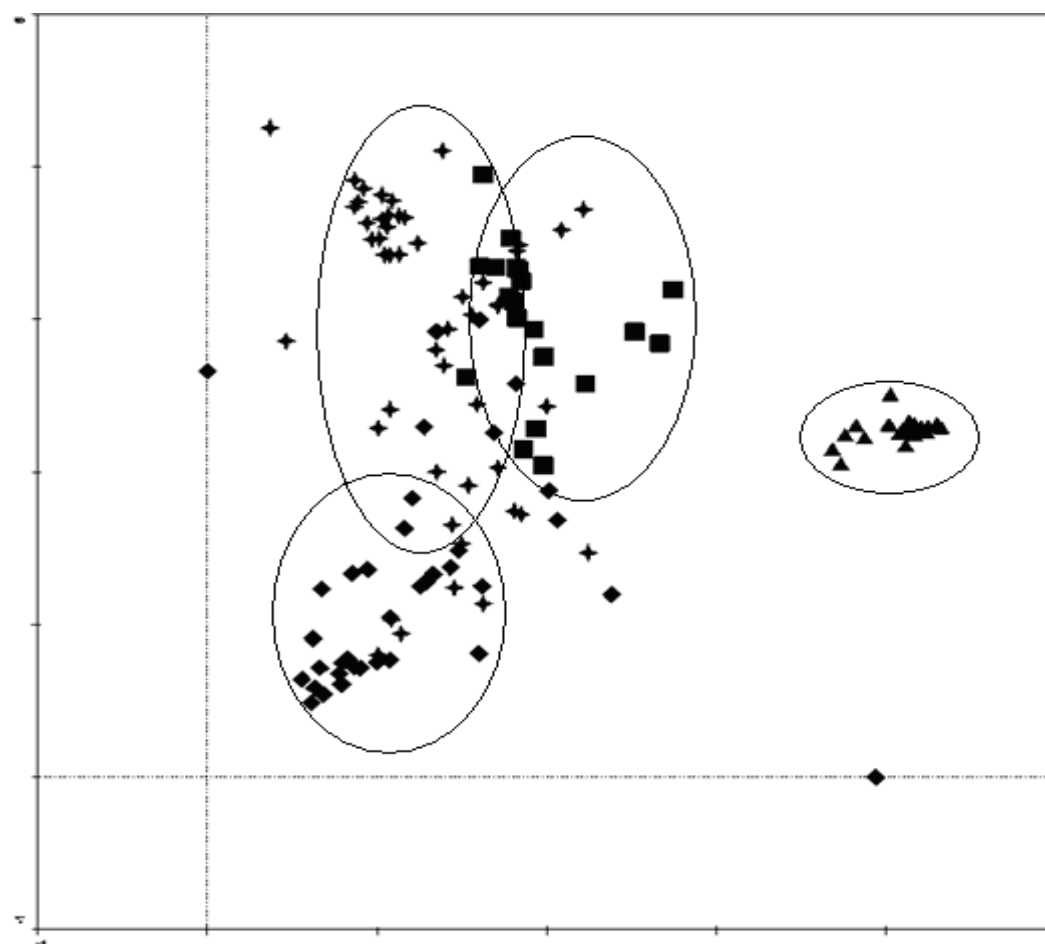
Seed density of the within-patch significantly correlated to seed density of the between-patch ( $P < 0.01$  and  $r = 0.65$ ).

**Table 1.** Species in the seed bank and the aboveground vegetation which occurred in within-patch and between-patch.

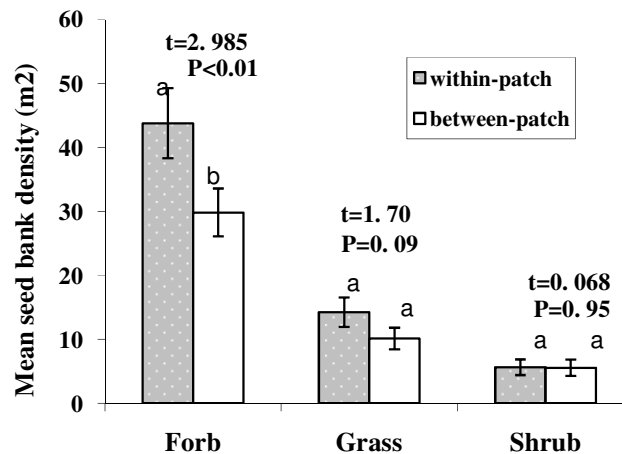
Species which occurred in between-patch		Species which occurred in within-patch	
Vegetation	Seed bank	Vegetation	Seed bank
<i>Allyssum campestre</i> L. Rothm.	<i>Achillea millefolium</i> L.	<i>Arum maculatum</i> L.	<i>Achillea millefolium</i> L.
<i>Anthemis brachystephana</i> Boiss.	<i>Allyssum campestre</i> L. Rothm.	<i>Astragalus aegobromus</i> L.	<i>Alopecurus pratensis</i> L.
<i>Astragalus aegobromus</i> L.	<i>Allyssum campestre</i> L. Rothm.	<i>Berberis intergerima</i> Bunge	<i>Arenaria montana</i> Sieber
<i>Astragalus tribuloides</i> Delile	<i>Arenaria montana</i> Sieber	<i>Bromus brevis</i> Steud.	<i>Asperula odorifera</i> L.
<i>Bromus brevis</i> Steud.	<i>Artemisia aucheri</i> Boiss.	<i>Bromus inermis</i> Leyss.	<i>Asphodelus aculis</i> Des.
<i>Bromus inermis</i> Leyss.	<i>Asparagus officinalis</i> L.	<i>Bromus tomentellus</i> Boss.	<i>Capsella bursa</i> L.
<i>Bromus tectorum</i> L.	<i>Bromus tectorum</i> L.	<i>Coronilla varia</i> L.	<i>Cardaria draba</i> L. Desv.
<i>Capsella bursa</i> L.	<i>Capsella bursa</i> L.	<i>Crypsis aculeate</i> L.	<i>Carex brunnea</i> Thunb.
<i>Cyperus helferi</i> L.	<i>Capsella bursa</i> L.	<i>Erodium cicutarium</i> L.	<i>Chenopodium alba</i> L.
<i>Erodium cicutarium</i> L.	<i>Carex brunnea</i> Thunb.	<i>Geum rivale</i> L.	<i>Cousinia wilhelmina</i> Boiss.
<i>Gagea lutea</i> L.	<i>Cousinia wilhelmina</i> Boiss.	<i>Lactuca virosa</i> L.	<i>Cyperus helferi</i> L.
<i>Gentiana aquatica</i> L.	<i>Cyperus helferi</i> L.	<i>Lathyrus pratensis</i> L.	<i>Dactylis glomerata</i> L.
<i>Ixilirion tataricum</i> Fisch. & C.A.Mey	<i>Cyperus helferi</i> L.	<i>Lepidium latifolium</i> L.	<i>Digitalis lanata</i> L.
<i>Lepidium latifolium</i> L.	<i>Digitalis lanata</i> L.	<i>Mentha pulegium</i> L.	<i>Festuca ovina</i> L.
<i>Myosotis sylvatica</i> V. Blue	<i>Erigeron Canadensis</i> L.	<i>Nepeta persica</i> Boiss.	<i>Gagea lutea</i> L.
<i>Onobrychis cornuta</i> Desv.	<i>Gagea lutea</i> L.	<i>Onobrychis cornuta</i> Desv.	<i>Galium aparina</i> L.
<i>Polygonum persicaria</i> L.	<i>Gagea lutea</i> L.	<i>Poa pratensis</i> L.	<i>Geranium rotundifolium</i> L.
<i>Stellaria holostea</i> L.	<i>Galium aparina</i> L.	<i>Salvia officinalis</i> L.	<i>Lactuca virosa</i> L.
<i>Taraxacum officinale</i> Web.	<i>Geranium rotundifolium</i> L.	<i>Scrophularia laneolata</i> L.	<i>Lactuca virosa</i> L.
	<i>Minuarita kashmirica</i> Mattf.	<i>Thymus vulgaris</i> L.	<i>Lathyrus pratensis</i> L.
	<i>Oxalis acetosella</i> L.	<i>Verbascum thapsus</i> L.	<i>Lathyrus pratensis</i> L.
	<i>Parietaria officinalis</i> L.	<i>Verbena officinalis</i> L.	<i>Lotus corniculatus</i> L.
	<i>Phlomis russeliana</i> Boiss.	<i>Viola occulata</i>	<i>Marrubium officinale</i> L.
	<i>Pilosella officinarum</i> L.		<i>Minuarita kashmirica</i> Mattf.
	<i>Plantago lanceolata</i> L.		<i>Oxalis acetosella</i> L.
	<i>Plantago major</i> L.		<i>Parietaria officinalis</i> L.
	<i>Poa bolbusa</i> L.		<i>Phlomis russeliana</i> Boiss.
	<i>Polygonum aviculare</i> L.		<i>Pilosella officinarum</i> L.
	<i>Polygonum persicaria</i> L.		<i>Plantago lanceolata</i> L.
	<i>Polygonum persicaria</i> L.		<i>Plantago major</i> L.
	<i>Potentilla reptans</i> L.		<i>Poa annua</i> L.
	<i>Ranunculus ficaria</i> L.		<i>Polygonum aviculare</i> L.
	<i>Scrophularia laneolata</i> L.		<i>Polygonum persicaria</i> L.
	<i>Sonchus oleraceus</i> L.		<i>Potentilla reptans</i> L.
	<i>Stachys lanata</i> Benth.		<i>Poterium sanguisorba</i> Boiss.
			<i>Ranunculus ficaria</i> L.
	<i>Stellaria holostea</i> L.		<i>Rumex acetosa</i> L.
	<i>Stellaria holostea</i> L.		<i>Sanguisorba minor</i> Scop.
	<i>Tragopogon pratensis</i> L.		<i>Scrophularia laneolata</i> L.
	<i>Trifolium repens</i> L.		<i>Scrophularia laneolata</i> L.
	<i>Urtica dioica</i> L.		<i>Senecio aureus</i> L.
			<i>Setaria viridis</i> L.
			<i>Stachys lanata</i> Benth.
			<i>Stellaria holostea</i> L.
			<i>Trifolium pretense</i> L.
			<i>Trifolium repens</i> L.
			<i>Urtica dioica</i> L.
			<i>Viola occulata</i> L.
			<i>Viola occulata</i> Lehmann.
			<i>Zizia aurea</i> L.



**Figure 2.** Average Sørensen index ( $\pm$ SE) in within-patch and between-patch. Small successive letters indicate significant differences between within-patch and between-patch in each depth, separately.



**Figure 3.** Detrended correspondence analysis (DCA) of species composition of aboveground vegetation and soil seed bank. ▲ = Within-patch vegetation; ◆ = Within-patch seed bank; ■ = Between-patch vegetation and, ✦ = Between-patch seed bank.



**Figure 4.** Variation of forb, grass, and shrub seed bank density in between- and within-patches. Small successive letters indicate significant difference among the within-patch and between-patch for each functional group, separately.

## DISCUSSION

Our study area has been grazed mostly by sheep for many years, leading to development of bare ground gaps due to the intensive grazing (five animals per hectare). Although the most abundant seed bank was related to non-palatable species in the within-patch (e.g. *Senecio aureus*), the seeds of palatable species germinated in the greenhouse too. Therefore, it can be concluded that shrubby patches play important role not only for conservation of standing vegetation but also for saving soil seed bank of palatable species. The results showed that some palatable species (e.g. *Sanguisorba minor*) existed in the aboveground vegetation in the within-patch. They were also found in the soil seed bank of the within-patch, while they were absent in both aboveground vegetation and soil seed bank of the between-patch plots. Therefore, it can be concluded that patch can act as a protection for palatable species in the overgrazing sites, playing an important role in conservation of these species. The shrubs act as effective seed traps and may provide safe micro-sites for certain suppressed species (such as palatable

species) to germinate and establish by providing refuge in the overgrazing sites (Flores and Jurado, 2003). The lack of desirable propagules of some species such as palatable species in the between-patch can have some possible causes, e.g. reduced seed set through grazing livestock, a low amount of seed arriving at the site, or the lack of safe site (Kinloch and Friedel, 2005; Solomon *et al.*, 2006; Erfanzadeh *et al.*, 2010a; Erfanzadeh *et al.*, 2010b). Nevertheless, seed density of the between-patches was highly correlated to seed density of the within-patches, suggesting the relevance of the patch effect. The patch effect extends to the surrounding bare matrix creating a seed bank gradient in density (Aguiar and Sala, 1994) and composition (Caballero *et al.*, 2008). In this study, twenty-six species occurred in the soil seed bank of both the within- and the between-patches. The plants in the patch dispersed their seeds mainly in the vicinity of maternal plants and, to a lesser extent, in the surrounding bare area. It seems that the seed bank composition variability in bare areas was controlled by patches in the vicinity (Caballero *et al.*, 2008). Previous studies showed that seeds of the target species arrive to all locations of



the vegetation patches and the bare soil among patches (Bonvissuto and Busso, 2007).

The result of DCA showed that the species composition of the vegetation was very different from that in the seed bank at the within-patch. The composition of the between-patch did not show such obvious differentiation among seed bank and aboveground vegetation. The lower degree of similarity between the species composition of vegetation and seed bank in the within-patch can be explained by the minor contribution of the dominant shrubby species to the formation of the seed bank such as *Berberis integerrima*. Díaz-Villa et al. (2003) reported that the similarity between soil seed bank and aboveground vegetation in the woody patches was lower than that in the grassland patches. However, the degree of similarity between soil seed bank and aboveground vegetation is predicted to increase with grazing, due to the greater relative abundance of annuals in the vegetation (Chamber, 1993; Matus et al., 2005; Ma et al., 2010). We suppose that the intensity of disturbance in the between-patch is higher than within-patch, resulting in the increase of the similarity between species composition of both community components.

The results showed that total seed density of the within-patches was much higher than the total seed density of the between-patches. According to some reports, in arid and semiarid habitats, seed bank density was much higher inside patches than the surrounding areas (Pugnaire and Lázaro, 2000; Marone et al., 2004). In overgrazing sites, shrubs accumulated large and diverse soil seed banks beneath their canopy which were different in composition from seed banks of the open matrix in dry habitats (Dreber and Esler, 2011; Dreber et al., 2011). These differences could be explained by a very high amount of seed input (by seed producing and trapping) within patches, seed source effect (increasing both soil seed bank and aboveground vegetation abundance),

and the capability of patches to trap seeds from surrounding environment.

Differences between seed composition of the within-patches and the between-patches help clarify some patterns of germination and recruitments of herbaceous vegetation in our study site. Previous studies (Marone et al., 2000; Marone et al., 2004) showed that forb seed density were often higher under woody plants and positively correlated with the cover of woody vegetation, whereas the seed density of grasses were less pronounced by woody vegetation. These results are consistent with seed composition over the within- and between-patches in this study. *Senecio aureus* (forb), *Minuartia kashmirica* (forb) and *Urtica dioica* (forb) had the highest seed bank density in the within-patch with 15.49, 8.86, and 4.40%, respectively. In the between-patch, the most abundant species in the seed bank belonged to *Minuartia kashmirica* (forb), *Festuca ovina* (grass) and *Bromus tectorum* (grass) with 17.84%, 5.06% and 4.08%, respectively.

This study showed that the limited number of woody patches, which have remained in the study area, could play an important role in conservation of herbaceous species (i.e. palatable and non-palatable species) by their positive effect on manager to take a program for conservation of these woody patches. Sometimes, it has been observed that people use the fire to change the structure of woody patch into the grassland while the number of the patches is very low per hectare. However, restricting grazing in some part of the study area could clarify whether these shrubby patches are a consequence of overgrazing or not, a topic that is necessary to be studied in the future.

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## نقش لکه های بوته ای در حفظ پوشش گیاهی بوسیله بانک بذر خاک در یک رویشگاه چراشده کوهستانی، شمال ایران

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### چکیده

در سالهای اخیر تاثیر گیاهان پرستار بر گیاهان زیراشکوب در رویشگاههای مختلف جهان به کرات مطالعه شده است. در تمامی این مطالعات تاثیر گیاهان پرستار بر پوشش روزمینی سایر گیاهان مطالعه، در حالیکه بر بانک بذر خاک نادیده گرفته شده است. در این تحقیق جهت آگاهی از نقش پرستاری گیاهان چوبی بر بانک بذر خاک، ترکیب و تراکم بانک بذر خاک بین لکه های بوته ای موجود در علفزارهای شمالی کشور ایران (البرز) با بیرون این لکه ها مقایسه گردید. در مجموع ۲۰ لکه چوبی انتخاب گردید و سپس نمونه های خاک از داخل و بیرون لکه ها جهت تخمین بانک بذر آن برداشت شد. نتایج نشان داد که تراکم بانک بذر خاک بویژه پهن برگان در داخل لکه ها به مقدار چشمگیری بیشتر از خارج لکه ها بود. به هر حال، تراکم بانک بذر بیرون لکه های بوته ای به شدت با تراکم بانک بذر داخل لکه ها همبستگی نشان داد که حاکی از "اثر لکه" بود. تشابه بانک بذر خاک با پوشش روزمینی در داخل لکه ها کمتر از بیرون آنها بود. این مطالعه نشان داد که در منطقه مورد مطالعه لکه های محدود باقیمانده از گونه های بوته ای و درختچه ای نقش مهمی را در حفاظت گونه های علفی و خوشخوراک و جلوگیری از انقراض آنها با تاثیر مثبتی که بر روی بانک بذر آنها در خاک دارد، بازی می کنند. بنابراین به مرتعدار توصیه می شود تعداد و وسعت این لکه ها را حفظ و حتی با بازکاشت گونه های چوبی افزایش دهد.