

Article

Germinable Soil Seed Bank in Biancana Badlands

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Abstract: Seed banks are important for understanding vegetation dynamics and habitat regeneration potential. Biancana badlands are vanishing landscapes where recurring and non-recurring management has been advocated to restore vegetation. Here, we investigated germinable seed bank structure and composition of a biancana badland in central Italy and evaluated the relationship between the standing vegetation and soil seed bank. We identified four land cover classes in five biancana badlands of Tuscany (central Italy) and collected data from 132 vegetation plots and 660 soil cores. We recorded 117 species in the standing vegetation. The seedlings that emerged from the soil samples, mostly annual species, numbered 183 and belonged to 31 taxa (392.5 seedlings/m⁻² on average across the four land cover classes). Standing vegetation showed an aggregated spatial pattern with distinct communities while the seed bank showed a less aggregated spatial pattern. The similarity between the seed bank and standing vegetation was low. In contrast with the features generally found for disturbed and pioneer communities, but in line with seed bank characteristics of other badlands, the seed bank was particularly poor in species.

Keywords: badlands; biodiversity; grasslands; restoration; succession; shrub encroachment

1. Introduction

Seed banks, which include all viable seeds in the soil in a given area [1], are important for understanding vegetation dynamics [2–4] and are considered an indicator of the likelihood of restoration success [5–11]. Plant communities of arid and semi-arid regions, including those from large areas in the Mediterranean basin, maintain important seed banks, especially in annual communities [12], and are the habitats with the highest diaspore richness and density [13]. Despite abundant literature on the subject, some key questions, such as the relationships between the seed bank and spatial structure of vegetation, remain unclear [14–16]. It is important to know how the seed bank and standing vegetation interact in order to correctly manage and restore natural ecosystems [17,18]. A recent review of European plant community seed bank characteristics indicates some general trends: The number of live seeds in the soil and similarity between the standing vegetation and seed bank are high in unstable disturbed communities; and similarity between communities seems to be higher in the seed bank than in standing vegetation [10]. Restoration of target communities therefore seems to be more feasible for early successional plant communities and for heathland than for late successional plant assemblages [10].

In badland environments, soil erosion affects vegetation at all stages of plant life, directly by removing seeds, litter, or whole plants by runoff, or indirectly by modifying soil properties necessary for seed germination and seedling establishment [19]. In a semi-arid badland of south-east Spain (Alicante Province), García-Fayos and Recatalá [20] and García-Fayos et al. [21] investigated the size of the soil seed bank, seed rain, and seed removal by erosion to construct a seed balance that proved to be positive. Seed bank densities were low (273 seeds/m²) but enough for plant cover to develop: On badland

slopes, after 40 min of simulated rainfall, only 4% of seeds were lost [22] (Cerdá and García-Fayos, 1997). Seed bank and seedling dynamics were also investigated in humid badlands in the Pyrenees [23]. Here, the presence of buried diaspores was unexpectedly high (1396 seeds/m²) considering the low vegetation cover, and was dominated by small, slightly heterometric seeds. Seedling recruitment on badland slopes was low, and this was attributed to the lack of suitable 'safe sites' for seed germination and seedling survival [22].

On the biancana badlands of southern Tuscany, the distribution of plant communities is extremely patchy and influenced by erosion, soil moisture, and soil salinity [24–31]. Pioneer grasslands occur on morphologies with extreme soil conditions, for example, high salt content, soil erosion, and deposition, such as the southern slopes and pediments of biancana. *Bromus erectus* grasslands, a habitat of European concern (Festuco-Brometalia priority habitats [32]), occur on biancana domes, where soil conditions are less extreme and the soil is more developed. Grasslands with different degrees of shrub cover are found on more stable morphologies with greater water availability [26,28]. Biancana badlands are threatened by soil reclamation for agriculture and re-vegetation due to the reduction in the erosion rate associated with cessation of traditional management [31,33,34]. Recent studies have shown a decrease of 0.9 ha year⁻¹ in bare or sparsely vegetated spots: If the process continues at this rate, in 35 to 40 years, all biancana domes will be completely re-vegetated [31]. In this context, recurring and non-recurring management was advocated to restore badland vegetation [34].

The aim of this research was to investigate germinable seed bank structure and composition of biancana badlands in central Italy. In detail, our aims were: (i) To quantify the germinable soil seed bank of different plant communities of biancana badlands; (ii) to verify the presence of valuable grassland species in the soil; and (iii) to evaluate the relationship between standing vegetation and germinable soil seed bank communities. As suggested by the results of previous research on semi-arid badland environments, we postulated that biancana badlands have poor germinable soil seed banks.

2. Materials and Methods

2.1. Study Sites

The study area is located in the municipality of Asciano (centroid coordinates 43°16'35" N, 11°25'56" E, Datum WGS84), in the landscape known as Crete Senesi, Siena Province (Tuscany, Italy). The sedimentary lithology consists of Pliocene marine clays, rich in salts, such as sodium chloride, calcium carbonate, and sulphates (pH between 7.6 and 8.8) [26]. The study area is part of the Natura 2000 network of Special Conservation Areas [32]. Climate is transitional between subhumid and subarid, with a mean annual rainfall of 700 to 800 mm. The biancana badlands of the Crete Senesi are characterized by cascades of small domes/hummocks (Figure 1). Differently developed biancana soils are distributed spatially in patches and are reflected by the distribution of vegetation [26].

2.2. Vegetation and Seed Bank Sampling

Vegetation data on the study area were obtained in an intensive monitoring program [30]. We identified five biancana fields (those still with typical biancana forms and not merely residual semi-natural vegetation) that we divided into two to four zones (Zs), depending on their surface area [30]. We produced a land cover map of the biancana badlands in the study area using an object-oriented approach on a QuickBird multispectral image (acquisition date: 19 July 2004, spatial resolution: 2.44 m in the multispectral channels), corrected geometrically and radiometrically. Segments were generated automatically by a technique that aggregates neighbor pixels according to their spectral similarity using an agglomerative iteration process [35]. Segmentation was performed using eCognition software (eCognition 2001, Definiens Imaging, Munich, Germany). Finally, we attributed classes manually to each segment (polygon) according to vegetation physiognomy. The four land cover classes were: (i) Bare ground with little or no vegetation (less than 15%); (ii) sparsely and discontinuous herbaceous

cover (less than 30%); (iii) grassland; and (iv) overgrown grassland (shrub cover less than 20%). To classify the four land cover classes, we used the CORINE Land Cover legend with some revisions.

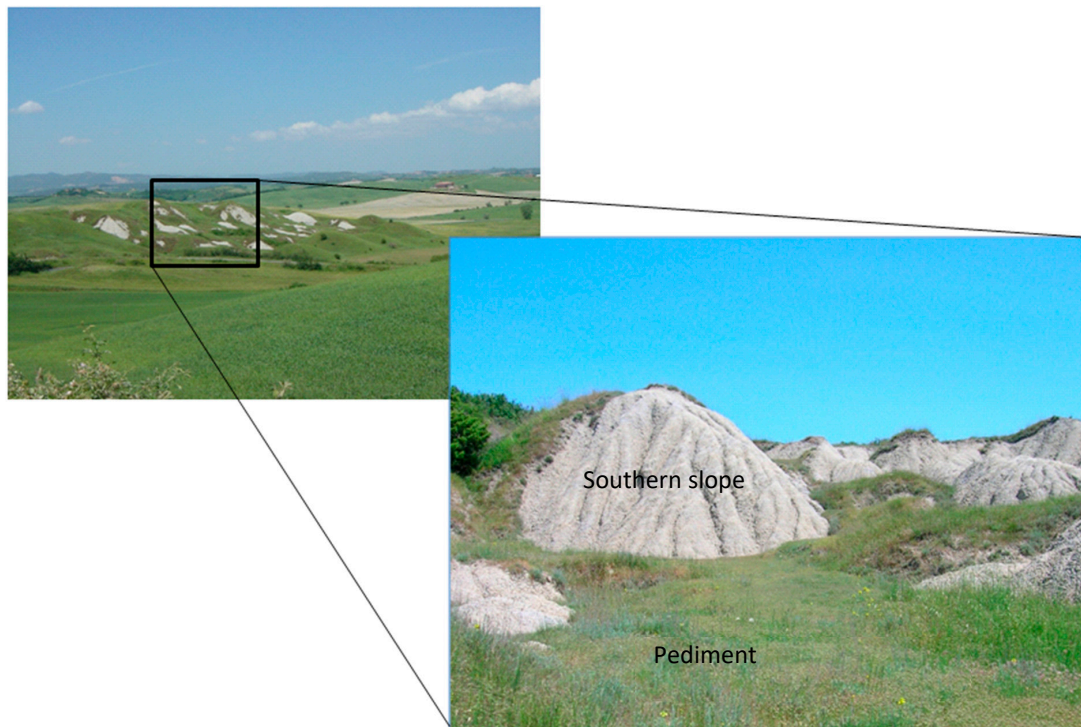


Figure 1. Example of a biancana field and singular biancana.

In each zone, we established three random 1×1 m plots representative of each of the four land cover classes (Cs), so that up to 12 plots were identified in zones where all four land cover classes were present. The number of plots in each badland was thus proportional to its surface area and heterogeneity, which was expressed by the land cover class. For example, in small biancana badlands, with two zones and three land cover classes per zone ($Z = 2, C = 3$), we sampled $3 \times 3 \times 2$ plots. The number of plots per badland varied from 18 to 48, for a total of 132 plots. In June 2006, the percentage cover of vascular plants in each 1×1 m plot was estimated using a point-quadrant method [36], with a density of 100 pins/m².

For seed bank sampling, we collected five soil cores (diameter 3 cm, depth 5 cm) for each of the 132 1×1 m plots established for the vegetation survey. The 660 cores included 180 for each of the three types, bare ground, sparsely vegetated plots, and grassland, and 120 for overgrown grassland. The area and volume of soil sampled were 1271.7 and 6358.5 cm³, respectively, for bare soil, sparsely vegetated plots, and grassland, and 847.8 and 4239 cm³, respectively, for overgrown grassland. Seed bank samples were taken in November 2012, when the main seed shedding was finished, in order to get as full a picture as possible of the soil seed bank [37]. The collected soil was dried and sieved through a 4-mm mesh to remove large stones, roots, bulbs, and tubers. Since previous studies showed that very few seedlings emerged from badland soil, for each land use, we mixed the soil from the five biancana fields and obtained a total of 22 replicates that were spread in 22 plastic trays (6 replicates for bare ground, sparsely vegetated plots, and grasslands and 4 replicates for overgrown grassland). The soil was spread in a layer 1 cm thick over a 3-cm layer of perlite and watered from above to simulate field conditions. The emergence test was started in a greenhouse on 6 December 2012. Temperatures were similar to spring and autumn conditions, the germination period in the field. The seedlings that emerged were counted, identified, and removed once a week. When identification was not possible, seedlings were transplanted into individual pots to continue growth. Five control trays of

pure substrate were tested at the same time for germination of airborne seeds. No seedlings emerged in the control trays.

We investigated the germinable soil seed bank as a reliable estimate of the viable seeds in a sample [38]. Germination was recorded for 10 months. No attempt was made to assess the number of ungerminated seeds remaining in the samples. The nomenclature of vascular plant species and habitat affiliation for each species were according to Pignatti [39]. We classified vascular plants according to life-form groups [40], namely therophytes, hemicryptophytes, geophytes, chamaephytes, and phanerophytes.

2.3. Data Analysis

We plotted the median number of seedlings and number of species per square meter for the germinable seed bank and median number of species per square meter for standing vegetation. To analyze the differences in the number of seedlings emerging and the number of species in standing vegetation and the seed bank among the different land cover types (four levels), we used univariate permutational analysis of variance based on Euclidean distance [41] of untransformed data. All tests were performed with 999 permutations of residuals under a reduced model [42]. Factors were considered statistically significant for $p < 0.05$. When the factor land cover type was significant by univariate analysis, it was investigated by a posteriori pair-wise comparison using the PERMANOVA t statistic and 999 permutations. The permutation approach gives a “distribution free” result, not constrained by many of the typical assumptions of parametric statistics [43]. We used the PERMANOVA routine in the computer program PRIMER v6 [44] with the add-on package PERMANOVA+ [45].

Species similarity within classes of land use between the soil seed bank and standing vegetation was calculated using the Sørensen similarity index [46]:

$$Q = 2c/(a + b),$$

where a and b are the number of species in the first (seed bank) and second (established vegetation) lists, respectively, and c is the number of species occurring in both lists. We analyzed the differences in community composition (presence of species and abundances) between the different land use classes of standing vegetation and germinable soil seed bank by redundancy analysis [47] to obtain community spatial patterns. We used the four land cover classes in constrained ordination as predictor variables against vegetation and seed bank response variables. The significance of the multivariate analysis was tested by the Monte Carlo permutation test (999 permutations). Multivariate analysis was performed using the software package CANOCO for Windows 4.5 [48].

3. Results

3.1. Species Richness in Standing Vegetation

A total of 117 species of vascular plants were recorded in standing vegetation: 29 on bare ground, 81 in sparsely vegetated plots, 94 in grassland, and 68 in overgrown grassland (Table 1). Sparsely vegetated and overgrown grassland plots showed the highest total number of species and bare ground the lowest (Figure 2a; Tables 1–3).

Table 1. List of recorded taxa (number of emerged seedlings) in the germinable soil seed bank of different land use classes.

Species	Bare Ground (n = 6)	Sparsely Vegetated (n = 6)	Grassland (n = 6)	Overgrown Grassland (n = 4)	Total Number of Seedlings	Habitat Affiliation	Biological Form
<i>Parapholis incurva</i> (L.) Hubbard	3	16	15	17	51	saline soil	T
<i>Anagallis arvensis</i> L.		2	12	5	19	garrigue, waste	T
<i>Avena fatua</i> L.		2	6	3	11	pasture, arable	T
<i>Artemisia cretacea</i> (Fiori) Pignatti	1	1	8		10	pliocene clay	Ch
<i>Parapholis strigosa</i> (Dumort) Hubbard	2	6	1		9	subsaline soil	T
<i>Daucus carota</i> L.			7	2	9	waste, dry pasture	Hb/T
<i>Hippocrepis unisiliquosa</i> L.		2	3	3	8	waste scrubland	T
<i>Sherardia arvensis</i> L.			5	2	7	garrigue, waste	T
<i>Xeranthemum cylindraceum</i> S. et S.			6		6	dry pasture, waste	T
<i>Coronilla scorpioides</i> (L.) Koch		1	3	2	6	waste, pasture	T
<i>Hedysarum coronarium</i> L.			3	3	6	clay soil	H
<i>Hypochaeris achyrophorus</i> L.			5	1	6	waste, dry pasture	T
<i>Carex flacca</i> Schreber				5	5	pasture	G
<i>Veronica persica</i> Poirlet			3	1	4	arable	T
<i>Melilotus sulcata</i> Desf.		2	1		3	arable, dry waste	T
<i>Convolvulus arvensis</i> L.			3		3	arable, waste	G
<i>Trifolium campestre</i> Schreber		2			2	dry waste	T
<i>Pallenis spinosa</i> (L.) Cass.		1		1	2	waste dry pasture	T/Hb
<i>Cynodon dactylon</i> (L.) Pers.				2	2	waste	G/H
<i>Scabiosa columbaria</i> L.				2	2	pasture	H
<i>Stellaria media</i> (L.) Vill.				2	2	human habitat	T
<i>Catapodium rigidum</i> (L.) Hubbard			1		1	waste, dry pasture	T
<i>Centaureum erythraea</i> Rafn			1		1	muds, scrubland	Hb/T
<i>Linum strictum</i> L.			1		1	scrubland, garrigue	T
<i>Rapistrum rugosum</i> (L.) All.			1		1	human habitat, dry waste	T
<i>Trifolium scabrum</i> L.			1		1	waste	T
<i>Leucanthemum vulgare</i> Lam.				1	1	human habitat pasture, scrubland	H
<i>Torilis</i> sp.				1	1	waste, human habitat	T
<i>Trifolium echinatum</i> Bieb.				1	1	waste	T
<i>Valerianella eriocarpa</i> Desv.				1	1	arable	T
<i>Vicia villosa</i> Roth				1	1	arable, dry waste	T
	Bare ground (n = 6)	Sparsely vegetated (n = 6)	Grassland (n = 6)	Overgrown grassland (n = 4)	Total		
Number of seedlings m ⁻²	41.18	275.22	676.26	660.53	329.5		
Total number of seedlings	6	35	86	56	183		
Total number of species in germinable seed bank	3	10	20	20	31		
Total number of species in growing vegetation	29	81	98	68	117		

T = therophyte, H = hemicryptophyte, Hb = biennial hemicryptophyte, G = geophyte, Ch = chamaephyte.

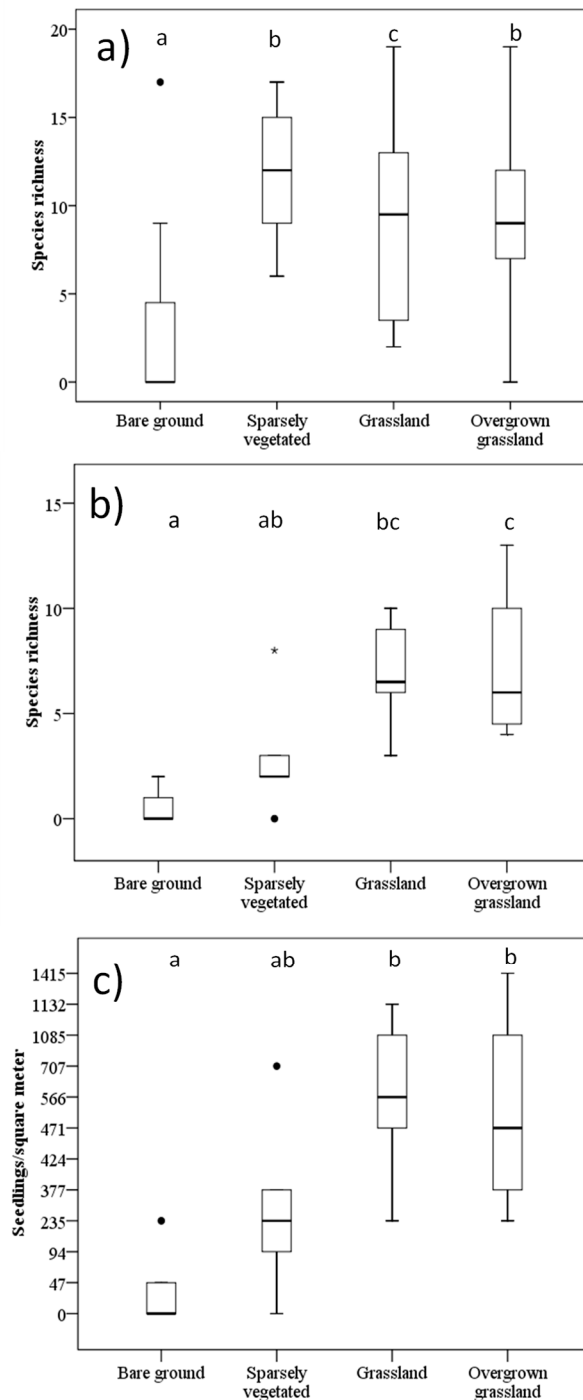


Figure 2. (a) Boxplots of number of species per square meter in standing vegetation for each land cover class: bare ground, sparsely vegetated plots, and grassland $n = 36$; overgrown grassland $n = 24$. (b) Boxplots of the number of species per replicate in the germinable soil seed bank for each land cover class: bare ground, sparsely vegetated plots, and grassland $n = 6$; overgrown grassland $n = 4$. (c) Boxplots of the number of seedlings per square meter per replicate in the germinable soil seed bank for each land cover class: bare ground, sparsely vegetated plots, and grassland $n = 6$; overgrown grassland $n = 4$. Asterisks indicate extreme values and the dot represents an outlier. Different letters indicate significant differences.

Table 2. Permutational ANOVA results for numbers of seedlings and number of species emerging in the 22 replicates.

Source of Variation	df	Vegetation		df	Seed Bank			
		Number of Species			Number of Seedlings		Number of Species	
		MS	F		MS	F	MS	F
Land use class	3	601.15	34.65 **	3	234.87	5.1 *	57.3	8.6 **
Residual	128	17.35		18	46.01		6.67	
Total	131			21				

* $p \leq 0.05$; ** $p \leq 0.01$ **Table 3.** Univariate pairwise comparisons between the four land use classes.

Land use class	Vegetation		Seed Bank	
	Number of Species	Number of Seedlings	Number of Seedlings	Number of Species
	<i>t</i>	<i>t</i>	<i>t</i>	<i>t</i>
Bare soil vs. sparsely vegetated	7.87 **	1.988	2.035	
Bare soil vs. grassland	10.94 **	4.379 **	6.348 **	
Bare soil vs. overgrown grassland	5.72 **	3.262 *	4.246 *	
Sparsely vegetated vs. grassland	2.2 *	1.882	2.418	
Sparsely vegetated vs. overgrown grassland	0.69	2.178	3.213 *	
Grassland vs. overgrown grassland	2.56 *	0.521	0.342	

* $p \leq 0.05$; ** $p \leq 0.01$.

3.2. Species Richness and Density in Germinable Soil Seed Bank

A total of 183 seedlings belonging to 31 taxa emerged from the soil seed bank (392.5 seedlings m^{-2}): 3 on bare ground, 10 in sparsely vegetated plots, 20 in grassland, and 20 in overgrown grassland (Table 1). The lowest median number of species was recorded in soil from bare ground and sparsely vegetated grasslands (Figure 2b, Table 3). The species with the highest number of emerged seedlings were *P. incurva* and *A. arvensis*.

The largest number of seedlings per square meter (1415.42) was found in an overgrown grassland plot and the smallest number in some plots of pioneer and ruderal grassland, where no seedlings emerged. The highest number of seedlings per unit area was found in grasslands and overgrown grassland and the lowest in bare ground plots (Figure 2c; Table 3). The most abundant species in the seed bank was the annual *P. incurva* followed by other therophytes (*A. arvensis*, *A. fatua*, *D. carota*, *H. biflora*, *P. strigosa*, and *S. arvensis*) and by the perennial *A. cretacea* (Table 1).

3.3. Relationship between the Standing Vegetation and Germinable Seed Bank and the Spatial Pattern of the Community Composition

According to the Sørensen index, the similarity between the standing vegetation and seed bank for the different land cover classes was 19% for bare ground, 16% for sparsely and discontinuous herbaceous cover, 24% for grasslands, and 27% for overgrown grassland. The species shared between standing vegetation and the seed bank were 3, 9, 16, and 12 for bare ground, sparsely vegetated ground, grassland, and overgrown grassland, respectively. All the species in the germinable seed bank were also present in the standing vegetation, except *C. rigidum*, *C. dactylon*, *S. media*, and *V. persica*.

The redundancy analysis ordination diagrams showed differences in the species composition of the standing vegetation and germinable soil seed bank between land cover classes (Figure 3a,b).

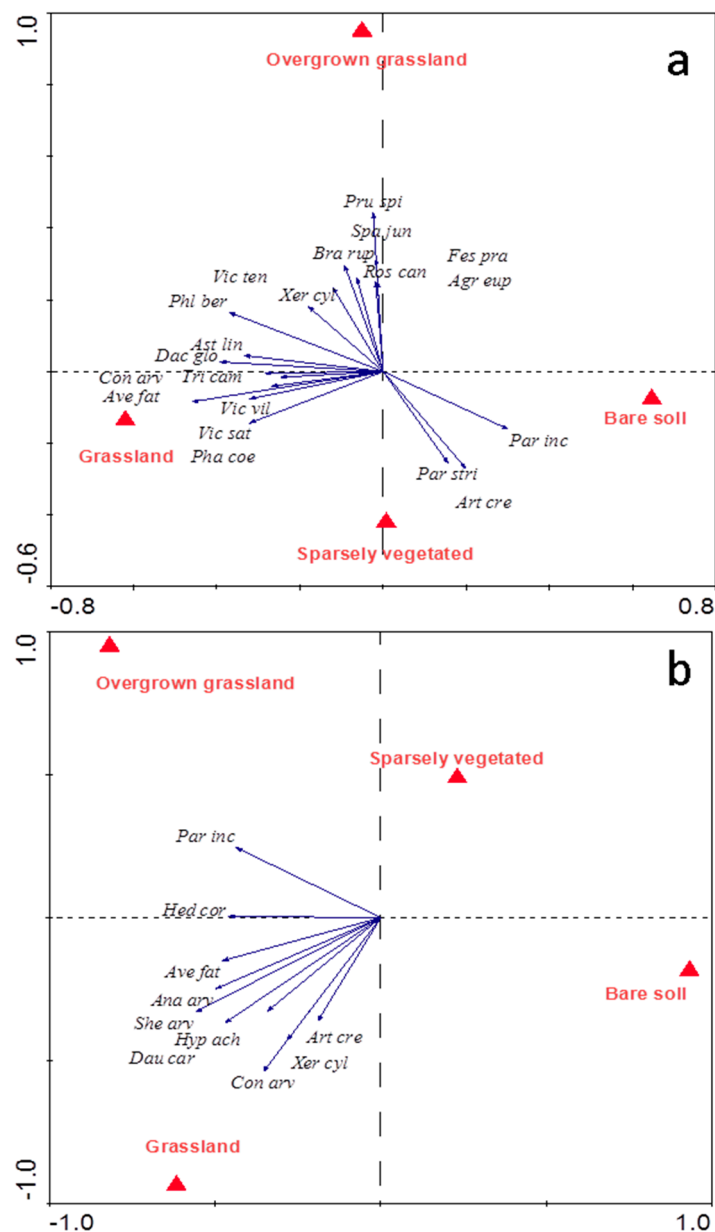


Figure 3. (a) Biplot based on redundancy analysis of vascular plant species abundance in the standing vegetation. Only species that showed at least 6% fit to the model are displayed. Species are coded as follows: Agr eup: *Agrimonia eupatoria* L.; Art cre: *A. cretacea*; Ast lin: *Aster linosyris* (L.) Bernh.; Ave fat: *A. fatua*; Bra rup: *Brachypodium rupestre* (Host) R. et S.; Con arv: *C. arvensis*; Dac glo: *D. glomerata*; Fes pra: *Festuca pratensis* Hudson; Par inc: *P. incurva*; Par str: *P. strigosa*; Pha coe: *Phalaris coerulea* Desf.; Phl ber: *Phleum bertolonii* DC.; Pru spi: *Prunus spinosa* L.; Ros can: *Rosa canina* L. sensu Bouleng.; Spa Jun: *Spartium junceum* L.; Tri cam: *T. campestre*; Vic sat: *Vicia sativa* L.; Vic ten: *Vicia tenuissima* (Bieb.) Sch. et Th.; Vic vil: *V. villosa*; Xer cyl: *X. cylindraceum*. (b) Biplot based on redundancy analysis of vascular plant species abundance in the soil seed bank. Only species that showed at least 20% fit to the model are displayed. Species are coded as follows: Ana arv: *Anagallis arvensis*; Art cre: *A. cretacea*; Ast lin: *A. linosyris*; Ave fat: *A. fatua*; Con arv: *C. arvensis*; Dau car: *D. carota*; Hed cor: *H. coronarium*; Hyp ach: *H. achyrophorus*; Par inc: *P. incurva*; She arv: *S. arvensis*; Xer cyl: *X. cylindraceum*. Arrows pointing in approximately the same direction as the triangles, which represent dummy land cover class variables, indicate a high positive correlation with that class and arrows pointing in opposite directions indicate a high negative correlation. Longer arrows indicate variables with more certain predicted correlations.

The first two ordination axes explained 7.7% and 14.9% of the total species variability for the vegetation data set and seed bank, respectively, but only the first canonical axis of the vegetation data set was significant ($p = 0.001$). Standing vegetation showed an aggregated spatial pattern with distinct communities characterized by different species composition. *P. strigosa*, *P. incurva*, and *A. caerulea* ssp. *cretacea* were the most abundant in the standing vegetation of bare soil and sparsely vegetated plots; *P. caerulea*, *A. fatua*, and *D. glomerata* showed the highest cover in grassland while *Prunus spinosa*, *Spartium junceum*, and *Brachypodium rupestre* were correlated with overgrown grassland. The germinable soil seed bank showed a less aggregated spatial pattern (Figure 3b). No species were exclusive to bare soil or sparsely vegetated plots, while *P. incurva*, *C. arvensis*, *A. fatua*, and *A. cretacea* were more frequent in grassland and overgrown grassland.

4. Discussion

4.1. Germinable Soil Seed Bank Composition and Density

In line with our hypothesis, the number of species and seedlings emerging in the trials was very low. Bare soil or sparsely vegetated sites had few seedlings (41.18 and 275.22 m⁻², respectively). A similarly small number of seeds was reported by García-Fayos and Recatalá [20] in Alicante badlands and by Kemp [49] in the North American Sonoran and Mojave deserts. Grasslands and overgrown grasslands showed similar soil seed bank compositions and did not differ in the number of seedlings or recorded species. Encroachment of grassland by spontaneous succession or afforestation generally causes a decrease in the number of grassland species in the soil seed bank [50–52]. Our results seem to differ from the latter studies, while agreeing with those of Milberg [53], Kalamees and Zobel [54], Maccherini and De Dominicis [8], and Bossuyt et al. [52], who failed to find any substantial changes in seed density or composition with grassland succession, at least in the early stages. The number of seedlings per square meter recorded in grassland and overgrown grassland was much lower than reported in other calcicolous grasslands [4,8,52,54–56]. The seed bank species composition of all communities was dominated by an annual species having mostly transient or short-term persistent seeds, similar to Bakker [57] and Thompson and Grime [58]. These annual species are typical of pioneer vegetation that colonizes biancana pediments and step-eroded areas of calanchi [25,26,28]. Indeed, the annual *P. incurva* was the most abundant species in all four communities considered in the present study. Annual species are more likely to produce a seed bank than perennial species in grasslands [55,59]. Soil seed banks of sandy grasslands have been similarly characterized by pioneer species [18,60]. In any case, valuable perennial grassland species were practically absent from the seed bank of the present study.

Our results indicated that in contrast with the features of pioneer communities [10], but in accordance with the findings of other semi-arid badlands, the seed bank was particularly poor in species. The scarcity of propagules in the soil may have two main causes: The intrinsic characteristics of soil and seed removal by erosion. The marine sedimentary deposits on which biancana badlands develop have a silty clay or clayey silt texture (USDA classification) and are rich in salts, particularly sodium. This gives the system dispersive characteristics that make badlands prone to overland flow and sediment transport, which may remove seeds from the soil surface and seed bank [19,61–66].

4.2. Spatial Pattern of Communities and Relationships between the Standing Vegetation and Seed Bank

The standing vegetation of the biancana badlands showed patchy distribution: Bare soil and sparsely vegetated plots hosted pioneer annual grassland communities with *A. cretacea*; grassland was characterized by ruderal and generalist species, such as *P. caerulea* and *A. fatua*; and overgrown grassland was characterized by typical grassland species, such as *F. pratensis* and *A. eupatoria*, and shrubs, including *P. spinosa*, *S. junceum*, and *R. canina*. The germinable soil seed bank showed a less aggregated spatial pattern. These results agree with the findings of Bossuyt and Honnay [10], Czarnecka [50], and Hanlon et al. [67], who found greater similarity in seed bank composition than in

the corresponding standing vegetation. Contrary to previous findings [10], the similarity between the seed bank and standing vegetation was lower for pioneer grassland than for other grasslands. Species characteristic of soil seed banks of pioneer communities were also common in that of grasslands and overgrown grasslands.

The absence of valuable grassland species in the seed bank and the scarcity of propagules in the soil suggest that the seed bank may be insufficient as a main source for increasing biodiversity when restoring pioneer vegetation and grasslands such as those investigated.

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