Article

Characterization of the seed bank of a grassland in southeastern Coahuila

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Abstract

The seed bank includes viable propagules present in the soil for variable periods of time, its study allows obtaining information on the richness, abundance and prediction of the presence of native species, when the area is impacted. In order to characterize the seed bank of an *Amelichloa clandestina* grassland in an area of 60 ha in southeastern Coahuila, 36 soil samples were collected in the 10 cm of the surface, in a quadrant of 12 × 12 cm. The samples were placed on aluminum trays and covered to prevent wind pollution. Periodic irrigation was applied. Species were counted at two-day intervals. The germination record was carried out for three months. The species richness is made up of 23 species belonging to 12 families. *A. clandestina* began the greatest germination after 48 days and was the species that had the highest seed germination with 1 030 (ind m^{-2}). In the grassland there is a high reserve of seed of *A. clandestina*, in addition to the fact that it is the dominant species and therefore the species richness of the grassland is low.

Keywords: diversity, opportunistic species, seed bank.

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Introduction

According to Marañón (2005), seed banks represent the first link in the regeneration cycle of a plant community, considering that the first condition to be met for a seed to be part of the seed bank is that it does not germinate immediately, these latency processes are part of the evolution to perpetuate the existence in forests, scrublands and grasslands. Thompson and Grime (1979); Archibold (1989) describe it as those seeds and fruits (achenes and caryopses), since they are a reserve of viable propagules present on or within the soil.

The importance of its structure and dynamics has been recognized in the ecology of plant communities by Fenner and Thompson (2005); nevertheless, there is still a lack of knowledge about the processes that regulate it. Several studies have described the structure and composition of the seed bank of the communities, such as the works by Caballero *et al*. (2003, 2008a) in Chinchon, Madrid, Spain. But the understanding of the processes involved such as dispersal, predation and persistence in the soil that affect its formation and dynamics, as well as its connection with vegetation, is still limited.

Experimental and field evidence proposes that seed banks of communities such as forest, grassland and wetland have a similar composition in annual species (Hopfensperger, 2007). Water availability is the most important factor affecting the dynamics of plant populations in annual communities, as it is a limiting resource in arid environments (Miranda *et al*., 2011). Water acts as a filter that determines the richness and diversity of plants in semiarid environments that affect seed germination, in addition to plant growth (Valladares *et al*., 2004).

All these changes occur in vegetation and affect seed bank characteristics (Caballero *et al*., 2008b). In semiarid areas degraded by intensive grazing, the positive effects that shrubs and scrublands have by significantly increasing the number of seeds trapped in the soil, improving the microclimate, promoting better conditions in production, diversity, abundance and germination of seeds of different plants, especially herbaceous plants, have been studied (Barnes *et al*., 2009; Erfanzadeh *et al*., 2014).

The richness and abundance of the seeds of the bank allows predicting which native plant species will colonize an area if vegetation is impacted, or the hydrological conditions present are modified (Cronk and Fennessy 2016). In addition, it indicates the possibility of future invasions of alien species that threaten habitats, affecting their structure, function and local dynamics (Alharthi *et al*., 2021). The analysis of seed banks reveals the potential stored in sediments and is therefore a useful tool in vegetation restoration programs (Espeland *et al*., 2010).

Their structural complexity and the need to quantitatively estimate the reserve stored in the soil haspromoted the creation and combination of several methods of analysis, which are concentrated in two groups: 1) separation methods; and 2) germination methods (Piudo and Cavero 2005). According to McFarland and Shafer (2011), the methods of separating seeds from the soil consist of separating them by flotation using a saline solution or washing from soil samples and passing them through a series of sieves of different mesh sizes, to reduce the volume of the sample and remove as much organic matter as possible, and then separate and observe all the seeds under the microscope.

Germination methods are based on the emergence of seedlings from soil samples, which are placed under controlled conditions to favor the germination of the largest number of viable seeds (Bernhardt *et al*., 2008). Small seeds are detected with this method, and they are the most used to analyze sediment banks (de Winton *et al*., 2000). Therefore, the objective of this work was to characterize a seed bank of a grassland dominated by Mexican ricegrass (*Amelichloa clandestina*) in southeastern Coahuila, Mexico. With the information obtained in this study, it is intended to generate information for its control.

Materials and methods

The soil studied was collected in the ranch 'Los Angeles', owned by the Antonio Narro Autonomous Agrarian University (UAAAN) for its acronym in Spanish, with an area of 7 000 ha. It is located 34 km southeast of the city of Saltillo, between 25° 04' 12" and 25° 08' 51" north latitude and 100° 58' 07" and 101° 03' 12" west longitude (Figure 1) with an altitude of 2 150 m. Land use is the grazing of cattle, in addition to horses. The dominant climate, according to the Köeppen classification system, modified by García (2004), is semiarid, with cool winter [BWhw(x') (e)], with average annual temperature fluctuating between 18 and 20 °C, with average annual rainfall of 350 mm, distributed mainly in summer and winter (López-Santos *et al*., 2008).

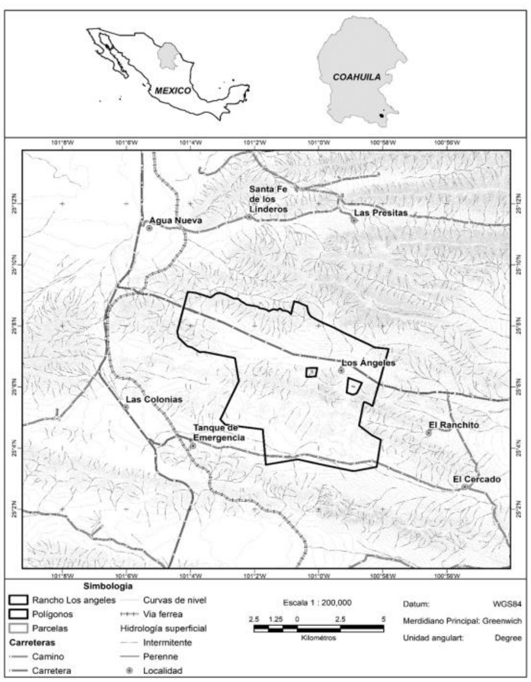

Figure 1. Geographical location of the study area in the ranch Los Angeles, Saltillo, Mexico.

The study was carried out in two agricultural areas abandoned in 2012, of 40 and 60 ha. After the suppression of agriculture, the Mexican ricegrass (*A. clandestina*) settled, with the advance of the succession, a dense grassland settled. The soils are of alluvial origin, they are in lowlands, which are part of the valley, the soils are deep with well-defined profiles and horizons, characteristic of grassland vegetation, the soils are brown and light reddish brown, they are soils of the Feozem calcaric type, in addition, the study area is surrounded by semidesert grassland.

In the two selected areas, 18 plots of 10 ×10 m were established, with 5 m of separation, where the three treatments were placed with their six repetitions. Two samples per each plot (36 total samples) were collected, on the 10 cm of the surface of the sediment (Liu *et al*., 2005). A metal quadrant 12 × 12 cm in diameter was used. The samples were temporarily stored in plastic bags and kept in a dark room, for further establishment in the greenhouse in May 2019. The collected samples were established in greenhouses, according to the methodology of Bernhardt *et al*. (2008).

Aluminum trays of 22 x 30 cm, perforated at the bottom for proper drainage, were used and covered with plastic to avoid contamination of species other than the seed bank. The irrigation was carried out every day at field capacity and the species were counted at two-day intervals to determine the density of each species and the days to germination were recorded in a period from May 18 to August 18, 2019.

Data were analyzed under a completely randomized experimental design, with 36 repetitions. To determine if there is a difference in the density and germination days of the seeds by species, an analysis of variance was performed with the PROC GLM procedure of SAS for Windows version 9.0 (SAS Institute Inc. Cary, North Caroline, USA). A comparison of means with Tukey's test was performed. After identifying the species, the diversity indices most used in ecology were calculated: Margalef, Shannon-Weaver (H), Simpson (D) and Pielou (J). Diversity as a unique value combines the parameters of specific richness and equity, fundamental factors that define the diversity of a community.

Results and discussion

In total, 708 seedlings germinated, which belong to 12 families, the dominant ones were: Asteraceae, Poaceae, Lamiaceae and Euphorbiaceae. Twenty-three species were recorded in the richness of the seed bank, 16 species are annual and seven are perennial. The density of the species found in the grassland presents a significant difference ($p < 0.05$). The results showed that the highest density occurred in *Amelichloa clandestina* followed by *Euphorbia serrula* Engelm., and species such as *Dyssodia papposa* (Vent.) Hitchc., *Erodium cicutarium* (L.) L'Hér., *Eruca vesicaria* (L.) Cav., *Pseudognaphalium roseum* (Kunth) Anderb and *Sonchus oleraceus* L., had low densities (Table 1).

Table 1. Average of plant density (ind m^{-2}) by species in the grassland seed bank.

Amelichloa clandestina	1 030 A	*Amaranthus blitoides*	6 B
Euphorbia serrula	60 B	*Marrubium vulgare*	6 B
Solidago velutina	48 B	*Rumex crispus*	4 B
Anoda cristata	21 B	*Laennecia coulteri*	4 B
Eragrostis mexicana	18 B	*Parthenium hysterophorus*	4 B
Eragrostis barrelieri	18 B	*Euphorbia exstipulata*	4 B

Salvia reflexa	17 B	*Pseudognaphalium roseum*	2 B
Glandularia bipinnatifida	13 B	*Erodium cicutarium*	2 B
Sanvitalia angustifolia	12 B	*Dyssodia papposa*	2 B
Disakisperma dubium	12 B	*Eruca vesicaria*	2 B
Argemone echinata	10 B	*Sonchus oleraceus*	2 B
Asphodelus fistulosus	8 B		
p > F	<0.05		<0.05

Equal capital letters between columns do not differ (*p*> 0.001).

Differences (*p*< 0.05) were observed on germination days of the species of the seed bank of the grassland. *D. papposa* was the species that germinated in the shortest time, with seven days on average, followed by *Parthenium hysterophorus* L. and *Disakisperma dubium* (Kunth) P. M. Peterson & N. Snow, with 9 and 11 days of average germination, respectively. The germination of *A. clandestina* was 48 days and the species that presented later germination, with 74 and 76 days, were *E. vesicaria* and *E. cicutarium* (Figure 2).

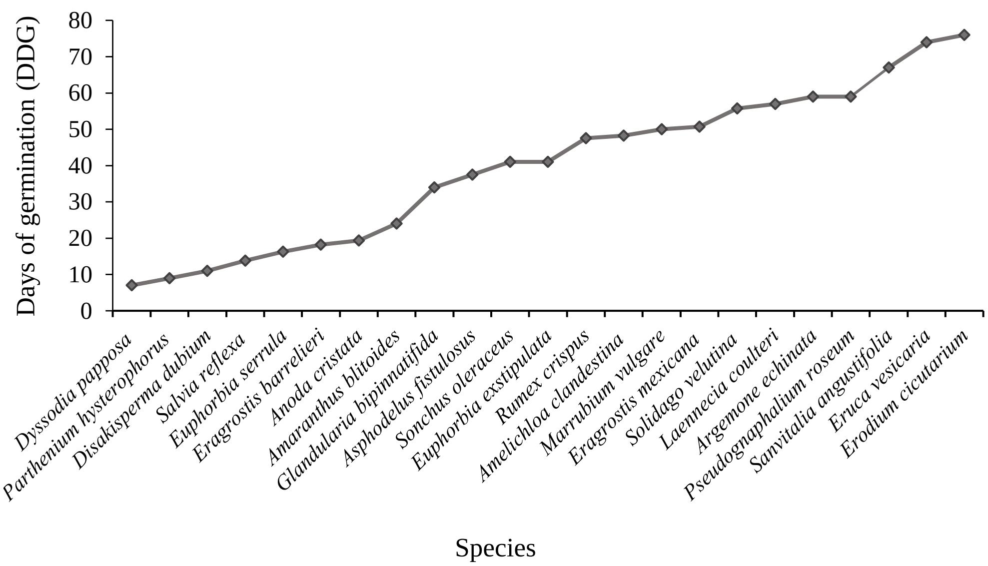

Figure 2. Average number of days to germination by species in the seed bank of a grassland, with dominance of *Amelichloa clandestina* in southeastern Coahuila, Mexico.

The composition, structure and diversity of the seed bank is a result of the disturbance of the area. This is, to a large extent, due to agricultural activities such as the clearing of native vegetation, it has generated that the seed bank includes a high amount of *A. clandestina* seeds and a low richness of species, with a total dominance of Mexican ricegrass.

The high amount of *A. clandestina* is due to the large seed production (Barkworth *et al*., 1989). The presence of cleistogamous spikelets in the Mexican ricegrass increased the number of seeds (Valdés-Reyna *et al*., 2015). This can be compared to what was reported by Dong *et al*. (2020), who recorded a high production of seed of *Ambrosia trifida* L., with a maximum result of 41 100 seeds m^{-2}. Seed banks are variable in space and time and are affected by several factors, including climate, vegetation, population demographics, plant density, dispersal strategy and seed predation (Parker, 1989).

Studies carried out in a secondary forest and a grassland report density of 147 seeds m^{-2} and 190 seeds m^{-2} and a richness between 21 and 28 species (Caicedo *et al*., 2018). On the other hand, in this study the density is higher with 1 030 (ind m^{-2}) and comparing results obtained by Cano *et al*. (2012) in the composition and abundance of the seed bank in a semiarid region in central Mexico, the richness of 23 species found in the grassland is lower than the 38 species found. The Shannon-Weaver species richness and abundance index (H) is shown in Table 2.

Table 2. Margalef, Shannon, Pielou and Simpson diversity indices for species. *Present in the seed bank.

Annual species*	Perennial species*	Wealth index (Margalef)	Diversity index (Shannon)	Equity index (Pielou)	Dominance index (Simpson)
16	7	3.352	0.995	0.317	0.347

A Shannon-Weaver diversity index of H’=1.8 was recorded in the secondary forest area and H’= 2.2 in the grassland area, compared to this study, which found a diversity of H’= 0.995 nats. The Shannon diversity index had low values for the seed bank, in its equity index and Margalef wealth index. In the dominance index it has a lower value, which indicates that, in the seed bank, *A. clandestina* is the dominant species of this grassland.

Species richness was low because *A. clandestina* is a species with large seed production and as seedling recruitment increases, species richness decreases. As a basis for the regeneration of plant community richness, the number of seedling recruitment is related to species richness (Houseman, 2014). Seed dispersal may be influenced by the behavior of dispersal agents and natural barriers that are important for spatial patterns of seed deposition (Myers and Harms, 2009).

In contrast, vegetation recruitment is influenced by abiotic factors, seedling characteristics, and competition (Baldwin *et al*., 2010). Species with high reproductive potential, high germination values, short life cycles and rapid growth with highly competitive efficiency, increase their population distribution in a short period of time, benefiting from the large presence of anthropogenic disturbances, become superior species and begin to be considered harmful, even if they are native, when they decrease local biodiversity, alter their ecological balance, cycle of nutrients, affect local biota and their ecosystem services, being particularly harmful in arid regions (Bonanomi *et al*., 2018; Wang *et al*., 2019; Abd El-Gawad *et al*., 2020; Alharthi *et al*., 2021).

Also, some previous studies conducted by Molina-Montenegro *et al*. (2015); Kenany *et al*. (2017); Alharthi *et al*. (2021) on the alterations caused in places with disturbances and dominated by invasive species indicate decreases in seed banks in the soil, allelopathic inhibition, exclusion of native species in canopies dominated by invaders, decrease in the efficiency of the dispersal capacity in seeds, modification of the composition of the soil, nutrients and its microbiota.

All these modifications directly reduce the floristic density and abundance in a short period of time and indirectly affect the local fauna, biogeochemical cycles and water runoff, impacting the succession of the invaded habitat in the short term. Studies conducted by Alharthi *et al*. (2021) recorded the decrease in abundance, richness and population distribution in seed banks related to alterations caused by invasive species in places with disturbances, as is the case of the alteration by the canopy of *Nicotiana glauca* in genera of *Euphorbia* and *Eragrostis* in Saudi Arabia.

The results are comparable with the densities found in the study area for the species of *Euphorbia serrula*, *E. mexicana* and *E. barrelieri*, species that are usually abundant in the areas surrounding the study area and highly representative of the Mexican semidesert (Duran, 1970; Bekele and Lester, 1981; Peterson and Giraldo-Canas, 2012). *Dyssodia papposa* also showed low levels of abundance within the grassland studied. It is a species highly studied for its rapid and continuous spread on the side of roads in different provinces in North America (Oldham and Klymko, 2011; Oldham *et al*., 2011).

The success of invasive plants can be evaluated using different dimensions, among the most important are the range of plant size, local abundance, impact with the abundance of native plants and their diversity, these species are less preferred by herbivores, this promotes their rapid colonization when there are changes or opening of clearings in the vegetation (Liao *et al*., 2021). *Amelichloa clandestina* has a height of 40-60, with rigid basal leaves with a sharp tip, caryopsis with three longitudinal ribs and persistent style bases, and cleistogamous axillary panicles in the basal sheaths of the leaves (Arriaga and Barkoth, 2006), its populations are dense, and these create a high spatial competition.

The height and size of shrub species have negative effects on the ability of plants to settle, due to the dense upper layer between the soil and their cover (canopy), causing a microhabitat with low light and temperature, causing some seeds of the bank to lose their viability (Yu *et al*., 2008). Bonanomi *et al*. (2018) have recorded the damage caused by early invasive plants in the plant succession of areas with disturbances and changes in their microhabitat, disturbances generated in root growth, in the wind current and increase in biomass in the spaces between plants.

Some species increase nitrogen nitrate content but decrease ammoniacal nitrogen by 29-4% in summer-autumn. There are currently no related studies on the chemical changes or allelopathic effects on soil caused by *A. clandestina*. Although *A. clandestina* presented latency in its seed, since it was the fourteenth species to initiate germination, this was the dominant species and most adapted to the arid conditions of the grassland.

According to Baskin and Baskin (2014), seed latency prevents or delays germination, and generally plays an important role in ensuring germination at the right time to maximize the likelihood that they settle successfully. Hu *et al*. (2014) mention that latency can be caused by the tissues surrounding the embryo, by the low growth potential of the embryo or by a combination of both, the lemma and palea are also important in the germination of seeds, since their elimination releases latency.

This coincides with other studies reporting that these bracts led to latency in grasses such as *Stipa viridula* (Fulbright *et al*., 1983), *S. tenacissima* (Gasque and Garcia 2003), *Leymus secalinus* (Zhu *et al*., 2007). Annual species such as *D. papposa*, *P. hysterophorus*, *S. reflexa* and *E. serrula* have a lower latency, since they are the species that germinated in the shortest time, which is consistent with a study by Figueroa *et al*. (2004) in a Mediterranean scrubland in central Chile.

The presence of species such as *Disakisperma dubium* in the surrounding natural grassland indicate that the seeds are transferred to this grassland through the wind and by animals, as mentioned by Cronk and Fennessy (2016). Annual and ruderal species such as *E. serrula*, *A. cristata*, *Salvia reflexa* Hornem, *Sanvitalia angustifolia* Engelm. Ex A. Gray, remain in the seed bank; however, their density is lower, which, according to Morlans (2005), indicates a greater advance in the process of vegetation succession.

Although several works have been carried out in semiarid areas, this is the first work where an estimate of the density and richness in the seed bank is made for a grassland invaded by *A. clandestina* in southeastern Coahuila. This study constructs an approach to understand the dynamics of areas abandoned by agriculture in semiarid regions of northeastern Mexico.

Conclusions

The seed bank presented a high number of viable seeds of *Amelichloa clandestina*, and the annual and ruderal species that are found have a lower density and the presence of perennial species such as *Solidago velutina* and *Disakisperma dubium* indicate a greater advance in the process of succession of vegetation and thus greater stability. In the grassland studied, *A. clandestina* is the dominant species and therefore the species richness is low.

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