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Analysis of composition and density of soil seed banks of *Prosopis juliflora* in Afar region rangelands, Northeast Ethiopia

By Wakshum Shiferaw, Sebsebe Demissew, Tamrat Bekele, Ermias Aynekulu, and Wolfgang Pitroff

On the Ground

- We analyzed the composition and spatial variations of soil seed banks of plant species and densities of soil seed banks in *Prosopis juliflora* invaded and noninvaded grasslands.
- Soil samples were collected from soil layers of 0 to 3, 3 to 6, and 6 to 9 cm.

The highest density of 1,037 ± 633 seedlings/m2 was recovered from a soil depth of 3 to 6 cm. But, the lowest density of 461 ± 315 seedlings/m2 was recovered from the depth of 0 to 3 cm.

- In noninvaded grasslands, the lowest density of 94 ± 32 seedlings/m2 was germinated in the soil depth of 0 to 3 cm. In *Prosopis juliflora* grasslands at the 3 to 6 cm, the density was 519 ± 257 seedlings/m2, but 1,484 ± 1,144 seedlings/m2 was in the noninvaded grasslands at the depth of 3 to 6 cm.
- Our findings have implications for recovery of rangelands after disturbances.

Keywords: Invasive species, *Prosopis juliflora*, Rangeland degradation, Soil seed bank, Ethiopia

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Introduction

Grazing is an important component of a secure livelihood in pastoral and agro-pastoral areas. However, overgrazing is a major cause of rangeland deterioration.[1] Rangeland degradation affects the size and species composition of soil seed banks that are vital for rangeland management.[2] Soil seed banks are viable seeds present in the soil or associated humus.[3,4] They represent retention of the present plant community and improvement of future plant communities.[5] Soil seed banks are a significant component in successional regeneration of ecosystems after disturbances. Buried viable seeds germinate to cover the disturbed and exposed soil surfaces.[6] The formation of soil seed banks is a strategy developed by plants to prevent germination under unfavorable soil and climate conditions.[7,8]

Analysis of soil seed bank composition and density is important for managing plant communities when they have been invaded by invasive or exotic species and to promote desired plant species.[9] Invasive species are the second greatest threat to global biodiversity loss next to land-use change that is introduced either intentionally or accidentally.[10] *Prosopis juliflora* is an invasive plant species indigenous to South America, the Caribbean, and Central America.[7] *Prosopis juliflora* was introduced intentionally to Ethiopia, specifically in the Afar region in the late 1970s.[11–14] Although *Prosopis juliflora* is used as fuelwood, for shade, and dry season fodder for rural populations, the threat of invasion in fertile agricultural lands and prime grazing lands, as well as the resulting loss of biodiversity, is becoming enormous.[15]

In Ethiopia, human and natural impacts on rangelands facilitated encroachments of invasive plants into rangelands that have become a threat to pastoral production systems.[11,16–20] Studying soil seed banks is vital to understanding the expansion of *Prosopis juliflora* into other land beyond pastoral land and to alleviating further invasion of this species. Land-use changes, competitive ecological advantages, and climate changes are key factors influencing the invasion of *Prosopis juliflora*.[20,21] When an invasive species becomes established, controlling it can often be difficult and eradicating it can be nearly impossible. Moreover, the impact on biodiversity and ecosystem processes is serious.[22]

Research on soil seed banks has been undervalued all over the world,[8] in part because of the challenge in isolating viable seeds from soil samples.[23] However, soil seed banks are an important component of ecosystem resiliency and represent

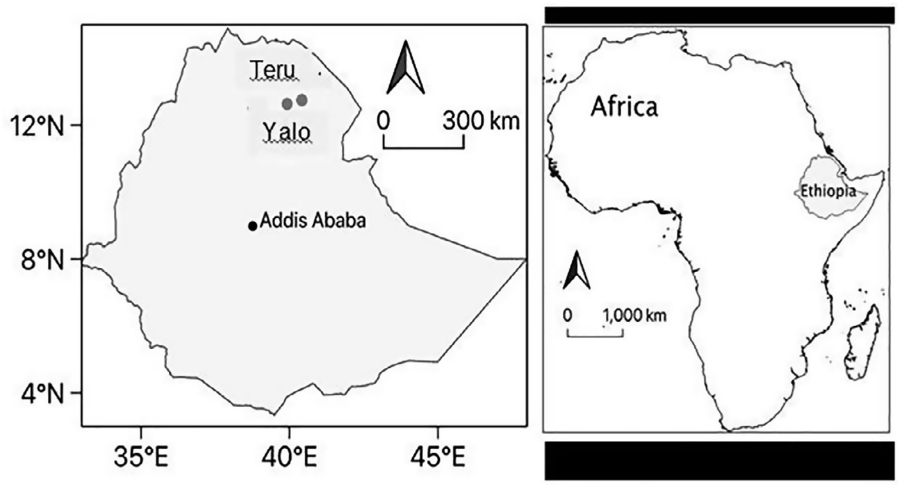

Figure 1. Map of Yalo and Teru districts in northern Ethiopia located in northeastern Africa.

the regeneration potential in many plant collections.[24] Additionally, the soil seed bank is essential for understanding species composition, capacity of storage, size, seasonal changes, and distribution patterns of plant species.[25] Research on soil seed banks can also provide information about past management practices and their impacts on current and future vegetation.[26]

The Afar region in Ethiopia is one of the hottest and driest places in the world. Although it is affected by climatic variability, it is an area where humans have been living since time immemorial, adapting their pastoralist activities to changing environmental conditions.[27] To date, few studies have investigated the soil seed banks in the Afar region.[19,28–30] However, no research has reported the status of soil seed banks in the districts of Teru and Yalo (Fig. 1). We aim to analyze the composition, spatial variation, and density of soil seed banks in grasslands invaded and not invaded by *Prosopis juliflora*. We attempt to answer the following questions: 1) what is the status of the density and species composition in soil seed banks? 2) what is the spatial variation in *Prosopis juliflora* in comparison to other native species in soil seed banks? 3) does *Prosopis juliflora* invasion modulate densities of species in soil seed banks in Teru and Yalo districts? and 4) what are the implications of the status of soil seed banks for the restoration of rangelands?

Materials and methods

Description of the study area

The Yalo district is located at 12°38′03.95″N to 13°0′.00″N and 39°55′.00″E to 40°18′00.0″E, and at an altitude of 354 m above sea level (1,161 feet above sea level). Whereas, Teru is located between 12°40′0″N to 12°45′0″N and 39°65′0.00″E to 40°25′.00″E, and at an altitude of 826 m above sea level (2,710 feet above sea level; Fig. 1).

Climate data were taken from the nearest Chifira Meteorology Station in the Afar region.[31] The mean annual temperature of our study sites was 27.5°C (81.5°F), and the mean minimum annual temperature was 16.8°C (61.7°F) and the mean maximum annual temperature 39.9°C (103.8°F). The annual precipitation in our study areas was 173 mm (6.8 inches; Fig. 2).

The dominant geological feature is the intense slicing of the Afar Neogene volcanics by early Pleistocene fault-belts.[32] Soil texture is typically sandy, which originated from Jurassic and Cretaceous limestone and other sedimentary rocks. According to the Food and Agricultural Organization, soil classification, and International Soil Reference and Information Centre-World Soil Information, the soil of the Afar Floristic region is Lithic, Eutric leptosols, and Eutricfluvisols.[33] *Acacia-Commiphora* woodland and bush vegetation types characterize the floristic region of our study sites. The characteristic herbaceous species consist of *Chrysopogon plumulosus, Sporobolus pellucidus, Dactyloctenium scindicum, Cymbopogon commutatus,* and

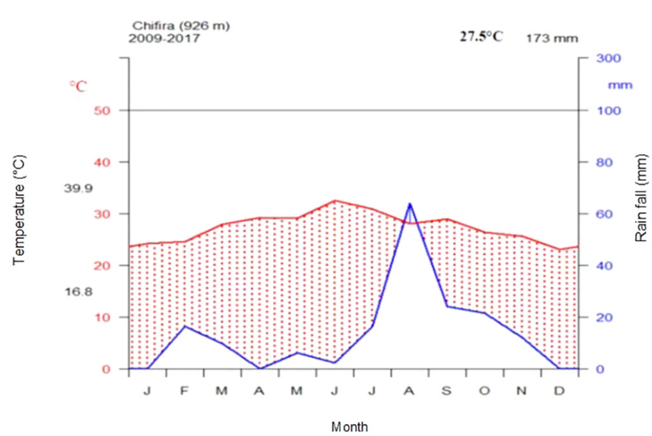

Figure 2. Climate of study sites (x-axis is month, y-axis is temperature [left] and rain fall [right]), red dotted color is deficit in rain fall, and blue solid line is high rain fall. Meteorological data is from the Chifira district during 2009 to 2017. Eight years of data were used to calculate annual rain fall (173 mm) and mean temperature for this area (27.5 °C).

Cynodon dactylon. The woody vegetation is mainly composed of *Acacia senegal, Acacia nubica, Acacia nilotica, Acacia tortilis, Acacia mellifera*, *Acalypha acrogyna*, *Cadaba rotundifolia, Dobera glabra, Grewia tenax, Salvadora persica, Balanites aegyptiaca,* and *Ziziphus spina-christi.*[33]

Sampling design

Site selection-Prosopis juliflora invasion sites were categorized during a preliminary reconnaissance survey. Our study sites were stratified into approximately homogeneous units based on amount of invasion of *Prosopis juliflora*, and the diameter size of individual *Prosopis juliflora* plants, and the existence of noninvaded grasslands (i.e., open grazing lands) for comparison of soil seed banks. The soil samples from soil seed banks were collected in August 2018 after the rainy season.

Plot layout and data collection-A total of 156 soil samples were collected in our study sites with 78 soil samples collected each in Teru and Yalo districts. Soil samples were taken from 0 to 3 cm, 3 to 6 cm, and 6 to 9 cm soil depths under *Prosopis juliflora* invaded and noninvaded grasslands. For each soil layer, about 1 kg of composite and representative soil samples were collected, placed in plastic bags and labeled.[25] Sampling was completed within 1 week to avoid differences between land use and any temporal bias in seed availability and composition.[34]

Seed germination and identification-The number of viable seeds in the soil was estimated using the seedling emergence technique in the greenhouse.[35] Soil from each sample was placed into plastic trays in the greenhouse in the Central Ethiopia Environment, Forestry and Climate Change Research Center, Addis Ababa, Ethiopia.

To prevent contamination of soil samples with nonexperimental seeds, trays were placed in a shade house located in an open site (<80% full sunlight) and covered by a layer of white plastic mesh (<0.5 mm aperture) and transparent nylon sheet. Temperatures fluctuated between 55 and 90°F. The trays were watered three times each week to keep the soil moist. Seedlings were identified and counted weekly for 6 months until emergence ceased.[30]

Seedlings were transplanted to other pots after they were identified with accession numbers. They were removed to minimize confusion with newly emerged plants and possible density effects on later germination.[19] Each seedling was identified using photos and plant specimens in the herbarium using published Flora of Ethiopia and Eritrea.[36–43]

Data analyses-Effects of *Prosopis juliflora* invasion, locations (i.e., districts) and soil depth on the density of soil seed banks was analyzed by SAS software using an analysis of variance (ANOVA) test. Significant differences among means was calculated using Duncan's multiple range tests for effects of habitats (invaded and noninvaded grasslands), soil depth, and location on soil seed bank densities.[44]

Results

Composition and density of soil seed banks across soil depths

The density of soil seed banks showed an increasing trend across soil depths in the Teru district, but did not show clear trends across soil depths in the Yalo district (Fig. 3A). Furthermore, *Prosopis juliflora*-invaded grasslands showed an increasing trend with greater soil depths, but in noninvaded grassland there was no clear trend across the soil depths. For the composition of soil seed banks in our study sites, 10 species of plants germinated with 90% being herbaceous species and 10% being woody species (Table 1). These plant species were distributed across 8 families of plants. Forty percent of these 10 species were found in both the 0 to 3 and 6 to 9 cm (0–1.2 and 2.4–3.5 inches) soil depths, which included *Galinsoga parviflora, Lipocarpha rehmannii, Physalis lagascae,* and *Eragrostis*

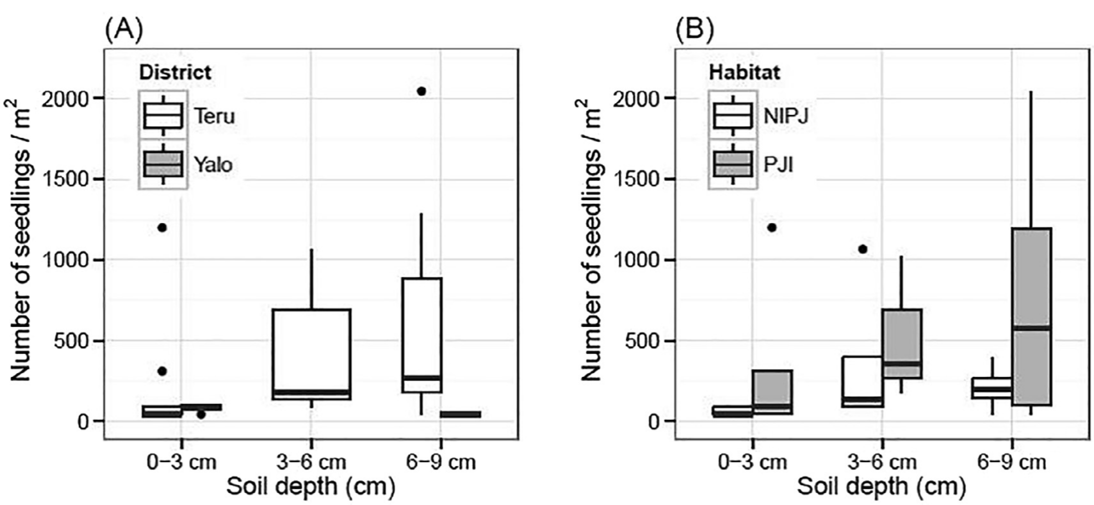

Figure 3. (A) Density of seedlings in soil seed banks of Teru and Yalo districts (B) Density of seedlings in soil seed banks in invaded and non-invaded grasslands by *Prosopis juliflora* in Teru and Yalo districts in the Afar region, Ethiopia by soil depth. NIPJ = non-invaded by *Prosopis juliflora* and PJI = *Prosopis juliflora* invaded grasslands.

Table 1. Plant species composition and density of soil seed banks in Teru and Yalo districts in the Afar region, Ethiopia for both invaded and noninvaded grasslands.

District	Soil depth (cm)	Species	Soil seed bank density (number of seedlings/m^2)	Taxonomic family
Teru	0–3	*Galinsoga parviflora* Cav.	44	Asteraceae
		Prosopis juliflora (Sw.) DC.	44	Fabaceae
		Lipocarpha rehmannii (Ridl.) Goetgh.	1,422	Cypercaeae
		Physalis lagascae Roem. & Schult.	133	Solanaceae
		Eragrostis cilcilianensis (All.) Vign. Ex Janchen	5,422	Poaceae
	3–6	*Lipocarpha rehmannii*	2,978	Cypercaeae
		Eragrostis cilcilianensis	6,000	Poaceae
	6–9	*Lipocarpha rehmannii*	3,778	Cypercaeae
		Galinsoga parviflora	44	Asteraceae
		Physalis lagascae	3,111	Solanaceae
		Eragrostis cilcilianensis	1,289	Poaceae
		Kalanchoe glaucescens Britten.	222	Crassulaceae
Yalo	0–3	*Galinsoga parviflora*	89	Asteraceae
		Bidens pilosa L.	133	Asteraceae
	3–6	*Oxalis anthelmintica* A. Rich.	356	Oxalidaceae
	6–9	*Brachiaria ovalis* Stapf	44	Poaceae
		Amaranthus thunbergii Moq.	89	Amaranthaceae

These data were collected at two locations during August 2018 after the main rainy season (soil seed bank density is measured as number seedlings per meter squared).

cilcilianensis. Twenty percent were found in both the 0 to 3 and 3 to 6 cm (0–1.2 and 1.2–2.4 inches) soil depths, which included *Lipocarpha rehmannii* and *Eragrostis cilcilianensis*. However, *Prosopis juliflora* seedlings were recorded in only the 0 to 3 cm soil depth (Table 1).

We found the highest density of seedlings in the soil seed bank was for *Eragrostis cilcilianensis* (6,000 seedlings/m^2 at 3–6 cm and 5,422 seedlings/m^2 at 0–3 cm) at the Teru district sites (Table 1). The lowest density of seedlings in soil seed banks was for *Galinsoga parviflora* (44 seedlings/m^2 at 6–9 cm), *Brachiaria ovalis* (44 seedlings/m^2 at 6–9 cm), and *Prosopis juliflora* (44 seedlings/m^2 at 0–3 cm).

Furthermore, Poaceae was the most frequently observed plant family across both districts, but other families that were observed included Crassulaceae, Oxalidaceae, and Amaranthaceae (Table 1).

Density of soil seed banks at Teru and Yalo districts

Our ANOVA results show that densities of seedlings in soil seed banks did not vary significantly by soil depth and habitats ($P > 0.05$; Table 2).

The mean density of seedlings in the soil seed banks in the Teru district (818 ± 267 seedlings/m^2) was significantly higher than in the Yalo district (119 ± 48 seedlings/m^2; Table 3). The mean density of seedlings in the soil seed banks in noninvaded grasslands (813 ± 375 seedlings/m^2) was significantly higher than in the invaded grasslands (545 ± 156 seedlings/m^2). Additionally, the mean density of seedlings in soil seed banks at the soil depth of 3 to 6 cm (1,037 ± 633 seedlings/m^2) was higher than at 0 to 3 cm (461 ± 315) and 6 to 9 cm soil depths (Table 3).

The densities of seedlings in soil seed banks in the Teru district (589 ± 418, 849 ± 324, and 1,122 ± 711 seedlings/m^2 at 0–3, 6–9, and 3–6 cm soil depths, respectively) were higher than in the Yalo district (119 ± 48, 44 ± 0, and 356 ± 0 seedlings/m^2 at 0–3, 6–9, and 3–6 cm soil depths, respectively; Fig. 3A).

Invasion of *Prosopis juliflora* and density of seedlings in soil seed banks

At a soil depth of 0 to 3 cm and in noninvaded grasslands, densities of seedlings in the soil seed banks were 94 ± 32

Table 2. Analysis of variance results showing effects of habitat (i.e., grasslands invaded or noninvaded by *Prosopis juliflora*), and soil depth (cm) at Teru and Yalo districts in Afar region, Ethiopia.

Explanatory variables	Source	DF	Sum of squares	Mean square	F value	Pr > F
Habitat and soil depth	Model	3	3,546,279.29	1,182,093.10	0.62	0.61
	Error	32	61,157,655.32	1,911,176.73	–	–
	Corrected Total	35	64,703,934.61	–	–	–
Location and soil depth	Model	3	3,710,235.08	1,236,745.03	0.63	0.60
	Error	31	60,871,104.21	1,963,584.01	–	–
	Corrected Total	34	64,703,934.61	–	–	–

Table 3. Statistical variations of seedling densities ($P < 0.05$) in soil seed banks in Teru and Yalo districts in the Afar region, Ethiopia.

Explanatory variable	Mean ± SE	Minimum	Maximum	
Location	Teru (N = 30)	818 ± 267^{a}	44	6,000
	Yalo (N = 6)	119 ± 48^{b}	44	89
Habitat	PJI (N = 15)	545 ± 156^{a}	44	2,044
	NIPJ (N = 21)	813 ± 375^{b}	44	6,000
Soil depth (cm)	0–3 cm (N = 16)	461 ± 315^{a}	44	5,067
	3–6 cm (N = 9)	$1{,}037 \pm 633^{b}$	89	6,000
	6–9 cm (N = 11)	776 ± 302^{c}	44	3,111

Note: Similar letters are statistically insignificant, but different letters show significant variations of variables.
PJI indicates *Prosopis juliflora* invaded grassland; NIPJ, noninvaded grassland; SE, standard error of the mean.

seedlings/m^2, and in grasslands invaded by *Prosopis juliflora* there were 828 ± 622 seedlings/m^2 (Fig. 3B). Moreover, the density of seedlings in the soil seed banks in the 3 to 6 cm soil depth was 519 ± 257 seedlings/m^2 under the invaded grasslands, but 1,296 ± 953 seedlings/m^2 in noninvaded grasslands. In the 6 to 9 cm soil depth and in invaded grasslands, densities of seedlings in soil seed banks were 1,098 ± 435 seedlings/m^2 and in noninvaded grasslands were 211 ± 73 seedlings/m^2 (Fig. 3B).

Discussion

Assessing soil seed banks are important for understanding ecological processes that conserve biodiversity. Moreover, soil seed banks are influenced by propagule pressure and invasive plant species.[2] We sought to understand the composition and density of seedlings in soil seeds banks by evaluating the persistence of seeds. Thus, soil seed banks with persistent seeds allow plants to disperse over time.[45] In our study, most of the species that germinated were herbaceous, such as grass species, potentially because of seed longevity in the soil and small seed size, which might escape detection by predators.

In the Teru district, the increasing trends in density of seedlings in the soil seed banks in relation to lower soil layers could be related to the size of the seeds with smaller seeds moving down to lower soil layers.[8] Moreover, density of seedlings in soil seed banks in the lower soil layers might be hindered from emergence by the lack of soil moisture in lower soil layers.[35,46] Disturbances such as grazing and harsh climatic conditions could also contribute to the lower density of seedlings in the soil seed bank in lower soil layers.[47] Furthermore, sediment loads from soil erosion at lower slopes and the periphery of streams could influence the higher densities of seedlings in soil seed banks in lower soil layers, particularly in the Teru district.[48]

In both districts, the density of germinated *Prosopis juliflora* seeds in the soil was low in comparison to other plant species. It is possible that animals may not be the main seed dispersal agents, which facilitate the germination of seeds through animal ingestion.[8,15,19] The density of seedlings in the soil seed banks in the Teru district compared with the Yalo district was low, which could be related to differing effects of grazing and human impact.[5,49] In fact, the number of soil seed banks recovered in both districts was low in comparison to other regional sites. This could be due to temporal and spatial variations in the status of seeds in the soil, the effects of variations in nutritional conditions, and potential of seeds sources in the districts.[50–52]

Moreover, lower rates of seed dispersal and unfavorable micro-climatic conditions (i.e., extreme high temperatures) could contribute to higher seed mortality rates and are possible reasons for the lower density and richness of seeds in the Yalo district than the Teru district.[53] The variations in the density of seedlings in the soil seed banks could also be due to variations in elevation between the districts. At higher elevations, the density of seedlings in the soil seed bank is lower than at lower elevations, which could be related to the effects of gravity and wind movement depositing more seeds at lower elevations.[54]

The density of seedlings in soil seed banks in the Teru district was greater than the density reported by Shiferaw et al.[4] in the soil seed banks of South Afar of Ethiopia and also greater than Ozaslan et al.[55] in Turkey. However, the densities of seedlings in soil seed banks in the Yalo district were lower than the aforementioned research in south Afar of Ethiopia and in southern Marmara, Turkey.

In the Teru district, densities of seedlings in soil seed banks were higher than those reported by Elsafori and Abdllah[6] in semiarid regions of Sudan. On the other hand, the number of species that emerged in both the Teru and Yalo districts was lower than those found by Tessema et al.[47] in a semiarid African savanna. Moreover, the number of species we observed in the soil seed banks was lower than those observed by Dreber[56] in the arid Nama Karoo rangelands in South Africa. Our findings on the predominance of herbaceous species and lack of woodland species are similar to what Baum et al.[57] found in northern Germany and central Sweden.

Our finding that the overall abundance and density of seedlings in the soil seed banks were lower in the invaded *Prosopis juliflora* grasslands than noninvaded grasslands could be due to the allelochemical effects of the *Prosopis juliflora* and the effects of shade on the native plant species, which could cause plants to not produce sufficient seeds for dispersal.[21,27,58] *Prosopis juliflora* is a highly colonizing and invasive thorn tree species that places vast amounts of seeds in the seed bank, unlike other species.[59] Moreover, the findings of Shiferaw et al.[19] in the Middle Awash of Ethiopia showed a greater density of the seeds in soil seed banks than in the Yalo district but lower than the Teru district.

In our study, the density of seedlings in soil seed banks in both districts was greater than the density found by Kebede and Coppock[30] in Northeastern Ethiopia in both the invaded and noninvaded sites of *Prosopis juliflora*. In both districts, the density of seedlings in soil seed banks was higher than densities reported by Andrade and Miranda[3] during November to May in woody savannas of Central Brazil.

We found lower soil seed bank densities and diversity compared with other similar agroecologies, which could be related to different factors, such as distance and concentration of the seed source (i.e., seed rain), seed dispersal, nature and activity of dispersal agents, and spatial heterogeneity of the parent plants in the field. These variations may also reflect differences among species in terms of seed longevity in the soil, modes of seed dispersal and subsequent movement, seed predation, and probable differences in the slope of the landscape and local edaphic conditions where the seeds land.[48]

Conclusions

In our study, a few number of plant species and their low density in the soil seed banks in the Yalo and Teru districts revealed limited opportunities for restoration of rangelands in the future. Furthermore, the low density of seeds in the soil bank, particularly in the Yalo district, could be indicative of high-grazing intensity, human impact, other disturbance factors, and other limitations of edaphic factors (e.g. moisture stress, extremely high temperatures, and low nutrients) in our study areas.

On the other hand, seeds of species such as *Eragrostis cilcilianensis*, *Galinsoga parviflora*, *Lipocarpha rehmannii*, *Physalis lagascae*, and *Kalanchoe glaucescens* in the Teru district, and those of species such as *Brachiaria ovalis*, *Amaranthus thunbergii*, *Oxalis anthelmintica*, and *Bidens pilosa* in the Yalo district were persistent in soil seed banks. The presence of these species highlights the potential for conservation and re-seeding of these species to restore rangelands in Ethiopia.

The invasion of *Prosopis juliflora* in our study and other studies revealed variations in densities of native plant species in the soil seed banks, which ultimately affected the species composition of the rangelands. To reverse this situation, conservation of grazing grasslands and management of the invasion of the *Prosopis juliflora* are vital to sustaining the grazing grasslands in the region. Thus, the long-term effects of *Prosopis juliflora* on soil seed banks in association with above-ground flora should be investigated and sample collections of deep soil seed banks should be recommended for future research.

Declaration of competing interest

None of the authors have a conflict of interest.

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Authors are from: Arba Minch University, College of Agricultural Sciences, Natural Resources Management, Arba Minch, Ethiopia; Addis Ababa University, College of Natural Sciences, Plant Biology and Biodiversity Management, Addis Ababa, Ethiopia; The World Agroforestry Centre (ICRAF), Nairobi, Kenya; College of Agriculture, Food and Natural Resources, University of Missouri, Columbia, MO, USA.