

## **Research** Article

# Study of the Soil Seed Bank Composition in Arjo-Diga Humid Afromontane Forest under Different Land Use Types and Its Implications for the Restoration of Degraded Lands in Western Ethiopia

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The soil seed bank present in forests serves as a crucial indicator of soil resilience following disturbances. The objective of this study was to investigate the composition, density, and vertical distribution of the soil seed bank in the Diga District of Western Ethiopia across four land use types: forestland, grassland, bare land, and shrubland. Soil samples were collected from plots measuring 225 square centimeters with a depth of 9 cm. A total of 108 soil samples were collected, representing the four land use types. Each plot was sampled at three different soil layers: 0-3 cm, 3-6 cm, and 6-9 cm depths. From the soil seed bank, 51 plant species were identified, with 46 (90.2%) being herbs, 3 (5.88%) trees, and 2 (3.92%) shrubs. The Asteraceae (13.30%), Poaceae (11.53%), and Fabaceae (9.61%) families exhibited the highest species composition, accounting for 38.44% of the total. Species richness varied significantly across all land use types and soil layers (p < 0.05), with shrubland having the highest species richness and bare land having the lowest count. The total seed bank density in the soil across all plots was 92153 seeds/m<sup>2</sup>, with the highest density observed in the top 3 cm of the soil layer. The first and second soil layers exhibited a high Jaccard similarity coefficient of 0.76. Forestland and shrubland displayed the highest Jaccard coefficient of similarity (0.72), while the lowest was observed between bare land and shrubland (0.43). There was greater species similarity between the first and second soil layers (0.76) and lower similarity between the middle and bottom layers (0.32). The study found limited similarity between aboveground vegetation and the soil seed bank due to the low regeneration of woody species. Forestland and shrubland exhibited a high Jaccard similarity coefficient to aboveground vegetation, while grassland had the lowest similarity coefficient. Based on the findings, it can be concluded that the shrubland land use types had high soil seed bank composition and density. Therefore, the conservation of shrubs in the Arjo-Diga Forest should be considered for the restoration of degraded areas, taking into account the soil seed bank in the shrubland ecosystem.

#### 1. Background of the Study

Globally, forest cover was reduced to 420 million hectares between 1990 and 2020 through conversion to agricultural land and other uses [1, 2]. Tropical forests in particular continue to be destroyed by natural and human intervention [3–6]. As a result, 75% of tropical forests are being cleared, degraded, and converted to cropland [6–8] and human habitation, which is common in the tropics, e.g., Ethiopia [9, 10]. In these cases, while soil seed banks have the potential to recruit regeneration, they are continuously eliminated by weed control and eventually completely depleted [11, 12].

Ethiopia is recognized as one of the most biodiverse countries in Africa due to its diverse climate, topography, and soil variability [13]. However, many natural resources have been reduced and overused through degradation for decades. This degradation is the result of rapid population growth, extensive forest clearance for cultivation, and livestock grazing [14]. Forest fires and illegal harvesting of forest products also reduced the forest area [15-17]. Recent land use changes are likely to affect biodiversity and the ability of plant species to regenerate or recover in rural ecosystems [16, 18, 19]. In addition, the increase in the human population has led to the depletion of the soil seed bank [20, 21]. When the population grows rapidly, land is needed for agriculture, urbanization, and development, resulting in the loss of native vegetation and soil seed banks [22]. Likewise, changes in land use resulting from human population growth in the Chilimo forests and the Buska Mountain forests of Ethiopia led to the depletion of seeds naturally preserved in soil seed banks [23]. Therefore, a nature-based solution to land degradation has long been considered a cost-effective solution for remediation and vegetation restoration to increase the sustainability of degraded land [24, 25]. As a result, a soil seed bank study has been proposed as a promising method for vegetation restoration.

Soil seed banks are viable seeds present on the soil surface or buried in the soil [26, 27] and can serve as a "memory" of past plant communities by containing seeds from previous vegetation in the area and enhancing future plant communities [28]. It is widely believed that the soil seed bank contains native seeds for native vascular plants and provides the majority of seedlings for natural vegetation restoration [29]. However, the feasibility of vegetation restoration using the soil seed bank is highly dependent on seed density and species composition. The density and composition of the soil seed bank have been influenced by disturbances such as humans, grazing, and fires [30]. Soil seed bank density may have increased or decreased with increased human disturbance [20]. Livestock grazing alters the composition of the soil seed bank due to continuous grazing during the flowering and seeding periods [31] and can provide better opportunities for seed germination through trampling manure pellets [32], which can result in minimizing seed retention in the seed bank. Therefore, understanding the effects of grazing on the seed bank is crucial for conservation and grazing management in the tropics [33]. For this reason, the effect of fire on the soil seed bank in various grassland areas has been extensively studied [34, 35], and it has been reported that fire can affect vital processes such as flowering, fruiting, and seed germination.

Obtaining adequate information on natural forest restoration potential as well as threat factors is now urgently needed to implement appropriate forest management measures that could minimize forest loss [36, 37]. Therefore, determining the state of the seed bank is crucial for ecological stability and the restoration of degraded forests. Current habitat destruction and fragmentation (land use change) in the study area poses a significant challenge, as it threatens seed formation and maturation as well as seedling production under vegetation. In addition, no previous ecological survey of the soil seed bank in the area was conducted.

The current study was conducted in the Arjo-Diga Forest in Diga District, Western Ethiopia, in different land use types that help to establish conservation approaches for both the present and the future. Therefore, the objectives of this study were (1) to study the horizontal and vertical distribution and density of soil seed banks under different land use types and soil layers and (2) to assess the similarity in species composition between the soil seed bank and the standing vegetation of the Arjo-Diga Forest, Western Ethiopia.

#### 2. Materials and Methods

2.1. The Study Area. Arjo-Diga Forest is located in Diga District, East Welega Zone, Oromia Regional State. It is located 234 kilometers southwest of Addis Ababa between latitudes of  $9^{\circ}59'00''$  and  $9^{\circ}6'30''$  and longitudes of  $36^{\circ}18'30''$  and  $36^{\circ}24'30''$  with altitudinal ranges between 1360 and 2,220 meters above sea level (Figure 1).

The study area features a diverse landscape that includes plains, slopes, gentle slopes, and steep hills [38]. Three main types of soil are present in the study area: *Dystrian Nitrosols*, *Dystrian Gleysols*, and *Orthoclastic Acrisols* [38]. Geologically, the study area is characterized by Precambrian rocks of high origin and migmatites [39]. The monthly maximum and minimum temperatures in the study area are recorded as 25.7°C and 10.2°C, respectively. The mean annual rainfall in the study area is 1997 mm, following a unimodal rainfall pattern. Monthly rainfall data show that the highest precipitation occurs in July, June, and August, while the period from November to February is relatively dry.

Jaleta and Debella [40] classified the study area into six different land use types, including arable land (71%), grassland (11%), dense forest (10%), bushes and shrubs (2%), woodland (5%), and bare land (1%). The vegetation type of the study area is humid Afromontane forest characterized by broadleaf evergreen plant species. The lowland area also includes solid lowland bamboo thickets and *Combretum* species.

2.2. Site Selection, Sampling, and Data Collection. Four land uses, i.e., forestland, shrubland, grassland, and bare land, were used for the collection of soil samples. Due to frequent and uncontrolled disruptions, cultivated and rural areas were not included in the present study because cultivation has no role in the restoration of vegetation [41]. Soil samples were collected in late October 2018 at the end of the growing season (after seed dispersal). To compare the seed bank flora with the standing vegetation in the Arjo-Diga Forest, a total of 36 plots with standing vegetation were randomly selected (three successive vertical soil layers \* 36 plots). A plot of size 15 cm × 15 cm was purposefully laid in each land use type following the methods in [22]. Soil samples were collected from five smaller subplots measuring  $15 \text{ cm} \times 15 \text{ cm}$  (one from the center and four from the corner) of each of the 36 sample plots. The soil samples were collected from three successive layers: the top (0-3 cm), middle (3-6 cm), and bottom (6-9 cm), using a marked metal rod following the methods in [20]. Soil from similar layers was mixed to form a composite, and approximately one kilogram of composite



FIGURE 1: Map of Ethiopia showing the Oromia Region and the study district.

soil samples was transferred to cotton bags and kept separately in plastic trays. Finally, a total of 108 soil samples (3 layers  $\times$  4 land use types  $\times$  9 sample replicates) were collected and transported to the Ethiopian Biodiversity Institute (EBI) greenhouse for seed germination by the germination method.

2.3. Seedling Emergence Methods. Soil samples were airdried and sieved with a 2 mm·mesh to remove plant matter, including roots, leaves, and stones. Soil samples were then spread in plastic trays, perforated at the bottom, watered to three days per week, and incubated in the greenhouse for seed germination. Seedlings that were easy to identify by their scientific names were counted and recorded following standard methods [42]. Seedlings that were difficult to identify were transplanted into pots and grown until they produced diagnostic characteristics that were easy to identify [43]. Seedling emergence was recorded for at least six months [44]. All specimens were pressed, dried, and identified from the Flora of Ethiopia and Eritrea and deposited in the National Herbarium, Addis Ababa University, Ethiopia.

2.4. Data Analysis. Soil seed bank density, species composition, abundance, and vertical distribution in each land use type and soil layer data were organized in an Excel spreadsheet (version 2010). The composition and density of the seeds in the soil were obtained from the combined data from the germination experiment. Soil seed bank density was calculated based on the number of seeds recovered from the soil samples. On the other hand, the vertical distribution of seeds was obtained from the combination of similar layers and converted to density of seeds/m<sup>2</sup> according to the methodology applied in [45]. In this study, R software version 4.2.3 was utilized to investigate the impact of land use types and soil layers on the mean species diversity (H'), species richness (S), and evenness (E) of soil seed banks. A two-way ANOVA was used to investigate how different types of land use and soil layers affect soil seed bank density. In addition, the mean differences in soil seed bank species compositions among the land use and aboveground vegetation were computed using one-way ANOVA, with a post hoc Tukey HSD test with a significance level of 0.05.

The similarity of soil seed bank composition among different land uses and soil layers was examined using the Jaccard similarity coefficient [46]. The coefficient is calculated using the following equation:

$$Sj = \frac{a}{a+b+c},$$
(1)

where  $S_j = Jaccard$  similarity coefficient; a = number of species shared by both types of land use and soil layers; b = number of species that are only found in the soil layer or land use type b; and c = number of species unique to land use type c/soil layer c. The coefficient has a value from 0 to 1, where 1 indicates complete similarity and 0 shows dissimilarity.

#### 3. Result

3.1. Seedling Composition. In this study, a total of 51 plant species belonging to 44 genera and 24 families were identified in the soil seed bank (supplementary appendix 1). The

number of species varied across land use types within each family. Among the 24 families recorded in the soil seed bank, shrubland had 17 families (31 species), forestland had 14 families (27 species), grassland had 13 families (24 species), and bare land had 11 families (22 species) (refer to Figure 2). Similarly, among the 24 families recorded in the soil layers, the first upper layer (0–3 cm) had 22 families (45 species), the middle layer (3–6 cm) had 19 families (33 species), and the bottom layer (6–9 cm) had 17 families (28 species) (supplementary appendix 2).

The three most common families in the study area were Asteraceae, Poaceae, and Fabaceae, which contributed 9 (17.30%), 6 (11.53%), and 5 (9.61%) species to the soil seed bank, respectively. Conversely, 32.7% of species were contributed by the families Cyperaceae, Euphorbiaceae, Acanthaceae, Amaranthaceae, Caryophyllaceae, Rubiaceae, and Tiliaceae, while 1.92% of species were contributed by the remaining 14 families (supplementary appendix 1).

Herbs were represented by the highest number of species in all land use types (46, 90.2%), followed by trees (3, 5.88%) and shrubs (2, 3.92%) (supplementary appendix 1). The number of species collected from different land use types ranged from 22 to 31. Shrubland had the highest number of species (31 species), followed by forestland (28 species). However, the bare land soil had the lowest number of species (22 species) in the soil seed bank. Achyranthes aspera, Hypoestes triflora, Ficus sur, Ipomoea eriocarpa, and Mitracarpus hirtus were found only in shrubland and forestland soil samples. In addition, Oldenlandia herbacea only germinated from soil samples taken from bare land soil samples. In contrast, Eragrostis schweinfurthii and Hygrophila schulli were discovered exclusively from soil samples from grassland land use types. Vernonia auriculifera, Croton macrostachyus, Entada abyssinica, Ficus sur, and Calpurnia aurea were woody species recovered from forest and shrubland land use types.

3.2. Species Diversity, Richness, and Evenness of Seedlings in Different Land Use Types. According to this study, species richness was significantly different in all land use types in the soil seed banks (p < 0.05), but the Shannon–Wiener diversity index and Shannon evenness index were not (p > 0.05) (Table 1). In shrubland ( $6 \pm 0.39$ ), higher species richness was observed among seedlings. However, bare land had the lowest species richness ( $3.81 \pm 0.31$ ). The same table shows that forestland ( $2.52 \pm 0.14$ ) had the largest Shannon–Wiener diversity index, followed by grassland ( $2.42 \pm 0.9$ ), shrubland ( $2.40 \pm 0.9$ ), and bare land ( $2.39 \pm 0.08$ ). The highest evenness value was found in grassland ( $0.78 \pm 0.03$ ), while the lowest evenness value ( $0.71 \pm 0.04$ ) was observed in forestland.

3.3. Soil Seed Bank Density for Different Land Use Types. The present study investigated the variations in soil seed bank density among different land use types, as outlined in supplementary appendix 3. The results revealed that shrubland exhibited the highest mean soil seed bank density ( $1320.2 \pm 2.1$ ), while forestland had the lowest mean soil seed bank density ( $539.9 \pm 2.3$ ). The corresponding table also



FIGURE 2: Number of families and species of soil seed bank in four land use types in the Arjo-Diga Forest.

indicated that the maximum and minimum numbers of seedlings per square meter were observed in shrubland and forestland, respectively.

Additionally, the two-way ANOVA results presented in Table 2 demonstrated that different land use types had a significant and strong impact on soil seed bank density (F = 15.901, p < 0.001) across soil layers. However, the analysis indicated that the interaction between land use types and soil layers did not have a statistically significant effect on soil seed bank density (F = 0.952, p > 0.462).

The result of Tukey's pairwise comparisons of means of soil seed density revealed significant differences between land use types 3-1, 3-2, and 4-3 (p < 0.001). However, there was no significant difference in soil seed bank density between land use types 2-1, 4-1, and 4-2 (Table 3).

According to this study, soil layers with depths of 0-3 cm, 3-6 cm, and 6-9 cm contained 45, 33, and 28 seedling species, respectively (Table 4). In the 0-3 cm soil layer, herbaceous species accounted for 91.1% of the seedling population compared to trees (6.7%) and shrubs (2.2%). Herbaceous species accounted for 90.9% of the seedling population in the 3-6 cm soil layer, compared to 6% for trees and 3% for shrubs. Furthermore, herbaceous species accounted for 82.14% of the seedling population in the 6-9 cm soil layer, while shrub species (two species) and tree species (three species) accounted for 7.15% and 10.71% of the seedling population, respectively.

The results of the soil seed density analysis indicated a significant decrease in soil seed bank density as the soil layer depth increased, as shown in Table 5. Specifically, the top layer (0-3 cm) exhibited the highest density, with 39,333 seedlings per square meter. The 3–6 cm and 6–9 cm bottom layers followed with slightly lower densities. Among the herbaceous species, the 0–3 cm layer had the maximum density of 144,687 seedlings per square meter. In contrast, the layer of 6–9 cm had the lowest density, with 21,220 seedlings per square meter.

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Land use types	R	H'	E
Forestland	$4.81 \pm 0.31a$	$2.52 \pm 0.14a$	$0.71 \pm 0.04a$
Shrubland	$6 \pm 0.4b$	$2.40 \pm 0.9b$	$0.73 \pm 0.02a$
Grassland	$5 \pm 0.39b$	$2.42 \pm 0.09 b$	$0.78 \pm 0.03a$
Bare land	$3.21 \pm 0.34c$	$2.39 \pm 0.08b$	$0.74\pm0.02a$

TABLE 1: Species richness, diversity, and evenness of soil seed bank across four land use types.

H' = Shannon–Wiener diversity index, R = species richness, and E' = Shannon evenness; the same letters indicate nonsignificant differences at p < 0.05.

TABLE 2: Two-way ANOVA analysis of soil seed bank density in soil layers and land use type.

Source of variation	Df	Sum Sq	Mean Sq	F value	Pr (>F)	Sig.
Land use types	3	3274	1091.3	15.901	1.80e - 08	* * *
Soil layers	2	1473	736.6	10.732	6.22e - 05	* * *
Land use types * soil layers	6	392	65.3	0.952	0.462	
Residuals	96	6589	68.6			

\*\*\* Significant at p < 0.001.

TABLE 3: Tukey's pairwise comparisons of four land use types based on soil seed bank density.

Explanatory variable	Land use types	Estimate	Std. Error	t value	Pr (> t )	Sig.
	2-1	-3.0130	2.4538	-1.228	0.610628	
	3-1	-13.5126	2.4538	-5.507	< 1e - 04	* * *
Soil and hank donsity $(aada/m^2)$	4-1	-0.2647	2.4538	-0.108	0.999548	
son seed bank density (seeds/m)	3-2	-10.4995	2.4538	-4.279	0.000242	* * *
	4-2	2.7483	2.4538	1.120	0.678069	
	4-3	13.2478	2.4538	5.399	< 1e - 04	***

\*\*\* Significant at p < 0.001.

TABLE 4: Number of individual seedlings and density across soil layers.

Plant habit	Soil layers					
	C	)–3 cm	3–6 cm		6–9 cm	
	No. of species	Density (seeds/m <sup>2</sup> )	No. of species	Density (seeds/m <sup>2</sup> )	No. of species	Density (seeds/m <sup>2</sup> )
Tree	1	44	1	44	1	44
Shrub	1	44	1	44	0	0
Herb	43	93245	30	30266	27	21176
Total	45	39333	33	31600	28	21220

The frequency of soil seed bank species in soil samples taken from each of the three soil layers can vary significantly from layer to layer (Table 5). In the 0-3 cm soil layers, Ageratum conyzoides, Cardamine trichocarpa, Commelina benghalensis, Centella asiatica, Cyperus fischerianus, Oplismenus hirtellus, and Veronica javanica represented a high number of seedlings (>13 seedlings each). Adenostemma mauritianum, Eragrostis schweinfurthii, Oldenlandia herbacea, and Triumfetta brachyceras had the lowest number of seedlings (<2 seedlings each). Cyperus fischerianus, Oldenlandia herbacea, and Oplismenus hirtellus were dominant in the 3-6 cm soil layer with seedlings (>6 each), while Eleusine indica, Laggera crispata, and Mitracarpus hirtus together accounted for 8.1%. Cyperus fischerianus, Veronica javanica, and Centella asiatica were the dominant seedlings in the 6-9 cm soil layers, accounting for approximately

55.5%, 52.7%, and 38.8%, respectively. Furthermore, *Achyranthes aspera*, *Psilotrichum elliotii*, *Euphorbia rothiana*, *Triumfetta rhomboidea*, *Cynoglossum lanceolatum*, *Eragrostis schweinfurthii*, *Tagetes minuta*, and *Persicaria nepalensis* only germinated from the 0–3 cm soil layers. *Ipomoea eriocarpa* and *Hygrophila schulli* were found only at a soil depth of 6–9 cm. In contrast, *Hypoestes triflora* was found only from the 3–6 cm soil layers (appendix 2).

Soil seed bank species richness was significantly different across soil layers (p < 0.05) (Table 6). However, the Shannon diversity index and species evenness showed no significant differences between soil layers (p > 0.05). The 0–3 cm soil layer had a mean Shannon diversity index higher than that of the other soil layers ( $2.46 \pm 0.1a$ ). In addition, the 6–9 cm soil layer had the highest mean species uniformity of seedlings ( $0.77 \pm 0.02a$ ) compared to the other soil layers. In the

Soil depth (cm)	Species with high seedling frequency	% frequency	Species with low seedling frequency	% frequency
	Ageratum conyzoides	22 (61.1%)	Adenostemma mauritianum	1 (2.7%)
	Cardamine trichocarpa	13 (36.1%)	Eragrostis schweinfurthii	1 (2.7%)
	Commelina benghalensis	14 (38.8%)	Oldenlandia herbacea	1 (2.7%)
0-3	Centella asiatica	21 (58.3%)		
	Cyperus fischerianus	25 (69.4%)	Triture for the land during the	1(2.70/)
	<b>Oplismenus</b> hirtellus	20 (55.5%)	Iriumjetta brachyceras	1 (2.7%)
	Veronica javanica	19 (52.7%)		
	Cyperus fischerianus	9 (25%)	Eleusine indica	1 (2.7%)
	Nicandra physalodes	5 (13.8%)	Laggera crispata	1 (2.7%)
	Oldenlandia herbacea	7 (19.4%)	Mitracarpus hirtus	1 (2.7%)
3-6	Oplismenus hirtellus	6 (16.6%)		
	Ipomoea teteraphyla	5 (13.8%)		
	Plantago lanceolata	6 (16.6%)		
	Ageratum conyzoides	5 (13.8%)		
	Centella asiatica	14 (38.8%)	Dichrocephala integrifolia	1 (2.7%)
	Ageratum conyzoides	11 (30.5%)	Cardamine trichocarpa	1 (2.7%)
6–9	Cyperus fischerianus	19 (52.7%)	Hypoestes triflora	1 (2.7%)
	<b>Oplismenus</b> hirtellus	13 (36.1%)	Nicandra physalodes	1 (2.7%)
	Veronica javanica	20 (55.5%)	Phyllanthus ovalifolius	1 (2.7%)

TABLE 5: Frequencies of seedling species in the soil seed bank in three soil layers in the Arjo-Diga Forest.

TABLE 6: Species richness, diversity, and evenness of soil seed bank across soil layers.

Soil layers (cm)	R	H'	E
0-3	$5.61 \pm 0.32b$	$2.46 \pm 0.1a$	$0.72 \pm 0.03a$
3-6	4.69 ± 0.36ab	$2.43 \pm 0.08b$	$0.73 \pm 0.02a$
6–9	$4.44 \pm 0.38a$	$2.41\pm0.09\mathrm{b}$	$0.77 \pm 0.02b$

H' = Shannon–Wiener diversity index, R = species richness, and E = Shannon evenness; the same letters indicate nonsignificant differences at p < 0.05.

topsoil layer, species uniformity had the lowest value  $(0.72 \pm 0.03a)$ . In most cases, the diversity of seedlings across soil levels decreased with increasing soil layer.

A pairwise comparison of soil layers for significant differences was conducted using Tukey's linear contrast hypothesis. The results of the pairwise comparisons of the mean indicated significant differences in seed density between the 3-6 cm and 0-3 cm soil layers, as well as between the 6-9 cm and 0-3 cm soil layers. However, no significant differences were observed between the 6-9 cm and 3-6 cm soil layers, as shown in Table 7.

3.4. Similarity in Species Composition between Land Use Types, Soil Layers, and Standing Vegetation. In terms of species composition, forestland and shrubland had the highest Jaccard similarity coefficient value (0.72), followed by forestland and grassland (0.62), while bare land and shrubland had the lowest similarity value (0.43) (supplementary appendix 4).

The degree of Jaccard similarity between the soil seed bank layers, on the other hand, showed that the first and second layers were more similar. Conversely, the first and third layers showed the least similarity (supplementary appendix 5).

According to the study, there were 234 different species of aboveground plants in the main plots. Only 65 plant species were present in both the standing vegetation and the soil seed bank (supplementary appendix 6). The soil seed bank contained only 9.1% of the woody plants that made up the aboveground vegetation. However, the standing vegetation contained all herbaceous species found in the soil seed bank. In addition to common species present in both the seed bank and aboveground flora, 234 aboveground species and 80 seed bank-identified species were used as comparisons for the Jaccard and between the soil seed bank and standing species. However, the soil seed band and aboveground vegetation in the shrubland had a comparatively higher similarity index than the other land use types. The output of one-way ANOVA showed the variation in soil seed bank species composition and standing vegetation. Accordingly, the findings revealed that there was a significant difference between the land use types as well as a notable distinction between the seed bank and existing vegetation (Table 8).

#### 4. Discussion

4.1. Species Composition in the Soil Seed Bank. The study of species composition, distribution, and density of soil seed banks in different land uses reflects in part the history of the standing vegetation [47]. Our results showed that the soil seed bank contains a high proportion of herbaceous species and a low proportion of woody species. The dominance of herbaceous species and species of the Asteraceae and Poaceae plant families in the soil seed bank could be due to species seeds being small, light, and winged [48]. This characteristic makes seeds more durable and dispersible through a variety of mechanisms, contributing to their high species composition in the soil seed bank [42, 49, 50].

Explanatory variable	Pairwise comparison of soil layers	Estimate	Std. Error	<i>t</i> value	Pr (> t )	Sig.
	3-6 cm vs. 0-3 cm	-7.138	2.329	-3.064	0.00771	**
Seed density (seeds/m <sup>2</sup> )	6–9 cm vs. 0–3 cm	-8.383	2.329	-3.599	0.00141	* *
	6–9 cm vs. 3–6 cm	-1.245	2.329	-0.534	0.85461	

TABLE 7: Pairwise comparisons of the mean soil seed density between soil layers.

\*\*Significant at p < 0.01.

TABLE 8: Analysis of variance (ANOVA) showed a significant difference in species composition between land use types (of soil seed bank) and standing species (significance at p < 0.05).

Multiple mean comparison						
Number of species in above and below ground composition per						
181	nd use types					
	lukey HSD					
Species in seed bank (belowground) Compared with Mean Sig						
	Shrubland above	2.542	0.089			
	Forestland	3.375*	0.024			
Shrubland	Forestland below	4.771*	0.002			
	Grassland above	3.313*	0.027			
	Grassland below	1.792	0.231			
	Grassland above	-1.521	0.309			
	Forestland below 1.458		0.329			
Grassland	Shrubland above	-1.083	0.468			
	Shrubland below 3.313		0.027			
	Forestland above	2.979*	0.047			
	Forestland above	Forestland above -1.396				
	Shrubland 2.542*		0.001			
Forestland	Shrubland below	-4,771*	0.002			
	Grassland above	-1.458	0.329			
	Grassland below	-2.979*	0.047			

\*The mean difference is significant at the 0.05 level.

Another possible reason is that herbaceous species have a better chance of recovering from disturbance because they produce many seeds quickly and spread over long distances [4, 43]. In contrast, only five woody species (e.g., *Vernonia auriculifera*, *Croton macrostachyus*, *Entada abyssinica*, *Ficus sur*, and *Calpurnia aurea* seeds) emerged from the soil seed bank of shrubland and forestland use types in this study. This number is lower than the number of woody species identified in the Arjo-Diga Forest aboveground vegetation survey because of the difficulty of incorporating their larger seeds into the soil to form seed banks [51], and they are more susceptible to fungal infections and predators [52]. The results of this study were consistent with soil seed bank studies in Ethiopia [45, 49, 53–56]. Larger, moist seeds of woody species are adapted for rapid germination, seedling formation, and survival under a forest canopy [57, 58]. Similarly, Senbeta and Teketay [3] reported that the lack of soil seed banks for woody plants affects the number of seedling populations on the forest floor.

4.2. Soil Seed Bank Species Richness and Density among Land Use Types. The results showed that both species richness and soil seed density were highest in shrubland land use types. This could be due to shrubland creating suitable conditions for the growth of many seeds under their canopies by increasing the moisture content of the soil [59] and encouraging plants to produce large numbers of viable seeds [60]. Similarly, previous work by the authors of [61, 62] has shown that shrubs have a significant impact on the movement of wind or water in an area and thus can trap seeds or act as a barrier to movement. Shrubs can also serve as perches for birds to transport and deposit seeds [63], resulting in increased seed deposition. In addition, by improving accessible soil conditions, shrublands can create favorable conditions for herbaceous plants under their canopy, which can facilitate the colonization and growth of herbaceous species [64, 65] and can increase seed production of these species. In addition, shrubs act as effective seed traps and can provide safe microsites for the germination and establishment of certain suppressed species (e.g., palatable species) by providing refuge in overgrazing areas [61]. Therefore, this study emphasizes the need to conserve and restore shrublands, which are crucial for sustaining biodiversity and ecosystem function. In contrast, bare land has lower soil species richness and seed bank density because there is no mature aboveground vegetation that can produce and deposit seeds in the soil. Additionally, bare land is susceptible to erosion, which can remove soil and seeds from the site [66]. Soil disturbances due to natural processes such as landslides or human activities can further reduce soil seed species richness and seed bank density by damaging the soil surface and removing seeds [67]. Our findings suggest that shrubland serves as a nursery, in comparison to other land use types, for maintaining the species richness and diversity of the soil seed bank.

4.3. Vertical Distribution of the Soil Seed Bank among Soil Layers. Our study found that viable soil seed density, species richness, and the Shannon diversity index decreased significantly with increasing soil depth, with the highest values observed in the layers. This could be due to several factors, including greater availability of nutrients, light, and moisture content, that promote seed germination in the upper soil layers [66, 68, 69]. Furthermore, the upper soil layers are dominantly covered with herbaceous species and hence have high diversity attributes (i.e., richness, diversity, and evenness) due to the better chances of recovery from the soil seed banks [23, 49].

Although the vertical distribution of soil seed banks varies by species, the overall pattern suggests that the seed density and seed richness of soil seed banks were highest in the top 3 cm of soil and gradually decreased with depth. The high seed density in the top layer of soil can be attributed to several factors, such as light availability, oxygen, temperature, and moisture [70]. These conditions promote successful germination and the establishment of seeds in the upper soil layers. This general trend toward higher seed bank density in the soil and higher species richness in the upper soil layers is commonly observed in our study. However, it is important to remember that species can vary because different plants have different seed characteristics and dispersal abilities. Some species may have adaptations that allow their seeds to survive and germinate at deeper soil depths, while others may have specific requirements for shallower soil depths [71]. In our study, some woody plants, such as Vernonia auriculifera, Croton macrostachyus, Entada abyssinica, Ficus sur, and Calpurnia aurea, sprouted from the 3-6 cm and 6-9 cm soil layers. This is associated with several adaptive strategies, such as the ability to sprout from dormant buds underground or long-lived seeds, which allow them to survive and thrive in different soil conditions.

4.4. Similarity in Species Composition among Land Use Types and Aboveground Vegetation. Despite differences in species composition in the soil seed bank, different land use types shared many common species, including Ageratum conyzoides, Anagallis arvensis, Cardamine trichocarpa, Centella asiatica, Cyperus brevifolius, Cyperus fischerianus, Digitaria abyssinica, Eleusine indica, Galinsoga parviflora, Laggera crispata, Oplismenus hirtellus, and Veronica javanica. The highest Jaccard similarity coefficient between forest and shrubland could be because both land use types have a similar number of common plant species and have similar ecological conditions, such as climate, soil type, and topography. The lowest Jaccard similarity coefficient was observed between shrubland and bare land use types due to variation in soil nutrient content and microclimate between these land use types [72-74].

Low similarities in species composition between the soil seed banks and the aboveground vegetation are associated with poor regeneration of woody species from soil seed bank samples. In this study, it was found that only 9.1% of woody species occur in both seed banks and aboveground vegetation. These findings suggest that the seeds of woody species are rapidly decomposed and heavily eaten by small mammals, leading to a significant deterioration in the composition of the seed bank in the soil [67, 75, 76]. Another possible reason for the different species compositions between soil seed banks and aboveground vegetation is that most woody plants produce seeds that sprout a few days or weeks after dispersal [75]. In addition, seedling emergence methods underestimate the density of the soil seed bank due to seed dormancy and the specific environmental conditions needed for germination [65, 77], which leads to a decrease in the density of the seed bank. This result is also consistent with the results of several studies conducted in Ethiopia [49, 53, 78].

Understanding soil seed banks is important for maintaining and repairing degraded ecosystems [29]. For example, seeds buried in the ground cannot fully regenerate the standing plant from anthropogenic or natural damage. This has significant management implications. Similarly, the Arjo-Diga Forest has been degraded as a result of charcoal production, animal grazing, expansion of agricultural land, and tree felling for housing. Consequently, most tree and shrub species have a minimal regenerative capacity [3]. Therefore, the soil seed bank would be essential to recover the previously existing vegetation that has been degraded.

4.5. Soil Seed Bank and Its Impact on Biodiversity Conservation. The presence of viable seeds in the soil is an important trait for making conservation measurements because it indicates the plant's ability to recover. Herb seedlings abound in the research area. Because herbaceous vegetation produces large number of long-lived seeds, it has a better chance of recovery [49, 53]. Few seedlings of woody species have been found in soil seed banks. This indicates that trees and shrubs have low regeneration potential, which could be related to various disturbances, such as humans, livestock grazing, and fires, in the study area. Therefore, planting native seedlings and preserving existing trees are crucial for tree recovery. This also applies to bare land, which has a low seedling density and a low regeneration potential.

#### **5. Conclusions**

The study examined soil seed banks in different land use types and their potential for natural plant regeneration. Herbaceous seedling density was high across all land use types due to small, light, and winged seeds. However, only a few woody species such as *Vernonia auriculifera*, *Croton macrostachyus*, *Entada abyssinica*, *Ficus sur*, and *Calpurnia aurea* seeds have emerged from the soil seed bank of shrubland and forestland use types. This is primarily due to the challenge of incorporating their larger seeds into the soil to form seed banks. Active restoration strategies, such as no-till practices and planting native species in nurseries, were recommended.

Shrubland had high species richness, diversity, and seed density due to its role as a bird perch, facilitating seed deposition. Conserving and restoring shrubland ecosystems were emphasized for biodiversity and ecosystem functioning. Bare land had low species richness and seed density, attributed to erosion susceptibility. Vegetation establishment was suggested to improve the soil seed bank.

Vertical distribution of seed banks varied with soil layers, influenced by seed properties and environmental conditions. Dissimilarities were found between aboveground vegetation and soil seed banks, indicating challenges in natural regeneration. Establishing nurseries and producing seedlings were recommended for sustainable management of native plant species.

In conclusion, the study highlights the importance of soil seed banks for natural plant regeneration. Active restoration, shrubland conservation, vegetation formation in bare areas, and nursery establishment were identified as effective strategies. Understanding seed bank dynamics and its relationship with aboveground vegetation is crucial for ecosystem management and biodiversity conservation.

#### **Data Availability**

The data supporting the findings of this study are available upon request from the corresponding author.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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#### **Supplementary Materials**

This section consists of list of identified soil seed bank species in Diga district, percentage frequency of soil seed bank species and their density across soil layers, mean ( $\pm$ SD) densities (m<sup>-2</sup>) of soil seed recovered from different land use types, similarity of the Jaccard coefficient in species composition between land cover types, and Jaccard's coefficient of similarity (JCS) among soil layers and between the soil seed bank and aboveground vegetation. (*Supplementary Materials*)

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