

Full Length Research Paper

Assessment of soil seed bank from six different vegetation types in Kakamega forest, Western Kenya

Jennifer N. Mukhongo^{1*}, J. I. Kinyamario¹, R. M. Chira¹ and W. Musila²

¹School of Biological Sciences, University of Nairobi, P.O Box 30197-00100, Nairobi, Kenya.

²National Museums of Kenya, P.O Box 40658-00100, Nairobi, Kenya.

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Kakamega forest, the only rainforest in Kenya, has faced extensive fragmentation and degradation over the last decades. Slow recovery of degraded areas is due to slow or no natural regeneration. An assessment was conducted to ascertain the contribution of soil seed bank in forest regeneration within six sites in Kakamega Forest. Sites investigated were the natural forest, plantation, shrubland, secondary grassland, natural glade, and burnt glade. Soil sampling was done from three stratified depths of 0 to 5, 5 to 10 and 10 to 15 cm in each site. Soil seed bank was determined by seedling emergence technique and total seed counts. Laboratory experiments on seed viability were done in a germination chamber at 20°C. Soil seed banks in all the six vegetation types were mainly dominated by herbaceous species. There was a high seed density in the upper layers for all the sites except for natural forest and burnt glade. Seed viability tests revealed low seed viability for the seeds from all the sites. It was concluded that natural regeneration is slowed by low woody species which ranged from 5.7 for natural glade to 48.4% for natural forest soil seed bank, and a low seed viability that ranged between 1.3 for plantation to 33.8% for grassland. It is therefore important to consider other ways of forest restoration other than the soil seed bank.

Key words: Seed bank, herbaceous species, seed viability, seedling emergence.

INTRODUCTION

Kakamega forest has been under severe anthropogenic pressure over the years, with its closed canopy cover declining progressively (Mitchell, 2004; KEFRI, 2006). The forest is highly fragmented and degraded due to overdependence of the adjacent local communities on the forest. Some agents resulting in degradation include selective logging, tree felling and overgrazing (Fashing et al., 2004; Mitchell, 2004). This resulted in the forest vegetation change to a mosaic different vegetation type (Althof, 2005). However, recovery of the degraded areas is slow which threatens the existence of this ecosystem (Mitchell, 2004). Several factors can delay or stop regeneration in degraded sites such as; low soil fertility, soil compaction, lack of seeds (due to lack of plants that

supply new seeds), depleted seed banks (Uhl and Jordan, 1984), unfavourable microclimatic conditions (Brown and Lugo, 1994; Guarigata et al., 1995), emergence of aggressive herbaceous growth and high seed and seedling predation (Nepstad et al., 1991).

A soil seed bank is a major initiator of regeneration in a natural forest (Abdella et al., 2007). The study on soil seed banks is very crucial in conservation because seeds on germinating form part of a future generation (Van der Valk et al., 1989). The aim of this study was to investigate the contribution of soil seed bank in facilitating natural regeneration in Kakamega forest. Specific objectives were; 1) to determine the soil seed bank composition of six different vegetation sites in Kakamega forest; 2) to determine the vertical distribution of the soil seed bank and 3) to determine the soil seed bank viability. The hypothesis for these objectives was: H_0 : Kakamega forest soil seed bank is diversely constituted, viable and is evenly distributed along vertical strata.

*Corresponding author. E-mail: jennifanafla@yahoo.com. Tel: 0722417866.

MATERIALS AND METHODS

Study area

Kakamega forest lies between latitudes 0°10' and 0°21' N and longitudes 34°47' and 34°58' E. It is located in Western Kenya at the altitude of 1500 to 1700 m (Mitchell, 2004). The forest is considered as the remnant of the lowland Congo Basin rainforests of Central Africa (Kokwaro, 1988). It was gazetted in 1933 with a total of 18,300 ha, out of which only about 10,000 ha is still remaining as an indigenous closed canopy forest.

The forest receives precipitation of 1960 mm per year, with an average temperature of 10.6°C (rainy season) and 27.7°C (dry season) (Farwig and Bohning-Gaese, 2008). Kakamega forest has high plant diversity with over 400 plant species, 112 trees, 62 shrubs, 58 climbers and 114 herbs, many of which are of Congolese lowland forest affinities (Kokwaro, 1988; Althof, 2005). Also, the forest exhibits a high degree of endemism and rarity in its fauna with an estimated 10 to 20% of the fauna endemic to this region (Mitchell, 2004). Kakamega forest soils are heterogeneous in nature, acidic, strongly weathered and low in minerals (Musila, 2007).

The study was conducted over a period of eight months. Six sites were identified for the study based on Lung (2004) classification. They were: natural forest - forest of lowest disturbance level and dense canopy older than 50 years; plantation forest - plantation of Cypress trees, *Cupressus lusitanica*, grown as monoculture up to 35 m; shrubland - bush areas less than 10 m in height, interspersed with grass and other herb plus very young secondary forest of less than 10 years; grassland - grass with single bushes or trees; natural glade - grass, partially of natural origin, used as meadows and roof thatching; burnt glade- this was part of the natural glade that had been burnt down one month to sampling. The parameters investigated included; amounts of soil seed bank, seedling emergence rates, plant species and seed viability.

Soil sampling

Soil samples were collected between the months of April and May 2008. Three transects of 100 m were established in each sites. Along each transect, three quadrats of 5 × 5 m were systematically established at equal distances from each other. A vegetation survey was done on life forms in all sampled quadrats prior to soil sampling. Each quadrat was further sub-divided into 25 sub-quadrats of 1×1 m from which randomly selected 10 sub-quadrats were sampled. Soil samples were taken from the centre of each sub-quadrat at three different depths of 0 to 5, 5 to 10 and 10 to 15 cm, using a 20 × 20 × 5 cm metal cube. The metal cube was driven into the ground using a mallet and a small trowel was used to scoop the soil from the metal cube. Soil samples were then put in labeled plastic paper bags and transported to a greenhouse for soil seed bank analysis.

Soil seed bank analysis

A total of 1620 soil samples were collected from the six sites, with 270 samples from each site. Samples were classified into odd or even numbers based on the quadrat from which they were collected. The odd numbered samples were weighed to get a weight of 1 kg, which was germinated in germination trays for seedling emergence in a greenhouse. Roots, tubers, and bulbs were picked and discarded to avoid any growth from sprouts. Germination trays bottoms were perforated to prevent water logging. Germinated seedlings were counted and identified from two weeks onwards. Identification to species level was done in collaboration with the National Museums of Kenya, while those not identified in the field were taken to the East African Herbarium for

identification.

Even numbered samples were dried in the greenhouse within a temperature range of 15 to 30°C. The dried samples were sieved using a 2 mm mesh sieve and re-weighed for dry weights. Coarse soil samples that remained on the sieve were screened for seeds and the seeds were hand picked and used in carrying out seed viability tests. The fine soil samples were re-weighed and representative sub-samples of 30 g were taken for screening, using a dissecting microscope to check for seeds that could not be picked using the naked eye. Each sample was spread on a Petri-dish and seeds were identified using a microscope at ×1000 magnification. The number of observed seeds was counted using a counter.

Seed viability tests

Seed viability tests were done using the procedures of the International Seed Testing Association (ISTA) (Steve et al., 2007). Seeds that had been hand picked from the coarse samples were soaked in warm water (40°C) for 15 min to break seed dormancy. They were later germinated in a 1% agar in a Petri-dish inside a germination chamber at room temperature (20°C). Seeds picked from a single sample were all germinated in one Petri-dish. Germination experiments went on for 14 days and scoring was done every two days. After the 14 days, the non-germinated seeds were subjected to another treatment of temperature rise in an oven with a constant temperature of 25°C for another 7 days. Raising of the temperature was to further break any seed dormancy. Scoring was done for the 7 days and seeds that did not germinate by then were treated as unviable.

Statistical analysis

Data on seedling emergence, screened seeds, percentage viability and life forms (from both the vegetation survey, screened seeds and emerging seedlings) were tested for normality prior to any statistical test using the Kolmogorov-Smirnov test. Variables that did not conform to normal distribution were logarithmically [$X' = \log(X+1)$] or Arcsin transformed before applying parametric analysis tests. All statistical analyses were done using STATISTICA (2004 Edition; Version 7.0 statSoft Inc.; Tulsa; USA). Variables of different sites were compared using analysis of variance (ANOVA) and post-hoc Scheffe test of significance, which indicates the actual differences. All the statistical analyses were evaluated at the $p < 0.05$ level of significance.

RESULTS

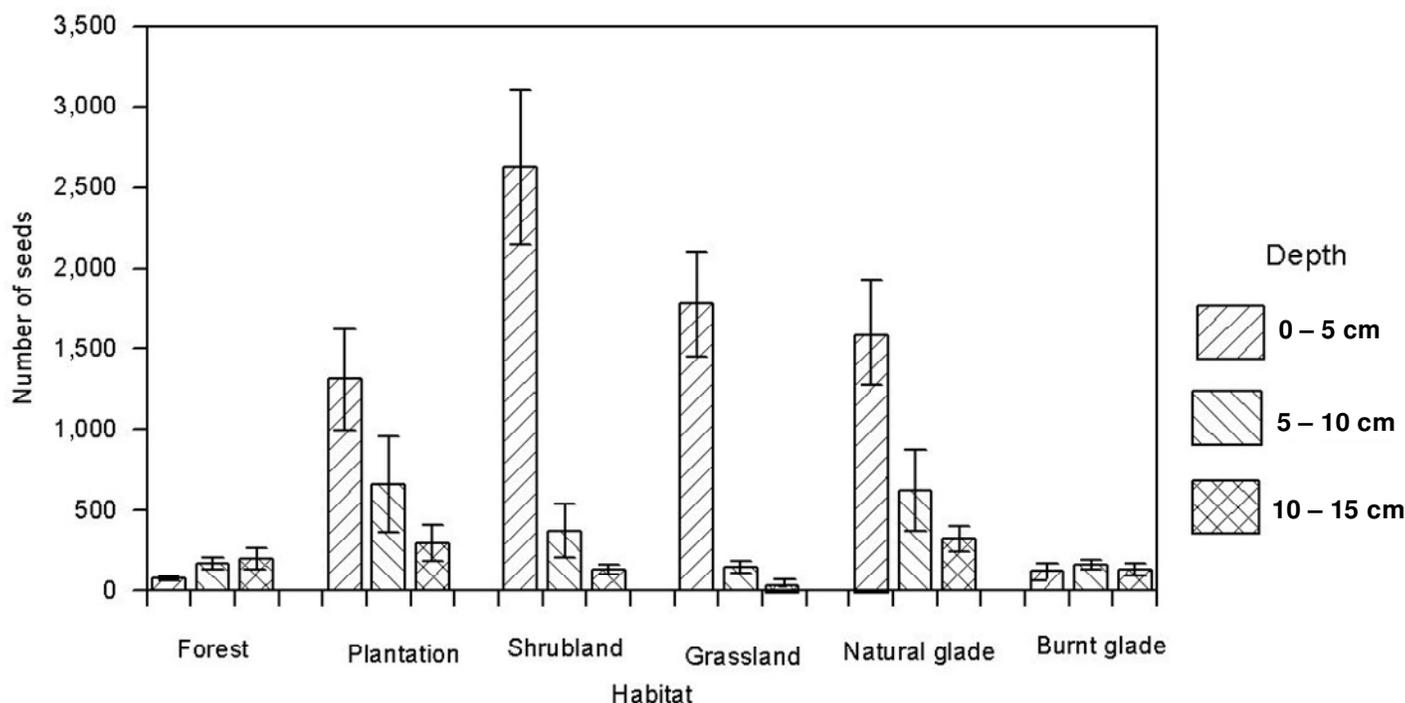
Soil seed bank stratification and species composition from the six sites

A total of 15,676 seedlings emerged from the six sites, with grassland having the highest number of seedlings, followed by shrubland, plantation, natural glade and burnt glade, while natural forest had the least number (Table 1). There was significant difference (ANOVA: $F_{2, 5} = 6212.4$, $P < 0.05$) in emerging seedlings from the three stratified sampling depth categories for all the six sites, with most seedlings observed in the upper soil depth (0 to 5 cm). However, Scheffe test did not reveal any difference among the three depths of the various sites ($P > 0.05$).

There was a decline in seed density with depth in all

Table 1. Summary on seedling emergence distribution in the three stratified depths from the six sites.

Site	Soil depth (cm)			Grand Total
	0 to 5	5 to 10	10 to 15	
Forest	159	149	96	404
Plantation	843	450	257	1550
Shrubland	2579	1399	1053	5031
Grassland	3120	1879	1358	6357
Natural glade	568	370	255	1193
Burnt glade	547	331	263	1141
Grand Total	7816	4578	3282	15676

**Figure 1.** Number of seeds for each depth per sites from the screening of the six sites (n = 810).

sites, except from burnt glade and natural forest (Figure 1). Secondary grassland sites recorded the highest seed density in 0 to 5 cm depth, and numbers reduced with depth. A further post-hoc Scheffe test revealed no significant difference ($P > 0.05$) in seed numbers with depths.

A comparison of total seed count and seedling emergence revealed a significant difference ($P < 0.05$). Out of the 15,676 emerged seedlings, 15,060 were from 43 distinct taxa (species and genera). The remaining 616 were too small in size, died before identification and hence were classified as unidentified (Table 2). The vegetation survey (Table 3a), revealed significant difference (ANOVA $F_{2, 5} = 17.32$ $P < 0.05$), in life forms from the soil seed bank life forms (Table 3b), for the six

sites. However, a further Scheffe test did not detect any difference in life forms between standing vegetation and soil seed bank ($P > 0.05$).

Seed viability

There was a significant difference (one sample t-test $t = 3.43$ $P < 0.05$) in percentage seed germination for the six sites. Seed viability was highest in the grassland and lowest in the plantation (Figure 2). Burnt glade germination percentage was higher than that of natural glade. Soil seed bank viability ranged between 1.3 for plantation to 33.8% for grassland. Temperature increase from 20 to 25°C resulted in a germination increase of 5.2

Table 2. Summary of individual species numbers found in each of the six habitats.

Species	Author	Site					
		Forest	Plantation	Shrubland	Grassland	Natural glade	Burnt glade
<i>Acacia abyssinica</i>	Benth.spp. <i>calophylla</i> Brena	-	3	12	-	19	13
<i>Acalypha racemosa</i>	Baill. (A. Paniculata miq)	-	14	-	-	-	-
<i>Achyranthes aspera</i>	L. Devil's Horse whip	-	11	-	-	8	73
<i>Biophytum pertersianum</i>	Klotzsch	-	-	-	-	8	22
<i>Celtis africana</i>	Burm.f.	2	-	-	-	-	-
<i>Celtis durandii</i>	Bak.	4	-	-	-	-	-
<i>Chamaescrista hildebrandtii</i>	(Vatke) Lock (Cassia hildebrandtii Vatke	-	-	26	-	2	1
<i>Commelina benghalensis</i>	L.	-	274	-	-	94	-
<i>Commelina species</i>	L.	-	-	-	3	4	3
<i>Conyza sumatrensis</i>	(Retz.) E.H. Walker (c.floribunda H.B.K., Erigeronfloribundum (H.B.K) SCH.Bip.)	287	-	25	1	11	2
<i>Cupressus lusitanica</i>		-	4	-	-	-	-
<i>Cyperus species</i>	L.	-	-	-	-	1	-
<i>Desmodium adscendens</i>	(sw.) DC.	-	32	-	-	6	-
<i>Digitaria abyssinica</i>	Stapf	1	1	36	115	29	51
<i>Dissotis senegalensis</i>	(Guill. & Perr.) Triana	-	-	-	-	17	-
<i>Dorstenia brownii</i>	Rendle	-	47	5	7	5	-
<i>Ficus species</i>	L.	4	-	-	-	-	-
<i>Ficus sur</i>	Forssk.	2	4	-	-	-	-
<i>Fimbristylis dichotoma</i>	Vahl	6	35	2032	747	698	350
<i>Funtumia africana</i>	(Benth.) Stapf	1	-	-	-	-	-
<i>Galinsoga parviflora</i>	Cav.	-	-	4	773	4	-
<i>Glycine wightii</i>	(Wight & Arn.) Verdc	-	1	6	6	1	2
<i>Harungana madagascariensis</i>	Poir.	-	11	-	-	-	-
<i>Hydrocotyle mannii</i>	Hook.f.	1	134	2088	80	82	399
<i>Justicia flava</i>	Vahl	-	2	-	10	-	-
<i>Kyllinga alba</i>	Nees	-	-	145	197	17	9
<i>Lantana camara</i>	L.	6	443	7	10	-	-
<i>Leonotis nepetaefolia</i>	(L.) Ait.f. (inc. L. AFRICA (P.Beauv.)Briq.)	-	14	269	3748	44	172
<i>Maesa lanceolata</i>	Forssk.	-	3	-	-	-	-
<i>Oxalis corniculata</i>	L. (O. radicata A. Rich.)	-	5	19	3	1	5
<i>Phyllanthus acidus</i>	L.	1	42	175	228	102	14
<i>Phyllanthus fischeri</i>	Pax	-	3	-	-	-	-
<i>Plectranthus species.</i>	H.	1	-	-	-	-	-
<i>Polyscias fulva</i>	(Hiern) Harms (P.ferruginea (Hiern)Harms)	1	-	-	-	-	-

Table 2. Continue.

<i>Polystachya</i>	Hook	58	120	41	214	8	4
<i>Prunus africana</i>	(Hook.f.) kalkm.	-	3	-	-	-	-
<i>Psidium guajava</i>	L.	-	19	-	-	10	-
<i>Trema orientalis</i>	(L.) Bl.	32	169	12	-	-	3
<i>Urera lobota</i>	L.	-	30	26	39	-	2
<i>Zanthoxylum gillettii</i>	(De wild.) Waterm.	2	-	-	-	-	-
Unidentified		9	127	241	-	194	45

for grassland, 2.3 for forest, 0.7 for shrubland and 0.2% for plantation. However, seeds from natural and burnt glade did not respond positively to temperature increase.

DISCUSSION

Species composition and seed viability across the six sites

There was little similarity in life form composition between the vegetation survey and the soil seed bank for the six vegetation sites. The vegetation survey recorded a high percentage of trees from all the six sampled sites as compared to seedling emergence. This is in agreement with the studies by Thomas (2000) and Valbuena et al. (2001), who found little similarity between soil seed bank species and above ground vegetation. However, there are exceptions as found mainly in annual-dominated communities that often contain early succession species, but do not represent late or dominant succession species (Robert, 2000). This difference may be due to seed dispersal mechanisms, seed burial and predation or even decomposition. The soil seed banks of various sites were generally dominated by the herbaceous species, making the soil seeds less reliable for natural regeneration of the forest. This could be

attributed to easy dispersal ability of herbaceous species by various dispersal agents such as wind and water because of their light weight. Given that many herbs and shrubs have small-sized seeds, they tend to produce seeds in large quantities and end up dominating the soil seed bank (Obiri et al., 2005).

These results concur with Liu et al. (2000) and Oke et al. (2006), whose findings indicated that herbaceous species dominated the soil seed banks, while only a few tree species were capable of accumulating long-lived seeds in the soil. Most of the tree species seeds are large, hence more vulnerable to predation than small seeds (Obiri et al., 2005). Degraded natural forest sites are mostly invaded by non-tree plants such as ferns, vines, grasses and shrub at the expense of tree species, which may take decades to recruit (Bernhardt and Ulbel, 2000). This implies that herbaceous species have better chances of recovery than tree species from the soil seed banks in the event of disturbance (Bernhardt and Ulbel, 2000). This also concurs with results of Bernhardt and Ulbel (2000) and Abdella et al. (2007), who found that seed bank density and dominance by weeds increases with continuous farming.

The small numbers of graminoids in secondary grassland could be attributed to the fact that their seeds, which are small in size, accumulated in the

0 to 5 cm soil depth and therefore most of them may have germinated by the time sampling was done. These results concur with Markus et al. (2002), whose findings revealed that grass seeds occur in the 0 to 3 cm depth and therefore easily germinated when conditions are favourable. Most of the non-grass herbs in this site were garden weeds which could have been brought by the Shamba system practices in the early nineties (Kokwaro, 1988). Shrubland soil seed bank had large percentage of graminoid seeds. However, our vegetation survey revealed high number of shrubs and trees species in this sites (Table 3). This indicates that with reduction of anthropogenic impacts, this site is likely to regenerate naturally. The burnt glade soil seed bank was dominated by other non-graminoid herbaceous species seeds unlike the natural glade. This could be attributed to suppression of the graminoids by the fire, prompting other herbaceous species to dominate. This in essence implies that the effects of burning can alter the composition of species; this practice could be useful in encouraging vegetation diversity. Burnt glade high seed viability could be attributed to the fact that burning contributed to breaking seed dormancy hence enhancing seed viability.

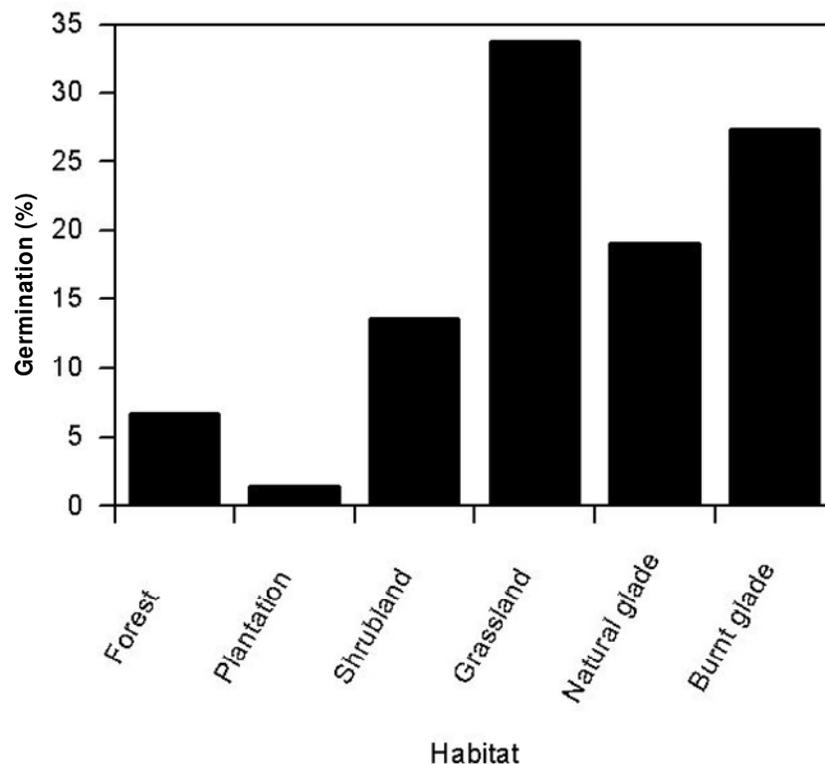
Natural forest soil seed bank was dominated by seeds of herbaceous species, contrary to the findings of Leckie et al., (2000), which revealed

Table 3a. Life form percentages of standing vegetation in sampled sites for the six sites.

Life form	Grassland (%)	Shrubland (%)	Burnt glade (%)	Natural glade (%)	Plantation (%)	Forest (%)
Climber	1.0	3.0	5.6	4.3	13.8	15.1
Fern	1.0	0	0.3	0	2.4	1.4
Grass	40.1	30.3	22.1	14.6	2.4	0.7
Other herbs	35.4	38.4	50.9	59.4	19.2	10.9
Orchid	0	0	0	0	0.3	0
Shrub	8.3	16.2	12.4	16.0	23.0	23.5
Tree	14.1	12.1	8.8	5.7	38.8	48.4

Table 3b. Life form percentages of seed emergence in sampled sites for six sites.

Life form	Grassland (%)	Shrubland (%)	Burnt glade (%)	Natural glade (%)	Plantation (%)	Forest (%)
climbers	0.1	0.1	0.2	0.1	0.1	0
graminoids	19.4	43.9	35.9	55.8	4.4	1.6
other herb	14.4	41.6	42.7	17	33.3	69.4
shrubs	66.1	9.3	16	11.1	40.1	15.8
trees	0	0.5	1.3	2.1	13.9	11
Unidentified	0	4.6	3.8	14	8.2	2.2

**Figure 2.** Seed viability (%) for the six sites.

forest seed bank was dominated by seeds of woody species. However, studies by Znikov (1983) revealed that while the number of herbaceous species remained relatively constant in different years, the number of

woody species fluctuated widely. This could be caused by impaired light penetration due to tree canopy cover. The plantation soil seed bank was dominated by shrubs, of which a greater proportion was the invasive species

Lantana camara. These could have set in after logging of the primary forest, just before the re-planting with *C. lusitanica*. This might have been contributed to by seed dispersal and light penetration due to canopy gaps, which provided favourable conditions for colonization. It was generally found that the seeds from all the sites had a viability that ranged from 1.3 to 33.8%. Therefore, viable soil seed bank and the above ground vegetation composition variations could be attributed to low seed viability.

Spatial distribution of seed density in soil stratum

There was variation in seed density for total seed count and seedling emergence experiments. The variation was attributed to seed viability of each site; seedling emergence experiment only took into account viable seeds while the total count incorporated both viable and unviable seeds. There was a clear trend of number of seeds decreasing in density with depth from the secondary grassland, natural glade, plantation, and shrubland. This is consistent with studies by Sandrine (2006) and Abdella et al. (2007), who found that seed density decreased with depth. High seed density in the 0 to 5 cm depth profile is attributable to organic matter accumulation on the soil surface of the sites. Putwain and Gillham (1990) emphasized that a high percentage (about 96%) of seed banks of heather and other sedge species was from the top 50 mm layer and was due to high litter accumulation.

The low seed density in the burnt glade in 0 to 5 cm depth could be attributed to destruction of above ground matter by the fire. The trend in seed density within mid-depths (5 to 10 cm) and lower depths (10 to 15 cm) followed the same trend as in the other sites because the fire could not penetrate deep into the soil. Natural forest recorded the highest number of seeds in the 10 to 15 cm depth followed by 5 to 10 cm depth, but was lower in the 0 to 5 cm depth. This could be attributed to seasonality because at the time of sampling, the forest floor was observed to have a lot of seedlings which might have germinated from within the 0 to 5 cm depth. Fewer seeds found within the 5 to 10 cm depth as compared to 10 to 15 cm depth could be an indication that there was some secondary seed dispersion taking place in the soil either through below ground organisms or by spatial variability of percolating soil water, though this need further investigation. This could also be as a result of impaired seed germination due to increased depth; hence seeds accumulated in lower soil depths instead of germinating (Thomas, 2000; Gutterman et al., 2007). Increased soil depths may impair seed germination by altering moisture, air, light and temperature, hence making seeds to remain in a state of dormancy for long (Thomas, 2000; Gutterman et al., 2007). In addition, Abdella et al. (2007) demonstrated that average depth of seeds in the soil indicated their distribution and longevity in the soil. The

distribution of seeds in various soil depths is also attributable to several biotic factors such as the activity of ants, mole rats and other soil micro-fauna (Forget et al., 1998).

Conclusion

Soil seed banks for the six sites were generally depleted to an extent that such sites like the burnt glade and natural glade, could not effectively support seedling germination and establishment adequately. However, with a reduction in anthropogenic activities in the shrubland, this site is likely to regenerate naturally. Seed depth stratification in the various sites decreased with depth, with more seeds found in the top layer. Deeper layers had the least number of seeds in all the sites with an exception of the natural forest and the burnt glade. The difference in seed viability percentage and species composition between the natural glade and burnt glade imply that burning could enhance forest regeneration. There was variation between the above ground vegetation life form and the soil seed bank life forms in the six sites. From the results therefore, it can be concluded that soil seed bank of Kakamega forest is depleted and of low viability, and is therefore not adequately reliable for natural regeneration of the degraded forest areas. Human intervention is hence needed to accelerate the rate of forest recovery through seedling planting and alleviation of anthropogenic impacts in the forest.

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