Scientific Research Publishing

Open Journal of Ecology, 2023, 13, 435-453
https://www.scirp.org/journal/oje
ISSN Online: 2162-1993
ISSN Print: 2162-1985

# Soil Seed Bank Characteristics of the Mbe and Nguela Shrub Savannas, and Implications for the Reforestation, Republic of Congo

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**How to cite this paper:** Douh, C., Moussoumbou, C., Mabengo, B.C., Malonga, L.M., Nsongola, G., Miafouna, T., Mahoua, A.J., Ndzaï, S.F. and Koubouana, F. (2023) Soil Seed Bank Characteristics of the Mbe and Nguela Shrub Savannas, and Implications for the Reforestation, Republic of Congo. *Open Journal of Ecology*, **13**, 435-453.
https://doi.org/10.4236/oje.2023.137027

**Received:** April 4, 2023
**Accepted:** July 7, 2023
**Published:** July 10, 2023



## Abstract

The soil seed bank is an important source of restoration and resilience of disturbed ecosystems. This study evaluates the regeneration potential through the soil seed bank of the shrub savannas of Nguela and Mbe in order to predict the eventual dynamics. Three plots of 0.25 ha subdivided into four sub-plots of 0.015 ha have been installed in each savannah. In total, 48 samples of each savannah, *i.e.* 96 samples of both savannas, have been taken from the soil layers, 0 - 5 cm, 5 - 10 cm, 10 - 15 cm and 15 - 20 cm. Species diversity and abundance of the soil seed bank have been assessed after germination. The results reveal 167 seedlings belonging to 23 species in the Mbe savannah and 144 seedlings belonging to 14 species in the Nguela savannah. The total densities of the germinated seeds were respectively 463.63 seeds/m$^2$ and 400 seeds/m$^2$. Nevertheless, the 20 cm deep layers have illustrated themselves compared to the superficial layers with densities of 16.29 seeds/m$^2$ and 21.66 seeds/m$^2$, respectively, in the savannas of Mbe and Nguela. Herbaceous species largely dominated, with percentages of 91% and 100%, respectively, in the savannas of Mbe and Nguela. Alone, the *Trema orientalis* (L.) Blume species has been identified as woody species in the Mbe savannah. The greatest specific richness has been obtained in the first five centimeters of soil, with 21.73% and 28.57% of exclusive species, respectively, in the savannas of Mbe and Nguela. The results reveal that restoration through the soil seed bank would be limited to a single woody species found (*T. orientalis*). Consequently,

the study suggests silvicultural interventions based on planting or enrichment techniques for sustainably managed savannas exposed to anthropogenic disturbances.

## Keywords

Soil Seed Bank, Shrub Savannas, Restoration, Anthropogenic Disturbances, Republic of Congo

## 1. Introduction

Savannas constitute one of the largest biomes of the world, with about 20% of the earth's surface [1]. They cover about 65% in Africa, 60% in Australia and 45% in South America [2]. In Central Africa, the savannas are heterogeneous and occupy an area estimated at 154.4 million hectares [3].

The relative questions about savannas have aroused various scientific works which evoke the anthropogenic origin of the savannas in African regions [4]. Farmers have cleared the forest to cultivate and feed themselves, and the frequent practice of the culture in the same spaces would have led to a process of savanization [5]. Nonetheless, some works on the soil seed bank in African savannah areas have been carried out [6] [7] [8]. The results of this work reveal an average density estimated at 21.000 seeds/$m^2$. Furthermore, other authors in eastern Ethiopia have demonstrated a density of 14.2 seeds/$m^2$ [9]. In the Republic of Congo, some works on the floristic composition of the savannas have been carried out in the western part and in the Congolese basin [10] [11] [12]. Other works have been carried out in the Niari valley, in the Teke plateaus and on the Atlantic coast [13] [14] [15] [16] [17].

To our knowledge, studies on the dynamics of regeneration (process at the base of the reconstitution) integrate sparsely the savannah zones and, even less, the "soil seed bank" component, which constitutes a reservoir of seeds and reflects the floristic composition of the vegetation from a place at a given time [18].

The soil seed bank is a natural solution for the conservation of biodiversity in disturbed environments [19]. In addition, it is considered as an important mechanism for the natural regeneration of ecosystems in the process of ecological succession of wooded ecosystems [20] [21] [22] [23] [24]. It generally encompasses all viable seeds buried in the soil, as well as those found on the surface, including in the litter and humus [23]. Also, the ability of plants to persist at the state of seeds in the savannah soil bank is a less well-documented research section in Central Africa, and even less in the Republic of Congo. True seed reservoirs of the plant communities and the soil seed bank can be considered as a potential for regeneration and an indicator of resilience facing disturbances [25] [26] [27].

Knowledge of the density and the diversity of the savannah soil seed bank

could provide significant information on the history of savannah disturbances, but also and above all, on the potential resilience of these ones facing disturbances [28].

In addition, in the Republic of Congo, studies dedicated to the germination of the savannah soil seed bank, the floristic composition and the density of the seeds stored in the soil layers of these ones yet remain unknown or even unexplored. Consequently, their inclusion is, therefore, necessary in order to better characterize the soil seed bank of the savannas in the Republic of Congo.

Insofar as it is currently difficult to predict the regeneration potential and resilience capacity of savannas across the soil seed bank, the present study aims to evaluate the natural regeneration potential through the soil seed bank of the two geographically distinct savannas in order to understand the dynamics of floristic potential. To achieve this, the following research hypotheses are formulated: 1) the depth of sampling of the soil samples significantly influences the germination of seeds in the two savannas studied; 2) the floristic composition and the density of the soil seed bank differ considerably from one savannah to another; 3) the biological diversity of the soil seed bank varies in terms of the savannas and the layers of soil sampled.

## 2. Material and Methods

### 2.1. Study Sites and Soil Sampling

The study was carried out in two shrub savannas, namely: the savannah of Nguela, close to the locality of Nguela, which is called in the manuscript by "NG", and the savannah of Mbe, close to the locality of Mbe, which is also called in the manuscript by "MB". Geographical coordinates of the two savannahs are respectively, S04˚336'42" and 014˚87'474"E, S03˚18'14" and 15˚53'51"E. The Nguela savannah "NG" is located in the sub-prefecture of Kinkala (Pool Department) close to the tourist site called "Trou de Dieu" at 11 km from Kinkala and 60 km from Brazzaville (**Figure 1**). However, the Mbe savannah "MB" is located to more than 150 km from Brazzaville in the northern part of Pool Department (**Figure 1**). The Nguela savannah "NG" is subject to the Sudano-Guinean type climate [4] [29] [30]. Average annual temperatures oscillate around 25˚C. The annual rainfall varies from 1200 to 1400 mm/year and undergoes great variations according to the years [31]. On the other hand, the climate of the Mbe savannah is of the Guinean sub-equatorial type, and is characterized by rainfall ranging from 1600 to 2100 mm/year [15]. Average annual temperatures are between 23˚C and 25˚C. The soils of the Nguela savannah "NG" generally belong to the category of ferralitic soils. Overall, it's about the Inkisi sandstones and the polymorphic sands [32]. However, the soils of the Mbe "MB" savannah are generally sandy to sandy clay [15].

Nonetheless, the vegetation of the two shrub savannas (Nguela and Mbe) is dominated by species such as: *Bridelia ferruginea* Benth., *Hymenocardia acida* Tul., *Annona arenaria* Thonn.

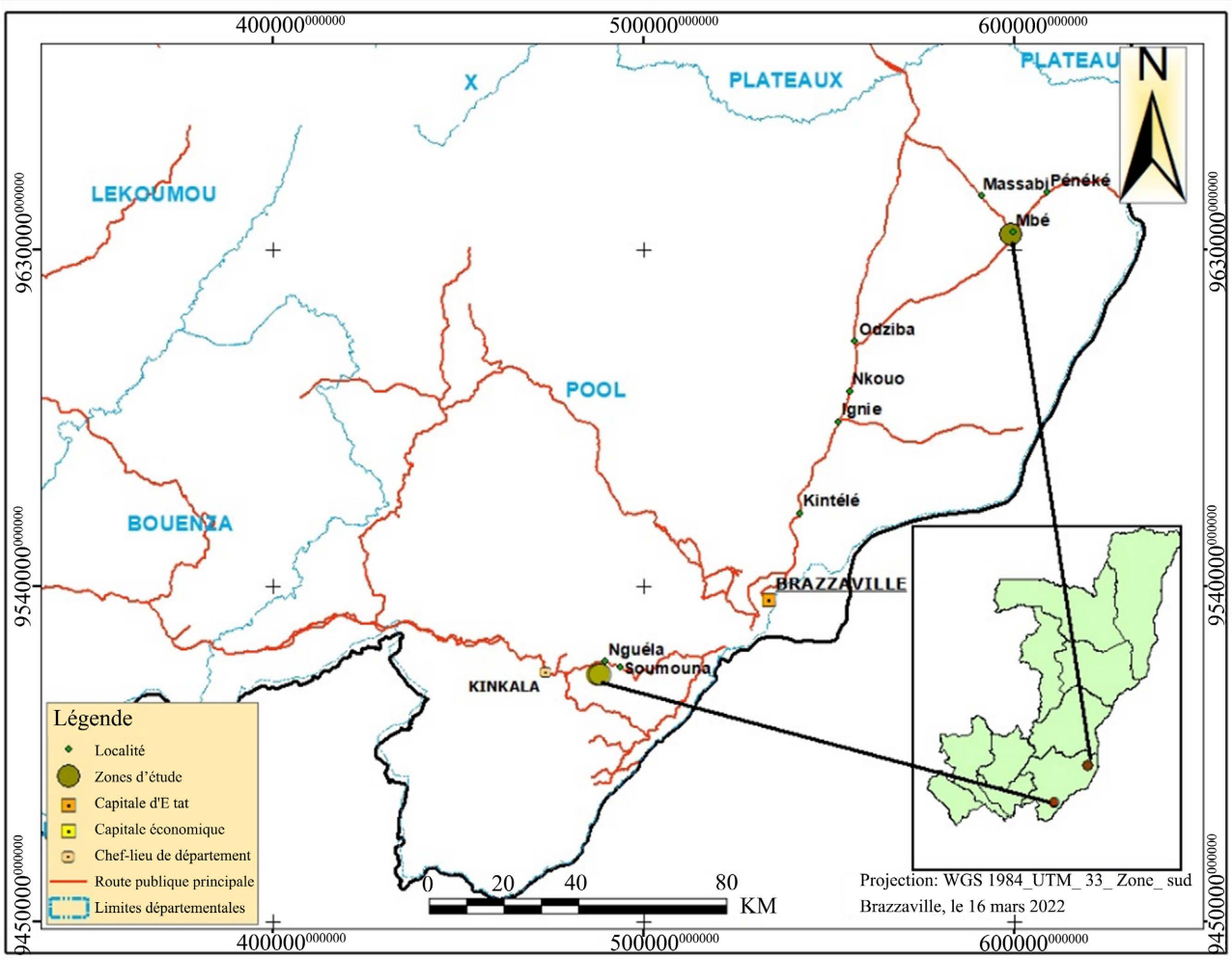


**Figure 1.** Location of the Nguela "NG" and Mbe "MB" savannas.

Soil samples were collected in January and March 2021, respectively for the shrub savannas of Nguela "NG" and Mbe "MB". On each of the two savannas, the soil samples were collected in three non-contiguous plots of 25 m × 25 m, installed by a double decametre following the orientations of the compass (North-South and East-West). Each plot has been divided into four sub-plot of 12.5 m × 12.5 m, *i.e.* an area of 156.25 $m^2$ inside which soil samples were collected. The soil has been collected using a parallelepipedic box in woode measuring 10 cm long × 10 cm wide × 5 cm deep. Twelve collection points per savannah were established in the center of each sub-plot. Four superimposed soil layers were collected per point: 0 - 5 cm, 5 - 10 cm, 10 – 15 cm and 15 - 20 cm. Each sample was composite, that is to say from of mixture of three unit taking realized on the summits of an equilateral triangle of 1 m side, *i.e.* a volume per layer and per point of 1.500 $cm^3$ [27] [33] [34]. The three units taking from each sampling point were putting in a plastic bag, which contained informations: type of plot and sub-plot, number of the collection point and the level of taking of the soil layer corresponding either to the savannah of Nguela "NG" and/or savannah of Mbe "MB". A total volume of 36,000 $cm^3$ has been obtained for the two sa-

vannas (NG and MB), *i.e.* 2 × 18,000 $cm^3$ per savannah, over a total sampling area of 0.72 $m^2$ (2 × 0.36 $m^2$ per savannah). Three weeks after collecting the soil samples, they were sented to Brazzaville and more precisely to the departmental nursery of SNR (Service National de Reboisement) for germination tests.

## 2.2. Estimation of Viable Seeds

The number of viable seeds in the soil samples has been estimated by the germination method [27] [35] [36]. The soil samples have been sieved with a 2 mm mesh size item in order to eliminate crude pieces of plants and coarse mineral elements (e.g. leaves, pieces of wood and stones). A total of 2 blank trays (containing only sterilized substrate) per savannah have been used to verify eventual contamination of the soil samples; no germination was noted from the blank samples at the end of the observation period. The germination was carried out in a nursery at the SNR (Service National de Reboisement) site to Brazzaville in a nonforest zone in order to prevent contamination of the soil samples by seeds of the surrounding vegetation. The shadehouse's relative light intensity was about 30% - 40% of full sunlight. Each soil sample was spread out over a sterilized (by heat) sand substrate in a way that the average depth of the sample should not exceed 1 cm [20] [27]. Watering was almost daily. The monitoring of the germination has been performed five times a week (every 4 - 5 days). When their development stage allowed it, the seedlings were removed of each blank and transplanted individually in polyethylene bags in order to favour their growth and identification. As all the germinations took place over the course of the first 12 weeks, the experiment was stopped after 16 weeks (*i.e.* 4 months of experimentation). Voucher specimens were collected and species were identified by botanists (Gilbert Nsongola, Isaac Dzombo). We followed the taxonomy of the Geneva Herbaria Catalogue (http://www.ville-ge.ch/musinfo/bd/cjb/chg).

## 2.3. Data Analysis

To assess the effect of soil samples collection depth on seeds germination, the Generalized Linear Model (GLM) in terms of the explanatory variable "soil collection depth", followed by a classification according to Student-Newman-Keurls (SNK) between the average number of germinated seeds per soil layer within the two savannas (NG and MB) were realized using Statistical Package for the Social Sciences (SPSS) version 22.0 software [37]. To identify the characteristics of the species observed in the soil seed bank, we classified species in terms trees, shrubs, herbaceous and undetermined. For describe the abundance of seeds of two savannas (NG and MB), the following parameters were used: the absolute density *AD* (seeds/$m^2$), the relative density *RD* (%, number of seeds of a given species/the total number of seeds for all species), the relative frequency *RF* (%, proportion of samples containing the given species) and the species Importance Value Index (*IVI*) computed as the sum of *RD* and *RF* [24] [34] [38] [39] [40]. For identify the indicator species of each savannah from the seedlings of the soil bank, we computed the "Indicator Value Index" (*INDVAL*), using the

labdsv package implemented in the R environment [41]. Significance was set at $p < 0.05$. This index is defined as follows [42]:

$$INDVAL = A_{ij} \times B_{ij} \times 100$$
$$A_{ij} = N_{individuals\ ij} / N_{individuals\ i} \quad (1)$$
$$B_{ij} = N_{sites\ ij} / N_{sites\ j}$$

$INDVAL$ = the Indicator Value of species in site group $j$;

$A_{ij}$, is a measure of specificity (based on the abundance of species $i$);

$N_{individuals\ ij}$, is the mean number of individuals of species $i$ in the sites of group $j$;

$N_{individuals\ i}$, is the number of individuals of species $i$ in all groups;

$B_{ij}$, is a measure of fidelity (based on incidence of species $i$);

$N_{sites\ ij}$, is the number of sites in the group $j$ where species $i$ is present;

$N_{sites\ j}$, is the total number of sites in that group. Here, there were two groups constituted by the two savannah, NG and MB. The undetermined species were excluded from the computation.

In terms of species diversity, we first computed the observed species richness, *Sobs*. But as *Sobs* is very dependent on the sampling effort and is considered as an unreliable indicator of the total species richness [43], we also computed estimated species richness using two of the most recommended estimators: the bias-corrected Chao2, *Schao*2 (based on incidence) and Jackknife1, *Sjack*1 (based on abundance) [44] [45] [46] [47], using the program EstimateS 9.1.0 [48]. Finally, the specificity of layers in terms of species found exclusively in each layer was determined by comparing these "exclusive" species with the total number of species found in the layers (*%Sexcl*).

Rarefaction curves were derived from the observed and estimated species richness to evaluate the representativeness of our sampling effort. Finally, within both savannas, viable seeds density per soil layer was computed cumulating the data of each point per soil layer. The differences of mean seed densities between the two savannas have been tested using a parametric student's t-test at the 5% threshold, the normality of the data having been verified by the Shapiro-Wilks and Kolmogorov-Smirnov tests [37] [49]. Nonetheless, for compare the floristic composition of soil seed bank between the two savannas (NG and MB), we performed a Nonmetric Multidimensional Scaling (NMDS) based on seeds abundance data (12 collection points per savannah). NMDS, applied with the R package MASS [50], makes no assumptions about the data [51], and is considered among suitable methods for graphical representation of floristic ordination [52] [53]. The undetermined species were not taken into account in the ordination. The samples that did not provide any germination were also excluded from the analysis.

Shannon's (H') and Pielou's Equitability (E) indices were respectively used to evaluate the specific diversity and the equitable distribution of individuals from the soil seed bank between the two savannas and the soil layers [54] [55]. In ad-

dition, to study the variations of diversity indices between different soil layers, we used the nonparametric Kruskall-Wallis test with post-hoc pairwise multiple comparisons between the means for a probability value of p-value < 0.05.

## 3. Results

### 3.1. Influence of Soil Sampling Depth on the Seeds Germination of the Mbe (MB) and Nguela (NG) Savannas

Table 1 demonstrates that the explanatory variable "soil sampling depth" did not have a significant effect (p-value = 0.694) on the germination at the level soil layers of the Mbe (MB) savannah. Nonetheless, the most important average was found in the 0 - 5 cm layer (3.25 ± 3.08) followed by 15 - 20 cm (2.56 ± 2.30), 5 - 10 cm (2.37 ± 1.85) and finally 10 - 15 cm layers cm (2.31 ± 2.46) (Table 1). Also, the act of exploring the deeper soil layers did not significantly influence the germination of the Nguela (NG) savannah soil seed bank (p-value = 0.662) (Table 2). The most important average was found in the 5 - 10 cm (2.561 ± 1.71) layer, followed by 15 - 20 cm (3.44 ± 1.75), 10 - 15 cm (2.19 ± 1.68) and finally 0 - 5 cm (1.88 ± 1.50) (Table 2).

Table 1. Generalized Linear Model (GLM) on the germination of the soil seed bank of the Mbe (MB) savannah. SCE: sum of the squares of the deviations; ddl: degree of freedom; CM: mean square; F: Fisher-Snedecor statistical test; p-value: critical value.

| Dependent variable: germination | | | | | |
|---|---|---|---|---|---|
| **Source** | **SCE** | **ddl** | **CM** | **F** | **p-value** |
| Corrected model | 8.875 | 3 | 2.958 | 0.485 | 0.694 |
| Constant | 441 | 1 | 441 | 72.27 | 0.000 |
| Soil layer | 8.875 | 3 | 2.958 | 0.485 | 0.694 |
| Error | 366.125 | 60 | 6.102 | - | - |
| Total | 816 | 64 | - | - | - |
| Corrected total | 375 | 63 | - | - | - |

Table 2. Generalized Linear Model (GLM) on the germination of the soil seed bank of the Nguela (NG) savannah. SCE: sum of the squares of the deviations; ddl: degree of freedom; CM: mean square; F: Fisher-Snedecor statistical test; p-value: critical value.

| Dependent variable: germination | | | | | |
|---|---|---|---|---|---|
| **Source** | **SCE** | **ddl** | **CM** | **F** | **p-value** |
| Corrected model | 4.422 | 3 | 1.474 | 0.533 | 0.662 |
| Constant | 328.516 | 1 | 328.516 | 118.69 | 0.000 |
| Soil layer | 4.422 | 3 | 1.474 | 0.533 | 0.662 |
| Error | 166.063 | 60 | 2.768 | - | - |
| Total | 499 | 64 | - | - | - |
| Corrected total | 170.484 | 63 | - | - | - |

### 3.2. Floristic Composition and Density of the Soil Seed Bank of the Mbe (MB) and Nguela (NG) Savannas

We recorded 167 seedlings in the Mbe (MB) savannah and 144 seedlings in the Nguela (NG) savannah, with respective average densities of 20.17 ± 34.77 seedling/$m^2$ and 28.57 ± 4 6.06 seedling/$m^2$ (Table 3). There were no significant differences between the mean densities of the two savannas (test T de student p-value = 0.242). In the Mbe (MB) savannah, the seedlings belonged to 23 species with 21 herbaceous species (91%), 1 tree species (5%) and 1 undetermined species (4%). The only tree species identified was *Trema orientalis* (L.) Blume.

The most abundant herbaceous species were *Erigeron sumatrensis* Retz (133.33 seedling/$m^2$), *Cyperus cyperoides* (L.) Kuntze (100 plantule/$m^2$) and *Oldenlandia corymbosa* L. (83.33 seedling/$m^2$) (Table 3). However, in the Nguela (NG) savannah, the seedlings belonged to 14 species with 14 herbaceous species (100%). No tree, liana and/or indeterminate species were observed in the soil seed bank of the Nguela (NG) savannah. Nonetheless, the most abundant herbaceous species were E. sumatrensis (152.32 seedling/$m^2$), *C. cyperoides* (97.22 seedling/$m^2$) and to a lesser extent, *Spermacoce ruelliae* DC (61.11 seedling/$m^2$) (Table 3). Regarding the indicator species, the Nguela (NG) savannah displayed only one indicator species, it is the *S. ruelliae* species (IndVal = 0.48%). On the other hand, in the Mbe (MB) savannah, no indicator species was revealed (p-value > 0.05) (Table 3). Nonetheless, the floristic composition of the soil seed bank of the two savannas illustrated using the NMDS, demonstrates that there is a spatial structuring of the samples confirming that the species from the two savannas are globally similar (Figure 2).

Concerning the specific richness at the level of the soil layers, the Mbe (MB) savannah displays a relatively high specific richness compared to the soil layers of the Nguela (NG) savannah which seem to regress progressively with the depth of soil (Table 4).

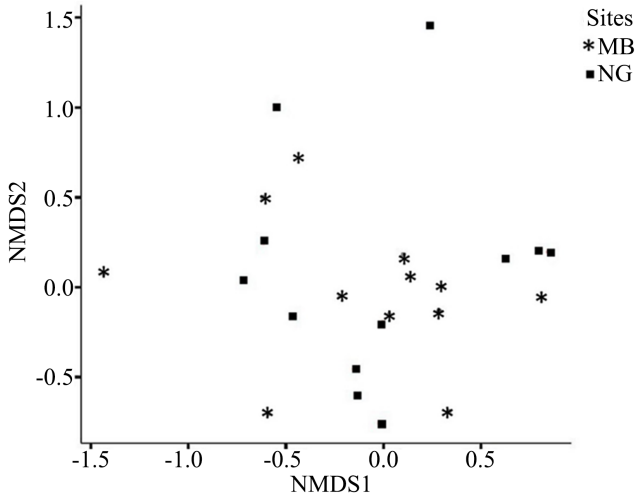


Figure 2. Two-dimensional Nonmetric Multidimensional Scaling (NMDS) ordination of soil samples of the two studied savannas (MB: Mbe savannah and NG: Nguela savannah).

**Table 3.** Composition and characteristics of the soil seed banks of the two savannas, the Mbe (MB) savannah and Nguela (NG) savannah. *AD* = Absolute Density, *RD* = Relative Density, *RF* = Relative Frequency, *IVI* = species Importance Value Index, INDVAL = Indicator Value Index and its p-value.

| Taxons | Famille | *Number of seeds* | *AD* (n/m²) | *RD* (%) | *FR* (%) | *IVI* | *IndVal* (%) | *p-value* |
|---|---|---|---|---|---|---|---|---|
| **Mbe (MB) savannah** | | | | | | | | |
| **Herbaceous species (21 species)** | | | | | | | | |
| *Ageratum conyzoides* L. | Asteraceae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.08 | Ns |
| *Chamaecrista mimosoides* (L.) Greene | Fabaceae-caesalpinioideae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.08 | Ns |
| *Chromolaena odorata* (L.) R. M. King & H. Rob. | Asteraceae | 4 | 11.11 | 2.4 | 0.52 | 2.91 | 0.13 | Ns |
| *Coleus monostachyus* (P. Beauv.) | Lamiaceae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.08 | Ns |
| *Cyperus cyperoides* (L.) Kuntze | Cyperaceae | 36 | 100 | 21.55 | 4.65 | 26.2 | 0.42 | Ns |
| *Cyperus difformis* L. | Cyperaceae | 2 | 5.56 | 1.2 | 0.26 | 1.46 | 0.17 | Ns |
| *Cyperus esculentus* L. | Cyperaceae | 3 | 8.33 | 1.8 | 0.39 | 2.18 | 0.06 | Ns |
| *Cyperus* sp. | Cyperaceae | 3 | 8.33 | 1.8 | 0.39 | 2.18 | 0.08 | Ns |
| *Erigeron sumatrensis* Retz. | Asteraceae | 48 | 133.33 | 28.73 | 6.2 | 34.9 | 0.48 | Ns |
| *Euphorbia hirta* L. | Euphorbiaceae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.01 | Ns |
| *Fimbristylis hispidula* (Vahl) Kunth | Cyperaceae | 6 | 16.67 | 3.59 | 0.77 | 4.37 | 0.25 | Ns |
| *Melastomastrum* sp. | Melastomataceae | 3 | 8.33 | 1.8 | 0.39 | 2.18 | 0.17 | Ns |
| *Oldenlandia corymbosa* L. | Rubiaceae | 30 | 83.33 | 17.95 | 3.87 | 21.8 | 0.41 | Ns |
| *Paspalum scrobiculatum* L. | Poaceae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.08 | Ns |
| *Paspalum* sp. | Poaceae | 7 | 19.44 | 4.18 | 0.9 | 5.1 | 0.33 | Ns |
| *Phyllanthus amarus* Schum. & Thonn. | Phyllantaceae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.03 | Ns |
| *Phyllanthus urinania* L. | Phyllantaceae | 4 | 11.11 | 2.4 | 0.52 | 2.91 | 0.25 | Ns |
| *Schwenckia americana* L. | Solanaceae | 3 | 8.33 | 1.8 | 0.39 | 2.18 | 0.25 | Ns |
| *Spermacocé latifolia* Aubl. | Rubiaceae | 4 | 11.11 | 2.4 | 0.52 | 2.91 | 0.25 | Ns |
| *Spermacoce ruelliae* DC. | Rubiaceae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.08 | Ns |
| *Triumfetta rhomboidea* Jacq. | Malvaceae-tilioideae | 1 | 2.78 | 0.6 | 0.13 | 0.73 | 0.08 | Ns |
| **Tree and shrub species (1 species)** | | | | | | | | |
| *Trema orientalis* (L.) Blume | Cannabaceae | 2 | 5.56 | 1.2 | 0.26 | 1.46 | 0.08 | Ns |
| **Undetermined species (1 species)** | | | | | | | | |
| *Rubiaceae* sp. | Rubiaceae | 4 | 11.11 | 2.4 | 0.52 | 2.91 | - | - |
| **Total** | | 167 | 463.89 | 100 | - | - | - | - |
| **Nguela (NG) savannah** | | | | | | | | |
| **Herbaceous species (14 species)** | | | | | | | | |
| *Bidens oligoflora* (Klatt) Wild | Asteraceae | 1 | 2.78 | 0.69 | 0.17 | 0.87 | 0.08 | Ns |

**Continued**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Chromolaena odorata* (L.) R. M. King & H. Rob. | Asteraceae | 1 | 2.78 | 0.69 | 0.17 | 0.87 | 0.13 | Ns |
| *Cyperus cyperoides* (L.) Kuntze | Cyperaceae | 35 | 97.22 | 24.32 | 6.08 | 30.3 | 0.42 | Ns |
| *Cyperus esculentus* L. | Cyperaceae | 1 | 2.78 | 0.69 | 0.17 | 0.87 | 0.06 | Ns |
| *Digitaria leptorhachis* (Pilg.) Stapf | Poaceae | 2 | 5.56 | 1.39 | 0.35 | 1.74 | 0.08 | Ns |
| *Dissotis brazzae* Cogn. | Melastomataceae | 1 | 2.78 | 0.69 | 0.17 | 0.87 | 0.08 | Ns |
| *Eragrostis tenella* (L.) P. Beauv. ex Roem. & Schult. | Poaceae | 2 | 5.56 | 1.39 | 0.35 | 1.74 | 0.08 | Ns |
| *Erigeron sumatrensis* Retz. | Asteraceae | 57 | 158.32 | 39.58 | 9.9 | 49.4 | 0.52 | Ns |
| *Euphorbia hirta* L. | Euphorbiaceae | 5 | 13.89 | 3.47 | 0.87 | 4.34 | 0.21 | Ns |
| *Fimbristylis hispidula* (Vahl) Kunth | Cyperaceae | 2 | 5.56 | 1.39 | 0.35 | 1.74 | 0.25 | Ns |
| *Murdannia simplex* (Vahl) Brenan | Commelinaceae | 1 | 2.78 | 0.69 | 0.17 | 0.87 | 0.08 | Ns |
| *Oldenlandia corymbosa* L. | Rubiaceae | 12 | 33.32 | 8.33 | 2.08 | 10.4 | 0.41 | Ns |
| *Phyllanthus amarus* Schum. & Thonn. | Phylantaceae | 2 | 5.56 | 1.39 | 0.35 | 1.74 | 0.25 | Ns |
| *Spermacoce ruelliae* DC. | Phyllantaceae | 22 | 61.11 | 15.29 | 3.82 | 19.1 | 0.48 | 0.028 |
| **Total** | | 144 | 400 | 100 | - | - | - | - |

**Table 4.** Soil seed bank characteristics for different soil layers in the two savanah (Mbe and Nguela savannas). *Sobs* = observed species richness; *Schao*2 = specific richness estimated according to the Chao2 approach; *Sjack*1 = estimated species richness following Jackniffe 1 approach; *%Sexcl* = percentage of exclusive species in each soil layer; Abund. = the number of seeds per unit area (mean ± SD).

| Soil layers | Mbe (MB) savannah | | | | | Nguela (NG) savannah | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Sobs* | *Sexclu* (%) | *Schao*2 | *Sjack*1 | *Abund.* (seeds/m$^2$: *mean* ± *SD*) | *Sobs* | *Sexclu* (%) | *Schao*2 | *Sjack*1 | *Abund.* (seeds/m$^2$: *mean* ± *SD*) |
| 0 - 5 cm | 17 | 21.73 | 23.09 | 18.92 | 6.48 ± 5.14 | 10 | 28.57 | 10.71 | 11.92 | 8.33 ± 6.66 |
| 5 - 10 cm | 12 | 8.69 | 13 | 15.25 | 8.11 ± 7.39 | 8 | 7.14 | 11.5 | 10.85 | 15.87 ± 11.79 |
| 10 - 15 cm | 8 | 0 | 10.98 | 9.2 | 9.34 ± 8.21 | 8 | 7.14 | 10.98 | 9.8 | 12.15 ± 10.39 |
| 15 - 20 cm | 7 | 0 | 9.25 | 11.02 | 16.29 ± 18.48 | 5 | 0 | 6.03 | 6.59 | 21.66 ± 20.44 |

The species richness values observed at the level of the 0 - 5 cm layers of the Mbe (MB) and Nguela (NG) savannas are respectively 17 to 10 species. On the other hand, the 15 - 20 cm layers include 7 and 5 species, respectively in the Mbe (MB) savannah and that of Nguela (NG) (Table 4). Nonetheless, the Jackknife1 estimator displays 18.92 and 11.92 species for the 0 - 5 cm layers and 9.25 and 6.03 species for the 15 - 20 cm layers, respectively for the Mbe (MB) and Nguela (NG) savannas (Table 4). Also, the average densities show that the 15 - 20 cm layers have a relatively high average density (16.29 ± 18.48 seeds/m$^2$ for the Mbe (MB) savannah and 21.66 ± 20.44 seeds/m$^2$ for the Nguela (NG) savannah).

However, the relatively low average densities are those of the 0 - 5 cm layers which displays 6.48 ± 5.14 and 8.33 ± 6.66, respectively for the Mbe (MB) and Nguela (NG) savannas (Table 4). Finally, the student's t-test reveals no significant difference in the variations of the average densities of the layers of Mbe (MB) ($p = 0.707$) as well as those of Nguela (NG) ($p = 0.564$) (Table 4).

### 3.3. Soil Seed Bank Biological Diversity

In the two savannas and at the level of the soil layers, the values of the Shannon index varies from 1.19 to 2.41 and of 1.08 to 1.88, respectively in the layers of 0 - 5 cm and 15 - 20 cm, in the Mbe (MB) and Nguela (NG) savannas (Table 5). On the other hand, those of Equitability of Pielou vary from 0.61 to 0.80 and 0.67 to 0.81, respectively in the two savannas (Table 5). Overall, the values of Shannon index are relatively higher in the Mbe (MB) savannah. However, those of Equitability are slightly high in the Nguela (NG) savannah (Table 5). Nonetheless, the variations of the diversity indices between soil layers by the Kruskall Wallis test has allowed to demonstrate that there are no significant differences (p-value > 0.05) between the soil layers of the two savannas studied (Figure 3).

Table 5. Biological diversity of the two savannas in terms of the soil layers.

| Sol layers | Mbe (MB) savannah | | | Nguela (NG) savannah | | |
|---|---|---|---|---|---|---|
| | Abundance | Shannon index | Equitability index | Abundance | Shannon index | Equitability index |
| 0 - 5 cm | 51 | 2.41 | 0.8 | 30 | 1.88 | 0.81 |
| 5 - 10 cm | 38 | 2.02 | 0.79 | 40 | 1.61 | 0.83 |
| 10 - 15 cm | 37 | 1.88 | 0.78 | 35 | 1.64 | 0.79 |
| 15 - 20 cm | 41 | 1.19 | 0.61 | 39 | 1.08 | 0.67 |

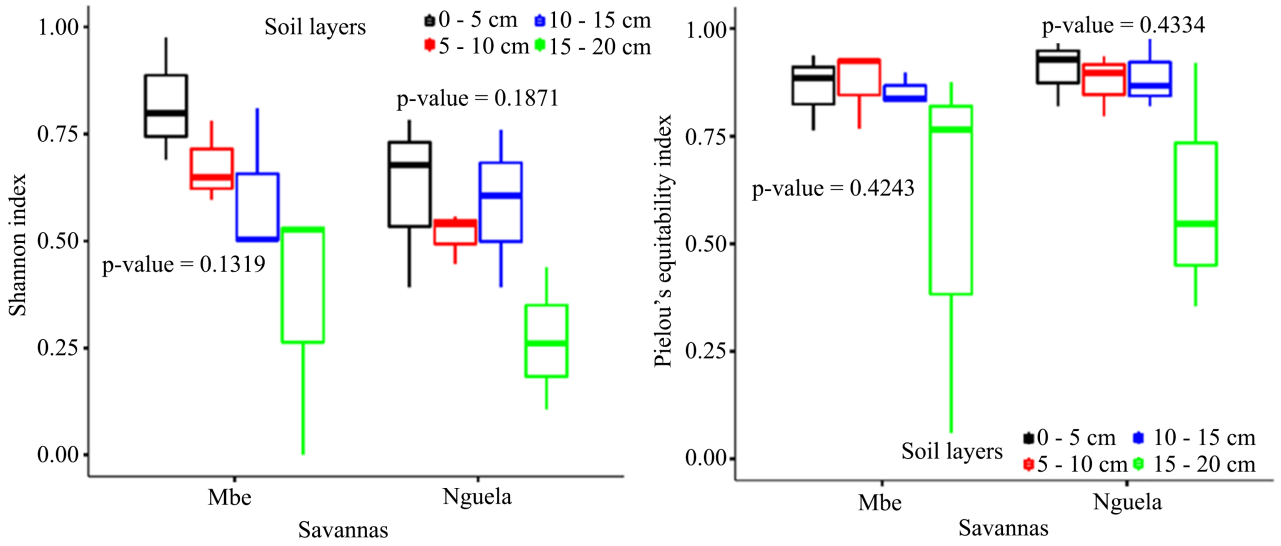


Figure 3. Variation of diversity indices in terms of the soil layers of the two savannas (Mbe and Nguela).

## 4. Discussion

### 4.1. Soil Seed Bank Germination Varies in Terms of the Savannas and Soil Layers

In the present study, seed germination showed up differently from one savannah to another. Overall, the germination number was relatively higher in the first soil layers (0 - 5 cm and 5 - 10 cm) than those the deeper layers (10 - 15 cm and 15 - 20 cm). The higher germination number of the superficial layers could be explained by the stay of the seeds in the soil bank which probably initiated the breaking of dormancy allowing water to reach the seed embryos. Nonetheless, we could admit hypothesis that the seeds of deep layers of the soil were still dormant, translating by a relatively late pace of germination. More, the deeper the seeds are buried, more, they ensure their stay of viability in the soil bank [23]. What seems relatively surprising in the present study, is the relatively significant number of germinated seeds in the deepest soil layer (15 - 20 cm), compared to other layers in the two savannas studied.

This could reflect a significant decrease in the soil seed bank in the first layers for the benefit of the 15 - 20 cm layer. This observation contradicts the conclusions of authors who discuss on the germination [56] [57]. These authors estimate that germination decreases with soil depth because the oxygen concentration would be relatively low in the deep layers. Our results do not agree with those who have worked in the shrub savannah of Sanguie in the western region of Burkina Fasso [58]. These authors demonstrate significant seed bank germinations in the first soil layers compared to the deeper layers.

This difference could be explained by the fact that the savannas of Mbe (MB) and Nguela (NG) are savannas that undergo recurrent disturbances from different origins [15]. Nonetheless, these disturbances have a direct effect on the soil layer and could destroy the seed bank of the superficial layer of 0 - 5 cm. Nevertheless, this discovery coincides with works which demonstrates that fire can damage a considerable proportion of seeds located in the litter layer, 0 - 5 cm or even 5 - 10 cm [59]. The authors point out that at a certain temperature deemed fatal to seeds, fire can have an effect up to approximately 6 centimeters in the soil bank.

### 4.2. The Density and the Floristic Composition of the Soil Seed Bank Differs in Terms of the Savannas and Soil Layers

Seed density in the present study varied slightly compared to site. Thus, it was 400 seeds/$m^2$ and 463.88 seeds/$m^2$, respectively in the savannas of Nguela (NG) and Mbe (MB). This density seems low compared to that reported in the savannah ecosystems of Ghana [20]. The authors demonstrate that the soil seed bank was abundant and varied between 100 and 700 seeds/$m^2$. Likewise, in the Serengeti savannas inTanzania, the soil seed bank was abundant, reaching up to 3000 seeds/$m^2$ [6].

This difference could be explained by the fact that previous studies only took

into account a single layer of soil [6] [20], respectively 0 - 4 cm and 0 - 5 cm which are considered as the layers more abundant in floristic species. The vertical distribution of seeds in the soil shows that the densities were relatively low in the first three layers of soil up to the last (15 - 20 cm). This discovery does not concur with some works realized on the soil seed bank in savannah.

For example, in the wooded savannas of West Africa, other works demonstrates that the highest densities were in the upper layers of soil [56] [60] [61]. These observations do not concur with the hypothesis reported in the literature namely that the majority of seeds are located in the upper soil layer [7] [56]. Nonetheless, the results on seed density obtained in this study could be explained by the fact that the 15 - 20 cm layer had more viable seeds than other layers. Moreover, this could reveal that the conditions and factors of germination were favorable for the germination of the seeds of this layer.

This study has demonstrated that there is the presence of a species of dense forests exclusively in the Mbe (MB) savannah. This is *Trema orientalis* (L.) Blume in the 0 - 5 cm layer. *T. orientalis* is a fast-growing species growing in previously disturbed areas and forest edges [62]. The authors point out that it is a pioneer species that can grow on relatively poor soils and can be used to reforest disturbed forest areas by providing shade and protection for young plants. Species like *Phyllanthus urinaria* L. and *Schwenckia americana* L. are annual species living in fields, open forests and disturbed habitats [63] [64]. Consequently, the presence of these species in the soil seed bank of the Mbe (MB) savannah could be explained, on the one hand, by the fact that *S. americana* would be the result an transformation of forests in shrub savannas. This hypothesis concurs with of others works who demonstrates that the savannah would be the result of forest degradation inherent to human activities [56] [61].

The evaluation of viable seeds in the soil bank of the two savannas displays 100% herbaceous species in the Nguela (NG) savannah and 91% herbaceous species in the Mbe (MB) savannah. Our results on the dominance of herbaceous species corroborate with other works in the shrub savannas [9] [61] [65]. These work shows that herbaceous species largely dominate in the soil seed bank of shrub savannahs rather than wooded species. The impoverishment of woody species in the soil seed bank would indicate a limited possibility of the resilience of degraded semi-arid ecosystems by the soil seed bank [58] [63]. Nonetheless, other studies demonstrate that the lack of seeds of woody species in the soil bank suggests that the regeneration of such species would be inhibited by the loss of large-diameter individuals in the surrounding vegetation [66]. Thus, the high proportion of herbaceous species in the soil seed bank can be explained by the fact that: 1) herbaceous species produce many small seeds compared to large seeds. They have a high probability to escape at the predation by seed-eating animals; 2) seeds of herbaceous species gradually accumulate in the soil bank, and can remain viable for several decades compared to other types of seeds [67] [68]. The size of the seed and its longevity are negatively correlated, which could justify the main factor of absence of seeds of woody species [69].

This study has clearly demonstrated a regeneration deficit in the Mbe (MB) and Nguela (NG) shrub savannas from the soil seed bank despite the presence of a pioneer species, *Trema orientalis*, in the Mbe (MB) savannah. In the Nguela (NG) savannah, there are species that dominate by their recoveries, the other existing species, this is the case of *Cyperus cyperoides* (L.) Kuntze and *Erigeron sumatrensis* Retz largely dominated on all the other species with absolute densities of 97.22 seeds/$m^2$ and 158.33 seeds/$m^2$, respectively. On the other hand, in the Mbe (MB) savannah, the species display recoveries of the same importance.

This could be explained by the fact that the majority of the species identified in this study have relatively nearby similar absolute densities. Finally, Sorensen's similarity coefficient demonstrated that there is considerable similarity between the two shrub savannas studied, which suggests that in the two savannas, the species identified in the soil bank are globally similar and the inter-savannah biodiversity is very low, even almost zero.

## 5. Conclusion

The present study consisted of evaluating the natural regeneration potential through the soil seed bank of the two geographically distinct savannas in order to understand the dynamics of floristic potential. The results highlighted the role that the soil seed bank could play in the natural regeneration of savannas. We have shown that the characteristics of the soil seed bank of two savannas are globally similar. We can overall remember that: 1) herbaceous species were quantitatively more important in the soil seed bank of the two savannas; 2) the species from the soil seed bank of the two savannas are globally similar; 3) only one species of wood has been found in the soil seed bank of the Mbe savannah, this is Trema orientalis. The high abundance of herbaceous species in the two savannas and the presence of a single woody species clearly demonstrate the need to preserve these savannas' ecosystems in the face of anthropogenic disturbances. Consequently, reforestation to improve the regeneration of wooded species within the two savannas is all the urgently needed [70] [71].

## Acknowledgements

The authors thank the Marien N'GOUABI University (Republic of Congo), and the local populations of Nguela and Mbe for their contributions to the realization of the study. Finally, we wish to thank Bijoux Toumba-Paka, Julienne Bonazaba and Isaac Dzombo for their various contributions and advice.

## Authors' Contributions

The authors C.D., C.M., and B.C.M. each have provided assistance in the preparation and reading of this article. L.M.M., G.N., T.M. and A.J.M. participated in the initiation, collection, analysis and processing of field data. S.F.N. and F.K. contributed effectively to the scientific orientation of the manuscript.

## Conflicts of Interest

The authors declare that they have no competing interests.

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