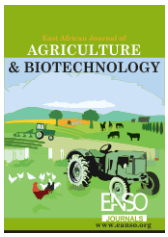

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Original Article

Effect of Two Years of Conservation Agricultural Practices on Weed Seed Banks Evaluated Using Three Techniques

Otim Godfrey Anyoni[1,2], David Ocan[3], Alfred Kumakech[2], Susan Tumwebaze[4]& Alfred Obia[3]*

[1] Uganda Martyrs University, P. O. Box 5498, Kampala, Uganda.
[2] Ngetta Zonal Agricultural Research and Development Institute, (NARO), P. O. Box 295, Entebbe, Uganda.
[3] Gulu University, P. O. Box 166, Gulu, Uganda.
[4] Makerere University, P. O. Box 7062, Kampala, Uganda.
* Author for Correspondence ORCID ID: https://orcid.org/0000-0003-1861-6228; Email: Otimgw@gmail.com

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ABSTRACT

Understanding the soil seed bank is very important when developing integrated weed management technologies and detecting the influence of crop management practices on weeds. In conventional weed control, farmers tend to focus on the above-ground, yet the above-ground vegetation is related to the below-ground soil seed bank. Several soil seed bank estimation methods have been used to estimate the soil seed bank, but varying results have been obtained under different field management practices and soil depths. In this study, we compared three methods for estimating the soil seed bank: seedling emergence method and two direct seed extraction methods (floatation and cloth bag) for determining weed seed density and diversity from different soil depths under conservation farming practices. The three methods had significant differences in estimating weed densities from the soil seed bank ($p<0.001$). The greenhouse seedling emergence method had a mean number of 5.06, cloth bag had 4.07 while floatation method had the lowest number of 3.38 per 300g of soil. The mean highest weed density was obtained from soil depth of 0-15 cm (5 weeds/300g vs 1.6 weeds/300g of soil from 15-30 cm depth). For soil seed bank diversity, cloth bag method had the highest mean value followed by greenhouse emergence and lastly floatation method with Simpsons diversity index of 2.72, 1.79 and 1.31, respectively. Shannon Weiner diversity index followed the same pattern for the three methods. The methods had different sensitivity to density and diversity and therefore greenhouse emergence method should always be combined with cloth bag method. The greenhouse emergency method detected a total of 26 weed species, cloth bag detected 22 weed species and Floatation method detected 18 weed species. Despite, the greenhouse emergence method detecting more species than the cloth bag at 26 and 22 weed species, respectively, it had a lower Simpson's diversity index than the cloth bag method due to lower species evenness. Sampling of the entire soil plough layer of 0-30 cm depth for disturbed agricultural soils may produce the best results. Seasons significantly influenced soil seed bank diversity and not soil seed bank density where second season (B) significantly increased soil seed bank diversity. Soil cover practice through intercropping maize with soybean significantly reduced soil seed bank density and not diversity in both minimum and conventional tilled plots. This positive influence on weed density and diversity is a good indicator for integration into a weed management program.

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INTRODUCTION

Soil seed banks are important in understanding the influence of "disturbance", like crop management practices on weed density and diversity (Andreasen et al., 2018; Padonou et al., 2022a). One of the most important weed management strategies yet so much neglected is to reduce the soil seed bank (Kumari, 2018). The soil seed bank defines the weed seed reserve present on the soil surface and scattered throughout the soil profile (Hossain & Begum, 2016). It is composed of recently shed seeds and older seeds that have persisted in the soil for a long time (Thompson et al., 1993). The soil seed bank as well includes tubers, bulbs, rhizomes, and other vegetative structures. Cropping practice highly influence the soil seed bank and information on its influence on the soil seed bank is important in developing an integrated weed management strategy (Buhler et al., 1997; Forcella et al., 2003; Netto et al., 2022). Knowledge of the soil seed bank content can help farmers project and manage the potential impacts of weed competition on crop yield and quality (Dekker, 2011). Eliminating weed seed "deposits" in the soil seed bank is the best approach to ease future weed management. Initial weed populations are directly related to the soil seed bank populations (Menalled & Schonbeck, 2011). The higher the soil seed bank density, the higher the weed infestation in the crop.

Agricultural systems world over have become more vulnerable to biotic stress and less sustainable due to the selection of homogenous crop genotypes that have high reliance on pesticides. The world trend now is inclined toward sustainable agricultural practices; including weed management under conservation agriculture such as minimum tillage or even no-till systems (Feledyn-Szewczyk et al., 2020; Tataridas et al., 2022; Teixeira & Basch, 2022). These conservational agricultural practices are reflected in many studies (Carpio et al., 2020; Feledyn-Szewczyk et al., 2020; Teixeira & Basch, 2022), and results from the impact of these practices on the density and diversity of weed flora and soil seed bank are inconclusive (Feledyn-Szewczyk et al., 2020). The integration of conservation agricultural practices such as soil cover, crop rotation and diversification together with minimum tillage may contribute to weed management.

The transition to conservation agriculture from conventional tillage is likely to affect the weed dynamics due to minimum tillage, soil cover and rotation practices. The extent to which this shift to CA may affect the weed density and diversity in both below and above ground is unknown. Hossain et al. (2021), reported that strip tillage and mulching decreased weeds and increased weed diversity in the soil seed bank under intensive rice-based crop rotation. Weed

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management in CA depends on herbicides and soil cover practices through mulching, diverse crop rotation, and cover crops (Adeux et al., 2022). The effects of these soil cover practices on weed density and diversity in CA systems are not well understood (Cordeau, 2022). Cropping systems that rely on diverse crop rotation and ecological weed management rather than depending on intense herbicide use appeared to increase weed diversity (Adeux et al., 2019; Cordeau, 2022; Ulber et al., 2009). New weed management strategies must focus on the optimization of non-chemical alternatives and the provision of desirable agroecosystem services by enhancing biodiversity and securing farmer income (Tataridas et al., 2022). A special focus should be placed on how these sustainable crop cultivation practices influence the weed seed density and diversity in the soil seed bank (MacLaren et al., 2020).

Quantification of abundance and diversity of weed seed in the soil may be influenced by the depth of soil sampling but seed banks typically are confined to the surface and upper 30 cm of soil, although some perennial plants maintain seeds in above-ground seed banks (Forcella et al., 2003). Soil sampling usually is a necessary component of soil seed bank studies, however, the obvious question that may arise is how many and what size of soil sample should be taken? It should be kept in mind however that if the goal of the study is to characterize the seed flora and density completely, as in weed community analysis, the number of cores required is higher because the less common species will be sampled at a lower level of precision than the more common species.

Upon sampling the soils for weed seeds quantifications, several methods exist and their efficiency is further complicated by soil type, crop management practices, and depth of soil sampling (Mesgaran et al., 2007a). In most of the soil seed bank studies, soil sampling has been done at a depth of 0-5 cm and 0-10 cm (Mukhongo et al., 2011; Padonou et al., 2022b) with Ranjit et al. (2007) reporting highest pressure of weed seeds at soil depths of 5-10 cm.

Two methods commonly used to estimate the soil seed bank are the direct seed extraction (that includes various sieving sub-methods) and germination methods. Germination method is where the seed bank is assessed through the identification and enumeration of seedlings that emerge from the soils under controlled conditions (Gonzalez & Ghermandi, 2012). A combination of direct seed extraction and germination methods is recommended for estimating the size of the viable soil seed bank due to the problems inherent to each method (Price et al., 2010), such as challenges of seed dormancy in seedling germination method or very tiny seeds in the direct seed extraction methods by sieving. The sieving, floatation and cloth bag sub-methods in the seed extraction method were found to have no significant difference in seed recovery, although the cloth bag sub-method was more efficient at 75%, followed by sieving at 67% and floatation at 61% (Mesgaran et al., 2007a). Padonou et al. (2022b) reported that greenhouse germination method was the most frequently used method at 60.2% while the use of sub-methods of direct extraction is way lower (sieving at 23.9% and floatation at 15.9 %). In this study, three methods were used for studying the soil weed seed bank including two direct seed extraction methods (floatation and cloth bag methods) and the greenhouse germination technique. The objective of this study is to compare three soil seed bank estimation techniques for determining weed seed density and species composition.

The study had three hypotheses: 1) minimum tillage in combination with soil surface cover using legume intercropping and crop residue will reduce weed seed density and maintain diversity. 2) Soil depth of 0-15 cm is a more representative sampling depth compared to 15-30 cm and 0-30 cm in determining soil seed bank density, species composition and detecting changes in the soil seed bank due to the conservation agricultural practices applied. 3) Cloth bag technique is more accurate compared to floatation and greenhouse germination techniques for determining weed seed density, species composition and detecting changes in the soil seed bank due to the

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conservation agricultural practices applied. To address the hypotheses, three soil seed bank estimation techniques were used to determine the seed density and diversity from soil samples taken from experiments at varying depths where several conservation agricultural practices were compared to conventional tillage practices. In general, soil cover techniques reduced weed seed density while maintaining diversity irrespective of soil seed bank estimation techniques. Therefore, conservation agricultural practices may constitute an important element of sustainable weed management strategy.

MATERIALS AND METHODS

Study Site

The study location was at Ngetta Zonal Agricultural Research and Development Institute (Ngetta ZARDI) in northern Uganda for a duration of 3 seasons. Ngetta ZARDI is located at 02°.29573'N; 032°.92092'E at an elevation of 1180 meters above sea level and experiences an average daily temperature of 25 ℃ and a maximum temperature of 29 °C. The climate is described as moist, sub-humid, with a mean annual rainfall of 1,639 mm, bi-modally distributed from March-June (season A) and August-December (season B) (Kumakech et al., 2014). The soil at the experimental site is sandy loam (Sand 73%, Silt 11%, Clay 16%), average pH of 6.4, organic matter content of 2.5%, P 20 ppm, K 506 ppm, Ca 1089 ppm, Mg 317 ppm (Anyoni et al., 2023)

Experimental Design and Crop Management

The experimental design used was split plot design with tillage practice as the main plot factor in two treatments of ox-ploughing and ox ripping. The subplot factor is soil cover in five treatments (sole maize with mulch, sole maize, two lines of soybean in between maize line, one line of soybean in between maize line, sole soybean) (*Table 1*). Specifically, soil cover practices such as mulching at 6 t/ha was used in this study as recommended (Fonteyne et al., 2020; Kumari, 2018; Mani et al., 2016; Uwah & Iwo, 2011). Also, two intercropping patterns where soybean acted as a cover crop in maize were studied as conservation agricultural practice to be compared with sole maize without mulch and sole soybean as control practices. Plots size was 4 m x 4 m with a 2 m border in between plots within a block, and 2.5 m between blocks. The experiment had a total of 30 plots. The field was slashed, weeds left to sprout for two weeks and after a non-selective herbicide glyphosate, was applied at a rate of 4 l/Ha.

Table 1: Experimental layout.

Ox-plough tillage	Minimum tillage rip line	Ox-plough tillage	Minimum tillage rip line	Ox-plough tillage	Minimum tillage rip line
Replicate 1	**Replicate 1**	**Replicate 2**	**Replicate 2**	**Replicate 3**	**Replicate 3**
3	2	3	5	4	1
4	1	4	1	1	2
2	5	5	2	3	4
1	3	1	3	5	5
5	4	2	4	2	3

Note: *1 = Mulched Maize, 2 = Control no mulch, 3 = Two lines of soybean in between one line of maize, 4 = One line of soybean in between one line of maize, 5 = Pure soybean.*

Soil Sampling

Soil sampling was done using auger with a 5 cm diameter and 15 cm length. This size of soil core was representative to detect available seeds, but small enough not to burden the researcher with too much soil. It is therefore recommended for soil seed bank studies (Forcella et al., 2003). The diagonal transect design was used for soil sampling within the experimental plots as recommended (Colbach et al., 2000).

Soil samples for seed bank estimation were collected just before the start of rains between February -March for season A and July for season B, just before planting. Soil seed bank samples

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were taken at times that follow seed shed, but precede seed germination. Sampling soil seed banks after seedling emergence when the seasonal rains have started has little value in theory or in practice (Forcella et al., 2003).

In this study, estimates of soil seed bank density and diversity obtained from the direct seed extraction techniques (floatation and cloth bag) were compared with estimates obtained from the greenhouse germination technique. After performing 1st and 2nd season primary tillage, five soil cores per experimental unit were taken at a depth of 0-15 cm, 15-30 cm and 0-30 cm and bulked, respectively. By quarter sampling, a composite sample of approximately 2000 grams each from 0-15 cm, 15-30 cm and 0-30 cm depth were obtained for each experimental unit. Composite samples were placed in polythene bags, maintained at a temperature of 5 °C in a refrigerator. The sample was then picked from the refrigerator allowed to gain room temperature, after which a clean working samples were prepared by sieving the soil to remove debris and breaking up cores with 6.35 mm mesh sieve as earlier reported by Thompson et al. (1993). The resulting sample weighed 1,800 g for each experimental unit, after being mixed and split using a riffle-type, soil-splitting apparatus.

The field soil sample was divided into three parts of 300 g, 300 g and 900 g, respectively per plot, a size above the minimum weight of 100 g recommended for determination of seed bank species composition (Colbach et al., 2000; Forcella, 1992). The first and second part of 300 g was processed for direct seed extraction procedure using the floatation method, similar to that described by Ball and Miller (1989); Ter Heerdt et al. (1996) and cloth bag method as presented by Mesgaran et al. (2007), respectively. The third sample of 900 g was divided into 3 replicates, and each placed in a temperature and light-controlled greenhouse from where seeds were allowed to germinate.

Floatation Method

Direct seed extraction in the first sample was performed by placing 300 g of soil samples into a 10 L plastic bucket. After, a 200 ml solution containing 10 g of sodium hexametaphosphate, 5 g of sodium bicarbonate and 25 g of magnesium sulphate was mixed in 200 ml of tap water and added in the bucket containing 300 g of soil sample and stirred continuously for 2 minutes. This solution facilitated the density separation of seeds and organic matter from the soil mineral fraction. After stirring, the slurry was allowed to settle for approximately 60 seconds causing the soil mineral fraction to settle and the seed and organic matter fraction to float. The floating fraction contained extracted seeds that was decanted off and caught on a fine mesh sieve of 0.297 mm. This flotation/separation procedure was repeated three times using the same batch of magnesium sulphate, sodium hexametaphosphate solution. The remaining soil mineral fraction was discarded after completion of the third mixing and separation operation. The organic debris and seed fraction remaining after extraction was washed from the sieve onto a filter paper on a Buchner suction funnel to draw excess water. The seed/detritus residue was left on the filter paper until dry and then transferred to vials for storage until identification and counting.

Seeds were counted and identified into species by placing them under a 10X magnification glass. Available seed identification resources were used to support identification. Viability of the seed was determined by applying gentle pressure to each seed with forceps. The seed resisting pressure was “apparently viable” and recorded. The method of determining apparent viability has been used by several scholars (Ball & Miller, 1989). A determination of apparent viability was sufficient to make appropriate study comparisons and determine soil seed bank density and diversity.

Cloth Bag Method

Briefly, 300 g of sample was poured into the cloth bag (with a mesh opening of 0.25 mm) and washed under running tap water, allowing clay particles and most of the sand to be washed away. The organic debris, seed fraction and sand particles >0.25 mm remaining after extraction was washed from the cloth bag onto a filter paper on a

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Buchner suction funnel to draw excess water. The seed, organic debris residue, and sand >0.25 mm was left on the filter paper until dry and then transferred to vials for storage until identification and counting. Apparent viability of the weed seeds was determined as described in the floatation method above

Seedling Germination Method

In the seedling germination method, a total of 900 g of soil sample was used but divided into three 300 g samples. The three replicates were incubated to stimulate germination by placing a layer of vermiculite on the tray surface to allow easy drainage, then covered with fabric for easy cleaning, watering and drainage. The 300 g of soil for each replicate was spread on the tray at 2-3 cm thick soil layer to facilitate enough aeration. The soil was kept moist by applying 50 ml of water per day. A spatula was used to do light soil turning so as to stimulate seed germination by improving soil aeration. Weed seedlings were removed once identified. If difficult to identify it was transplanted to another pot to grow until its features enabled, it to be identified. Common weed identification resources were used to aid weed identification (Botha, 2001), including software guides and online websites. Identified weeds were removed to avoid competition. Weed identification was done in a well-lit room. A magnifying glass 10X supported the identification of seedling features.

Data Collection

Weed seeds count and species identification was done on weed seeds that were sieved and floated using the two methods of the direct seed extraction. Also, emerged weed seedlings in the germination method were identified (species), counted and recorded for each 300 g sample at three, six, nine and twelve weeks after placement of the soil in germination tray. The cycle was repeated for another 12 weeks for a total of 9 months. Data was collected for three seasons and analysed across seasons. The Shannon-Weiner diversity index, H and Simpsons diversity index, D were computed.

$$H = -\sum_{1=i}^{s} p_i \ln p_i \quad [1]$$

$$D = \Sigma \text{ (sum) of } Pi^{2} \quad [2]$$

Where P_i = Fraction of the entire population made up of the species, i (proportion of a species i relative to total number of species present, not encountered), s = numbers of species encountered.

Here, a high value of H would be representative of a diverse and equally distributed community and lower values represent less diverse community. The Shannon index is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled. The Simpson index is a dominance index because it gives more weight to common or dominant species. In this case, a few rare species with only a few representatives do not affect the diversity.

Statistical Analysis

Analysis of variance (ANOVA) F-test was used to compare the three-soil seed bank estimation methods and to determine the effect of tillage and soil cover practices on soil seed densities (N) and diversity as measured by Shannon Weiner (H) and Simpson's (D) diversity indices over three seasons. Data on appropriate soil sampling depth for assessment of soil seed bank density and diversity was analysed using ANOVA. Tukey's test was used to find means that are significantly different. Pareto charts were used to enumerate the species detected in the three estimation methods at different soil depths. Pareto chart is a quality analytical tool that helps one focus on the contribution of a specific component to the broader response or y-variable.

RESULTS

Soil Seed Bank Density

The three methods used in estimating soil seed banks produced significantly different seed density estimates (*Table 2*; $p<0.001$). The greenhouse germination method had a mean of 7.6 seeds per 300 g of soil, which was significantly

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higher than the cloth bag (N=5.3) and floatation method (N=4.9) at 0-30 cm depth. The cloth bag and floatation method didn't show a significant difference in estimating seed densities ($p>0.05$; *Table 2*). The seed density from soil depth of 0-15 cm was significantly higher than seed density from 15-30 cm depth at an average of 5.03 seeds and 1.55 seeds/300 g of soil, respectively for the two tillage practices ($p<0.05$; *Table 3*). All three seed bank estimation methods detected higher seed densities in the 0-15 cm soil layer, compared to 15-30 cm by an average of threefold. However, seed density from soil depth of 0-30 cm was not significantly different from seed density obtained from soil depth of 0-15 cm ($p>0.05$; *Tables 2* and *3*). This trend was observed for all the three methods of soil seed bank estimation tested in this study.

Seedling emergence soil seed bank estimation method provided the highest estimate of soil seed bank density (7.58; *Table 2*). The seedling emergence soil seed bank estimation method generally had a higher soil seed bank density estimate compared to the cloth bag and floatation method under different tillage and soil cover practices (*Tables 4* and *5*). The two maize-soybean intercropping patterns significantly reduced soil seed bank density (2.75/300g of soil; Table 5). *Table 6* shows the effect of soil cover practices and season on the soil seed density. It shows that sole crops were not able to significantly reduce the soil seed bank density compared to the intercrops, especially one line of soybean in between maize reduced the soil seed bank density to 1.85/300 g of soil, compared to sole maize mulched and not mulched at 6.59 and 5.48, respectively per 300 g of soil. The sole mulched maize was not able to reduce the soil seed bank as expected. Also, the intercrops were more effective in reducing the soil seed bank density compared to sole soybeans.

Table 2: Comparison of the mean seed density, Shannon Weiner and Simpson's diversity indices per 300 g soil sample across the three-soil seed bank estimation methods.

Estimation method	Soil depth (cm)	Mean values[1]		
		Seed density/300g (N)	Shannon Weiner index (H)	Simpsons index (D)
Seedling emergence	0-15	6.24^{ab}	0.88^{a}	2.10^{cd}
	15-30	1.35^{d}	0.21^{c}	0.75^{f}
	0-30	7.58^{a}	0.92^{a}	2.5^{bc}
Floatation	0-15	3.4^{cd}	0.43^{bc}	1.4^{def}
	15-30	1.76^{d}	0.22^{c}	0.9^{f}
	0-30	4.9^{bc}	0.56^{b}	1.7^{cde}
Cloth bag	0-15	5.41^{bc}	1.1^{a}	3.7^{a}
	15-30	1.53^{d}	0.34^{bc}	1.2^{ef}
	0-30	5.3^{bc}	0.96^{a}	3.3^{ab}

[1]Tukey pairwise comparisons, means for each soil depth that do not share a letter within estimated parameter along each column are significantly different.

Table 3: Comparison of the mean seed density, Shannon Weiner and Simpsons diversity indices per 300g soil sample at three soil depth levels under two tillage practices as assessed using means of the three methods.

Tillage Method	Soil depth (cm)	Seed density/300g (N)	Shannon Weiner index (H)	Simpsons index (D)
Conventional tillage	0-15	5.15^{a}	0.88^{a}	2.57^{a}
	15-30	1.64^{b}	0.24^{b}	0.93^{b}
	0-30	5.61^{a}	0.79^{a}	2.51^{a}
Minimum tillage	0-15	4.92^{a}	0.72^{a}	2.25^{a}
	15-30	1.46^{b}	0.26^{b}	0.92^{b}
	0-30	6.25^{a}	0.84^{a}	2.45^{a}

Tukey pairwise comparisons, means that do not share a letter within estimated parameter along column are significantly different.

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Table 4: Comparison of the weed seed density and diversity indices per 300 g soil sample as influenced by tillage method.

Tillage practice	Estimation method	Mean values[1]		
		Seed density/300 g (N)	Shannon Weiner index (H)	Simpsons index (D)
Conventional tillage	Seedling emergence method	4.89^{ab}	0.68^{a}	1.78^{b}
	Floatation method	3.31^{b}	0.41^{b}	1.33^{b}
	Cloth bag method	4.19^{ab}	0.82^{a}	2.84^{a}
Minimum tillage	Seedling emergence method	5.23^{a}	0.65^{a}	1.81^{b}
	Floatation method	3. 44^{b}	0.40^{b}	1.29^{b}
	Cloth bag method	3.96^{ab}	0.77^{a}	2.60^{a}

[1]Tukey pairwise comparisons, means that do not share a letter within estimated parameter along each column are significantly different.

Table 5: Comparison of the mean seed density, Shannon Weiner and Simpsons diversity indices per 300 g soil sample from five soil cover practices.

Soil cover practice	Estimated parameter[1]		
	Seed density/300 g (N)	Shannon Weiner index (H)	Simpsons index (D)
Sole maize with mulch	5.15^{a}	0.64^{ab}	2.14^{ab}
Sole maize	4.91^{a}	0.73^{a}	2.24^{ab}
2 lines of soybean in-between maize	3.69^{ab}	0.50^{b}	1.54^{b}
1 lines of soybean in-between maize	2.75^{b}	0.54^{ab}	1.67^{ab}
Sole soybean	4.35^{a}	0.69^{ab}	2.10^{ab}

[1]Tukey pairwise comparisons, means that do not share a letter within estimated parameter are significantly different.

Table 6: Soil seed bank density estimates/300 g of soil in descending order obtained from different soil cover practices over three seasons.

Season (2019B, 2020A & 2020B)	Soil cover practice	Soil seed density[1]
2019B	Sole maize and mulch	6.59 ^{a}
2019B	Sole maize	5.48 ^{ab}
2020B	Sole soybean	5.28 ^{ab}
2020A	Sole maize	5.00 ^{abc}
2020A	Sole maize and mulch	4.67^{abc}
2020B	Sole maize	4.26 ^{abc}
2020B	Sole maize and mulch	4.20^{abc}
2019B	Sole soybean	4.04^{abc}
2020A	2 Lines soy-maize	4.02^{abc}
2019B	2 Lines soy-maize	3.89 ^{abc}
2019B	1 Line soy-maize	3.74 ^{abc}
2020A	Sole soybean	3.72^{abc}
2020B	2 Lines soy-maize	3.15 ^{bc}
2020B	1 Line soy-maize	2.67 ^{bc}
2020A	1 Line soy-maize	1.85 ^{c}

Note: [1]Tukey pairwise comparisons, means that do not share a letter are significantly different

Soil Seed Bank Species Diversity Measured by Shannon Weiner Index (H) and Simpson's Index (D)

The cloth bag soil seed bank estimation method generally provided higher estimates of the soil seed bank diversity compared to the floatation and seedling emergence methods ($p<0.001$; *Tables 2*

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and *4*). In determining Shannon Weiner diversity index (H), the cloth bag and seedling emergency estimation methods were significantly higher compared with the floatation method (P<0.001). However, in determining Simpsons diversity index (D) cloth bag estimation method had a significantly higher value (p<0.001; *Table 5*) compared with the seedling emergency and floatation method. For the 0 – 15 cm and 0 – 30 cm soil depths, all the three seed bank estimation methods were not significantly different in estimating Shannon and Simpson diversity index (p>0.05; *Table 2*). The 0-15 cm soil layer contained a total of 34 weed species compared to 19 weed species only in the 15-30 cm soil layer, as estimated from the mean of all the three soil seed estimation methods.

Direct seed extraction method by cloth bag provided the highest estimate of soil seed bank diversity (D) in both conventional and minimum tillage plots, respectively (2.84 and 2.6; *Table 4*). Soil seed bank diversity in the control plot of sole maize without mulch did not differ significantly with all the soil cover practices and tillage method implying that the soil seed bank was not altered by these practices; *Table 5*.

Soil seed bank diversity as estimated by Shannon wiener and Simpsons indices was determined by seasons rather than the soil cover practices; *Table 7*. The surface cover through intercrops had a lower soil seed bank diversity compared to sole crops, though these were not statistically significant (p>0.05; *Table 5*).

Table 7: Soil seed bank Simpsons diversity index estimates in descending order obtained from five soil cover practices over three seasons.

	Soil cover practice	Simpsons index[1]
2019B	Sole maize	3.03[a]
2019B	Sole maize and mulch	2.82[ab]
2020B	Sole soybean	2.46[abc]
2019B	Sole soybean	2.44[abc]
2019B	1 Line soy-maize	2.17[abcd]
2020B	Sole maize and mulch	2.14[abcd]
2020B	Sole maize	2.10[abcd]
2020B	1 Line soy-maize	1.74[bcd]
2019B	2 Lines soy-maize	1.70[bcd]
2020B	2 Lines soy-maize	1.61[bcd]
2020A	Sole maize	1.60[bcd]
2020A	Sole maize and mulch	1.46[cd]
2020A	Sole soybean	1.39[cd]
2020A	2 Lines soy-maize	1.31[cd]
2020A	1 Line soy-maize	1.11[d]

Note: [1]Tukey pairwise comparisons, means that do not share a letter are significantly different

Table 8: Composition of the soil seed bank in 300 grams of soil as determined by the three estimation methods (0-30 cm).

Class of weeds	Family	Weed species	Seed size (mm)[1]	Frequency by estimation method		
				Greenhouse	Floatation	Cloth bag
Broad leaved	Amaranthaceae	*Amaranthus hybridus*	1.3	168	11	2
	Fabaceae	*Acacia mimisoides*		152	32	55
	Fabaceae	*Desmodium intortum*	1.5-2	136	89	101
	Fabaceae	*Centrosema pubescens*	4	34	0	71
	Euphorbiaceae	*Euphorbia heterophylla*	3	18	0	30
	Fabaceae	*Medicago polymorpha*	3	18	4	0
	Amaranthaceae	*Amaranthus retroflexus*	1	12	43	3

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Class of weeds	Family	Weed species	Seed size (mm)[1]	Frequency by estimation method		
				Greenhouse	Floatation	Cloth bag
	Rubiaceae	*Richardia brasiliensis*	2.5-1.8	0	0	6
	Rubiaceae	*Richardia grandiflora*	2	8	0	14
	Amaranthaceae	*Amaranthus thunbergii*	1.3	8	0	29
	Malvaceae	*Abelmoschus*	5.5	6	3	4
	Asteraceae	*Bidens pilosa*	1-11	2	0	19
	Solanaceae	*Solanum nigrum*	2	2	0	39
	Asteraceae	*Tagete minuta*	6	2	4	0
	Asclepiadaceae	*Asclepias*	5-7	1	0	28
	Fabaceae	*Aeschynomene abyssinica*		0	5	10
	Amaranthaceae	*Amaranthus spinosus*	1	0	4	5
	Solanaceae	*Datura stramonium*	3	0	2	0
	Commelinaceae	*Commelina benghalensis*	1.3-3	0	2	28
	Asteraceae	*Ageratum conyzoides*	0.5-2	0	0	13
Grasses	Poaceae	*Cynodon dactylon*	0.7-1.1	64	2	0
	Poaceae	*Sorghum halepense*	3.5	66	64	0
	Poaceae	*Panicum maximum*	1-2.1	114	16	30
	Poaceae	*Cynodon aethiopicus*	2.5-3	44	6	0
	Poaceae	*Dactyloctenium aegytium*	1.2	19	0	0
	Poaceae	*Urochloa mosambicensis*	1.3-2	13	4	0
	Poaceae	*Sporobolus pyramidalis*	0.5-1	11	3	0
	Poaceae	*Elymus repens*	0.2-1	8	0	0
	Poaceae	*Sorghum Bicolor*	2-6	4	0	1
	Poaceae	*Eleusine jaegeri*	1-2	3	0	34
	Poaceae	*Megathyrmus maximus*	2-4	2	0	0
	Poaceae	*Rottboellia cochinchinensis*	2-4	2	0	24
	Poaceae	*Bracharia*	1.1-2	0	2	0
	Poaceae	*Vulpia myuros*	0.75-4	0	0	2

[1]*The size range defines average length and width of weed seeds that are not circular in shape.*

DISCUSSION

In this study, it was found that different tillage and soil surface cover practices affect soil weed seed density and diversity and the effects change with season (*Table 3*). Conservation tillage and surface cover practices in general reduced soil weed seeds density and maintained diversity, with values being higher for samples from surface soil layer (0 – 15 cm) than deeper layer of 15 – 30 cm. The three methods used to study the density and diversity of weed seeds in soil gave different values requiring that the appropriate choice of method needs to be kept in mind when soil weed seeds are being studied. As such, the farming practices, soil depth, seasons and measurement approaches are important elements of studying soil weed seeds and developing sustainable weed management strategies.

The greenhouse germination method gave a good indication of the weed seed density compared to the other two methods and was able to detect some of the weed seeds below 0.5 mm size; Table 8, that

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was not detected by floatation and cloth bag method. This is because the germination method provided the highest density and provided a great outlook of germinable weed seeds. In order to obtain better results, the soil sample has to be maintained for an extended period, which in this case was 9 months. The disadvantage with greenhouse germination method is that it requires enough space and seeds that remain dormant and those that need specific environmental condition for germination cannot be detected. This may have resulted in underestimation of the species evenness, given that fewer seeds may have been detected due to dormancy causing lower diversity index although the species richness was high. However, both the Shannon Wiener and Simpson's diversity index takes into account species evenness. Weed seed size, dormancy, sieve size, soil type may lead to variations in soil seed bank estimation methods by affecting their efficiencies (DeMalach et al., 2021). Soil seed bank estimation by floatation and cloth bag methods involves filtering through filter paper and cloth, respectively. This means to be isolated and identified, weed seed size is a key factor. In the greenhouse germination, seed dormancy and conditions for germination may affect weed seed count. The Simpson's diversity index is more sensitive to species evenness in the community, whereas the Shannon diversity index is more sensitive to species richness (Travlos et al., 2018). Despite, the greenhouse emergence method detecting more species than the cloth bag at 26 and 22 weed species, respectively, it had a lower Simpson's diversity than cloth bag method due to lower species evenness. The cloth bag soil seed bank estimation method was more sensitive than the greenhouse emergency method to detect soil seed bank diversity as defined by Simpson and Shannon Weiner indices. The floatation method only detected 18 weed species, amounting to only 69% of the greenhouse germination method and only 32% of weed diversity compared to the greenhouse germination method. In the floatation method, filter paper was used to decant seed and floating organic matter residues, leaving small weed seeds to remain on the filter paper. However, their identification may have remained a challenge due to being mixed with organic residues.

The 0-15 cm soil layer contained 84% of the total weed seed density and 94% of the weed species observed in comparison with the 0-30 cm soil layer. Two weed species *Megathyrmus maximus* and *Cyperus rotundas* were not found in the 0-15 cm soil depth out of the 36 weed species detected, probably because *Cyperus rotundas* is vegetatively propagated and could not be detected in the two methods using direct seed extraction. The vegetatively propagated weeds have high chances of being sorted out as organic debris during preparation of the working sample. Also, *Megathyrmus maximus* could have had difficulties in germination due to seed dormancy or displaced from the 0-15 cm soil layer during tillage. Soil disturbance due to tillage tends to burry some seed below, since tillage practice may push the seed below 15 cm soil depth. Several studies have indicated that weed seed density and diversity decreased with increasing soil depth (Price et al., 2010; Yang et al., 2021). In line with this, a study from the Mediterranean grassland reported that 98.9% of seed that germinated were within 1 cm depth soil layer and the emergence percentage declined significantly with depth (Traba et al., 2004). This is more so because the soil is undisturbed, unlike in tilled agricultural fields.

The plots under conventional tillage especially those under sole maize was the most disturbed by tillage during weeding by use of hand hoe. In the plots under intercrops with soybean and sole soybean plots, weeding was mainly done by hand pulling through spot weeding, and similarly, for plots under minimum tillage. The soil depth of 15-30 cm in conventional tilled soils tended to have a higher relative ratio of soil seed density between 15-30 cm to 0-30 cm soil layer. This could have been influenced by tillage during land preparation and weeding. The fields used for this experiment were previously under conventional tillage and may be the major reason for the presence of weeds seeds in the 15-30 cm soil layer in the minimum tillage plots. Intercropping helps to reduce the intensity of tillage as seen from the lower soil seed

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densities in the 15-30 cm soil layer found in intercropped plots. Tillage affects the soil seed bank density and diversity compared to fields that are never tilled (Auškalnienė et al., 2018). In a study on different tillage practices that varied soil disturbance at different soil depths, the distribution of surface seeds through the soil profile was associated with the level of soil disturbance (Chauhan et al., 2006). In this study, tillage with ox-plough was done by using inversion-type ox-mouldboard ploughing (two times) at a depth of 15-25 cm, followed by harrowing and planting using a hand hoe at depth of 18-20 cm for the conventional tillage method. Soil cover through intercropping reduced the soil seed bank density probably due to suppression through competition for resources and allelopathy of above ground weeds (Sharma et al., 2021). This significantly reduced the soil seed bank density without altering the soil seed bank diversity, since the soil seed diversity under intercrops were not significantly different compared to sole maize (*Table 5, 6*).

Sole maize with mulching had the highest soil seed bank density probably because the mulch could have introduced additional weed seeds into the soil seed bank or the mulching rate of 6 t/ha may not be sufficient enough in the northern agroecology to be able to significantly suppress weeds. Seasons significantly affect soil seed bank diversity due factors such as aboveground vegetation, environment, water regime. The use of integrated weed management options has become very important to control weeds, specially to maintain ecological balance for co-existence to avoid obnoxious weeds and herbicide-resistant weeds.

CONCLUSION

The greenhouse emergence method emerged as the best method by providing the highest estimate of the soil weed seed density followed by the cloth bag and floatation, respectively. However, when it comes to weed diversity using Shannon Weiner and Simpson's diversity indices, the cloth bag method ranked highest followed by the greenhouse germination technique and lastly floatation method. For accurate results, sampling should be done on the 0-30 cm soil layer for disturbed agricultural land through tillage and 0-15 cm for undisturbed land. This allows accurate estimate of the soil seed bank as the below-ground weed seeds is related to the above-ground weed contributing to the development of an integrated weed management program. Soil cover through intercropping highly influenced soil seed bank density and not diversity, especially when practised under minimum tillage. Seasons highly influences soil seed bank; soil seed bank diversity was high in second season (B) compared to first season (A). The intercropping patterns where soybean was used as a soil cover significantly reduced the soil weed seed bank density and not diversity under minimum tillage. We recommend soil weed seed bank quantification to be integrated into a sustainable weed management control program and also the intercropping pattern with one line of soybean between maize line. This is because it reduced soil weed seed bank, yet less costly to implement than the other intercropping pattern mentioned in this study to produce similar results.

AUTHOR CONTRIBUTIONS

Otim Godfrey Anyoni developed the concept note, implemented and conducted data analysis. Dr. Obia Alfred and Associate professor Susan Tumwebaze contributed to the review of the research concept note and write up of manuscript. Kumakech Alfred contributed to revision of manuscript, Ocan David gave input to the concept note. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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