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Risk of weed seed and seedling emergence from poultry litter

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Abstract

In areas surrounding large poultry industries, poultry litter is often an alternative nitrogen fertilizer for crop production. However, farmers who have not used poultry litter in the past have concerns regarding potential weed seed contamination. A survey was conducted to determine the occurrence of germinable weed seed in poultry litters ($n = 61$) submitted by growers and industry representatives across North Carolina. In a 9:1 potting media:poultry litter mix, a single grass seed germinated from the 61 surveyed poultry litters, equating to 0.3 viable seeds 100 g^{-1} poultry litter. Viable seed content averaged 1.1 seeds 100 g^{-1} litter using the extractable seedbank method on 25% of the litters from the survey, much higher than the grow out method, and the majority of seeds found were *Amaranthaceae*. A growth chamber experiment was then conducted and demonstrated that there was a negative relation between poultry litter application and weed seedling emergence. There was a 65%, 75%, and 85% reduction in *Senna obtusifolia* (L.) H.S. Irwin & Barneby, *Setaria pumila* (Poir.) Roem. & Schult., and *Amaranthus palmeri* S. Watson germination, respectively, from the control to highest application rate of poultry litter (26.9 Mg ha^{-1}). A laboratory study showed that poultry litter leachates can decrease seed radicle length and integrity and is likely due to osmotic or salinity stress. The weed seed content in litter as well as the negative impact of poultry litter and its leachates on weed seedling emergence make it unlikely that poultry litter applications will significantly increase seedbanks above levels commonly observed in agricultural fields.

1 | INTRODUCTION

Poultry production has increased rapidly over the past 30 years in the United States, especially in North Carolina (NASS, 2021). With 30 million turkeys and 961 million broilers in 2020, poultry production in North Carolina has resulted in the widespread availability of poultry litter in the state. Poultry litter is a valuable fertilizer for crop production (Savala et al., 2016; Sosinski et al., 2022). However, there exist environmental concerns due to overapplication of poultry litter and increased soil test phosphorus concentrations, resulting in potential negative environmental impacts from phosphorus loss to the environment (Gatiboni et al., 2020). Transportation of poultry litter to areas farther from the area of production is often encouraged to avoid further accumulation of phosphorus in areas of production, but a concern of farmers who do not utilize poultry litter is the potential for weed seed introduction to the farm through the application of poultry litter (Pelletier et al., 2001).

Research suggests poultry litter specifically has a lower risk of contamination of weed seeds in comparison to other livestock manures due to the efficiency of the digestion process in poultry (Harmon & Keim, 1934; Rasnake, 1995). However, few studies have investigated weed seed occurrence from a variety of poultry litters generated in the region; instead, most of the work has focused on the potential contamination of feed and likelihood of residual viable seed in resulting manure (Harmon & Keim, 1934; Oswald, 1908). Additionally, those studies that tested multiple litters either sampled from few varying sources of litter when determining the effects of poultry litter on weed introduction and infestation in field trials, which does not account for variability in the content of viable weed seeds among manure sources (McGrath, 2009; Rasnake, 1995; Sumner et al., 2002). Since not all viable seeds within the litter will germinate over the study period, estimates of the impact of poultry litter application on weed infestation could be understated. Therefore, the goals of this study were to (1) capture the variation in viable weed seed found in poultry litters generated across the state of North Carolina to assess the risk of weed seed transport through land application of poultry litter and (2) determine how poultry litter application rate could affect weed seedling germination and emergence.

Core Ideas

- Poultry litters (n = 61) were assessed for weed seed contamination using germinable seedbank methods.
- Average viable seed concentration was 1.1 seeds 100 g^{-1} litter using a wet-sieving extractable seedbank method.
- Weed seeds were identified in 1.6% and 40% of poultry litters using grow-out and extractable seedbank methods, respectively.
- Application rate negatively affected the emergence of weed seed.
- Osmotic or salinity stress is likely a factor in seedling emergence when applying poultry litter.

2 | MATERIALS AND METHODS

2.1 | Experiment 1: Poultry litter survey

2.1.1 | Survey sample collection

Poultry litters were submitted in the fall of 2021 from 61 individual poultry farms located across North Carolina. No identifying information was collected to protect anonymity of the participating producers. Producers and industry representatives collected and submitted litter samples for assessment, and samples were stored in coolers until transport to the laboratory in Raleigh, NC. Approximately 1 kg was collected at each location. In North Carolina, it is recommended that poultry litter samples are a pool of subsamples collected from 10 to 12 locations in the house and that stockpiled waste be subsampled from at least 10 locations in the pile to a depth of 46 cm (Kulesza et al., 2021). Once transported to the laboratory, samples were refrigerated at 4°C until further analysis.

2.1.2 | Germinable seedbank study

An initial test comparing germination of seeds in either potting media or poultry litter showed complete inhibition of germination when seeds were planted into poultry litter alone (0% germination) as a result of a phytotoxic effect from the litter. Consequently, a second test was conducted evaluating seed germination at decreasing ratios of potting media to poultry litter to determine a safe threshold for seed germination. Five ratios (dry-weight basis) of potting media:poultry litter were selected (4:0, 3:1, 2:2, 1:3, and 0:4), and germination of three different species [*Lolium perenne* (L.), *Brassica napus* (L.), and *Triticum aestivum* (L.)] was tested. Each mixture (15 g), spiked with seeds, received 20 mL of deionized water to maintain moisture adequate for germination. The results of the second test showed a decline in germination as poultry litter inclusion increased, and even in a 3:1 potting media to poultry litter mix, germination was affected. Therefore, to ensure uninhibited germination for any seeds within the poultry litters collected, a dilution ratio of 9:1 potting media to poultry litter was selected as the safe threshold for seed germination for poultry litter treatments.

Germination studies were conducted using 20 g of a 9:1 potting media:litter mixture replicated five times on all 61 submitted litters. Litter was mixed with the potting media and added to a 100 mm by 25 mm (diameter by depth) Petri dish. The mixture was moistened with 25 mL of deionized water to ensure adequate conditions for germination, covered with parafilm, and incubated in a Conviron G30 growth chamber (Conviron) for 21 days with a constant relative humidity of 65% and alternating temperature of 18–24°C every 12 h. Moisture was maintained gravimetrically by weighing each dish and adding water as needed. At 2, 4, 6, 8, 10, 13, 17, and 21 days, germinated seeds were counted and removed from the Petri dishes. As positive controls, potting media alone and four of the potting media-litter mixes were spiked with three different weed species to verify the conditions were adequate for germination. Either 30 *Senna obtusifolia* (L.) H.S. Irwin & Barneby, 50 *T. aestivum*, or 50 *Brassica nigra* (L.) W.D.J. Koch seeds were added to individual Petri dishes at the beginning of the germination study and monitored over 21 days.

2.1.3 | Extractable seedbank study

The extractable seedbank presence in poultry litter samples (Reinhardt & Leon, 2018) was determined using a wet-sieving extractable seedbank method adapted from Wilson et al. (2022) on 25% of the samples (n = 15). There were 3, 20 g subsamples collected from 15 randomly selected poultry litters, and each sample was rinsed through a series of 7.6-cm-diameter sieves with 2.8-, 1.0-, and 0.425-mm mesh openings using deionized water. The material captured on each sieve was rinsed onto a filter paper with deionized water and dried at 35˚C until analysis. Once dried, the total weight of the material collected on each sieve size was measured, and seeds were counted and classified using a dissecting microscope and removed for assessment of viability. Seed viability was determined using the imbibed crush test, as described by Borza et al. (2007). Seeds were placed in a Petri dish containing a moist filter paper. After 24 h, seeds were crushed with forceps. If there was resistance to pressure prior to collapse and no indication of degradation, the seeds were considered viable.

2.1.4 | Statistical analysis

For the germination study, analysis of variance was conducted on the response of positive controls using a fixed effect model where weed species, substrate (potting media: litter mix or potting media alone), and their interactions were fixed effects. Data were analyzed using JMP Pro 15.2.

2.2 | Experiment 2: Poultry litter effect on weed seedling emergence

A pot experiment was conducted in a climate-controlled growth chamber at the North Carolina State University Phytotron to investigate the effects of poultry litter application rate on weed seedling emergence and early growth variables of selected weeds. Pots were placed in a growth chamber under ceramic metal halide lamps that provided 375 μmol m^{-2} s^{-1} of photosynthetically active radiation in a 14-h photoperiod. A diurnal temperature regime was programmed to 30˚C light and 20˚C dark with a relative humidity of 75%.

Soil from an agricultural field in Goldsboro, NC, was used as the potting medium. The soil was obtained from the Ap-horizon (approximately 15-cm deep) and is classified as a Kenansville loamy sand (loamy, siliceous, subactive, and thermic Arenic Hapludult). Initial soil characterization indicated an average pH value of 5.0, cation exchange capacity of 5.9 $cmol_c$ kg^{-1}, and P, K, Ca, and Mg concentrations of 386, 52, 562, and 84 mg kg^{-1}, respectively.

Poultry litter used for this experiment was obtained from a broiler farm in Randolph County, NC, analyzed for nutrient content (Table 1), and stored in a refrigerator at 4˚C until 3 weeks prior to the initiation of the experiment.

Three weed species were selected for this experiment: *Amaranthus palmeri* S. Watson, *Setaria pumila* (Poir.) Roem. & Schult., and *S. obtusifolia*. Weed seeds for *A. palmeri* and *S. pumila* were obtained from Azlin Seed Service, while *S. obtusifolia* seed was collected from the Central Crops Research Station in Clayton, NC (35˚40′ N, 78˚29′ W) in October of 2021. *Senna obtusifolia* seed was scarified with sandpaper for 15 s prior to the initiation of the experiment to reduce physical dormancy and increase germination. All seeds were stored in a refrigerator at 4˚C until the initiation of the experiment.

There were five treatments: poultry litter applied at 6.7, 13.6, and 26.9 Mg ha^{-1} (fresh-weight basis), chemical N fertilizer (340 g N kg^{-1}; 26% polymer-coated urea plus 8% ammonium sulfate) applied at a N rate of 120 kg ha^{-1}, and a negative control treatment that did not receive any poultry litter or fertilizer. The poultry litter treatments were selected to represent moderate, high, and very high application rates and provided plant-available N rates equivalent to 101, 205, and 406 kg ha^{-1} when assuming 50% of the total nitrogen is plant available.

Treatments were applied to pots (1650 mL, with a height of 15 and 14 cm top diameter) arranged in a completely randomized design replicated four times, resulting in a total of 60 pots. Pots were placed 20 cm apart from each other and were randomly rearranged weekly to avoid any bias due to location within the chamber. The experiment lasted 42 days, and two runs were conducted. Two-hundred seeds of *A. palmeri* and *S. pumila*, and 100 seeds of *S. obtusifolia* were planted at a depth of approximately 1 cm into the soil before poultry litter and chemical fertilizer treatments were applied by broadcasting on the soil surface. No other nutrients were supplied to the experimental units at any time during the experiment. All pots were maintained at 70% field capacity via capillary action by placing a tray under each pot and filled with 150 mL of DI water daily. All pots were surface misted daily for the first 14 days in order to prevent crusting of the top layer of soil and litter treatments.

Seedling emergence was evaluated every 2 days during the course of each experimental run. Emerged seedlings were counted and removed by hand. At the end of each experimental run, total seedling emergence for each treatment was determined as the summation of all emerged seedlings from 2 to 42 days after planting. The experiment was terminated at day 42 of each run, and the results are presented as the percent of emerged seeds out of the total number of seeds planted.

2.2.1 | Statistical analyses

Data were analyzed by weed species in JMP Pro 15.2. Analysis of variance was conducted with poultry litter rate, experimental run, and their interaction as fixed effects. If the

TABLE 1 Initial characterization of the poultry litter used in the growth chamber experiment.

pH	DM ($g\ kg^{-1}$)	C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	NH4-N ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)
8.5	775	245	23.4	5	18.4	19.3	3.8	6.1	439	389	415

Note: Dry matter concentration determined at 105˚C.
Abbreviation: DM, dry matter.

poultry litter rate by run interaction was significant ($p < 0.05$), experimental runs were analyzed separately. Percent germination for each species was log transformed to address normality. Results were fitted for linear and quadratic models, and the regression models were selected based on significance and best fit of the regression.

2.3 | Experiment 3: Toxicity of poultry litter leachates

A concentrated leachate solution was generated using a 1:4 poultry litter:deionized water mixture that was shaken for 5 min and filtered with a number 1 filter paper (Fisher Scientific). There were six poultry litter solution treatments of varying concentrations, with 0%, 1%, 5%, 10%, 20%, or 40% stock solution diluted with deionized water. Three seed types were selected to represent a variety of seed sizes and seed coat thicknesses: *Brassica alba* (L.) Rabenh, *T. aestivum*, and *L. perenne*. Fifty seeds of each species were placed on a filter paper in Petri dishes (60 mm by 15 mm) with 2 mL of the treatment solution, and each treatment was replicated 10 times. Dishes were kept in a germination chamber, alternating between lights on and 24˚C and lights off and 18˚C every 12 h for 7 days. At the conclusion of the study, percent germination, viability of remaining seeds, radicle length, and abnormal radicles were determined. Viability of remaining seeds was determined using an imbibed crush test, and abnormal radicles were identified visually. Radicle length was measured on five random seedlings per Petri dish, and the study was conducted twice.

To determine if the high electrical conductivity/ion concentration was the cause of seedling toxicity, leachate solutions were treated with Amberlite (Sigma-Aldrich), a mixed bed ion exchange resin, which removes ions from solution. Amberlite is a mixture of strong acid and strong base exchange resins, which allows for anion and cation removal. Each solution (25 mL) was treated with 5 g of Amberlite, shaken for 5 min, and filtered. Germination studies were conducted twice on all treatments with 10 replicates using *B. alba* as indicator species to determine the effect of ion removal on germination, viability of remaining seeds, radicle length, and abnormal radicles, as mentioned previously. Data were analyzed in JMP Pro 15.2. Analysis of variance was conducted, considering poultry litter solution, experimental run, and their interactions as fixed effects. Results were fitted for linear and quadratic models, and the regression was selected based on significance and best fit of the regression.

TABLE 2 Seed types and viability of seeds collected from 15 poultry litters collected across North Carolina.

Seed types	Total seed count	Number of viable seeds	Viability (%)
Amaranthus spp.	37	6	16.2
Poaceae	8	2	25.0
Polygonum sp.	6	1	16.7
Brassica spp.	1	0	0.0
Rumex sp.	1	1	100.0
Senna obtusifolia	1	0	0.0
Total	54	10	18.5

3 | RESULTS AND DISCUSSION

3.1 | Experiment 1: Poultry litter survey

Over the 21-day germination period, one grass seed germinated from the 61 poultry litter samples evaluated, which equates to 0.3 viable seeds 100 g^{-1} of material on average for the germinable seedbank study.

When germination was compared for positive controls, there were differences among weed species, but there was no effect of growth media (litter mix vs. potting media alone), indicating there was no inhibitory effect on seed germination with the 10-fold dilution of poultry litter in potting media. There was a higher germination rate for *T. aestivum* (96.5%) as compared to *S. obtusifolia* (89.1%) and *B. nigra* (88.5%).

In the extractable seedbank, a total of 54 seeds were retrieved from the 15 litters, with an overall viability of 18.5% (Table 2). Total weed seed content ranged from 0 to 20 seeds 100 g^{-1} litter, with 12 of the litters containing at least 1.7 seeds 100 g^{-1} litter. The extractable seedbank analysis of the weed seed content indicated a much higher estimate of viable weed seeds in poultry litters, with those containing viable seeds ranging from 1.7 to 5 seeds 100 g^{-1} litter or 1.1 seeds 100 g^{-1} litter on average, as compared to the 0.3 viable seeds 100 g^{-1} in the germinable seedbank study.

Amaranthus spp. were the most frequently occurring weed seeds, found in 11 of the 15 litters but viable in only 5 of the 11 litters. *Amaranthus* spp. accounted for 68.5% of the total seeds

TABLE 3 Emergence of *Amaranthus palmeri*, *Setaria pumila*, and *Senna obtusifolia* under different rates of poultry litter (x Mg ha^{-1}) in a growth chamber experiment.

Variable	Species	Run	Regression	R^2	p value
Seedling emergence (%)	*A. palmeri*	Combined	$y = 26.79 - 0.791x$	0.18	0.0056
	S. pumila	Combined	$y = 7.677 - 0.5847x + 0.0143x^2$	0.22	0.0372
	S. obtusifolia	1	$y = 32.79 - 3.592x + 0.08907x^2$	0.68	<0.0001
	S. obtusifolia	2	$y = 10.21 + 0.169x$	0.07	0.5497

and 60% of the viable seeds within the 15 litters. The next most frequently occurring weed seeds were Poaceae and *Polygonum* sp., which were found in 7 and 4 litters, respectively. Viable Poaceae seeds were identified in two of the litters, and a viable *Polygonum* sp. seed was found in only one litter. *Brassica* spp., *Rumex* sp., and *S. obtusifiolia* were each found in one litter, but *Rumex* sp. was the only viable seed of the three.

3.2 | Experiment 2: Poultry litter effect on weed seedling emergence

In general, seedling emergence declined in response to poultry litter rate for all three weed species (Table 3). A negative linear relationship was found between *A. palmeri* seedling emergence and poultry litter rate, decreasing from 26% germination in the control to 4.6% germination in the highest rate. These results coincide with what has also been observed in other greenhouse studies examining manure rate effects on germination of *Amaranthus rudis* J.D. Sauer from composted swine manure (Menalled et al., 2005) and *Amaranthus retroflexus* (L.) from composted dairy cattle manure (Amisi & Doohan, 2010), particularly at high application rates.

There was a significant rate effect on seedling emergence for *S. pumila* (p = 0.0096). A decreasing quadratic model was found to be significant (p = 0.0372) with a low-quality fit (R^2 = 0.22; Table 3). The highest emergence rate for *S. pumila* was in the non-treated control (7.6%) and declined to 2.3% at 26.9 Mg ha^{-1} of poultry litter. The extremely low seedling emergence for this species may be attributed to the variability in its germination response to temperature as it relates to dormancy stage (Dekker, 2003). In the future, different temperature regimes or longer studies might lead to better germination.

There was a significant rate × run interaction for *S. obtusifolia* (0.0001), and regression analysis indicated a negative quadratic relationship for run 1 (p <0.0001; Table 3) and no relationship in run 2. In run 1, the emergence was highest in the non-treated control (34.5%), and it decreased to 0% at 26.9 Mg ha^{-1} of poultry litter.

In general, poultry litter treatments, especially the two highest rates, exhibited lower seedling emergence than the N fertilizer control, suggesting that N was not likely the driving factor in emergence inhibition (data not shown). It has been well documented in the literature that animal manures (fresh and composted) can have phytotoxic effects toward weed seed germination (Amisi & Doohan, 2010; Larney & Blackshaw, 2003; Milotić & Hoffmann, 2016). Various chemical and biological attributes of the manures have been studied, including phenolic compounds, volatile organic acids, fatty acids, ammonia, salts, and increased microbial activity; however, no one characteristic was identified as the sole independent variable explaining the phytotoxicity.

Physical seed characteristics such as size and seed coat may also have potential effects on seedling emergence and dormancy, affecting moisture and oxygen uptake and the necessity of scarification for germination. In the case of *S. obtusifolia*, seed germination is stimulated by mechanical or chemical abrasion to its hard, waxy, and impervious seed coat (Creel et al., 1968), which also has the potential to protect the seed from phytotoxic compounds. In a laboratory experiment investigating the effects of vinasse (a by-product of ethanol production from plant biomass) on weed seed germination and growth, Soni et al. (2014) found that *S. obtusifolia* germination was the least affected compared to the small-seeded species *A. palmeri* and *Digitaria ciliaris* (Retz.) Koeler when planted in field soil mixed with increasing rates of vinasse, which has a high electrical conductivity. Because poultry litter can also have a high electrical conductivity, it is likely these salts had an effect on the germination of these species in the growth chamber study and that seed size and seed coat thickness played a role in their response.

3.3 | Experiment 3: Toxicity of poultry litter leachates

There was an interaction between leachate concentration, species, and experimental run for germination (p = 0.007; Table 4). In Run 1, there was a negative quadratic relationship between germination and poultry leachate concentration for *B. alba* and negative linear responses in *T. aestivum* and *L. perenne*. In Run 2, there were negative linear responses to poultry leachate concentration on germination in all three species. Despite the negative effect on germination, there were no clear responses in the viability of non-germinated

TABLE 4 Germination and presence of abnormal radicles in *Brassica alba*, *Triticum aestivum*, and *Lolium perenne* germinated in varying concentrations of poultry litter leachate.

Variable	Species	Run	Regression	R^2	*p* value
Germination (%)	*B. alba*	1	$y = 88.89 + 0.49x - 0.0136x^2$	0.13	0.0232
	T. aestivum	1	$y = 87.91 - 0.6518x$	0.37	<0.0001
	L. perenne	1	$y = 93.19 - 0.8983x$	0.30	<0.0001
	B. alba	2	$y = 94.17 - 0.3311x$	0.35	<0.0001
	T. aestivum	2	$y = 81.04 - 0.4899x$	0.12	<0.0001
	L. perenne	2	$y = 87.82 - 0.3650x$	0.11	<0.0001
Abnormal radicles (%)		Combined	$y = 11.18 + 1.494x$	0.29	<0.0001
Radicle length (mm)	*B. alba*	Combined	$y = 24.87 - 0.4771x$	0.23	<0.0001
	T. aestivum	Combined	$y = 66.70 - 1.162x$	0.37	<0.0001
	L. perenne	Combined	$y = 31.55 - 0.5067x$	0.13	<0.0001

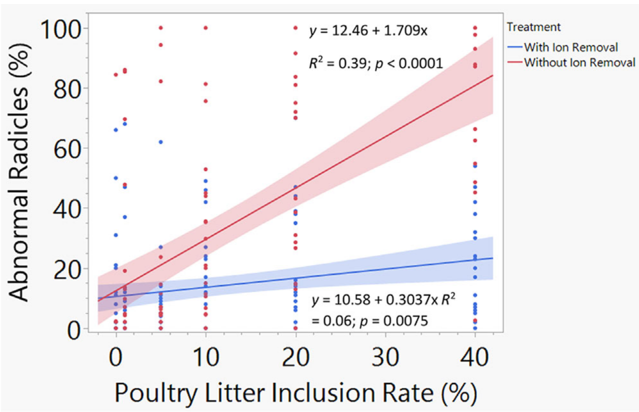

FIGURE 1 Percentage of abnormal radicles in *Brassica alba* treated with solutions with or without ion-removing resins at varying poultry litter leachate inclusion rates.

seeds when the imbibed crush test was performed on remaining seeds, suggesting that the toxicity of poultry leachates was acting on exposed tissues of seedlings (Leon & van der Laat, 2018). In fact, there was a positive relation between poultry leachate concentration and the percent of abnormal radicles ($p < 0.0001$; Table 4), which increased from 6% to 70% for 0% to 40% leachate concentration, respectively. Furthermore, radicle length in all species exhibited a negative relation with poultry leachate concentration ($p < 0.0001$; Table 4).

Considering the negative effect poultry litter leachates had on seedlings, it was evaluated whether the toxicity was caused by osmotic stress due to the high electrical conductivity of these materials in *B. alba*. Ion removal (IR) reduced the electrical conductivity of the solutions, which diminished radicle stunting and the occurrence of abnormal radicles. In IR solutions, the percent of abnormal radicles increased from a mean of 11% in water to 21% at 40% inclusion rate, which is far lower than that of the ion-containing solutions, with 82% at 40% inclusion rate (Figure 1). These results suggest that the concentration of ions in soil solution is likely a major driver of negative responses on weed seedling growth and establishment, as documented in the poultry litter survey and the growth chamber experiments.

3.4 | Practical considerations

The results indicated that the presence of viable weed seeds in poultry litter is possible and should not be completely discounted as a concern for receivers of these materials. However, the 0.3–5 viable seeds 100 g^{-1} of poultry litter detected here is much lower than viable seeds in dairy manure, which had an average of 7.5 seeds 100 g^{-1} when assessed using similar methods (Pleasant & Schlather, 1994). Additionally, application rates of dairy manure are much higher than poultry litter, which would increase the potential weed seed contamination and make poultry litter a much more attractive option in terms of risk should farmers have a choice between manure sources.

When litter is applied at the common rate of 6.7 Mg ha^{-1}, the anticipated seed distribution equates to 7.5 seeds m^{-2}, which would add an equivalent of <1.3% of the weed seedbank densities reported within the literature (Forcella et al., 1992; Jensen, 1969; Reinhardt & Leon, 2018; Roberts, 1981). Total viable seedbank densities ranged from 600 to 102,000 seeds m^{-2} in agricultural fields of Nebraska and Illinois, respectively (Forcella et al., 1992), and Jensen (1969) found a range of 600–496,000 seeds m^{-2} in a survey of fields in Denmark. Reinhardt and Leon (2018) found a mean seed density of 58 seeds 100 g^{-1} soil from a research station in Florida. Using a standard bulk density of a hectare furrow slice (2200 Mg), this would equate to over 1.7 billion seeds ha^{-1} in the top 20 cm of soil when using extractable seedbank methods similar to those used in this study. While the addition of poultry litter is unlikely to dramatically increase weed pressure, the high number of *Amaranthus* spp. seeds in poultry litter would be a major concern for growers without existing *Amaranthus* infestations, as species of this genus can produce hundreds of thousands of seeds per plant, or in regions with concern for the spread of herbicide resistance. Special attention should be paid to changes in weed communities when litter is applied in these situations.

It is not possible to know whether the viable seeds detected in the poultry litter survey went through the digestive gut of the animals in the poultry farms from where they were collected or if those seeds contaminated the bedding or litter after its production due to exposure to dispersal agents (e.g., animals and agricultural equipment) during storage, transportation, or handling. The research conducted by Harmon and Keim (1934) provided robust evidence that very few weed seeds survive being consumed by poultry, even including species with hard seed coats [e.g., *Abutilon theophrasti* Medik. and *Convolvulus arvensis* (L.)].

Based on our preliminary studies, seed germination in poultry litter is very unlikely unless it is greatly diluted, as required in the germinable seedbank study. Furthermore, the growth chamber and leachate experiments showed that poultry litter reduces weed seedling emergence. Therefore, the presence of viable seed in poultry litter from these germination and growth chamber studies should be considered with caution because this does not necessarily mean that weed seed germination and seedling establishment are certain in the field, where weather conditions and agricultural management might play important roles in determining those processes.

The results of the present study suggest that the use of poultry litter as a fertilizer likely presents a low risk of increasing weed seedbanks in agricultural fields. Future research should focus on the potential sources of weed seed contamination, with a focus on bedding sources, vegetation management surrounding animal housing and manure storage areas, and management options that minimize the percentage of viable weed seeds that germinate when a poultry litter is contaminated.

AUTHOR CONTRIBUTIONS

Stephanie B. Kulesza: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—original draft. **Ramon G. Leon**: Conceptualization; formal analysis; methodology; resources; writing—original draft; writing—review and editing. **Stephanie C. Sosinski**: Data curation; formal analysis; investigation; methodology; writing—original draft; writing—review and editing. **Grace M. Kilroy**: Investigation; methodology; writing—review and editing. **Brittani Meis**: Investigation; writing—review and editing. **Miguel S. Castillo**: Formal analysis; writing—review and editing. **Melissa L. Wilson**: Methodology; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Amisi, K. J., & Doohan, D. (2010). Redroot pigweed (*Amaranthus retroflexus*) Seedling emergence and growth in soils amended with composted dairy cattle manure and fresh dairy cattle manure under greenhouse conditions. *Weed Technology*, *24*, 71–75.

Borza, J. K., Westerman, P. R., & Liebman, M. (2007). Comparing estimates of seed viability in three foxtail (*Setaria*) species using the imbibed seed crush test with and without additional tetrazolium testing. *Weed Technology*, *21*, 518–522.

Creel, J. M., Hoveland, C. S., & Buchanan, G. A. (1968). Germination, growth, and ecology of sicklepod. *Weed Science*, *16*, 396–400.

Dekker, J. (2003). The foxtail (*Setaria*) species-group. *Weed Science*, *51*, 641–656.

Forcella, F., Wilson, R. G., Renner, K. A., Dekker, J., Harvey, R. G., Alm, D. A., Buhler, D. D., & Cardina, J. (1992). Weed seedbanks of the US corn belt: Magnitude, variation, emergence, and application. *Weed Science*, *40*, 636–644.

Gatiboni, L., Osmond, D., Hardy, D., & Kulesza, S. (2020). *Starter phosphorus fertilizer and additives in North Carolina soils: Use, placement, and plant response. SoilFacts Publication AG-439-75*. North Carolina Cooperative Extension Service. North Carolina State University.

Harmon, G. W., & Keim, F. D. (1934). The percentage and viability of weed seeds recovered in the feces of farm animals and their longevity when buried in manure. *Agronomy Journal*, *26*, 762–767.

Jensen, H. A. (1969). Content of buried seeds in arable soil in Denmark and its relation to the weed population. *Dansk Botanisk Arkiv*, *27*, 1–56.

Kulesza, S. B., Hicks, K., & Sharara, M. (2021). *Waste analysis (Soil Facts Publication AG-439-33). North Carolina Cooperative Extension Service*, North Carolina State University.

Larney, F. J., & Blackshaw, R. E. (2003). Weed seed viability in composted beef cattle feedlot manure. *Journal of Environmental Quality*, *32*, 1105–1113.

Leon, R. G., & van der Laat, R. (2018). Herbicidal and seed dormancy induction activity of fermentation residual vinasse. *Weed Science*, *66*, 317–323.

McGrath, S. R. (2009). *Poultry litter as a nutrient source for low input forage systems* (master's thesis). Virginia Polytechnic Institute and State University.

Menalled, F. D., Buhler, D. D., & Liebman, M. (2005). Composted swine manure effects on germination and early growth of crop and weed species under greenhouse conditions. *Weed Technology*, *19*, 784–789.

Milotić, T., & Hoffmann, M. (2016). Reduced germination success of temperate grassland seeds sown in dung: consequences for post-dispersal seed fate. *Plant Biology*, *18*, 10380–1047.

[NASS] USDA National Agricultural Statistics Service. (2021). *NASS–Quick stats*. USDA National Agricultural Statistics Service. https://data.nal.usda.gov/dataset/nass-quick-stats

Oswald, E. I. (1908). *The effect of animal digestion and fermentation of manures on the vitality of seeds* (Bulletin 128). The Maryland Agricultural Experiment Station, University of Maryland.

Pelletier, B. A., Pease, J., & Kenyon, D. (2001). *Economic analysis of Virginia poultry litter transportation*. Virginia Agricultural Experiment Station (Bulletin 01–1). Virginia Polytechnic Institute and State University.

Pleasant, J. M., & Schlather, K. J. (1994). Incidence of weed seed in cow (*Bos* sp.) manure and its importance as a weed source for cropland. *Weed Technology*, *8*, 304–310.

Rasnake, M. (1995). *Weed seed in poultry litter: Should farmers be concerned?* Soil Science News and Views. https://uknowledge.uky.edu/pss_views/100/

Reinhardt, T., & Leon, R. G. (2018). Extractable and germinable seedbank methods provide different quantifications of weed communities. *Weed Science*, *66*, 715–720.

Roberts, H. A. (1981). Seed banks in soils. *Advances in Applied Biology*, *6*, 1–55.

Savala, C. E. N., Crozier, C. R., & Smyth, T. J. (2016). Poultry manure nitrogen availability influences winter wheat and yield components. *Agronomy Journal*, *108*, 864–872. https://doi.org/10.2134/agronj2015.0355

Soni, N., Leon, R. G., Erickson, J. E., Ferrell, J. A., Silveira, M. L., & Giurcanu, M. C. (2014). Vinasse and biochar effects on germination and growth of Palmer amaranth (*Amaranthus palmeri*), sicklepod (*Senna obtusifolia*), and southern crabgrass (*Digitaria ciliaris*). *Weed Technology*, *28*, 694–702.

Sosinski, S., Castillo, M., Kulesza, S., & Leon, R. (2022). Poultry litter and nitrogen fertilizer effects on productivity and nutritive value of crabgrass. *Crop Science*, *62*, 2537–2547.

Sumner, D. R., Hall, M. R., Gay, J. D., MacDonald, G., Savage, S. I., & Bramwell, R. K. (2002). Root diseases, weeds, and nematodes with poultry litter and conservation tillage in a sweet corn–Snap bean double crop. *Crop Protection*, *21*, 963–972.

Wilson, M. L., Brusa, A., Christensen, H., Strack, S., Alto, E., Allen, L. F., Cortus, S. D., Moderman, C., & Becker, R. L. (2022). Comparison of methods to recover amaranth weed seeds from manure. *Agricultural & Environmental Letters*, *7*, e20065.

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