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Integrated weed management for reduced weed infestations in sustainable cropping systems

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Integrated weed management for reduced weed infestations in sustainable cropping systems

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Editorial: Integrated weed management for reduced weed infestations in sustainable cropping systems

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Editorial on the Research Topic

Integrated weed management for reduced weed infestations in sustainable cropping systems

Summary

Weeds are a major biotic constraint of agricultural systems worldwide interfering with crop production and resource use efficiency (Oerke, 2006; Colbach et al., 2020). Chemical control is a cost- and time-effective weed management method and for that reason remains as the most widely and frequently used method to sustain agricultural productivity and food security in the current era. However, repeated use of a limited number of herbicide active ingredients in non-diversified crop rotations enhances the selection of herbicide-resistant weed biotypes. The over-reliance on chemical weed control has led to shifts in weed communities (Mahaut et al., 2019) which are now becoming dominated by highly competitive and herbicide-resistant prone species able to cause significant yield losses (Adeux et al., 2019b). Widespread herbicide resistance (Heap, 2023) accompanied by the increasing concern of herbicides entering the food chain and/or impacting the environment has created a tremendous demand for alternative weed management methods.

Alternative weed management practices that reduce weed populations indirectly lowers selection pressure thus helping delay the evolution of further herbicide resistance. Controlling weeds during the critical period of weed removal is paramount for achieving the full yield potential of any crop (Zimdahl, 1988; Colbach et al., 2020). In conservation tillage with cover cropping, research on the critical period of weed removal is warranted to further elucidate cover crop weed suppressive attributes and efficient utilization of herbicides (Kumari et al.). Preventive weed control measures include all the possible means that restrict the entry and establishment of weeds in an area. Cultural control is an ecological method of weed control in which good crop management methods are followed to stimulate rapid crop growth and canopy closure (Petit et al., 2018). Cultivar selection, planting time, seeding rate and method, fertilizer rates, and water management are some of the critical agronomic management decisions that can not only impact yield but also the time of crop and weed emergence thus crop-weed interactions (Kaur et al., 2018). The use of organic or plastic mulch is another alternative weed control strategy, mainly for specialty crops (Schonbeck, 1999). Physical weed control measures such as burning, flame weeding, and soil steaming can be used to effectively control weed emergence and growth through plant or seed exposure to high temperatures. An integrated approach incorporating soil steaming, cover crops, and mulching can result in reduced herbicide reliance and effective weed control (de Oliveira et al.).

Complete reliance on any one weed management practice, either chemical or non-chemical, may fail within a relative short time due to the rapid evolutionary ability of weeds (Neve et al., 2009). Integrated weed management (IWM) relies on a combination of multipronged measures deployed in a compatible manner aimed at reducing weed populations while sustaining the crop yield potential (Swanton and Weise, 1991; Kudsk, 2022). In IWM systems, cultural, mechanical, biological, and/or chemical strategies can be deployed to reduce weed seed germination, establishment, crop-weed competition, and the influx of weed seeds into the soil seedbank. While non-chemical weed management options are largely exploited in organic agricultural systems, the use of bioherbicides is an area that warrants further research (Cordeau et al., 2016; Triolet et al., 2020). The use of biocontrol agents can also be exploited for control of troublesome weeds such as *Puccinia punctiformis* for control of *Cirsium arvense* (Chichinsky et al.).

IWM decision-making process relies on environmental information, weed biology and ecology to control weeds in the most economical and sustainable possible way (Sanyal, 2008). Various methods, such as weed seed predation with granivorous fauna-ants, selective weeding of escaped weed plants, uprooting/hand pulling of weeds before seed setting, mechanical weed seed harvest, chaff lining, etc., can be used to prevent the spread and seedbank enrichment of weeds. The dispersal of weed seeds and vegetative propagules allow their territorial expansion (Benvenuti, 2007; DiTommaso et al., 2018). Dispersal of weed seeds is facilitated by many dispersal agents including wind, water, soil, crops, manure, and animals. However, ensiling conditions, livestock ingestion, and manure management can reduce weed seed viability thus be effective integrated non-chemical weed management options (Asaduzzaman et al.).

Weed distribution and management surveys can be important decision-support tools to identify common weed management challenges and the short- and long-term impact of IWM and other practices on weed populations. For instance, information gathered from the survey by Butts et al. provided direct insights into current rice weed management practices and a better understanding of current concerns. Crop-weed competition modelling can help defining the relationship between crop yield loss and weed density (or biomass) accounting for specificities of the weed species, crop and location. The shifts in weed spectrum and weed emergence time may affect the yield density equation, and improved knowledge of weed emergence periodicity may be used to enhance management tactics (Brown et al.). Models of crop-weed interference can contribute to improved weed management strategies and evaluation of weed control programs (Singh et al., 2020; Colbach et al., 2021).

Simplified cropping systems/rotations create and maintain a favorable environment for annual weeds whose emergence and growth phenology are similar to these crops, and its diversification may contribute to effective weed control. Practicing the same cropping sequence year after year leads to the simplification of management practices, including herbicide programs, which may eventually result in increased weed pressure threatening the sustainability of crop production. Crop diversification is an important component of IWM programs (Adeux et al., 2019a), and is one of the three principles in conservation agriculture systems (FAO, 2021; Cordeau, 2022). Differences in crop phenology and diverse management tactics can lead to a net loss in weed seed population density and composition in the soil seed bank, and reduced weed biomass (Liebman and Gallandt, 1997; MacLaren et al., 2020) (Nguyen and Liebman, Nguyen and Liebman). Cropping systems affected the germination patterns of most of the weed species due to differential selection pressures of IWM practices followed in these systems (Cordeau et al.). Rotating crops with dissimilar life cycles, or crops which require different agronomic practices, can help interrupt the weed life cycle. A change in the crop facilitates the change in planting time of the crop and use of different weed control practices along with herbicide rotation; thus, provide effective management of a particular weed species. Long-term cropping system experiment can be a powerful tool to compare the short and long-term outcomes of IWM strategies.

Besides providing effective weed suppression (Osipitan et al., 2018; Rouge et al., 2023), well-managed cover crops perform other ecological functions such as accumulating soil organic carbon, moderating soil temperature, improving water infiltration, improving water storage, reducing soil erosion, and reducing nitrate leaching. However, few studies showed that cover crops had no effect on weeds in the subsequent crops when cover crop did not accumulate enough biomass to impact weeds emergence through a mulch effect, or when cover crops were terminated by tillage and/or when in-crop weed management relied on herbicides, and concluded that intensive weed management could override the potential effect of cover crops on weeds in the subsequent crops (Adeux et al., 2021).

Conclusions

Weeds pose a major challenge to the sustainability of agricultural production systems, causing significant crop yield and economic losses. Chemical weed control tactics play a major role in weed management, maintaining the productivity of diverse cropping systems, reducing yield losses and facilitating conservation agriculture. However, limiting the reliance on a unique management lever, regardless its efficacy or cost, is critical for the sustainability of all cropping systems. IWM aims to diversify weed management strategies mainly by the means of non-chemical control methods, so that reliance on herbicides can be reduced (Shaner, 2014). IWM strategies involve a combination of physical, chemical and biological tools in an integrated way, without excessive reliance on any single measure. IWM can be a successful approach for managing the herbicide-resistant weeds and sustain crop production and global food security. Innovative and feasible IWM systems may be designed for diverse production situations that can reduce weed infestations and environmental impacts, and prolong the use of herbicides. Further improvement in the implementation of IWM approach requires support from governmental agencies, extension services, social scientists, marketing professionals, the crop protection manufacturing and distribution industry, along with weed scientists and farmers.

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Legacy Effects of Contrasting Long-Term Integrated Weed Management Systems

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To reduce reliance on herbicides and maintain crop productivity, integrated weed management (IWM) seeks to optimize synergies between diverse sets of weed management practices combined at the cropping system scale. Nevertheless, data on weed community response to the long-term implementation of IWM practices remain scare. Here, we assessed the effects of four IWM systems with contrasting objectives and practices (S2: transition from superficial tillage to conservation agriculture; S3: no-mechanical weeding; S4: mixed mechanical and chemical weeding; S5: herbicide-free; all with 6 year rotations) compared to a conventional reference (S1: herbicide-based with systematic plowing and a 3 year rotation) on taxonomic and functional weed community composition and structure after 17 years of continuous implementation. We examined the legacy effects of these systems with a uniformity trial consisting of winter wheat managed uniformly across the systems as well as with a novel *in situ* weed seedbank approach involving tilled strips. We found that resulting weed communities in IWM systems were more species rich (species richness from 1.1 to 2.6 times greater) and more abundant (total density from 3.3 to 25 times greater) than those observed in the reference system, and differed in term of taxonomic and functional composition. In addition, we found that, when systems shared the same weed species, germination patterns of two thirds of the species differed between systems, highlighting the selection pressures some IWM practices exert on weeds. We showed that analyzing the superficial germinable seedbank *in situ* with tilled strips could provide a comprehensive view of resulting weed communities and be helpful in developing cropping systems that foster agroecological weed management.

Keywords: seedbank, uniformity trial, no-till, herbicide reliance, weed community, functional trait, germination pattern

INTRODUCTION

Effective weed management is recognized as crucial for the ecological intensification of agriculture (Petit et al., 2015) because weeds can generate severe yield losses (Oerke, 2006) and current approaches to weed management rely heavily on herbicides. To reduce herbicide reliance and maintain crop productivity, integrated weed management (IWM) strategies aim to both disrupt weed population dynamics and reduce weed interference by coherently combining a diversity of chemical, physical, and cultural weed management practices at the cropping system scale (Harker, 2013). Ideally, these practices are combined in ways that optimize synergistic interactions among practices by targeting weeds in complementary ways and at different stages in their life cycles (Ryan et al., 2011). In doing so, effective IWM strategies keep weed populations below economic thresholds while maintaining or enhancing weed diversity (Liebman and Gallandt, 1997). However, there are surprisingly few examples of long-term research comparing different combinations of IWM practices on weed communities, which could enable the identification of successful IWM approaches and facilitate decision making (Harker and O'donovan, 2013). An effective IWM strategy must diversify selection pressures in time to avoid shifts toward weed communities dominated by competitive species (Adeux et al., 2019b) or those adapted, either via evolution or morphological or phenological plasticity, to avoid or resist management (Menalled et al., 2016). The degree to which contrasting IWM strategies can shift the ecology and/or biology of arable weeds over the long-term is largely unexplored but of critical importance to move toward more sustainable alternatives to herbicides (Neve et al., 2018).

Long-term cropping system experiments can be powerful tools with which to compare the short and longer-term outcomes of contrasting IWM strategies aimed at controlling weeds while reducing herbicide reliance (Adeux et al., 2019a). Lechenet et al. (2017) reviewed experimental designs for cropping system experiments intended to test pest management principles. They concluded that to ensure an efficient comparison of cropping systems, the most complete design requires the implementation of (i) temporal replicates of the cropping systems where all the crops of the crop sequence are cultivated each year (i.e., all entry points), and (ii) spatial replicates where all entry points are replicated within blocks the same year. Unfortunately, most experimental designs of cropping system experiments do not include both aspects because of space or labor issues and are therefore prone to misinterpret the temporal dynamics of the response variables (Lechenet et al., 2017). Diversified crop sequences appear as a critical component of IWM across a diversity of situations (Weisberger et al., 2019) because each crop and its associated practices will act as a set of filters that can disrupt different phases of the weed species' life cycle (Derksen et al., 2002). However, when cropping system experiments lack temporal replicates, weeds are rarely assessed across a common baseline, i.e., the same crop cultivated the same year in all plots. To overcome this issue, previous studies have investigated either the weed seedbank as a method for revealing the effects of past practices (Bàrberi and Lo Cascio, 2001) or the weed flora emerging in a reference crop as part of a uniformity trial (Brown and Gallandt, 2018). Rarely are both of these approaches implemented together (Jernigan et al., 2017). Uniformity trials—in which a standard agronomic treatment is applied across all plots at the end of a cropping system experiment in order to partition of variability due to previous cropping system effects, without any confounding effects—can provide critical insight on the legacy effects associated with the integration of practices across diverse crop rotations (Jernigan et al., 2017).

The objective of this study was to quantify weed seedbank and emerged weed communities after 17 years of five contrasting IWM strategies in a long-term cropping system experiment. The nature and magnitude of these legacy effects were assessed in a reference crop (winter wheat) managed uniformly across the cropping system experiment in year 18. We hypothesized that weed seedbanks would be more diverse (both in term of taxonomic and functional diversity) and more abundant in the IWM systems compared to the reference system due to the higher diversity of crop functional types (winter annuals, summer annuals, etc.) in the IWM systems coupled with incomplete control of weeds in each crop. Secondly, we hypothesized that weed community composition would differ both taxonomically and functionally among the contrasting IWM systems, due to differences in their overarching management strategies (e.g., balance between chemical, physical, and cultural approaches to weed management). Finally, we hypothesized that some weeds would exhibit shifts in their germination phenology (manifesting in differences in the timing and duration of peak emergence from the soil seed bank) across contrasting IWM systems due to different selection pressures imposed by each system.

MATERIALS AND METHODS

Experimental Site and Set-Up

The field experiment was conducted at the INRAE experimental farm in Bretenière (47°14′11.2″ N, 5°05′56.1″ E), 15 km southeast of Dijon, France. The experiment was set up as a randomized complete block design (two blocks separated by 1 km) and included five cropping systems with contrasting IWM objectives and practices. The reference cropping system (S1) was characterized by a 3-year oilseed rape—winter wheat—winter barley rotation, systematic moldboard plowing in summer-autumn, and herbicides as the sole curative weed management tool. S1 is a typical grain-based cropping system of the Burgundy region, designed to maximize financial return. The other four cropping systems (S2, S3, S4, and S5) were designed to mimic contrasting agronomic pathways that farmers might implement to reduce herbicide reliance and resulted in a more complex 6-year rotation. The complete crop sequence is detailed in **Supplementary Table 1**. Crop rotations in S2, S3, S4, and S5 systems were made of three winter sown crops (winter wheat, winter barley, triticale, or faba bean), autumn sown oilseed rape, one spring crop (oat, sugarbeet, faba bean, lupin, spring barley, or mustard) and one summer sown crop (maize, sorghum, soybean, or sunflower). Hence, winter wheat and oilseed rape, the two most common crops of the region, were present throughout the five CS. Sugar beet was only cropped in S4 (up to 2006 when the nearby sugar refinery plant closed). In S5, perennial forage crops such as alfalfa were included in order to manage Canada thistle (*Cirsium arvense*) or bitter dock (*Rumex obtusifolius*). Similarly, in S3, companion crops (such as faba bean, lentil, vetch, flax) were intercropped in oilseed rape to cover interrows before winter.

System S2 represented a typical transition from a reduced tillage system (i.e., no inversion tillage from 2001–2010) to no-till conservation agriculture (2010–2017). S2 was designed to reduce labor requirement and time consuming operations. In contrast, systems S3, S4, and S5 all implemented moldboard plowing every 2 years on average over the 2001–2017 period. Herbicides were used as the sole method of direct weed control in S2 and S3. This choice was made in coherence with the strategy of minimum soil disturbance in S2 and to reflect the wish of certain farmers to not invest in mechanical weeding tools in S3. Weed management relied on mechanical tools and herbicides in S4 and only on mechanical tools in S5 (Adeux et al., 2019a). In addition, the four alternative systems also implemented a wide array of preventive and cultural weed management tools such as false seedbed technique, delayed sowing of winter cereals, and higher seeding rates. S4 aimed to be the typical IWM system, resorting preferentially to preventive measures, and mechanical weeding. However, applications of specialized herbicides on target species remained possible when weather conditions were not suitable for mechanical weeding or to control weeds with low sensibility to mechanical weeding.

The set of decision rules characterizing each of the five cropping systems was replicated on two blocks, resulting in 10 plots of 1.7 ha each on average. A complete description of the long-term cropping system experiment (crop sequence—see **Supplementary Table 1**—and associated management, including tillage intensity/frequency, herbicide use/types, mechanical weeding, etc.) implemented from 2000 to 2017 is available in Adeux et al. (2019a, 2022) and synthesized in **Table 1**.

In 2017–2018, a uniformity trial with winter wheat was established across the experimental site in order to examine the legacy effects of the previous cropping systems. Soil preparation prior to sowing winter wheat consisted in stubble cultivation to 8 cm deep (10/10/17) and rotary harrowing, also 8 cm deep (13/10/17). All fields were sown with winter wheat (variety "Nemo") at a rate of 350 seeds.m^{-2} and a sowing depth of 3 cm (15/10/17). Sowing was performed with a mechanical seed drill (Amazone D9) set at a 13.8 cm row spacing. Nitrogen fertilization was split into two applications, i.e., 50 kg N.ha^{-1} on 22/02/18 and 116 kg N.ha^{-1} (+ 33 kg S.ha^{-1}) on 10/04/18. Septoria leaf spot was controlled on 07/05/18 with 50 g.ha^{-1} of benzovindiflupyr, 40 g.ha^{-1} of cyproconazole, and 375 g.ha^{-1} of chlorothalonil. Wheat yellow rust was controlled on 16/05/18 with 50 g.ha^{-1} of benzovindiflupyr. Weed control in winter wheat was performed late (one application of herbicide on 22/03/18) so as to allow full expression of the autumn- and spring-emerging weed flora and consisted in a tank mixture of 9 g.ha^{-1} of mesosulfuron, 60 g.ha^{-1} of diflufenican, 3 g.ha^{-1} of iodosulfuron, and 25 g.ha^{-1} of amidosulfuron.

Measurements

Weed Seedbank Assessments

During the cropping system experiment phase (i.e., 2001–2017), the soil seedbank was assessed in 2001, 2002, 2003, 2005, and 2010, and not repeated afterwards, by collecting 10 soil samples per field each year (each sample being a composite of 10 soil cores, 4.5 cm diameter, 30 cm depth) from the same 100 m² zone in each field each year. Each core was split into superficial (0–10 cm depth) and deep (10–30 cm) soil horizons before pooling. The weed seeds were extracted from the soil samples using a sieving method (with different sieves until 400 μm) and then submitted to a germination bioassay in greenhouse (Mahé et al., 2021). The emerged seedlings of each species were identified at the species level (when possible) and counted. After 1 year of succeeding cohorts, the residual seeds were identified and counted under a dissecting scope. Based on the diameter of the soil probe, seedbank density was then expressed as seed.m^{-2}, both in the superficial and deep soil horizons.

Legacy effects of the cropping systems on the germinable soil seedbanks were assessed *in situ* during the uniformity trial (i.e., 2017–2018) using a false seedbed technique. Seedling emergence from the superficial seedbank was stimulated by shallowly tilling (10 cm depth) two strips per field (4 m wide, about 200 m long) every 6 weeks during the autumn and spring growing seasons (except when weather conditions were unsuitable for implementing tillage, **Supplementary Figure 1**). In total, six sessions of tillage were implemented (05/08/2017, 14/09/2017, 29/10/2017, 13/02/2018, 12/04/2018, 25/05/2018; **Supplementary Table 2**). Weed surveys, in which all emerged seedlings within 10 0.36 m² quadrats per strip were identified and counted, were performed immediately prior to each tillage session, i.e., approximatively 6 weeks after the previous tillage (12/09/2017, 25/10/2017, 06/12/2017, 09/04/2018, 18/05/2018, 18/06/2018; **Supplementary Table 2**) so as to allow the maximum number of individuals of each species to emerge (Cordeau et al., 2017b).

Weed Communities in the Uniformity Trial

The legacy effect of cropping systems on weed communities was also investigated by assessing emerged weed flora in a winter wheat crop uniformity trial. Weed community composition was assessed before weed control (08/02/18) in eight 16m² zones per plot. No herbicide or mechanical weeding was done in autumn or early spring, prior to the weed survey, to capture maximum weed diversity. Weeds were identified at the species level (when possible) and the abundance of each species in the 16 m² area was estimated visually using the scale of abundance developed by Barralis (1976) with sux classes (one individual, <1 individuals m^{-2}, 1–2, 3–20, 21–50, and 51–500 individuals.m^{-2}). Total weed abundance was computed using the center of each class (0.06, 0.5, 1.5, 11.5, 35.5, and 275 individuals.m^{-2}, respectively) to allow the aggregation of individual species' abundances. Species richness was computed as the number of weed species per 16 m^2 zones. Community weighted means (CWM, average value of a given attribute weighted by the relative abundance of each species) were also computed on three attributes [height, seed mass, specific leaf area (SLA)] reflecting weed community response to past agricultural practices (Storkey et al., 2010; Gaba et al., 2017).

Statistical Analysis

All analysis were carried out at the soil sample (seedbank in the cropping system experiment), quadrat (superficial germinable seedbank in uniformity trial) or 16 m² zone level (weed survey

TABLE 1 | Differences in terms of farming practices between experimented cropping systems (over the 2012–2017 period).

Farming practices	Experimented cropping system effect ($df = 4$)	S1	S2	S3	S4	S5
Plowing frequency	$F = 47.67$, $P = 0.001$	0.83 ± 0.00c	0.00 ± 0.00a	0.42 ± 0.12b	0.42 ± 0.12b	0.67 ± 0.00bc
Average number of false seedbed operations year^{-1}	$F = 7.24$, $P = 0.041$	1.75 ± 0.12ab	0.00 ± 0.00a	2.58 ± 0.59b	2.67 ± 0.24b	2.08 ± 1.30ab
Frequency of delayed sowing of winter cereals	$F = 12.53$, $P = 0.016$	0.00 ± 0.00a	0.75 ± 0.35b	0.83 ± 0.24b	1.00 ± 0.00b	0.58 ± 0.12ab
Average HTFI year^{-1}	$F = 32.31$, $P = 0.003$	1.34 ± 0.18bc	1.97 ± 0.18c	0.82 ± 0.26ab	0.50 ± 0.16a	0.00 ± 0.00a
Average HTFI before sowing year^{-1}	$F = 639.7$, $P < 0.0001$	0.02 ± 0.02a	1.05 ± 0.06b	0.02 ± 0.03a	0.01 ± 0.01a	0.00 ± 0.00a
Average number of mechanical weeding operations year^{-1}	$F = 37.38$, $P = 0.002$	0.00 ± 0.00a	0.00 ± 0.00a	0.17 ± 0.24a	1.83 ± 0.00b	2.92 ± 0.58b
Average nitrogen fertilization kg N year^{-1}	$F = 29.14$, $P = 0.003$	154 ± 8b	94 ± 6a	96 ± 5a	109 ± 2a	79 ± 12a
Proportion of autumn-sown crops	$F = 6.00$, $P = 0.055$	0.67 ± 0.04b	0.50 ± 0.04ab	0.50 ± 0.04ab	0.50 ± 0.04ab	0.42 ± 0.04a
Number of crops	$F = 8.60$, $P = 0.03$	3.00 ± 0.35a	5.00 ± 0.35ab	5.50 ± 0.35b	5.00 ± 0.35ab	5.50 ± 0.35b
Number of sowing periods	Perfect fit*	2.00 ± 0.00a	4.00 ± 0.00b	4.00 ± 0.00b	4.00 ± 0.00b	4.00 ± 0.00b

Effects were determined through F-tests on linear models. Values (observed means ± standard error) were computed over the rotation and standardized at the annual scale. Cropping systems sharing identical letters are not significantly different at $P < 0.05$.

*Perfect fit denotes a model where each level of the factor shows no variability, i.e., $R^2 = 1$.

in uniformity trial) with the [*lme4*] package of the R software version 3.3.2 (R Development Core Team, 2019). Linear mixed-effects models were used to analyse continuous response variables [$\log_{10}$ $(x + 1)$ transformed weed density, CWM variables] whereas mixed Poisson regression with a log-link was used to analyse whole, non-negative response variables (species richness) and mixed beta-regression with a logit-link were used to analyse proportion data (percent of seeds in the top soil horizon). All response variables were regressed against cropping system, and sometimes in interaction with year (seedbank in the cropping system experiment) or the weed survey session (superficial germinable seedbank). Field and block were considered as random effects in all models to account for the design of the experiment and the sampling design (see the 17-year crop sequences in **Supplementary Table 1**). Significance of cropping system effects were assessed through type III Wald Chi-squared tests using the ANOVA function of the [*car*] R package. Contrasts between the cropping systems were adjusted using the [*emmeans*] R package. Except for beta-regression, the quality of the model was assessed using marginal R^2 accounting for the fixed effects (R^2m) and conditional R^2 accounting for the fixed and random effects (R^2c).

Partial canonical correspondence analysis (pCCA) was performed to visualize and assess cropping system legacy effects on the superficial germinable weed seedbank (all sessions pooled) and emerged weed community composition before weed control in winter wheat. Field and field:block effects were partialled out to highlight cropping system effects. Only species with frequencies of occurrence ≥5% of the quadrats (superficial germinable seedbank) or zone (emerged weeds) were retained and were described by their abundance. Significance of cropping system effects was assessed using permutation-based ANOVA ($N = 999$ permutations).

To test for differences in species emergence patterns in the superficial germinable seedbank of the uniformity trial, kernel density weighted by the relative abundance of each species at each session was estimated using the *density* function and the approach described in several recents studies (Perronne et al., 2014; Bourgeois et al., 2019). We computed the dissimilarity of emergence distribution using the *overlapTrue* function from the [*overlap*] package. In order to test the significance of the dissimilarities between each pair of systems where the same species was observed, we used a randomization method to determine whether the overlap between species emergence was significantly lower than the null hypothesis (random layout of the weed community between systems). The randomization procedure was implemented by generating random permutations of the variable "cropping system" to randomize the weed emergence in each quadrat at each session. Thus, we generated 10,000 random distributions and implemented a one-tailed direct test of significance for the non-random structure. *P*-values were estimated as the proportion of random distributions having a value of overlap lower than the observed overlap.

RESULTS

Seedbank Dynamics During the Cropping System Experiment

A total of 53 species were observed in the soil seedbank from 2001 to 2010. The five most abundant species, representing 82% of total abundance, were *Alopecurus myosuroides, Solanum nigrum, Anagallis arvensis, Chenopodium album,* and *Amaranthus retroflexus*. The majority of species (i.e., 50) were found in both the superficial (0–10 cm) and deep soil horizon (10–30 cm). Only *Veronica agrestis* was found solely in the deep soil horizon

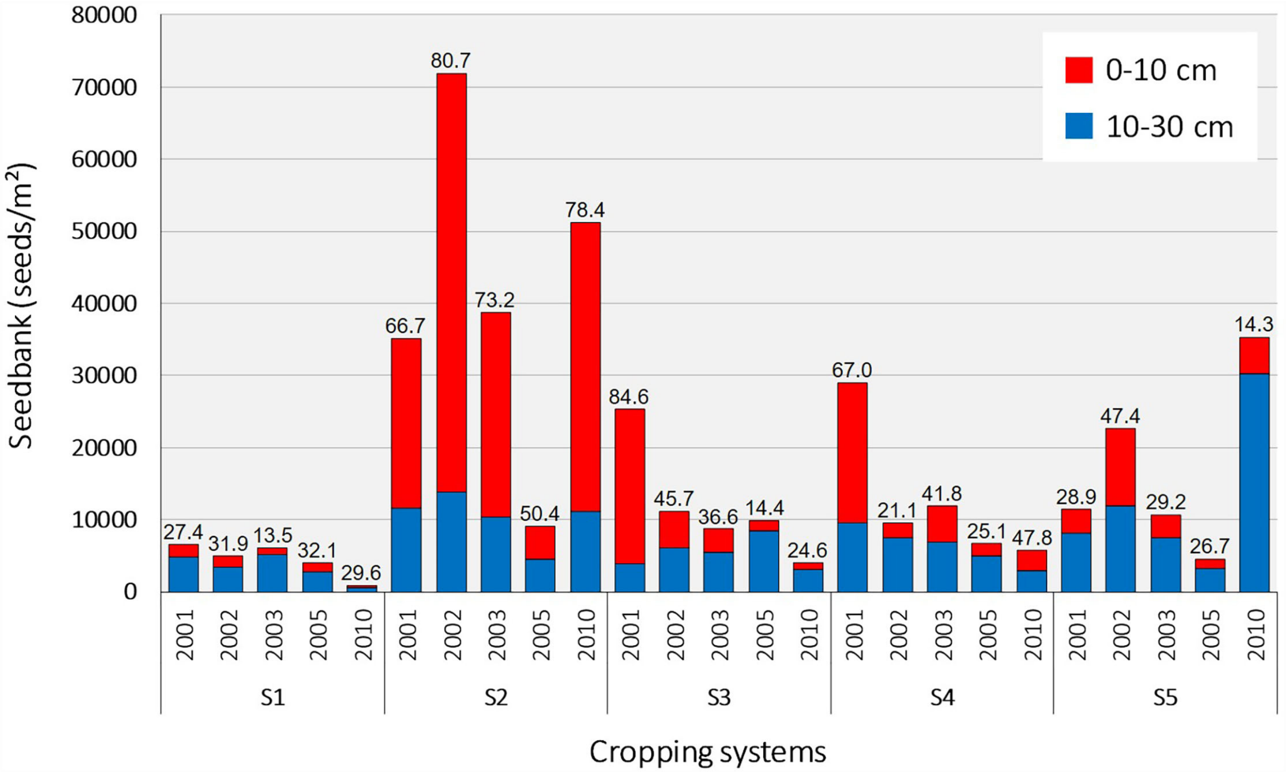

FIGURE 1 | Superficial (red) and deep (blue) weed seedbank (total number of seeds.m^{-2}) assessed during the cropping system experiment (2001–2017) testing four integrated weed management systems (S2–S5) compared a reference system (S1). Figures on top of the bars represent the percentage of seeds located in the top horizon (red bar, 0–10 cm). Only the data collected from 2001 to 2010 were presented because no investigation of the seedbank was done afterwards.

(10–30 cm) and *Medicago* sp. and *Epilobium* sp. were found exclusively in the superficial horizon (0–10 cm). Total weed seedbank abundance (**Figure 1**) varied by system; however, the effect of system depended on the year (system: $df = 4$, $\chi^2 = 27.1$, $P < 0.001$; year: $df = 4$, $\chi^2 = 4.9$, $P = 0.30$; system-by-year interaction: $df = 16$, $\chi^2 = 559.9$, $P < 0.001$). Similarly, the proportion of seeds located in the superficial soil horizon varied by systems ($df = 4$, $\chi^2 = 95.85$, $P < 0.001$), year ($df = 4$, $\chi^2 = 24.6$, $P < 0.001$), and the interaction between both factors ($df = 16$, $\chi^2 = 337.8$, $P < 0.001$), averaging 50–80% in the S2 no-plow system compared to 10–50% in the other plowing-based systems (except in S3 and S4 in the first year). A total of 45 species were observed the last year that seedbank data were collected in the cropping system experiment (i.e., 2010). The top five most frequent species (in decreasing order) were *A. arvensis*, *Galium aparine*, *C. album*, *S. nigrum,* and *A. myosuroides*. Seedbank species richness in 2010 was higher in all alternative IWM systems (S2–S5) than the S1 reference (**Table 2**). Total seed density in 2010 varied by systems (**Table 2**) and was highest in the no-plow (S2) and herbicide-free (S5) systems. The proportion of the seedbank located in the superficial soil horizon was higher in S2 than in the plowing-based systems.

Legacy Effect of Cropping Systems in the Uniformity Trial

Legacy Effect on the Germinable Superficial Seedbank

A total of 59 species were observed in the seedbank strips over the 2017–2018 growing season. The most frequent species observed (in decreasing order) were *A. myosuroides, C. album, Fallopia convolvulus, S. nigrum,* and *Chenopodium polyspermum* and represented 58.6% of total abundance observed in the tilled strips over the season. Of the 59 weed species, 33 species were also observed in the 2010 seedbank samples. Species richness and total weed density varied by cropping system (**Table 2**). Specifically, seedbanks in all four IWM systems had higher species richness and were more abundant compared to the reference system (S1). Seedbank community composition also varied by cropping system (**Figure 2A**, *P*-value = 0.002). The first pCCA axis separated systems according to a tillage gradient. The no-plow (S2) system was associated with spring/summer germinating dicots (*A. arvenis*, *Kickia* sp., *C. polyspermum*) and one grass species (*Echinocloa crus-galli*), whereas the plowing-based (S3) system was associated with autumn-germinating species (*Veronica persica*, *Veronica herderifolia*, *Viola arvensis*). The second axis discriminated cropping systems according to

TABLE 2 | Weed seedbank measured in 2010 during the cropping system experiment (S1–S5 conducted from 2001 to 2017) and *in situ* weed seedbank and emerged weed communities measured during the winter wheat uniformity trial (2017–2018).

Experiment/dataset	Response variable	χ^2	Pr (> χ^2)	S1	S2	S3	S4	S5	R^2m	R^2c
Cropping system experiment	Species richness in the seedbank in 2010 (nb species/soil sample)	30.9	**<0.001**	5.9 ± 2.1(a)	15.1 ± 2.6(b)	10.1 ± 1.5(b)	12.4 ± 3.7(b)	11.7 ± 2.7(b)	0.49	0.58
	Total weed seedbank density in 2010 (seeds/m²)	19.4	**<0.001**	859 ± 360(a)	51,168 ± 18,126(b)	4,036 ± 2,842(a)	5,764 ± 2,528(a)	35,215 ± 35,083(ab)	0.58	0.86
	Percentage of seeds in the top 0–10 cm soil horizon in 2010	21.8	**<0.001**	28.1 ± 11.8(a)	67.6 ± 13.0(b)	38.5 ± 23.1(a)	41.9 ± 20.6(ab)	31.1 ± 14.5(a)	–	–
Superficial germinable seedbank in 2017–2018	Total weed species richness (nb species/quadrat) over the season	43.4	**<0.001**	4.3 ± 2.6(a)	12.9 ± 1.8(b)	8.5 ± 2.3(b)	9.7 ± 2.8(b)	10.9 ± 1.9(b)	0.54	0.64
	Total weed density (plants/m²) over the season	47.1	**<0.001**	49.7 ± 55.9(a)	534.5 ± 299.1(c)	109.4 ± 53.0(b)	236.2 ± 198.7(bc)	314.2 ± 135.9(bc)	0.63	0.76
Uniformity winter wheat trial in 2017–2018	Species richness before weeding (nb. species/16 m²)	36.6	**<0.001**	3.9 ± 1.9(a)	10.2 ± 2.3(c)	7.9 ± 1.8(bc)	7.7 ± 2.2(bc)	6.4 ± 1.9(b)	0.44	0.46
	Total weed density before weeding (plants/m²)	17.7	**<0.001**	3.8 ± 4.9(a)	95.1 ± 126.9(b)	39.1 ± 22.0(b)	38.9 ± 65.1(b)	19.2 ± 17.1(ab)	0.44	0.65
	CWM weed height	15.9	**<0.01**	39.5 ± 10.6(ab)	46.3 ± 5.9(b)	33.7 ± 4.9(a)	39.0 ± 7.6(ab)	38.1 ± 8.1(ab)	0.23	0.28
	CWM weed SLA	33.1	**<0.001**	25.6 ± 3.8(a)	28.1 ± 2.6(ab)	27.6 ± 3.3(ab)	31.6 ± 5.1(bc)	36.2 ± 5.9(c)	0.44	0.51
	CWM weed seed mass	2.8	0.51	3.09 ± 1.93	2.45 ± 0.68	2.30 ± 1.06	2.73 ± 2.14	1.78 ± 1.32	0.08	0.27

Mean ± standard error, CWM, community weighted (by density) mean; R²m, marginal R², effect of the fixed effects; R²c, conditional R², effect of the fixed and random effects. Significant terms at P < 0.05 are highlighted in bold. Systems are not different at P < 0.05 if they share the same letters.

their herbicide use, the herbicide-free (S5) system was associated with perennials species (*Rumex* sp. and *C. arvense*) and summer-germinating annuals (*C. album, S. nigrum*).

When accounting for the different survey sessions (**Figure 3**), weed density varied by system ($df = 4$, $\chi^2 = 104.2$, $P < 0.001$), session ($df = 5$, $\chi^2 = 78.1$, $P < 0.001$), and the interaction between both ($df = 20$, $\chi^2 = 358.1$, $P < 0.001$). Weed density ranged from 0 to 830.5 plants/m² and averaged 41.4 plants/m². Overall, total weed density was highest in the spring/summer sessions. This was particularly the case for the no-plow (S2) and herbicide-free (S5), which were the only systems to show the highest abundance in all of the last three sessions. Systems S1 and S2 had the lowest and highest total densities at each of the six sessions, respectively. Species richness (**Figure 3**) ranged from 0 to 10 species/0.36 m² quadrat, averaged 2.4 species and varied by system ($df = 4$, $\chi^2 = 62.5$, $P < 0.001$), session ($df = 5$, $\chi^2 = 65.7$, $P < 0.001$), and the interaction between both factors ($df = 20$, $\chi^2 = 12.38$, $P < 0.001$). Species richness in all four IWM systems was higher than the reference (S1) in spring sessions.

Legacy Effect on Emerged Communities in Winter Wheat

In the uniformity trial, a total of 38 weed species were observed prior to herbicide application for weed control in winter wheat. The most abundant species, representing 58.8% of total abundance, were *A. myosuroides, Veronica hederifolia, G. aparine, V. persica,* and *Stellaria media*. Similar to what we observed in the superficial seedbank, *A. myosuroides* was not associated with a particular system, while other species showed varying levels of association with specific systems (**Figure 2**). Species richness ranged from 1 to 14 species per 16 m² zone and averaged 7.25 species per zone. Species richness varied by cropping system (**Table 2**), with the lowest richness observed in the reference system (S1) and the highest in S2. Total weed density prior to weed control ranged from 0.2 to 319.4 plants.m^{-2} and averaged 39.2 plants.m^{-2}. Total weed density varied by cropping system (**Table 2**), and was higher in the four IWM systems compared to the reference system, where weed density was very low (averaging 3.8 plants.m^{-2}).

Weed community composition varied by cropping system (**Figure 2B**, *P*-value = 0.002, partial variance explained by axis 1 and 2 = 22.3%). The first pCCA axis (accounting for 12.9% of the partial variance) discriminated the systems according to tillage intensity, whereas the second axis (accounting for 8.4% of the partial variance) separated systems according to the herbicide use. Several species showed clear associations with system S2, including the perennial species *C. arvense*, and *Asteraceae* taxa (*Sonchus asper* and *Lapsana communis*), resulting in a community with a higher CWM height than S3 (**Table 2**). Species associated with system S3 were mostly autumn-germinating short-cycle prostrate species such as *V. arvensis, Aphanes arvensis, Senecio vulgaris, V. persica,* and *V. hederifolia,* resulting in a community of low height (**Table 2**). Species associated with S5 were fewer; however, the high density and frequency of *S. media*, *Rumex* sp., and *Fumaria officinalis* (**Figure 2B**) resulted in a community with a high SLA (**Table 2**).

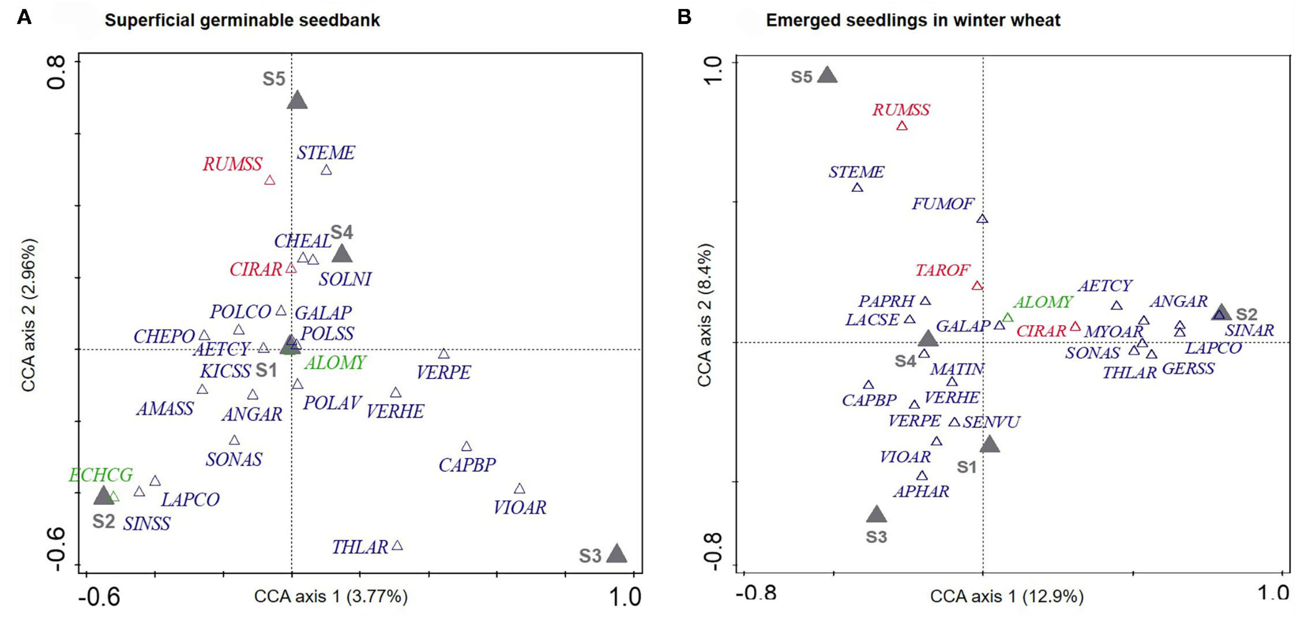

FIGURE 2 | Partial canonical correspondence analysis (pCCA) highlighting the effects of cropping systems (S1–S5) on community composition of the superficial germinable weed seedbank (**A**, *P*-value = 0.002, explained variation = 9.4%) and in the subsequent winter wheat uniformity trial (**B**, *P*-value = 0.002, explained variance = 22.3%) after partialling out the sampling events **(A)** and field **(A,B)** effects. Only the most frequent species (*N* = 24, green: annual grass; blue: annual dicots, red: perennial dicots) are represented and named according to their EPPO codes (https://gd.eppo.int/).

Despite differences in soil disturbance between systems, no differences were found in the CMW seed mass.

Legacy Effect of Cropping System on Germination Patterns

A total of 21 weed species were found in the tilled strips of at least two of the cropping systems, allowing us to compare the distribution of their emergence between cropping systems over the growing season (**Figure 4**; **Supplementary Table 3**). In total, 14 out of the 21 species exhibited distributions of emergence that varied between one or more systems (**Figure 4**). For example, across the five cropping system treatments, a total of 3,814 individuals of *A. myosuroides* germinated over the season (counted prior to six tillage events). The period of germination for *A. myosuroides* was wider in systems S5 and S3 than in system S4 (**Supplementary Table 3**). This pattern was also observed for *G. aparine*, with early germination concentrated in autumn in S1, delayed but still concentrated in autumn in S4, and generally extended over a longer period including spring and summer in systems S3 and S5. Known for being capable of germinating all season, the germination patterns of *S. media* and *V. persica* also varied by system (**Figure 4**), with more frequent germination occurring in autumn in systems S4 and S3, compared to an extended period of germination across the whole season in system S5. Seedlings of *Amaranthus hybridus* were observed at many tillage timings over the growing season in the no-plow system (S2) but were only observed in the spring/summer periods in the tillage-based S4 and S5 systems (**Figure 4**). The opposite pattern was observed for *A. retroflexus*, i.e.. only observed in spring/summer in system S2, whereas it was observed emerging over a wider period in autumn in system S5. This difference in emergence periodicity between S2 and the tillage-based systems was also observed for *C. arvense*. Few significance differences were observed for autumn-germinating species (i.e., *V. hederifolia*) or spring/summer-germinating species (e.g., *S. nigrum, C. polyspermum, C. album*). When differences in seedling emergence periodicity were found between S5 and another cropping system for a given species, the species germinated later in S5 (except for *A. retroflexus*).

DISCUSSION

Legacy Effects of IWM Systems on Weed Species Richness and Abundance

We found that 17 years of continuous implementation of IWM resulted in germinable seedbank and emerged weed communities that were more species rich and more abundant than those observed in the reference system, validating our first hypothesis. Higher weed density was also observed in the IWM systems during the initial 17 years of the cropping-system experiment phase; however, this higher weed abundance was not associated with a loss in crop productivity (Adeux et al., 2019a). We hypothesize that even though weed management met its primarily objective, i.e., to prevent annual crop yield losses, it was not as efficient as it was in the reference system. The reduced use of herbicides in the IWM systems, even when replaced by a combination of alternative weed management practices, allowed

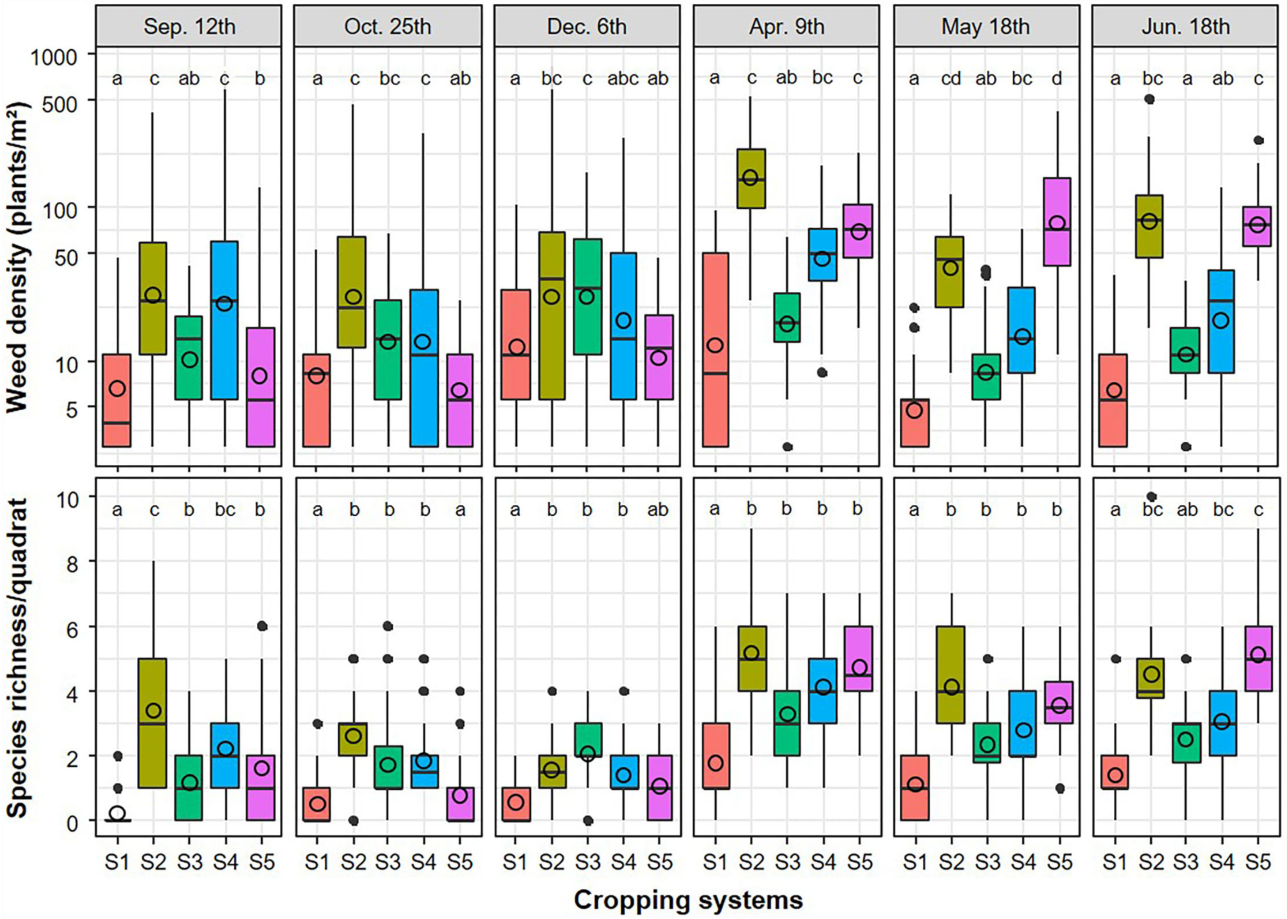

FIGURE 3 | Total weed density and species richness observed in the tilled strips (i.e., *in situ* superficial germinable seedbank) of the cropping systems (S1–S5) by sampling event (implemented approximately 6 weeks after each tillage event). Weed density model quality: $R^2m = 0.51$, $R^2c = 0.59$; Species richness model quality: $R^2m = 0.60$, $R^2c = 0.65$. Within a sampling event, mean values (open circles) for each systems are not different at $P < 0.05$ if they share the same letters.

certain species to complete their life cycles and shed seeds, leading to a more abundant weed seedbank.

Higher weed species richness in the four IWM systems is congruent with many previous studies (synthesized by Clements et al., 1994), and is partially related to IWM systems having higher diversity of crop types compared to the reference system. Increasing crop diversity through the inclusion of spring and summer crops in the rotation, with the aim of managing autumn-germinating weeds such as *A. myosuroides*, extended the ecological niche (Mahaut et al., 2019), allowing spring- and summer-germinating species such as *S. nigrum*, *A. arvensis*, or species capable of germinating all year round such as *S. media*, to establish. Sowing period plays a major role in structuring weed communities within (i.e., delayed sowing, Fried et al., 2012) and across (Fried et al., 2010; Gunton et al., 2011) crops. Differences in herbicide use between the IWM systems likely had little or no effect on species richness, as previously reported (Mahn and Helmecke, 1979; Derksen et al., 1995).

High species richness in the S2 system was likely related to the decrease in soil disturbance as it transitioned to a strict conservation agriculture system (superficial tillage from 2001 to 2010 and no-till from 2010 to 2017). Indeed, conservation agriculture relies on three fundamental pillars, namely diversified crop rotation, permanent soil cover and absence of soil disturbance (Hobbs et al., 2008). While herbicide use in S2 was similar to S1 (Adeux et al., 2019a), S2 mainly relied on glyphosate applications during the summer fallow period. No-till results in weed seeds remaining on the soil surface, a condition deemed unfavorable to weed seed germination, due to poor seed:soil contact (Cordeau et al., 2015), and increased weed seed mortality (Nichols et al., 2015). However, many studies have reported higher weed pressure under no-till than under plowing (Cardina et al., 2002; Adeux et al., 2019a), likely because permanent no-till systems provide a stable habitat for a new suite of adapted species (Armengot et al., 2016; Cordeau et al., 2020). In our study, the seedbank assessed in system S2 in 2010, i.e., before the no-till phase, was 60 times more abundant than in the reference system (S1) and 1.5 times more abundant than in the herbicide-free system (S5). In addition, 50–80% of the seedbank in S2 was concentrated in the top soil horizon, and the last 7 years

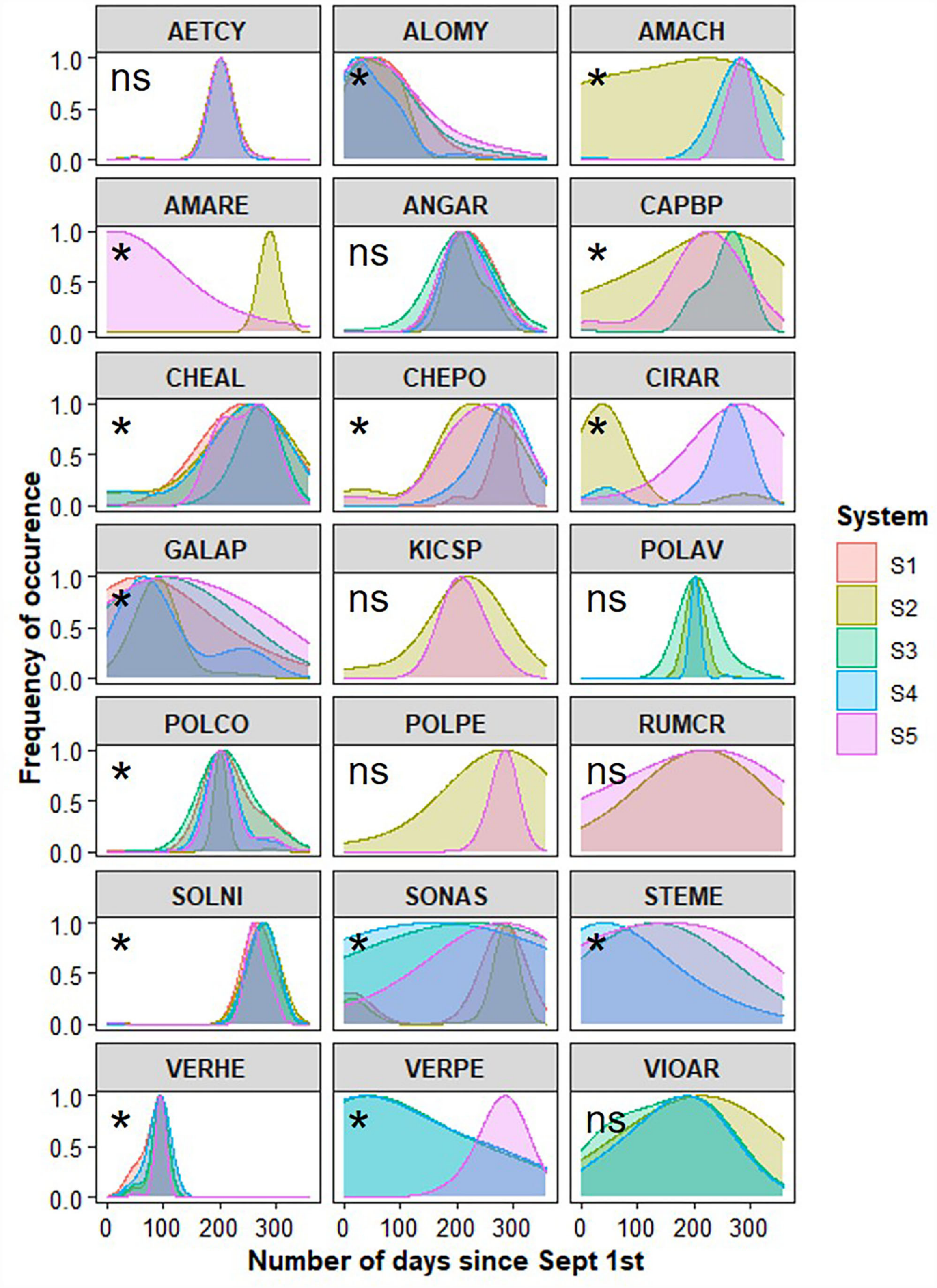

FIGURE 4 | Distribution of emerged seedlings per weed species (named by EPPO code) in each cropping system (S1–S5) during the superficial germinable seedbank experiment in which strips were tilled every 6 weeks over the growing season. Significance of overlap between pairs of systems was tested with a randomization procedure (*at least one overlap is significantly different, ns: no significant difference in overlap, see detailed overlap values and significance in **Supplementary Table 3**).

of no-till phase likely amplified this phenomenon (Chauhan et al., 2006; Vasileiadis et al., 2007; Cordeau et al., 2020). Finally, studies have shown that mulch can suppress weed emergence in no-till cover-crop based systems such as implemented in S2 in the last phase. However, low cover crop productivity during the summer fallow periods of the cropping system experiment did not allow to generate a weed suppressive mulch. The small amount of cover crop residues were incorporated to the soil by the soil fauna during the subsequent crops before having a chance to accumulate.

Weed seedbanks are often assumed to reflect past farming practices. Nevertheless, recent farming practices might have a disproportionate effect on observed seedbanks, compared to practices more distant in time, especially for weed species exhibiting transient seedbanks. Weed species persistence in the soil seedbank is hence a key trait to account for when investigating relationship between farming practices and weed seedbanks.

Legacy Effects of IWM Systems on Taxonomic and Functional Weed Community Composition

Our study showed that the implementation of contrasting IWM systems over a 17 year period shifted weed community composition, revealed by taxonomic and functional differences in both seedbank and emerged weed communities in winter wheat of the uniformity trial, validating our second hypothesis. Half of the species observed in the germinable seedbank assessed by the tilled strips were observed 7 years earlier in the seedbank, probably due to their high persistence (Lutman et al., 2002). These results highlight that shifts in weed community composition are slow, probably due to the weak filtering effects of many IWM farming practices. Assembly rules in weed community ecology state that each set of farming practices will act as a set of filters on weed species traits (Booth and Swanton, 2002). Tillage, cash crop and direct weed control are often considered to be major filters of weed community composition (Légère et al., 2005; Ryan et al., 2010; Fried et al., 2012).

We found that the cropping systems tested were first discriminated by tillage use (first CCA axis), with S2 associated to perennial and/or *Asteraceae* taxa, as shown by previous studies (Trichard et al., 2013). Our cropping systems were then discriminated by herbicide use (first CCA axis), favoring species of low stature able to germinate all year round. Higher intensity of herbicide use and higher diversity of herbicide spectrum was found to be associated to shorter flowering duration and late germination, respectively (Fried et al., 2012).

We found a higher CWM height in S2 than in S3, which is intuitive because high tillage intensity is often related to a trait syndrome of annual life history, short stature, small seed size, and early flowering (Fried et al., 2012). The high CWM height in S2 can also be explained by weed species competing for light with the cover crop during the summer fallow period. Canopy height is considered to be a reliable proxy for competitive ability for light, especially for cereal crops (Seavers and Wright, 1999) because competitive outcomes are strongly influenced by hierarchies in resource capture between crop and weeds at crop canopy closure (Adeux et al., 2019b).

We observed a higher CWM for SLA in the herbicide-free S5 system, compared to the other systems. Specific leaf area is an indicator of the efficiency by which leaf biomass is allocated to the production of leaf area (Cavero et al., 2000; Storkey, 2005). Two allocation strategies have been observed in weeds in the literature (Storkey, 2005): a shade-tolerance syndrome that characterizes small-statured weed species with high SLA values, as was observed in system S5, and a shade-avoidance syndrome, which is the most common response in the context of competitive hierarchies among plants, as we observed in system S2. We hypothesize that the repeated mechanical weeding in system S5 stimulated weed germination late in season, which resulted in weed seedlings needing to grow in the shade of the crop.

Finally, contrary to our expectation, the CWM of seed mass did not differ among systems. This was surprising because previous studies have reported that seed mass negatively correlates with tillage intensity (Ghersa and Martinez-Ghersa, 2000; Albrecht and Auerswald, 2009; Storkey et al., 2010). We hypothesize that the relative lack of weed species diversity and high abundance of the relatively large-seeded species *G. aparine* in the S1 system (most intense tillage) contributed to the lack of a similar correlation in our study.

Evidence That IWM Can Lead to Shifts in Emergence Patterns Within Weed Species

We found that for two thirds of the weed species analyzed, continuous implementation of IWM practices resulted in shifts in their emergence patterns, even after simply 17 years. We observed three general patterns: (i) weed species known to germinate in autumn (e.g., *A. myosuroides* and *G. aparine*) extended their germination period in the no-plow S2 system; (ii) phylogenetically-related species belonging to the same genus (e.g., *A. retroflexus* and *A. hybridus*) often had divergent patterns of emergence, even in the same system; and (iii) species known to germinate all year round (e.g., *V. persica* and *S. media*) exhibited emergence patterns that were restricted to late in the season in the herbicide-free S5 compared to S3 and S4 systems. We discuss each of these patterns below.

For the 17 year duration of the IWM cropping system experiment, both *A. myosuroides* and *G. aparine* were observed to occur nearly exclusively in the winter crops (Adeux et al., 2019a). Our *in situ* seedbank approach (i.e., tilled strips) revealed that these species emerged in high abundance in spring and summer in some of our systems. *A. myosuroides* has been noted to germinate when temperatures rise above 0°C (Colbach et al., 2002) and emerge in two phases (Naylor, 1972), with the highest peak occurring in autumn (∼80%) and a lesser peak in spring (∼20%). We attribute the higher proportion of *A. myosuroides* seeds germinating in spring, in part, to the selective effect of 17-years of repeated false seedbeds coupled with delayed sowing—a typical IWM practice (Rasmussen, 2004)—in the tillage-based IWM systems (S3, S4, S5), which likely selected against the autumn-emerging cohorts. Secondly, environmental conditions may also have played a role. Indeed, the winter of the uniformity trial was mild and *A. myosuroides* emerged in a period of the year where usually, already emerged seedlings are in vegetative rest and seeds wait for favorable conditions in the spring to germinate when temperature increases (Colbach et al., 2002). In addition, in the years preceding the uniformity trial, weather conditions prior to cereal harvest *i.e.* when *A. myosuroides* shed seeds (in June 2016 and 2017, **Supplementary Figure 2**), were hot and dry, conditions known to decrease seed dormancy (Colbach et al., 2002; Menegat et al., 2018). These conditions may have led to early germination (e.g., August). However, summer and autumn 2017 (uniformity trial) were hot and dry (**Supplementary Figure 1**), conditions which are not favorable for germination (Colbach et al., 2002), thus shifting germination to the following winter and spring, and explaining the stretch of the germination period. That weather conditions likely played a role is also supported by the fact that, while the soil seedbank is considered to reflect the effect of past farming history, expression of the germinable seedbank tends to more strongly reflect the effects of more recent farming practices non-persistent seeds such as *A. myrosuroides* (Moss, 1985). Emergence of *G. aparine* is expected to occur from October to January, with few individuals emerging in spring (Taylor, 1999), despite plasticity in the timing of seed germination (Kutsch and Kappen, 1991). *G. aparine* seeds are relatively large and not adapted to germinate on the soil surface and in dry conditions (Cordeau et al., 2018). We hypothesize that the continuous no-till phase in S2 (2010–2017) delayed the *G. aparine* germination period to more favorable conditions. Indeed, during winter and early spring, natural burial occurs (Benvenuti, 2007), particularly in our clay soils, due to changes in soil structure in response to freeze/thaw and humectation/desiccation phases. In addition, conservation agriculture systems, such as S2, are known to harbor a high diversity of organisms, such as earthworms (Smith et al., 2008), which are active in winter/spring and capable or burying seeds (Smith et al., 2005), which could favor germination of *G. aparine*.

A. retroflexus and *A. hybridus* differed in their germination patterns in the no-plow S2 system. Germination of *A. retroflexus* occurred strictly in summer, while germination of *A. hybridus* occurred throughout the season. This was unexpected given their phylogenetic similarity, as well as the fact that the literature does not indicate major differences in germination requirements or phenology between the species (Weaver and McWilliams, 1980; Costea et al., 2004). While *A. hybridus* tends to germinate at lower temperatures, both species exhibit a variable dormancy and polymorph germination as a result of maternal, genetic and environmental factors (Costea et al., 2004). Since their seeds were persistent in the soil seedbank (Costea et al., 2004; Steckel et al., 2007), we hypothesize that *A. retroflexus* and *A. hybridus* seeds were produced before and after the transition to the strict no-till phase in 2010, respectively. Thus, *A. retroflexus* seeds persisted in the soil seedbank and remained adapted to germinate late in season, whereas *A. hybridus* seeds were located close to the soil surface, exposed to environmental conditions, and thus acquired the capacity to germinate at different timings of the year. Unfortunately, we cannot confirm this hypothesis by looking at the seedbank in 2010 because *Amaranthus* species were not discriminated at the species level.

Finally, we observed species such as *V. persica* and *S. media*, known to germinate all year round, which exhibited a germination periodicity that was restricted to late in the season is some systems. This was particularly evident when comparing germination patterns of these species in the herbicide-free S5 system with the herbicide-based S3 and the typical IWM S4 system (i.e., implementing mechanical weeding and herbicides later in season if needed). Indeed, repeated mechanical weeding over the crop season in S5 controlled weeds but also stimulated emergence (Bond and Grundy, 2001). We hypothesize that the last mechanical weeding stimulated the emergence of weeds, which thereafter were uncontrolled other than the suppressive effect of the crop canopy (Van Der Meulen and Chauhan, 2017). This was not the case in S3 and S4 because weeds were managed with herbicide all year round (in S3) or at the last weeding (in S4), thus eliminating weeds without stimulating new emergence. Thus, we hypothesize that *V. persica* and *S. media* had to germinate late in season and shed seeds before the primary tillage implemented after crop harvest to maintain their populations in S5 over time, explaining their restricted germination period.

To conclude on the shift of emergence patterns, we hypothesize that (i) weeds have experienced selective pressures that resulted in shifts in their emergence and that (ii) there were differences in biotic and abiotic environments during the last couple of years of the cropping system experiment that resulted in differences being observed in the uniformity year only. High densities of certain weed species observed during the uniformity trial may result from specific weather conditions that occurred during the last years of the cropping system experiment (**Supplementary Figure 2**). Nevertheless, we argue that shifts in emergence timing can be related to past selective pressures, at least for species with persistent seedbanks.

Methods to Assess the Legacy Effects of Past Cropping Systems on Weeds

A primary rationale for this study was to assess the legacy effects of four contrasting cropping systems managed with IWM principles in comparison to a reference system, on the weed seedbank and on weed communities emerging in a subsequent, uniformly managed winter wheat crop. The seedbank was evaluated *in situ*, using a novel "tilled strips" approach in which strips of soil were disturbed every 6 weeks in order to stimulate weed seedling emergence over the period of investigation, as suggested by previous studies (Cordeau et al., 2017a,b,c). Half of the species observed in the tilled strips were also observed in the seedbank analyzed with a sieving method 7 years earlier. Considering the low persistence of some autumn-germinating grass species we observed (Lutman et al., 2002), we consider the tilled strips method to be effective in reflecting the germinable seedbank resulting from the past cropping system treatments. While the tilled strips method is less demanding in human labor compared to other methods (Mahé et al., 2021), it does preclude the ability to grow and harvest a crop during the study period, something to consider if similar approaches are to be adopted on farmers' fields. Jernigan et al. (2017) proposed a succession of two uniformity trials, one initiated in autumn with oat and one in spring with sorghum/millet, so as to harvest forage cover crops while assessing the legacy effect of past cropping systems at two crucial cropping periods.

To examine the legacy effects of the previous cropping systems on the emerging weed community, we managed uniformly, i.e., with the same practices (e.g., primary and secondary tillage, seeding date and rate, weeding tactics), a sole crop of winter wheat. Deciding exactly which farming practices to implement during the uniformity trial, so as to most effectively reveal the legacy effects of the past systems, was no easy task. Mouldboard plowing was excluded because it would have buried the seedbank accumulated in the superficial horizon in the no-plow S2 system (Colbach et al., 2000), thus masking the legacy effects of that system (Cordeau et al., 2020). Following the same principles as implemented over the past 17 years (Adeux et al., 2019a), e.g., systematic plowing in the reference-S1 and no-plow in S2, would have led to confounding factors, since differences in weed communities may have resulted from the combined effects of past practices (the one we wanted to assess) and the tillage practices of the uniformity trial. We thus decided to superficially till all fields, which was efficient to reveal the legacy effect, but probably resulted in an over estimation of total weed density in the no-plow S2 system compared to the other systems (Blanco-Canqui and Wortmann, 2020; Cordeau et al., 2020).

Finally, the legacy effects were studied with weed surveys implemented before weeding only because no differences were observed after spring herbicide treatment (data not shown here, but confirmed by a joint experiment on the same site:year in the no-plow S2 system, Cordeau et al., 2020). This highlights the tremendous capacity of herbicides to homogenize initially contrasted weed flora and the difficulty to link agronomic practices and weed observations, when the latter are made after weeding (Colbach et al., 2020).

CONCLUSION

We assessed the legacy effects of IWM cropping systems implemented over 17 years on the superficial germinable weed seedbank and emerged weed flora in a uniformly managed winter wheat trial conducted in year 18. We concluded that the resulting weed communities in IWM systems were more species-rich and more abundant than those observed in the reference system, and differed in terms of taxonomic and functional composition. In addition, we found that, when systems shared the same species, germination patterns of two-thirds of the weed species differed between systems. Increasing the diversity of agroecological levers to manage weeds in IWM systems allows for decreased herbicide use (Adeux et al., 2019a; Colbach et al., 2020) while limiting yield loss (Adeux et al., 2019a) and diversifying weed communities. Finally, our new method to assess the superficial germinable seedbank *in situ* with tilled strips was inexpensive and effective in revealing the legacy effects of IWM systems on weed communities. We showed that the long-term effect of IWM systems may shift the emergence patterns of some weed species. We discuss that diversified crop rotation, no-till, repeated false seed bed and delayed sowing may be the main IWM practices responsible for this shift. This information can be used to forecast future weed community dynamics and redesign cropping systems to move toward more agroecological systems that provide a more robust portfolio of ecosystem services while limiting disservices (Hunter et al., 2017; Petit et al., 2018; Vanbergen et al., 2020).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, upon request.

AUTHOR CONTRIBUTIONS

SC, NM-J, PF, and GA designed the study. SC and NM-J funded the experiment. SC, AB, HB, EV, NM-J, and GA planned data collection. SC, AB, HB, EV, and GA collected the data. SC, AB, and GA analyzed the data. All authors were involved in the interpretation of the results and contributed to writing the original version of the manuscript and improving the subsequent ones.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2021.769992/full#supplementary-material

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Impact of Cropping System Diversification on Vegetative and Reproductive Characteristics of Waterhemp (*Amaranthus tuberculatus*)

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Corn- and soybean-dominated cropping systems create and maintain a favorable environment for summer annual weeds whose emergence and growth phenology are similar to these annual summer crops. Cropping system diversification can be an effective approach for controlling noxious weeds without increasing reliance on chemical herbicides. Diversification may be especially important for managing waterhemp, a dioecious, summer annual weed that is becoming increasingly prevalent in the US Corn Belt due to its life history characteristics and herbicide resistance profile. Compared to corn and soybean, alfalfa and oat emerge and establish earlier and are thus more competitive with warm-season weeds like waterhemp. Knowledge of vegetative and reproductive characteristics in a range of crop environments can be valuable for planning weed management strategies. However, most of the relevant characteristics for a population dynamics model were available in corn and soybean monocultures. We examined the relationship between waterhemp's aboveground mass and fecundity under four crop species' presence within three crop rotation systems: a 2-year sequence of corn and soybean; a 3-year sequence of corn, soybean, and oat intercropped with red clover; and a 4-year sequence of corn, soybean, oat intercropped with alfalfa, and alfalfa. All the rotation systems were treated with conventional or reduced rates of herbicides. We established eighteen linear equations to predict waterhemp's fecundity from dried aboveground mass in each crop and associated crop management program since measuring the latter allows for quicker estimation of fecundity compared to counting seeds on each individual plant. Rotation system and crop phase within rotation system had significant effects on all the response variables but weed control regime on some. The sex ratios at maturity were slightly female-biased in oat and alfalfa. Mature waterhemp plants were larger in corn and soybean than in oat and alfalfa. Oat and alfalfa were planted earlier than corn and soybean and successfully competed for resources against waterhemp despite the absence of herbicide or interrow cultivation. Frequent hay cuts in alfalfa served as physical weed control and contributed to suppressing waterhemp and other weeds substantially.

Keywords: waterhemp (*Amaranthus tuberculatus* (Moq.) J. D. Sauer), cropping system diversification, fecundity, integrated weed management, reproductive potential, Midwestern—United States, agroecolgy, sex ratio

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INTRODUCTION

Cropping system diversification can contribute to the effective suppression of noxious weeds such as velvetleaf (*Abutilon theophrasti* Medik.) (Westerman et al., 2005), giant foxtail (*Setaria faberi* Herrm.) (Liebman et al., 2014), giant ragweed (*Ambrosia trifida* L.) (Liebman and Nichols, 2020), and other species while complementing the effects of herbicides and physical weed control practices (Davis et al., 2012; Weisberger et al., 2019). Much of the effectiveness of diversified cropping systems for weed suppression can be attributed to differences among crop species in their phenologies and the management techniques applied to them. Differences in crop phenology and diverse management tactics can lead to a net loss in weed seed population density in the soil seed bank (Liebman and Gallandt, 1997; MacLaren et al., 2020) resulting from reductions in weed fecundity and increased consumption of weed seeds by granivores. The present study focuses on fecundity and other relevant individual- and population-level reproductive and vegetative characteristics of waterhemp [*Amaranthus tuberculatus* (Moq.) J. D. Sauer] in three rain-fed cropping systems differing in crop species richness and management practices.

Waterhemp is a dioecious, summer annual, dicotyledonous species that has been listed as one of the five most noxious weeds in row crops in the US based on the number of times this species appears in the literature (Johnson et al., 2009) and by growers' concern across 22 states in the US (Prince et al., 2012). At least 54 waterhemp populations are resistant to up to five herbicide modes of action as of 2021 (Heap, 2021). As a dioecious species, populations of waterhemp are expected to express a 1:1 male:female ratio (Grant, 1959; Costea et al., 2005; Heneghan and Johnson, 2017). However, waterhemp has three characteristics that favor female-biasedness under conditions of no stress, according to a study of 243 dioecious species excluding waterhemp (Field et al., 2013): (1) the male sex is heterogametic (Montgomery et al., 2021); (2) the species has abiotic pollination and seed dispersal; and (3) the fruits are non-fleshy (Costea et al., 2005). A stressed waterhemp population tends to be female-biased, with up to ten females per male (Pratt and Clark, 2001), which is consistent with the general pattern of sexually differentiated stress tolerance in herbaceous plants (38 species, excluding waterhemp, Juvany and Munné-Bosch, 2015). The sex ratio plasticity of waterhemp suggests that a stressed population, which is characterized by low density, may allocate available resources to produce more female offspring as an effort to increase population density, as observed in its close relative, Palmer amaranth (*A. palmeri*) (Korres and Norsworthy, 2017; Mesgaran et al., 2019). Knowing how sex ratios may deviate from parity under different biotic and abiotic conditions could inform how a waterhemp population might progress from one generation to the next.

Waterhemp management is agronomically challenging because of a suite of life history characteristics, including a persistent soil seedbank (Davis, 2008), an extended seedling emergence pattern (Buhler and Hartzler, 2001), high relative growth rate, high fecundity (Heneghan and Johnson, 2017), and rapid herbicide resistance development (Tranel, 2021). One year of prolific seed production can replenish a declining seedbank with more seeds than existed in the seedbank (Davis, 2008). Failing to control waterhemp can cause up to 43% yield loss in soybean [*Glycine max* (L. Merr.)] (Hager et al., 2002) and 74% yield loss in corn (*Zea mays* L.) (Steckel and Sprague, 2004). The accumulated mass and density of a weed population reflect the relative competitiveness against crops and the favorability of the environment, which, in turn, could signal the effectiveness of weed management throughout the season. From a planning perspective, population density at maturity and plant fecundity are useful for estimating the density of new seeds produced and potentially added to the soil seedbank, and for adjusting weed management regimes accordingly (Buhler et al., 1997). Waterhemp's fecundity has been studied in corn and soybean crops (Menalled et al., 2004; Nordby and Hartzler, 2004) but not in other crops' or in an extended crop rotation system. Alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and oat (*Avena sativa* L.) are cool-season crops that can be grown in rotation sequences with corn and soybean, whereas waterhemp is a summer annual weed. Compared to corn and soybean, alfalfa, red clover, and oat seedlings emerge and establish earlier. Alfalfa, red clover, and oat also emerge and establish earlier than a number of summer annual weed species, including waterhemp (Horak and Loughin, 2000; Buhler et al., 2008).

In the present study, we examined the population aboveground mass, density and sex ratio, and the relationship between waterhemp's female size and fecundity when the weed grew in association with five crop species (corn, soybean, oat, red clover, and alfalfa) arranged in three rain-fed cropping systems. Assessing waterhemp characteristics in the presence of oat intercropped with red clover or alfalfa and alfalfa grown as a sole crop as well as corn and soybean could help to fill the gap of information concerning waterhemp performance in extended rotations. We hypothesized that the sex ratio of a waterhemp population deviated from parity depending on the environment's favorability, but how much and to which direction the shift would occur would depend on how much the studied population was suppressed. In addition to informing management, individual- and population-level characteristics would provide useful contextual details for sex ratio comparison. Counting seeds for waterhemp fecundity assessment is time-consuming and laborious, so it would be convenient to extrapolate fecundity from plant mass. We hypothesized that regression relationships with which to predict fecundity from plant mass could be identified but that such relationships would differ among treatments, due to differences in crop phenology, crop-weed competition, and management practices.

MATERIALS AND METHODS

Experiment Design

Empirical measurements of waterhemp biomass and fecundity were made in 2018 at Iowa State University's Marsden Farm in Boone County, Iowa, USA, (42° 01'N, 93° 47'W, 333 m above sea level). The site description and crop management practices were

TABLE 1 | Crop phases of the three rotation systems present in a replicate block in 2018 and 2019.

	2-year	2-year	3-year	3-year	3-year	4-year	4-year	4-year	4-year
2018	corn	soybean	corn	soybean	oat/red clover	corn	soybean	oat/alfalfa	alfalfa
2019	soybean	corn	soybean	oat/red clover	corn	soybean	oat/alfalfa	alfalfa	corn

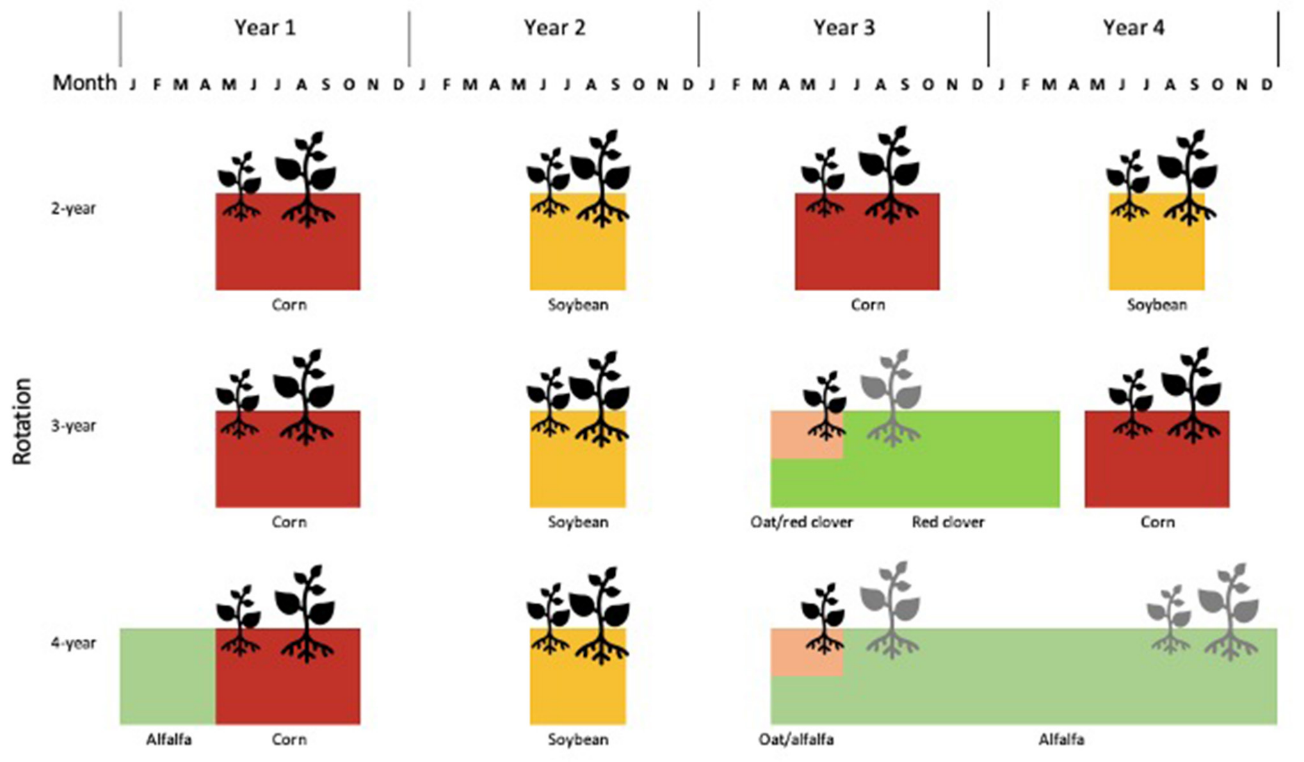

FIGURE 1 | Conceptual diagram of the three rotation systems compared within the experiment. A cycle of four calendar years is shown. Crops are color-coded and displayed for the approximate months that they were present. Emergence and establishment of common waterhemp plants are illustrated with black symbols. Grey plants shown in oat intercropped with red clover or alfalfa's first year were physically controlled by crop harvest operations. Red clover was not harvested but kept as a living cover crop until senescence. Grey plants shown in alfalfa's second year were physically suppressed three to four times by hay harvest. Alfalfa's hay was harvested when approximately 5% of the plants flowered.

described by Liebman et al. (2021). The experiment was initiated in 2001 on a 9-hectare field to compare the performance of three different crop rotations. In 2008, the experiment was reorganized to allow comparison of two contrasting weed management regimes in each of the three rotation systems. Since 2001, within each of the four blocks, plots were randomly assigned to one of the crop phases within one of three rotations.

In the present study, the experiment comprised of 72 experimental units, each 9 x 84 m. The experimental units (eu) were arranged in a randomized complete block split-plot design with four replications. The experiment was two-way factorial, with crop rotations comprising main plots and weed management regimes comprising split plots. In any year, all crop phases within the same rotation were present in different plots in the same replicate block to avoid confounding effects of the year with those of the treatments (Payne, 2015). The 2-year, 3-year, and 4-year crop sequences included in the study are shown in **Table 1**. The conceptual diagram for the experiment is shown in **Figure 1**. Alfalfa in this experiment was sown with oat. Oat was harvested for grain in 2018 and for hay in 2019 and alfalfa was retained over the winter and used as a hay crop the following year. Oat in our experiment was intended for grain harvest but was harvested for hay in 2019 due to severe hail damage to the crop and subsequent weed infestation. In the conventional weed management regime, herbicide was broadcast on the whole area that was planted to corn, whereas in the low herbicide regime, herbicide was applied in 38-cm bands over corn rows, and interrow areas were cultivated. Even though corn was the only crop that received the contrasting weed management regimes, all other crops were identified with the weed management applied to the corn phase (hereafter referred to as *corn weed management*), to which they followed. To improve weed control efficacy, herbicides used for soybean differed between 2018 and 2019.

Details concerning crop genotypes and management practices are shown in **Table 2**.

Sample Collection

Samples for all response variables were collected at least 3 m in from the border of each eu to avoid the possible edge effects. For each response variable, samples were collected from eight quadrats per eu to account for the patchiness of the weed populations. In 2018, the sex ratio was assessed by scouting the whole eu to obtain higher degrees of freedom. The date of sample collection and total sampled area for population- and individual-based measurements are shown in **Table 3**.

Population Sex Ratio

In 2018, we scouted each eu until 100 or all the available plants (if the number of plants available was fewer than 100) were sexed to obtain greater degrees of freedom. We determined the sex of 252, 1999, 2426, and 895 waterhemp plants in alfalfa, oat, soybean, and corn, respectively. In 2019, eight quadrats per eu were marked at the beginning of the season and fixed until crop harvest for a census. Overall, 413, 1331, 0, and 553 waterhemp plants were sexed in alfalfa, oat, soybean, and corn, respectively. Zero observations in all the soybean eu's resulted from high herbicide efficacy, so the 2019 data was imputed (**Appendix B**).

Population Aboveground Mass and Density

The quadrats were randomly placed in a 4 x 2 grid at the sampling date and were non-overlapping with the 2019 census quadrats. The number of plants and the total dried biomass was tallied by eu.

Individual Female Aboveground Mass and Fecundity

The maturation of waterhemp seeds can take 20 days from pollination (Bell and Tranel, 2010). We harvested female waterhemp plants as close to crop harvest as possible to maximize the number of mature seeds on mother plants. Prior to sample collection in an eu, the whole area was visually inspected to estimate the difference in plant sizes. Specimens were then collected to best capture the range of within-eu variance. Given the time and labor constraints, we planned to collect eight intact plants from each eu, which was equivalent to 576 plants in total. Plants had to be identifiable per Uva et al. (1997). By the time the seeds reached maturity, 389 intact plants were collected and processed. No intact plants were collected from two eu's. Plant specimens were contained individually in tightly knitted fabric bags to prevent seed loss. The detailed procedure for seed cleaning and counting is provided in **Appendix A**.

Model Fitting and Selection

Waterhemp survival in soybean was the greatest among all crops in the experiment in 2018 but the least in 2019 because of the use of different herbicide active ingredients. Given year-to-year differences in the sampling scheme and herbicide efficacy, the two years of data were thus analyzed separately for all the response variables in R version 4.1.2 (R Development Core Team, 2021). The data was curated with the `tidyverse` package version 1.3.1 (Wickham et al., 2019).

In 2018, 2% of the sex ratio data was missing due to zero observations in one eu, so complete case analysis, in which eu's of known sex ratio were retained while unknown sex ratio were removed from the data set, was used. In 2019, 22% of the sex ratio data was missing, so the data were imputed with predictive mean matching (PMM) method with the `mice` package version 3.14.0, (van Buuren and Groothuis-Oudshoorn, 2011) to optimally replace missing data with meaningful values without altering the observed sex ratios (**Appendix B**). Any model that involved the imputed data was fitted on all the produced (imputed) data sets, and the results were pooled (White et al., 2011).

Block was included in all models as a fixed factor because blocks were used to control the different field conditions across sections, and thus to reduce variance between eu's (Dixon, 2016). All the models were first fitted full, with block, corn weed management and crop identity, the interaction of corn weed management and crop identity, and covariates when applicable. Crop identities are the combination of crop species and the rotation to which they belonged. The within-eu variation was random and absorbed in the random error term in each model equation. The fitted models were linear (`lm`), generalized linear (`glm`), or generalized least square (`gls`) depending on the data structure and the nature of the response variable. The response and quantitative variables were appropriately transformed as needed to obtain homogeneous variances. Half of the minimum, non-zero value among all the observations was added to all the observations before ln-transformation to replace zeros. Response variables that were all non-zero were ln-transformed without adjustment. The goodness of fit of each model was assessed with diagnosis plots and mean squared error (MSE) of the variance.

The marginal means of each response variable were estimated with the `emmeans` function from the `emmeans` package version 1.7.2 (Lenth et al., 2022) to accommodate non-integer and unequal degrees of freedom among groups. Marginal means were averaged over blocks for post-ANOVA or post-ANCOVA contrasts and over factors whose effects were non-significant. Degree of freedom adjustment was done with the Satterthwaite method for the `gls` and Kenward-Roger method for the `glm` and `lm` models. ANCOVA (analysis of covariance) was applied to examine the effect of treatments on the relationship between female aboveground biomass and fecundity and between population sex ratio and biomass or density at maturity. ANCOVA combines regression and ANOVA (analysis of variance) to improve precision in mean estimation as compared to ANOVA estimation (Yang and Juskiw, 2011). Type III sums of squares error were calculated with the `emmeans`'s `joint_tests` function to accommodate unbalanced data with interaction when occurred and to avoid misleading assessment of factors' effects based on their sequential order in the model.

Population Sex Ratio at Maturity

A logistic regression model was fitted with the `glm` command and `family = quasibinomial(link = logit)` argument specification to analyze sex ratio. The `quasibinomial` family with follow-up F-test was used to accommodate overdispersion and `logit` link function

TABLE 2 | Crop varieties, and dates and rates for management operations in 2018 and 2019.

Rotation	Crop	Hybrid or cultivar	Planting date	Harvest date	Seed density	Crop density	Interrow	Cultivation	Herbicide (kg ai/ha)
2018 season									
					seeds m^{-2}	plants m^{-2}	cm		
All	corn	Epley 1420	May. 8	Oct. 30	8	8	76	low: Jun. 4; conv: none	low: tembotrione (0.054); conv: PRE thiencarbazone methyl (0.037), isoxaflutole (0.093); POST: mesotrione (0.105), nicosulfuron (0.053)
All	soybean	Latham 2758 R2	Jun. 3	Oct. 29	35	18	76	none	flumioxazin (0.096); POST: glyphosate as potassium salt (1.540), lactofen (0.140)
					kg m^{-2}	plants m^{-2}	cm		
3- and 4-year	oat	INO9201	Apr. 24	Jul. 20	0.009	225 (3-year) and 236 (4-year)	20	none	none
3-year	red clover	Mammoth Red	Apr. 24		0.002	187	20	none	none
4-year	alfalfa	55H94	Apr. 12, 2017	Jun. 4, Jul. 9, and Sep. 10	0.002	154	20	none	none
2019 season									
					seeds m^{-2}	plants m^{-2}	cm		
All	corn	Epley 1730	Jun. 3	Nov. 6	8	8	76	none	low: tembotrione (0.0054); conv: PRE: thiencarbazone methyl (0.037), isoxaflutole (0.093); POST: mesotrione (0.105), nicosulfuron (0.053)
All	soybean	Latham 2758 R2	Jun. 10	Oct. 18	35	31	76	none	PRE: flumioxazin (0.096); POST: glufosinate ammonium (0.594), clethodim (0.136)
					kg m^{-2}	plants m^{-2}	cm	none	none
3- and 4-year	oat	INO9201	Apr. 16	Jul. 22 and 24	0.009	366 (3-year) and 330 (4-year)	20	none	none
3-year	red clover	Mammoth	Apr. 16		0.002	219	20	none	none
4-year	alfalfa	WS Leafguard	Apr. 24	Jun. 7, Jul. 12, Aug. 26,	0.002	176	20	none	none

Soybean germination in 2018 was lower than in 2019 because of poor drainage in the soil. Oat and red clover were intercropped in the 3-year system. Oat and alfalfa were intercropped in the third year of the 4-year system and alfalfa was overwintered after oat harvest.

TABLE 3 | Sampling dates and areas in 2018 and 2019.

		2018		2019	
Measurement	**Crop**	**Collection date**	**Sampled area (m^2)**	**Collection date**	**Sampled area (m^2)**
Population above-ground mass	corn	Sep. 11, 12, 13	148.3	Sep. 17, 18, 19	148.3
	soybean	Sep. 17, 19, 21	148.3	Sep. 30	148.3
	oat	Sep. 27; Oct. 4, 15, 16, 18, 19	16	Sep 23, 25, 26; Oct. 3, 4, 7	17.9
	alfalfa	Sep. 26, 27; Oct 16, 19	16	Sep 24, 25; Oct. 3, 7	17.9
Population density	corn	Sep. 11, 12, 13	148.3	Sep. 17, 18, 19	148.3
	soybean	Sep. 17, 19, 21	148.3	Sep. 30	148.3
	oat	Sep. 27; Oct. 4, 15, 16, 18, 19	16	Sep 23, 25, 26; Oct. 3, 4, 7	17.9
	alfalfa	Sep. 26, 27; Oct 16, 19	16	Sep 24, 25; Oct. 3, 7	17.9
Population sex ratio	corn	Sep. 10, 11, and 12	729	Sep. 17, 18, and 19	148.3
	soybean	Sep. 17 and 30	729	Sep. 30	148.3
	oat	Oct. 4, 15, and 18	729	Sep. 24, 25, 26, 30, Oct. 1 and 2	17.9
	alfalfa	Nov. 1	729	Oct. 3 and 4	17.9
Individual female above-ground mass	corn	Oct. 18, 19, 22, 24, and 25	729	Sep. 17, 18, and 19	148.3
	soybean	Oct. 4, 15, and 18	729	Sep. 30	148.3
	oat	Oct. 29, 30, 31, Nov. 1	729	Sep. 24, 25, 26, 30, Oct. 1 and 2	17.9
	alfalfa	Nov. 1	729	Oct. 3 and 4	17.9
Individual female fecundity	corn	Oct. 24 and 25	729	none	none
	soybean	Oct. 4, 15, and 18	729	none	none
	oat	Oct. 29, 30, 31, Nov. 1	729	none	none
	alfalfa	Nov. 1	729	none	none

transformed the sex ratio using the natural logarithm (ln) (Crawley, 2013).

$$S_{ijk} = Binomial\,(N_{ijk}, \pi_{ijk})$$

$$ln\frac{\pi_{ijk}}{1-\pi_{ijk}} = \mu + b_k + \alpha_i + \gamma_j + \alpha_i\gamma_j + \epsilon_{ijk} \tag{1}$$

where,

S_{ijk} is the number of female plants among all the N_{ijk} plants in block kth under crop identity ith and corn weed management jth,
$ln\frac{\pi_i}{1-\pi_i}$ is the logit transformation of S_{ijk},
μ is the overall mean female proportion, the intercept,
α_i is the effect of the i^{th} crop identity,
γ_j is the effect of the j^{th} corn weed management,
b_k is the block effect,
$\alpha_i\gamma_j$ is the interaction effect of crop identity and corn weed management, and
ϵ_{ijk} is the random error.

Population Aboveground Mass and Density

A linear regression model was fitted with the `lm` command on each of the two variables, population aboveground mass or stand density. The general model equation for these response variables is

$$Y_{ijk} = \mu + b_k + \alpha_i + \gamma_j + \alpha_i\gamma_j + \epsilon_{ijk} \tag{2}$$

where,

Y_{ijk} is either the ln-transformed population aboveground mass or ln-transformed stand density in block kth under crop identity ith and corn weed management jth, and other terms as defined in Equation (1).

ANCOVA of Population Sex Ratio, Aboveground Mass, and Density

The regression of sex ratio against population density or biomass was extended from the ANOVA of sex ratio Equation (1).

$$S_{ijk} = Binomial\,(N_{ijk}, \pi_{ijk})$$

$$\begin{aligned} ln\frac{\pi_{ijk}}{1-\pi_{ijk}} &= \mu + b_k + \alpha_i + \gamma_j + \alpha_i\gamma_j + \delta D_{ijk} + \alpha_i D_{ijk} + \gamma_j D_{ijk} \\ &\quad + (\alpha_i\gamma_j)D_{ijk} + \epsilon_{ijk} \end{aligned} \tag{3}$$

where,

δ is the effect of the covariate,
D_{ijk} is the natural log-transformed population stand density in block k^{th} under crop identity i^{th}, and corn weed management j^{th}, the covariate, and other terms as defined in Equation (1).

Individual Female Aboveground Mass and Fecundity

A compound symmetric linear regression model (with `nlme` package's `gls` command was first fitted for each of the response variables, individual aboveground mass and fecundity to accommodate negative variance that occurred when the within-eu variance was larger than the between-eu variance and correlated errors occurred within blocks (version 3.1-153, Pinhero et al., 2022). The `corCompSymm` argument in the `gls` command was specified by identifying unique combinations of block and treatment. The model in this exercise is of the same form as the model in Equation (2):

$$Y_{ijkl} = \mu + b_k + \alpha_i + \gamma_j + \alpha_i\gamma_j + \epsilon_{ijkl} \tag{4}$$

where,

Y_{ijkl} is either the ln-transformed aboveground mass or ln-transformed number of seeds of female plant l^{th} in block k^{th} under crop identity i^{th} and corn weed management j^{th},

ϵ_{ijkl} is the random error, and other terms as defined in Equation (1).

Individual Female Aboveground Mass and Fecundity relationship

The regression of individual plant fecundity against individual plant aboveground mass was combined from the ANOVA of each (Equation 4) to establish a relationship between the two variables:

$$\begin{aligned} Y_{ijkl} = \mu + b_k + \alpha_i + \gamma_j + \alpha_i\gamma_j + \beta X_{ijkl} + \alpha_i X_{ijkl} + \gamma_j X_{ijkl} \\ + (\alpha_i\gamma_j)X_{ijkl} + \epsilon_{ijkl} \end{aligned} \tag{5}$$

where,

TABLE 4 | ANOVAs of crop identity and corn weed management effects on waterhemp population aboveground mass and stand density.

			2018				2019			
			Population aboveground mass		Population stand density		Population aboveground mass		Population stand density	
Source of variation	df1	df2	*F*.value	*p*.value	*F*.value	*p*.value	*F*.value	*p*.value	*F*.value	*p*.value
Crop ID	8	51	21.23	<0.0001	27.45	<0.0001	42.14	<0.0001	84.03	<0.0001
Corn weed management	1	51	0.41	0.5241	0.87	0.3555	1.23	0.2730	0.30	0.5889
Crop ID x Corn weed management	8	51	0.40	0.9139	0.96	0.4736	1.35	0.2415	0.63	0.7486

Crop identity was the only influential factor on both population aboveground mass and stand density in 2018 and 2019.

TABLE 5 | Rotation system and crop species effects on population aboveground mass and stand density.

	2018				2019			
	Population aboveground mass		Population stand density		Population aboveground mass		Population stand density	
Contrast	Ratio	*p*.value	Ratio	*p*.value	Ratio	*p*.value	Ratio	*p*.value
(A) - Rotation system effects								
C2 vs C3	12.54	0.1237	2.38	0.2990	0.84	0.9680	1.19	0.9284
C2 vs C4	23.12	0.0430	1.84	0.5435	10.61	0.0054	14.45	<0.0001
C3 vs C4	1.84	0.8798	0.78	0.8983	12.65	0.0026	12.11	<0.0001
S2 vs S3	6.42	0.3151	2.07	0.4258	6.27	0.0369	2.60	0.1265
S2 vs S4	2.45	0.7597	1.29	0.8976	6.27	0.0369	2.60	0.1265
S3 vs S4	0.38	0.7298	0.62	0.6963	1.00	1.0000	1.00	1.0000
O3 vs O4	0.82	0.8774	1.78	0.3235	0.14	0.0096	0.29	0.0132
(B) - Crop species effects								
oat vs soybean	3.15	0.5040	47.18	<0.0001	2,580.00	<0.0001	1330.00	<0.0001
oat vs corn	5,795.38	<0.0001	222.88	<0.0001	85.30	<0.0001	32.60	<0.0001
oat vs alfalfa	831.33	<0.0001	29.84	<0.0001	8.34	0.0071	2.39	0.1712
soybean vs corn	1,840.55	<0.0001	4.72	0.0001	0.03	<0.0001	0.02	<0.0001
soybean vs alfalfa	264.02	<0.0001	0.63	0.7660	0.00	<0.0001	0.00	<0.0001
corn vs alfalfa	0.14	0.2509	0.13	0.0005	0.10	0.0014	0.07	<0.0001

Some zero ratios are due to rounding.

C2: corn in the 2-year rotation; C3: corn in the 3-year rotation; C4: corn in the 4-year rotation; S2: soybean in the 2-year rotation; S3: soybean in the 3-year rotation; S4: soybean in the 4-year rotation; O3: oat in the 3-year rotation; and O4: oat in the 4-year rotation.

Y_{ijkl} is the ln-transformed number of seeds of plant l^{th} in block k^{th} under crop identity i^{th} and corn weed management j^{th}, X_{ijkl} is the ln-transformed dried aboveground mass of plant l^{th} in block k^{th} under crop identity i^{th}, and corn weed management j^{th}, the covariate, and other terms as defined in Equation (1).

If the MSE of the model described in Equation (5) were smaller than that of the model described in Equation (4), the former model would be considered better for estimating the number of seeds produced by an individual waterhemp plant.

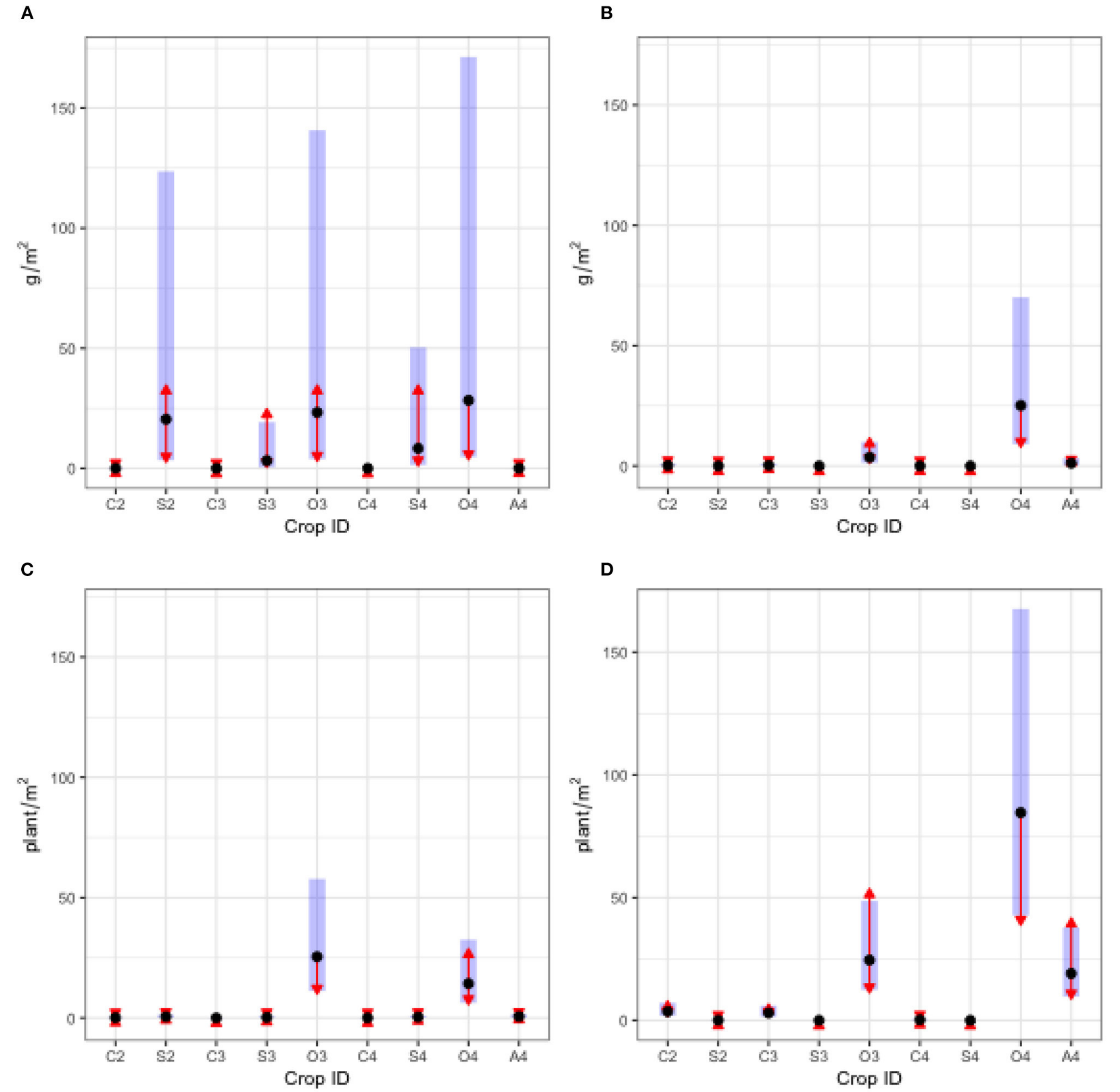

FIGURE 2 | Waterhemp population aboveground mass and stand density averaged over corn weed managements. The abbreviations on the *x*-axis are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged (C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; and A4, alfalfa in the 4-year rotation). The black dots are estimated marginal means. The blue bars are 95% confidence intervals. The red arrows reflect the comparison among means. Overlapping arrows indicate non-significant differences. **(A)**, 2018 population above ground mass; **(B)**, 2019 population above ground mass; **(C)**, 2018 population stand density; **(D)**, 2019 population stand density.

We tested the assumption that all the regression lines were parallel. Violation of this assumption required an individual regression line for each treatment. To test model robustness, samples in each treatment were pooled across four blocks and divided into four size-based subsets. Samples from each subset were then randomly placed into the testing and training sets using the 80 testing : 20 training ratio. A model was considered to perform well if the data points in the testing set blended well with the data points from the training set. A robust model could be used to predict plant fecundity with new biomass data.

RESULTS

Using `ggResidpanel` version 0.3.0 (Goode and Rey, 2019), `boot` version 1.3-28 (Canty and Ripley, 2021), and two customized functions for model diagnosis, no predictable pattern in the plots of residuals vs. predicted values suggests that the analysis models fit the data well (Details are provided in Nguyen and Liebman, 2022a). In all rotations, all the crop yields were comparable to those of Iowa and Boone County where the experiment is situated (Hunt et al., 2020; Nguyen and Liebman, 2022b). Tables were compiled with `kableExtra` version 1.3.4 (Zhu et al., 2021). Figures were made with `emmeans` version 1.7.2 (Lenth et al., 2022) and `ggplot2` version 3.3.5 (Wickham et al., 2016).

Population Aboveground Mass and Stand Density

In both years, population aboveground mass and stand density were strongly influenced by crop identity (**Table 4**). The rotation system in which a crop was grown also affected population aboveground mass and stand density, although not consistently between years (**Table 5**).

In 2018, population aboveground mass in the same crop species was comparable across rotations except for corn grown in the 2-year (C2) vs. 4-year rotation (C4) (p-value = 0.043). In 2019, population aboveground mass in the same crop was different across rotations, except for corn in the 2-year (C2) vs. 3-year rotation (C3) (p-value = 0.968) and soybean in the 3-year (S3) vs. 4-year rotation (S4) (p-value = 1). Averaged across rotations, population aboveground mass was comparable in 2018 for corn vs. alfalfa (p-value = 0.2509) and soybean vs. oat (p-value = 0.504), but 10- to 5795.38-fold different in the other ten pairs of comparison (p-values < 0.01).

In 2018, population stand density in the same crop species was comparable across the rotations. In 2019, population stand density in the same crop species was comparable for soybean (p-values = 0.1256 and 1) and C2 vs. C3 (p-value = 0.9284), but significantly different for the other corn comparisons and for oat in the 3-year (O3) vs. 4-year rotation (O4). Averaged over rotations, population stand density was comparable in 2018 between soybean and alfalfa (p-value = 0.766), but 5- to 1330-fold different in the other eight pairs of comparison (p-values < 0.001).

In 2018, population aboveground mass was the highest in soybean and oat (**Figure 2A**) because soybean weed management was ineffective and herbicide was intentionally not applied in oat. The legacy of an ineffective weed management program in 2018 soybean plots was observed in 2019 oat plots where population aboveground mass and stand density were the highest among all the crop identities (**Figure 2B**). High stand density in 2019 oat plots was also due to uneven oat establishment. The change in 2019 in weed management for soybean substantially reduced the waterhemp pressure on soybean (**Figures 2B,D**).

TABLE 6 | ANOVAs of crop identity, herbicide, and covariate effects on population sex ratio using 2018 data.

Source of variation	df1	df2	*F*.value	*p*.value
(A) no covariate. Residual deviance = 165.9, dispersion = 3.32.				
Crop ID	8	Inf	8.45	<0.0001
Corn weed management	1	Inf	0.01	0.9317
Crop ID x Corn weed management	8	Inf	0.46	0.8862
(B) with population aboveground mass covariate. Residual deviance = 104.3, dispersion = 3.24.				
Crop ID	8	Inf	1.02	0.4155
Corn weed management	1	Inf	0.00	0.9601
Population aboveground mass	1	Inf	1.51	0.2198
Crop ID x Corn weed management	8	Inf	0.71	0.6847
Crop ID x Population aboveground mass	8	Inf	1.04	0.4038
Corn weed management x Population aboveground mass	1	Inf	2.85	0.0916
Crop ID x Corn weed management x Population aboveground mass	8	Inf	1.24	0.2713
(C) with population stand density covariate. Residual deviance = 82.12, dispersion = 2.54.				
Crop ID	8	Inf	0.96	0.4679
Corn weed management	1	Inf	0.93	0.3346
Population stand density	1	Inf	2.46	0.1169
Crop ID x Corn weed management	8	Inf	1.46	0.1675
Crop ID x Population stand density	8	Inf	1.71	0.0896
Corn weed management x Population stand density	1	Inf	5.16	0.0231
Crop ID x Corn weed management x Population stand density	8	Inf	2.36	0.0155

*With population aboveground mass covariate included **(B)**, crop identity was the only influential factor on population sex ratio. With population stand density covariate included **(C)**, sex ratio responded differently in each treatment and stand density combination.*
A zero F.value is due to rounding.

Population Sex Ratio

Population stand density was included to improve the precision of estimates of population sex ratios (**Tables 6A,C**). The population sex ratio in 2018 differed significantly among treatments, at different population stand densities within each treatment (p-value = 0.0155, **Figure 3** and **Table 6**). Therefore, sex ratios in the same treatment were evaluated at four population densities, i.e., 1, 5, 50, and 500 plants/m^2, to illustrate that three-way interaction (**Figure 3**). Female-biasedness was more likely if a waterhemp population was grown in oat

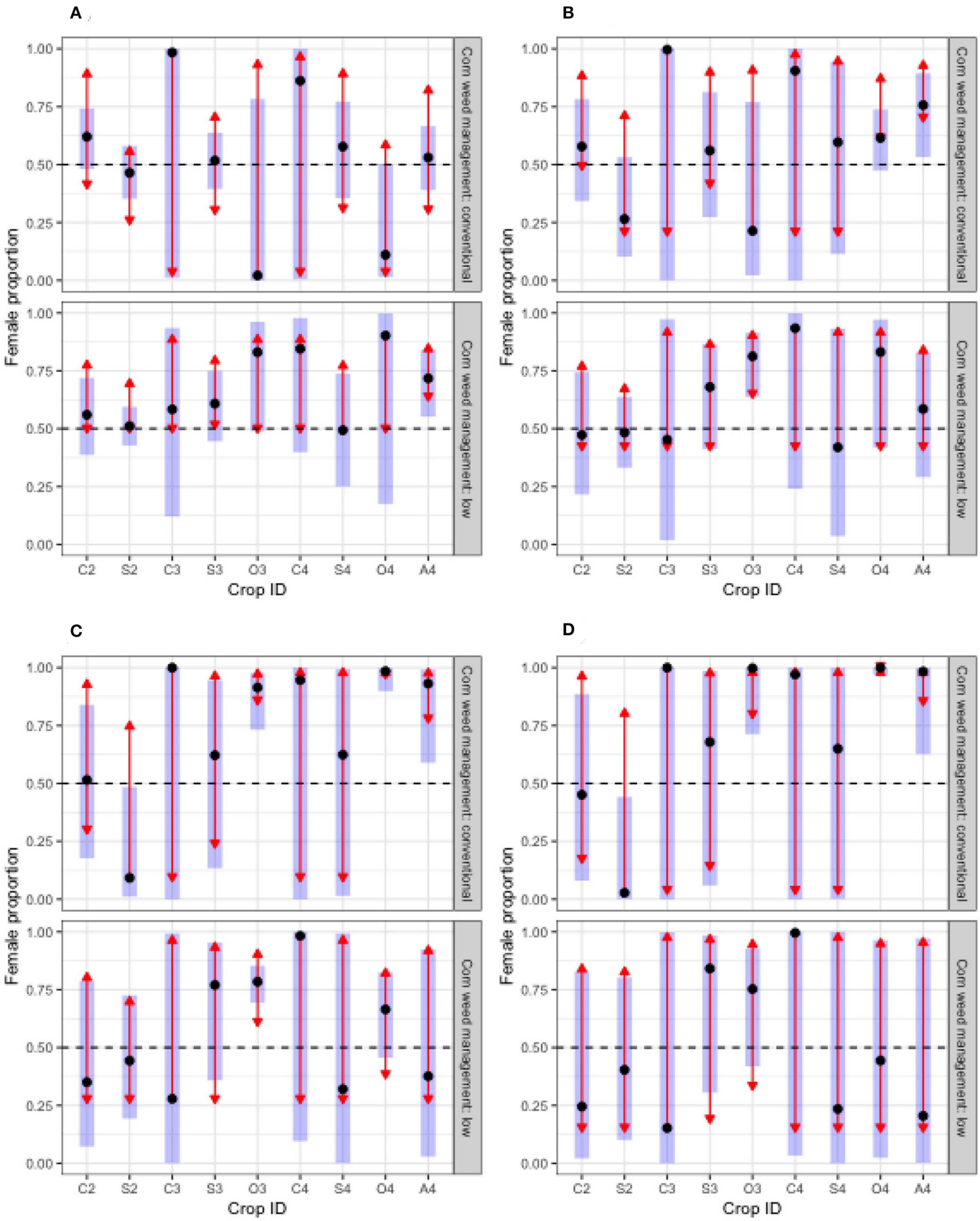

FIGURE 3 | Waterhemp population sex ratios under 54 combinations of experimental treatments and population stand densities. The abbreviations on the *x*-axis are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged (C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; and A4, alfalfa in the 4-year rotation). The dashed lines mark sex ratio parity. The black dots are estimated marginal means. The blue bars are 95% confidence intervals. The red arrows reflect the comparisons among means. Overlapping arrows indicate non-significant differences. **(A)**, 1 plants/m^2; **(B)**, 5 plants/m^2; **(C)**, 50 plants/m^2; **(D)**, 500 plants/m^2.

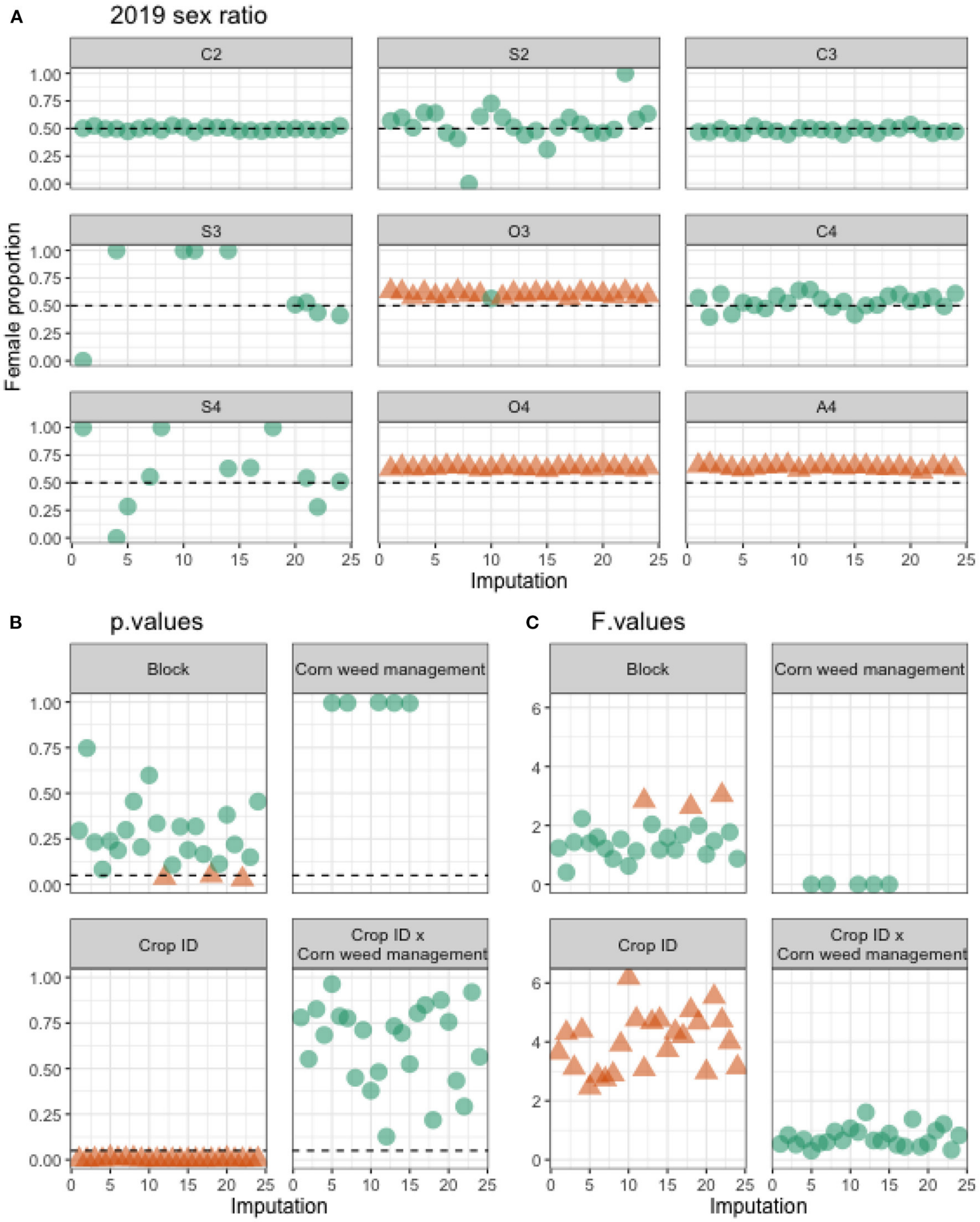

FIGURE 4 | Waterhemp population sex ratios under nine crop identities averaged over two Corn weed management regimes using 2019's 24 imputed data sets **(A)**. The abbreviations on the **(A)**'s sections are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged (C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; and A4, alfalfa in the 4-year rotation). The dashed lines mark sex ratio parity in **(A)** and level of confidence in **(B)**, respectively. The blank spaces are nonestimable values. The triangulars and circles in **(A)** represent female-biased and even populations assessed at alpha = 0.05, respectively. F-ratios for sources of variation are shown in **(C)**.

TABLE 7 | Rotation system and crop species effects on individual female aboveground mass and fecundity.

	Female individual aboveground mass				**Individual fecundity**			
	Corn weed management: conventional		**Corn weed management: low**		**Corn weed management: conventional**		**Corn weed management: low**	
Contrast	**Ratio**	***p*.value**	**Ratio**	***p*.value**	**Ratio**	***p*.value**	**Ratio**	***p*.value**
(A) - Rotation system effects								
C2 vs C3	2.62	0.1335	4.29	0.0064	3.95	0.0820	7.29	0.0032
C2 vs C4	2.12	0.3402	2.27	0.1613	3.00	0.2367	2.55	0.2288
C3 vs C4	0.81	0.9302	0.53	0.4070	0.76	0.9240	0.35	0.2253
S2 vs S3	0.18	0.0076	0.33	0.2005	0.07	0.0010	0.47	0.6323
S2 vs S4	0.70	0.7885	0.76	0.9068	0.60	0.7451	1.19	0.9782
S3 vs S4	3.81	0.0268	2.27	0.3553	8.45	0.0045	2.51	0.4525
O3 vs O4	0.93	0.8695	0.39	0.0457	0.62	0.4363	0.27	0.0321
(B) - Crop species effects								
Soybean vs corn	8.64	<0.0001	35.10	<0.0001	17.51	<0.0001	96.74	<0.0001
Soybean vs oat	36.55	<0.0001	30.29	<0.0001	110.44	<0.0001	55.97	<0.0001
Soybean vs alfalfa	128.16	<0.0001	133.62	<0.0001	5423.32	<0.0001	6,857.12	<0.0001
Corn vs oat	4.23	0.0001	0.86	0.9616	6.31	0.0001	0.58	0.4904
Corn vs alfalfa	14.83	<0.0001	3.81	0.0099	309.73	<0.0001	70.88	<0.0001
Oat vs alfalfa	3.51	0.0324	4.41	0.0062	49.11	<0.0001	122.51	<0.0001

C2: corn in the 2-year rotation; C3: corn in the 3-year rotation; C4: corn in the 4-year rotation; S2: soybean in the 2-year rotation; S3: soybean in the 3-year rotation; S4: soybean in the 4-year rotation; O3: oat in the 3-year rotation; and O4: oat in the 4-year rotation.

and alfalfa. None of the waterhemp populations grown in corn and soybean expressed gender biasedness. It is unclear whether the corn weed management program had a significant effect on gender biasedness given the magnitude of the variance (**Figure 3**).

We defined a useful imputed data set to be a set that resulted in fully estimable marginal means for sex ratio comparison across all treatments, which was achievable with non-zeros in female and male categories in at least one replication among the four blocks for the missing observations in the 2019 original sex data. Unlike the 2018 data, the sex ratio in 2019 was analyzed without the covariates because none of the covariates improved the goodness of fit for the analysis model. With $m = 24$, five imputed data sets were useful (**Appendix B**). The significance and influence of treatment factors and their interaction in the imputed data sets for waterhemp sex ratio in 2019 were consistent with those of the 2018 data (**Figure 4**). In 21 out of 24 sets, sex ratio in 2019 was affected by crop identity (**Figures 4B,C**). Female biasedness was observed in oat and alfalfa but not in corn and soybean (**Figure 4A**).

Individual Female Aboveground Mass and Fecundity

Individual female aboveground mass and fecundity were affected by rotation, crop species, and corn weed management (**Table 7**). Crop identity was more influential on female aboveground mass and fecundity than corn weed management regime, but the effect of crop identity differed between corn weed management regimes (**Tables 8A,B**). Differences in relative female size and fecundity across rotation by herbicide treatments were attributed to the relative size and fecundity differences when the waterhemp populations grew in different crops' presence.

Individual female aboveground mass was comparable in most pairwise comparison of the same crop species in different rotations, except S2 vs. S3 (p-value = 0.0076) and S3 vs. S4 (p-value = 0.0268) that followed corn under conventional weed management and C2 vs. C3 (p-value = 0.0064) under low weed management. Averaged over rotations, individual female aboveground mass was 3.51- to 133.62-fold different across each pair of comparison (p-values < 0.05), except for corn under low weed management vs. the succeeding oat (p-value = 0.9616).

Individual fecundity was comparable in most pairwise comparison of the same crop species in different rotations, except S2 vs. S3 (p-value = 0.001) and S3 vs. S4 (p-value = 0.0046) that followed corn under conventional weed management and C2 vs. C3 under low weed management (p-value = 0.0032), and O3 vs. O4 that followed corn under low weed management (p-value = 0.0321). Averaged over rotations, individual fecundity was comparable between corn under low herbicide and oat in the same system (p-value = 0.4904) but was 6.31- to 6857.12-fold different in other pairs of comparison (p-values ≤ 0.0001).

TABLE 8 | ANOVAs for the effects of crop identity, corn weed management, and female aboveground mass on individual female aboveground mass **(A)**, fecundity **(B)**, and fecundity with aboveground mass covariate **(C)**.

Source of variation	df1	df2	F.value	p.value
(A) - Individual female aboveground mass. MSE = 2.02				
Crop ID	8	46.56	48.83	<0.0001
Corn weed management	1	158.23	13.57	0.0003
Crop ID x Corn weed management	8	73.81	2.36	0.0255
(B) - Individual fecundity. MSE = 3.43				
Crop ID	8	41.67	72.13	<0.0001
Corn weed management	1	146.29	14.64	0.0002
Crop ID x Corn weed management	8	63.87	2.98	0.0067
(C) - Individual fecundity with individual aboveground mass covariate. MSE = 1.01				
Crop ID	8	67.84	16.53	<0.0001
Corn weed management	1	312.01	2.92	0.0886
Biomass	1	349.07	483.09	<0.0001
Crop ID x Corn weed management	8	151.00	1.66	0.1136
Crop ID x Biomass	8	300.15	2.99	0.0031
Corn weed management x Biomass	1	349.20	2.84	0.0931
Crop ID x Corn weed management x Biomass	8	333.06	2.49	0.0122

Each combination of crop identity and corn weed management affected female aboveground mass and fecundity differently.

TABLE 9 | Means and SEs for estimated linear regression of waterhemp fecundity index [ln(seeds + 1)] vs. biomass index [ln(gram + 0.005)] intercepts and slopes, accompanied by the R^2 values of each equations.

Effect		Intercept		Slope		R^2
Crop ID	Corn weed management	Estimate	Std.error	Estimate	Std.error	
C2	conventional	6.07	0.18	1.24	0.08	0.89
C2	low	5.88	0.22	1.22	0.11	0.78
S2	conventional	6.30	0.31	1.14	0.11	0.89
S2	low	7.07	0.22	0.97	0.07	0.96
C3	conventional	5.86	0.25	1.26	0.14	0.83
C3	low	5.11	0.35	0.66	0.21	0.33
S3	conventional	7.25	0.44	0.96	0.09	0.84
S3	low	4.89	0.82	1.47	0.20	0.78
O3	conventional	5.73	0.24	1.29	0.22	0.60
O3	low	5.64	0.21	0.60	0.18	0.29
C4	conventional	5.90	0.60	1.26	0.29	0.60
C4	low	6.04	0.16	1.41	0.10	0.90
S4	conventional	7.57	0.41	0.75	0.12	0.67
S4	low	7.33	0.56	0.74	0.19	0.58
O4	conventional	6.05	0.18	1.01	0.16	0.66
O4	low	6.29	0.14	0.92	0.13	0.70
A4	conventional	3.06	0.67	0.80	0.35	0.21
A4	low	1.97	0.43	0.50	0.20	0.23

*The abbreviations in the Crop ID column are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged. R^2 values were calculated from the whole data set (training and testing sets), and thus, were slightly different from those in **Figure 5**.*

C2: corn in the 2-year rotation; C3: corn in the 3-year rotation; C4: corn in the 4-year rotation; S2: soybean in the 2-year rotation; S3: soybean in the 3-year rotation; S4: soybean in the 4-year rotation; O3: oat in the 3-year rotation; O4: oat in the 4-year rotation; and A4 alfalfa in the 4-year rotation.

Effects of Weed Management Regimes and Rotations on Female Aboveground Mass and Fecundity Relationship

Since the treatment effects were statistically significant for both female aboveground mass and fecundity (**Table 8**), we proceeded with finding the slopes and intercepts for each linear regression of fecundity against biomass. Different slopes were specified by including interaction terms between the covariate and treatment factors. A regression slope for each treatment was necessary. The training and testing sets' data points were well mingled indicated that the established equations were robust (**Figure 5**). That the equations in **Table 9** could predict waterhemp fecundity parsimoniously from dried aboveground mass using the relevant context of crop and crop management. The presented means and SEs for the estimated intercepts and slopes were established from the whole data set.

DISCUSSION

Results of this study indicate that waterhemp was affected by crops and crop management in multiple ways, including a reduction in individual biomass and fecundity to the point of non-existence as occurred in 2019 soybean plots. Despite the 2018 and 2019 data being overdispersed, which resulted in high residual deviance, the significance of treatment effects was consistent. Crop identity was the most influential factor for all responses. Some covariation relationships were observed: population stand density affected sex ratio and female aboveground mass was a reliable predictor for fecundity.

Waterhemp is a small-seeded species that is more sensitive to environmental stress than larger-seeded species (Harbur and Owen, 2004). In the present study, the number of stress and mortality factors likely increased as crop diversity increased temporally and spatially (Martin and Felton, 1993). Stress and mortality factors arose from the strategic cropping system designs that employ crops of different phenology, management requirements, and relative competitiveness with weeds (Liebman and Janke, 1990; Liebman and Dyck, 1993).

The two summer annual row crops in our study, corn and soybean, differed from the cool season and perennial crops, oat, red clover, and alfalfa with regard to the strongest selection pressure against weeds: herbicides. In corn (C2, C3, and C4), weeds were controlled with broadcast herbicide (conventional), or a combination of banded herbicide (38-cm strips on top of crop rows) and interrow cultivation. In soybean (S2, S3, and S4), weeds were controlled with broadcast herbicide as in conventional corn, with different active ingredients. In contrast, in the O3, O4, and A4 treatments, no herbicide or cultivation was applied, but those three crops were strategically introduced to the 3-year and 4-year rotations for their potential allelopathic and shading effects (Liebman and Dyck, 1993; Singh et al., 2003). The spring establishment of O3 and O4 and overwintering of A4 treatments gave the crops a headstart for resource competition against waterhemp, a summer annual weed that emerged later (Hartzler et al., 2004). The timing of oat harvest in late July matched waterhemp's early reproductive stage (Horak and Loughin, 2000; Buhler et al., 2008) and the resulting mechanical damage at this stage reduced the weed's reproductive potential. Intercropping oat and alfalfa can produce stronger weed suppression than might be achieved by each species grown as a sole crop (Lanini et al., 1992), whereas the effects of intercropping oat with red clover can be more variable (Samson et al., 1990). For established alfalfa in the 4-year rotation, three to four hay cuts per crop season also served as a significant means of physical control and to reduce waterhemp reproductive potential.

High waterhemp population stand densities in oat resulted from highly productive plants in the preceding corn and soybean phases of the rotation and signaled abundant replenishment of soil seedbanks. Dyke and Barnard (1976) found that a clover and cereal intercrop substantially reduced weed emergence whereas Heggenstaller et al. (2006) found that a triticale (*x Triticosecale* Wittmack) and red clover intercrop increased weed seedling recruitment. Taking these findings with the present study's observation that higher waterhemp population stand densities and lower waterhemp aboveground mass were found in small grain and forage crops than in row crops, it is possible that cold-tolerant crops can be used to stimulate and induce fatal germination to deplete the soil seedbank (Davis and Liebman, 2003; Gallandt et al., 2005). Eventually, as more mortality and stress factors are imposed on emerged weeds *via* various control methods, such as allelopathy and mechanical damage *via* crop harvest, those emerged plants might be expected to contribute fewer seeds to the soil seedbank.

Waterhemp populations in three of the treatments, O3, O4, and A4, were slightly female-biased. Waterhemp populations in other treatments were even in sex ratio, which might be attributed to a more stressful conditions in small grain and forage crops than in row crops. A larger data set might help reducing the variance in sex ratio and provide a clearer understanding of the effect of corn weed management program on waterhemp sex ratio in subsequent oat and alfalfa phases. Systematic analysis is needed to identify the contribution of each stressor on waterhemp development and population dynamics. The 2019 sex ratio data were imputed without 2018 input but returned consistent conclusions on treatment effects, as compared to 2018. This consistency suggests an acceptable precision of the analysis model and the imputation algorithm. Since `pmm` seeks to fill in missing values with placeholders without changing the overall mean, it is reasonable to assume that the sex ratios in soybean eu's were even. The high herbicide efficacy in soybean was the strongest selection pressure on the exposed waterhemp populations.

Our analysis indicated that female aboveground mass could be used to predict fecundity parsimoniously. The strong evidence of the significant interaction effect of weed management regime and crop identity on waterhemp fecundity justified the use of separate equations for each treatment. Since different sources of stress were introduced in the small grain and forages than in row crops, we attributed female-biasedness and lower fecundity in forages than in row crops to female herbaceous plants outperforming males under abiotic and biotic stresses (Juvany and Munné-Bosch, 2015). The stand density and sex ratio data in this study does not provide sufficient information to establish a relationship between them, as was established between individual female biomass and fecundity. It would be helpful to explore the population stand density and sex ratio relationship with a bigger data set.

In the present study, using mother plant reproduction potential (aboveground mass) gives a rough estimate of the number of seeds being added to the soil seedbank. Additionally, the total number of seeds produced at the end of the season in each treatment depended on the parent plant density and population sex ratio. The possibility of post-harvest seed loss due to seed predators under different ground cover conditions adds to the complexity of seedbank dynamics. Red clover that remained after oat harvest and alfalfa living mulch may enhance granivore activities (Davis and Liebman, 2003; Gallandt et al., 2005). Heggenstaller et al. (2006) found increased predation of velvetleaf (*Abutilon theophrasti* Medik) and giant foxtail (*Setaria faberi* Herrm) seeds in more diverse cropping systems than in shorter corn-soybean rotations. Overwintering crops such as alfalfa delayed pigweed (*Amaranthus quitensis* H.B.K.) emergence (Huarte and Arnold, 2003) and can exude allelochemicals for weed suppression (Miller, 1996). Compared to the bare ground after corn and soybean production, the post-harvest environment in oat and alfalfa may induce more seed loss due to predation (Gallandt et al., 2005). Waterhemp was not included in the Heggenstaller et al. (2006) study, but waterhemp seeds are preferred over other species' seeds by field crickets and ground beetles (van der Laat et al., 2015) so it is likely that the small grain and forage crops in the present study enhanced waterhemp seed predation.

The key take-away messages from this present study are that: (1) waterhemp reproductive potential can be effectively suppressed without herbicide with the integration of cool season crops into the cropping system and (2) female waterhemp plants may have higher survival rate than males under the same conditions, which was reflected by higher female: male ratios.

More investigation is needed to determine how soil seedbank dynamics contribute to population dynamics in different scenarios, such as how female-biasedness could be potentially helpful to replenish a seedbank, whether sexual unevenness in a generation causes sexual unevenness in freshly produced seeds, and how those biases contribute to long-term population changes and competitiveness against crops. In the near-total control situation as occurred in the 2019 soybean plots, as the number of fresh seeds added to the soil seedbank can be considered negligible, other factors, such as sex ratio, population stand density, and plant size are of less practical concern. A more practical investigation would be to see how different levels of control efficacy translate into medium- and long-term population changes, because no herbicide is totally invulnerable to the

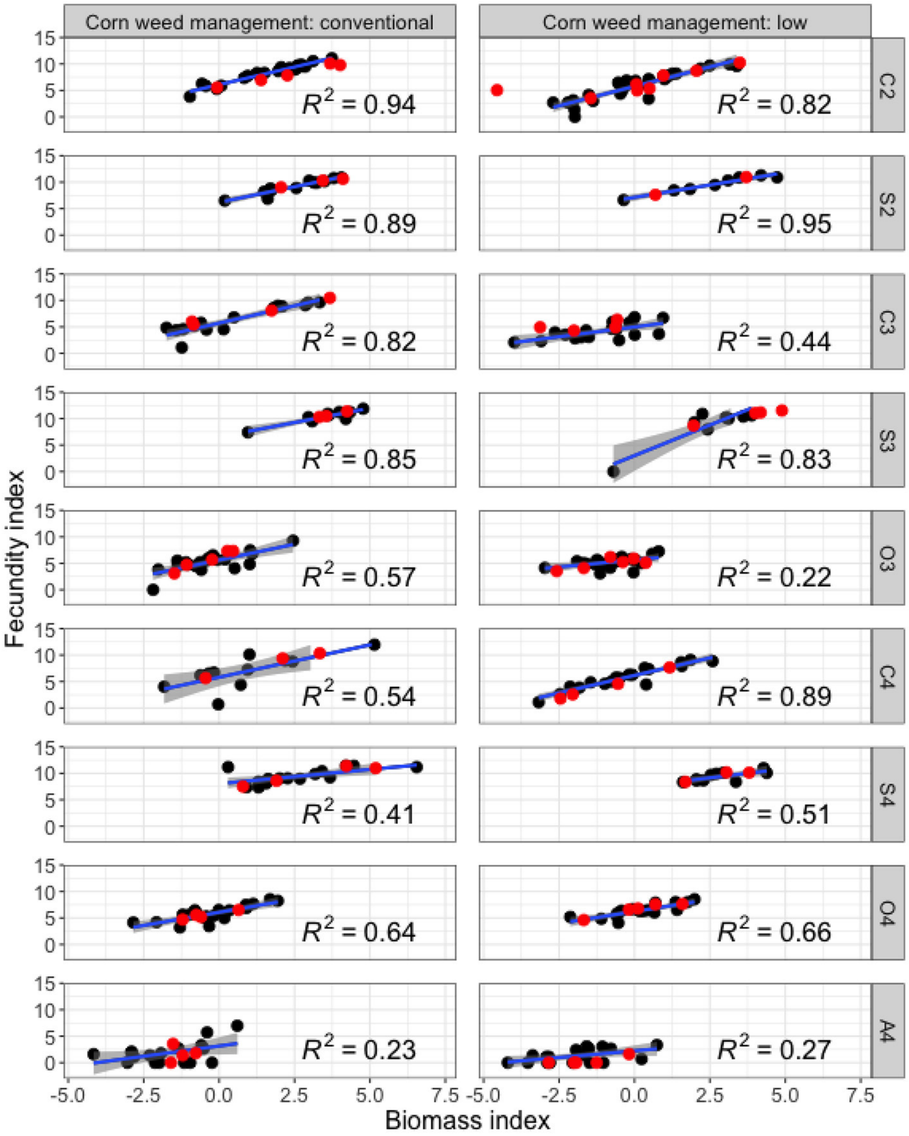

FIGURE 5 | Estimation of fecundity using aboveground mass. The abbreviations on the right-hand-side *y*-axis are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged (C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; and A4, alfalfa in the 4-year rotation). The black and red dots are values from training and testing sets, respectively. Each regression line was plotted for one crop identity by herbicide treatment using the training set. R^2 values were calculated from the training set only, and thus, were slightly different from those in **Table 9**. Biomass index = ln(gram biomass + 0.005) and Fecundity index = ln (seeds + 1).

evolution of resistance in weed populations. It would also be useful to see how populations would change once resistance occurred and how various control methods might contribute to resistance management.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s. The data from this present study is available at doi: 10.25380/iastate.18190784.

AUTHOR CONTRIBUTIONS

ML conceptualized and designed the experiment. HN collected the data and wrote the original draft of the manuscript. ML and HN finalized the manuscript. Both authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2022.811359/full#supplementary-material

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Weed Community Composition in Simple and More Diverse Cropping Systems

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Weed communities in three cropping systems suitable for the Midwestern USA were studied from 2017 to 2020 to examine how crop diversification and the intensity of herbicide use affected weed community diversity, stand density, and aboveground mass. A baseline 2-year cropping system with corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) grown in alternate years was diversified with cool-season crops, namely oat (*Avena sativa* L.), red clover (*Trifolium pratense* L.), and alfalfa (*Medicago sativa* L.) in 3-and 4-year systems. Herbicide was not applied in the cool-season crops. Changing weed management regime from broadcast to banded application and interrow cultivation in corn and omitting herbicide in cool-season crops of the 3- and 4-year rotations resulted in an overall reduction of herbicide a.i mass. The reduction in the mass of herbicide active ingredients was associated with increases in weed stand density, aboveground mass, and community diversity. Increased weed abundance under herbicide mass reduction was not associated with crop yield loss. In the cool-season crops phases of the 3- and 4-year rotations, weed emergence was increased but weed growth was not, as compared with the warm-season crop environments. The dominance of aggressive weed species such as common waterhemp (*Amaranthus tuberculatus* (Moq ex DC) J.D. Sauer) and common lambsquarter (*Chenopodium album* L.) tended to be greater in corn and soybean phases of the rotations than in oat, red clover, and alfalfa.

Keywords: weed community composition, diversity, evenness, richness, Midwestern-United States, agroecology, integrated weed management

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INTRODUCTION

The composition of weed communities found in agricultural fields is strongly affected by the types of crops grown and their attendant management practices (Mohler, 2001; Légère et al., 2005; Culpepper, 2006; Smith and Gross, 2007). The US Corn Belt is dominated by monocultures and short-term rotations of corn and soybean (Center for Spatial Information Science and Systems, 2021). In response to simplified crop management customized for corn and soybean, weed communities have shifted to domination by aggressive summer annual species including common waterhemp (*Amaranthus tuberculatus* (Moq ex DC) JD Sauer), Palmer amaranth (*Amaranthus palmeri* S. Wats), giant ragweed (*Ambrosia trifida* L.), common lambsquarter (*Chenopodium album* L.), and wooly cupgrass (*Eriochloa villosa* (Thunb) Kunth) (Owen, 2008; Kruger et al., 2009; Reddy and Norsworthy, 2010). Improved understanding of how management practices influence weed community composition could inform weed managers whether crop losses to weed competition are likely to occur and whether a weed community is shifting toward dominance by species that are more aggressive toward crops (Liebman, 2001).

Cropping system diversification strategies that are designed to reduce reliance on external inputs, including herbicides, can balance productivity, profitability, and environmental quality goals (Davis et al., 2012; Hunt et al., 2017, 2019, 2020; Bowles et al., 2020; Tamburini et al., 2020; Beillouin et al., 2021). They can also increase cropping systems' overall resilience to growing environmental adversity (Bowles et al., 2020) and can be effective in suppressing weeds (Weisberger et al., 2019). Increased crop species richness within crop sequences coupled with diversification of management practices applied to maximize crop and minimize weed resource acquisition, are expected to challenge weeds with large sets of stress and mortality factors compared to simple cropping systems (Liebman and Gallandt, 1997; Liebman and Staver, 2001; Westerman et al., 2005).

Storkey and Neve (2018) hypothesized that a more diverse weed community can be less competitive toward crops and weed seedbank diversity can be used as an indicator of cropping system sustainability. Nonetheless, few studies have examined weed community composition in rotations with crop species other than corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and wheat (*Triticum aestivum* L.), especially in fully phased settings, in which all crop phases within a rotation are present each year to control for year to year variations in weather conditions and management efficacy (Payne, 2015). Davis et al. (2005b) studied weed aboveground and underground community shifts in four row-crop systems under four combinations of weed management and tillage regimes and found a strong negative relationship between crop yield and weed diversity, density, and total biomass; individual responses of only common waterhemp and common lambsquarter were reported. Smith and Gross (2007) compared a monoculture of corn with 2- and 3-year rotations of corn with soybean and winter wheat, with or without cover crops and found that crop rotation and diversity had weak effects on weed community composition, whereas the cover crop in a particular rotation played an important role in weed species diversity. Increased reliance on glyphosate-based weed management has caused weed floras to shift to dominance by hard-to-control species (Owen, 2008), but it is unclear whether reduction in herbicide use would cause the same problem. Liebman et al. (2021) provided empirical evidence to support the hypothesis that seedbank diversity could be used as an indicator of cropping system sustainability (Storkey and Neve, 2018).

This study was pursued to address the current gap of information concerning weed community density and aboveground mass responses to the filtering effects of different crop and weed management programs (Ryan et al., 2010; Fried et al., 2012). We studied three different cropping systems suitable for the US Corn Belt. The baseline system was a conventional corn—soybean system. We diversified that baseline system with oat (*Avena sativa* L.), red clover (*Trifolium pratense* L.), and alfalfa (*Medicago sativa* L.). Conventional broadcast herbicide and reduced herbicide management regimes were applied in a split-plot manner to corn phases of the three rotations. We hypothesized that diversified cropping systems, with reduced use of chemical herbicides, would provide weed control equal in effectiveness to the conventional approaches applied in the 2-year corn and soybean system. We assessed weed control efficacy by measuring weed aboveground mass and population densities. Additionally, we measured crop yields, positing that differences in weed aboveground mass and density could be reflected in differences in crop yields. Next, we hypothesized that the weed communities in the more diverse cropping systems would be more diverse, more even, and more species-rich than those in the 2-year corn and soybean system, reflecting a broader range of crop species and their attendant management practices in the more diverse rotations. Finally, we hypothesized that including oat, red clover, and alfalfa in rotations with corn and soybean would reduce the density and aboveground mass of noxious weed species in corn and soybean when the rotations cycles returned to corn and soybean.

MATERIALS AND METHODS

Empirical measurements of weed community composition were made from 2017 to 2020 at Iowa State University's Marsden Farm in Boone County, Iowa, USA, (42° 01'N, 93° 47'W, 333 m above sea level). All soil types present at the site are Mollisols (Chen et al., 2014). A detailed description of the experiment site and crop management can be found in Liebman et al. (2021) and the field layout and experiment design are provided in Nguyen and Liebman (accepted). Briefly, a randomized complete block, split-plot design with four replications was used to study three different crop rotation systems (2-, 3-, or 4-year; the crop sequence in each rotation was presented in Table 1 of Nguyen and Liebman, accepted). The main-plot factor, i.e., the crop identity, was represented by crop species and the rotation system in which it occurred (C2-corn in the 2-year rotation, C3-corn in the 3-year rotation, C4-corn in the 4-year rotation, S2-soybean in the 2-year rotation, S3-soybean in the 3-year rotation, S4-soybean in the 4-year rotation, O3 - oat in the 3-year rotation, and O4- oat in the 4-year rotation, and A4-alfalfa in the 4-year rotation). The split-plot factor, i.e., the weed management regime applied in the corn phase (corn weed management), was represented by herbicide level (conventional—pre- and post-emergent herbicides broadcast over the whole corn area, or low—post-emergence herbicides banded 38 cm wide on top of corn rows). The reduction of herbicide mass in the low herbicide treatment was supplemented by interrow cultivation. Details concerning crop genotypes and weed management regimes are provided in **Table 1**.

Volunteer crops from a preceding crop season, such as a volunteer corn plant in a soybean plot or a soybean plant in an oat plot, were not considered weeds. Data were collected for individual weed species aboveground mass and density, community weed biomass and density, and crop yield. Weeds were surveyed 4–6 weeks before corn and soybean harvests, and 2–3 weeks after oat harvest or the last hay cut of the season. The passage of a few weeks between oat and alfalfa harvest and weed surveys allowed physically damaged plants in those crops to grow back to recognizability. Weed aboveground samples were

TABLE 1 | Crop variety or hybrid and management from 2017 to 2020 field seasons.

Year	Activity or input	Low herbicide	Conventional herbicide	Low herbicide	Conventional herbicide
		Corn	**Corn**	**Soybean**	**Soybean**
2017	Hybrid or variety	Epley E1420	Epley E1420	Latham L2758 R2	Latham L2758 R2
	Planting date	9-May	9-May	16-May	
	Interrow cultivation date	Jun. 7	Jun. 7	none	none
	Harvest date	Oct. 19	Oct. 19	Oct. 19	
	Herbicides applied (kg ai./ha)	POST: tembotrione (0.049) applied May 31, interrow cultivated Jun. 7	PRE: thiencarbazone methyl (0.037), isoxaflutole (0.093)	PRE: flumioxazin (0.109); POST: glyphosate as potassium salt (1.249), acifluorfen (0.224)	PRE: flumioxazin (0.109); POST: glyphosate as potassium salt (1.249), acifluorfen (0.224)
	Total (kg a.i./ha)	0.049	0.13	1.581	1.581
	Weed sampling date	Sep. 5 and 6	Sep. 5 and 6	Sep. 6, 7, and 8	Sep. 6, 7, and 8
2018	Hybrid or variety	Epley E1420	Epley E1420	Latham L2758 R2	Latham L2758 R2
	Planting date	8-May	8-May	Jun. 3	Jun. 3
	Interrow cultivation date	Jun. 4	none	none	none
	Harvest date	Oct. 30	Oct. 30	Oct. 29	Oct. 29
	Herbicides applied (kg ai./ha)	POST: tembotrione (0.054)	PRE: thiencarbazone methyl (0.037), isoxaflutole (0.092); POST: mesotrione (0.105), nicosulfuron (0.053)	PRE: flumioxazin (0.096); POST: glyphosate as potassium salt (1.540), lactofen (0.140)	PRE: flumioxazin (0.096); POST: glyphosate as potassium salt (1.540), lactofen (0.140)
	Total (kg a.i./ha)	0.054	0.287	1.776	1.776
	Weed sampling date	Sep. 11, 12, and 13	Sep. 11, 12, and 13	Sep. 17, 19, 20, and 21	Sep. 17, 19, 20, and 21
2019	Hybrid or variety	Epley E1730	Epley E1730	Latham 2684 L (Liberty Link)	Latham 2684 L (Liberty Link)
	Planting date	Jun. 3	Jun. 3	Jun. 10	Jun. 10
	Interrow cultivation date	none, due to weather adversity	none	none	none
	Herbicides applied (kg ai./ha)	POST: tembotrione (0.049)	PRE: thiencarbazone methyl (0.037), isoxaflutole (0.092); POST: mesotrione (0.105), nicosulfuron (0.053)	PRE: flumioxazin (0.096); POST: glufosinate ammonium (0.594), clethodim (0.136)	PRE: flumioxazin (0.096); POST: glufosinate ammonium (0.594), clethodim (0.136)
	Total (kg a.i./ha)	0.049	0.287	0.826	0.826
	Weed sampling date	Sep. 17 and 18	Sep. 17 and 18	Sep. 30	Sep. 30
2020	Hybrid or variety	Epley E1730	Epley E1730	Latham 2684 L (Liberty Link)	Latham 2684 L (Liberty Link)
	Planting date	Apr. 23	Apr. 23	13-May	13-May
	Interrow cultivation date	Jun.8	none	none	none
	Harvest date	Oct. 2	Oct. 2	Sep. 23	Sep. 23
	Harvest date	Nov. 6	Nov. 6	Oct. 18	Oct. 18
	Herbicides applied (kg ai./ha)	POST: tembotrione (0.051)	PRE: thiencarbazone methyl (0.037), isoxaflutole (0.092); POST: mesotrione (0.105), nicosulfuron (0.053)	PRE: flumioxazin (0.096); POST: glufosinate ammonium (0.594), clethodim (0.136)	PRE: flumioxazin (0.096); POST: glufosinate ammonium (0.594), clethodim (0.136)
	Total (kg a.i./ha)	0.051	0.287	0.826	0.826

(Continued)

TABLE 1 | Continued

Year	Activity or input	Low herbicide	Conventional herbicide	Low herbicide	Conventional herbicide
	Weed sampling date	Sep. 14 and 15	Sep. 14 and 15	Sep. 16	Sep. 16
		Oat	**Oat**	**Alfalfa**	**Alfalfa**
2017	Hybrid or variety	IN09201	IN09201	Leafguard	Leafguard
	Planting date	Apr. 12	Apr. 12	Mar. 29, 2016	Mar. 29, 2016
	Stubble clipping	Aug. 7 in O3 and O4 and Sep. 11 in O4	Aug. 7 in O3 and O4 and Sep. 11 in O4	Aug. 10, 2016	Aug. 10, 2016
	Harvest date	Jul. 17	Jul. 17	Jun.6, Jul. 7, Aug. 7, and Sep. 11	Jun.6, Jul. 7, Aug. 7, and Sep. 11
	Weed sampling date	Sep. 25, 27, 28, and 29	Sep. 25, 27, 28, and 29	Sep. 25, 27, 28, and 29	Sep. 25, 27, 28, and 29
2018	Hybrid or variety	IN09201	IN09201	Leafguard	Leafguard
	Planting date	Apr. 24	Apr. 24	Apr. 12, 2017	Apr. 12, 2017
	Stubble clipping	Sep. 11	Sep. 11	Sep. 11, 2017	Sep. 11, 2017
	Harvest date	Jul. 20	Jul. 20	Jun. 4, Jul. 9, and Sep. 10	Jun. 4, Jul. 9, and Sep. 10
	Weed sampling date	Sep. 26, Oct.4, 15, 16, 18, and 19	Sep. 26, Oct.4, 15, 16, 18, and 19	Sep. 26, Oct.4, 15, 16, 18, and 19	Sep. 26, Oct.4, 15, 16, 18, and 19
2019	Hybrid or variety	IN09201	IN09201	Leafguard	Leafguard
	Planting date	Apr. 16	Apr. 16	Apr. 24, 2018	Apr. 24, 2018
	Stubble clipping	none	none	none	none
	Harvest date	Jul. 24 and 29	Sep. 24 and 29	Jun. 7, Jul. 12, Aug. 26, 2019	Jun. 7, Jul. 12, Aug. 26, 2019
	Weed sampling date	Sep. 23, 24, 25, and 26, Oct. 3, 4, 7, and 8	Sep. 23, 24, 25, and 26, Oct. 3, 4, 7, and 8	Sep. 23, 24, 25, and 26, Oct. 3, 4, 7, and 8	Sep. 23, 24, 25, and 26, Oct. 3, 4, 7, and 8
2020	Hybrid or variety	IN09201	IN09201	Leafguard	Leafguard
	Planting date	Apr. 2, May 7*	Apr. 2, May 7*	Apr. 16, 2019	Apr. 16, 2019
	Stubble clipping	none	none	none	none
	Harvest date	Jul. 24	Jul. 24	Jun. 2, Jul. 6, and Aug. 17	Jun. 2, Jul. 6, and Aug. 17
	Weed sampling date	Sep. 23, 24, and 29, Oct. 2, 6, 7, and 8	Sep. 23, 24, and 29, Oct. 2, 6, 7, and 8	Sep. 23, 24, and 29, Oct. 2, 6, 7, and 8	Sep. 23, 24, and 29, Oct. 2, 6, 7, and 8

Corn was planted at 12,950 seeds/ha, soybean at 56656 seeds/ha, oat at 80.7 kg/ha, red clover and alfalfa at 19.1 kg/ha. PRE and POST herbicide in corn and soybean refers to pre-emergence and post-emergence, relative to weed emergence. No herbicide was applied in oat, red clover, and alfalfa. "Belle" (in 2017) or "Mammoth" (in 2018–2020) red clover was intercropped with oat in the 3-year rotation (O3). Alfalfa was intercropped with the oat phase in the 4-year rotation (O4) and was overwintered to the following year as a sole crop (A4). Oat was replanted in 2020 due to poor germination.

collected from eight quadrats arranged in a 4 x 2 grid throughout each experimental unit (eu). The sample grid was randomized every year in such a way that quadrats were at least 3 m away from plot borders to avoid any edge effect.

Individual Weed Species Abundance

All the same-species plants from each eu were clipped, enumerated, dried, and weighed at ~0% moisture together to make single data points per eu. The total surveyed area was 18.5 m^2/eu (8 x 2.3 m^2) in corn and soybean and 2.2 m^2/eu (8 x 0.28m^2) in oat and alfalfa. Plants were identified to species as guided by Uva et al. (1997). Plant counts and dried weights were converted to plants m^{-2} and g m^{-2}.

Weed Community Abundance

Weights and counts of individual weed species from each eu were tallied for community abundance.

Ecological Indices

Weed community diversity is the combination of two indices. The community evenness index ranges from 0 to 1, with higher values indicating higher evenness (Alatalo, 1981). The species richness index is a count of the number of species observed. The presence of rare species in low abundance decreases the overall evenness of a weed community (Pielou, 1984; Stirling and Wilsey, 2001). Studying all three indices, i.e., diversity, evenness, and richness, generates a more complete description of a community than any one of the indices (Morris et al., 2014). Simpson's diversity, evenness, and richness indices were calculated in terms of stand density and aboveground mass in each eu. We evaluated eighteen weed communities, corresponding to nine crop identities crossed with two weed management regimes in corn.

Let:

S represent species richness (i.e., the number of species presented),
n_i represent density of the i*th* species (plants m^{-2}),
N represent density of all presented species (plants m^{-2}),
b_i represent aboveground mass of the i*th* species (g m^{-2}),
B represent aboveground mass of all species, g m^{-2}, and
p_{i_d} and p_{i_b} represent the proportional of density or aboveground biomass of the i*th* species.

Community diversity was evaluated with Simpson's index, $Simpson's\ D = \frac{1}{D} = \frac{1}{\sum p_i^2}$, because it is less sensitive to sample size and is useful to describe evenness (Nkoa et al., 2015). Simpson's evenness index was calculated with $\frac{\frac{1}{D}}{S}$. The p_i component in Simpson's diversity and evenness indices here was calculated with stand count ($\frac{n_i}{N}$) or biomass ($\frac{b_i}{B}$). Ideally, only one richness index is needed because it is the number of species presented. However, two ABUTH (*Abutilon theophrasti*) plants that were found in 2019 were too light to register on a scientific scale, resulting in zero weight for the species' aboveground mass. Therefore, the richness index was calculated for both stand and aboveground mass. The evenness index was thus calculated with the relevant richness index with regards to stand count and aboveground mass.

Crop Yields

Six 84-m long rows of corn and soybean (383 m^2) were harvested from each eu, whereas for oat and alfalfa, whole plots were harvested (i.e., two adjacent subplots combined, 1,530 m^2). Yields were adjusted to moisture concentrations of 155 g H_2O kg^{-1} for corn, 130 g H_2O kg^{-1} for soybean, 140 H_2O kg^{-1} for oat grain, and 150 g H_2O kg^{-1} for alfalfa.

Model Fitting

Block, crop identity, weed management regime applied to the corn phase of a rotation (corn weed management), and the interaction of crop identity and corn weed management were considered fixed factors; year and the interaction between year and the fixed factors were considered random factors; and the residual was random by default. Block was treated as a fixed factor to control for the different field conditions across sections and reduce the variance between eu's (Dixon, 2016).

R version 4.1.2 (R Development Core Team, 2021) was used for all data organization, manipulation, analysis, models diagnosis, and result presentation. Statistical tests were evaluated at an $\alpha = 0.05$ level of significance. All the response variables were natural logarithm (ln) transformed to ensure homogeneity of variance. For each response, the minimum non-zero value was added to zero values before transformation). Type III sums of squared error were calculated with the `emmeans` package's `joint_tests` function to accommodate unbalanced data with interaction (version 1.7.2, Lenth, 2022). Results were back-transformed for presentation. Degree of freedom adjustment was done with Satterthwaite's method. *P*-values adjustment was done with Tukey's method.

Stand diversity, stand evenness, stand richness, aboveground mass diversity, aboveground mass evenness, aboveground mass richness, community aboveground density, community aboveground mass, individual species density, and individual species aboveground mass were analyzed separately with a linear mixed-effects model, using the `lmer` function in the `lme4` package (version 1.1–27.1, Bates et al., 2015) according to the following model.

$$R_{ijklm} = \mu + B_i + C_j + H_k + CH_{jk} + Y_l + BY_{il} + YC_{lj} + YH_{lk} + YCH_{ljk} + BYC_{ijl} + \epsilon_{ijkl} \tag{1}$$

where,

R is one of the aforementioned responses,
μ is the overall mean,
B is the block,
Y is the year,
C is the crop identity,
H is the corn weed management,
CH is the interaction between crop identity and corn weed management,
BY is the block within a year,
YC is the interaction between crop identity and year,
YH is the interaction between year and corn herbicide,

YCH is the interaction between year, crop identity, and corn weed management,
BYC is the interaction between block, year, and crop identity, and
ϵ_{ijkl} is the residual.

The crop identity term in the right-hand side of the model (Equation 1) represents the main-plot effect of the experiment, which comprises of the crop species and the rotation to which it belonged. In this present study, "cropping system" is the combination of "rotation system" (2-, 3-, and 4-year) and herbicide regime in corn (low or conventional); and crop type represents growing condition, so corn and soybean were grouped as warm-season crops, whereas oat and alfalfa were grouped as cool-season crops. With this model, we tested the following three sets of hypotheses for treatment effects on weed community stand diversity, community stand evenness, community stand richness, community aboveground mass diversity, community aboveground mass evenness, community aboveground mass richness, community aboveground density, and community aboveground mass:

1) The response variables increased as cropping system diversity increased.
2) In the same crop species the response variables differed between cropping systems.
3) In the same crop species the response variables differed between different crop types within a given cropping system.

The first set of hypotheses was tested by contrasting the responses in the 2-year rotation with those in the average of the 3- and 4-year rotations and the responses in the 3-year rotation with those in the 4-year rotation. The second set of hypotheses was tested by contrasting the responses in the same crop species within different rotations. The third set of hypotheses was tested by contrasting the average responses in the warm-season crops between rotations, in the cool-season crops between rotations, in the warm-season vs. cool-season crops within the same rotation, and between the warm-season crops and the cool-season crop(s) averaged over rotations.

The same sets of contrasts used to evaluate weed community ecological indices, weed community aboveground mass, and weed community stand density were applied to data concerning the stand density and aboveground mass of the seven most abundant weed species to test for the treatment effects on those species:

4) The response variables differed between rotations for the same crop species, differed between rotations, and differed between crop type within a given cropping system.

The fourth set of hypotheses was tested by contrasting individual weed species density and aboveground mass (a) in the 2-year rotation vs. the average of 3- and 4-year rotations and in the 3- vs. 4-year rotation, (b) in the same crop species or type between rotations, (c) in different crop types within the same rotation, and (d) in different crop types averaged over rotations.

TABLE 2 | Contrasts of rotation effect (expressed by Crop ID) on crop yields.

	ANOVA				**Comparison**		
Source of variation	**df1**	**df2**	***F***	***p***	**Contrast**	**Ratio**	***p***
(A) Corn							
Crop ID	2	6	3.19	0.1138	C2 vs. C3	0.94	0.1882
Corn weed management	1	3	0.32	0.6088	C2 vs. C4	0.93	0.1278
Crop ID x Corn weed management	2	6	2.20	0.1914	C3 vs. C4	0.99	0.9507
(B) Soybean							
Crop ID	2	6	8.22	0.0191	S2 vs. S3	0.96	0.5499
Corn weed management	1	3	0.18	0.7018	S2 vs. S4	0.86	0.0181
Crop ID x Corn weed management	2	6	0.62	0.5677	S3 vs. S4	0.90	0.0670
(C) Oat							
Crop ID	1	2	1.14	0.3979	O3 vs. O4	0.91	0.3979

The abbreviations on the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred. Corn weed management: low herbicide or conventional. Crop ID: crop species and the cropping system in which it occurred: C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, oybean in the 2-year rotation; S3, oybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation.

A different linear mixed-effects model was used to analyze corn, soybean, and oat yields (`lme4` version 1.1-27.1, Bates et al., 2015):

$$R_{ijkm} = \mu + B_i + C_j + H_k + CH_{jk} + Y_l + BY_{il} + YC_{lj} + YH_{lk} + YRH_{lij} + BYC_{ilj} + \epsilon_{ijkl} \quad (2)$$

where,
R is the individual crop yield, and
all the terms in the right-hand side of the model are as defined in Equation (1).

As each crop species was fitted with a model, the crop identity represents the rotation effect only. With this model (Equation 2), we tested the hypothesis that the yield of the same crop species (corn, soybean, and oat) did not differ between rotations. Crop yields were then contrasted between rotations to examine the magnitude of any significant difference.

RESULTS

A lack of any obvious bias in plots of residuals vs. predicted values suggested that the analysis models fit the data well. Diagnosis plots made with `ggResidpanel` (version 0.3.0, Goode and Rey, 2019) are available in Model Diagnosis.

How Did Rotation System and Corn Weed Management Affect Crop Yields?

Results of the experiment indicated that crop diversification and reduced use of herbicides were not associated with lower crop yields (**Table 2**). Averaged over 4 years, soybean was the only crop whose yield was affected by rotation ($p = 0.0191$, **Table 2**).

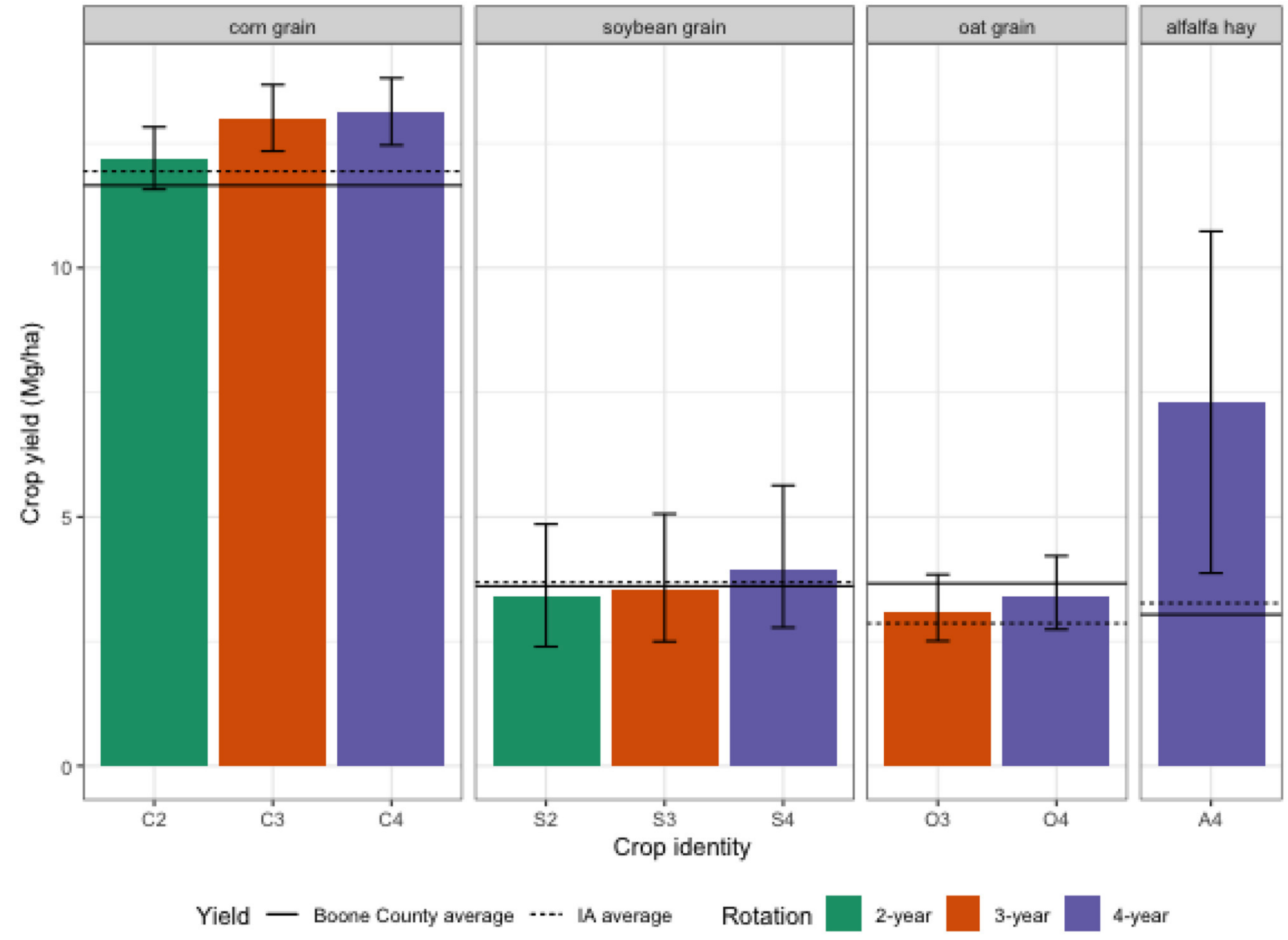

FIGURE 1 | Mean crop yields by rotation from 2017 to 2020. The color-coded bars show crop yields (Mg ha^{-1}) in the experiment plots. The error bars show the 95% confidence intervals. The solid horizontal lines show mean yields for Iowa and dashed lines show mean yields for Boone County. Corn, soybean, and alfalfa yields in the experiment were averaged over 4 years, oat grain yields in the experiment were averaged over 2017, 2019, and 2020 because in 2018 oat was harvested for hay. Boone County and Iowa hay yields were averaged over 2017 and 2018 because 2019 and 2020 yields were not available at this writing.

Soybean yield was 16% higher in the 4-year rotation than in the 2-year rotation ($p = 0.0181$). Crop yields in the experiment were as high or higher than the averages for the state of Iowa and Boone County (**Figure 1**).

How Did Rotation System, Crop Species, and Corn Weed Management Affect Community Ecological Indices?

Crop identity (i.e., rotation system x crop phase combination) affected weed community stand density evenness ($p = 0.0064$) and richness ($p = 0.0123$, **Table 3C**) and aboveground mass diversity ($p = 0.0007$, **Table 3A**), evenness ($p = 0.0003$, **Table 3B**), and richness ($p = 0.013$). For all the differences in ecological indices, crop types were more influential than rotations, with larger differences found between crop types than between rotations (**Figure 2**, **Tables 4**, **5**).

In general, the hypothesis that "weed communities in the more diverse cropping systems are more diverse" was supported.

Averaged over crop phases within each rotation system (**Table 4A**), the weed community stand diversity index for the 3-and 4-year rotation systems was comparable with that in the 2-year rotation ($p = 0.0535$ and $p = 0.1575$, respectively). For the individual crops (**Table 4B**), the weed stand density diversity index was comparable among rotations ($p > 0.05$). For different crop types (**Table 4C**), the weed community stand density diversity index in the average for the cool-season crops (O3, O4, and A4) was 1.2-fold greater than that in the average for the warm-season crops (C2, S2, C3, S3, C4, and S4) ($p = 0.0145$), but similar between the warm-season and cool-season crops in the same rotations ($p = 0.4666$ and $p = 0.0987$, respectively). The weed stand density diversity index was similar between oat and alfalfa ($p = 0.7762$).

Averaged over crop phases within the same rotation (**Table 5A**), the weed community aboveground mass diversity index was different between the 2-year rotation and the average of the 3- and 4-year rotations ($p = 0.0148$), and between the 3- and 4-year rotations ($p = 0.0209$). Averaged over the corn

TABLE 3 | ANOVAs of crop identity, corn weed management, and their interactive effects on weed community ecological indices.

Source of variation	df1	df2	Stand density		Aboveground mass	
			F	***p***	***F***	***p***
(A) Community diversity						
Crop ID	8	24	1.25	0.3116	5.22	0.0007
Corn weed management	1	3	0.21	0.6804	0.47	0.5439
Crop ID x Corn weed management	8	24	0.54	0.8182	1.35	0.2659
(B) Community evenness						
Crop ID	8	24	3.66	0.0064	5.87	0.0003
Corn weed management	1	3	0.24	0.6589	0.01	0.9414
Crop ID x Corn weed management	8	24	0.74	0.6547	0.47	0.8632
(C) Community richness						
Crop ID	8	24	3.23	0.0123	3.19	0.0130
Corn weed management	1	3	1.32	0.3330	1.59	0.2959
Crop ID x Corn weed management	8	24	0.71	0.6803	0.86	0.5635

Corn weed management: low herbicide or conventional. Crop ID: crop species and the cropping system in which it occurred: C2,corn in the 2-year rotation, C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; A4, alfalfa in the 4-year rotation.

and soybean phases within the same rotation (**Table 5A**), the weed community aboveground mass diversity index was similar between rotations ($p = 0.4217$ and $p = 0.2426$, respectively). For the individual crops (**Table 4B**), the weed community aboveground mass diversity index was comparable between rotations, except for oat ($p = 0.0351$). For different crop types (**Table 4C**), the weed community aboveground mass diversity index in the cool-season crops average was 1.3-fold greater than that in the warm-season crops averages, overall ($p < 0.0001$), and was 1.23-fold and 1.27-fold greater in the cool-season than that in the warm-season crops in the 3-year ($p = 0.034$) and 4-year rotation ($p = 0.0037$), respectively. The weed community aboveground mass diversity index was comparable between oat and alfalfa ($p = 0.2583$).

The hypothesis that "weed communities in the more diverse cropping systems are more even" was partially supported (**Figures 2B,E**). However, a lower community evenness index can occur because the presence of rarer species decreases the overall evenness index (Stirling and Wilsey, 2001). More details to support this concept are presented later (**Figures 3C,D**).

Averaged over crop phases within the same rotation (**Table 4A**), the weed community stand density evenness index in the 2-year rotation was 1.6-fold greater than that in the average of the 3- and 4-year rotations ($p = 0.006$), but comparable between the 3- and 4-year rotations ($p = 0.2802$). Averaged over the corn and soybean phases within the same rotation (**Table 4A**), the weed community stand density evenness index was comparable between rotations ($p = 0.1539$ and $p = 0.5031$, respectively). For the individual crops (**Table 4B**), the weed community stand density evenness index was comparable between rotations ($p > 0.05$). For different crop types (**Table 4C**), the weed community stand density evenness index in the cool-season crops average was half of that in the warm-season crops average ($p = 0.0002$) and half of that in the cool-season and warm-season crop in the 4-year rotation ($p = 0.0012$), but similar between the warm-season and cool-season crops in the 3-year rotation ($p = 0.4418$). The weed community stand density evenness index was comparable between oat and alfalfa ($p = 0.8986$).

Averaged over crop phases within the same rotation (**Table 5A**), the weed community aboveground mass evenness index in the 2-year rotation was 1.65-fold greater than that in the average of 3- and 4-year rotations ($p = 0.0012$), but similar between the 3- and 4-year rotations ($p = 0.0802$). Averaged over the corn and soybean phases within the same rotation (**Table 5A**), weed community aboveground mass evenness index was comparable between rotations ($p = 0.1081$ and $p = 0.8682$, respectively). For the individual crops (**Table 4B**), the weed community aboveground mass evenness index was comparable between rotations ($p > 0.05$), except for oat ($p = 0.0189$). The weed community aboveground mass evenness index in the warm-season crops average was twice that of the cool-season crops average ($p < 0.0001$). The weed community aboveground mass evenness index in the warm-season crops was twice that of the cool-season crops in the 4-year rotation ($p = 0.0002$), but comparable between the warm-season and cool-season crops in the 3-year rotation ($p = 0.141$), and between oat and alfalfa ($p = 0.5911$).

The hypothesis that "the weed communities in the more diverse cropping systems are more species-rich" was supported.

Averaged over crop phases within the same rotation (**Table 4A**), the weed community stand density richness index was comparable in the 2-year rotation and in the average of the 3- and 4-year rotations ($p = 0.1819$), but the stand density richness index in the 3-year was 0.77 that of the 4-year rotation ($p = 0.0257$). Averaged over the corn and soybean phases within the same rotation (**Table 4A**), weed community aboveground mass richness index was comparable between the 2-year rotation and the 3- and 4-year rotations average ($p = 0.7996$) and between the 3- and 4-year rotations ($p = 0.3469$). For individual crops (**Table 4B**), the weed community stand density richness index was comparable between rotations (p > 0.05). For different crop types (**Table 4C**), the weed stand density richness index in the cool-season crops average was 1.33-fold greater that of the warm-season crops average ($p = 0.0003$). Within the 4-year rotation, the weed stand density richness index in the cool-season was 1.58-fold greater than that in the warm-season crops ($p = 0.0034$). The weed stand density richness was comparable between the warm-season and cool-season crops in the 3-year rotation ($p = 0.0725$) and between oat and alfalfa ($p = 0.9499$).

The same patterns of difference and similarity of weed community richness index calculated with aboveground mass was observed (**Table 5**).

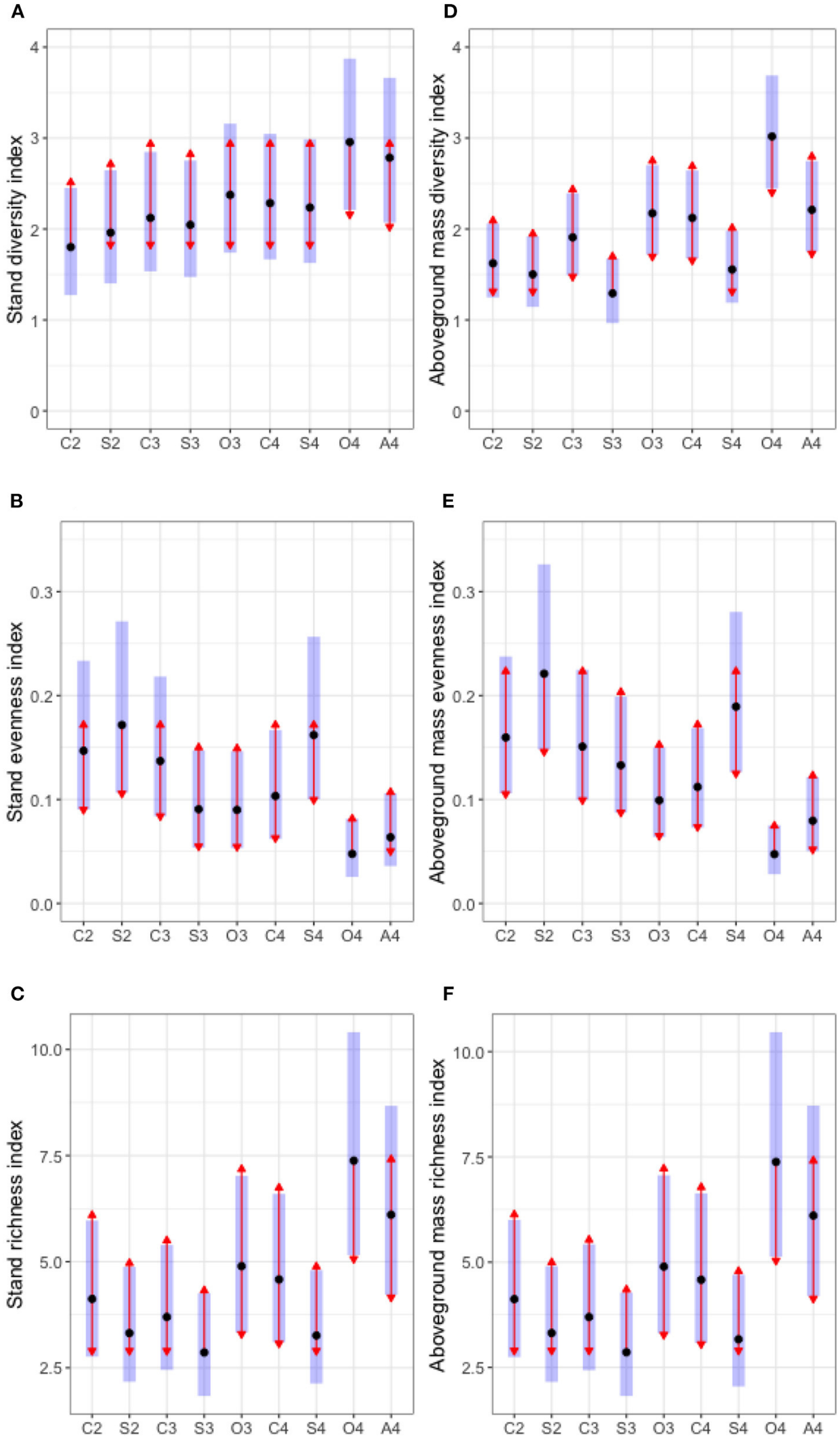

FIGURE 2 | Weed community stand diversity **(A)**, evenness **(B)**, richness **(C)**, community aboveground diversity **(D)**, evenness **(E)**, and richness **(F)**. The abbreviations on the x-axis are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred (C2-corn in the 2-year rotation, C3-corn in the 3-year rotation, C4- orn in the 4-year rotation, S2-soybean in the 2-year rotation, S3-oybean in the 3-year rotation, S4-soybean in the 4-year rotation, O3-oat in the 3-year rotation, O4-oat in the 4-year rotation, and A4-alfalfa in the 4-year rotation). The black dots are estimated marginal means. The blue bars are 95% confidence intervals. The red arrows reflect comparisons among means. Overlapping arrows indicate non-significant differences.

TABLE 4 | Weed stand density ecological indices contrast significance.

	Diversity index		Evenness index		Richness index	
Contrast	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***
(A) Rotation system effects						
[(C2+S2)/2] vs. [(C3+S3+O3+C4+S4+O4+A4)/7]	0.85	0.0535	1.60	0.0060	0.86	0.1819
[(C3+S3+O3)/3] vs. [(C4+S4+O4+A4)/4]	0.90	0.1575	1.18	0.2802	0.77	0.0257
[(C2+S2)/2] vs. [(C3+S3+C4+S4)/4]	0.91	0.2749	1.28	0.1539	1.03	0.7996
[(C3+S3)/2] vs. [(C4+S4)/2]	0.95	0.5824	0.88	0.5031	0.87	0.3469
(B) Rotation system effects within individual crops						
C2 vs. [(C3+C4)/2]	0.88	0.2836	1.20	0.4406	1.00	0.9985
C3 vs. C4	0.95	0.7231	1.28	0.3757	0.84	0.3966
S2 vs. [(S3+S4)/2]	0.94	0.6331	1.36	0.2065	1.06	0.7212
S3 vs. S4	0.94	0.6711	0.60	0.0746	0.91	0.6260
O3 vs. O4	0.85	0.2716	1.66	0.0757	0.70	0.0912
(C) Crop type effects						
[(O3+O4+A4)/3] vs. [(C2+S2+C3+S3+C4+S4)/6]	1.20	0.0145	0.55	0.0002	1.53	0.0003
O3 vs. [(C3+S3)/2]	1.09	0.4666	0.83	0.4418	1.38	0.0725
[(O4+A4)/2] vs. [(C4+S4)/2]	1.19	0.0987	0.49	0.0012	1.58	0.0034
[(O3+O4)/2] vs. A4	0.97	0.7762	1.03	0.8986	0.99	0.9499

The abbreviations on the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred. C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; A4, alfalfa in the 4-year rotation.

TABLE 5 | Weed aboveground mass ecological indices contrast significance.

	Diversity index		Evenness index		Richness index	
Contrast	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***
(A) Rotation system effects						
[(C2+S2)/2] vs. [(C3+S3+O3+C4+S4+O4+A4)/7]	0.85	0.0148	1.65	0.0012	0.86	0.1967
[(C3+S3+O3)/3] vs. [(C4+S4+O4+A4)/4]	0.87	0.0209	1.27	0.0802	0.78	0.0309
[(C2+S2)/2] vs. [(C3+S3+C4+S4)/4]	0.95	0.4217	1.28	0.1081	1.04	0.7694
[(C3+S3)/2] vs. [(C4+S4)/2]	0.91	0.2426	0.97	0.8682	0.88	0.3930
(B) Rotation system effects within individual crops						
C2 vs. [(C3+C4)/2]	0.87	0.1425	1.20	0.3825	1.00	0.9985
C3 vs. C4	0.93	0.5084	1.31	0.2780	0.84	0.4035
S2 vs. [(S3+S4)/2]	1.03	0.7219	1.36	0.1543	1.08	0.6801
S3 vs. S4	0.90	0.3166	0.72	0.1905	0.93	0.7075
O3 vs. O4	0.79	0.0351	1.83	0.0189	0.70	0.0957
(C) Crop type effects						
[(O3+O4+A4)/3] vs. [(C2+S2+C3+S3+C4+S4)/6]	1.30	<0.0001	0.51	<0.0001	1.54	0.0003
O3 vs. [(C3+S3)/2]	1.23	0.0340	0.73	0.1410	1.38	0.0766
[(O4+A4)/2] vs. [(C4+S4)/2]	1.27	0.0037	0.48	0.0002	1.60	0.0032
[(O3+O4)/2] vs. A4	1.11	0.2583	0.89	0.5911	0.99	0.9506

The abbreviations on the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred. C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; A4, alfalfa in the 4-year rotation.

General Description of the Weed Flora

Overall, 34 weed species were identified during the 4 years of data collection (**Table 6**). Seven weed species, SETFA (*Setaria faberi*), AMATA (*Amaranthus tuberculatus*), CHEAL (*Chenopodium album*), DIGSA (*Digitaria sanguinalis*), ECHCG (*Echinochloa crus-galli*), SETLU (*Setaria glauca*), and TAROF (*Taraxacum officinale*) made up 94.4% of the total weed density and 94.0% of the total weed biomass (**Figures 3C,D**).

How Did Rotation, Crop Species, and Corn Weed Management Affect Weed Community Density and Growth?

Crop identity affected weed community stand density ($p < 0.0001$) and weed community aboveground mass ($p = 0.0057$), but corn weed management and its interaction with crop identity did not affect weed community stand density or biomass ($p > 0.05$) (**Tables 4**, **5**). Weed community stand density and

TABLE 6 | List of weed species (in alphabetical order) found from 2017 to 2020 field seasons.

Bayer code	Scientific name	Life cycle
(A) Dicotyledon species		
ABUTH	*Abutilon theophrasti* Medicus	Annual
AMARE	*Amaranthus retrofelxus* L.	Summer annual
AMATA	*Amaranthus tuberculatus* (Moq.) Sauer var. *rudis*	Summer annual
AMBEL	*Ambrosia artemissifolia* L.	Erect, branching, summer annual
ARFMI	*Arctium minus* (Hill) Bernh.	Biennial
CHEAL	*Chenopodium album* L.	Erect summer annual
CIRAR	*Cirsium arvense* (L.) Scop.	Rhizomatous perennial
CIRVU	*Cirsium vulgare* (Savi) Tenore	Biennial
EPHHT	*Euphorbia humistrata* Engelm. ex Gray	Mat-forming summer annual
EPHMA	*Euphorbia maculata* L.	Mat-forming summer annual
EUPHY	*Eupatorium hyssopifolium* L.	Summer annual
MORAL	*Morus alba* L.	Perennial shrub
PHYSU	*Physalis subglabrata* Mackenz. and Bush	Rhizomatous perennial
PLAMA	*Plantago major* L.	Rosette-forming perennial
POLPE	*Polygonum perfoliatum* L.	Spiny summer annual vine
POLPY	*Polygonum pensylvanicum* L.	Ascending much-branched summer annual
POROL	*Portulaca oleracea* L.	Prostrate mat-forming summer annual
SOLPT	*Solanum ptycanthum* Dun.	Erect branching summer annual
SONAR	*Sonchus arvensis* L.	Rhizomatous perennial
TAROF	*Taraxacum officinale* Weberin Wiggers	Tap-rooted perennial
(B) Monocotyledon species		
AGRRE	*Elytrigia repens* (L.) Nevski	Rhizomatous perennial
BROTE	*Bromus tectorum* L.	Summer or winter annual
CCHPA	*Cenchrus longispinus* (Hack.) Fern.	Summer annual
CONAR	*Convolvulus arvensis* L.	Rhizomatous perennial
CYPES	*Cyperus esculentus* L.	Rhizomatous perennial
DACGL	*Dactylis glomerata* L.	Clump-forming perennial
DIGSA	*Digitaria sanguinalis* (L.) Scop.	Summer annual
ECHCG	*Echinochloa crus – galli* (L.) Beauv.	Summer annual
ERBVI	*Eriochloa villosa* (Thunb.) Kunth	Erect summer annual
FESSP	*Festuca* spp.	Clump-forming perennial
PANCA	*Panicum capillare* L.	Summer annual
PANDI	*Panicum dichotomiflorum* Michx.	Summer annual
SETFA	*Setaria faberi* Herrm.	Clump-forming, erect summer annual
SETLU	*Setaria glauca* (L.) Beauv.	Clump-forming, erect summer annual

aboveground mass in each crop identity category, averaged over blocks, years, and corn weed management regimes, are presented in **Figures 3A,B**. Contributions by the dominant species are presented in **Figures 3C,D**. Contrasts for the effects of rotation systems, rotation system within individual crops, and crop types on community stand density and aboveground mass are shown in **Table 7C**.

Weed community density and aboveground mass of the 3- and 4-year systems averages were comparable to those of the 2-year system ($p = 0.058$ and $p = 0.9451$, respectively; **Table 7B1**). The weed community density in the 4-year rotation was 2.5-fold greater than that in the 3-year rotation ($p = 0.0368$), but the community aboveground mass was comparable between the 3- and 4-year rotations.

For the individual crops (**Table 7B2**), increased rotation diversity tended to decrease weed density and aboveground mass in corn and soybean and increase weed abundance in oat, but these changes were not significant ($p = 0.6354$ and $p = 0.4041$ for corn, $p = 0.1834$ and $p = 0.0739$ for soybean, and $p = 0.3955$ and $p = 0.335$ for oat). The patchiness of weeds, which was reflected in the high standard error values, might have caused the lack of significance for these inconclusive trends.

For different crop types, weed community density and aboveground mass were comparable between the warm-season crops (corn and soybean) and between the cool-season crops (oat and alfalfa) (**Table 7B3**). Overall, the average weed community density in the cool-season crops was 26-fold greater than that in the warm-season crops ($p < 0.0001$), and the average weed community aboveground mass in cool-season crops was 16-fold greater than that in warm-season crops ($p = 0.0001$). In the 3-year rotation, the weed stand community stand in oat (O3) was 11.5-fold greater than the average in corn and soybean (C3 and S3) ($p = 0.0012$), but the weed community aboveground mass was comparable between O3 and the average of the C3 and S3 phases ($p = 0.1502$). In the 4-year rotation, the weed community stand density in the average of oat and alfalfa (O4 and A4) was 36-fold greater than the average of the corn (C4) and soybean (S4) phases ($p < 0.0001$), and the average weed biomass for the O4 and A4 phases was 29-fold greater than that for the C4 and S4 phases ($p < 0.0001$).

How Did Rotation, Crop Species, and Corn Weed Management Affect Individual Weed Species Abundance?

The hypothesis that "including oat and alfalfa in rotations with corn and soybean will reduce the density and aboveground mass of noxious weed species in corn and soybean" was partially supported. Crop identity affected individual density of seven most abundant weed species but corn weed management affected that of two weed species only, i.e., DIGSA and SETFA ($p = 0.0189$ and $p = 0.0196$, resepectively; **Table 8**. Among those seven weed species, the aboveground mass of four (CHEAL, DIGSA, SETFA, and TAROF) were affected by crop identity, but none was affected by corn weed management (**Table 8**). The magnitude of difference in stand density and aboveground mass were the most pronounced between crop types (**Table 9**). The main-plot effects concerning crop identity on individual species responses are elaborated below.

The cool-season crops were responsible for AMATA stand density differences, but those differences were not strong enough to be apparent between rotation averages. AMATA stand density and

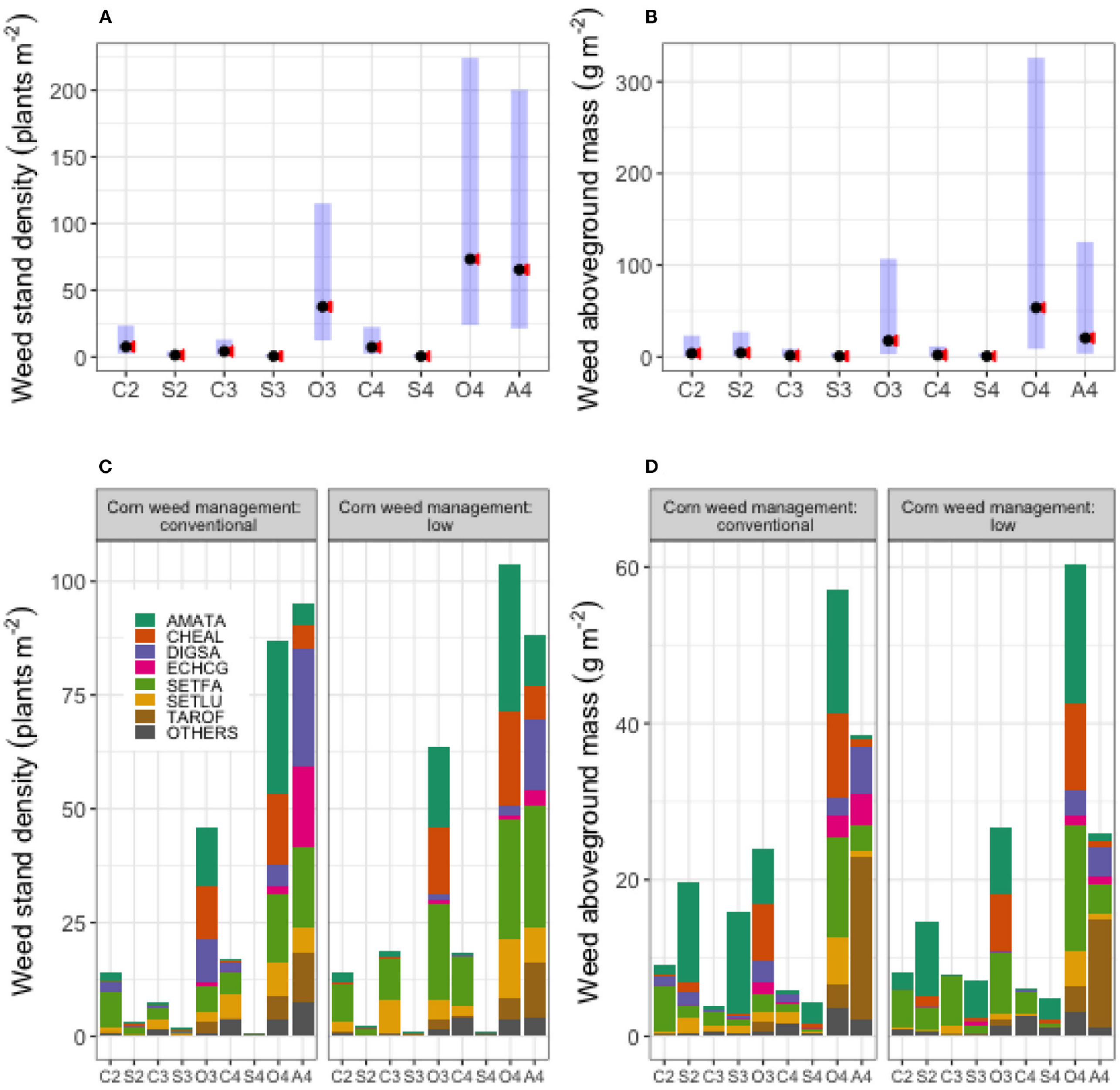

FIGURE 3 | In **(A,B)** weed community stand density and aboveground mass were averaged over four blocks, 4 years, and two corn weed management regimes; the black dots are estimated marginal means; the blue bars are 95% confidence intervals; the red arrows reflect the comparisons among means; overlapping arrows indicate non-significant differences. In **(C,D)** the contribution of the seven most abundant weed species and the rarer species (species ordered eighth and above grouped in OTHERS) in each crop identity, averaged over four blocks and 4 years, are ordered alphabetically. The abbreviations on the x-axis are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred (C2-corn in the 2-year rotation, C3-corn in the 3-year rotation, C4-corn in the 4-year rotation, S2-soybean in the 2-year rotation, S3-soybean in the 3-year rotation, S4-soybean in the 4-year rotation, O3-oat in the 3-year rotation, O4-oat in the 4-year rotation, and A4-alfalfa in the 4-year rotation). The less abundant weed species which made up 6% of the whole community are grouped in OTHERS. The means displayed on **(A,B)** were estimated marginal means, calculated based on the analysis model (with `emmip` (version 1.7.2, Lenth, 2022) function) but the means displayed on **(C,D)** were arithmetic means, calculated from the data so they are slightly different.

aboveground mass were comparable among all rotation systems averaged over crop phases ($p > 0.05$), among rotations for the same crop species ($p > 0.05$), and within the same crop type across rotations ($p > 0.05$). Averaged over the same crop types (warm-season or cool-season), AMATA stand density in cool-season was 12.25-fold greater than that in warm-season crops ($p = 0.0001$), but AMATA aboveground mass was comparable in cool-season and warm-season crops ($p = 0.0906$). Within the

TABLE 7 | Community density and aboveground mass ANOVA and contrasts.

(A) ANOVA			**Stand density**		**Aboveground mass**	
Source of variation	**df1**	**df2**	***F***	***p***	***F***	***p***
Crop ID	8	24	12.22	<0.0001	3.74	0.0057
Corn weed management	1	3	2.13	0.2402	0.02	0.8900
Crop ID x Corn weed management	8	24	1.66	0.1613	0.99	0.4660
(B) Contrasts			**Ratio**	***p***	**Ratio**	***p***
(B1) Rotation system effects						
[(C2+S2)/2] vs. [(C3+S3+O3+C4+S4+O4+A4)/7]			0.42	0.0580	0.96	0.9451
[(C3+S3+O3)/3] vs. [(C4+S4+O4+A4)/4]			0.40	0.0368	0.42	0.1712
(B2) Rotation system effects within individual crops						
C2 vs. [(C3+C4)/2]			1.38	0.6354	2.30	0.4041
C3 vs. C4			0.59	0.4969	0.73	0.7853
S2 vs. [(S3+S4)/2]			2.49	0.1834	6.25	0.0739
S3 vs. S4			1.19	0.8248	1.04	0.9731
O3 vs. O4			0.51	0.3955	0.33	0.3350
(B3) - Crop type effects						
[(C2+S2)/2] vs. [(C3+S3+C4+S4)/4]			1.85	0.2032	3.79	0.0665
[(C3+S3)/2] vs. [(C4+S4)/2]			1.69	0.3426	3.54	0.1274
[(O3+O4+A4)/3] vs. [(C2+S2+C3+S3+C4+S4)/6]			26.10	<0.0001	16.00	0.0001
O3 vs. [(C3+S3)/2]			11.50	0.0012	4.29	0.1502
[(O4+A4)/2] vs. [(C4+S4)/2]			35.90	<0.0001	28.70	0.0003
[(O3+O4)/2] vs. A4			0.80	0.7440	1.49	0.6870

The abbreviations in the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred. C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; A4, alfalfa in the 4-year rotation.

same rotation, AMATA stand density was 11-fold ($p = 0.0143$) and 23-fold ($p = 0.0003$) greater in the cool-season than in the warm-season crops overall averages, but AMATA aboveground mass was comparable in these crop environments ($p = 0.2355$ and $p = 0.0493$, respectively).

The cool-season crops, especially oat were responsible for CHEAL stand density and aboveground mass differences between rotation averages. CHEAL stand density and aboveground mass were 4-fold ($p = 0.008$) and 5-fold ($p = 0.199$) greater in the average of the 3- and 4-year rotations than in the 2-year rotation, but comparable between the 3- and 4-year rotations ($p = 0.9195$ and $p = 0.6114$, respectively). CHEAL stand density and aboveground mass were comparable between rotations for the same crop species ($p > 0.05$) and within the warm-season crops ($p > 0.05$). CHEAL stand density and aboveground mass were 38-fold ($p < 0.0001$) and 204-fold ($p < 0.0001$) greater in the cool-season crops than in the warm-season crops overall averages; 67-fold ($p < 0.0001$) and 571-fold ($p < 0.0001$) greater in the cool-season crop than in the warm-season crops average of the 3-year rotation; and 37-fold ($p < 0.0001$) and 232-fold ($p < 0.0001$) greater in the cool-season crop than in the warm-season crops average of the 4-year rotation. CHEAL stand density and aboveground mass were 11-fold ($p = 0.0001$) and 96-fold ($p = 0.0001$) greater in oat than in alfalfa.

The cool-season crops, especially alfalfa were responsible for DIGSA stand density and aboveground mass differences between rotation averages. DIGSA stand density in the average of the 3- and 4-year rotations was two-fold greater than in the 2-year rotation ($p = 0.0072$) and 5-fold greater in the 4-year rotation than in the 3-year rotation ($p < 0.0001$). DIGSA aboveground mass was comparable between the 2-year and the average of the 3- and 4-year rotations ($p = 0.1098$), but 14-fold greater in the 4-year than in the 3-year rotations ($p = 0.0001$). DIGSA stand density and aboveground mass were comparable between rotations for the same crop species ($p > 0.05$), except for oat ($p = 0.0062$ and $p = 0.0032$). DIGSA stand density and aboveground mass were 10- and 27-fold greater in the cool-season crop averages than in the warm-season crops averages, 20-fold ($p = 0.0001$) and 103-fold ($p = 0.0001$) greater in the cool-season crops than in the warm-season crops of the 4-year rotation, but comparable between cool-season and warm-season crops of the 3-year rotation ($p = 0.0603$ and $p = 0.3924$, respectively). DIGSA stand density and aboveground mass were 14-fold ($p = 0.0001$) and 33-fold ($p = 0.0001$) greater in alfalfa than in oat.

ECHCG responses generally were similar to those of AMATA. ECHCG stand density and aboveground mass were comparable between all rotation averages ($p > 0.05$), between rotations for the same crop species ($p > 0.05$), within the same crop type between rotations ($p > 0.05$), and within the 3-year rotation ($p > 0.05$). Averaged over the same crop types, ECHCG stand density and aboveground mass were 4-fold ($p = 0.0003$) and 10-fold ($p = 0.0012$) greater in the cool-season than in the warm-season crops. Within the 4-year rotation, ECHCG stand

TABLE 8 | Treatment effects on the stand density and aboveground mass of the seven most abundant weed species, listed alphabetically.

			Stand density		Aboveground mass	
Source of variation	**df1**	**df2**	***F***	***p***	***F***	***p***
(A) AMATA						
Crop ID	8	24	3.72	0.0058	1.52	0.2016
Corn weed management	1	3	0.73	0.4566	4.19	0.1333
Crop ID x Corn weed management	8	24	0.96	0.4886	1.09	0.4052
(B) CHEAL						
Crop ID	8	24	22.06	<0.0001	15.53	<0.0001
Corn weed management	1	3	2.10	0.2430	0.56	0.5097
Crop ID x Corn weed management	8	24	1.59	0.1808	1.07	0.4180
(C) DIGSA						
Crop ID	8	24	15.52	<0.0001	8.14	<0.0001
Corn weed management	1	3	21.52	0.0189	16.44	0.0270
Crop ID x Corn weed management	8	24	1.25	0.3126	0.78	0.6237
(D) ECHCG						
Crop ID	8	24	2.61	0.0328	2.20	0.0645
Corn weed management	1	3	5.80	0.0952	4.84	0.1150
Crop ID x Corn weed management	8	24	1.16	0.3615	1.04	0.4348
(E) SETFA						
Crop ID	8	24	8.78	<0.0001	4.22	0.0028
Corn weed management	1	3	20.91	0.0196	13.96	0.0334
Crop ID x Corn weed management	8	24	0.70	0.6892	1.04	0.4371
(F) SETLU						
Crop ID	8	24	3.09	0.0154	1.33	0.2774
Corn weed management	1	3	4.44	0.1257	3.28	0.1681
Crop ID x Corn weed management	8	24	1.11	0.3930	0.83	0.5875
(G) TAROF						
Crop ID	8	24	49.63	<0.0001	35.81	<0.0001
Corn weed management	1	3	0.61	0.4914	0.33	0.6067
Crop ID x Corn weed management	8	24	0.74	0.6553	1.20	0.3382
(H) OTHERS						
Crop ID	8	24	4.76	0.0014	2.35	0.0503
Corn weed management	1	3	1.99	0.2533	2.27	0.2288
Crop ID x Corn weed management	8	24	0.07	0.9997	0.43	0.8939

All the other weeds species were grouped into OTHERS. Corn weed management: low herbicide or conventional. C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; A4, alfalfa in the 4-year rotation.

density and aboveground mass were 5-fold ($p = 0.0014$) and 18-fold ($p = 0.0031$) greater in the cool-season than in the warm-season crops.

The cool-season crops were responsible for SETFA stand density and aboveground mass differences, but those differences were not strong enough be apparent between rotation averages. SETFA stand density and aboveground mass were comparable between all rotation averages ($p > 0.05$), between rotations for the same crop species ($p > 0.05$), within the warm-season crops between rotations ($p > 0.05$), and within the cool-season crops ($p > 0.05$). Averaged over the same crop types, SETFA stand density and aboveground mass were 10-fold ($p < 0.0001$) and 15-fold ($p = 0.0008$) greater in the cool-season than in the warm-season crops. Within the same rotation, SETFA stand density and aboveground mass were 11-fold to 23-fold greater in the cool-season than in the warm-season crops (**Table 9**).

SETLU stand density and aboveground mass were comparable in most pairs of comparison ($p > 0.05$), except that SETLU stand density was 2.5-fold greater in the cool-season crops average than in the warm-season crops average ($p = 0.0404$).

The cool-season crops, especially oat were responsible for TAROF stand density and aboveground mass differences between rotation averages. TAROF stand density and aboveground mass in the 3- and 4-year rotations average were 4-fold ($p < 0.0001$) and 14-fold ($p < 0.0001$) greater than those in the 2-year rotation. TAROF stand density and aboveground mass in the 3-year rotation were and 5-fold ($p < 0.0001$) and 20-fold ($p < 0.0001$) greater than those in the 4-year rotation. TAROF

TABLE 9 | Contrast of stand density and aboveground mass of the seven most abundant weed species.

	AMATA		CHEAL		DIGSA		ECHCG		SETFA		SETLU		TAROF	
Contrast of the main-plot effect	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***	**Ratio**	***p***
(A) Stand density														
(A1) Rotation system effects														
[(C2+S2)/2] vs. [(C3+S3+O3+C4+S4+O4+A4)/7]	0.74	0.6105	0.28	0.0008	0.42	0.0072	0.57	0.1170	0.64	0.3011	0.50	0.1569	0.24	<0.0001
[(C3+S3+O3)/3] vs. [(C4+S4+O4+A4)/4]	0.81	0.7077	0.97	0.9195	0.21	<0.0001	0.55	0.0834	0.49	0.0927	0.44	0.0827	0.19	<0.0001
[(C2+S2)/2] vs. [(C3+S3+C4+S4)/4]	2.45	0.1746	1.37	0.3889	1.14	0.6798	0.98	0.9584	1.86	0.1906	0.70	0.4944	0.95	0.8129
[(C3+S3)/2] vs. [(C4+S4)/2]	1.76	0.4533	1.45	0.3823	0.69	0.3213	0.97	0.9384	0.75	0.5877	0.74	0.6234	0.84	0.5105
(A2) Rotation system effects within individual crops														
C2 vs. [(C3+C4)/2]	2.33	0.3598	1.42	0.4995	0.93	0.8818	0.97	0.9497	1.56	0.5010	0.56	0.4277	1.02	0.9547
C3 vs. C4	1.65	0.6368	1.31	0.6510	0.54	0.2466	0.89	0.8579	0.49	0.3501	0.49	0.3990	0.87	0.6923
S2 vs. [(S3+S4)/2]	2.58	0.3065	1.33	0.5837	1.40	0.4658	0.99	0.9915	2.21	0.2337	0.88	0.8628	0.88	0.6958
S3 vs. S4	1.87	0.5543	1.60	0.4312	0.88	0.8088	1.04	0.9444	1.14	0.8620	1.14	0.8780	0.82	0.5914
O3 vs. O4	0.32	0.2890	0.74	0.6212	0.21	0.0062	0.46	0.2130	0.59	0.4848	0.33	0.2006	0.09	<0.0001
(A3) Crop type effects														
[(O3+O4+A4)/3] vs. [(C2+S2+C3+S3+C4+S4)/6]	12.25	0.0001	38.15	<0.0001	10.11	<0.0001	3.60	0.0003	9.85	<0.0001	2.48	0.0404	24.33	<0.0001
O3 vs. [(C3+S3)/2]	10.94	0.0143	67.07	<0.0001	2.43	0.0630	1.94	0.2248	11.32	0.0010	1.05	0.9435	4.33	0.0001
[(O4+A4)/2] vs. [(C4+S4)/2]	23.36	0.0003	36.99	<0.0001	20.08	<0.0001	4.82	0.0014	11.63	0.0001	2.96	0.0798	53.81	<0.0001
[(O3+O4)/2] vs. A4	3.71	0.1606	10.75	0.0001	0.07	<0.0001	0.49	0.1954	1.17	0.8068	0.37	0.1812	0.17	<0.0001
(B) Aboveground mass														
(B1) Rotation system effects														
[(C2+S2)/2] vs. [(C3+S3+O3+C4+S4+O4+A4)/7]	3.10	0.3402	0.21	0.0199	0.36	0.1098	0.35	0.1417	0.93	0.9245	0.46	0.3588	0.07	<0.0001
[(C3+S3+O3)/3] vs. [(C4+S4+O4+A4)/4]	1.30	0.8168	1.33	0.6414	0.07	0.0001	0.32	0.1040	0.56	0.4497	0.39	0.2420	0.05	<0.0001
[(C2+S2)/2] vs. [(C3+S3+C4+S4)/4]	9.26	0.0893	2.30	0.2315	1.60	0.4852	0.89	0.8841	3.54	0.1566	0.58	0.5502	0.86	0.7608
[(C3+S3)/2] vs. [(C4+S4)/2]	2.83	0.4799	2.43	0.2676	0.54	0.4264	1.00	0.9958	0.94	0.9537	0.89	0.9148	0.67	0.4810
(B2) Rotation system effects within individual crops														
C2 vs. [(C3+C4)/2]	7.45	0.2696	2.21	0.4167	1.06	0.9499	1.02	0.9882	2.81	0.4070	0.48	0.5668	0.94	0.9237
C3 vs. C4	1.78	0.7802	1.70	0.6372	0.40	0.3994	0.69	0.7630	0.39	0.5131	0.50	0.6404	0.85	0.8309
S2 vs. [(S3+S4)/2]	11.50	0.1821	2.39	0.3720	2.40	0.3571	0.79	0.8252	4.47	0.2329	0.71	0.7847	0.80	0.7378
S3 vs. S4	4.50	0.4709	3.49	0.2708	0.73	0.7772	1.44	0.7687	2.27	0.5667	1.59	0.7516	0.54	0.4336
O3 vs. O4	0.14	0.3486	0.53	0.5666	0.03	0.0032	0.10	0.0768	0.29	0.3941	0.12	0.1539	0.01	<0.0001
(B3) Crop type effects														
[(O3+O4+A4)/3] vs. [(C2+S2+C3+S3+C4+S4)/6]	6.11	0.0906	204.44	<0.0001	27.29	<0.0001	9.56	0.0012	15.00	0.0008	2.05	0.3316	389.81	<0.0001
O3 vs. [(C3+S3)/2]	8.70	0.2355	571.14	<0.0001	2.26	0.3924	2.54	0.3920	22.34	0.0180	0.47	0.5554	19.10	0.0002
[(O4+A4)/2] vs. [(C4+S4)/2]	20.20	0.0493	231.64	<0.0001	102.80	<0.0001	17.54	0.0031	22.79	0.0045	3.18	0.2706	1482.81	<0.0001
[(O3+O4)/2] vs. A4	28.24	0.0724	94.46	0.0001	0.03	0.0008	0.64	0.6762	5.38	0.1818	0.43	0.5132	0.05	0.0001

Weed species are listed alphabetically. The abbreviations on the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occurred. C2, corn in the 2-year rotation; C3, corn in the 3-year rotation; C4, corn in the 4-year rotation; S2, soybean in the 2-year rotation; S3, soybean in the 3-year rotation; S4, soybean in the 4-year rotation; O3, oat in the 3-year rotation; O4, oat in the 4-year rotation; A4, alfalfa in the 4-year rotation.

stand density and aboveground mass were comparable among the warm-season crops between rotations and within the same crops between rotations ($p > 0.05$), except in oat ($p < 0.0001$). TAROF stand density and aboveground mass were 24-fold ($p < 0.001$) and 390-fold ($p < 0.0001$) greater in cool-season than in warm-season crop averages, 4-fold ($p = 0.0001$) and 20-fold ($p = 0.0002$) greater in oat than in corn and soybean averages in the 3-year rotation, and 54-fold ($p < 0.0001$) and 1,483-fold ($p < 0.0001$) greater in the cool-season crops than in the warm-season crops in the 4-year rotation. TAROF stand density and aboveground mass were 6-fold ($p < 0.0001$) and 20-fold ($p = 0.0001$) greater in oat than in alfalfa.

DISCUSSION

Diversification of cropping systems led to increased weed community aboveground mass and stand density, increased weed community diversity and species richness, and decreased weed community evenness. Increased weed abundance was not associated with reduced crop yield. Crop identity in the present experiment had the strongest influence on the response variables. This observation is consistent with previous studies in which crop identity showed the strongest influence on weed community characteristics (Légère et al., 2005; Smith and Gross, 2007). The observation that crop yields were not correlated with increased weed aboveground mass suggests that low amounts of weed biomass can be tolerated, rather than the commonly desired weed-free condition (Zimdahl, 2012). Tolerating greater weed abundance can create some risks of resurgence by formerly prevalent weed species or outbreak of highly adapted introduced species under favorable conditions (Mohler, 2001). Consequently, weed growth and weed community composition should be monitored frequently to keep weed infestations at tolerable levels and to detect risks for future seasons. As weeds develop resistance to herbicides, weed eradication is likely to be increasingly impractical for technical, financial, and environmental reasons (Stewart et al., 2011; Brookes and Barfoot, 2013), making the monitoring of weed communities a critically important component of weed management.

Ryan et al. (2010) found that weeds growing in a preceding crop phase of a sequence affected the subsequent seedbank more strongly than the seedbank influenced the emerged weed flora; the investigators attributed this a filtering effect of crop management on weed seed production by mixed-species communities. The four years of data presented here did not reveal any weed species that might become aggressive in the presence of oat, red clover, and alfalfa. Following the critical period for weed control concepts described by Knezevic et al. (2002), weed control measures were applied in corn and soybean at their early establishment stages, but were not necessary in oat's early establishment because the most abundant weed species in this experiment site were summer annuals, whose emergence and establishment are synchronized with corn and soybean. Planting oat and red clover after soybean (in the 3-year rotation), instead of circling back to corn (as in the 2-year rotation), disrupted life cycles of those summer annual weeds. An extended disruption was also imposed in the 4-year rotation with the oat/alfalfa intercrop in year 3 and established alfalfa in year four. Frequent hay cuts severely suppressed weed species with erect stature, such as AMATA, CHEAL, and ECHCG, but did not significantly affect other species such as TAROF, SETFA, and SETLU. TAROF is a low stature weed, which was not as severely suppressed in alfalfa and oat as were AMATA, CHEAL, and ECHCG. SETFA and SETLU are clump-forming species that are less likely to be affected by harvest machinery. In oat, AMATA, CHEAL, ECHCG, SETFA, and SETLU, like most of the summer annual weeds at the experiment site, were in their early vegetative stages at oat harvest (Buhler and Hartzler, 2001; Cordeau et al., 2017). By the weed sampling dates, those weeds were physically severed once by the oat harvest combine, or twice by additional stubble clipping if the weed pressure was deemed high.

Tolerating higher amount of weeds might increase the risk of crop damage if weeds can serve as alternative hosts to pathogens (Wisler and Norris, 2005; Mohler and Johnson, 2009). However, soybean sudden death syndrome (SDS), caused by the soil-borne pathogen *Fusarium virguliforme* (Hartman et al., 2015), had its incidence and severity reduced due to cropping system diversification within the present experiment (Leandro et al., 2018). Among the currently recognized *Fusarium virguliforme* alternative hosts that were present at the experiment site, crops, such as alfalfa and red clover are considered symptomatic while weeds such as lambsquarter and pigweed asymptomatic (Kolander et al., 2012). Taking the findings of Kolander et al. (2012) and Leandro et al. (2018) together, it is more likely that crops played more important roles than weeds in SDS outbreaks and that cropping system diversification can control the risk of SDS outbreak effectively.

Differences in weed responses to cropping systems and management practices were more pronounced in aboveground mass than in stand density (**Tables 4**, **5**), which implied that rotation significantly affected weed growth but not weed emergence. These observations matched the general pattern reported by Weisberger et al. (2019). We attributed the observed community composition shift to the differences in crop phenology and required management practices between the warm-season crops (corn and soybean) and the cool-season crops (oat and alfalfa) (Gaba et al., 2014; Weisberger et al., 2019). In the present study, the magnitude of difference in sowing dates between soybean and oat seeded with red clover or alfalfa (60 days), as compared to that of corn and soybean (14 days), could be the largest contributor to reductions of weed density.

We considered the weed management programs in the 3- and 4-year rotations effective because the crop yields at our experiment site were comparable between rotations (**Table 2**) and to averages for the state of Iowa and Boone County (**Figure 1**). In the 2-year rotation, the net saved amount of herbicide between the low and conventional herbicide regimes was 13% as soybean plots were all treated with conventional weed management practices. The mass of herbicide active ingredients was reduced further in the 3- and 4-year rotations as corn and soybean were supplemented with oat, red clover, and alfalfa. For example, a 3-year rotation with corn under the low herbicide regime saved 42% of herbicide active ingredients as compared to the 2-year rotation with corn under conventional weed management; and the 4-year rotation with corn under low herbicide weed management saved 57% of herbicide active ingredients as compared to the 2-year rotation with corn under conventional weed management. We also considered two weed management programs for the same crop equally effective because the crop yields were not significantly different between corn weed management regimes. In the corn phase of the rotation systems, a transition from conventional to low herbicide weed management reduced the mass of herbicide active ingredients by 80% over 4 years because herbicide was applied in a band half of the area planted to corn.

Weed community aboveground mass composition and individual aboveground mass responses to cropping system diversification suggested that the weed communities that were dominated by few competitive species in the corn and soybean phases of the 2-year rotation could be shifted to have more of the rarer, less aggressive species. Community shifts to rarer, less aggressive weed species were reflected in the significant differences in ecological indices between cool-season and warm-season crops. The reduction of herbicide use, especially during oat and alfalfa phases of the rotation allowed some rarer species to grow, and thus, higher species richness and lower evenness were observed in oat and alfalfa than in corn and soybean. Community evenness indices in warm-season crops were higher than those in cool-season crops because fewer weed species were found in corn and soybean. The experimental units with high evenness index values had species of similar abundance and competitiveness, such as AMATA and CHEAL. Although an even weed community is desirable because of reduced chances that one or a few species are dominantly competitive (Adeux et al., 2019), weed communities could also be evenly dominated by a few weed species like AMATA, with high competitiveness, high reproduction potential, and quick herbicide resistance development. Thus, careful monitoring is required.

It is noteworthy that the relative abundance of the top seven species appeared more even in oat and alfalfa than in corn and soybean (**Figure 3**). Weeds can emerge in pulses in response to changes in soil conditions (e.g., temperature and moisture), so emergence after weed control measures have been applied and any residual effects have dissipated could result in successful establishment. Among the seven most abundant species in this experiment, five were influenced more strongly by crop identity than by corn weed management (**Table 8**). This observation is consistent with previous findings that emphasized the role of crops in weed community shifts (Davis et al., 2005b; Smith and Gross, 2007; Owen, 2008; Fried et al., 2012).

Due to labor constraints, only eight quadrats were evaluated per experimental unit (eu), and the samples in the eight quadrats within the same eu were tallied to make one data point. By using Simpson's ecological indices, we have limited the sensitivity of the responses to sample size (Nkoa et al., 2015). With eight quadrats randomly spaced within an eu, we sought to control for the patchiness of weed communities (Cardina et al., 1997), but the list of weed species presented in this manuscript is likely to not be exhaustive of species at the experiment site. We suggest, however, that the responses of dominant weed species, which are more agronomically important than the rarer species, were representatively assessed because the effects of spatially separated blocks on responses were non-significant. Also due to labor constraints, individual plant weight was not assessed, so we could not explore how community evenness was affected by individual plant size and whether there was any relationship or coincidence between evenness and individual plant reproductive potential.

A community that is dominated by AMATA, CHEAL, DIGSA, ECHCG, SETFA, and SETLU is more concerning than one dominated by TAROF, as determined by the frequency that those species are regarded as problematic (Kruger et al., 2009; Prince et al., 2012), their seedbank persistence characteristics (Buhler and Hartzler, 2001; Davis et al., 2005a), and their invulnerability to the strongest control measures (Mohler, 2001; Culpepper, 2006). Further investigation of AMATA, CHEAL, DIGSA, ECHCG, SETFA, and SETLU population dynamics, including emergence patterns, survival throughout crop season, and reproductive potentials under various cropping systems could help guide efforts to regulate the timing of their emergence, limit their growth and reproductive potentials, and eventually deplete their seedbanks. The reproductive potential of AMATA was reduced substantially in cool-season crops as compared to warm-season crops (Nguyen and Liebman, accepted). Taking the finding of Nguyen and Liebman with those of Gaba et al. (2014) and Weisberger et al. (2019), it is likely that the cool-season crops in the present study served to deplete the soil seedbank by inducing seed loss through weed emergence and granivore activities (van der Laat et al., 2015), while reducing reproduction potential through growth suppression. As demonstrated for SETFA (Davis et al., 2003), retrospective analyses applied to aggressive weed species can contribute to understanding species responses to management practices and to tailoring management tactics and timing to target them.

Overall, we conclude that by monitoring aboveground weed communities, a track record of species aggressiveness and collective response to management is available, and thus, it could be easier to control risks of weed resurgence and outbreak. Coupling knowledge of aboveground weed communities with that of weed seedbank composition and abundance would further improve our ability to predict and manage weed communities (Forcella et al., 1992; Menalled et al., 2001; Forcella, 2003; Davis et al., 2005b).

DATA AVAILABILITY STATEMENT

The data from this present study is available at https://doi.org/10.25380/iastate.19111376.v1.

AUTHOR CONTRIBUTIONS

ML conceptualized and designed the experiment. HN collected the data and wrote the original draft of the manuscript. ML and HN finalized the manuscript. Both authors contributed to the article and approved the submitted version.

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Arkansas Rice: Herbicide Resistance Concerns, Production Practices, and Weed Management Costs

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An online survey to better understand current weed management practices and concerns in Arkansas rice was distributed in the fall of 2020. A total of 123 respondents from across the Arkansas rice growing region returned the survey covering a total of 236,414 rice hectares, representing about 40% of the planted Arkansas rice hectares in 2020. The most problematic weeds were *Echinochloa crus-galli* (L.) P. Beauv. (ECG), *Cyperus* spp., and *Oryza sativa* L. (weedy rice), respectively, in flooded rice, and ECG, *Amaranthus palmeri* S. Wats., and *Cyperus* spp., respectively, in furrow-irrigated rice. Most respondents (78%) reported high concern with herbicide-resistant weeds, and crop rotation (>74%) was the most common strategy listed to control and mitigate the development of herbicide-resistant weeds. A chi-square test of homogeneity showed that strategies implemented to control herbicide-resistant weeds and mitigate the evolution of herbicide-resistant weeds were not dependent on occupation type (farmer, consultant, or industry rep) nor on years of involvement in rice production. Respondents failed to control ECG 44% of the time with their first postemergence herbicide. After initial herbicide failure, 53% of respondents stated two additional herbicide applications were required to control ECG escapes while another 21% of respondents stated it was never controlled. The average ECG population at 2020 harvest was between 0.1 and 1.0 plant m^{-2} according to 44% of the respondents; however, 41% of respondents indicated an ECG density of 2 to 10 plants m^{-2} at 2020 harvest. The reported annual average cost of herbicides for rice weed control was \$266.40 ha^{-1} with ECG accounting for 81% of the total cost. Average yield loss attributed to ECG was estimated to be 505–959 kg ha^{-1} (economic loss of \$134–254 ha^{-1}). However, yield loss in the most heavily infested fields was estimated to be 757–1,464 kg ha^{-1} (economic loss of \$200–387 ha^{-1}). Effective, non-chemical approaches to weed management were ranked as the least important current research or educational effort, indicating a paradigm shift in rice producers' weed control line of thought is needed with dwindling herbicide options due to herbicide resistance.

Keywords: barnyardgrass, furrow-irrigated rice, integrated weed management (IWM), survey, weed competition, yield loss

INTRODUCTION

Rice (*Oryza sativa* L.) has a production of ∼480 million metric tons of milled rice annually and feeds more than half of the world population (Muthayya et al., 2014). The United States (US) produced 10.3 million metric tons of rough rice in 2020, and this production is accomplished mainly in the four regions of the Arkansas Grand Prairie, Mississippi Delta (parts of Arkansas, Mississippi, Missouri, and Louisiana), Gulf Coast (Texas and Southwest Louisiana), and Sacramento Valley of California (USDA-ERS, 2022). With more than 56% of the US long-grain crop (USDA-ERS, 2022), Arkansas is the leading rice producer in the country (Rouse et al., 2018). Rice production accounts for more than US$1 billion yearly in Arkansas and is a main contributing factor to its economy (USDA-NASS, 2022). However, weed competition is particularly detrimental to rice production with yield reductions > 50% (Ziska et al., 2015). *Echinochloa crus-galli* (L.) P. Beauv. (ECG) can provoke more than 55% grain yield reduction (Zhang et al., 2017) while competition from *Oryza sativa* L. (weedy rice) can induce up to 72% reduction in the number of filled grains (Martin and Tanzo, 2015). Additionally, rice weed species can decrease land value (Ottis and Talbert, 2007), increase the soil seedbank (Bagavathiannan et al., 2011), and lead to price dockages because of contaminated rice seed. Rice growers in Arkansas rely heavily on herbicides for weed management (Rouse et al., 2018; Barber et al., 2022). However, chemical weed control and alternative integrated weed management strategies in rice production systems have significantly evolved throughout the years as well as rice herbicide traits and weed spectrum.

Since the 1950s, herbicides have been used in US rice production systems to selectively manage major weeds such as *Echinochloa* spp, *Oryza sativa* L., *Diplachne* spp., *Sesbania herbacea* (P. Mill) McVaugh, and *Aeschynomene virginica* (L.) B.S.P. Propanil was introduced in Arkansas in 1959 as the first highly effective and primary herbicide for weed control in rice (Rouse et al., 2018). It was continually used for 3 decades until the development of propanil-resistant ECG in 1990 (Heap, 2022). Today, ECG resistance to multiple herbicide sites-of-action has been documented (Barber et al., 2022; Heap, 2022), leading to numerous control failures across the state when chemical control strategies are solely used.

Improving weed control in complex and dynamic weed communities requires integrated approaches to weed management (Norsworthy et al., 2012). The aforementioned escalation of herbicide resistance in important rice weeds (Heap, 2022) increased the interest in more diverse weed management tactics (Owen et al., 2015). Integrated weed management (IWM), a combination of multiple weed control methods (cultural, mechanical, biological, and chemical) (Harker and O'Donovan, 2013), is meant to help growers make informed weed-management decisions and diversify strategies based on scientific knowledge (Swanton et al., 2008). As a holistic approach, IWM provides crops a competitive advantage over weeds and reduces selection for herbicide resistance. Strategies commonly used in rice include prevention (weed-free certified seeds, clean equipment, control of volunteer-weeds in ditches, fence lines and field edges) (Norsworthy et al., 2012; Riar et al., 2013a), herbicide-resistant trait technology (Clearfield®, FullPage®, Provisia®, MaxAce®), cultural practices (cultivar selection, rotation, cover crops, planting date, irrigation management), and mechanical practices (tillage). But the level of implementation of IWM strategies and the barriers to adoption of certain IWM strategies must be evaluated for defining future research opportunities (Swanton et al., 2008).

Weed management surveys are important decision-making tools that help to improve our comprehension of the levels of adoption of production practices and the short- and long-term impact of these practices on weed populations (Norsworthy et al., 2013). They are essential for identifying the most problematic weeds and shifts in the weed spectrum and for setting future research and educational priorities (Norsworthy et al., 2007). Weed management surveys have been conducted in Arkansas in the past to evaluate growers' and consultants' perception of problematic weeds and identify weed management challenges (Norsworthy et al., 2007, 2013; Burgos et al., 2021). However, herbicide resistance has increased in Arkansas since 2011 and weed management practices have changed. A holistic evaluation of the current weed management practices, problematic weeds, costs of weed control and suggested areas of scientific research that will help growers and stakeholders to improve their ongoing management strategies is needed. Therefore, the objective of this research was to assess changes in production practices, shifts in general weed management strategies and weed spectrums, herbicide resistance concerns, and current weed management costs in Arkansas rice.

MATERIALS AND METHODS

A survey was established and distributed to better understand current weed management practices and concerns in Arkansas rice. The online survey was conducted using the Qualtrics survey platform (Qualtrics, Provo, UT 84604 USA) and was distributed through multiple vectors in the fall of 2020. A link to the survey and short description were direct-emailed to 106 members of the Arkansas Agricultural Consultant's Association and 126 Arkansas County Extension Agricultural Agents. Additionally, the survey link was distributed and publicized through multiple online media sources. The survey was available online for one month, and all respondents remained anonymous. Specific survey questions can be found in **Supplementary Material S1**.

The survey included 30 questions divided into four sections detailing respondents' demographics, general rice weed management strategies and economics, herbicide resistance, and ECG. The first section comprised demographic and background information such as employment description, years involved in rice production, county location, and number of rice hectares under supervision. The second section focused on general rice weed control details such as the prevalence of and reasons for continuous rice hectares, cost of average rice herbicide programs, and the most problematic weed species. The premise of the third section was to gather information regarding respondents' perception, concern, and mitigation strategies of herbicide-resistant weeds. The fourth section involved an in-depth investigation of ECG to evaluate the prevalence and average densities of ECG in Arkansas rice hectares, perceived herbicide resistance, and effective strategies for the successful control of this problematic weed species.

Data collected from the online survey software were directly imported into a spreadsheet software (Microsoft® Excel® for Office 365, version 2002, Redmond, WA 98052) for analysis (Shaw et al., 2009). One question requested survey participants to provide the three most problematic weeds in flooded and furrow-irrigated rice and to rank them based on importance, with 1 being the most important. Weeds listed as the #1, #2, and #3 most problematic were awarded 3, 2, and 1 points, respectively, and points were summed. Greater total points indicated the respective weed species was more consistently listed as a top problematic weed species in Arkansas rice hectares. Several questions permitted respondents to provide more than one answer resulting in a total number of weed species responses greater than the number of individual respondents. In these instances, the number of observations (n) presented refer to the number of specific individual respondents to the respective question.

Another question requested survey participants to provide two areas of weed management research that would benefit their operation's profitability and/or overall weed control. These results were summarized in two separate ways. First, each response was analyzed for singular keywords or short phrases to provide a broad spectrum look at respondent's perceived needs. These keywords were then analyzed for word frequency and a word cloud was generated using the "tm", "SnowballC", and "wordcloud" packages in R 3.5.1 statistical software (R Core Team, 2018). Secondly, responses to this open-ended question were grouped into broader categories of research to provide a more generalized view for future research directions.

A chi-square test of homogeneity comparing respondents' primary occupation and years of active involvement in rice production with strategies implemented to control or mitigate herbicide-resistant weeds was performed using proc freq in SAS v9.4 (SAS Institute, Cary, NC 27513). Not all respondents provided an answer to every survey question. The total number of observations (n) are included for each survey question presented in the results and discussion sections.

RESULTS

Demographics

A total of 123 responses were received encompassing 34 out of 39 rice-producing counties, and accounting for 236,414 hectares out of the 583,152 harvested rice hectares (40.5%) in Arkansas for 2020 (Hardke, 2021) (**Table 1**). Of the 123 respondents, 55, 45, 19, and 4 respondents reported their primary occupation as farmer, consultant, industry representative, and "other", respectively (**Table 1**). Respondents that selected "other" each fell into more than one of the designated categories. Most survey participants (75%) had been actively involved in rice production for 11 years or more ($n = 122$) (data not shown). Fifty percent had been actively involved in rice production for 21 years or more. Only 5% of survey participants had been active in rice production for <6 years.

TABLE 1 | Occupation and rice hectares of respondents collected from a rice weed management survey conducted in 2020 in Arkansas, USA ($n = 123$).

Occupation	Primary occupation		Rice hectares	
	Frequency	**Percent %**	**Hectares**	**Percent %**
Farmer	55	45	32,563	14
Consultant	45	37	168,861	71
Industry rep	19	15	32,946	14
Other	4	3	2,044	1
			Total = 236,414	

Problematic Rice Weeds and Perceived Weed Research Needs

A total of 105 responses were returned for flooded rice and 92 responses were returned for furrow-irrigated rice regarding the most problematic weed species in the respective production systems. Results revealed that in flooded rice the most problematic weeds were ECG, followed by *Cyperus* spp., and *Oryza sativa* L., respectively, while in furrow-irrigated rice they were, in order of importance, ECG, *Amaranthus palmeri* S. Wats., and *Cyperus* spp. (**Figure 1**). ECG recorded the largest total number of points in flooded (292 of a possible 315) and furrow-irrigated rice (236 of a possible 276) indicating nearly every respondent considered ECG to be the #1 most problematic weed species in rice, regardless of growing environment. *Cyperus* spp. included *Cyperus iria* L., *Cyperus esculentus* L., *Cyperus difformis* L., *Cyperus flavicomus* Michx., and the generic term "sedges". *Oryza sativa* L., the third most problematic weed in flooded rice, was the sixth most problematic weed in furrow-irrigated rice. *Diplachne* spp. were perceived as the fourth most problematic weeds in flooded rice and fifth most problematic in furrow-irrigated rice. Other problematic weeds reported in flooded rice by respondents were *Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster, *Aeschynomene virginica* (L.) B.S.P., *Amaranthus palmeri* S. Wats., *Sesbania herbacea* (P. Mill) McVaugh, and *Digitaria* spp. In furrow-irrigated rice, other reported weeds included *Digitaria* spp., *Sesbania herbacea* (P. Mill) McVaugh, *Sorghum halepense* (L.) Pers., *Eleusine indica* (L.) Gaertn., and *Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster. Therefore, the top two problematic broadleaf weeds in flooded rice were *Amaranthus palmeri* S. Wats. and *Aeschynomene virginica* (L.) B.S.P., while the top two problematic broadleaf weeds in furrow-irrigated rice were *Amaranthus palmeri* S. Wats. and *Sesbania herbacea* (P. Mill) McVaugh.

Results from the question that asked respondents to provide two areas of weed management research that would benefit their operation's profitability and/or overall weed control are presented in **Figure 2**. Singular keywords or phrases detected most frequently in responses indicated research needs including "*Echinochloa-crus-galli*" and "MOA" (modes-of-action),

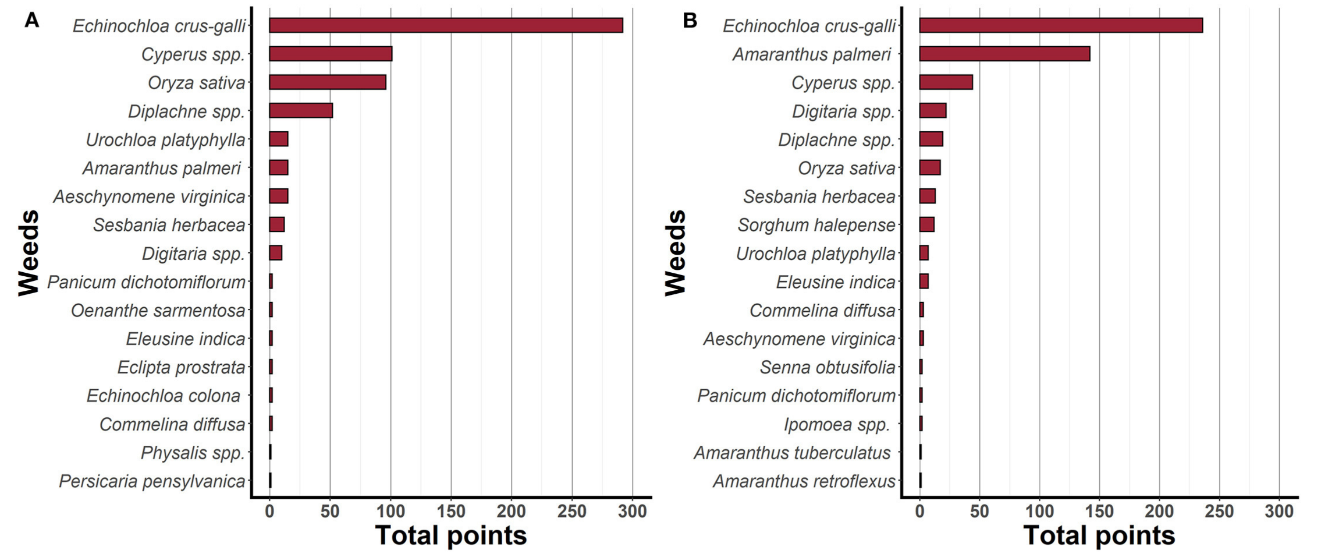

FIGURE 1 | Most problematic weed species reported in **(A)** paddy rice ($N = 105$) and **(B)** furrow-irrigated-rice ($N = 92$). Weeds listed as the #1, #2, and #3 most problematic were awarded 3, 2, and 1 points, respectively. Greater total points indicate the respective weed species was more consistently listed as a top problematic weed species in Arkansas rice hectares. **(A)** *Cyperus* spp. included: *Cyperus* spp. (56), *Cyperus iria* L. (38), *Cyperus esculentus* L. (3), *Cyperus flavicomus* Michx. (2), and *Cyperus difformis* L. (2). **(B)** *Cyperus* spp. included: *Cyperus* spp. (34), *Cyperus iria* L. (6), *Cyperus difformis* L. (3), and *Cyperus esculentus* L. (1).

followed by "residual", "resistance", "preemergence", and "grass" (**Figure 2A**). The most generalized common area of weed science research requested by survey respondents was "Control of *Echinochloa crus-galli*" (27 responses) (**Figure 2B**). "Preemergence or residual herbicide effectiveness" and "development of new modes of action or chemistry" each received 21 responses as an important area of research that would benefit rice production. "Herbicide resistance" (16 responses) was the only other reported research need by survey participants to receive a minimum of 10 responses [excluding the catch-all "other" category that included broad-spectrum topics such as biology, agronomy, identification, etc. (12 responses)].

Twelve current research or educational efforts were also rated by importance on a scale of 1 to 5, (where 1 = not important, 2 = slightly important, 3 = moderately important, 4 = very important, and 5 = extremely important). Respondents perceived development of new herbicide options as very important with the highest average ranking of 4.79 (**Table 2**). One important comment made by a survey participant was, "We need novel solutions to resistance issues. Nothing is working!" However, effective, non-chemical approaches to weed management were ranked as the least important current research or educational effort with an average ranking of 2.97 (slightly to moderately important).

Herbicide-Resistant Weeds

Ninety responses were returned regarding the rate of concern for herbicide-resistant weeds (data not shown). Seventy-eight percent of survey participants reported high concern with herbicide-resistant weeds while 21 and 1% of respondents reported moderate and slight concern, respectively. Among farmer respondents, 67% reported high concern with herbicide-resistant weeds while 86% of consultants, 83% of industry representatives, and 100% of other respondents reported high concern with herbicide-resistant weeds (data not shown).

Eighty-four percent of survey respondents also indicated they are managing herbicide-resistant weeds (excluding ECG) currently in their rice hectares (**Table 3**). When asked to provide the weeds (excluding ECG) and herbicides to which they were resistant, 39% of the respondents (44) reported *Cyperus iria* L. as resistant to acetolactate synthase (ALS)-inhibitors and synthetic auxin herbicides (**Table 4**). *Oryza sativa* L. received the second highest number of responses (19 responses, 17%) and was believed to be resistant to ALS-inhibiting herbicides. Third was *Diplachne* spp. (13%), thought to be resistant to acetyl-CoA carboxylase (ACCase)-inhibiting, Photosystem II (PSII)-inhibiting, and ALS-inhibiting herbicides. Fourth was *Amaranthus palmeri* S. Wats. (12%) believed to be resistant to synthetic auxins, PSII-inhibitors, protoporphyrinogen oxidase (PPO)-inhibitors, and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)-inhibitor.

Eighty-six responses were returned for the question, "Are you implementing any strategies to minimize the occurrence of new herbicide-resistant weeds or spread of resistance?" Thirteen percent of the total number of respondents were not implementing any strategy to minimize the occurrence of new herbicide-resistant weeds or spread of resistance (**Table 3**).

Seventy-two survey participants that indicated they suspected herbicide-resistant weeds in their rice fields responded to, "What strategies are you using to control herbicide-resistant weeds?" A chi-square test of homogeneity revealed that strategies implemented to control the herbicide-resistant weeds were not

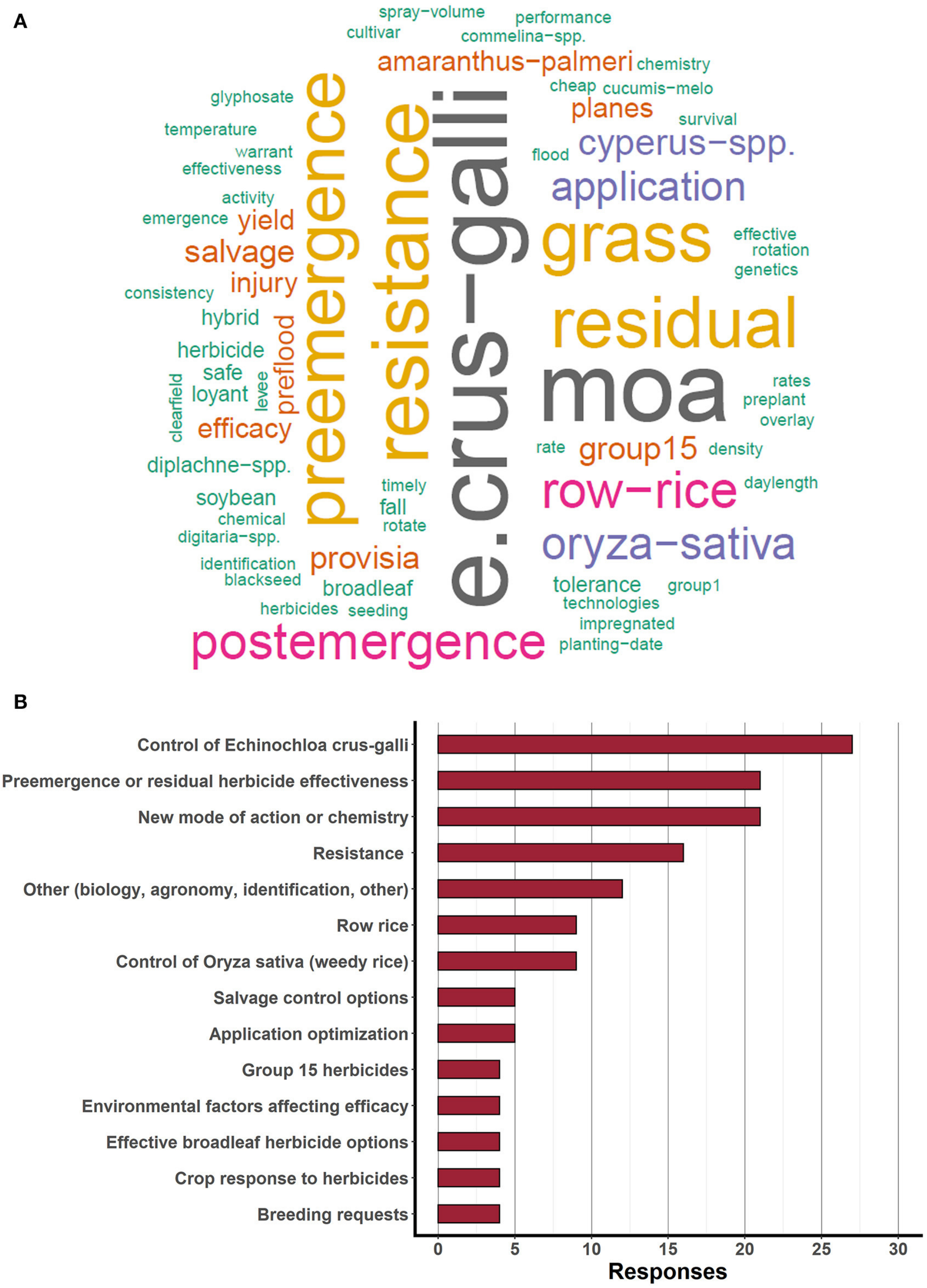

FIGURE 2 | Results for perceived areas of weed management research needs that would benefit rice producers' operation's profitability and/or overall weed control as reported by survey respondents. **(A)** WordCloud analysis of singular keywords or phrases generated from responses for rice weed management research needs. Words with a similar size and color received a similar number of responses. The larger the word, the more responses that keyword received [i.e., *Echinochloa-crus-galli* and MOA (mode-of-action) were the top two responses]. Some respondents provided more than one keyword resulting in a total number of observations greater than individual respondents ($n = 124$). **(B)** Groupings of perceived areas into broader categories of research to provide a more generalized view for future research directions ($n = 76$).

dependent on occupation type (chi-square = 2.9, $P = 0.82$) nor on number of years of involvement in rice production of survey participants (chi-square = 7.3, $P = 0.69$). Therefore, averaged across all respondents, the most commonly used method reported for managing herbicide-resistant weeds was crop rotation (85%, either alone or in conjunction with

TABLE 2 | Importance of current research or educational efforts as rated by survey respondents on a scale of 1–5, where 1 = not important, 2 = slightly important, 3 = moderately important, 4 = very important, and 5 = extremely important.

Research or educational effort	Mean ranking
Development of new herbicide options	4.79
Control strategies for herbicide-resistant weeds	4.46
Rice tolerance to new herbicides	4.27
Strategies to reduce the occurrence and spread of resistant weeds	4.20
Herbicide resistance screening program [ex. *Echinochloa crus-galli* (L.) P. Beauv., annual *Cyperus* spp.]	4.09
Impact of uncontrolled weeds on rice yields and overall economics	4.02
Expansion of weed control options in row rice	4.00
Performance of current herbicides	3.98
Economical weed control programs	3.93
Application optimization (nozzles, spray volume, adjuvants, etc.)	3.85
Impact of off-target herbicide movement and injury to rice	3.56
Effective, non-chemical approaches to weed management	2.97
	***n* = 91**

TABLE 3 | Respondents' suspicion of the existence of herbicide-resistant weeds [excluding *Echinochloa crus-galli* (L.) P. Beauv.] in the rice fields they farm or scout and whether they were implementing strategies to minimize the occurrence of herbicide resistance evolution or spread of resistance.

	Answer	Responses	Percent %
Suspect herbicide-resistant weeds [excluding *Echinochloa crus-galli* (L.) P. Beauv.]	Yes	76	84
	No	14	16
		***n* = 90**	
Employing strategies to minimize the occurrence of herbicide resistance evolution or spread of resistance	Yes	75	87
	No	11	13
		***n* = 86**	

other strategies) (**Table 5**). Eighty-one percent of respondents indicated the use of alternative herbicides either alone or in combination with other methods to manage herbicide-resistant weeds (**Table 5**). However, 5% of survey participants were using solely alternative herbicides to manage herbicide-resistant weeds (data not shown). Seed (trait) selection (38%), weed seedbank management (31%), and earlier rice planting (24%), were the next most commonly reported methods for managing herbicide-resistant weeds (**Table 5**).

Although crop rotation was listed as a primary method for managing herbicide-resistant weeds, 48% of respondents indicated 10% or more of their reported hectares were in continuous rice (3 consecutive years or more) (**Figure 3**). When weighted by number of hectares reported, 18% of the total hectares were designated as continuous rice (data not shown). Multiple reasons were provided by respondents as limitations for rice rotation to other crops (**Figure 4**). Twenty-nine percent of respondents indicated field/soil type was the main limitation for rice rotation to other crops, followed by zero-grade fields (28%) and commodity prices/profitability (23%), respectively.

In addition to strategies utilized for managing already established herbicide-resistant weeds, survey participants were asked what strategies, if any, were being implemented to mitigate the evolution of new herbicide-resistant weeds and the spread of current herbicide resistance. Similar to management strategies for current herbicide-resistant weeds, the most commonly implemented mitigation strategy was crop rotation (54%) followed by the use of multiple mode-of-action mixtures and overlapping residuals, each with 25% of respondents indicating use (**Table 5**). One interesting note, of the 16 strategies that were reported to mitigate herbicide resistance, half involved the use of or improvement of chemical control strategies (**Table 5**).

Echinochloa crus-galli

Respondents ($n = 85$) reported that 92% of their rice hectares were infested with ECG equating to 174,323 of a possible 189,522 reported hectares (data not shown). Additionally, 72 survey respondents indicated they believed they had herbicide-resistant ECG on their farm ($n = 84$, 86%) (**Table 6**).

Respondents listed nine different herbicide sites-of-action in which they believed ECG to be resistant to in their rice hectares (**Table 6**). Most respondents indicated they believed their ECG to be resistant to PSII-inhibitors (WSSA Group 5) (80%), quinclorac synthetic auxin (WSSA Group 4) (80%) and ALS-inhibitors (WSSA Group 2) (79%) (**Table 6**). Furthermore, 87% of respondents perceived ECG to be multiple-resistant to three sites-of-action or more (**Table 6**).

Survey participants reported that their first postemergence herbicide application for ECG control often failed. Fifty-two percent of respondents indicated that the first postemergence application failed > 40% of the time with an overall average of 44% of the time (**Figure 5**). Therefore, almost half of the first postemergence herbicide applications fail to successfully control ECG. After this initial failure from the first postemergence herbicide application, most respondents (53%) believed that two additional postemergence applications were required to effectively control the ECG escapes (**Figure 5**). Even more concerning, 17 respondents (21%) replied "I never control it" after the initial herbicide failure. When asked to rank the importance of the factors in causing failure of herbicides to control ECG on their farm or scouted hectares on a 1–5 scale (where 1 = not important, 2 = slightly important, 3 = moderately important, 4 = very important, and 5 = extremely important), survey participants perceived herbicide resistance, herbicide selection and weed size at application to be very important (with ranks of 4.28, 4.22, and 4.06, respectively) (**Table 7**). They also perceived lack of adequate coverage and environmental conditions as moderately to very important with ranks of 3.80 and 3.72, respectively.

ECG densities in the absence of herbicide use (germination potential within a given year based on the soil seedbank) were described to be between 11 and 107 plants m^{-2} on average by more than half of the respondents (51%) (**Table 8**). Although the seedbank is plentiful and germination potential is high, 44% of survey respondents thought ECG densities were from 0 to 1

TABLE 4 | Weed species [excluding *Echinochloa crus-galli* (L.) P. Beauv.] suspected of herbicide resistance as reported by survey respondents and the herbicide site-of-action and WSSA Group # to which they are suspected resistant to.

Weeds	Responses	Site-of-action	WSSA Group #
Cyperus iria L.	44	ALS-inhibitors, Synthetic auxins	2, 4
Oryza sativa L.	19	ALS-inhibitors	2
Diplachne spp.	15	ACCase-inhibitors, ALS-inhibitors, PSII-inhibitors	1, 2, 5
Amaranthus palmeri S. Wats.	13	ALS-inhibitors, Synthetic auxins, PPO-inhibitors, PSII-inhibitors, EPSPS-inhibitor	2, 4, 5, 9, 14
Cyperus esculentus L.	6	ALS-inhibitors	2
Persicaria pensylvanica (L.) M. Gomez	4	ALS-inhibitors, PPO-inhibitors	2, 14
Digitaria spp.	4	ACCase-inhibitors, ALS-inhibitors	1, 2
Cyperus difformis L.	3	ALS-inhibitors	2
Sesbania herbacea (P. Mill) McVaugh	2	PPO-inhibitors	14
Urochloa platyphylla (Munro ex C. Wright) R.D. Webster	1	ACCase-inhibitors	1
Eclipta prostrata (L.) L.	1	ALS-inhibitors	2
Cyperus flavicomus Michx.	1	Synthetic auxins	4
	***n* = 64**[a]		

[a] *The number of individual respondents was 64; however, respondents were permitted to provide more than one answer resulting in a total number of weed species responses greater than the number of individual respondents. #, number.*

plants m^{-2} at rice harvest in 2020, while 41% believed they were from 2 to 10 plants m^{-2} (**Table 8**).

Survey respondents indicated very similar strategies were employed for ECG control as the aforementioned herbicide-resistant weeds control (**Table 5**). The chi-square test of homogeneity revealed that strategies implemented to manage ECG were not dependent on occupation type of survey participants (chi-Square = 13.1, $P = 0.16$) nor on number of years of involvement in rice production (chi-Square = 12.6, $P = 0.81$). Most survey participants (81%) were using crop rotation alone or in combination with other strategies to manage ECG and alternative herbicides (69%) was the second most commonly used tactic alone or in combination with other strategies to manage ECG (**Table 5**). Other non-chemical ECG control strategies including seedbank management (24%) and earlier planting dates (30%) were only moderately used.

Cost of Rice Weed Control

The average cost of chemical weed control for reported rice hectares was evaluated during the survey. The perceived average cost of chemical control in rice weighted by hectares reported per respondent was \$266.20 ha^{-1} ($n = 113$) (**Figure 6**). When asked, "What percent of your overall herbicide expense this year was for ECG control?", survey participants estimated ∼81% of the total herbicide cost was attributed to ECG which equated to an average cost of \$215.90 ha^{-1} (data not shown).

Survey participants were also asked to evaluate the average yield loss on their farms attributed to ECG. Most participants estimated rice yield loss attributed to ECG of 455–959 kg ha^{-1} (29%) and 203–454 kg ha^{-1} (29%) (**Table 9**). Using the average price of \$264.50 per metric ton of rough rice in 2020 (USDA-NASS, 2022), the corresponding economic loss would be \$121–254 ha^{-1} and \$54–120 ha^{-1} for 455–959 kg ha^{-1} and 203–454 kg ha^{-1}, respectively (**Table 9**). Survey participants were also asked to evaluate the yield loss in their most heavily infested rice field. Most participants (29%) estimated yield loss of 708–1,464 kg ha^{-1} and 22% of respondents perceived the estimated yield loss to be 1,465–2,221 kg ha^{-1}. The corresponding economic loss would be \$188–387 ha^{-1} and \$388–588 ha^{-1} for 708–1,464 kg ha^{-1} and 1,465–2,221 kg ha^{-1}, respectively.

DISCUSSION

Problematic Rice Weeds and Perceived Weed Research Needs

The prevalence of ECG as the most detrimental weed in Arkansas rice is consistent with previous survey data that reported it as the most problematic weed in rice production systems (Norsworthy et al., 2007, 2013). ECG was detected in the seedbank of most major cropping systems of Arkansas, and it has a prolonged emergence period from mid-April to late September that contributes to the weeds' success (Bagavathiannan et al., 2011). *Cyperus* spp. have drastically risen on the Arkansas rice problematic weeds list since the previous survey was conducted in 2011 (Norsworthy et al., 2013). In the previous survey, *Cyperus esculentus* L. was reported as the 8th most problematic weed, and *Cyperus iria* L. was not ranked in the top 20; data from the current survey conducted nearly a decade later resulted in the combination of *Cyperus* spp. being the 2nd most problematic weed species to combat in rice hectares. The presence and spread of ALS-inhibitor-resistant *Cyperus esculentus* L. and *Cyperus iria* L. (Norsworthy et al., 2007) might be a major reason why *Cyperus* spp. are considered so problematic in Arkansas rice.

Oryza sativa L. has held constant as a top four problematic weed species in Arkansas rice as it was previously ranked third in importance and the fourth problematic weed in 2011 (Norsworthy et al., 2013), and more recently, Burgos et al. (2021) also reported it as the third most problematic weed. With infestation levels up to 1,076 plants m^{-2} (Burgos et al.,

TABLE 5 | Strategies reported from respondents being used to control herbicide-resistant weeds and *Echinochloa crus-galli* (L.) P. Beauv. and to mitigate the evolution of herbicide-resistant weeds[a].

		Other herbicide-resistant weeds	***Echinochloa crus-galli* (L.) P. Beauv.**
		Responses	**Responses**
Strategies used to control herbicide-resistant weeds	Crop rotation	61	68
	Alternative herbicides	58	58
	Seed (trait) selection	27	35
	Seedbank management	22	20
	Earlier planting dates	17	25
	Cover crops	3	2
	Fall deep tillage	3	1
	Other[b]	2	3
	None	1	3
	Pinpoint flood	0	1
		***n* = 72**	***n* = 84**
Strategies used to mitigate the evolution of herbicide-resistant weeds	Crop rotation	35	
	Multiple modes-of-action mixtures	16	
	Overlapping residuals	16	
	Rotating chemistries	11	
	Technology/trait rotation	9	
	Start with preemergence herbicide	7	
	Start clean	6	
	Weed seed prevention	6	
	Full use rate	5	
	Application emphasis	4	
	Early flooding	3	
	Fallow rotation	3	
	Sanitation	2	
	Tillage reduction or elimination	2	
	Early planting	1	
	Flushing to activate herbicides	1	
		***n* = 65**	

[a] *The number of individual respondents for controlling herbicide-resistant weeds and Echinochloa crus-galli (L.) P. Beauv. was 72 and 84, respectively; for mitigating the evolution of herbicide-resistant weeds the number of individual respondents was 65; however, respondents were permitted to provide more than one answer resulting in a total number of responses greater than the number of individual respondents.*

[b] *Other responses included: Overlapping residuals, preventing emergence, and spraying Echinochloa crus-galli (L.) P. Beauv. when small.*

2021), multiple reasons might explain its prevalence including resistance to ALS-inhibiting herbicides and the morphological similarity to the rice crop. The prevalence of *Amaranthus palmeri* S. Wats. in furrow-irrigated rice as the second most troublesome weed is not surprising as it is one of the two most troublesome weeds in Arkansas soybean [*Glycine max* (L.) Merr.] (Riar et al., 2013b) and furrow-irrigated production removes the cultural practice of flooding as a management tactic. Also, *Amaranthus palmeri* S. Wats. has evolved resistance to eight different sites-of-action in Arkansas (Barber et al., 2022; Heap, 2022), making it persistent and difficult-to-control across cropping systems. Soybean is the most common rotational crop with rice (Burgos et al., 2021); therefore, the rise of occurrence and herbicide resistance in soybean cropping systems is likely to promote the increase of *Amaranthus palmeri* S. Wats. infestations in rice fields (Norsworthy et al., 2013).

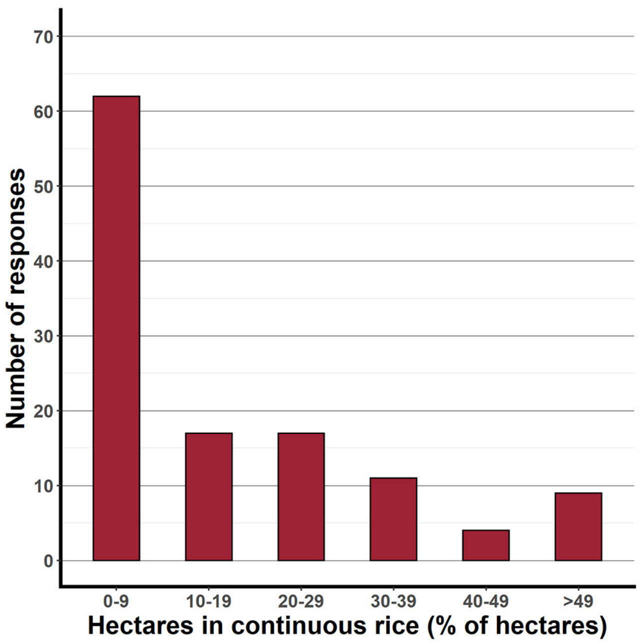

FIGURE 3 | Percentage of continuous rice hectares for 3 consecutive years or more (*n* = 120).

Although *Diplachne* spp. dropped from the second most problematic weed species in Arkansas rice in 2011 (Norsworthy et al., 2013) to the fourth and fifth most problematic species in flooded and furrow-irrigated rice, respectively, in the present survey, their occurrence of remaining in the top five most problematic weeds indicates persistence and difficult-to-manage nature. In 2006, *Aeschynomene virginica* (L.) B.S.P. and *Persicaria* spp. were the two most problematic broadleaf weeds (Norsworthy et al., 2007), while *Aeschynomene virginica* (L.) B.S.P. and *Amaranthus palmeri* S. Wats. were the two most problematic broadleaf weeds in 2011 (Norsworthy et al., 2013). Excluding *Amaranthus palmeri* S. Wats. in furrow-irrigated rice production, minimal broadleaves were reported in the present survey as truly problematic likely due to the morphological difference from rice and the presence of multiple effective herbicide options (Barber et al., 2022).

The most common area of weed management research reported to benefit the respondent's operation profitability and/or overall weed control was "Control of *Echinochloa crus-galli*" which was similar to the 2011 survey where ECG control was the most common weed research area recommended by respondents

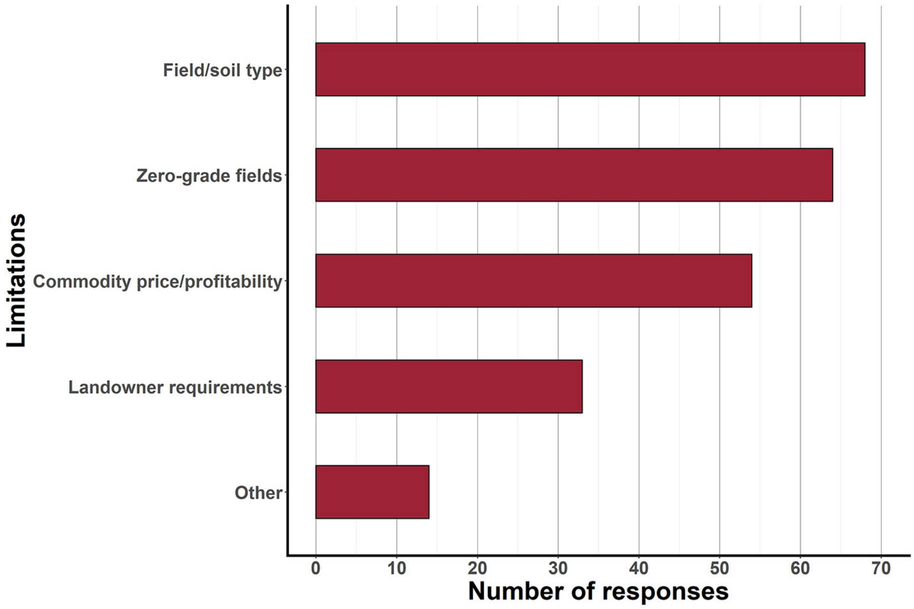

FIGURE 4 | Reported limitations for crop rotation from rice to other cropping systems (*n* = 118, 233 total responses).

(Norsworthy et al., 2013). Major reasons for this request might include the high infestation levels of ECG when left uncontrolled, reduced commodity price when rice seeds are contaminated with ECG seeds, and the presence of ECG seedbank in most Arkansas cropping systems (cotton, soybean, rice). The request of "pre-emergence herbicides" as a major area of research might be due to failure of postemergence control of weeds in the previous growing seasons, the slower evolution of resistance to some soil applied herbicides, and better understanding the probability for rice injury, causes, and yield loss potential.

The request for research of new modes-of-action or chemistry is likely due to the escalation of herbicide resistance to the existing modes-of-action, the lack of introduction of new herbicides, continued failed attempts at successful weed control, and the continued search for an easy method of weed control. Even though discovery of herbicides with new mechanisms-of-action may help to manage herbicide-resistant weeds, changes in the patterns of herbicide use are required to reduce herbicide resistance evolution (Gaines et al., 2021). Ranking effective, non-chemical approaches to weed management as the least important current research or educational effort, is an indication that a paradigm shift in rice producers' weed control line of thought is needed with dwindling herbicide options due to herbicide resistance.

Herbicide-Resistant Weeds

High herbicide resistance concerns reported in the present research are similar to survey results from nearly a decade ago. Norsworthy et al. (2013) also reported moderate or high concern regarding herbicide resistance from 98% of participants, with resistant weeds suspected in rice fields scouted by 88% of consultants. In Arkansas, resistance has been documented in several major weeds that respondents reported including *Oryza sativa* L. (ALS-inhibitors), *Cyperus iria* L. (ALS-inhibitors), *Cyperus difformis* L. (ALS-inhibitors), *Echinochloa colona* (L.) Link (ALS-inhibitors, PSII-inhibitors, and synthetic auxins), *Cyperus esculentus* L. (ALS-inhibitors), and *Persicaria pensylvanica* (L.) M. Gomez (ALS-inhibitors) (Heap, 2022). However, there have been no confirmed cases of resistance for many of the other herbicides and weeds listed by survey participants which implies that control failure in these cases might be due to factor(s) other than resistance (Norsworthy et al., 2012).

Although respondents provided some alternative weed management strategies to chemical control methods, more efforts (research, educational, and on-farm implementation) are needed to diversify strategies. Breeding of more competitive cultivars, robotic systems for weed control, and use of RNA to silence key weed genes through the process of RNA interference (RNAi) are all potential future options to enhance rice weed management efforts (Westwood et al., 2018). "Breeding efforts" were minimally requested by survey participants as important future weed management research needs (**Figure 2**). However, breeding new rice cultivars with a competitive advantage over weeds (Shrestha et al., 2020) might help to reduce selection pressure. Additional cultural methods pertaining to breeding or cultivar selection include selection of hybrid rice lines with greater tillering and taller growth characteristics (Shivrain et al., 2009), selection of more competitive rice cultivars and optimizing agronomic conditions (Gealy and Duke, 2017), and full-season cultivars that maximize the period with crop cover (Reddy and Norsworthy, 2010).

Earlier planting dates have been implemented as a strategy to combat herbicide-resistant weeds (**Table 5**), but could be

TABLE 6 | Herbicide resistance in *Echinochloa crus-galli* (L.) P. Beauv. across Arkansas rice hectares as reported by survey respondents[a].

	Answer	Responses
Do you suspect herbicide-resistant *Echinochloa crus-galli* (L.) P. Beauv.?	Yes	72
	No	12
		***n* = 84**

	Herbicide	WSSA Group #	Responses
What herbicides do you suspect resistance to?	Photosystem II inhibitor	5	57
	Synthetic auxin (quinclorac)	4	57
	ALS-inhibitor	2	56
	ACCase-inhibitor	1	33
	EPSPS-inhibitor	9	29
	DOXP synthase inhibitor	13	21
	Synthetic auxin (florpyrauxifen-benzyl)	4	19
	Microtubule inhibitor	3	14
	Lipid synthesis inhibitor	8	11
			***n* = 71**[b]

	# of sites-of-action	Responses
Reported multiple resistance in *Echinochloa crus-galli* (L.) P. Beauv.	One	1
	Two	8
	Three	24
	Four	14
	Five	10
	Six	7
	Seven	1
	Eight	2
	Nine	4
		***n* = 71**

[a] Florpyrauxifen-benzyl and quinclorac were treated as different sites-of-action.

[b] The number of individual respondents was 71; however, respondents were permitted to provide more than one answer regarding herbicides that they suspected resistance to resulting in a total number of responses greater than the number of individual respondents. #, number.

adopted to a wider extent, especially as a strategy to mitigate the evolution of herbicide resistance development. By planting rice earlier, it provides the crop a competitive advantage by emerging and growing prior to the optimum emergence window for some of our most problematic rice weed species like ECG, *Panicum dichotomiflorum* Michx., and *Amaranthus* spp. (Werle et al., 2014). Additionally, winter flooding, seed burial depth, and the stale seedbed technique can help to reduce the size of soil seedbank and weed infestation (Franca et al., 2020). Preventive measures such as weed-free certified seeds and equipment sanitation are also essential (Norsworthy et al., 2012; Riar et al., 2013a). Crop rotation is crucial and has been previously recommended as a key strategy for controlling herbicide-resistant ECG (Norsworthy et al., 2007) and *Oryza sativa* L. (Norsworthy et al., 2007; Burgos et al., 2021). The most common rotations in Arkansas are rice–soybean, rice–soybean–corn (*Zea mays* L.), and rice–fallow–soybean (Burgos et al., 2021) with soybean being the most compatible rotational crop with rice because it allows for alternative herbicide programs to be utilized and increases ease of controlling grass species in a broadleaf crop (Burgos et al., 2008).

Although crop rotation has great benefits for weed management and many survey respondents indicated they used this strategy for the management of herbicide-resistant weeds (**Table 5**), nearly 1/5th of reported rice hectares were under continuous rice production ($\geq$3 years) which is a concerning practice affecting successful long-term weed management. Proportions of different rice varieties grown were not assessed in the present survey, but a previous survey reported imidazolinone-resistant rice was planted on 64% of the planted rice hectares, 42% of which was treated exclusively with an ALS-inhibiting herbicide for grass control (Norsworthy et al., 2013). Therefore, the probability of continuous rice hectares receiving repetitive herbicide treatments annually is high, resulting in the continued selection for herbicide-resistant weeds. Continued education efforts must be implemented to warn against the overuse of specific herbicide technologies and demonstrate the importance of integrated weed management strategies for long-term weed management success.

In Arkansas, several factors such as decreased labor, ease of management, and potentially fewer input costs increased the interest in precision-leveling fields to zero grade (Hardke, 2021). Unfortunately, zero-grading land constrains growers to a continuous rice production system due to limited water movement inhibiting other non-flooded crop production (Hardke, 2018). Although this practice provides some benefits to Arkansas growers, the crop rotation limitation and resulting monoculture agricultural system establishes a weed spectrum that thrives in that specific environment and quickly adapts to the repeated similar management strategies.

Echinochloa crus-galli

ECG was reported as the most problematic weed species in both flooded and furrow-irrigated rice hectares (**Figure 1**), with 92% of respondents indicating the presence of ECG in their rice fields. With the occurrence of ECG populations with multiple resistance in the midsouthern U.S. (Barber et al., 2022; Heap, 2022), it is logical that a large proportion of the rice weed control budget would be directed toward ECG control. This may also partially explain the high proportion of reported postemergence herbicide failures, in addition to the inconsistency in control observed from herbicides selectively targeting ECG, a very morphologically similar weed species to rice.

In Arkansas, research has documented ECG resistance to six sites-of-action (when considering quinclorac and florpyrauxifen-benzyl as two separate sites-of-action within synthetic auxins): ACCase-inhibitors (WSSA Group 1), ALS-inhibitors (WSSA

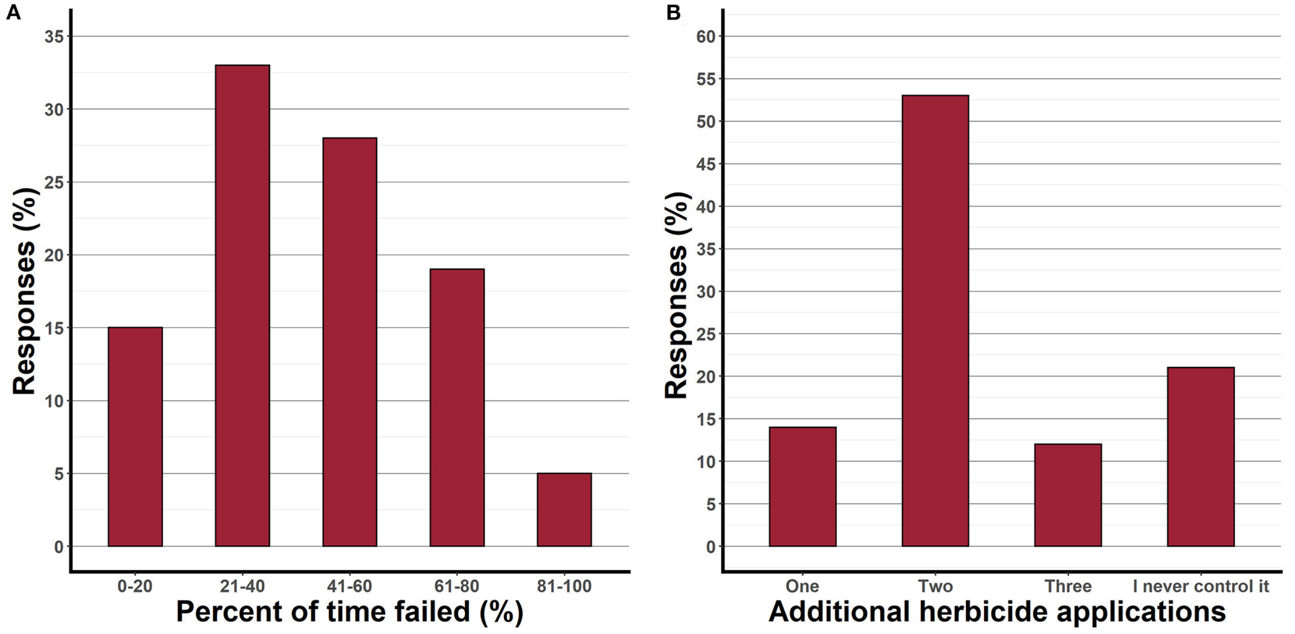

FIGURE 5 | Responses for **(A)** percent of the time that respondents failed to effectively control *Echinochloa crus-galli* (L.) P. Beauv. with the first postemergence herbicide application ($n = 100$) and **(B)** the average number of additional applications required for *Echinochloa crus-galli* (L.) P. Beauv. control if the initial application fails ($n = 81$).

TABLE 7 | Importance of factors causing herbicide failure on *Echinochloa crus-galli* (L.) P. Beauv. control as reported by survey respondents[a].

		Mean response
Importance of factors causing herbicide failure	Resistance	4.28
	Herbicide selection	4.22
	Weed size at application	4.06
	Lack of adequate coverage	3.80
	Environmental conditions	3.72
		***n* = 81**

[a] *Importance was ranked by respondents on a 1–5 scale, where 1 = not important, 2 = slightly important, 3 = moderately important, 4 = very important, and 5 = extremely important.*

Group 2), synthetic auxin (quinclorac, WSSA Group 4), synthetic auxin (florpyrauxifen-benzyl, WSSA Group 4), PSII-inhibitor (WSSA Group 5), and DOXP-inhibitor (WSSA Group 13) (Barber et al., 2022; Heap, 2022). Present survey results indicated respondents believed ECG was resistant to a total of nine different sites-of-action (**Table 6**). Therefore, either there are undocumented instances of herbicide resistance to additional sites-of-action within the state of Arkansas, or these herbicide failures are the result of other factors such as suboptimal environmental conditions, application errors, weed size, or herbicide selection (**Table 7**). The lack in implementation of alternative, diverse weed management strategies (**Table 5**) compromise the sustainability of current weed management options.

TABLE 8 | Reported *Echinochloa crus-galli* (L.) P. Beauv. densities if herbicides were to not be applied (germination potential within a given year based on the soil seedbank) and the actual *Echinochloa crus-galli* (L.) P. Beauv. density present in respondents' 2020 rice crop at harvest.

	Density Plants m^{-2}	Responses #	Percent %
Which of the following densities best describes the *Echinochloa crus-galli* (L.) P. Beauv. population on your farm if herbicides were to not be applied?	0–1	2	2.5
	2–10	13	16.0
	11–107	41	50.6
	108–1,075	19	23.5
	>1,075	6	7.4
		***n* = 81**	
Which of the following densities best describe the *Echinochloa crus-galli* (L.) P. Beauv. population in your 2020 rice crop at harvest?	No ECG present	3	3.7
	0–1	36	44.4
	2–10	33	40.7
	11–107	8	9.9
	108–1,075	1	1.2
	>1,075	0	0.0
		***n* = 81**	

#, number.

Postemergence herbicide failures on ECG were common as reported by survey respondents, and twenty-one percent of survey participants said "I never control ECG" following failure of the first postemergence application (**Figure 5**). In addition

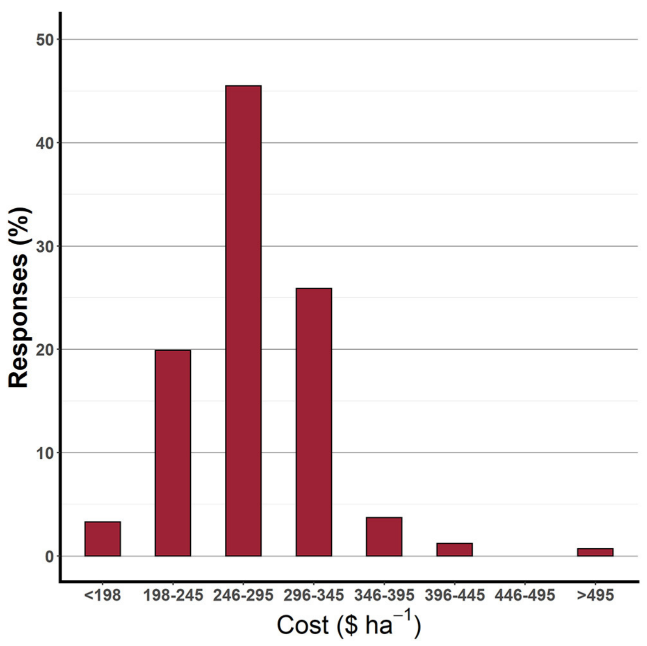

FIGURE 6 | Estimated rice weed control cost ha^{-1} weighted by hectare reported per respondent (n = 113).

TABLE 9 | Estimated average rice yield loss attributed to *Echinochloa crus-galli* (L.) P. Beauv. in Arkansas rice fields and estimated yield loss in most heavily infested fields (maximum potential loss) as reported by survey respondents.

	Estimated yield loss kg ha^{-1}	Value of yield loss[a] US \$ ha^{-1}	Responses	Percent %
Average	0–202	0–53	22	26.8
	203–454	54–120	24	29.3
	455–959	121–254	24	29.3
	960–1,464	255–387	9	11.0
	1,465–1,969	388–521	2	2.4
	≥1,970	≥522	1	1.2
			***n* = 82**	
Heavily-infested fields	0–707	0–187	17	20.7
	708–1,464	188–387	24	29.3
	1,465–2,221	388–588	18	22.0
	2,222–2,979	589–788	13	15.9
	2,980–3,736	789–988	4	4.9
	3,737–4,493	989–1,189	3	3.7
	≥4,494	≥1,190	3	3.7
			***n* = 82**	

[a] *The value of yield loss in dollars was calculated using an average price of rough rice = \$264.5 per metric ton (USDA-NASS, 2022).*

to inducing unacceptable yield loss for the simultaneous rice crop, ECG escapes can produce up to 39,000 seeds plant^{-1} (Bagavathiannan et al., 2012) that will increase the soil seedbank and compromise subsequent growing seasons. In Arkansas rice fields, the ECG seedbank was previously predicted to contain an average of 6,000 seeds m^{-2} with up to 215,000 seeds m^{-2} (Bagavathiannan et al., 2011). With 41% of respondents estimating 2–10 ECG plants m^{-2} at the 2020 harvest, this has the potential to increase the soil seedbank by 78,000–390,000 seeds m^{-2}. A primary recommendation for reducing the risks of herbicide resistance is the use of a diversified approaches to weed management that target the reduction of seed production and the number of weed seeds in the soil seedbank (Norsworthy et al., 2012).

Cost of Rice Weed Control

The high concern for herbicide-resistant weeds reported by respondents in their Arkansas rice hectares is alarming from multiple standpoints. However, one often overlooked facet of herbicide resistance is the significant increase in weed control costs as additional reactive management strategies are required to be implemented (Llewellyn et al., 2002) resulting in an average additional cost of \$65.60 ha^{-1} that can reach up to \$98 ha^{-1} (Norsworthy et al., 2013). Other additional estimated costs associated with herbicide-resistant weeds are crop yield loss, decreased commodity prices due to weed-seed contamination, and reduced land values (Norsworthy et al., 2012).

Season-long interference of ECG with densities between 1 and 20 plants m^{-2} reduced rice grain yield up to 301 kg ha^{-1} per ECG plant (Stauber et al., 1991) equating to approximately a \$79 ha^{-1} loss for each additional ECG plant present with 2020 rough rice prices (USDA-NASS, 2022). This ECG density range was reported by >41% of respondents at 2020 Arkansas rice harvest (**Table 8**). Additionally, heavy infestations of ECG are known to reduce land value by removing 60–80% of nitrogen from the soil (Ottis and Talbert, 2007). In addition to ECG impacts on yield, land value, and the soil seedbank, this weed species is likely to contaminate rice seed at harvest leading to price dockages for the producer.

Although chemical weed control options tend to be simpler and offer increased short-term economic returns, the prevalence of postemergence herbicide failures reported by respondents (**Figure 5**) would significantly increase this cost. Furthermore, after an initial failure from the first postemergence herbicide application, most respondents (53%) believed that two additional postemergence applications were required to effectively control ECG escapes (**Figure 5**), resulting in an added expense of ∼\$150 ha^{-1} (Barber, personal communication).

Alternative integrated weed management practices are often more laborious to enact and may increase the immediate weed management expense. However, when shifting focus from short-term to long-term economics, and the potential for widespread, multiple site-of-action resistance rendering herbicides non-viable, chemical costs of weed control will drastically increase (Davis and Frisvold, 2017). The implementation of integrated weed management strategies for both the management of current herbicide-resistant weeds and the mitigation of future evolution of herbicide resistance is one of the most effective and economical long-term strategies we currently have today.

Respondents in the present survey indicated non-chemical weed management practices as the least important current research effort (**Table 2**); therefore, educational campaigns to enhance adoption are required with an emphasis placed on illustrating the economic benefits of integrated weed management strategies (Llewellyn et al., 2004).

CONCLUSIONS

ECG is the most problematic weed in Arkansas rice. Other major weeds such as *Cyperus* spp., *Oryza sativa* L., *Diplachne* spp., and *Amaranthus palmeri* S. Wats. are also problematic. Overreliance on chemical weed control cannot provide sustainable control of these weeds as herbicide resistance has been widely documented and is of high concern as indicated by survey respondents. Integrated weed management strategies are required to reduce selection pressure and improve long-term weed management success. However, effective, non-chemical approaches to weed management were ranked as the least important current research or educational effort, indicating a paradigm shift in rice producers' weed control line of thought is needed with dwindling herbicide options due to herbicide resistance. Educational efforts must be established highlighting the long-term weed management and potential economic return benefits by being proactive to implement diversified strategies rather than reactive. Information gathered from the survey provided direct insights into current rice weed management practices and a better understanding of current concerns with making accurate and efficient weed management decisions. Additionally, the information provided will be used to prioritize research and Extension outreach efforts moving forward to address stakeholder needs more effectively.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Arkansas Institutional Review Board (IRB). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TB contributed to survey development, survey conduction and management, analysis, and writing of manuscript. KK contributed to analysis and writing of manuscript. JN contributed to survey development, analysis, and editing of manuscript. LB contributed to survey development and editing of manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2022.881667/full#supplementary-material

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REVIEW
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Improving Weed Management Based on the Timing of Emergence Peaks: A Case Study of Problematic Weeds in Northeast USA

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We reviewed the timing of the peak rate of emergence for 15 problematic weed species as well as ways to use this knowledge to improve control. Much of the previous literature modeled emergence based on growing-degree-days. For these models, we input average temperature data from several zones of Northeast USA. Within species, model-predicted peak emergence in the warmest and coolest zones differed by an average of 39 days. Also within species, there was some variation between models, likely reflecting different conditions in study locations and population-level differences that will need to be addressed in future modelling efforts. Summarizing both observed and modelled results, emergence typically peaked early-season for barnyardgrass, Canada thistle, common lambsquarters, common ragweed, giant foxtail, large crabgrass, perennial sowthistle, and smooth crabgrass. Emergence typically peaked mid-season for hairy galinsoga, mouseear chickweed, and red sorrel. Emergence typically peaked late-season for annual bluegrass. Several species emerged in a protracted manner, including common chickweed, quackgrass, and redroot pigweed. With this improved knowledge, farmers may target key problematic species of a particular field in several ways. Weed seedling control efforts can be timed at the highest densities or most vulnerable phenological stage. Residual herbicides and suppressive mulches can be timed to maximize effectiveness prior to their breakdown. And if management flexibility allows, crop selection and associated planting dates may be adjusted to improve crop competition or facilitate seedbank depletion through timely bare fallow periods. Such improvements to weed management based on timing of emergence will likely become even more impactful as predictive model reliability continues to improve.

Keywords: emergence periodicity, seedbank, tillage, annual weed, ecologically-based management, stale seedbed

INTRODUCTION

The timing of weed emergence is among the most important variables determining how species respond to management (Ryan et al., 2010; Cordeau et al., 2017c). This is especially pertinent for weeds germinating from seed, which are most vulnerable to management at the time of emergence (Mohler, 2001). The timing of emergence can also correlate with other biological traits of weeds that impact management success, such as seed weight, cotyledon type, and photosynthetic pathway (Cordeau et al., 2017b). Therefore, improved knowledge of weed emergence periodicity may be used to enhance management tactics (Bastiaans et al., 2008; Norsworthy et al., 2012; Reinhardt Piskackova et al., 2021). For example, the timing of weed control efforts (Forcella, 1999; Batlla et al., 2020), crop planting dates (Royo-Esnal et al., 2018; Sousa-Ortega et al., 2020), and seedbank depletion tactics (Nordell and Nordell, 2009) may all be adjusted to disadvantage problematic weed species.

But emergence periodicity varies greatly between species, reflecting contrasting patterns of dormancy, germination requirements, and pre-emergence growth rates, which are mediated by many abiotic factors (Batlla et al., 2020) so that even within species, empirically observed patterns of emergence can range widely (Cordeau et al., 2017b; Cordeau et al., 2017a). To improve management, modeling efforts have attempted to predict cumulative emergence for individual species, typically using Weibull or Gompertz functions (Royo-Esnal et al., 2020) with growing-degree-days (Forcella et al., 1997; Renner et al., 1999; Sousa-Ortega et al., 2020) or cooling-degree-days (Taylor et al., 2021) calculated using base temperatures required for germination of each modeled species (Masin et al., 2012). Addition of base water potential (Cao et al., 2011; Šoštarčić et al., 2021) or other edaphic parameters (Archer et al., 2006; Davis et al., 2013) may be used to further improve model accuracy.

In previous work investigating emergence periodicity at several sites in Northeast USA, Cordeau et al. (2017b) tilled new plots every two weeks and recorded weed densities by species. It was observed that some weed species exhibited prominent emergence peaks (**Figure 1**), which may be targeted for improved management. Emergence peaked early for most summer annual species, including common lambsquarters (*Chenopodium album* L.), common ragweed (*Ambrosia artemisiifolia* L.), giant foxtail (*Setaria faberi* Herrm.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and smooth crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.). But redroot pigweed (*Amaranthus retroflexus* L.), barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.], and hairy galinsoga (*Galinsoga quadriradiata* Cav.) demonstrated a delayed emergence peak compared to the other summer annuals. Winter annuals, including common chickweed [*Stellaria media* (L.) Vill.] and annual bluegrass (*Poa annua* L.), as well as perennials, including mouseear chickweed (*Cerastium fontanum* Baumg.), perennial sowthistle (*Sonchus arvensis* L.), red sorrel (*Rumex acetosella* L.), and quackgrass [*Elymus repens* (L.) Gould] generally exhibited later or bimodal emergence peaks. The exception was the perennial, Canada thistle [*Cirsium arvense* (L.) Scop.], which emerged early, and for an extended period.

This review further examines the emergence periodicity of these 15 species – many of which have been cited by farmers as the most problematic weeds of not only Northeast USA (Jabbour et al., 2014), but also Midwest USA (Gibson et al., 2006), and Europe (Bourgeois et al., 2019). For each species, we review the empirically-observed and model-derived emergence peaks and provide an updated synthesis of emergence-based management recommendations applicable to Northeast USA and similarly temperate regions.

REVIEW METHODS

We conducted a literature search using ProQuest and Google Scholar with search terms “weed,” AND “emergence,” AND “timing,” “periodicity,” OR “phenology.” Additional searches replaced “weed” with the scientific name of each of our 15 species. Species entries in the series, *The Biology of Canadian Weeds*, were also reviewed. Pertinent sources cited in the resulting articles were also reviewed. No geographic limitations were imposed on our search so we could contextualize the emergence of each species over a broad range of locations. Since many of the empirically-based emergence periodicity results were qualitative, we presented them in table form (**Table 1**). This literature search also provided the basis for our discussion of management tactics that can be improved using knowledge of emergence periodicity.

Some results of our literature search predicted 50% cumulative emergence based on growing-degree-days. This timing typically coincides with the peak, or greatest rate of emergence (Royo-Esnal et al., 2020). To determine the timings associated with the growing-degree-days required for 50% emergence, we used weather data representing four prominent USDA Plant Hardiness Zones found in Northeast USA (USDA, 2012). Specifically, from coldest to warmest, we used weather data representing Zones 4a, 5a, 6a, and 7a, with average annual minimum temperatures of -34.4 to -31.7°C, -28.9 to -26.1°C, -23.3 to -20.6°C, and -17.8 to 15.0°C, respectively. Within each zone, we selected three representative weather station sites from the Network for Environment and Weather Applications (https://newa.cornell.edu) to encompass a wide geographic range. Zone 4a included Milan, NH, Morrisville, VT, and Saranac Lake, NY. Zone 5a included Bangor, ME, Ceres, NY, and Saratoga Springs, NY. Zone 6a included Cabot, PA, Geneva, NY, and Northborough, MA. Zone 7a included Atlantic City, NJ, Philadelphia, PA, and Riverhead, NY. Growing-degree-days were calculated in accordance with each prior study, but using mean daily maximum and minimum temperature data from 2018 to

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Abbreviations: annual bluegrass, *Poa annua* L.; barnyardgrass, *Echinochloa crus-galli* (L.) Beauv.; Canada thistle, *Cirsium arvense* (L.) Scop.; common chickweed, *Stellaria media* (L.) Vill.; common lambsquarters, *Chenopodium album* L.; common ragweed, *Ambrosia artemisiifolia* L.; giant foxtail, *Setaria faberi* Herrm.; hairy galinsoga, *Galinsoga quadriradiata* Cav.; large crabgrass, *Digitaria sanguinalis* (L.) Scop.; mouseear chickweed, *Cerastium fontanum* Baumg.; perennial sowthistle, *Sonchus arvensis* L.; quackgrass, *Elymus repens* (L.) Gould; redroot pigweed, *Amaranthus retroflexus* L.; red sorrel, *Rumex acetosella* L.; smooth crabgrass, *Digitaria ischaemum* Schreb. *ex* Muhl.

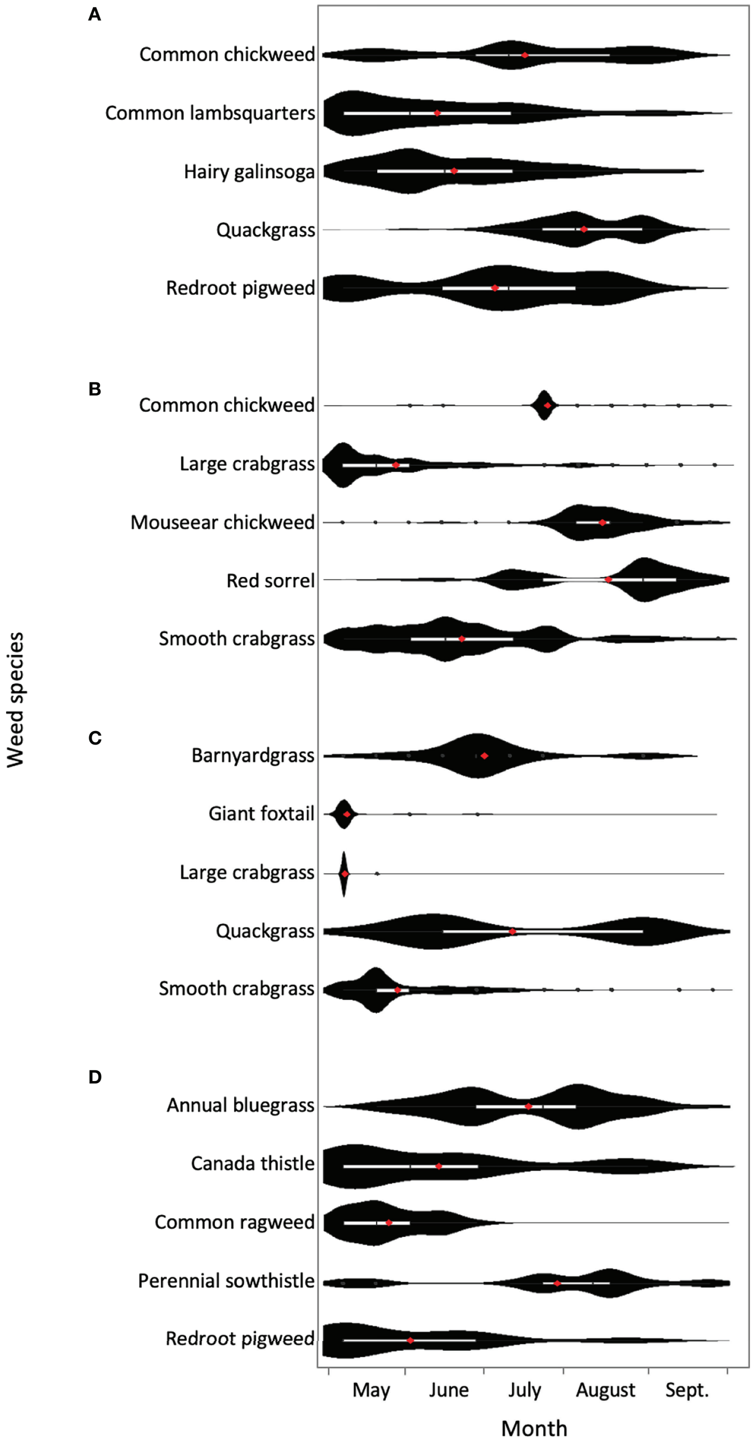

FIGURE 1 | Violin plots of emergence of the five most abundant weeds following tillage on different dates and locations; Old Town, ME **(A)**; Durham, NH **(B)**, Big Flats, NY **(C)**, and Aurora, NY **(D)** (adapted from Cordeau et al., 2017b). Box plots (white) are presented within violin plots (black) and means represented by red dots.

2020 over the three locations representing each zone. If no start date was provided, we began the accumulation on January 1. One of the prediction models required additional information on the soil conditions (Archer et al., 2006). For this model we input typical conditions for Northeast USA – chisel plowed loam following corn, with wet initial conditions, and adequate rainfall. Predicted dates for 50% (peak) emergence were graphed by species and model (**Figure 2**).

TABLE 1 | Comparison of peak emergence of target weeds from previous studies.

Species (Latin name)	Location	USDA plant hardiness zone*	Observed peak emergence	Reference
Annual bluegrass (*Poa annua* L.)	California, USA	9	November 5	Shem-Tov and Fennimore, 2003
	Illinois, USA	6	Spring and autumn	Branham, 1991
	Legnaro, Italy	8	Late February	Masin and Macolino, 2016
	New York, USA	6	June 23 and Aug. 4	Cordeau et al., 2017b
	Maryland, USA	7	Late September to mid October	Kaminski and Dernoeden, 2007
	Scotland, UK	7	September	Lawson et al., 1974, as reported by Mohler, 2001
	Tennesssee, USA	7	Mid October	Taylor et al., 2021
Barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.)	Arkansas, USA	7	Late May	Bagavathiannan et al., 2011
	Central Czech Republic	6	May and June	Jursík et al., 2014
	Massachusetts, USA	6	June, but through September	Vengris, 1965
	New York, USA	6	June 23	Cordeau et al., 2017b
	Northern Greece	7	April 20	Vasileiadis et al., 2016
	Ontario, Canada	5	June	Maun and Barrett, 1986
Canada thistle (*Cirsium arvense* (L.) Scop.)	Idaho, USA	6	March and April	Hodgson, 1955
	Montana, USA	4	Early May	Hodgson, 1964
	New York, USA	6	April 28	Cordeau et al., 2017b
	North Dakota, USA	4	Early May	Donald, 2000
Common chickweed (*Stellaria media* (L.) Vill)	England, UK	8	Early spring or late fall	Roberts and Dawkins, 1967
	Maine, USA	5	July 7	Cordeau et al., 2017b
	New Hampshire, USA	6	July 21	Cordeau et al., 2017b
	New York, USA	6	Mid September	DiTommaso, 2016
	Scotland, UK	7	August to October	Lawson et al., 1974, as reported by Mohler, 2001
Common lambsquarters (*Chenopodium album* L.)	Central Czech Republic	6	March and April	Jursík et al., 2014
	Maine, USA	5	April 28	Cordeau et al., 2017b
	Mid-Atlantic USA	8	May 11 to June 1	Myers et al., 2004
	Minnesota, USA	4	Late April	Harvey and Forcella, 1993
	New York, USA	6	Mid May	DiTommaso, 2016
	Northern/central Italy	8	Late April	Masin et al., 2012
	Ontario, Canada	5	Early June	Roman et al., 2000
	Quebec, Canada	4	May 30	Leblanc et al., 2004
	Scotland, UK	7	May	Lawson et al., 1974, as reported by Mohler, 2001
	Wisconsin, USA	4	June 11	Buhler et al., 1996
Common ragweed (*Ambrosia artemisiifolia* L.)	Illinois, USA	6	April and May, none after June 1	Stoller and Wax, 1973
	Mid-Atlantic, USA	8	April 7 to May 1	Myers et al., 2004
	Nebraska, USA	5	April 28	Barnes et al., 2017
	New York, USA	6	Early May	DiTommaso, 2016
	New York, USA	6	May 12	Cordeau et al., 2017b
	New York, USA	6	Prior to June 9	Dickerson, 1968
	Ontario, Canada	5	90% emergence prior to June 15	Bassett and Crompton, 1975
Giant foxtail (*Setaria faberi* Herrm.)	Mid-Atlantic, USA	8	Sites ranged May 2 to May 24	Myers et al., 2004
	New York, USA	6	April 28	Cordeau et al., 2017b
	Ohio, USA	6	May 17	Cardina et al., 2007
	Wisconsin, USA	4	June 8	Buhler et al., 1996
Hairy galinsoga (*Galinsoga quadriradiata* Cav.)	Central Czech Republic	6	June and July	Jursík et al., 2014
	Maine, USA	5	May 26	Cordeau et al., 2017b
	Multiple locations	-	May and June, but continues throughout growing season	Warwick and Sweet, 1983
	New York, USA	6	Early October (delayed due to drought)	DiTommaso, 2016
Large crabgrass (*Digitaria sanguinalis* (L.) Scop.)	Arkansas, USA	8	Spring	King and Oliver, 1994
	Legnaro, Italy	8	Mid to late May	Masin et al., 2005
	Mid-Atlantic, USA	8	Sites ranged May 18 to June 8	Myers et al., 2004
	New Hampshire, USA	6	April 28	Cordeau et al., 2017b
	New York, USA	6	April 28	Cordeau et al., 2017b
	Ohio, USA	6	June 1	Cardina et al., 2011

(Continued)

TABLE 1 | Continued

Species (Latin name)	Location	USDA plant hardiness zone*	Observed peak emergence	Reference
Mouseear chickweed (*Cerastium fontanum* Baumg.)	New Hampshire, USA	6	August 4	Cordeau et al., 2017b
Perennial sowthistle (*Sonchus arvensis* L.)	New York, USA	6	July 21 and August 18	Cordeau et al., 2017b
	Uppsala, Sweden	5	Late April (from rhizomes)	Hakansson, 1969
	USA and Canada	5	Late May (from seed)	Lemna and Messersmith, 1990
Quackgrass (*Elymus repens* (L.) Gould)	Canada and USA	5	Early spring (from seed)	Werner and Rioux, 1977
	England, UK	8	Spring and autumn	Williams, 1971
	Maine, USA	5	August 4 and September 1	Cordeau et al., 2017b
	New York, USA	6	June 9 and September 1	Cordeau et al., 2017b
Red sorrel (*Rumex acetosella* L.)	New Hampshire, USA	6	September 1	Cordeau et al., 2017b
	Victoria, Australia	9	Autumn (from seed)	Amor, 1985
Redroot pigweed (*Amaranthus retroflexus* L.)	Central Czech Republic	6	Late April and May	Jursík et al., 2014
	Maine, USA	5	July 7 and August 18	Cordeau et al., 2017b
	New York, USA	6	April 28	Cordeau et al., 2017b
	Wisconsin, USA	4	June 8	Buhler et al., 1996
Smooth crabgrass (*Digitaria ischaemum* Schreb. *ex* Muhl.)	Maryland, USA	8	Early June	Fidanza et al., 1996
	New Hampshire, USA	6	June 9	Cordeau et al., 2017b
	New York, USA	6	May 12	Cordeau et al., 2017b
	Ohio, USA	6	May 10	Cardina et al., 2011

**Approximated based on USDA (2012) for sites in USA and Magarey et al. (2008) for all other sites.*

Many of these studies reported 50% cumulative emergence, which is not necessarily equivalent to peak emergence, but often represents the most rapid rate of emergence.

COMPARISON OF EMERGENCE PEAKS FROM PREVIOUS RESEARCH

The previous empirically-based emergence results (**Table 1**) were generally in agreement as to the season of peak emergence for a given species. But within such general trends, peak emergence varied by study, likely reflecting differences in growing-degree-day accumulation between sites.

Model-based predictions of peak emergence using weather data from Northeast USA (**Figure 2**) were in general agreement with empirically observed results. Spring-emerging species were predicted to emerge earlier in the warmer modelled zones. Within each species, the mean difference in predicted peak emergence between warmest and coolest modeled USDA Plant Hardiness Zones of Northeast USA was 39 days.

Results by Species

In this section, we will draw from empirical observations (**Table 1**) and model-based predictions (**Figure 2**) to summarize the typical emergence patterns of our 15 reviewed species. Although the climate of Northeast USA is primarily composed of USDA plant hardiness zones 4 through 7, our review found several previous results from zones 8 and 9, which we include for further context (**Table 1**).

Annual bluegrass generally exhibited peak emergence in autumn, but spring, summer, and even winter emergence peaks were also observed (**Table 1**, **Figure 2**). Emergence models predicted that warmer climates would experience earlier spring and later autumn emergence of annual bluegrass compared with colder climates (**Figure 2**). These results reflect the winter annual life cycle of annual bluegrass. Indeed, Håkansson (2003) reports that it germinates whenever dormancy has been broken and environmental conditions are adequate.

Barnyardgrass demonstrated emergence peaks in April or May from studies conducted in warm climates (**Table 1**). But in cooler climates, a June emergence peak was more common. For example, modelling efforts based in Minnesota and Iowa, USA showed a mean peak emergence of June 1 (**Figure 2**). Though emergence may occur for an extended period (Vengris, 1965; Gołębiowska and Kieloch, 2016), barnyardgrass phenological traits differ by location, and cold winter conditions likely select against late and untimely emergence (Martinková et al., 2021).

Canada thistle typically exhibited emergence peaks in early to mid-spring in studies from northern USA (**Table 1**, **Figure 2**). Emergence is mostly from overwintering root stock rather than seed (Hodgson, 1964), which likely contributes to vigorous early growth and establishment success.

Common chickweed has shown emergence peaks in spring, summer, and autumn (**Table 1**, **Figure 2**), reflecting its relatively weak dormancy (Grundy et al., 2003). Likewise, its emergence can be protracted over most of the growing season (**Figure 1**).

Common lambsquarters demonstrated emergence peaks mostly in mid-spring (**Table 1**, **Figure 2**). Earlier or later emergence was generally observed in warmer or colder climates, respectively. Common lambsquarters has distinct dormancy-breaking requirements (Grundy et al., 2003), but emergence can be somewhat protracted (**Figure 1**, Gołębiowska and Kieloch, 2016).

Common ragweed was generally the earliest emerging of the annual weeds we reviewed, with peaks mostly in early to mid-spring (**Table 1**, **Figure 2**). In several studies emergence was observed to curtail sharply in June (**Table 1**, **Figure 1**).

FIGURE 2 | Predicted time to 50% (peak) weed emergence based on growing-degree-day models. Emergence was modelled from a range of USDA Plant Hardiness Zones representing most of Northeast USA. Zones include 4a (circles), 5a (squares), 6a (triangles), and 7a (diamonds). Vertical lines represent the mean predicted time to 50% emergence across presented data for each species. Models were not available for all the species we investigated.

Giant foxtail emergence peaks were mostly in mid-spring (**Table 1**, **Figure 2**). Cordeau et al. (2017b) observed that emergence occurred over a short period, resulting in a prominent emergence peak (**Figure 1**).

Hairy galinsoga demonstrated emergence peaks mostly in late spring and summer (**Table 1**), congruent with anecdotal results from Jernigan et al. (2017). Though hairy galinsoga has no seed dormancy (Warwick and Sweet, 1983), it does require adequate soil conditions for germination, as evident in the emergence peak observed in autumn following a summer drought (**Table 1**).

Large crabgrass emerged in mid to late spring (**Table 1**, **Figure 2**). Cordeau et al. (2017b) found that emergence peaked sharply at one site, but was slightly protracted at another (**Figure 1**). In both cases, emergence was earlier than expected based on other findings from similar latitudes. This could perhaps be explained by the six-week window between tillage and sampling, which may have skewed results.

Mouseear chickweed demonstrated a mid-summer emergence peak (**Table 1**), but only one study was found reporting emergence periodicity.

Perennial sowthistle emerged primarily in mid spring in two of three studies (**Table 1**), reflecting its initiation of rhizome shoot elongation at that time (Torssell et al., 2015). But Cordeau et al. (2017b) found an emergence peak in summer, which may possibly be explained by spring field preparation with a glyphosate application that may have set back early-emerging perennial sowthistle.

Quackgrass exhibited emergence peaks in spring, spring and autumn, or summer and autumn (**Table 1**). But based on the experimental design of these studies, some of the summer and autumn emergence likely represents regrowth from earlier-emerged plants.

Red sorrel emergence, which is primarily from rhizomes, peaked in summer, whereas emergence from seed peaked in autumn (**Table 1**, **Figure 2**).

Redroot pigweed demonstrated a range of emergence peaks from spring to summer (**Table 1**, **Figure 2**). This protracted emergence pattern of *Amaranthus* species has been well-described in the literature (Jha and Norsworthy, 2009; Teasdale and Mirsky, 2015; Chahal et al., 2021; Reinhardt Piskackova et al., 2021).

Smooth crabgrass emergence peaks were primarily in May and June, similar to large crabgrass (**Table 1**, **Figure 2**).

MANAGEMENT RECOMMENDATIONS

In this section, we will provide a synthesis of management tactics that may be improved with knowledge of weed emergence. The suite of applicable management tactics depends on the season and pattern of emergence of the target weed species. For example, direct control of weed seedlings (**Figure 3A**), overlapping residual herbicides (**Figure 3B**), residue mulch (**Figure 3C**), and delayed planting (**Figure 3D**) are most applicable to early- and mid-season weeds. Early planting to improve crop competition (**Figure 3E**) is most applicable to mid- and late-season weeds. And feasibility of fallow or cover cropped periods (**Figures 3F, G**) may vary by season and

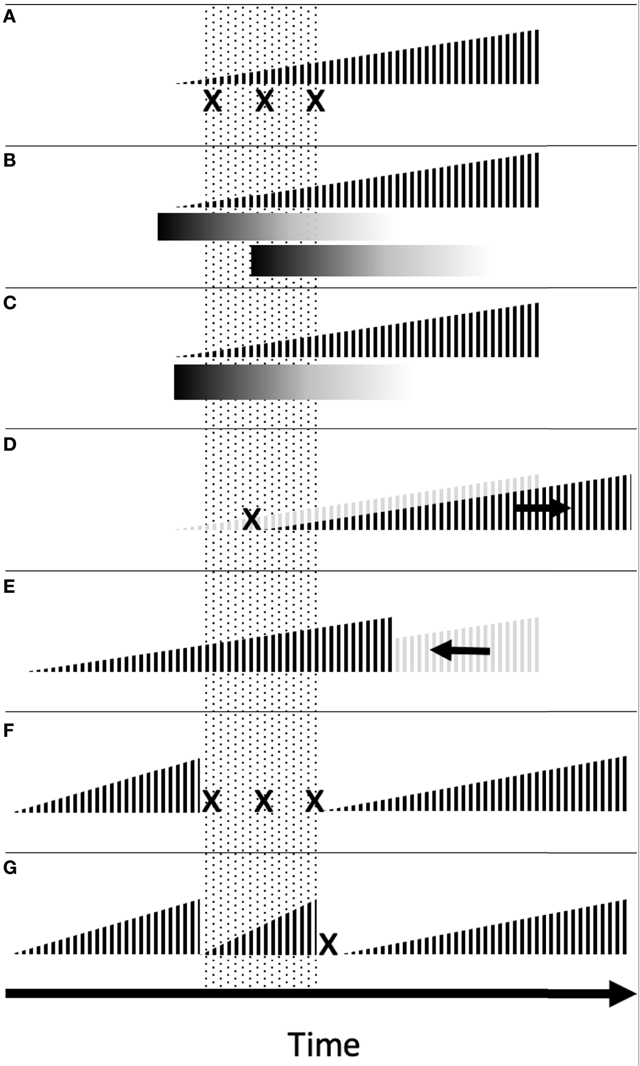

FIGURE 3 | Depiction of tactics that may be used to target a hypothetical weed emergence peak (dotted area) occurring shortly after the typical crop planting. If the planting date or crop rotation is not flexible, frequent control of weed seedlings **(A)**, overlapping residual herbicide applications **(B)**, establishment of residue mulch **(C)**, or some combination thereof may be used to target the peak emergence period. If the planting date or crop rotation are flexible, the peak emergence period may be targeted by delaying planting to allow pre-plant control of part of the flush **(D)**, planting early at high density to expedite crop canopy closure **(E)**, or adjusting rotation to allow for a bare fallow **(F)** or short-duration crop that concludes prior to weed seed production **(G)**. Height of vertical bars represents the relative crop maturity. Physical or chemical weed seedling control actions are symbolized with an "x." Gradients represent the breakdown of residual herbicides or residue mulch over time.

cropping system (**Figure 4**). Therefore, we present the following management recommendations by season of weed emergence. Within each season, management tactics are organized in ascending order of management flexibility required, in accordance with **Figure 3**. Although many of the studies of emergence-based management tactics were conducted outside of Northeast USA, most were conducted in similarly temperate climates, and their conclusions are applicable to this review.

Weeds With Early-Season Peak Emergence

Many of the species we reviewed exhibit emergence peaks in the early part of the growing season – typically April and May (**Table 1**) – including barnyardgrass, Canada thistle, common lambsquarters, common ragweed, giant foxtail, large crabgrass, perennial sowthistle, and smooth crabgrass. These weeds are especially problematic since they have the potential to compete with spring-planted crops for the entire growing season.

Direct control of early-season weed seedlings with herbicides or cultivation should be guided by weed emergence to maximize effectiveness (Forcella, 1999; Zimdahl, 2013). Farmers relying on a single post-emergence herbicide application should time it well after peak weed emergence so that the greatest number of weeds are sprayed (Fidanza et al., 1996; Masin et al., 2005; Archer et al., 2006). But for early emerging summer annual weeds, like common lambsquarters, larger weeds can survive herbicide applications, so earlier timings may be more effective in such cases (DeGreeff et al., 2018). Likewise, weed seedling control using cultivation is most effective in the early "white thread" stage, especially for intra-row control (Gallandt et al., 2017), so efficiency of cultivation could be maximized if conducted around peak emergence periods of problem weeds.

Timing of early-season residual herbicide applications is also of great importance. A premature application may allow the chemical to break down prior to peak emergence, whereas a late application may not affect emerged weeds. Application and planting dates can be adjusted to optimize residual herbicide effectiveness (Culpepper et al., 2004; Webster et al., 2009; Rosario-Lebron et al., 2019). Though preplant applications of residual herbicides may not be needed if most emergence occurs prior to seedbed preparation, they may be used prior to stale or false seedbed periods to bolster seedbank depletion (Coleman et al., 2016).

Cover crop residue, or residue applied in the form of hay or straw mulch, can effectively reduce weed emergence *via* physical (Carrera et al., 2004) or allelopathic mechanisms (Weston and Duke, 2003). Timing of cover crop termination for creation of grown-in-place mulch may interact with many other factors to affect weed biomass following termination (Wayman et al., 2015). Teasdale and Mirsky (2015) noted that weeds emerging prior to termination have an advantage in this system, with giant foxtail becoming the dominant weed when a hairy vetch (*Vicia villosa* Roth.) cover crop was terminated with rolling, but in disk-killed hairy vetch, smooth pigweed (*Amaranthus hybridus* L.) eventually dominated, likely due to warmer soil temperatures caused by tillage, which may have stimulated germination of this later-emerging species. Roller-crimped cereal rye (*Secale cereale* L.) can provide effective weed control in organic soybean [*Glycine max* (L.) Merr.], but common ragweed often escapes suppression since it emerges before the rye is rolled into a

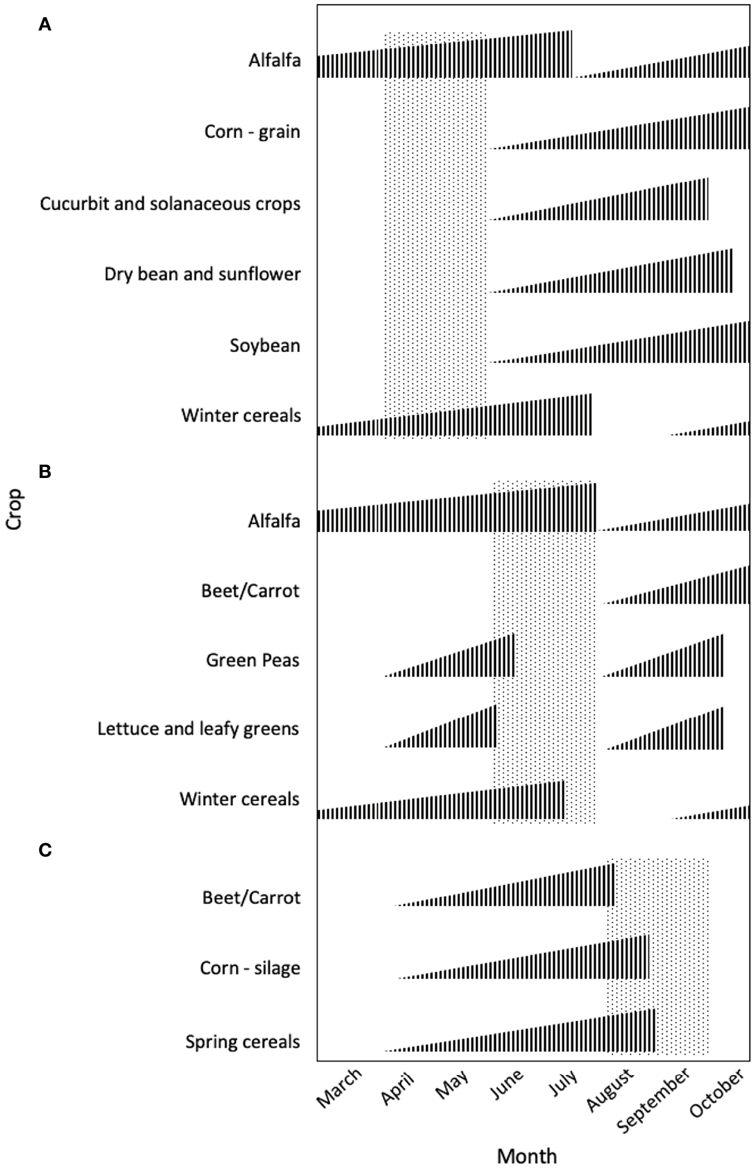

FIGURE 4 | Examples of crops that may be shifted to avoid of peak weed emergence periods occurring in the early (**A**, April-May), middle (**B**, June-July), or late (**C**, August-September) growing season of the cool, temperate climate of Northeast USA. Height of vertical bars represents the relative stage of crop growth.

suppressive mulch (Wallace et al., 2018). Thus, fields dominated by the earliest-emerging weeds should not rely on roller-crimped cover crops for weed control since they need to be terminated at anthesis, which is typically mid to late spring in Northeast USA. On small-scale farms, hay or straw mulch is sometimes used, and may be applied before planting robust crops or well after planting to ensure crops have achieved a sufficient height to withstand the mulching (Brown and Gallandt, 2018). When considering the best timing of mulch application relative to the peak emergence of problem weeds, farm managers should consider that it is most effective soon after application, before it decomposes (Law et al., 2006).

Delayed planting may be used to avoid peak emergence of problematic early-season weeds (Gill and Holmes, 1997). This would allow delayed usage of tillage or burndown herbicides to control most of the targeted species before the crop is planted (Chahal et al., 2021). Royo-Esnal et al. (2018) found that by delaying planting dates, they were able to avoid 50-100% of the emergence of their target weed. Common ragweed was less abundant in corn (*Zea mays* L.) and soybean crops when planting dates were delayed (Wallace et al., 2018). Delaying soybean planting date to June can decrease weed density at the time of post-emergence herbicide application (DeWerff et al., 2015). Delayed planting can also extend the activity and improve the control provided by pre-emergence herbicides (Rosario-Lebron et al., 2019). The timing of crop planting can also be adjusted to avoid crop losses due to the timing of weed competition (Mohler, 2001; Knezevic et al., 2002). Delaying planting may reduce the yield potential in some crops, which must be weighed against weed management benefits. Since crops differ in planting dates and associated management (Liebman and Gallandt, 1997), planting date may factor into crop or variety choice. Crops that can be planted in late-spring to avoid early-season weeds include corn, cucurbits, dry beans (*Phaseolus* spp.), solanaceous crops, soybeans, and sunflowers (*Helianthus* spp.) (**Figure 4**). Alternatively, alfalfa (*Medicago sativa* L.) and winter cereals may be planted the previous year to establish a competitive advantage over weeds with early-season peak emergence.

Bare fallow periods, including false or stale seedbeds, are some of the most powerful methods to reduce the weed seedbank (Gallandt, 2006), especially if the timing corresponds with the emergence peak of targeted weed species. Fallow periods are perhaps most feasible during the spring since more than one tillage pass or burndown herbicide application is sometimes necessary to prepare for planting, and spacing out these events allows emergence flushes between control timings. Although it is best to finish the sequence with minimal disturbance, such as with herbicides or flaming (Caldwell and Mohler, 2001), initiating with shallow tillage set to the germination depth of the target weed species can enhance the depletion of the seedbank (De Cauwer et al., 2019). But while tillage may affect the emergence of some species, the timing and magnitude of common ragweed emergence has been shown to be unaffected (Barnes et al., 2017). In such cases, it would be best to allow sufficient time for emergence rather than expecting an early peak due to tillage.

Weeds With Mid-Season Peak Emergence

Weeds with mid-season emergence peaks in June or July, included hairy galinsoga, mouseear chickweed, and red sorrel. These species may not be exposed to early season weed control efforts. Furthermore, opportunities to control these mid-season weeds may be curtailed if canopy closure or crop height restrict tractor entry into fields. Likely for these reasons, mid-season weeds can be the most abundant weeds in corn crops (Fried et al., 2020).

Direct control of mid-season weed seedlings through post-emergence herbicides or cultivation may be possible until the "layby" stage of the crop, immediately prior to canopy closure. Use of overlapping residual herbicides at this stage would further reduce emergence until full crop canopy closure (Culpepper et al., 2004).

Early planting of spring-planted crops may be used to establish a size advantage over mid-season weeds that can be used to improve control (Mohler, 2001). This size advantage is especially important for keeping weeds as small as possible for late post-emergence herbicide applications (Reinhardt Piskackova et al., 2021) and improving the selectivity of intra-row cultivation (Gallandt et al., 2017). The effect of early planting can perhaps be strengthened by additional weed suppressive tactics. Increasing crop planting density can be used to hasten canopy closure and facilitate mid-season shading (Mohler, 1996). Residue mulches (Brown and Gallandt, 2018; Wallace et al., 2018) or living mulches (Hartwig and Ammon, 2002; Westbrook et al., 2021) can provide further shading. Due to increased crop competition, late-emerging barnyardgrass and common lambsquarters had no effect on corn yield (Gołębiowska and Kieloch, 2016). Thus, planting earlier can perhaps be used to ensure mid-season weeds emerge after the critical period for weed control, thereby nullifying their threat – but only if seed production is prevented (Reinhardt Piskackova et al., 2020). For example, to reduce barnyardgrass seed production by 99%, rice crops required a 23-day advantage (Singh et al., 2017).

When planting dates were delayed, Wallace et al. (2018) found that composition of weeds with mid-season emergence periodicity increased in organic no-till corn and soybeans. They suggest integration of winter grains or perennial forages to reduce seedbanks of these species. Alternatively, in specialty crop systems, cool season crops that are established before or planted after peak emergence may avoid mid-season weeds entirely (DeVore et al., 2011). Such crops include beets, carrots, green peas, leafy greens, or lettuce (**Figure 4**).

Bare fallow periods targeting the seedbank of mid-season weeds may be difficult for farmers to implement in the middle of the growing season but could be integrated with cover cropping to simultaneously satisfy other pest management or soil health goals (Nordell and Nordell, 2009). Aligning the timing of cover crop seedbed preparation with the peak emergence of problem weeds would encourage weed seed germination, while termination of the cover crop prior to weed seed production would ensure a seedbank reduction (**Figure 3G**). Bare fallow periods can also be used in conjunction with cool season crops (**Figure 4**). Bare fallow periods in the mid to late season are especially important when targeting mid-season perennial weeds, such as quackgrass (**Table 1**), to exhaust the carbohydrate reserves in their rhizomes (Ringselle et al., 2020).

Weeds With Late-Season Peak Emergence

Weeds with peak emergence in late summer and autumn included the winter annual species, annual bluegrass. Weeds emerging at this timing provide minimal competition with established spring-planted crops (Knezevic et al., 2002; Cordeau et al., 2015; Gołębiowska and Kieloch, 2016). In interviews about problematic weeds, organic vegetable farmers made almost no mentions of winter annuals (Jabbour et al., 2014), reflecting the control of winter annuals by spring tillage. But late-season weeds can be problematic in winter cereals (Royo-Esnal et al., 2018). And in no-till systems, autumn-established winter annuals can grow too large the following spring to control with post-emergence herbicides (Wallace et al., 2019).

Autumn-applied burndown herbicides can reduce overwintering weed densities the following spring (Hasty et al., 2004). And autumn-applied residual herbicides in conjunction with winter cover crops can reduce the emergence of winter annual weeds (Walters et al., 2007) and keep them at a manageable height the following spring (Wallace et al., 2019). Interseeding may be used to expedite cover crop establishment and improve late season weed suppression, but compatibility with other post-harvest weed control tactics may be limited.

As grain corn or soybean crops mature and leaves wither, the open canopy can allow successful establishment of weeds with late-season emergence peaks. Harvest of these crops often occurs in late autumn, forcing farm managers to decide whether to terminate the weeds in autumn, or use the weeds as winter soil cover (Jabbour et al., 2014) but risk their escape in spring (Wallace et al., 2018).

Shifting spring-planted crops earlier (**Figure 4**) can allow for a late-season bare fallow period, which would target the seedbank of winter annuals, and help exhaust carbohydrate reserves of perennial weeds to minimize their survival over winter (Andersson et al., 2013). Late fallow periods may also necessitate a delay in autumn-planted crops. This could perhaps be offset with denser seeding rates. Royo-Esnal et al. (2018) found that by delaying winter cereal planting dates, they were able to avoid most emergence of their target weed. But in a dry year, weed emergence was delayed, and an earlier planting date would have been preferable.

Weeds With Protracted Emergence

Rather than emerging in a short, easily manageable cohort, several species exhibited a protracted emergence pattern. These included common chickweed, quackgrass, and redroot pigweed. The protracted emergence of *Amaranthus* species is well-known (Jha and Norsworthy, 2009; Chahal et al., 2021). Common lambsquarters (Gołębiowska and Kieloch, 2016) and hairy galinsoga (Jernigan et al., 2017) may also sometimes display protracted emergence. This emergence pattern allows these species to avoid early-season control efforts. Although late emerging weeds may not affect crop yield (Knezevic et al., 2002), they should still be controlled from a seedbank management standpoint (Norris, 1999; Brown and Gallandt, 2018) and because they are subject to sublethal herbicide doses, which can select for resistance (Norsworthy et al., 2012). Perennial weeds may also appear to have a protracted emergence due to their survival and resprouting after successive control efforts (Ringselle et al., 2020).

Control of weeds with protracted emergence requires an extended effort. We suggest a multifaceted strategy that includes as many of the following tactics as is feasible. In chronological order: densely planting crops that quickly close canopy (Mohler, 1996); overlapping residual herbicides to extend control through canopy closure (Chahal et al., 2018); ensuring seedling control is as effective as possible by timing control efforts while weeds are less than 10 cm tall (Reinhardt Piskackova et al., 2020) and using a final pass as late as possible (Crow et al., 2015); minimizing seed production of escapes using hand removal, mowing, rescue herbicide applications (Hill et al., 2016), electrical discharge systems, or artificial pollination (Lidor-Nili and Noivirt-Brik, 2017); using harvest weed seed control if the escapes have already produced seed (Walsh et al., 2012); avoiding post-harvest tillage to allow for seed predation (Birthisel et al., 2015); and moldboard plowing to bury remaining seeds below germinable depth (Mohler, 1996).

Management of Multiple Problem Weeds

As seen in **Figure 1**, farms often contain species with different weed emergence patterns. Therefore, farmers may need to prioritize targeting the emergence peaks of the most problematic species. Ideally, the most competitive or difficult-to-control weeds would be avoided entirely by adjusting planting dates or crop selection to allow for a bare fallow (**Figure 3F**) or short-term cover crop (**Figure 3G**). Lower priority weeds could be addressed with adjusted in-crop weed control (**Figures 3A–C**) combined with tactics that improve the competitive advantage of the crop (**Figures 3D, E**). Fields with profuse weed emergence over the entire growing season may need to be taken out of production for a full year to allow for extended bare fallow periods (Nordell and Nordell, 2009) or rapid succession cover crops that are incorporated before weeds have time to set seed (Sarrantonio and Gallandt, 2003; Gallandt, 2006; Mirsky et al., 2010).

Conversely, fields may have weed communities dominated by species with similar phenology, reflecting the history of management (Ryan et al., 2010). For example, an organic vegetable rotation and a conventional corn and alfalfa rotation resulted in two different weed communities with very different phenology (Mohler et al., 2018). Likewise, corn and soybean are more conducive to summer annual broadleaf weed emergence than wheat and alfalfa (Goplen et al., 2017). Therefore, in some cases it may be possible to target all of the problematic weeds by temporarily switching to a cropping system that allows for new management timings – such as the occasional rotation of spring versus autumn-planted crops.

OUTLOOK

Variation in Peak Emergence Within Each Species

From our review of previous research, the peak emergence of each species varied by study (**Figure 1**, **Table 1**). Indeed, for a given weed species, emergence timing is expected to differ by location due to different weather conditions (Royo-Esnal et al., 2020). But there was also variation between modelling results of a given species when we imposed identical weather conditions (**Figure 2**). This may be due to other differences between study sites where models were validated, such as soil texture (Leblanc et al., 2004), surface residue (Oreja et al., 2020), crop canopy (Cardina et al., 2011), or type of tillage employed (Mohler, 2001). Additionally, depth of burial can influence not only the time required by a seedling to reach the soil surface (Gonzalez-Andujar et al., 2016), but also the accumulation of conditions required to break seed or bud dormancy (Cao et al., 2011). Furthermore, within a species there may be genetic-based differences in emergence between populations resulting from varying selection pressure in different microclimates (Dorado et al., 2009; Papiernik et al., 2020; Liu et al., 2021). For example, barnyardgrass emergence differed based on the elevation of the seed collection site (Martinková et al., 2021) and common lambsquarters emergence varied by the slope of the landscape where seeds developed (Papiernik et al., 2020).

While Grundy et al. (2003) used a single thermal time model to sufficiently predict cumulative emergence of common chickweed from different sites brought to a single location, similar common garden experiments have found differences in emergence periodicity between populations (Papiernik et al., 2020; Martinková et al., 2021). Likewise, for large crabgrass (Dorado et al., 2009) and annual ryegrass (*Lolium rigidum* Gaud., Sousa-Ortega et al., 2019), single models could accurately predict emergence at single but not multiple locations, suggesting that different models should be developed for different regions.

But models may only require minor adjustments to accurately predict emergence in new regions. For example, the Italian emergence model, AlertInf (Masin et al., 2012), was modified with the base temperature and water potential required for germination of barnyardgrass in Croatia, to effectively predict emergence in Croatian corn (Šoštarčić et al., 2021). To further improve emergence predictions, other site-specific factors may need to be incorporated into models to maximize their accuracy and utility to farmers (Royo-Esnal et al., 2020). Inclusion of real-time *in-situ* information may be useful as well, such as phenological indicators (Cardina et al., 2007; Cardina et al., 2011), degree of crust formation, shading provided by the crop, and weed emergence scouting data. Models may also need to incorporate an adjustment factor to account for genetic tendencies of some populations within a species to emerge earlier or later than predicted. Farmers may achieve a simpler version of such a process by comparing their observed emergence to model predicted emergence and adjusting the prediction accordingly in subsequent years.

Considerations for Future Modelling Efforts

Other improvements to emergence models may be gained by improving the parameter estimates through statistically independent sampling and reducing the time between sampling events (Gonzalez-Andujar et al., 2016). For example, work of Cordeau et al. (2017b) was based on statistically independent samples as each sample through the season was from a new plot. But sampling occurred six weeks after each plot was tilled, which was ideal for investigating cohort effects resulting from different tillage timings, but was too imprecise for emergence modeling.

For this review, we focused on peak emergence or 50% cumulative emergence. The two terms are similar since the rate of emergence is theoretically highest at 50% cumulative emergence (Royo-Esnal et al., 2020). But the terms are not interchangeable since the greatest empirically observed emergence peak may occur before or after 50% cumulative emergence if the distribution of emergence is skewed by drought or other random variables. In cumulative emergence models, emergence peaks would be represented by large, predicted flushes of emergence resulting from favorable weather, which may or may not bring the cumulative total above 50%. Such predictions of large flushes may be just as useful for planning purposes as cumulative emergence because weed seedling control will need to be prioritized while the cohort remains small. Thus, farmer-oriented emergence models should allow for incorporation of weather forecasts to provide corresponding weed emergence forecasts.

We have made the case for managing weeds based on their emergence peaks, but accurate predictions of the start, end, or any other point on the emergence continuum can also be useful for management purposes. For example, to maximize efficiency, weed seedling controls with nearly 100% efficacy should not be conducted until most of the weeds have emerged (Fidanza et al., 1996; Masin et al., 2005; Archer et al., 2006), whereas controls that are only effective on small weeds should be initiated when relatively few have emerged (Oriade and Forcella, 1999).

Feasibility of Adjusting Management Based on Weed Emergence

We have presented a range of techniques to improve weed management based on knowledge of peak emergence (**Figure 3**). Certainly, slight adjustments to the timing of seedling control may be more feasible for many farmers than making drastic changes to the cropping system. But farmers are very responsive to adjusting their management based on the biology and timing of their most problematic weeds (Jabbour et al., 2014) and will sometimes go to extreme efforts to regain control. Since most of the tactics we present embody a "weed smarter, not harder" approach, they may be easily adopted by farmers.

For farmers unable to shift the timing of their planting, it may still be possible to alter the edaphic environment to shift weed emergence earlier or later. Prior to planting, weed emergence may be expedited through practices that increase soil warming, such as tillage (Travlos et al., 2020) or solarization. Timing of irrigation may also be used to alter weed emergence (Kanatas et al., 2021). Conversely, delaying the emergence of problematic weeds may be achieved through limiting absorption of sunlight *via* surface residue management (Oreja et al., 2020) or crop canopy establishment.

The feasibility of emergence-based management may be impacted by other factors warranting future research. Such as the weed community shifts that may occur after long-term emergence-based management. Or changing weather patterns that will likely lengthen the growing season to the benefit of many weed species (Peters and Gerowitt, 2015). The increased rainfall expected in Northeast USA may decrease the number of days when soils are dry enough to support tractor-drawn equipment, thereby increasing the importance of access to ample equipment and labor to conduct timely weed control efforts (Birthisel et al., 2021). Likewise, timely control may be challenging on large-scale farms since it may require several days to cover the entire acreage. But perhaps fields or microclimatic areas with consistently early weed emergence could be managed first, and so forth, to allow control timing to mirror emergence trends.

Overall, previous studies were generally in agreement as to the season of peak emergence for each species. Variation in observed results likely reflected the climatic conditions of each study site. But our use of identical weather data in previous models also showed variation in emergence trends within species, highlighting the importance of local edaphic conditions and population-level differences. Nonetheless, prediction accuracy will improve as models become more advanced (Royo-Esnal et al., 2020) and farmers can further improve prediction reliability based on their own ground truthing and adjustments to model predictions in subsequent years. Furthermore, even our broad characterizations of peak emergence for each species (i.e., "late spring") may be used to improve management. Depending on management flexibility, knowledge of the season and pattern of weed emergence be used to improve the timing of control efforts, enhance crop competition, or expedite weed seedbank depletion.

AUTHOR CONTRIBUTIONS

BB conducted the literature search. All authors were involved in the interpretation of the results. BB, EG, and SC wrote the initial draft. All authors were involved in improving the subsequent drafts. All authors contributed to the article and approved the submitted version.

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Seed viability of feathertop Rhodes grass (*Chloris virgata* Sw.) reduced by silage, digestion, and sheep rumen digestion

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Weed seeds can be spread by different vectors, and seed dispersal is an important mechanism for the weed to persist. Weed seeds passaging through the digestive tract of a ruminant animal is expected to result in reduced viability. Two separate experiments were conducted to determine the germinability and viability of the mature seeds of feathertop Rhodes grass (*Chloris virgata* Sw.) after exposure to four treatments, that is, 3 months in silage, 48 h in the rumen of steers, silage plus digestion, and passing through the digestive tract of sheep. Our results showed that three different treatments (silage, digestion, and silage plus digestion) can inhibit 90%–100% of the seed germination of feathertop Rhodes grass. Both silage and digestion reduced seed viability by 65%–90%, depending on the population. Silage followed by digestion reduced viability by 80%–97%. The sheep feeding study showed that total viable seeds from the daily recovery of feces for 12 consecutive days after ingestion was only 0.084% and 0.022% in the 2020 and 2021 experiments, respectively. In comparison with the untreated control, the seed viability of feathertop Rhodes grass was reduced by more than 99.9% after feeding through sheep, indicating that the spreading of feathertop Rhodes grass seeds *via* sheep feces is minimal. These results indicate that silage, digestion, silage followed by digestion, and the ingestion of mature seeds are effective non-chemical weed management options for an integrated weed management package for feathertop Rhodes grass.

KEYWORDS

germination, seed viability, silage, digestion, ruminant, sheep

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Introduction

Weeds are a problem in agriculture and were recently estimated to cost $3.3 billion in Australian cropping systems alone (Llewellyn et al., 2016). The primary method for weed control in most broadacre agricultural systems involves herbicide application, accounting for over 80% of weed control costs in Australia (Llewellyn et al., 2016). However, the threat to the efficacy of herbicides and to conservation tillage systems is the evolution of herbicide-resistant weeds (Heap, 2021). This is particularly the case where the repeated use of a single herbicide active ingredient (or active ingredients with the same mode of action) results in high selection pressure and accelerates the rate of herbicide resistance (Powles and Yu, 2010). To prolong the use of herbicides, future weed management will necessarily include non-chemical weed control strategies in combination with tactical herbicide use to manage herbicide resistance.

Annual weed species can frequently germinate early in the growing season and continue to germinate throughout the season, enabling them to maximize seed production and fitness across a broad range of environmental conditions (Sans and Masalles, 1997). The viable weed seeds result from weed survivors that reach maturity or can be imported into the field *via* other sources. Hence, the prevention of the replenishment of the weed seedbank and the reduction of viable weed seeds entering a field are critical to successful weed management. Often, annual weeds are highly palatable to grazing ruminants (Marten and Andersen, 1975) and can have high nutritive value (Moyer and Hironaka, 1993; Nashiki et al., 2005). Livestock can be used to directly consume and control weeds during fallow years and during fallow periods after harvest and before planting the next crop (Landau et al., 2006; Schoenbaum et al., 2009). Grazing livestock ingest various types of seeds when grazing mature weed plants or seeds scattered on the soil surface (Olson et al., 1997; Michael et al., 2006; Schoenbaum et al., 2009).

Passage through the digestive tract of ruminants (e.g., sheep and cattle) reduces the viability of several grass weeds seeds and legume seeds, thereby reducing the number entering the weed seed bank (Piggin, 1978; Stanton et al., 2002; Kneuper et al., 2003; Wang et al., 2017). However, some seeds are still viable after passing through the digestive tract of ruminants (Piggin, 1978; Stanton et al., 2002; Kneuper et al., 2003; Wang et al., 2017). These viable seeds can be transmitted by livestock, which is a mechanism of seed spread (endozoochory) utilized by many plant species. Weed seed spread by livestock is of most concern in regions of the world such as Australia with extensive grazing systems (Hogan and Phillips, 2011). The proportion of undamaged seeds is related to the seed coat, with hard-seeded species less affected by digestion by ruminant species and with less damage resulting from ingestion by cattle than sheep (Gardener et al., 1993a; Gardener et al., 1993b; Stanton et al., 2003; Michael et al., 2006; Haidar et al., 2010). Nevertheless, the role of the animal digestive tract on seed viability and the spread of weed seeds has not been sufficiently clarified for one of Australia's most important weeds—feathertop Rhodes grass (*Chloris virgata* Sw.).

The production of silage is a common practice in many countries including Australia, providing farmers with a reserve of livestock feed that can be used strategically for production or for ameliorating seasonal feed shortages (Piltz et al., 2017). Silage is produced when forage is mechanically harvested, stored anaerobically, and fermented to produce acids that preserve the forage while anaerobic conditions are maintained (Piltz and Kaiser, 2004). Weeds and weed seeds present at the time of harvest are ensiled with the forage, and often, it is assumed that the silage renders most of these weed seeds non-viable (Kaiser et al., 2004). However, several studies reported that while most weed seeds are destroyed by silage, a small percentage remained viable (Blackshaw and Rode, 1991; Mayer et al., 2000; Piltz et al., 2017). Silage is produced for the specific purpose of feeding livestock; therefore, these viable seeds can potentially be spread by livestock in feces *via* ingestion and passage through the digestive tract.

Feathertop Rhodes grass is a major weed in both cropping and non-cropping situations in Australia. This annual species is very difficult to control, and no single weed management option provides adequate control. Currently, feathertop Rhodes grass control relies heavily on a few registered herbicides, particularly those with glyphosate and acetyl CoA carboxylase (ACCase) inhibitors, resulting in the rapid evolution of resistance to these key agrochemicals (Widderick, 2020; Heap, 2022). Therefore, an integrated approach is required for the effective management of this weed. Pastures and forage crops inevitably contain weed seeds, and a reduction in the number of viable seeds following ingestion or silage may present an effective control strategy. However, there are no reported data on feathertop Rhodes seed survival after silage and/or digestion. Two separate experiments were conducted to determine the effect of silage, digestion in cattle, and their combined effect on the germination and viability of different populations of feathertop Rhodes grass. Additionally, we wanted to determine the percentages of the recovery of the seed viability of feathertop Rhodes grass seeds after passing through the digestive tract of sheep.

Materials and methods

Experiment 1: Impact of silage, digestion (ruminal digestion) and silage plus digestion

Seeds packets and silage preparation

A total of two and four populations of feathertop Rhodes grass were used for this study in 2020 and 2021, respectively (Table 1). Additionally, in 2021, annual ryegrass (*Lolium rigidum*) was used as a quality control and a comparison with previous studies.

TABLE 1 Feathertop Rhodes grass populations were tested against four different seed-damaging treatments (untreated control, silage, digestion, silage plus digestion) in 2020 and 2021.

Population	Location seed collections	2020	2021
FELT 04/20	East Darling Downs, QLD	–	Tested
HOLB 01/20	Holbrook, NSW	Tested	Tested
STURT/16-17	Wagga Wagga, NSW	Tested	Tested
MUTT 04/20	Wagga Wagga, NSW	–	Tested

Four treatments: (1) untreated (control), (2) silage, (3) digestion, and (4) silage plus digestion were employed in both years of the study. The seeds (n = 50) of each population were placed in bags made from white polyester monofilament (53 ± 10-µm pore size) used for *in sacco digestion* studies (Bar Diamond®). The pore size was small enough to contain all the seeds but still allows for the passage of water, gases, and microorganisms through the mesh (Haidar et al., 2010). Separate bags were used for each population, and the bags were ensiled in plastic bag mini-silos made of 100-µm polyethylene, each containing between 6 and 10 kg of chopped lucerne (*Medicago sativa* L.) forage (Wilson and Wilkins, 1972).

The forage was chopped directly from pasture with an Iseki mower with a catcher and spread in a thin (approx. 5–10-mm) layer on black plastic to wilt in order to reach the target dry matter (DM) content. The bags of seeds were layered in the chopped forage to ensure that each bag was in contact with silage. The chopped forage was physically compacted and air evacuated from the bag with a household vacuum cleaner and the bag opening tied securely to obtain an airtight seal. Then, each bag was placed inside a second bag of the same type and the vacuuming and tying process was repeated. The bags were packed into 200-L drums surrounded by damp sand with a layer of damp sand on the top to maintain weight on the bags. The bags were stored for 3 months to ensure the completion of the silage process; all silages were opened at the same time in each experiment. Two bags of each population were placed into each mini-silo, with each mini-silo representing a replicate; there were three replicates in the study. Six bags of each populations were retained and stored at room temperature (without silage).

Seed digestion in cattle rumen

Upon opening the silage, one bag of each population from each silo was paired with a bag of the same population that has not been ensiled. Both bags were placed in the rumen of a mature Red Poll steer for 48 h, which is the equivalent length of time as the rumen phase when determining *in vitro* digestibility (Tilley and Terry, 1963). All bags from each mini-silo were placed in the rumen of the same steer, and bags from different mini-silos were placed in different steers. Three steers were fed a diet consisting of lucerne hay, oaten chaff, barley grain, and oat grain at 300, 300, 200, and 200 g kg^{-1} of the diet, respectively, on an as-fed basis. The steers were fed daily each morning an amount calculated such that intake was approximately 1.2 times maintenance requirements. Diets were fed for 10 days prior to commencement of the digestion study to ensure that the rumen had adjusted to a standard diet and then for 48 h during which digestion degradability was determined.

Germination and viability test

After silage and digestion treatments had been imposed, the recovered seeds were placed on Whatman No. 1 filter paper moistened with 4 ml of distilled water in a 9-cm petri dish. Petri dishes were sealed with Parafilm and incubated for 11 days at 25°C/15°C day/night temperatures with a 12-h photoperiod. The number of germinated seeds was recorded, and the remaining non-germinated seeds of feathertop Rhodes grass were tested for viability using the tetrazolium test previously described by Stanton et al. (2002).

Experiment 2: Seed viability after feeding through sheep

Only one population (MUTT 04/20) was used for this study due to the availability of a large number of seeds. The seeds (900 g) of this population were collected between April and May 2020 on a roadside in Gobbagombalin (146°34’201”E, 35° 07’606”S), Wagga Wagga, NSW 2650. Laboratory tests showed that seed had a 65% germination rate 7 months after collection. A previous feeding trial protocol (Stanton et al., 2002) was adopted with slight modification. Briefly, before the commencement of the trial, the sheep (n = 8) were individually penned and fed a diet consisting of lucerne and oaten chaff (1:1). The sheep were fed daily each morning an amount calculated such that intake is approximately 1.2 times the maintenance requirements (average 856 g head^{-1}.day). On 26 November 2020, the sheep were fed the standard diet mixed with 40 g of feathertop Rhodes grass seeds (approximately 135,800 seeds). Following the introduction of feathertop Rhodes grass seeds into the diet, the total feces were collected daily from day 1 to day 12 to determine the time taken from the ingestion to complete excretion of the seed. Fecal collection for each day occurred 24 h after feeding; hence, feces for day 1 were collected prior to feeding on day 2 and so on.

The feces sample was weighed and separated into three subsamples where one subsample (260 g approx.) was used to determine seed viability in a glasshouse. Other subsamples were air-dried and kept in a cool room for further testing to confirm the results. The third subsamples were used for a digestibility test (not reported here). To determine the percentages of seed viability in the feces, the first subsample was evenly spread onto a plastic seedling tray (32 cm × 28 cm × 6 cm), which was filled with a field soil (loamy soil). The feces were gently rubbed and lightly mixed with the surface soil. The trays were kept moist and monitored for 21 days. The trays were maintained under a glasshouse condition and irrigated as required. The average temperature, relative humidity, and light intensity of the glass house during the experimental period were 22–27°C, 70%–85%, and 217–363 lum/ft.^{2}, respectively. The total emerged seedlings were counted to estimate the seed viability and the speed with which feathertop Rhodes grass seeds passed through the gut of sheep. The cumulative seedling emergence (E_c) in both years' experiments were calculated by using a formula: $E_c = e_0 + \sum (e_{i+1} - e_i)$, where e_0 is the number of seedlings in first count, and e_i is the number of seedlings in count i. We were unable to extract the seeds from the feces through sieving or washing due to the tiny seeds (0.39 mg 100 seeds^{-1}).

In March 2021, the above experiment was repeated with the second subsample to validate the results obtained in 2020. An additional control treatment was included where 300 seeds of feathertop Rhodes grass were spread on a seedling tray under the same glasshouse conditions in 2021. The total emerged seedlings were counted in the same manner as described above.

Experimental design and statistical analysis

A randomized complete block design was employed for the germination and viability evaluation for experiment 1, where the three shelves of the incubator were considered as a blocking factor to minimize systemic errors. The R packages including agricolae (De-Mendiburu 2021) were also used on R Core Team (2022) for exploratory data analysis and data fitting in different models. The normality and distribution of data was verified by a Shapiro–Wilk normality test. The data variance was also examined by plotting the residuals to confirm the homogeneity of variance. The significant differences among treatment means were identified by Tukey's HSD (honestly significant difference) at $p<0.05$.

Given that different populations were employed, and there were some variations observed between years, the data from 2020 and 2021 were analyzed separately for experiment 1. Data were analyzed using linear mixed models with the population, treatment and interaction as fixed effects and the position within the incubator as the random effect.

A completely randomized design was employed with eight replications (sheep) for experiment 2. Data were pooled from two runs, as there was no time-by-treatment interaction as determined by ANOVA. A Pearson correlation coefficient value was calculated to see if there was any significant correlation between the seedling emergence rate in 2020 and 2021 for experiment 2. The significant r-value indicated that there was consistency between the 2020 and 2021 experiments.

Results

Experiment 1: Impact of silage, ruminal digestion, and silage plus digestion

The silage dry matter content and pH value were 396 and 505 g kg^{-1} and 5.3 and 5.1 in 2020 and 2021, respectively. The germinability of untreated (control) seeds varied significantly ($p<0.005$) between the populations of feathertop Rhodes grass (Table 2). Silage treatments resulted in 0% germination for two and four populations tested in 2020 and 2021, respectively. Additionally, in 2020, the digestion treatment reduced seed germination and only 2%–10% of the seeds of feathertop Rhodes grass were subsequently germinated.

The repeated study in 2021 showed that the recovered seed of all 4 populations tested failed to germinate after digestion or silage plus digestion. Compared to the untreated control, the viability of the four populations was reduced by 65%–95%, 73%–90%, and 80%–97% after silage, digestion, and silage plus digestion, respectively (Table 2). The total number of viable seeds significantly ($p<0.005$) differed between populations after being exposed to the three different seed-damaging treatments.

There was no significant ($p>0.005$) difference in viability between the three treatments (silage, digestion, and silage plus digestion) (Figure 1) in 2021. On average, the seed viability of the four populations declined by 72%, 70%, and 82% after silage, digestion, and silage plus digestion, respectively.

Experiment 2: Seed viability after feeding through sheep

The average amount of fresh feces of eight sheep from day 1 to day 12 is presented in Table 3. The fresh feces ranged from 695.25 to 968 g with an average of 789 g for days 1–12. During fresh feces collection, it was noticed that there were no issues with diet adaption with feathertop Rhodes grass in seven of the eight sheep. Sheep number 4 suffered from diarrhea, and the number of extracted feces was comparatively less than that of other sheep.

We observed that seeds in the feces emerged 4 or 5 days later than the untreated (control) seeds in 2021 (data not shown). Most of the seedling emergence (58%–62%)

TABLE 2 Seed germination and viability of four different populations of feathertop Rhodes grass after four different seed-damaging treatments.

Testing year	Population	Control		Silage		Digestion		Silage Plus Digestion	
		Germination (%)	Total viable seeds (%)	Germination (%)	Total viable seeds(%)	Germination (%)	Total viable seeds (%)	Germination (%)	Total viable seeds (%)
2020	STURT/16-17	54 (± 4.9)^{c}	62 (± 3.0)^{d}	0^{a}	11 (± 0.7)^{ab}	10 (± 3.61)^{a}	15 (± 1.5)^{b}	0^{a}	6 (± 0.33)^{a}
	HOLB 01/20	22 (± 3.3)^{b}	35 (± 1.4)^{c}	0^{a}	11 (± 0.6)^{ab}	2 (± 1.2)^{b}	08 (± 1.8)^{a}	2 (± 1)^{a}	9 (± 2.5)^{a}
	STURT/16-17	58 (± 5.6)^{bc}	74 (± 7.8)^{f}	0^{a}	5 (± 1.6)^{a}	0^{a}	23 (± 3.1)^{c}	0^{a}	3 (± 1.3)^{a}
2021	HOLB 01/20	50 (± 7.5)^{b}	48 (± 3.1)^{e}	0^{a}	35 (± 0.6)^{d}	0^{a}	27 (± 4.3)^{cd}	0^{a}	20 (± 3.2)^{bc}
	FELT 04/20	64 (± 5.3)^{c}	68 (± 6.1)^{f}	0^{a}	17 (± 0.8)^{bc}	0^{a}	22 (± 3.4)^{c}	0	17 (± 2.3)^{bc}
	MUTT 04/20	66 (± 4.9)^{f}	75 (± 5.2)^{bc}	0^{a}	20 (± 2.0)^{ab}	0^{a}	10 (± 2.4)^{c}	0^{a}	10 (± 3.0)^{ab}
	Annual ryegrass	64 (± 8.0)	77 (± 1.5)	0	19 (± 3.2)	0	40 (± 5.0)	0	10 (± 2.6)

The data show mean value (± standard error). The interaction between the population and treatment was significant in 2020 and 2021 (both p<0.05) and significant differences were identified by Tukey's HSD at p<0.05. Values within years with different superscript letters are significantly different. Annual ryegrass was included as a comparison as it previously showed that germination and viability declined after silage, digestion, and silage plus digestion treatments.

occurred from the feces collected during the first 3 days after seed intake in both years (Table 3). There was still limited seedling emergence from feces collected 12 days after feeding, being less than 1% and 6% of the total seeds emerged in 2020 and 2021, respectively. On average, the total number of emerged seedlings from day 1 to day 12 was 38.00 in 2020 and 9.80 in 2021, which is estimated at a survival rate of 0.08% and 0.022% in 2020 and 2021, respectively.

A correlation analysis showed (Figure 2) that there was a significant (*p*<0.001) correlation (*r* = 0.82***) in the seedling

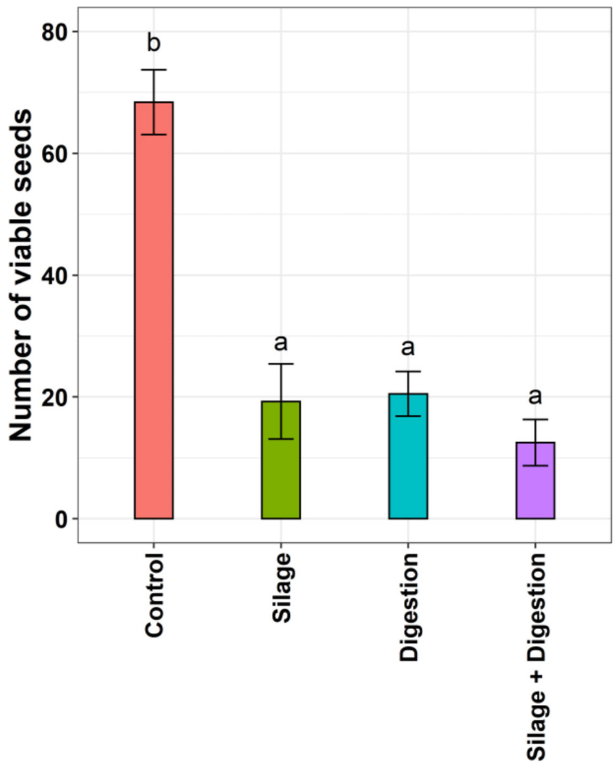

FIGURE 1
Average seed viability of four populations of feathertop Rhodes grass in response to different seed treatments in 2021. Vertical bar shows mean (± standard error) of germination. Different letters show significant difference as identified by Tukey's HSD at *p*<0.05.

TABLE 3 Average fresh feces (g) collected, the number of seedlings emerged/tray, and cumulative emergence (%) from day 1 to day 12.

Day after feeding	Feces (g)^{NS}	Average seedlings emerged/tray in 2020	Cumulative emergence (%) in 2020	Average seedlings emerged/tray in 2021	Cumulative emergence (%) in 2021
1	695.25 (± 74.9)	8.7 (± 1.3)^{a}	23.0	3.4 (± 1.2)^{a}	34.6
2	671.25 (± 77.3)	9.0 (± 2.6)^{a}	47.0	1.7 (± 0.9)^{a}	51.9
3	727.50 (± 98.5)	4.3 (± 1.3)^{ab}	58.0	1.0 (± 0.7)^{ab}	62.1
4	791.87 (± 95.6)	3.3 (± 1.6)^{ab}	67.0	0.7 (± 0.7)^{b}	69.2
5	851.37 (± 96.5)	5.0 (± 0.9)^{ab}	80.0	0.4 (± 0.3)^{b}	73.2
6	757.25 (± 93.2)	1.2 (± 1.1)^{ab}	83.0	0.4 (± 0.4)^{b}	77.2
7	759.38 (± 73.3)	2.0 (± 0.3)^{b}	88.0	0.3 (± 0.4)^{b}	80.2
8	968.13 (± 69.7)	0.7 (± 0.5)^{b}	90.0	0.0 (± 0.0)^{c}	80.2
9	789.25 (± 70.9)	2.6 (± 1.4)^{b}	97.0	0.5 (± 0.5)^{b}	85.3
10	858.0 (± 94.8)	0.6 (± 0.6)^{b}	99.0	0.5 (± 0.4)^{b}	90.4
11	845.63 (± 66.7)	0.1 (± 0.1)^{b}	99.3	0.3 (± 0.1)^{b}	93.4
12	757.75 (± 37.3)	0.5 (± 0.3)^{b}	100	0.6 (± 0.3)^{b}	100

The data show mean value (± standard error). Values in the same column with different letters are significantly different using Tukey's HSD at $p<0.05$. Seedling emergence significantly ($p<0.05$) varied between days after feces collection.
NS, Not Significant.

emergence pattern between the 2020 and 2021 experiments, indicating that both experiments achieved consistent results.

In comparison, 53.4% of the seed in the control treatment germinated in the trays under the same conditions. This showed that sheep rendered more than 99.9% feathertop Rhodes grass seeds unviable after passing through the digestive system.

Discussion

In our study, silage and digestion independently reduced germinability and viability compared with untreated control seeds for feathertop Rhodes grass, with the magnitude of the reduction varying with populations and in some cases between years. Our results were consistent with Piltz et al. (2017) who found that both seed damage treatments (silage and digestion) can significantly reduce the seed germinability and viability of a range of grass weed species including barley grass (*Hordeum* spp.), vulpia (*Vulpia* spp.), wild oat (*Avena fauta*), brome grass (*Bromus diandrus*), and annual ryegrass. The causes of seed death following ingestion by ruminant livestock are reasonably well understood (Hogan and Phillips, 2011). The damage of seed occurs during the digestive process in the rumen and the whole digestive tract. The extent of damage also depends on the degree of mastication, which varies between the species of an animal, with seeds being more extensively damaged by sheep and goats

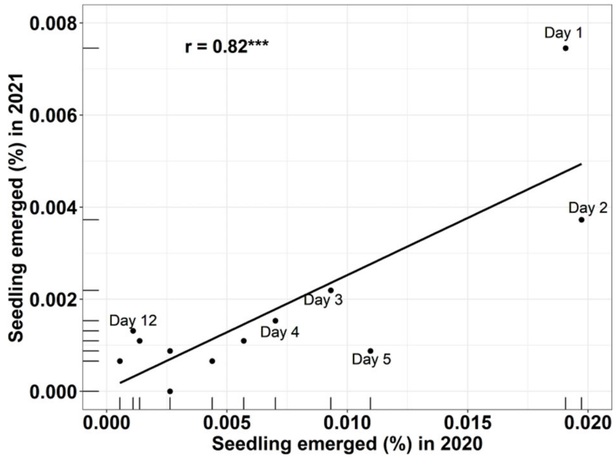

FIGURE 2
Correlation between seedling emergence (%) in sheep-feeding trial (experiment 2) in 2020 and 2021 at different days after feces collection. *** = highly significant.

than by cattle (Simao Neto et al., 1987). However, the mechanism(s) operating to reduce or eliminate seed viability during silage are not yet clear. Silage is a lactic acid fermentation process that is mainly used to preserve biomass and affects the seed viability of some plant species (Piltz et al., 2017; Hahn et al., 2021; Piltz et al., 2021). Lactic acid is the strongest of silage acids, and these acids are produced by this fermentation of plant sugars. Several acids produced by the fermentation are affected by the plant parameters such as forage dry matter content, water-soluble carbohydrate, and buffering capacity during the ensiling process (Müller and Bauer, 2006; Piltz and Kaiser, 2004).

The seed viability loss of feathertop Rhodes grass under silage varied between 65% and 95% dependent on populations in our research. The differences between the studies (2020 *vs*. 2021) of a population could be due to the possible effects of the surrounding silage substrate and to different initial seed qualities. The seed lot quality can vary strongly depending on factors such as seed age, plant population, and storage conditions (e.g., Hay and Probert, 2013; Weller et al., 2016;). For example, the viability of our untreated seeds and the seed size differed significantly between the populations of feathertop Rhodes grass (Asaduzzaman et al., 2022). The specific mechanisms by which a small proportion of seed of feathertop Rhodes grass survived after silage are not yet clear. Our studies suggest that a significant proportion of seed viability is reduced due to silage, which can be a suitable control measure for feathertop Rhodes grass. In addition to damaging seeds, silage appeared to have weakened the seed vigor of feathertop Rhodes grass as the proportion of germinable seeds to viable seeds was reduced. We think that it is unlikely that these viable seeds will develop into true or healthy seedlings. However, this assumption was not determined in our current study. Furthermore, weed seeds ensiled from pasture or crops during commercial production would likely be less mature; therefore, the impact from silage and digestion either alone or in combination on seed viability is possibly far greater.

In our study, the effect of digestion on the seed germinability and viability of feathertop Rhodes grass was almost the same as the observed effect by silage (Figure 1). Similarly, Stanton et al. (2002) in annual ryegrass who reported that 10.8% and 32.8% of the seed ingested was excreted by sheep and cattle, respectively, with 3.9% (sheep) and 11.9% (cattle) remaining germinable. They concluded that additional strategies were required to manage the excreted viable seeds. In our study, the impacts of silage and digestion were more effective in reducing the seed viability of feathertop Rhodes grass than annual ryegrass with the exception of only one population (HOLB 01/20) tested in 2021. These data support the use of silage production and grazing as part of an integrated control program for feathertop Rhodes grass. Generally, the reduction in germinability and viability following silage plus digestion was greater than for silage or digestion alone, which is expected as each treatment will potentially reduce seed integrity independently. The seeds of feathertop Rhodes grass are small with a thin seed coat; therefore, the tiny embryo can be easily damaged. Other studies have reported that generally, the seeds of grasses were more susceptible to damage during ensiling and/or digestion than the seeds of hard-seeded species (Blackshaw and Rode, 1991; Westerman et al., 2012; Hahn et al., 2021; Piltz et al., 2021). Additionally, previous research found a longer period in silage that resulted in a higher seed-killing efficacy (Waldo et al. 1973; Mayer et al., 2000; van Eekeren et al., 2006; Trolove and Dowsett, 2015; Simard and Lambert-Beaudet, 2016). However, most studies would indicate that 3 months of ensiling rendered most seeds unviable, and this is consistent with our findings.

Similarly, damage to seed depends on the degree of mastication, which varies between the species of animal, and on the length of time the seed remains in the digestive tract. Generally, the ability of excreted seed to germinate declines with time in the tract, although the rate of decline varies between plants species and animals (Hogan and Phillips, 2011). For example, sheep and goats caused more extensive damage on seed than by cattle (Simao Neto et al., 1987). Our study indicated a clear peak in seed excretion between 1 and 12 days after ingestion, with approximately 60% of viable seed recovered during days 1–3. In the case of 2020, which had higher numbers of excreted viable seed, there was 80% collection during days 1–5. Seed viability percentages after ingestion ranged from 0.022% to 0.084%. These percentages are lower than those reported in other studies involving small ruminants. For example, seed recovery percentages after ingestion were 0%–28% (Yu et al., 2012) and 10.4%–23.0% (Manzano et al., 2005) in sheep and 7.4%–17.4% in goats (Robles et al., 2005) for annual grass species. One of the possible reasons for the relatively low viability percentage in our studies is due to the small seed size (only 0.39 mg 100 seeds^{-1}) of feathertop Rhodes grass, which, we speculate, makes these seeds more prone to be damaged after ingestion. Additionally, the digestive tracts of smaller animals increase the likelihood of small seeds contacting the gut wall, which can damage the seed by abrasion (Razanamandranto et al., 2004). Additionally, Harvey (1981) used indirect comparisons and suggested that sheep are more effective than cattle in digesting seeds. However, we observed that some seeds were still viable at 12 days postfeeding after passing through the digestive tract. Consequently, some spread of viable seeds can still occur for a prolonged period after ingestion, and practically, farmers should monitor for the spread of feathertop Rhodes *via* this means into other areas.

Conclusion and practical implementation

Our study suggested that silage or digestion can significantly reduce both the seed germination and viability of feathertop

Rhodes grass. However, 5%–35% seeds of feathertop Rhodes grass can remain viable after silage depending on the population. The seed viability rate can be further reduced (by up to 90%) by the combined effect of silage plus digestion. Our research also revealed that the seed viability of feathertop Rhodes grass was reduced by feeding to sheep. Feeding to sheep reduced germinable seeds from the initial 65% to less than 0.1% in both studies. Approximately 60% of the germinable seeds were excreted by the sheep during the first 3 days after feeding. The seed viability of feathertop Rhodes grass was reduced by more than 99.9% after feeding through sheep, indicating that the spreading of feathertop Rhodes grass seeds through sheep tracts is minimal. However, a low level of germinable seeds (<10%) can still be detected at 12 days after feeding. Feathertop Rhodes grass plants should not be allowed to grow to the mature stage due to the massive seed production (Widderick et al., 2014). The light-weighted awny seeds can be spread by many mechanisms such as animal hide, feet, wind, water, and machinery. The use of sheep grazing should only be performed prior to seed head emergence to reduce the risk of potential spread. If sheep are suspected of having fed on mature plants, then ideally, these sheep should be quarantined for a week, which will allow more than 90% of seeds to be excreted. Effective grazing prior to seed head emergence provides an alternative to herbicides, thereby reducing selection pressure for herbicide resistance. Grazing can also buy time for effective control. For example, advanced weed plants under moisture stress are often less responsive to herbicide application. Therefore, grazing can keep stressed plants under control and allow for the grazed plants to have an herbicide applied at a later stage when the spraying conditions may be ideal for maximum control efficacy. Ecological weed management aims to subject weeds to multiple, temporally variable stresses, for which Liebman and Gallandt (1997) coined the term “many little hammers.” Based on the results of our study, we claimed that silage and digestion are an overlooked yet effective way to reduce feathertop Rhodes grass seed loads in a sustainable manner. By reducing the number and vigor of weed seeds, silage and digestion can help exclude feathertop Rhodes grass as well as other weeds from fields. The reduction of density and delaying of emergence can reduce the interference with crop growth or reproduction. Thus, silage and digestion or a combination of both can be some of the “little hammers” in ecological weed management in an integrated farming system.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by NSW DPI. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

MA, JP, EK, and HW contributed to the conception and the design of the study. AS, MH, MA, and JP contributed for the preparation of the silages. MA edited the manuscript, organized this project, analyzed the viability of the seeds, and performed all statistical analyses. JP, EK, and HW contributed for the revision of the manuscript. AS and MH helped for experiment set up and data collections. All authors contributed to the article and approved the submitted version.

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Conflict of interest

This study received funding from Grain Research Development Corporation (GRDC) and NSW Department of Primary Industries. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Cover crops and preemergence herbicides: An integrated approach for weed management in corn-soybean systems in the US Midwest

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Adoption of a fall established, high biomass cereal rye cover crop has potential to diversify weed management in corn and soybean production systems, reducing the selection pressure for resistance to postemergence herbicides. However, farmers and crop consultants express concern about limited weed suppression from an overwintering cover crop in areas where high biomass production is limited by cooler spring temperatures, such as in the Upper-Midwest U.S. Use of a preemergence herbicide, regardless of cover crop adoption, is a standard recommendation for improving early season weed control in corn and soybean. Field experiments were conducted at two sites in Wisconsin to assess the effects of six soil management practices (tillage, no-till, and four cereal rye cover crop termination timings/methods) with or without the use of a preemergence herbicide on weed suppression at the time of postemergence herbicide application and crop productivity. Results showed that cereal rye biomass increased > 6x between termination at the time of cash crop planting versus termination two weeks later. In corn and soybean, weed ground cover was lower for soil management with cereal rye cover crop terminated two weeks after cash crop planting (≤ 7% weed cover) compared to all other soil management practices (≥ 23% weed cover) when a preemergence herbicide was not used. Use of a preemergence herbicide resulted in low weed ground cover across treatments in corn (≤ 7% weed cover) and soybean (≤ 13% weed cover). Corn and soybean yield was not affected by preemergence herbicide treatments. Corn yield was lower at the south-central Wisconsin location for the soil management with a cereal rye cover crop terminated two weeks after cash crop planting (9.82 Mg ha^{-1}) compared to all other soil management practices (≥ 12.07 Mg ha^{-1}); at the southwest Wisconsin location, corn yield was greater for the conventional tillage treatment (14.28 Mg ha^{-1}) compared to all other soil management treatments (≤ 10.89 Mg ha^{-1}). Soil management did not affect soybean yield, although yields were different between locations with 3.44 Mg ha^{-1} at the south-central Wisconsin compared to 4.77 Mg ha^{-1} at the southwest Wisconsin location. These results indicate that in the absence of a high biomass cereal rye cover crop, preemergence herbicides are important for in-season weed control. Also, the inclusion of a late-terminated cereal rye cover crop in soybean should be considered as an effective management practice for reducing weed ground cover without affecting crop yield.

KEYWORDS

cereal rye, corn, soybean, soil management, preemergence herbicide, cover crop, integrated weed management, herbicide resistance management

Introduction

Corn (*Zea mays*) and soybean (*Glycine max*) are the most dominant crops typically grown in rotation in the Midwest U.S. In 2019, over 80% of the harvested corn and soybean area in the U.S. was in the Midwest (USDA, 2020). Herbicides are the main method of weed control for these crops and represent a major expense for farmers. Over 92% of the area in corn production and 98% of the area in soybean production in the Midwest received a herbicide application in 2018 (USDA, 2020). Two major challenges for farmers rotating these crops are resiliency of this production system (i.e. susceptibility to erosion, drought, pests) and herbicide resistance.

Resilient cropping systems are less prone to reduced crop productivity and environmental damage imposed by natural disturbances, which are becoming more common with climate change (Bennett et al., 2014; Liang et al., 2017; Bowles et al., 2018). Natural disturbances such as heat and moisture stress during crop pollination/grain fill periods from a warmer summer climate, or delayed crop planting from increased spring precipitation can result in significant crop yield loss (Kucharik and Serbin, 2008). Resilient cropping systems are better able to withstand or recover from disturbances such as increased frequency of extreme precipitation events and abnormal temperature fluctuations (Mishra et al., 2010; Pryor et al., 2014; Gaudin et al., 2015). Practices which improve cropping systems resiliency include adding perennial crops and diversifying crop rotations to increase the duration of living cover and reduce soil disturbance (Gaudin et al., 2015; Sanford et al., 2021); however, infrastructure and demand for cropping options beyond corn and soybean are limited. Adding cover crops to corn and soybean systems can provide many benefits which can help restore ecosystem functions that are diminished by conventional production practices, making current cropping systems more resilient (Blanco-Canqui et al., 2015). Use of a fall established cover crop has been shown to benefit summer annual cropping systems by sequestering soil organic carbon (Blanco-Canqui et al., 2013), moderating soil temperature (Teasdale and Mohler, 1993), improving water infiltration (Basche and DeLonge, 2019), improving water storage (Basche et al., 2016), reducing soil erosion (Blanco-Canqui et al., 2015), and reducing nitrate leaching (Tonitto et al., 2006).

Current crop production systems rely primarily on practices that are “techno-fixes” for weed control (i.e., herbicides, herbicide-tolerant crops, and tillage), meaning they are effective at solving problems in the short term but eventually result in delaying or transforming the problem so that it continues to be a long-term issue (Scott, 2011; MacLaren et al., 2020). Herbicides have benefitted crop production systems by allowing farmers to conserve soil with no-till soil management, although the simplification of crop production systems and dependence on herbicides for weed control has led to the widespread occurrence of evolved herbicide resistance (Young, 2006; Gaines et al., 2020; Heap, 2022). The continued documentation of multiple herbicide resistance mechanisms and cross resistance mechanisms is alarming (Owen et al., 2007; Yerka et al., 2013; Shergill et al., 2018). Even more concerning are cases of metabolic resistance which can impart resistance to herbicide sites of action that a population has not previously been exposed to (Nakka et al., 2017; Shyam et al., 2021). Reducing selection pressure and preventing weeds from producing seed are essential to postpone further herbicide resistance evolution.

Integrated weed management is an approach in which farm managers use multiple practices, technologies, and/or tools in a combined effort to reduce the selection pressure for resistance to any individual control used alone (Norsworthy et al., 2012; Harker and O’Donovan, 2013), maintaining efficacy of preferred control methods, such as herbicides. Controlling early season weeds is a good strategy to reduce the selection pressure on limited postemergence (POST) herbicide options

(Norsworthy et al., 2012). Two practices which have been shown to provide control of early season weeds when used separately are cover crops and preemergence (PRE) herbicides. Combining the use of PRE herbicides, a chemical weed control tool with soil residual activity, with cover crops, a physical/biological weed control tool, is one potential method to delay the selection pressure for resistance to POST herbicides. Previous research conducted in the eastern U.S. evaluating the integration of PRE herbicides and cover crops in corn and soybean has shown the potential for effective weed management when integrating these practices (Yenish et al., 1996; Bunchek et al., 2020). Additionally, research conducted in the Midwest indicates successful early-season weed control from a PRE herbicide or use of a cover crop (Ganie et al., 2017; Oliveira et al., 2017; Werle et al., 2017; Grint et al., 2022), but research studying the effects of combining these practices in a Midwest cropping system for integrated weed management to our knowledge remains limited. The effects of cover crops on crop productivity have been shown to be variable depending on geography, cropping system, and cover crop management practices used (Pantoja et al., 2015; Otte et al., 2019; Reed et al., 2019). Challenges of cover crop adoption in the U.S. Midwest include management complications (i.e. establishment and termination), infrastructure limitations (i.e. equipment availability), and uncertainty about the opportunity cost of adopting a cover crop (Roesch-McNally et al., 2018; Oliveira et al., 2019). Research in the Upper-Midwest U.S. is needed to provide farmers in Wisconsin and surrounding states with more information about management decisions and considerations for incorporating a cover crop into current cropping systems.

Field research was conducted to understand how soil management practices [tillage, no-till, and a cereal rye (*Secale cereale*) cover crop] and use of a PRE herbicide interact to influence weed control and crop productivity in corn-soybean systems. The addition of a cover crop in treatments without PRE herbicide use was hypothesized to improve weed control with increasing cover crop biomass accumulation when compared to soil management with tillage and no-till, and provide additional weed control when used with a PRE herbicide. Findings from this research can help farmers improve weed management and resiliency of their farms by optimizing the integration of a cereal rye cover crop and PRE herbicides into their cropping systems.

Materials and methods

Study establishment and management

Experiments were established in the fall of 2018 in Wisconsin at the University of Wisconsin-Madison Arlington Agricultural Research Station (43°18’36” N, 89°20’50” W; south-central WI) and the Lancaster Agricultural Research Station (42° 49’42” N, 90°47’25” W; southwest WI) (Table 1). Soils were classified as Plano silt loam (fine-silty, mixed, superactive, mesic, Typic Argiudoll) at Arlington and Fayette silt loam (fine-silty, mixed, superactive, mesic, Typic Hapludalf) at Lancaster. Each location had two experiments, one planted to corn and one planted to soybean, during the first growing season (2019) that were managed in a corn-soybean rotation in the subsequent years, allowing for a corn and soybean experiment to be present

TABLE 1 Field activity information for corn and soybean experiments with a cereal rye cover crop (CC) conducted in 2019 and 2020 growing seasons at the Arlington Agricultural Research Station and the Lancaster Agricultural Research Station in Wisconsin.

Arlington			**Lancaster**		
Crop	Activity	Date	Crop	Activity	Date
Corn	2018 CC Planting	October 1	Corn	2018 CC Planting	October 23
	2019 Crop Planting[a]	May 23		2019 Crop Planting[b]	May 23
	2019 POST	June 19		2019 POST	June 19
	2019 CC Planting	November 5		2019 CC Planting	November 10
	2020 Crop Planting[a]	May 21		2020 Crop Planting[a]	May 20
	2020 POST	June 18		2020 POST	June 17
Soybean	2018 CC Planting	October 16	Soybean	2018 CC Planting	October 23
	2019 Crop Planting[c]	May 23		2019 Crop Planting[d]	May 23
	2019 POST	June 25		2019 POST	June 25
	2019 CC Planting	November 5		2019 CC Planting	November 10
	2020 Crop Planting[e]	May 21		2020 Crop Planting[e]	May 20
	2020 POST	June18		2020 POST	June17

[a] corn hybrid NK® 9535-3220-EZ1 (Syngenta, Greensboro, NC)
[b] corn hybrid Pioneer® P9998AMXT (Corteva Agriscience, Johnston, IA)
[c] soybean variety Asgrow® AG21X7 (Bayer Crop Science, St. Louis, MO)
[d] soybean variety Asgrow® AG24X7 (Bayer Crop Science, St. Louis, MO)
[e] soybean variety Asgrow® AG18X0 (Bayer Crop Science, St. Louis, MO)
Corn planted at 80,300 seeds ha^{-1} and soybean planted at 345,900 seeds ha^{-1}.

at each location during every year of the study. Data for this study were collected from the 2019 and 2020 growing seasons. Fields were historically managed as a corn-soybean rotation under no-till soil management. Soil organic matter ranged from 2.9-3.5% at Arlington and 2.4-2.5% at Lancaster, whereas soil pH ranged from 6.1-6.5 at Arlington and 6.3-6.8 at Lancaster. Experiments for each crop at each site were established in a randomized complete block design with a six by two factorial treatment structure that included six soil management practices and two PRE herbicide treatments (inclusion or exclusion of a PRE herbicide). Soil management practices included conventional tillage (hereafter 'tillage'), no-till ('no-till'), and four cover crop termination timings/methods (early termination ['early termination'] two weeks prior to crop planting, plant termination ['plant termination'] at the time of crop planting, forage harvest ['forage harvest'] at the time of crop planting, and late termination ['late termination'] two weeks after crop planting). Each experiment had four replicated blocks in which experimental units (plots) were 3-m wide x 9.1-m long. The soil management practice by PRE herbicide treatment combination was maintained in each plot as crops were rotated between growing seasons in each experiment.

The cereal rye (Guardian Winter Rye, La Crosse Seed, La Crosse, WI) cover crop was drilled at 67 kg ha^{-1} (Smith et al., 2019) in the fall following harvest of the previous crop at a 3.2 cm seeding depth and 19 cm row-spacing (Table 1). Tillage was conducted in tillage treatments using a chisel-plow in the fall and field cultivator in the spring prior to crop planting. All experiments were broadcast fertilized annually in the spring, prior to crop establishment, according to Wisconsin fertilizer recommendations for optimum nitrogen, phosphorus, and potassium (Laboski and Peters, 2012). Fertilizer rates were calculated based on the requirements of soil managed with no-till for each experiment and the same rate was used for all treatments within an experiment. Arlington corn experiments were fertilized in the spring prior to planting with 336 kg ha^{-1} of dry urea (46-0-0) in 2019, and 364 kg ha^{-1} of dry urea and 224 kg ha^{-1} potash (0-0-60) in 2020. The Arlington soybean experiment in 2020 was fertilized prior to planting with 280 kg ha^{-1} potash (0-0-60). Lancaster corn experiments were fertilized in the spring prior to planting with 292 kg ha^{-1} dry urea with a nitrogen stabilizer (46-0-0) and 280 kg ha^{-1} of a dry phosphorus/potassium fertilizer blend (4-19-38) in 2019, and 292 kg ha^{-1} dry urea with a nitrogen stabilizer (46-0-0) and 295 kg ha^{-1} of a dry phosphorus/potassium fertilizer blend (4-19-38) in 2020. Lancaster soybean experiments were fertilized with 295 kg ha^{-1} of a dry phosphorus/potassium fertilizer blend (4-19-38) in the spring prior to planting in 2019 and 2020. Fertilizer sources used varied due to differences in availability at each location.

Glyphosate (1,262 g a.e. ha^{-1}, Roundup PowerMAX, Bayer Crop Science, St. Louis, MO) with ammonium sulfate (AMS) as an adjuvant (1% v/v) was used for chemical termination of all cover crop soil treatments and to control emerged weeds at the time of crop planting in no-till treatments. In the forage harvest treatment, aboveground biomass was harvested and removed from the plot area immediately before cash crops were planted. Glyphosate was applied for cover crop termination 2 weeks prior to crop planting in the early termination treatment, immediately after crop planting in the plant termination treatment, immediately after forage harvest and crop planting in the forage harvest treatment, and 2 weeks after crop planting in the late termination treatment. Glyphosate was not applied at the time of crop planting in the tillage treatments because established weeds were killed prior to crop planting with tillage. Crops were planted at 3.8 cm depth using a row-crop planter with 76-cm row-spacing (4 crop rows per plot). The PRE herbicides for each crop were applied immediately after crop planting to treatments that included a PRE herbicide. In corn, a PRE herbicide mix containing bicyclopyrone, mesotrione, and *S*-metolachlor (45, 179, and 1,604 g a.i. ha^{-1}, respectively; Acuron Flexi, Syngenta, Greensboro, NC) was used for treatments that included a PRE herbicide. In soybean, a PRE herbicide mix containing sulfentrazone and metribuzin (202 and 303 g a.i. ha^{-1}, respectively; Authority MTZ, FMC Corporation, Philadelphia, PA) was used for treatments that included a PRE herbicide. Herbicide applications for cover crop termination, no-till treatment burndown, and PRE herbicides were made using a CO_2 pressurized backpack sprayer and 3-m long boom with six TTI110015 nozzles (Spraying Systems Co., Wheaton, IL) calibrated at 140 L ha^{-1}. The POST herbicides were applied approximately 30 days after crop planting. For the POST application in corn, glyphosate (1,261.6 g a.e. ha^{-1}, Roundup PowerMAX, Bayer Crop Science, St. Louis, MO) plus dicamba and diflufenzopyr (140.2 and 56.1 g a.e. ha^{-1}, respectively; Status, BASF Corporation, Florham Park, NJ) with AMS (1% v/v) as an adjuvant were applied. For the POST application in soybean, glyphosate (1,261.6 g a.e. ha^{-1}, Roundup PowerMAX, Bayer Crop Science, St. Louis, MO) plus dicamba (558.9 g a.e. ha^{-1}, Xtendimax, Bayer Crop Science, St. Louis, MO) with a drift reducing agent (Intact, 0.5% v/v, Precision laboratories, Waukegan, IL) and a water conditioner (FS Certin, 1% v/v, GROWMARK FS, Bloomington, IL) were applied. A tractor mounted sprayer was used to apply POST herbicides for all treatments with a 6.1-m boom equipped with 12 TTI11003 nozzles (Spraying Systems Co., Wheaton, IL) calibrated to deliver 140 L ha^{-1} of spray solution.

Data collection

Monthly weather data (mean temperature and total precipitation) were collected from on-site weather stations. Normal (30-year) average temperature and total precipitation for each month (1988-2018) were estimated using historical daily weather data for 1 km grids at each field site using R software (*daymetr* package) (Thornton et al., 2016; Correndo et al., 2021).

Three subsamples of aboveground cereal rye biomass were collected at termination using a 0.09 m^2 quadrat placed randomly between the center rows of each plot not treated with a PRE herbicide to assess differences in cereal rye growth among termination timings. Biomass collection for plots treated with PRE herbicide were also sampled using the same sampling strategy to determine if PRE herbicide applied at crop planting influenced cereal rye biomass in the late termination treatment. All cereal rye subsamples were combined into a composite sample per plot, and forced air dried at 50°C for two weeks until constant dry biomass was achieved before being weighed.

Weed biomass, weed density, and visual estimation of overall weed ground cover between the center rows of each plot (0-100%, with 0% = absence of weed ground cover and 100% = complete ground coverage from weeds) were collected 28 days after crop planting to assess the level of weed infestation and control prior to POST herbicide application. However, weed biomass and density data were deemed impractical for analysis due to inconsistent weed pressure based on sampling size (2 random subsamples from each plot with a 0.09 m^2 quadrat). Therefore, visual estimation of weed ground cover was used to represent overall weed pressure in this study. It is suggested future researchers consider using a larger quadrat to obtain representative samples of sparse weed pressure. This sampling method, weed ground cover, was used for assessing weed pressure since populations of weed species were unevenly distributed and occurring at low frequency within studies (Teasdale et al., 2004). At Arlington, weed species present at the time of sampling included common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters (*Chenopodium album* L.), dandelion (*Taraxacum officinale* F.H. Wigg), yellow foxtail (*Setaria pumila* (Poir.) Roem. & Schult.), and giant foxtail (*Setaria faberi* Herrm.). At Lancaster, weed species present at the time of sampling included common lambsquarters, dandelion, waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), redroot pigweed (*Amaranthus retroflexus* L.), giant ragweed (*Ambrosia trifida* L.), purslane speedwell (*Veronica peregrina* L.), shepherd's-purse (*capsella bursa-pastoris* L. Medik.), eastern black nightshade (*Solanum ptychanthum* Dunal), hairy galinsoga (*Galinsoga quadriradiata* Cav.), yellow foxtail, and giant foxtail.

Crop grain mass and moisture content were measured from the center two rows of each plot at crop physiological maturity to assess crop productivity. A Gleaner K2 (AGCO Corporation, Duluth, GA) was used to harvest corn and an ALMACO SPC40 (ALMACO, Nevada, IA) was used to harvest soybean. Grain mass was standardized to 15.5% moisture for corn and 13% moisture for soybean.

Statistical analyses

Statistical analyses were performed using R software (version 4.1.1). A linear mixed model (*lme4* package) was fit to cereal rye biomass and crop yield data while a generalized linear mixed model with Template Model Builder with a beta distribution and logit link (*glmmTMB* package) was fit to weed ground cover data. The linear mixed model assumptions for a normal distribution and homogeneity of variance of residuals were assessed prior to analysis, and data for overall cereal rye biomass were log transformed to better meet assumptions of a normal distribution and homogeneity of variance. Data for each crop at each location were pooled between years and represented by a 'site' variable to test for differences between the two growing environments with distinct weed communities and soil type (Arlington versus Lancaster). Models were analyzed using ANOVA (anova function, *car* package for linear mixed models; Anova.glmmTMB function, *glmmTMB* package for generalized linear mixed models). Response variables were analyzed with separate models for each crop. In models for overall cereal rye biomass, weed ground cover, and crop yield, the experimental treatments and site were treated as fixed effects while block nested within site-year was treated as a random effect. Means were separated using the Dunn-Sidák correction (*emmeans* package) when interactions or fixed effects were significant ($P < 0.05$). When interactions involved site, separate models were fit to the data for each site with the experimental treatment that was a part of the interaction included as a fixed effect and replication nested within year as a random effect. Means were separated when the experimental treatment was significant in ANOVA ($P \leq 0.05$).

A linear mixed model was also used to assess if the PRE herbicide was associated with reduced cereal rye biomass in the late termination treatment. Separate models were fit to data for each crop that included PRE herbicide treatment and site as fixed effects and block nested within site-year as a random effect. ANOVA (*car* package) was performed on these models and means were separated using the Dunn-Sidák correction for significant interactions and fixed effects ($P \leq 0.05$).

Results

Weather

Total precipitation accumulated over the growing season (April-October) trended above the 30-year normal for Arlington 2019, Lancaster 2019, and Lancaster 2020 (Table 2). Precipitation trended lower than the 30-year normal for Arlington 2020. Average temperature across the growing season trended below the 30-year normal at both sites for 2019 and 2020.

TABLE 2 Monthly accumulated precipitation and average temperature during the growing season at the Arlington and Lancaster Ag Research Stations in WI.

Month	Arlington						Lancaster					
	Precipitation			Average Temp.[a]			Precipitation			Average Temp.[a]		
	2019	2020	Normal	2019	2020	Normal	2019	2020	Normal	2019	2020	Normal
	———-mm———-			———-°C———-			———-mm———-			———-°C———-		
April	77	37	111	7.5	6.0	7.4	60	37	110	7.9	5.3	8.3
May	172	113	118	12.6	12.9	14.1	143	139	121	12.9	13.6	14.7
June	141	110	148	18.6	20.1	19.5	119	198	152	19.3	16.3	20.1
July	118	142	114	22.7	22.3	21.6	161	131	129	22.8	28.0	22.1
August	153	97	118	18.9	19.7	20.5	81	94	118	19.7	22.0	21
September	146	76	94	17.6	14.3	16.2	472	186	102	18.3	18.7	16.7
October	158	111	80	7.2	6.2	9.2	130	93	78	9.9	4.6	9.7
Season[b]	965	686	783	15.0	14.5	15.5	1165	879	810	15.8	15.5	16.1

[a] Temp., Temperature

[b] Season, sum of precipitation or average temperature for the entire growing season

Monthly normal data collected from 1988-2018 (Thornton et al., 2016; Correndo et al., 2021).

Cover crop biomass

Timing of termination was significant in the models for cereal rye planted ahead of corn ($P = 2.0x10^{-16}$) and soybean ($P = 2.0x10^{-16}$). Site was also significant for cereal rye planted ahead of soybean ($P = 4.0x10^{-3}$) but not for corn (P = 0.14). For cereal rye planted ahead of corn, biomass increased between each termination timing, with 0.26 Mg ha^{-1}, 0.59 Mg ha^{-1}, and 4.08 Mg ha^{-1} for early termination, plant termination/forage harvest, and late termination treatment timings, respectively (Table 3). For cereal rye planted ahead of soybean, biomass increased between each termination timing, with 0.17 Mg ha^{-1}, 0.30 Mg ha^{-1}, and 2.59 Mg ha^{-1} for early termination, plant termination/forage harvest, and late termination treatment timings, respectively. Also, an average biomass accumulation of 0.40 Mg ha^{-1} was found at Arlington compared to 0.64 Mg ha^{-1} at Lancaster for cereal rye planted ahead of soybean.

The PRE herbicide treatment did not impact biomass accumulation for the late termination of cereal rye in either crop (Corn P = 0.54; Soybean P = 0.37). Average biomass for the cereal rye established in corn was 4.59 Mg ha^{-1} (SE ± 1.13) for treatments without a PRE herbicide and 4.92 Mg ha^{-1} (SE ± 1.13) for treatments with a PRE herbicide. Average biomass for the cereal rye established in soybean was 2.83 Mg ha^{-1} (SE ± 0.69) for treatments without a PRE herbicide and 2.45 Mg ha^{-1} (SE ± 0.69) for treatments with a PRE herbicide.

Weed ground cover

In corn, the interaction between soil management treatment and PRE herbicide treatment was significant ($P = 7.0x10^{-7}$), so means for all treatment combinations were compared. Site was not significant (P = 0.71). For treatment combinations without a

TABLE 3 Cereal rye cover crop aboveground biomass for corn (left) and soybean (Right) experiments conducted at Arlington and Lancaster Agricultural Research Stations in 2019 and 2020 Wisconsin growing seasons.

Corn					Soybean				
ANOVA[a]		Biomass			ANOVA[a]		Biomass		
			Mg ha^{-1}	± SE				Mg ha^{-1}	± SE
Termination	***	Early	0.26c	0.07	Termination	***	Early	0.17c	0.06
		Plant	0.59b	0.15			Plant	0.30b	0.11
		Late	4.08a	1.12			Late	2.59a	0.98
Location	ns	Arlington	0.75a	0.19	Location	**	Arlington	0.40b	0.14
		Lancaster	0.97a	0.24			Lancaster	0.64a	0.23

[a] significance code: P > 0.05 'ns' (non-significant), P < 0.05 '*', P < 0.01 '**', P < 0.001 '***'.

The termination timing corresponds to 'early' cover crop termination approximately 14 days prior to crop planting, 'plant' termination timing at the time of cash crop planting, and 'late' termination approximately 14 days after cash crop planting. Means between each terminations timing and locations were separated using the Dunn-Sidák correction when fixed effects were significant in ANOVA. Termination timings or locations with similar letters are not significantly different from each other at α=0.05.

PRE herbicide, the late termination (7% cover) treatment had less weed ground cover compared to all other treatments followed by the plant termination (23% cover), forage harvest (29% cover), no-till (38% cover), early termination (49% cover), and tillage (53% cover) treatments (Figure 1). Compared to the tillage treatment, weed ground cover was less in plant termination and forage harvest treatments when no PRE herbicide was used. When a PRE herbicide was used, weed ground cover was lower for all soil management treatments (except for the late termination treatment) compared to no PRE (Figure 1). When a PRE herbicide was used, weed ground cover was similar between the tillage (7% cover), no-till (7% cover), early termination (7% cover), plant termination (7% cover), forage harvest (7% cover), and late termination (6% cover) treatments.

In soybean, there was a two-way interaction between soil management treatment and location (P = 0.03), as well as a two-way interaction between soil management and PRE herbicide treatment (P = $1.9\text{x}10^{-4}$) (Figure 2). A separate model was fit to data for each location testing for differences in soil management. Soil management influenced weed cover at both Arlington (P = 0.02) and Lancaster (P = $9.9\text{x}10^{-3}$). At Arlington, soil management with the late termination treatment (9% cover) had lower weed ground cover compared to the tillage (27% cover), early termination (25% cover), and forage harvest (26% cover) treatments (Figure 2). Ground cover was similar between the tillage, no-till (21% cover), early termination, plant termination (21% cover), and forage harvest treatments. At Lancaster, weed ground cover for the late termination (8% cover) soil management treatment was lower than the no-till (22% cover) treatment, while ground cover was similar between the forage harvest (11% cover), plant termination (12% cover), early termination (16% cover), no-till, and tillage (20% cover) treatments.

To explore the soil management treatment x PRE herbicide treatment interaction in soybean, means were compared across all soil management by PRE herbicide treatment combinations. When no PRE herbicide was used, weed ground cover was lower in the late termination (6% cover) soil management treatment compared to all other treatments (Figure 3). There was more weed ground cover in the tillage (43% cover) treatment compared to the plant termination (20% cover) and forage harvest (20% cover) treatments, while ground cover was similar between the no-till (25% cover), early termination (32% cover), plant termination, and forage harvest treatments with no PRE herbicide (Figure 3). When a PRE herbicide was used, weed ground cover was similar between the tillage (9% cover), no-till (12% cover), early termination (10% cover), plant termination (10% cover), forage harvest (13% cover), and late termination (5% cover) treatments.

Crop yield

For corn yield, there was a two-way interaction between site and soil management treatment (P = $8.6\text{x}10^{-6}$) while PRE herbicide treatment had no effect on yield (P = 0.11). Individual models for each site were then fit to data to

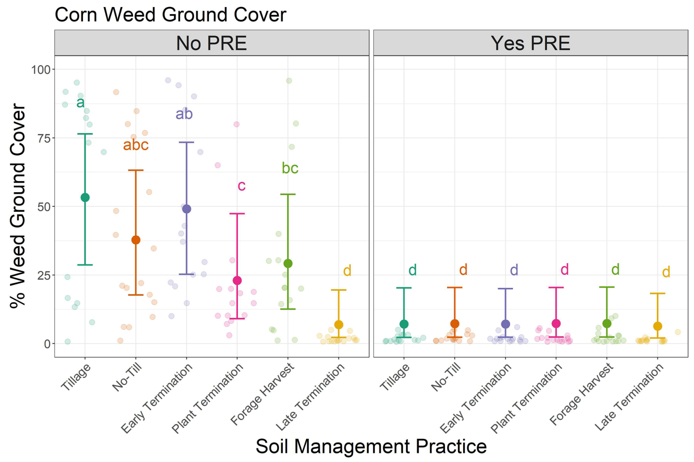

FIGURE 1
Visual estimation of % weed ground cover 28 days after pre-emergence herbicide (PRE) application in corn for pooled data across Arlington and Lancaster experiment locations in 2019 and 2020. Herbicide treatments included exclusion (No PRE) and inclusion (Yes PRE) of a PRE. Soil management practices include conventional tillage ('tillage'), no-till ('no-till'), cereal rye cover crop early termination 14 days before crop planting ('early termination'), cereal rye plant termination ('plant termination'), cereal rye forage harvest termination ('forage harvest') at the time of crop planting, and cereal rye late termination 14 days after crop planting ('late termination'). Based on ANOVA results, means were separated using the Dunn-Sidák correction for all soil management x PRE herbicide treatments. Jittered points represent actual data, centered solid points represent means, and error bars represent 95% confidence intervals. Treatments with similar letters are not different at α=0.05.

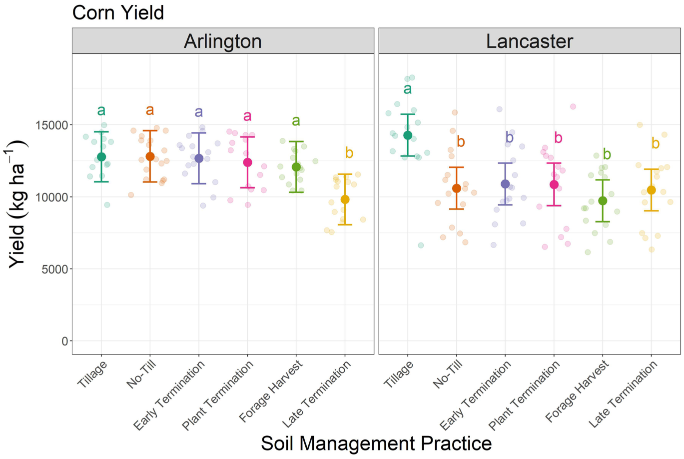

FIGURE 2
Visual estimation of % weed ground cover 28 days after planting in soybean for data pooled between pre-emergence herbicide treatments levels at Arlington and Lancaster experiment locations in 2019 and 2020. Soil management practices include conventional tillage ('tillage'), no-till ('no-till'), cereal rye cover crop early termination 14 days before crop planting ('early termination'), cereal rye plant termination ('plant termination'), cereal rye forage harvest termination ('forage harvest') at the time of crop planting, and cereal rye late termination 14 days after crop planting ('late termination'). Based on ANOVA results, means were separated using the Dunn-Sidák correction for all soil management x PRE herbicide treatments. Jittered points represent actual data, centered solid points represent means, and error bars represent 95% confidence intervals. Treatments with similar letters are not different at α=0.05.

evaluate if soil management influenced yield at each site. Yield was influenced by soil management at both Arlington (P = 7.1x10^{-12}) and Lancaster (P = 1.0x10^{-5}). At Arlington, soil management in the late termination treatment (9.82 Mg ha^{-1}) had reduced yield compared to the forage harvest (12.07 Mg ha^{-1}), plant termination (12.39 Mg ha^{-1}), early termination (12.67 Mg ha^{-1}), no-till (12.81 Mg ha^{-1}), and tillage (12.78 Mg ha^{-1}) treatments (Figure 3). At Lancaster, the tillage (14.28 Mg ha^{-1}) treatment had greater yield compared to the late termination (10.47 Mg ha^{-1}), forage harvest (9.72 Mg ha^{-1}), plant termination

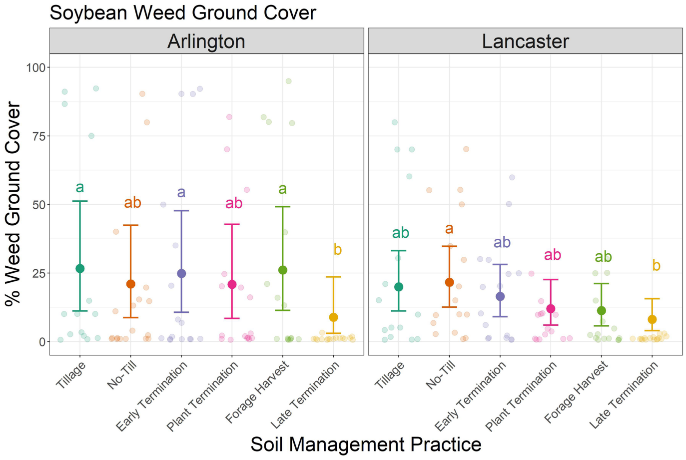

FIGURE 3
Visual estimation of % weed ground cover 28 days after pre-emergence herbicide (PRE) application in soybean for pooled data across Arlington and Lancaster experiment locations in 2019 and 2020. Herbicide treatments included exclusion (No PRE) and inclusion (Yes PRE) of a PRE. Soil management practices include conventional tillage ('tillage'), no-till ('no-till'), cereal rye cover crop early termination 14 days before crop planting ('early termination'), cereal rye plant termination ('plant termination'), cereal rye forage harvest termination ('forage harvest') at the time of crop planting, and cereal rye late termination 14 days after crop planting ('late termination'). Based on ANOVA results, means were separated using the Dunn-Sidák correction for all soil management x PRE herbicide treatments. Jittered points represent actual data, centered solid points represent means, and error bars represent 95% confidence intervals. Treatments with similar letters are not different at α=0.05.

(10.86 Mg ha^{-1}), early termination (10.89 Mg ha^{-1}), and no-till (10.60 Mg ha^{-1}) treatments (Figure 4).

For soybean yield, the site fixed effect was significant (P = $4.7x10^{-7}$), while soil management treatment (P = 0.12) and PRE herbicide treatment (P = 0.16) were found to not influence yield. The average soybean yield was 3.44 Mg ha^{-1} at Arlington and 4.77 Mg ha^{-1} at Lancaster (Figure 5).

Discussion

Cover crop biomass

Biomass produced by the cereal rye cover crop remained relatively low (< 0.3 Mg ha^{-1}) for the early and at plant cover crop termination timings, with biomass accumulation increasing drastically between the plant termination/forage harvest and late termination treatments for both corn and soybean phases of the rotation (Table 3). Despite herbicide injury on cereal rye leaf foliage from the soybean PRE herbicide (Grint, personal field observation), cereal rye biomass for the late termination treatment was not affected in either crop. There was no visual injury on cereal rye leaf foliage from the corn PRE herbicide (Grint, personal field observation). Future research should be conducted to assess the effects of more PRE herbicide products on cover crop growth to support farmers in selecting PRE herbicides that have effective activity on weeds when applied to a living cover crop while still allowing for continued cover crop growth if later termination is desired to increase the amount of cover crop biomass accumulated and the likelihood of achieving subsequent cover crop benefits. Other studies conducted in Wisconsin have reported similar cereal rye biomass production to that reported here with 0.28-3.44 Mg ha^{-1} with termination in early May and 2.30-6.00 Mg ha^{-1} for termination at the rye boot growth stage (West et al., 2020). This study would have benefited from detailed data on cereal rye growth stage at the time of cover crop termination. Previous research conducted in Wisconsin organic soybean production with a greater cereal rye seeding rate and delayed cereal rye termination until after the boot growth stage, indicates that there is potential to produce cereal rye biomass of 4.30-10.80 Mg ha^{-1} (Bernstein et al., 2011; Vincent-Caboud et al., 2019). Greater amounts of cereal rye biomass accumulation prior to crop planting have been observed in previous studies with 2.05-3.25 Mg ha^{-1} in Maryland (Otte et al., 2019), 7.39 Mg ha^{-1} in Virginia (Pittman et al., 2020), 4.97 Mg ha^{-1} in South Carolina (Norsworthy, 2004), 2.20-6.10 Mg ha^{-1} in Illinois (Ruffo et al., 2004), and 1.15-2.89 Mg ha^{-1} in Missouri (Cornelius and Bradley, 2017). The warmer spring growing conditions at these locations are favorable for a cereal rye cover crop to accumulate more biomass prior to the typical time of crop planting when compared to covers grown in the Upper-Midwest U.S. (Kukal and Irmak, 2018). Years of reduced or delayed cover crop biomass production from unfavorable fall and/or spring climate are common in the Midwest U.S. (Krueger et al., 2011; Pantoja et al., 2015; West et al., 2020), and should be considered when planning for an early cereal rye termination in this region. Cover crop benefits, such as weed suppression, have been shown to be influenced by the amount of cover crop biomass produced. According to a meta-analysis from corn-soybean studies

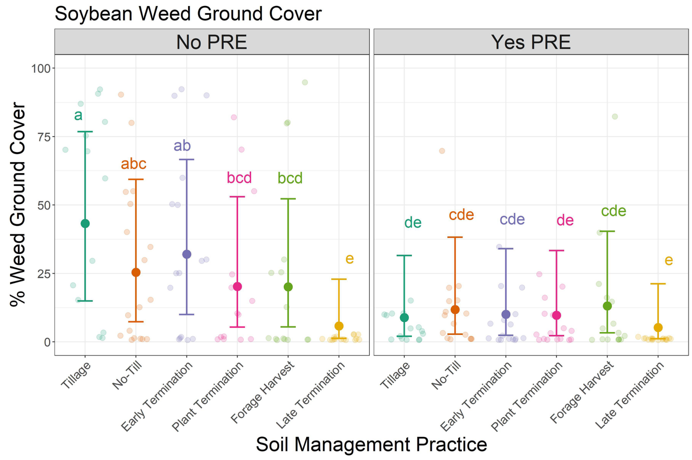

FIGURE 4
Corn grain yield for pooled data across pre-emergence herbicide treatment levels at Arlington and Lancaster experiment locations in 2019 and 2020. Soil management practices include conventional tillage ('tillage'), no-till ('no-till'), cereal rye cover crop early termination 14 days before crop planting ('early termination'), cereal rye plant termination ('plant termination'), cereal rye forage harvest termination ('forage harvest') at the time of crop planting, and cereal rye late termination 14 days after crop planting ('late termination'). Jittered points represent actual data, centered solid points represent means, and error bars represent 95% confidence intervals. Treatments with similar letters are not different at α=0.05.

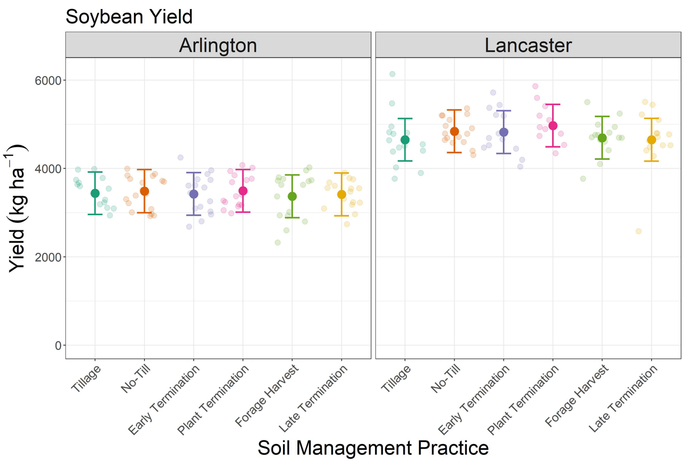

FIGURE 5
Soybean yield for pooled data across pre-emergence herbicide treatment levels at Arlington and Lancaster experiment locations in 2019 and 2020. Soil management practices include conventional tillage ('tillage'), no-till ('no-till'), cereal rye cover crop early termination 14 days before crop planting ('early termination'), cereal rye plant termination ('plant termination'), cereal rye forage harvest termination ('forage harvest') at the time of crop planting, and cereal rye late termination 14 days after crop planting ('late termination'). Jittered points represent actual data, centered solid points represent means, and error bars represent 95% confidence intervals. Yield was similar between all treatments at each location.

conducted in the Midwest U.S., at least 5 Mg ha^{-1} of cover crop biomass is needed for 75% reduction in weed biomass (Nichols et al., 2020). The average amount of cereal rye biomass produced in this study was below this threshold. Future research should be conducted to evaluate whether the amount of cereal rye cover crop biomass necessary to effectively suppress diverse weed communities at varying infestation levels change across geographies.

Weed control

Soil management impacted weed ground cover in both the corn and soybean phases of this study. When no PRE herbicide was used in corn, the lowest weed ground cover was in the late termination treatment while weed ground cover observed for the plant termination and forage harvest treatments lower than the tillage treatment (Figure 1). In soybean, lower weed ground was consistently observed in the late termination treatment compared to the tillage treatment at both locations (Figure 2). When no PRE herbicide was used in soybean, lower weed ground cover was observed for the late termination treatment compared to all other soil management treatments, while weed ground cover observed for the plant termination and forage harvest treatments was lower than tillage treatment (Figure 3). The inconsistent occurrence and variable density of weed species within experiments was limiting in this study to assess potential weed community impacts from soil management, however previous research conducted in Wisconsin observed greater weed biomass for soil management with tillage compared to a cereal rye cover crop in soybean (Bernstein et al., 2014) and greater density of common lambsquarters in chisel-plow corn compared to no-till soybean (Drewitz and Stoltenberg, 2018). Common lambsquarters was the most consistently observed species, however weed ground cover was not different between the tillage and no-till treatments in this study (Figures 1–3). Soil management that resulted in greater cereal rye biomass production such as occurred with the late, plant, and forage harvest cover crop termination timings/methods had lower weed ground cover compared to soil management with tillage in both crops (Figures 1–3). It is important to acknowledge that the action of chemical cover crop termination with glyphosate in this study likely contributed to weed control of emerged weeds for each termination timing thus impacting weed ground cover. Increased levels of cereal rye biomass accumulation have been shown to increase weed suppression from a cereal rye cover crop by delaying emergence of weeds, reducing emergence of weed seedlings, and reducing the amount of weed biomass (Mohler and Teasdale, 1993; Yenish et al., 1996; Bernstein et al., 2014; Cornelius and Bradley, 2017; Werle et al., 2017; Pittman et al., 2020). In addition, the cereal rye biomass from the late termination treatments was observed to persist longer into the growing season, and cereal rye residue from 2019 could still be found in the plots of the late termination treatments during the spring of 2020 (Grint, personal observation). As cereal rye matures, the carbon to nitrogen ratio has been shown to increase, prolonging the persistence of residue from delayed cereal rye cove crop terminations (Poffenbarger et al., 2015). The combination of increased cereal rye biomass and prolonged cereal rye residue persistence are ideal for weed suppression (Pittman et al., 2020).

Treatments containing a PRE herbicide often had lower weed ground cover in corn and soybean. In corn, there was lower weed ground cover for treatments combinations containing a PRE herbicide for all soil management treatments except for the late termination treatment, indicating that the use of a PRE herbicide did not provide additional control only when delayed termination of a high biomass cereal rye cover crop was implemented (Figure 1). In soybean, tillage and early termination treatment combinations which included a PRE herbicide had lower weed ground cover compared to tillage and early termination treatment combinations without a PRE herbicide (Figure 3). Previous research indicates the use of a PRE herbicide improves weed control when soil is managed with use of tillage, no-till, or a cover crop and can contribute to greater crop yield when weed pressure is high (Yenish et al., 1996; Reddy et al., 2003). A cereal rye cover crop has been shown to provide a similar level of weed suppression compared to use of a PRE herbicide in some cases and to reduce late season weed biomass when no PRE herbicide is used compared to soil management with tillage (Yenish et al., 1996). Previous research suggests that the use of a cover crop alone for early season weed suppression has the potential to increase selection pressure for resistance to POST herbicides by increasing the density of summer annual weeds after initial suppression (Bunchek et al., 2020). Our results do not support this suggestion, still the use of a cereal rye cover crop to reduce selection pressure for herbicide resistance should consider the influence of a cover crop on weeds prior to all potential herbicide applications. This study would have benefited from data on weed species, weed size, and weed density at the time of POST herbicide application to better assess how the interaction of PRE herbicide use with specific soil management strategies affects the risk for resistance to POST herbicides. The integration of a cover crop into a crop production system has the potential to reduce selection pressure for herbicide resistance by reducing weed size and density if long-term suppression occurs, but more research is needed to assess best management of a cereal rye cover crop used in conjunction with herbicides for weed control while reducing the selection pressure for herbicide resistance. Future research that

Crop yield

Different yield results between soil management practices were observed in corn phases between locations. At Arlington, corn yield was lower in the late termination treatment compared to all other soil management treatments (Figure 4). At Lancaster, corn yield was greater in the tillage treatment compared to all other treatments. Previous studies in Wisconsin have observed no difference in corn grain yield in a corn-soybean rotation between soil management with no-till and tillage (Pedersen and Lauer, 2003; Mourtzinis et al., 2017). No difference in corn yield between soil management with no-till and an early terminated cereal rye cover crop in this study contradicts research conducted in Iowa which reported a reduced corn yield when cereal rye was terminated approximately 7 days prior to corn planting (Pantoja et al., 2015). Similar corn yield between soil management with a cereal rye cover crop terminated 14 days before planting and cereal rye cover crop terminated at the time of crop planting in this study contradicts research in Iowa which found reduced corn yield when rye termination occurred < 10 days before crop planting (Acharya et al., 2017). Reduced corn grain yield in the late termination treatment at Arlington is supported by research conducted in Maryland, where greater amounts of cereal rye biomass production reduced corn grain yield compared to no cover crop, and Iowa, where termination 12 days after planting reduced corn grain yield (Otte et al., 2019; Acharya et al., 2022). Previous research assessing the long-term impact of using a cereal rye terminated prior to crop establishment has found no reductions in corn yield (Basche et al., 2016; Snapp and Surapur, 2018). Corn grain yield reduction from a cereal rye could be a result of less available nitrogen early in the growing season. Lower nitrogen content in corn plants has been observed with greater amounts of cereal rye biomass from delayed termination when compared to early termination timings and absence of a cover crop (Otte et al., 2019). Reduced soil nitrate at the time of corn planting with delayed cereal rye termination and nitrogen immobilization during peak timing for corn nitrogen demand have been observed (Crandall et al., 2005; Nevins et al., 2020). It is suspected that slow nitrogen release or nitrogen immobilization following late termination of cereal rye could limit available nitrogen for the corn crop, therefore reducing yield. Another potential cause of yield reduction in corn is cooler and drier soils from delayed cereal rye termination (Reed et al., 2019). Reduction of soil moisture by a cereal rye cover crop has been shown to negatively impact corn yield in semi-arid regions (Rosa et al., 2021). Unfortunately, not enough soil environmental data were collected across treatments in this study (i.e., soil moisture, soil temperature, nutrient availability) to support inferences about location differences that could have explained the lower corn yield in the late cover crop termination at Arlington versus no yield reduction in the same treatment at Lancaster when yield was compared to most of the other soil management treatments. Not enough research funds and labor were available at the time this study was established to collect such data.

Soybean yield remained consistent regardless of soil management practice being used for both locations (Figure 5). Previous research in Wisconsin comparing no-till soil management to tillage has found no yield difference (Mourtzinis et al., 2017), greater yield with tillage (Pedersen and Lauer, 2002; Arsenijevic et al., 2021), or greater yield with no-till (Pedersen and Lauer, 2003). Results from this study match previous research where no soybean yield difference was observed from a cereal rye cover crop terminated prior to crop planting compared to no-till (Pantoja et al., 2015), and no difference in soybean yield observed between a cereal rye termination after crop planting compared to termination prior to planting (Reed et al., 2019). Previous research in Nebraska also observed no difference in soybean yield between soil management with no-till, cereal rye termination prior to planting, termination at the time of planting, and termination 5-7 days after crop planting (McMechan et al., 2020, McMechan et al., 2021). Long-term research indicates soybean yield remains unaffected by use of a cereal rye cover crop when compared to no-till (Basche et al., 2016). Based on the results of this study and previous research, delayed termination of a cereal rye cover crop in soybean cropping systems can improve the potential of achieving weed suppression and other ecological benefits (i.e. reduced soil erosion, carbon sequestration, water infiltration) from cereal rye with less likelihood of reduced farmer income from yield reduction.

Integrated weed management and cropping system resiliency with conservation practices

Herbicides are currently a more efficient technology for achieving reliable weed control in cropping systems when compared to use of a cover crop alone (Yenish et al., 1996; Reddy et al., 2003; Bunchek et al., 2020). However, their effectiveness and wide-spread use results in strong selection pressure for the development of herbicide resistance (Young, 2006; Peterson et al., 2018). Novel cases of herbicide resistance are being reported at an alarming frequency (Heap, 2022), and likelihood of resistance to multiple herbicides is becoming more common with the development of metabolic resistance (Gaines et al., 2020; Han et al., 2021; Shyam et al., 2021). These are major concerns for the future use of herbicides as tools for weed control (Oliveira et al., 2020). Integrated weed management is needed to proactively manage weeds while relieving selection pressure for resistance and protecting future use of herbicides as weed management tools (Norsworthy et al., 2012; Hicks et al., 2018; MacLaren et al., 2020). The use of a cover crop has been shown to provide many environmental benefits to agricultural systems such as reduced soil erosion, reduced nitrate leaching, and improved soil water infiltration. An improved understanding of how best to use these soil management practices with herbicides can benefit integrated weed management and improve ecological sustainability of corn and soybean production systems.

Conclusion

In corn and soybean, delaying termination of a cereal rye cover crop until 14 days after crop planting to increase the amount of the cereal rye residue reduced the presence of weeds 28 days after crop planting when no PRE herbicide was used compared to all other soil management practices, and had similar weed suppression compared to when a PRE herbicide was used for all soil management practices. This finding supports our hypothesis that the addition of a cover crop in treatments without PRE herbicide use was expected to improve weed control with increasing cover biomass accumulation. Soil management with a late terminated cereal rye cover crop had lower corn yield at one site and had no effect on soybean yield at either site. These findings demonstrate delaying cereal rye cover crop termination to increase rye biomass suppressed weeds in Wisconsin and that PRE herbicides are effective for in-season weed control when a high biomass cereal rye cover crop is not present. Adoption of a late terminated cereal rye cover crop in soybean can reduce the presence of weeds with reduced risk of yield loss making this a beneficial practice for farmers to adopt in this region.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://github.com/kgrint/Cover-Crop-and-PRE-Herbicides-Publication.git.

Author contributions

RW designed the study and secured funding for the research. RD established the Arlington, WI studies. RD, KG, and NA managed the Arlington, WI studies. DHS, KG, and NA established and managed the Lancaster, WI studies. KG, MO, and RW analyzed the data. KG, RW, MO, DHS, FA, NA, and DES were involved with the interpretation of results, contributed to writing the original manuscript, and improving subsequent versions. All authors reviewed and approved the submitted article.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Effect of crimson clover on the critical period of weed control in conservation tillage corn

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An increasing number of herbicide-resistant weeds, in addition to troublesome weeds, pose a significant challenge for chemical weed control in corn. Simultaneously, high-biomass cover crop adoption has gained popularity among farmers as an efficient weed control strategy. While the critical period of weed control (CPWC) following conventional tillage has been well documented, there is little knowledge of CPWC following high residue cover crops in corn. A two-year field experiment was conducted to estimate the influence of a high biomass crimson clover cover crop and conservation tillage on the critical period of weed control (CPWC) in corn. The experiment was implemented in a split-plot design in which the main plots were conventional tillage (CVT), conservation tillage following winter fallow (CT + WF), and conservation tillage following crimson clover (CT + CC), and the subplot included multiple durations of weedy plots (estimation of critical timing of weed removal (CTWR), i.e., beginning of weed control) and weed-free plots (estimation of critical weed-free period (CWFP), i.e., end of weed control). The results described that the estimated duration of CPWC in three systems, included CT + CC, CT + WF and CVT equals 2.8 weeks, 3.5 weeks, and 4.9 weeks respectively in 2019. In 2020, the predicted value of CTWR under CT + CC equals 3.8 weeks after planting and the predicted values of CWFP were 5.1 and 5.7 weeks after planting under CT + WF and CVT systems, however, the model did not predict some values within the fitted 8 weeks of time. In conclusion, the presence of a crimson clover cover crop delayed the CTWR and caused the early beginning of CWFP and hence shortened CPWC in 2019. During most of the growing season, weed biomass production was less under CT + CC plots than CVT and CT + WF systems of weedy treatment in both years. While weed biomass production fluctuated in CT + CC, CVT and CT + WF systems in weed-free treatment.

KEYWORDS

critical weed-free period, critical timing of weed removal, conservation tillage, integrated weed management, relative yield, cover crop

1 Introduction

Corn (*Zea mays* L.) is one of the major grain crops cultivated worldwide, with the U.S. leading production globally. Corn has extensive uses, including food products and cooking oil, animal feed, industrial purposes, and ethanol production. Since the late 90s, potential corn yield losses have been increasing due to weed competition from herbicide-resistant and troublesome weed species (Chandler et al., 1984; Vissoh et al., 2004). Integrated weed management approach included the utilization of diverse herbicide modes of action and cover crops to decrease the selection pressure of herbicide resistance and control of glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) in corn (Wiggins et al., 2015). Therefore, the understanding of innovative strategies that reduce growers' reliance on herbicide should be adopted for increased weed control continues to be important. Best management practices to sustain or increase weed control included cultural, mechanical, and biological practices illustrated in the "Herbicide Resistant Weeds" section (Norsworthy et al., 2012). In the southeastern U.S., the adoption of conservation tillage utilizing high residue cover crops is increasing in corn and cotton (*Gossypium hirsutum* L.) production systems due to numerous advantages (Price et al., 2006; Price and Kelton, 2013; Reeves et al., 2005). Among other benefits, cover crops improve soil organic matter, nutrient cycling, and soil water conservation (Holderbaum et al., 1990; Sainju and Singh, 1997; Kaspar et al., 2001). Cover crops, including legumes, inhibit weed seed germination and seedling growth due to physical suppression and through allelopathic properties (Barnes and Putnam, 1983; Chase et al., 1991; Akemo et al., 2000; Teasdale and Mohler, 2000; Price et al., 2006; Price et al., 2008). Moreover, cover crops can also improve the soil's physical, chemical, and biological properties by increasing the soil organic matter content in case of grass cover crops with a higher C:N ratio and, nitrogen availability in case of leguminous cover crop species (Hubbard et al., 2013; Romdhane et al., 2019). The crimson clover (*Trifolium incarnatum*) contained N is an essential source of nitrogen for the succeeding crops. However, the rate of N disappearance was more rapid in conventional tillage than no-tillage system (Wilson and Hargrove, 1986). A study in Alabama suggested that conservation tillage with the utilization of crimson clover decreased the weed biomass and suppress the germination of early season weed species in corn. Further, lowest weed biomass recorded was 36 kg ha^{-1} corresponding to crimson clover biomass of 2453 kg ha^{-1} and the highest was 158 kg ha^{-1} corresponding to crimson clover biomass of 373 kg ha^{-1} (Saini et al., 2006). Hence, with the utilization of crimson clover in conservation tillage, it is necessary to establish the critical period of weed control (CPWC) parameters in an integrated weed management system to further understand cover crop weed suppressive attributes and efficient utilization of chemical herbicides (Swanton and Weise, 1991). Moreover, CPWC information is necessary and can be valuable in making decisions based on the need and timing of weed management (Hall et al., 1992; Van Acker et al., 1993). Also, cover crop seeding, and cultivation timing could be improved based upon CPWC knowledge.

The critical period of weed control (CPWC) is described as a 'window' of weed competition period during the crop growing season in which it is essential to control weeds to maintain crop potential yield (Swanton and Weise, 1991). CPWC has two independent components, including critical timing of weed removal (CTWR), which defines the beginning of the critical period from which weeds must be controlled and the maximum tolerance of the crop to the early emerging weeds without causing any unacceptable yield loss (>5%). While the critical weed-free period (CWFP) describes the end of weed control, to prevent considerable potential yield losses by late-emerging weeds (Knezevic et al., 2002; Williams et al., 2007; Korres and Norsworthy, 2015; Price et al., 2018). Thus, the weed interference duration in weedy plots represented CTWR and the weed-free duration in weed-free plots represented CWFP, with both parameters' length defined by 5% yield loss. Ultimately, weedy plots represented CTWR (beginning of weed control) and weed-free plots represented CWFP (end of weed control) and difference of CWFP and CTWR described the duration of CPWC.

The objective of this research was to evaluate the effect of a high residue crimson clover (*Trifolium incarnatum*) on the critical period of weed control in corn. Therefore, a field study was performed comparing a conservation tillage system with a clover cover crop (CT + CC) managed for maximum biomass, a conservation tillage system with winter fallow (CT + WF), and a conventional tillage (CVT) system on the CPWC.

2 Materials and methods

2.1 Location site

Field experiments were conducted in 2019 and 2020 at the E.V. Smith Research Center Field Crops Unit (32.4417° N, 85.8974° W) Shorter, Alabama. The soil characteristics at the research site were sandy loam, (coarse-loamy, siliceous, sub-active, thermic Paleudults) with pH 6.2 and 0.8% organic matter. The average temperature ranged from 18.1°C to 27.6°C and precipitation was 8.26 mm to 1.25 mm from April to August 2019. In 2020, the average temperature ranged from 17.27°C to 26.98°C and precipitation was 2.03 mm to 3.37 mm from April to August.

2.2 Experimental design

The study was conducted in a split-plot design with four replications. As previously stated, the three systems i.e., conservation tillage with a crimson clover cover crop (CT + CC), conservation tillage with winter fallow (CT + WF), and conventional tillage (CVT), were considered in the main plots. The durations of weedy plots described the beginning of weed removal (CTWR), and the durations of weed-free plots illustrated the end of weed control (CWFP). Hence, these durations in weedy and weed-free plots from 0 to 8 weeks after planting were considered in subplots.

2.3 Cover crop management and corn establishment

Crimson clover cultivar "Dixie" was seeded at a rate of 22.4 kg ha^{-1} using a grain drill. Termination of crimson clover was accomplished using a roller-crimper (Ashford and Reeves, 2003) followed by an application of glyphosate (Roundup Powermax®, Monsanto Company, St. Louis, MO) plus glufosinate (Liberty®, Bayer Crop Science, Research Triangle Park, NC) herbicides sprayed at the rate of 841 g ae ha^{-1} and 492 g ae ha^{-1} respectively. Within all plots, a KMC 4-row parabolic subsoiler (Kelly Manufacturing Company, Tifton, GA) was used to disrupt naturally occurring hard pans found at this location before planting corn in all treatments to prevent deep-tillage interaction. Subsequently, CVT plots were cultivated using three disks, and two field cultivator passes. Corn (Pioneer® 1197 YHR) was planted using a precision planter with the population set at 12950 seeds ha^{-1} on April 16, 2019, and April 27, 2020, respectively. A starter application of nitrogen, phosphorus, and potassium (NPK) fertilizer was applied at a rate of 45 kg ha^{-1} after planting corn. A tank mixture of glyphosate plus acetochlor (Warrant, Monsanto Company, St. Louis, MO) herbicide sprayed at the rate of 841 g ae ha^{-1} and 1682 g ae ha^{-1}, respectively, followed by hand hoeing, was utilized for weed control in a weed-free period and after weedy intervals using TDI 11004 nozzles. The corn was harvested on August 19, 2019, and August 27, 2019.

2.4 Data collection

Crimson clover biomass samples were collected randomly from a 0.25 m^2 area per plot before termination. The collected samples were placed in a forced air drier for 72 h at 65°C, and then the weight was recorded. Weed biomass was collected from a randomly selected 0.25 m^2 quadrat from weedy plots (CTWR) immediately before applying herbicides. For example, W2, i.e., two weeks weedy; herbicides sprayed at two weeks after planting and weed biomass collected just before application. Additionally, weed biomass collected once at the end of the growing season in the weed-free duration plots. Weed species inside the randomly selected area were cut at the soil surface, placed in a forced air drier for 72 h at 65°C, and then weighed.

2.5 Critical period for weed control estimation

A sigmoidal logistic model was fitted for the weedy periods (i.e., CTWR), while the Gompertz model was fitted for the weed-free periods (i.e., CWFP) in each winter fallow (CT + WF), conventional tillage (CVT), and cover crop treatments (CT + CC). The inverse prediction method applied at 95% relative yield to estimate the CTWR and CWFP (i.e., weeks on the x-axis). The estimation of CPWC components were the next steps under which there were not a relative yield reduction greater than 5%, as the acceptable yield losses (AYL) were considered at 5% for both curves Gompertz and logistic as described by Knezevic et al. (2002); Blankenship et al. (2003), and Price et al. (2018). Regression of relative yield was performed as a function of time for both CTWR and CWFP, and then nonlinear regression models were fitted to assess the CPWC, as illustrated by Knezevic et al. (2002); Williams et al. (2007) and Korres and Norsworthy (2015).

For the weedy periods to estimate CTWR, a logistic model with three parameters was fitted to relative corn yield under all three treatments.

$$y = \frac{\alpha}{1 + e^{-\left(\frac{x-x_0}{b}\right)}} \qquad \text{[Equation 1]}$$

Moreover, for the weed-free periods to evaluate CWFP, a Gompertz model with three parameters was fitted to relative corn yield under all three treatments.

$$y = \alpha\, e^{-e^{-\left(x-x_0|b\right)}} \qquad \text{[Equation 2]}$$

Where y is the relative corn yield, α is the asymptote, b is the slope of the curve, x_0 is the point of inflection, and x is time (i.e., weeks after planting).

Hence, the difference between CWFP and CTWR components described the CPWC estimation with a 5% acceptable corn yield loss in CT + CC, CT + WF, and CVT systems. As described previously, weed control experiments estimate the relation between weed interference timings and relative crop yield and then determine the CPWC.

The collected weed biomass was quantified as a function of critical timing of weed removal (CTWR) and the critical weed-free period (CWFP) for each CT + CC, CT + WF, and CVT system using equations 1 and 2 mentioned above, in which y represents weed biomass. A sigmoidal logistic model was fitted for various weed-free periods, while the Gompertz model was fitted for the weedy periods in CT + CC, CT + WF, and CVT systems to assess weed biomass.

2.6 Statistical data analysis

The ANOVA was applied to estimate treatment effects on actual and relative (percentage of long season weed-free period) corn yield data, and means were separated through Fisher's LSD at $\alpha=0.05$. The CPWC was estimated separately for each year due to significant treatments × year interaction. Sigma Plot 14.0 (Systat Software, San Jose, CA) and JMP Pro v. 13 (SAS Institute, Cary, NC) was used for the estimation of ANOVAs, inverse predictions, curve fitting regressions, and significance model parameters. The model parameters were utilized to support the predicted values of an explanatory variable (i.e., type of independent variable) CTWR and CWFP based on the response variable of relative corn yield. Coefficient of determination (R^2) was used to check the fitness of the regression model to the observed data. The comparisons between model parameters were used to evaluate the effect of experimental treatments, including CT + CC, CT + WF, and CVT, on weed biomass production.

3 Results and discussion

3.1 Crimson clover biomass and corn yield

At clover termination, the cover crop biomass was 4,204 kg ha^{-1} and 3,890 kg ha^{-1} in 2019 and 2020, respectively. The average yield following crimson clover was 7,575 kg ha^{-1}, winter fallow 6,478 kg ha^{-1}, and conventional tillage 7,400 kg ha^{-1} in 2019. The average yield following crimson clover was 8,253 kg ha^{-1}, winter fallow 7,224 kg ha^{-1}, and conventional tillage 7,280 kg ha^{-1} in 2020.

3.2 Critical period of weed control

Again, 5% acceptable yield loss (AYL) was considered to estimate the values of CTWR and CWFP as described by Blankenship et al. (2003) and Knezevic et al. (2002). In 2019, the predicted value of CTWR equals 2.5, 2.8, and 1.5 weeks after planting (WAP) for CT + CC, CT + WF and CVT systems, respectively (Figure 1 and Tables 1, 2). In addition, the predicted value of CWFP equals 5.3, 6.3, and 6.4 weeks after planting for CT + CC, CT + WF, and CVT, respectively (Figure 1 and Tables 1, 3). In 2019, based on the predicted values of CTWR for each system individually, the CTWR following the CT + CC system was delayed approximately 1.0 weeks compared with CVT system, while the beginning of CTWR under both CT + WF and CT + WF systems was in between second to third weeks (Figure 1 and Tables 1, 2). Additionally, comparing CT + CC system with CT + WF and CVT systems, the presence of crimson clover caused the early ending of CWFP at about 1.0 and 1.1 weeks respectively. However, the ending of CWFP under CT + WF and CVT systems were almost same during the weeks of 6 WAP.

In 2020, the predicted value of CTWR equals 3.8 WAP for CT + CC system. While the relative yield was above the threshold level of 95% for 8 weeks, so the model did not predict the CTWR value for CT + WF and CVT systems because curves were fitted only for 8 weeks (Figure 1 and Tables 1, 2). Moreover, the predicted values of CWFP equals 5.1, and 5.7 WAP for CT + WF and CVT, respectively, whereas for CT + CC system, the model did not predict the value due to greater than 95% relative yield during most of growing season (Figure 1 and Tables 1, 3). Hence, comparing the CVT system with CT + WF system, conventional tillage and winter fallow had almost same ending period during 5th weeks of timing (Figure 1 and Tables 1, 3).

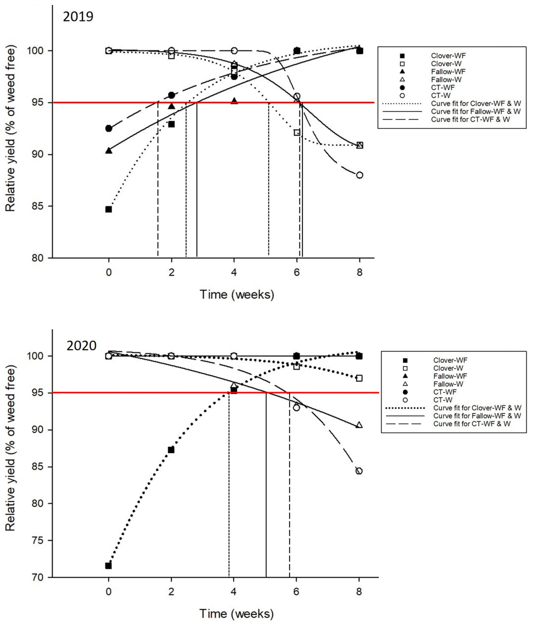

FIGURE 1
The critical period for weed control and its components (critical timing for weed control [CTWR, i.e., weedy] and critical weed-free period [CWFP, i.e., weed free]) for each of the conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020. Point estimates for CTWR and CWFP for CT + CC, CT + WF, and CVT treatments are presented in Tables 1–3. In Figure 1, WF depicted weed-free, and W represented weedy; Fallow referred to winter fallow (CT + WF), clover referred to cover crop (CT + CC), and CT depicted conventional tillage system (CVT).

TABLE 1 The estimation of points (i.e., inverse predictions), standard errors (SE) of inverse predictions, and confidence intervals (CI_{95}) corresponding to a 5% acceptable yield loss for the Logistic and Gompertz models used to estimate the beginning and end of the critical period in 2019 and 2020 for weed control in corn under three different tillage systems.

Model[a]	Tillage system[b]	Inverse prediction	SE	CI_{95} lower	CI_{95} upper
Year 2019					
Logistic (CTWR)	CT + CC	2.5	0.27	1.97	3.04
	CT+ WF	2.8	0.76	1.29	4.27
	CVT	1.5	0.27	0.97	2.03
Gompertz (CWFP)	CT + CC	5.3	0.81	3.68	6.89
	CT+ WF	6.3	0.21	5.93	6.75
	CVT	6.4	0.24	5.94	6.89
Year 2020					
Logistic (CTWR)	CT + CC	3.8	0.19	3.47	4.21
	CT+ WF	–	–	–	–
	CVT	–	–	–	–
Gompertz (CWFP)	CT + CC	–	–	–	
	CT+ WF	5.1	0.54	4.06	6.17
	CVT	5.7	0.43	4.87	6.55

[a] CWFP, critical weed-free period; CTWR, critical timing for weed removal.

[b] CT + CC, conservation tillage following a crimson clover cover crop; CT + WF, conservation tillage following winter fallow; CVT, conventional tillage without a cover crop.

TABLE 2 Statistics of the three-parameter logistic regression model fitted to relative corn yield to estimate the critical weedy period (CTWR) for each of the conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020.

Year 2019	Coefficient	Std error	t value	R^2
Clover				
α	90.89	0.195	61.732	0.997
b	-0.95	-12.339	-12.339	
x_o	5.34	6.732	34.492	
Fallow				
α	90.43	0.450	37.358	0.973
b	-1.317	0.197	-6.689	
x_o	6.438	0.172	16.729	
Conventional				
α	100.00	1.294	17.986	0.992
b	0.65	1.321	2.965	
x_o	6.03	1.956	12.836	
Year 2020	Coefficient	Std error	t value	R^2
Clover				
α	100.25	0.365	274.928	0.986
b	-2.31	0.845	-2.736	

(Continued)

TABLE 2 Continued

Year 2019	Coefficient	Std error	t value	R^2
x_o	15.85	2.748	5.768	
Fallow				
α	108.32	13.428	8.067	0.982
b	-9.91	9.305	-0.958	
x_o	23.22	10.219	2.272	
Conventional				
α	101.12	1.606	62.982	0.988
b	-2.12	0.678	-3.128	
x_o	11.59	1.097	10.564	

TABLE 3 Statistics of the three-parameter Gompertz regression model fitted to relative corn yield to estimate the critical weed-free periods (CWFP) for each conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020.

Year 2019	Parameter	Std error	t value	R^2
Clover				
α	101.07	0.961	105.190	0.992
b	2.24	0.402	5.584	
x_o	-3.66	0.620	-5.906	
Fallow				
α	105.83	12.366	8.558	0.9324
b	6.97	8.206	0.850	
x_o	-12.35	9.533	-1.295	
Conventional				
α	102.08	1.944	52.504	0.983
b	4.52	1.792	2.522	
x_o	-10.23	3.347	-3.055	
Year 2020	Parameter	Std error	t value	R^2
Clover				
α	101.51	0.861	117.873	0.997
b	2.10	0.179	11.704	
x_o	-1.83	0.165	-11.099	
Fallow				
α	100.00	0.00	98.345	0.996
b	0.045	0.045	9.876	
x_o	-3.156	0.00	-2.118	
				(Continued)

TABLE 3 Continued

Year 2019	Parameter	Std error	t value	R^2
Conventional				
α	100.00	0.00	99.877	0.998
b	0.087	0.001	10.036	
x_o	-2.165	0.00	-1.291	

We observed yield loss increased with the extent in time of weed infestation, and Gantoli et al. (2013) reported the same in the estimation of corn CPWC. Although our points of estimated critical period were not exact same among two years because of different weed pressure in two years (Figure 2). Some previous publications indicated that the CPWC differed remarkably when estimated in respect of days after planting or days after germination (Gantoli et al., 2013). Moreover, several corn studies have estimated the critical period of weed control, and there was great variability in the CPWC. The starting of the corn CPWC was more variable (3-14 leaf stage) than the end (14-leaf stage) in Canada (Hall et al., 1992). In contrast, Halford et al. (2001) illustrated that starting of the CPWC was more stable (around 6-leaf stage) than the end period (9-13 leaf stage or 24 to 46 DAE) in corn. Results reported by Evans et al. (2003) described that the starting of CPWC was estimated from germination up to the seven-leaf stage, while the end of the CPWC was estimated from seven-leaf stage up to anthesis in corn crop. A field experiment was conducted in Canada to compare the CPWC between conventional and no-till corn and summarized that the CPWC starting and ending period was earlier under a no-till system than in conventional tillage systems (Halford et al., 2001). In addition, the previous study concluded that the estimated value of CPWC in narrow-row spacing was different than wide rows spacing in corn due to higher competition for late-germinating weeds (Murphy et al., 1996). Thus, high-density corn planted in narrow row spacing would most likely decrease the end of the CPWC (Teasdale, 1998). However, Norsworthy and Oliveira (2004) concluded that there was no significant difference between light interception in narrow and wide row spacing of corn; hence CPWC and competition of late germination weeds were almost the same in these two systems.

3.3 Treatment effects on weed biomass production

The most common and troublesome weed species found in the southeastern United States cropping systems are Palmer amaranth (*Amaranthus* spp.), sicklepod [*Senna obtusifolia* (L.)], large crabgrass [*Digitaria sanguinalis* (L.) Scop.], morning glory (*Ipomoea* spp.), and nutsedges (*Cyperus* spp.) (Van Wychen, 2016). In 2019, weed removal needed to start before 150-200 kg ha^{-1} of weed biomass for all systems (Figure 2), based on the predicted values CTWR that started at approx. 3 WAP under CT + CC and CT + WF systems while approx. 1.5 WAP following the CVT system to prevent a yield loss greater than 5% in each system (Figure 2 and Table 4). In 2020 the recorded dry weight of weed biomass based on prediction value of CTWR (3.8 WAP)

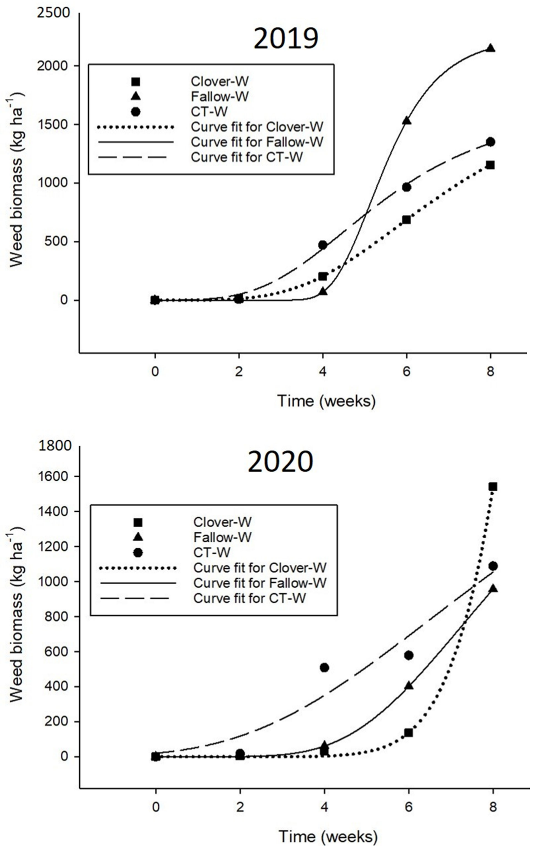

FIGURE 2
Weed biomass as a function of critical timing for weed removal CTWR (duration of weed interference with corn) for each of the conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020. Parameters of the models are presented in Table 4. W represented weedy; Fallow referred to winter fallow (CT + WF), clover referred to cover crop (CT + CC), and CT depicted conventional tillage (CVT).

for CT + CC treatment was 30 kg ha^{-1} approximately. Although weed biomass of CT + WF and CVT systems were approx. 60 and 400 kg ha^{-1} respectively (Figure 2 and Table 4) in between 3 to 4 WAP in 2019. In both years, the weed biomass increased as the critical timing of weed removal (CTWR) increased. However, results showed differences in point estimates between slope and inflection points under each system for both years due to difference in weed pressure among both years. It has been observed that weed density was lower in 2020 than in 2019 (Figures 2, 3 and Tables 4, 5).

The same strategy was followed in the case of the critical weed-free period (i.e., CWFP) following CT + CC, CT + WF, and CVT systems in both years (Figure 3 and Table 5). In 2019 the weed biomass was recorded during the predicted value of CWFP (5.3 WAP) following CT + CC treatment was approx. 100 kg ha^{-1}. However, in case of CT + WF and CVT systems, the recorded dry weight was approx. 50-60 kg ha^{-1} at 6 WAP (Figure 3 and Table 5). In 2020, the recorded weed biomass level at predicted value of CWFP following CT + WF treatment (i.e., 5.1 WAP) and CVT treatment (i.e., 5.7 WAP) was 50 kg ha^{-1} approximately.

Moreover, the recorded maximum production of weed biomass level in both weedy and weed-free plots following CT + CC (cover crop) treatment was lower as compared to CT + WF and CVT systems under both years (Figures 2 and 3, Tables 4 and 5). This is likely due to the cover crop inhibiting weed seed (mainly small, seeded weeds) germination and decreased growth through physical suppression and allelopathy in the conservation tillage system (Akemo et al.,

TABLE 4 Statistics for the three parameters Gompertz model used for fitting weed biomass production under various weedy periods for each of the conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020.

Year 2019	Coefficient	Std error	t value	R^2
Clover				
α	1716.68	4.856	353.498	0.998
b	2.38	0.008	279.455	
x_o	5.79	0.007	709.749	
Fallow				
α	2238.08	7.223	309.858	0.996
b	0.90	0.011	83.470	
x_o	5.13	0.009	531.994	
Conventional				
α	1603.97	155.229	10.333	0.997
b	2.02	0.381	5.312	
x_o	4.52	0.268	16.873	
Year 2020	Coefficient	Std error	t value	R^2
Clover				
α	1896.51	186.449	0.044	0.999
b	4.92	19.046	0.258	
x_o	15.73	53.124	0.296	
Fallow				
α	2008.49	41.324	48.603	0.999
b	2.58	0.045	57.201	
x_o	7.23	0.057	126.277	
Conventional				
α	2118.25	3142.11	0.674	0.965
b	4.22	5.304	0.795	
x_o	6.47	6.703	0.966	

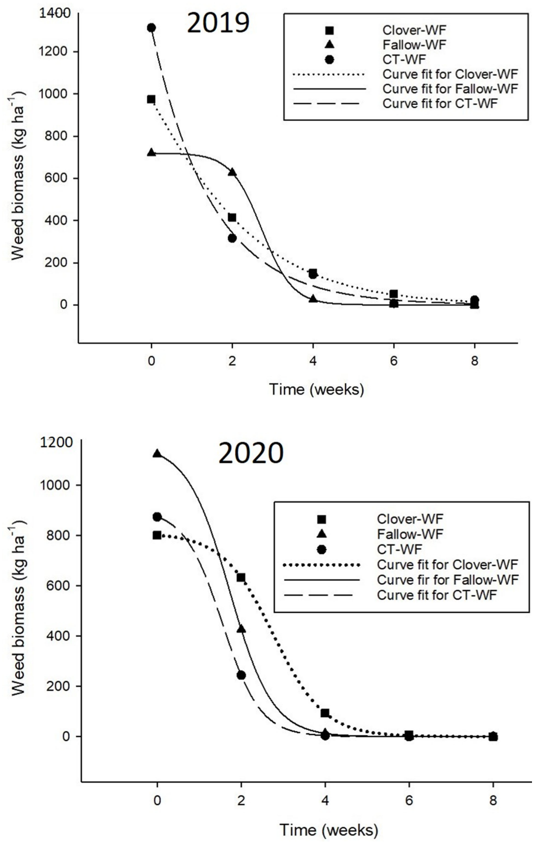

FIGURE 3
Weed biomass as a function of critical weed-free period CWFP for each of the conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020 experimental treatments. Parameters of the model are presented in Table 5. WF depicted weed-free; Fallow referred to winter fallow (CT + WF), clover referred to cover crop (CT + CC), and CT depicted conventional tillage (CVT).

2000; Haramoto and Gallandt, 2004; Korres and Norsworthy, 2015). The practical application for this research is to understand the critical period of weed control (CPWC) in row crops to maintain crop yield potential is a key point in the cropping system. In addition, it is very important to have knowledge about how different cultural practices, including cover crops among others, can influence the critical period for weed removal (CPWC) and weed biomass production. Estimation of critical period of weed control (CPWC) indicated that use of residual herbicides for weed control is required (Korres and Norsworthy, 2015). The use of effective POST herbicides could effectively control the problematic weed species, especially when the critical weed-free period is short Van Acker et al. (1993). A better understanding of the CPWC in different systems, including a high residue cover crop in corn, should help farmers to maintain yield and schedule appropriate weed control timing.

4 Conclusions

In general, a difference of CWFP (i.e., end of weed control) and CTWR (i.e., beginning of weed removal) estimated the CPWC (critical period of weed control, i.e., duration) as we discussed previously. In 2019, the cover crop system had a predicted value of critical timing of weed removal (i.e., starting time) equal 2.5 weeks after planting, and critical weed-free period (i.e., ending time) equal 5.3 weeks after planting, hence the estimated duration of critical period of weed control based on two components was 2.8 weeks. While for the winter fallow system the predicted values of critical timing of weed removal equal 2.8 weeks after planting and critical weed-free period equal 6.3 weeks after planting, hence the estimated duration of critical period of weed control based on two components was 3.5 weeks in 2019. For the conventional tillage system, we found that the

TABLE 5 Statistics for the three parameters logistic model used for fitting weed biomass production under various weed-free periods for each of the conservation tillage following a crimson clover cover crop (CT + CC), conservation tillage following winter fallow (CT + WF), and conventional tillage without a cover crop (CVT) treatment in 2019 and 2020.

Year 2019	Coefficient	Std error	t value	R^2
Clover				
α	2553.16	744.482	3.429	0.966
b	-1.73	0.153	-11.298	
x_o	-0.84	0.882	-0.949	
Fallow				
α	719.58	2.049	351.235	0.953
b	-0.38	0.007	-58.140	
x_o	2.73	0.014	19.275	

(Continued)

TABLE 5 Continued

Year 2019	Coefficient	Std error	t value	R^2
Conventional				
α	4569.27	1432.115	0.003	0.924
b	-1.49	0.677	-2.197	
x_o	-8.702	4.496	-0.018	
Year 2020	Coefficient	Std error	t value	R^2
Clover				
α	810.28	3.291	246.195	0.997
b	-0.60	0.008	-72.534	
x_o	2.77	0.014	196.926	
Fallow				
α	1161.62	0.606	1917.307	0.995
b	-0.51	0.002	-272.223	
x_o	1.72	0.002	1175.335	
Conventional				
α	903.41	0.261	3467.218	0.998
b	-0.45	0.009	-509.481	
x_o	1.55	0.001	1454.769	

estimated values of critical timing of weed removal equal 1.5 weeks after planting and critical weed-free period equal 6.4 weeks after planting, hence the determined duration of critical period of weed control based on two components was 4.9 weeks in the same experimental year. Therefore, the evaluated duration of critical period of weed control in three systems, including cover crop, winter fallow and conventional tillage had 2.8, 3.5, and 4.9 weeks respectively in 2019. The presence of crimson clover cover crop delayed the critical timing of weed removal and caused the early beginning of critical weed-free period and hence shortened critical period of weed control in the 2019 experimental year likely because of later weed emergence and suppression of growth thus a crimson clover cover crop will likely provide a significant competitive advantage to corn against troublesome weed species. In 2020, as we discussed above the model did not predict the critical timing of weed removal values for winter fallow and conventional tillage system since the relative corn yield is above the 95% threshold during most of the growing season. For the critical weed-free period the estimated values were 5.1 and 5.7 weeks after planting following winter fallow and conventional tillage systems, but no prediction following the cover crop system due to the same reason of a greater 95% relative yield in 2020. In conclusion, conservation tillage following crimson clover cover crop shortened the length of critical period of weed control in corn. Moreover, the end of weed control was almost similar (in between 5 to 6 weeks after planting) under winter fallow and conventional tillage systems depending on the weed pressure during the growing season. Also, the beginning of weed removal under cover crop treatment was quite stable from the 2.5 to 3.5 weeks after planting depending on weed density during growing season. Weed control during critical periods offered a significant benefit to corn against troublesome weeds and maintained relative corn yield.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

AP and AK: Outline the study and wrote the manuscript. NK, AG, SL, and AP: reviewed and editing. NK and AK: data analysis. All authors listed made a significant and intellectual contribution to the manuscript and approved the final manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Impact of *Puccinia punctiformis* on *Cirsium arvense* performance in a simulated crop sequence

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Cirsium arvense (Canada thistle) is a perennial weed that causes significant economic losses in agriculture. An extensive rhizomatous root system makes *C. arvense* difficult to manage, particularly in agricultural systems that use tillage as a primary management tool. There is a need for the development of integrated weed management toolsets that include *C. arvense* biological controls. *Puccinia punctiformis* (thistle rust) is an autoecious fungal pathogen that systemically infects *C. arvense*, with the potential to reduce host vigor over time. The goal of this study was to integrate the *P. punctiformis* biocontrol with a simulated annual cropping sequence in a greenhouse environment and evaluate *C. arvense's* above-and belowground biomass production, and its competitive ability. Repeated *P. punctiformis* inoculations produced systemically infected *C. arvense* stems in greenhouse pots over time. *Cirsium arvense* that was inoculated with *P. punctiformis* had 1.6 grams/pot ($p = 0.0019$) less aboveground biomass and 5.6 grams/pot ($p < 0.001$) less belowground biomass, compared to the non-inoculated (control). *Puccinia punctiformis* and crop competition interacted additively to lower aboveground ($p<0.001$) and belowground ($p<0.001$) *C. arvense* biomass more than individual use of either the biocontrol or competition alone. The aboveground competition intensity of *C. arvense* in a mixed crop sequence, relative to non-inoculated *C. arvense* grown in a monoculture, was moderately impacted by the *P. punctiformis* biocontrol ($p = 0.0987$). These results indicate that systemic infection can reduce biomass production and the competitive ability of *C. arvense*. Overall, *P. punctiformis* can be integrated into competitive annual cropping sequences with the potential to reduce *C. arvense* vigor over time.

KEYWORDS

crop competition, *Puccinia punctiformis*, agriculture, integrated weed management (IWM), Canada thistle, thistle rust, biocontrol

1 Introduction

Cirsium arvense (L.) Scop. (Canada thistle) is a problematic weed that causes large economic losses in agriculture, driving the need for integrated weed management tools that include biological control agents (Orloff et al., 2018). *Cirsium arvense* can be found throughout temperate climates of the world, where it exists as a perennial herb that reproduces through an extensive rhizomatous root system and wind dispersed seeds (Tiley, 2010). Clonal rhizomes make *C. arvense* resilient to disturbance, particularly in tilled organic cropping systems that do not use synthetic herbicides for weed management (Moore, 1975). Organic producers in the Northern Great Plains region of the United States generally depend on tillage as a primary weed management tool, however this practice increases soil erosion due to wind and water and depletes soil organic matter over time (Lenhoff et al., 2017). Additionally, tillage can disperse vigorous *C. arvense* rhizomes, causing a rapid increase of the weed's population (Tiley, 2010). As a result, *C. arvense* has become a serious management problem within organic cropping systems, where alternative management tools need to be explored (Tautges et al., 2016; Orloff et al., 2018).

The use of competitive annual crops is another common approach used to manage weeds in organic cropping systems (Bullock, 1992; Liebman and Dyck, 1993). Competitive crops can disrupt weed growth by reducing resource availability and niche dominance of weed species (Liebman and Dyck, 1993). However, the difficult nature of reducing *C. arvense* rhizomes, particularly in organic agriculture (Tautges et al., 2016; Orloff et al., 2018), has led to a search for alternative and integrated tactics, including biocontrol agents that inhibit root development (Berner et al., 2013; Cripps et al., 2014). The use of biocontrol agents can be challenging due to a lack of host specificity, varied responses to environmental conditions, and mismanagement. However, continued exploration of biocontrols for *C. arvense* has the potential to yield low-cost, long-term, host-specific options that can be integrated into existing weed management toolsets (Berner et al., 2013).

Puccinia punctiformis (F. Strauss) Rohl. (thistle rust) is a heterotrophic fungal pathogen of *C. arvense* that acts as a long-term systemic parasite (Buller, 1950; Menzies, 1953; Berner et al., 2013; Kentjens et al., 2023). As a parasite that consumes resources and weakens the root structure (Buller, 1950; Menzies, 1953), *P. punctiformis* is specific to *C. arvense* (Berner et al., 2013; Kentjens et al., 2023) and has been identified in temperate habitats around the globe (Berner et al., 2013; Kentjens et al., 2023). Once established in the roots, infected *C. arvense* can develop chlorotic leaf tissue with lesions, elongated stems, and growth irregularities which can reduce fitness and cause death (Buller, 1950; Berner et al., 2013). Diseased stems act as aboveground carriers for *P. punctiformis* spores, appearing as orange to dark-red pustules on leaves, where the fungus completes most of its five-stage heterothallic life cycle during summer months, eventually producing transmissible teliospores (Buller, 1950; Menzies, 1953; Kentjens et al., 2023). Teliospore-bearing thistle leaves senesce and abscise as precipitation and temperatures decline, where they can contact healthy *C. arvense* rosettes through wind or mechanical dispersion, leading to long-term systemic infection in new *C. arvense* hosts under ideal environmental conditions (French and Lightfield, 1990; Berner et al., 2013).

Puccinia punctiformis' impact on *C. arvense* abundance has been well documented (French, 1990; Thomas et al., 1994; Berner et al., 2013; Cripps et al., 2014; Kentjens et al., 2023). However, to our knowledge, the effects of integrating the *P. punctiformis* biocontrol with a competitive crop sequence on *C. arvense* growth have not been studied. We addressed this gap in knowledge using greenhouse experiment, which assessed the impact of *P. punctiformis* on *C. arvense* growth and competitiveness. Specifically, our questions were: 1) What is the probability of observing *P. punctiformis* infected *C. arvense* over time, and does the percentage of infected *C. arvense* stems increase over time? 2) How does *P. punctiformis* affect *C. arvense* above- and belowground biomass, and does crop competition interact with the effects? 3) Using a relative competition intensity index (RCI), is the competitive ability of *C. arvense* reduced when *P. punctiformis* is integrated into a sequence of competitive annual crops? We hypothesized that the integration of *P. punctiformis* with a competitive crop sequence would lead to a significant reduction in above- and belowground *C. arvense* biomass, compared to individual effects from *P. punctiformis* or crop competition when used alone.

2 Materials and methods

2.1 Experimental design

A greenhouse study with three independent trials was conducted at the Montana State University Plant Growth Center in Bozeman, Montana, between 2020 and 2022. A nested full factorial (2 x 2) design was used to assess the integration of *P. punctiformis* and crop competition. The primary treatment was *P. punctiformis* inoculation, with two levels: *C. arvense* inoculated with *P. punctiformis* (n = 20) and non-inoculated *C. arvense* grown as a control (n = 20). Nested within each level of the inoculation treatment was a competition treatment, split into two levels: *C. arvense* grown in monoculture (n = 10) and *C. arvense* grown in competition with a common crop species (n = 10; Supplementary Figure 1).

The competition treatment was a four-phase crop sequence that incorporated common crops used by organic farmers in the dryland areas of the Northern Great Plains. The sequence included the following four phases, with seeding depths and seeding rates scaled for greenhouse pots: 1) Fallow: 1-gram *C. arvense* rhizome planted ~ 10 cm deep; 2) spring wheat: 100 kg/hectare planted ~ 5 cm deep (18 plants/pot); 3) forage pea: 89 kg/hectare planted ~ 5 cm deep (8 plants/pot); and 4) safflower: 33 kg/hectare planted ~ 3 cm deep (2 plants/pot). *Cirsium arvense* rhizomes were planted in the approximate center of each pot during the first phase. Crops were planted in a manner that provided approximately equal space between individuals, with at least 5 cm of distance from pot edges.

Two separate greenhouse spaces were used to prevent movement of *P. punctiformis* spores between the *P. punctiformis* inoculated treatment and the non-inoculated (control) treatment.

Internal greenhouse temperatures for both spaces were set at a range of 18°C to 26.5°C during the day, and 10°C to 24°C at night. To ensure consistent lighting, passive solar lighting with supplemental 1000-watt metal halide lamps, set to 12 hour intervals, were used throughout the course of the study.

2.2 *Cirsium arvense* and *Puccinia punctiformis* establishment

Cirsium arvense rhizomes were acquired from naturally occurring populations in Gallatin County and Hill County, Montana during the summer of 2019. Rhizomes were maintained in greenhouse pots and used as the source of rhizome transplants for the study. Pots (25.4 cm diameter x 20.3 cm deep) were sown with 1-gram cuttings of *C. arvense* rhizome and randomly assigned to a treatment. Rhizomes were planted into a pasteurized soil mixture consisting of equal parts (by volume) of loam soil, washed sand, and Canadian sphagnum peat moss. Pots were watered every two days or as needed, for ten seconds per pot using the shower setting on a conventional garden hose wand. A soluble all-purpose fertilizer (20-20-20 NPK) was added to pots bi-weekly, by mixing 2.5 ml of fertilizer with 3.8 L of water in a watering can, and hand watering for ten seconds per pot. *Cirsium arvense* was grown for an average of 4.5 months during the first phase (fallow) in all treatments, which was approximately timed with the development of flower buds in all pots. In subsequent phases of each trial, *C. arvense* was allowed to grow until harvest at the maturity stage of the crop within each crop phase.

Puccinia punctiformis inoculum was collected from naturally occurring populations of infected *C. arvense* in Gallatin County, Montana and prepared as described by Berner et al. (2013). Systemically infected *C. arvense* stems were harvested in the autumns of 2020 and 2021, and dried in paper bags in a dark room at ambient temperatures. From the dried stems, leaf tissue containing signs of teliospores were gathered, and ground into a coarse powder inoculum using a household blender. The ground teliospore-bearing inoculum was immediately used or stored for future use in a -80°C freezer. Inoculation methodology followed Berner et al. (2013), where 5 ml of dry rust inoculum was dispersed evenly on the crowns of *C. arvense* rosettes at the beginning of each phase, for a total of four inoculations per pot in each trial. Cripps et al. (2014) estimated that the concentration of teliospores, using the same methodology, was 1.14×10^7 teliospores g^{-1}. The inoculated rosettes were misted with deionized water once a day for three days post inoculation to maintain humidity for spore germination. This method was repeated after the harvest of each phase and subsequent regrowth of *C. arvense*, for a total of four inoculations per pot in each trial.

2.3 Data collection

To address our first question, the density of *C. arvense* stems with signs of systemic *P. punctiformis* infection was recorded from each pot at the termination of each crop phase. *Cirsium arvense* stems were identified as systemically infected when spore structures were observed on leaves and stems. To address our second and third questions, *C. arvense* and crop stems were counted and cut at soil level at the termination of each crop phase. To obtain dry weight, the harvested biomass was oven dried for 72 hours at ~40.5°C and weighed to the nearest 0.01-gram. After each harvest, pots containing thistle rhizomes were left undisturbed and the next crop phase was seeded into pots assigned to the mixed competition treatment. After the aboveground harvest of final the crop phase (safflower) of each trial, *C. arvense* rhizome biomass was removed from the soil of each pot, cleaned of soil and residue with cool water, dried for 72 hours at ~40.5°C, and weighed to the nearest 0.01-gram *Cirsium arvense* pots assigned to the monoculture level of the competition treatment were harvested using the same methodology and at the same time as the mixed pots.

2.4 Data analysis

The probability of observing systemic *P. punctiformis* infection in pots was calculated at each phase in the crop sequence and was modeled using a generalized linear mixed effects model with a binomial distribution ("glmer" function in the R-Package "lmerTest"; Kuznetsova et al., 2017). The fixed effect in this model was crop phase, and pot ID was included as a random effect to account for repeated observations within each pot over the three trials. Model selection followed a backwards selection from a full model containing all potential explanatory variables using a 'Drop in Deviance' test (Ramsey and Schafer, 2012). Model overdispersion was checked by calculating the sum of squared Pearson residuals and comparing it to the residual degrees of freedom, and assumptions homoscedasticity, normality, or influential observations were visually assessed (Ramsey and Schafer, 2012).

The percentage of *C. arvense* stems with signs of systemic *P. punctiformis* infection within the inoculated treatment was calculated out of the total density of *C. arvense* stems per pot and was modeled using a linear mixed effects model ("lmer" function in the R-Package "lmerTest"; Kuznetsova et al., 2017). The fixed effects and random effects in this model were the same as previously described. Explanatory variables were backwards selected from a full model containing all potential explanatory variables ("step" function in the R-Package "lmerTest"; Ramsey and Schafer, 2012). Model assumptions of homoscedasticity, normality, and influential observations were visually assessed (Ramsey and Schafer, 2012).

Differences in *C arvense* above- and belowground biomass was evaluated using separate linear mixed effects models. In the model for aboveground biomass, the fixed effects were inoculation treatment, competition treatment, and crop phase, with pot ID as a random effect. In the model for belowground biomass, the fixed effects were inoculation treatment and competition treatment, with trial as a random effect to account for repeated observations within each trial. In both models, explanatory terms were selected, and assumptions were checked using methods described previously.

To assess the competitive ability of *C. arvense*, a relative competition intensity (RCI; Weigelt and Jolliffe, 2003) was used to evaluate the impacts of competition between the *P. punctiformis* inoculated and non-inoculated (control) treatments was calculated as:

$$\mathrm{RCI} = \frac{monoculture - mixed}{monoculture} \times 100$$

Where "monoculture" was the aboveground biomass of *C. arvense* from the non-inoculated (control) monoculture treatment, and "mixed" was the aboveground biomass of the mixed pots for either the *P. punctiformis* inoculated or non-inoculated (control) treatment. $\mathrm{RCI}^{\mathrm{control}}$ was calculated using aboveground biomass from the control monoculture and mixed pots that were not inoculated with *P. punctiformis*. $\mathrm{RCI}^{\mathrm{inoculated}}$ was calculated using aboveground biomass from the non-inoculated (control) monoculture and the aboveground biomass from the mixed pots in the *P. punctiformis* inoculated treatment. An RCI value ≤ 0 indicates that *C. arvense* grown in mixed pots produced as much or more aboveground biomass compared to *C. arvense* grown in a monoculture. In contrast, RCI > 0 indicates that aboveground biomass of *C. arvense* was reduced when grown in mixed pots, and RCI = 100 indicates that no aboveground *C. arvense* biomass was produced in the mixed treatment.

The relationship between $\mathrm{RCI}^{\mathrm{control}}$ and $\mathrm{RCI}^{\mathrm{inoculated}}$ was evaluated using a linear mixed effects model, with fixed effects of inoculation treatment and crop phase, and pot ID included as a random effect. Model selection was completed by comparing all potential models with an Extra Sums of Squares F-Test. All model assumptions were visually assessed.

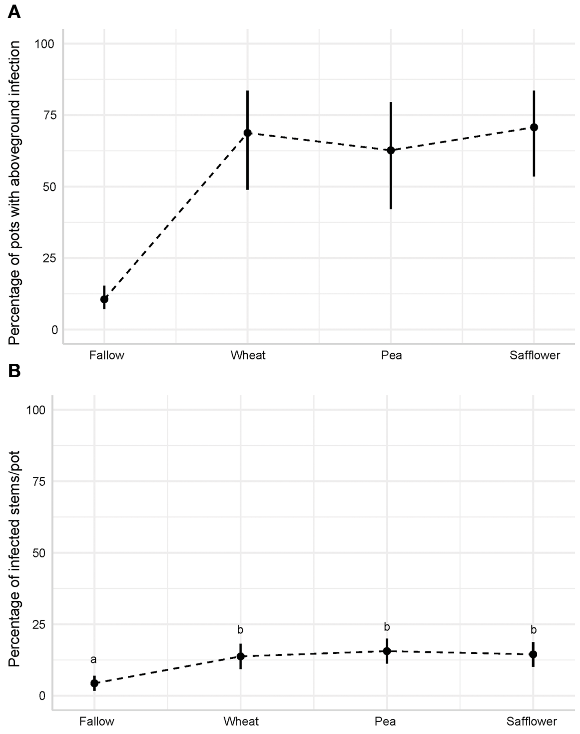

FIGURE 1
(A) Model predicted percentage of greenhouse pots with signs of systemically infected C arvense stems throughout the simulated crop sequence in the P. punctiformis inoculated treatment. **(B)** Model predicted percentage of systemically infected stems, out of the total C arvense stems produced per pot, in the P. punctiformis inoculated treatment throughout the simulated crop sequence. Letters (a & b) are representative of the statistical differences in percentage of infected stems/pot between crop phases, where phases that share the same letter are not statistically different.

3 Results

3.1 *Puccinia punctiformis* establishment

The overall frequency of *P. punctiformis* inoculated pots with systemically infected *C. arvense* stems over the three trials was 52% with no infection observed in the non-inoculated (control) treatment. Systemically infected *C. arvense* stems were observed in 15% of pots in the fallow phase, 65% of pots in the wheat phase, 60% of pots in the pea phase, and 67% of pots in the safflower phase (*F = 14.159; p<0.001*; Figure 1A). The percentage of *P. punctiformis* infected stems in the inoculated treatment, out of all *C. arvense* stems produced per pot, increased as the crop sequence progressed, with the largest increase occurring after the fallow phase *(F = 8.58; p<0.001)*. The overall mean percentage of *P. punctiformis* infected stems per pot was 12%. Out of all stems produced per pot, 4% were systemically infected in the fallow phase, 14% were systemically infected in the wheat phase, 16% were systemically infected in the pea phase, and 14% were systemically infected in the safflower phase (Figure 1B).

3.2 *Cirsium arvense* above-and belowground biomass

Cirsium arvense that was inoculated with *P. punctiformis* had (± SE) 1.6 (± 0.52) grams/pot less aboveground biomass compared to non-inoculated (control) *C. arvense* (*F = 9.965; p = 0.0020*). *Cirsium arvense* grown with crop competition produced (± SE) 3.1 ± 0.52 grams/pot less aboveground biomass than *C. arvense* grown in monoculture (*F = 36.396; p< 0.001*). *Cirsium arvense* biomass in the integrated *P. punctiformis* inoculated and crop competition treatment was (± SE) 4.8 ± 0.74 grams/pot less than *C. arvense* biomass in the monoculture, non-inoculated treatment (*t = 6.506; p<* 0.001; Figure 2, Table 1).

C. arvense rhizome biomass was 6.9 grams/pot in the *P. punctiformis* inoculated treatment and 12.5 grams/pot in the non-inoculated (control) treatment, after an average of 12.9 months of growth. Rhizome biomass in the *P. punctiformis* inoculated treatment was less than rhizome biomass in the non-inoculated (control) treatment (*F = 25.791; p< 0.001*). The estimated biomass of *C. arvense* rhizome in the inoculated treatment was (± SE) 5.6 ± 1.1 grams/pot less than in the control treatment. *Cirsium arvense* grown with crop competition produced (± SE) 2.7 ± 1.1 grams/pot less rhizome biomass than *C. arvense* grown in monoculture (*F = 6.211; p-value = 0.0141*). Rhizome biomass in the integrated *P. punctiformis* inoculated and crop competition treatment was (± SE) 8.3 ± 1.6 grams/pot less than rhizome biomass in the monoculture, non-inoculated (control) treatment (*t = 5.353; p< 0.001*; Figure 3, Table 2).

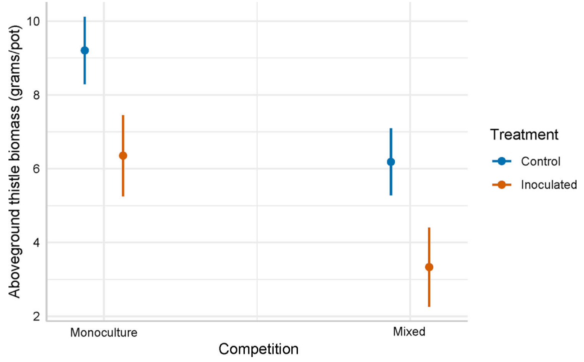

FIGURE 2
Model predicted aboveground *C. arvense* biomass (grams/pot) between the inoculated and non-inoculated (control). Inoculated and non-inoculated (control) *C. arvense* was either grown in a monoculture or grown with interspecific competition where *C. arvense* was mixed with a sequence of annual crops.

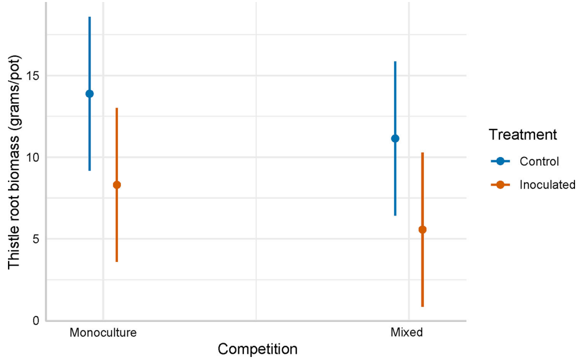

FIGURE 3
Model predicted belowground *C. arvense* biomass (grams/pot) between the inoculated and non-inoculated (control). Inoculated and non-inoculated (control) *C. arvense* was either grown in a monoculture or grown with interspecific competition where *C. arvense* was mixed with a sequence of annual crops.

3.3 *Puccinia punctiformis* impact on *Cirsium arvense* competition

Crop competition reduced aboveground biomass, with (± SE) 49.2% ± 5.9 biomass loss in the inoculated treatment, and (± SE) 39.2% ± 5.9 biomass loss in the non-inoculated (control) treatment, when compared against the monoculture index for growth in the non-inoculated (control) treatment. There was some evidence for a difference in RCI between the inoculated treatment and the non-inoculated (control) ($F = 2.816$, $p\text{-value} = 0.0987$). The relative competition of *C. arvense* varied between crop phases (wheat, pea, and safflower) in both the inoculated and control treatments ($F = 63.669; p < 0.001$). Crop competition reduced aboveground biomass by (± SE) 48% ± 5.9 in the wheat phase, (± SE) 71% ± 5.9 in the pea phase, and (± SE) 14% ± 5.9 in the safflower phase, when compared against the monoculture index for growth in the non-inoculated (control) treatment. Additionally, there was an interaction between the inoculation treatments and crop phases ($F = 3.329$; $p = 0.0393$). The RCI between the inoculation treatments increasingly separated as the crop sequence progressed, where the inoculated treatment lost (± SE) 24% ± 8.3 more biomass than the non-inoculated (control) treatment by the final safflower phase in the crop sequence (Figure 4, Table 3).

4 Discussion

Sustainable *C. arvense* management in organic cropping systems is a primary challenge in temperate regions around the globe. Integrated weed management strategies are needed to reduce the abundance, slow the spread, and minimize the impact of *C. arvense* in cropping systems over a long term (Liebman et al., 2001; Liebman and Davis, 2009; Davis et al., 2018; Orloff et al., 2018). In this study, we found that the integration of *P. punctiformis* and crop competition interacted to impact *C. arvense* biomass and competitive ability. Integrated weed management of *C. arvense* that combines the *P. punctiformis* biocontrol with crop competition can reduce *C. arvense* vigor but requires careful consideration for effective use within complex cropping systems.

Repeated inoculations of *C. arvense* rosettes with *P. punctiformis* yielded systemically infected *C. arvense* stems in all phases of the crop sequence. Inoculation of rosettes resulted in few systemically infected *C. arvense* stems in the first phase (3-4 months of growth) of the crop sequence, but incidence of infection increased over time. The slow development of systemically infected stems is consistent with the general development of plant pathogens, which often require an incubation period before infected plants develop symptoms (Agrios, 2005). Our findings are also consistent with literature that suggests that *P. punctiformis* mostly resides asymptomatically within *C. arvense* rhizomes (Bailiss and Wilson, 1969), especially during the initial stages of infection. In a study testing asymptomatic *C. arvense* rosettes in proximity to *P. punctiformis* inoculations, Berner et al. (2015) discovered that up to 60% of asymptomatic rosettes were positive hosts for *P. punctiformis*. Therefore, the success of our inoculations was likely greater than what was observed aboveground.

While systemically infected stems were observed in most inoculated greenhouse pots, the majority of stems produced were

TABLE 1 **ANOVA results for the *C. arvense* aboveground biomass response to *P. punctiformis* inoculation treatments (inoculated/non-inoculated) and competition treatments (monoculture/mixed).**

	df	SS	MS	F	p
Inoculation treatment	117.2	218.03	218.03	9.965	0.0020
Competition treatment	117.2	796.35	796.35	36.396	<0.001

TABLE 2 ANOVA results for the *C. arvense* root biomass response to *P. punctiformis* inoculation treatments (inoculated/non-inoculated) and competition treatments (monoculture/mixed).

	df	SS	MS	F	p
Inoculation treatment	115	932.98	932.98	25.791	<0.001
Competition treatment	115	224.68	796.35	6.211	0.0142

asymptomatic. This supports the conclusion that *P. punctiformis* is primarily a root pathogen (Berner et al., 2015; Kentjens et al., 2023) that remains latent until adequate resources are gathered from the host and environmental conditions are suitable for the emergence of spore bearing *C. arvense* stems (Mendgen and Hahn, 2002). The stabilization of infected *C. arvense* stems after the fallow phase reflects the host's capacity to support *P. punctiformis*, given the limitations of plant growth in greenhouse pots. Berner et al. (2015) and Watson and Koegh (1980) suggested that the robustness of infected *C. arvense* can be a factor that influences the development of systemically infected *C. arvense* stems, where a robust host is more likely to produce a relatively high abundance of infected stems, and systemic infection in a weaker host could produce fewer infected stems. It was concluded that systemic infection in a less robust host remains mostly asymptomatic and caused death more quickly than systemic infection in a robust host.

Cirsium arvense that was inoculated with the *P. punctiformis* biocontrol produced less belowground biomass compared to *C. arvense* that was not inoculated. Our results agree with the findings of Thomas et al.'s (1994) greenhouse experiment, where *P. punctiformis* inoculated *C. arvense* produced less root biomass than non-inoculated *C. arvense*. A weakened root system can directly impact aboveground biomass production, where root resources that would otherwise promote stem growth, are instead allocated to costly defense compounds, or become parasitized by *P. punctiformis* (Herms and Mattson, 1992; Thomas et al., 1994; Monson et al., 2022). This was demonstrated in our findings, where *P. punctiformis* inoculations yielded less aboveground biomass compared to *C. arvense* that was not inoculated, confirming that *P. punctiformis* inoculations can effectively impact the overall growth of *C. arvense*.

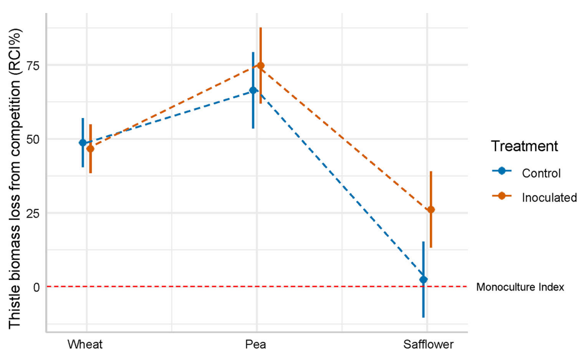

FIGURE 4
The relationship in aboveground *C. arvense* biomass loss in competition (RCI%) between the *P. punctiformis* inoculated and non-inoculated (control) treatments for the three crop phases for all three trials. There was no difference in RCI between the treatments or the crop phases. An RCI value ≤ 0 indicates that *C. arvense* grown in mixed pots produced as much or more aboveground biomass compared to *C. arvense* grown in a monoculture. In contrast, RCI > 0 indicates that aboveground biomass of *C. arvense* was reduced when grown in mixed pots, and RCI = 100 indicates that no aboveground *C. arvense* biomass was produced in the mixed treatment.

Competition with annual crops affected *C. arvense* aboveground growth, although the effects differed between crop species. Unexpectedly, peas were the most competitive annual crop species in the sequence, despite their relatively slow germination, shallow rooting depth, and open canopy (McKay et al., 2003). It is possible that wheat, a moderately competitive cereal species (Mason and Spaner, 2006), had a lasting impact on *C. arvense* vigor that wasn't evident until the following pea phase. The weak competitive qualities of peas may have facilitated a recovery in *C. arvense* vigor, becoming evident in the following phase, where safflower had the lowest relative competition intensity. However, safflower, known to be a weak competitor in the early stages of growth (Emongor and Oagile, 2017), was disadvantaged as the last crop in the sequence. It is possible that greenhouse pots with fully developed roots gave *C. arvense* a strong competitive advantage by the final phase of the crop sequence; seeding safflower directly into a dense and confined *C. arvense* root network likely impacted optimal safflower development.

When inoculated *C. arvense* was grown in mixed pots with interspecific crop competition, the biocontrol interacted additively with crop competition to further reduce above-and belowground biomass, more than individual impacts from the biocontrol or crop competition alone. Although *C. arvense* was never eradicated by the combination of *P. punctiformis* and crop competition, there was an interaction between the crop phases and the inoculation treatments, where the difference between the *P. punctiformis* inoculated and the non-inoculated (control) relative competition intensities gradually increased as the crop sequence progressed. As *P. punctiformis* inoculations did not immediately affect *C. arvense's* competitive ability, but increased through time, the effects appear to be associated with the establishment of infected *C. arvense* stems. The greatest impact on *C. arvense* competition emerged after aboveground disease incidence stabilized and persisted through time.

Although we didn't evaluate physiological responses of *C. arvense*, there is potential to accelerate disease establishment and increase the severity of *P. punctiformis* infection by stimulating hormonal responses (Clark et al., 2020), thus enhancing future integrations of the biocontrol. Overall, these results support our hypothesis and provide evidence in favor of integrated weed management as an effective strategy for *C. arvense* control (Demers et al., 2006; Liebman and Davis, 2009; Sciegienka et al., 2011; Davis et al., 2018; Orloff et al., 2018).

While crop competition is already a common integrated weed management practice (Pavlychenko and Harrington, 1934; Bullock, 1992; Liebman and Dyck, 1993; Liebman and Davis, 2009), there

TABLE 3 ANOVA results for the relative competition intensity (RCI) of *C. arvense* aboveground biomass response to *P. punctiformis* inoculation treatments (inoculated/non-inoculated), competitive crop phases (wheat/pea/safflower), and the interaction between inoculation treatments and crop phases.

	df	SS	MS	F	p
Inoculation treatment	58	2129	2129	2.816	0.9872
Crop phase	116	96261	48131	63.669	<0.001
Inoculation treatment *Crop phase	116	5034	2517	3.329	0.03927

remain practical challenges to the integration of the *P. punctiformis* biocontrol in field settings. Inoculum sourcing and mass production is limited by the inability to culture transmissible teliospores (Kentjens et al., 2023), creating a reliance on the harvest of teliospore bearing *C. arvense*. Limitations in inoculum ultimately reduce the scalability of the biocontrol under current sourcing methods. Most natural transmissions of *P. punctiformis* are limited to 12 meters from the source plant, with no transmissions occurring beyond 17 meters (Berner et al., 2015). Insect vectors or mowing have shown potential to transmit *P. punctiformis* and increase infection levels across fields (Demers et al., 2006; Wandeler and Bacher, 2006), however, careful cropping system management is required to facilitate effective spore distributions. The greenhouse environment simplifies biocontrol manipulations, but successful integration of *P. punctiformis* in a field setting will be dependent on variable environmental conditions and cropping system management that can influence survivability and germination of the biocontrol (French and Lightfield, 1990; Berner et al., 2013; Kentjens et al., 2023). Additionally, Thomas et al. (1994) found that *P. punctiformis* inoculations did not impact aboveground biomass production compared to non-inoculated *C. arvense*, suggesting inconsistent performance of the pathogen. Inconsistencies in the biocontrol's impact on *C. arvense* aboveground growth may be an indication of genetic variability within the host and pathogen populations, where disease severity can be determined by a range of resistance mechanisms in *C. arvense* or virulence factors in *P. punctiformis*. Despite inconsistencies and challenges, *P. punctiformis* has shown potential to increase *C. arvense's* vulnerability to integrated weed management tactics, making the biocontrol a viable management option.

5 Conclusion

The fungal biocontrol, *P. punctiformis* can be successfully integrated with crop competition as a *C. arvense* management tool. In this greenhouse study, inoculation of *C. arvense* rosettes with *P. punctiformis* teliospores caused an increase of symptomatically infected *C. arvense* stems over time, impacting above- and belowground *C. arvense* biomass production. Furthermore, *P. punctiformis* intensified the effects of crop competition when the biocontrol was integrated into a simulated crop sequence. While the use of *P. punctiformis* is possible in a greenhouse, successful integration of the biocontrol into a field setting will be dependent on a combination of environmental factors and deliberate cropping system management. *Puccinia punctiformis* is not a singular management solution for *C. arvense*, however it has potential to be integrated as a low-cost, and low-input biocontrol agent that can improve sustainable management of *C. arvense.*

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

DC was the primary author, who conducted the data collection, statistical analysis, and writing. CL assisted with experimental design, statistical analysis, and editing. JE wrote the initial grant proposal and edited the manuscript. FM guided the writing and edited the manuscript. TS supervised data collection, assisted with statistical analysis, guided the writing, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2023.1201600/full#supplementary-material

SUPPLEMENTARY FIGURE 1

Canada thistle growth was assessed within three levels of a competition treatment (crop monoculture, thistle monoculture, thistle & crop polyculture) that were nested into two levels of an inoculation treatment (control & thistle rust inoculated). Canada thistle was grown for 16 months in greenhouse pots, and evaluated for density and biomass within a 4-phase diversified crop rotation.

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An integrated weed management approach in tomato using soil steaming, mulching, and winter cover crops

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One of the most significant yield losses in tomato (*Solanum lycopersicum* L.) is due to weeds. Yellow and purple nutsedge, large crabgrass, and Palmer amaranth are the most troublesome weed species in tomato production throughout the southeastern United States. This study aimed to determine the impact of soil steaming, plastic mulching, and cover crops on weed suppression, tomato height, and fruit yield. The cover crops used were hairy vetch (*Vicia villosa*), crimson clover (*Trifolium incarnatum*), and cereal rye (*Secale cereale*). The study was conducted at the Mississippi State University Truck Crops Experiment Station in Crystal Springs, Mississippi, USA. The experiment used a completely randomized block design with three fall cover crop treatments, including fallow, and each was replicated three times and repeated in two years. Each plot was broadcasted with a mixture of yellow nutsedge (*Cyperus esculentus* L.), large crabgrass (*Digitaria sanguinalis* L.), barnyardgrass (*Echinochloa crus-galli)*, and Palmer amaranth [*Amaranthus palmeri* (S.) Watson] at a density of 20 plants m^{-2} for each weed species. Two days after sowing the weed seeds, the soil surface was steamed according to its assigned treatment until it reached 61°C for either 0, 5, or 20 min. After steaming, drip irrigation tubing was laid on each row, and covered by black, 0.0254-mm plastic mulch. Data were recorded in both years, including weed cover, plant height, and fruit yield. The lowest weed cover was observed at 5 min of soil steaming in mulched treatment, and the highest cover was noted at 0 min of soil steaming in the absence of mulching. Yellow nutsedge was the dominant weed species, even under steam and mulch treatments. The use of cover crops did not show a difference compared to fallow treatments. However, hairy vetch showed the lowest weed cover, followed by crimson clover. Tomato plants in steamed soil were up to 13 cm taller than those in unsteamed soils. Additionally, steaming at 5 or 20 min in combination with plastic mulch increased the marketable and cull yield. Soil steaming and mulching increased tomato plant height and yield while decreasing weed population and can, therefore, be effectively incorporated into an integrated weed management program in tomato.

KEYWORDS

sustainable agriculture, organic farming, weed control, pest management, integrated weed management, non-chemical

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most produced and consumed vegetables worldwide, and it requires considerable care in its production to prevent yield loss (Sunday et al., 2022). One factor that reduces tomato yield considerably is weed competition, which can be described as the competition between crops and weeds for vital resources such as water, nutrients, light, soil, and space (Giles et al., 2004). Various methods prevent, control, and exterminate weeds in the field. Still, one of the most time-efficient and effective methods to manage different weeds is by using herbicides (Manisankar et al., 2022). However, chemical control should be used judiciously to protect its efficacy while also ensuring the safety of surface water and environmental pollution. Doing so slows the evolution of resistant weeds that threaten herbicide efficacy, ultimately protecting farmer profits while minimizing herbicide's environmental impact. Rather than relying on chemical control alone, layering weed management strategies creates a more robust and sustainable approach (Naeem et al., 2022). Additionally, herbicide use is restricted in organic production and demands developing and optimizing alternative strategies.

Cover crops are primarily planted to improve soil structure, increase soil organic matter, enhance water viability, reduce erosion, control pests and diseases, and increase soil nitrogen rate, which is an essential component for different plants (Kenney et al., 2015), consequently increasing the yield (Finney et al., 2017). Cover crops control weeds by competing for resources and inhibiting weed development through allelochemical production, blocking stimuli for weed seed germination, or altering the soil microbial population to put weed species at a disadvantage (Teasdale et al., 2007). Some cover crop species release nitrogen faster than other species, such as hairy vetch (*Vicia sativa* L.) and cereal rye (*Secale cereal* L.). Hairy vetch is a legume with better nitrogen-fixing ability, deeper roots, and a lower C/N ratio that decays very rapidly in the soil. For these reasons, hairy vetch releases more nitrogen for immediate use and at a faster rate than cereal rye. Cereal rye is a grass species with fibrous roots extending to the soil and acting like a sponge soaking up water. The roots help anchor the plant to the ground and have greater C/N ratios resulting in a prolonged composting process (Brust, 2019). However, both species can decrease nitrogen fertilizer costs because they rapidly decompose and release nitrogen into the soil (Sievers and Cook, 2018). In Missouri, Cornelius and Bradley (2017) reported that cereal rye plus hairy vetch and cereal rye cover crops reduced the winter annual weed cover by 68 and 72%, respectively. Additionally, the same cover crop treatments substantially reduced (41%) early-season summer annual weeds. It has been reported that cover crops such as cereal rye exude phytotoxic allelopathic compounds that promote the suppression of different weeds (Schulz et al., 2013).

Another alternative to control weeds in a sustainable agricultural system is using synthetic materials or plant residues/waste on the soil, also known as mulching (Marıń-Guirao et al., 2022). One of the materials intensively used as mulch is plastic film. Mulch film improves soil temperature and moisture, providing a suitable environment for enzymes produced by the microorganism community and improving soil productivity. The additional advantage of mulching is improved weed management by preventing weed seed germination and blocking emerging seedlings' growth. Also, mulching blocks photosynthetically active radiation while allowing the infrared transmission to maintain the soil warm (Akhtar et al., 2018; Monteiro and Santos, 2022; Zhang et al., 2022).

Soil steaming is another strategy that can be used to effectively control weed emergence and growth through exposure to high temperatures. It has traditionally been used as a fumigant replacement in ornamentals and horticultural crops where some chemicals are not permitted (Fennimore and Goodhue, 2016; Baldoin et al., 2010). Soil steaming is shown to reduce weed seed germination by 50% at 62-68°C and 90% at 76-86°C (Bitarafan et al., 2022). Also, high temperatures damage plant membranes, resulting in cellular damage (Monteiro and Santos, 2022; Samtani et al., 2011; Fennimore et al., 2016). According to Bitarafan et al. (2022), no germination occurred in soil steaming with a target temperature of 99°C at 90, 180, or 540 s. A study on strawberry (*Fragaria ×ananassa*) production reported that steaming soil at 70°C for 20 min provides similar weed control efficacy as methyl bromide and chloropicrin treatments (Samtani et al., 2011).

Combining cover crops, soil steaming, and mulching for weed suppression may improve weed control in tomato. Consequently, this study aimed to test the presence and absence of cover crops and the effects of soil steaming, with and without plastic mulch, on weed suppression, tomato plant growth, and tomato yield.

Material and methods

Studies were conducted in the 2021-2022 growing season at the MSU Truck Crops Experiment Station at Crystal Springs, Mississippi, United States (lat. 31°56′45.8"N, 90°22′40.4" W). The experiment was conducted as a split-split plot design with year as the whole plot factor, the cover crops (crimson clover, cereal rye, hairy vetch, and fallow) as the split-plot factor, and the split-split plot factor being a factorial arrangement of steaming duration (0, 5, and 20 min), mulching (mulched, non-mulched), and weed species (barnyardgrass, large crabgrass, Palmer amaranth, and yellow nutsedge) in a randomized complete block, and each treatment combination had three replications. The cover crops were sowed in November 2020 and 2021 at a rate of 69, 84, and 84 kg/ha, respectively.

In the spring, the entire field was cut to a height of 13 cm and sprayed with glyphosate (Roundup Powermax®, 48.7% active ingredient) at a rate of 868 g/ha during the spring to control weeds. A week after herbicide application, the field was tilled to a depth of 10 cm. Twenty-four 0.6 m wide by 27 m long rows were formed to prepare the field for the tomato transplants. Each row was spaced 0.9 m apart. Twenty-four rows were formed per replication (block) and were broadcasted at a density of 20 plants m^{-2} for each weed species: yellow nutsedge (*Cyperus esculentus* L.), large crabgrass (*Digitaria sanguinalis* L.), Palmer amaranth [*Amaranthus palmeri* (S.) Watson] and barnyardgrass (*Echinochloa crus-galli*). These are the most problematic weed species in tomato (Webster, 2014). Weed seeds of large crabgrass, Palmer amaranth, and barnyardgrass and tubers (in the case of yellow nutsedge) were broadcasted on the soil surface. Immediately after broadcasting the seeds and tubers, a roller was used to endure good contact with the soil surface. Two days after sowing (DAS) the weed seeds, the soil surface in each plot was exposed to steaming until soil temperature reached 61°C at 10 cm deep, after which steaming was continued for 5 and 20 min based on the assigned steam treatment. Plots assigned for 0 min of steam were not steamed. The steam generator (Steam-Flo model SF-20, Sioux Corporation, Beresford, South Dakota, USA) used for this study is rated for 308 kg/hr steam output. To apply the steam, a permeable, woven, 5.30 cm nylon sleeve (Beresford, S. USA) was placed over the center of each plot assigned to be steamed and covered with a heat-resistant, three-ply, 0.1524-mm tarp with reinforced scrim (Beresford, S. USA) to capture the steam from the sleeve and allow it to penetrate the soil (Figure 1). Chains were placed around the perimeter of the tarp to hold the tarp in place during the steaming process and prevent steam loss through the sides of the tarp. The soil temperature was monitored at a depth of 10 cm by a temperature probe connected to a 4-channel temperature monitoring system (Signals 4-Channel, Thermoworks, American Fork, Utah, USA).

After steaming, drip irrigation tubing was laid on each row. As per soil test recommendations, 3 kg of 0-20-20 (N-P-K; P_2O_5 and K_2O; Bumper Crop, Schulenburg, TX) and 0.9 kg of 33-0-0 (50% CH_4N_2O and 50% $(NH_4)_2SO_4$; Bumper Crop, Schulenburg, TX) were applied to each row. The clay-loam soil had a pH ranging from 5.9-6.2 within the field. Twelve of 24 rows in each replication (block) were randomly assigned to be covered by black, 0.0254-mm plastic mulch. Each treatment combination was replicated three times for a total of 72 0.9 × 6-m subplots. A 3-m gap was provided between subplots.

FIGURE 1
Steaming system used in the experiment with a permeable nylon sleeve placed over the center of each plot **(A)** and covered with a heat-resistant, three-ply, 0.1524-mm tarp with reinforced scrim with chains placed around the perimeter of the tarp to hold the tarp and prevent steam loss through the sides of the tarp **(B)**.

The tomato cultivar Roadster was used throughout the study. It is marketed as a determinate fresh market, slicing tomato with 227 to 340 g of fruits. The tomato transplants were generated by sowing seeds into 72-cell plug trays filled with soilless potting media (Pro-mix BX; Rivière-du-Loup, Quebec, Canada) and grown in a greenhouse. Tomato transplants developed at least four true leaves at the time of transplant. Six tomato plants were planted 61 cm apart in each subplot in May 2021 and 2022.

Weed cover per plot (0.9 × 6-m) were collected based on the visual ground cover (%) ("weed cover" hereafter) of each weed species 10 weeks after transplanting (WAT) and rated using the scale 0 to 100%, where 0% is no weed present, and 100% is complete ground cover within the plot by weed. The weed cover was measured once for the whole plot at 10 WAT. The height (cm) of the tomato plants was measured using a ruler from the base of the plant to the tip of the uppermost leaf at 10 WAT. Tomato height was based on average of three plants per plot. Tomato were harvested from all of the plants and combined from each subplot before they were weighted (i.e., we weighed the fruits as a group and not as individual plants) and graded as marketable or unmarketable, and the total yield (t/ha) based on fresh weight was determined.

All results were run through LS-means in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. Data from the two years of the experiment were pooled if there was no significant experimental effect.

Results

Weed cover

Cover crops on weed cover

Cover crop treatments did not significantly differ in weed emergence compared to fallow treatment in both years. Although not statistically significant, hairy vetch in 2021 and crimson clover in 2022 resulted in the lowest weed cover.

Mulching on weed cover

We found no significant interactions between mulching and year (Table 1). Nevertheless, the mean cumulative 2-year weed cover (%) in mulched treatment significantly affected weed emergence ($P < 0.05$) compared to non-mulched treatment (Figure 2). Plastic mulch reduced weed cover by almost 50% compared with no mulch (Figure 2). Although the use of plastic mulching was not significant for Palmer amaranth and barnyardgrass, a significant improvement in the control of yellow nutsedge and large crabgrass population was found under mulch application. However, among the weed species, the cover of yellow nutsedge was the highest, followed by large crabgrass under mulched and non-mulched treatments. In the presence of mulching, the cover of yellow nutsedge was reduced by 50%, while the cover of large crabgrass was decreased by 64% (Figure 3).

Steaming on weed cover

The use of steaming significantly reduced the population of different weed species in both years (Table 1). In 2021 and 2022, the use of steaming at 5 and 20 minutes significantly reduced ($P < 0.05$) large crabgrass compared to 0 minutes. However, soil steaming did not significantly affect the population of barnyardgrass, and Palmer amaranth in either 5 or 20 minutes compared to 0 minutes. The major weed species in both years was yellow nutsedge. The 5- and 20-min steaming was less effective in controlling yellow nutsedge than unsteamed plots (Figure 4).

Steaming and mulching on weed cover

Regardless of soil steaming, the non-mulched treatment resulted in the highest weed cover compared to the mulched treatment. The highest weed cover was that of large crabgrass (63%) and yellow nutsedge (52%) at 0 and 20 mins of steaming, respectively, in the absence of mulching. In the presence of mulching, these values were reduced to 23 and 25%, respectively (Figure 5).

Yellow nutsedge cover was significantly higher at 5 and 20 minutes of steaming than 0 minutes, regardless of mulching. The presence and absence of mulching at 20 minutes increased the yellow nutsedge cover by 51 and 74%, respectively, compared to 0

TABLE 1 Analysis of variance for all the study parameters with respect to weed cover (%).

Parameter	Year	Degree of freedom		Effects Tests		
		Treatment	Year x Treatment	Mean Squares	F-Ratio	Treatment p-values
Year	2	1	2	514.3600	28.3494	< 0.001*
Steaming	2	2	4	2697.3700	14.1233	< 0.001*
Year*Steaming	2	2	4	123.7900	0.6482	0.5236
Cover crop	2	3	6	356.3800	1.8660	0.1349
Year*Cover crop	2	3	6	182.1800	0.9539	0.4146
Steaming*Cover crop	2	6	12	136.7800	0.7162	0.6368
Year*Steaming*Cover crop	2	6	12	103.1200	0.5399	0.7778
Mulching	2	1	2	9103.1200	47.6636	< 0.001*
Year*Mulching	2	1	2	229.2900	1.2000	0.2739
Steaming*Mulching	2	2	4	86.5500	0.4532	0.6360
Year*Steaming*Mulching	2	2	4	662.2900	3.4677	0.0322*
Cover crop*Mulching	2	3	6	119.7400	0.6270	0.5980
Year*Cover crop*Mulching	2	3	6	53.4000	0.2796	0.8401
Steaming*Cover crop*Mulching	2	6	12	85.6100	0.4482	0.8462
Year*Steaming*Cover crop*Mulching	2	6	12	57.2100	0.2995	0.9369

Parameters or interactions indicated by an asterisk (*) are significantly different ($P < 0.05$).

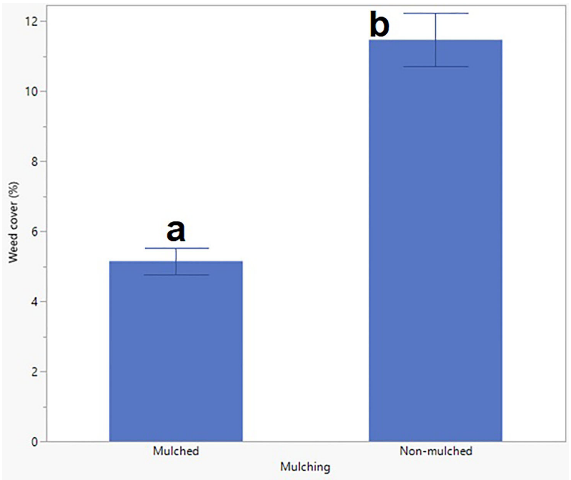

FIGURE 2
Mean cumulative 2-year weed cover (%) in mulched and non-mulched treatment collected based on the overall plot cover of each weed species 10 weeks after transplanting (WAT) and rated using the scale 0 to 100%, where 0% is no weed present, and 100% is complete coverage. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. Means followed by the same letter are not significantly different (*P* < 0.05).

minutes of soil steaming (Figure 5). An increase in the duration of soil steaming decreased large crabgrass cover and was lower in the presence of mulching. On the other hand, barnyardgrass and Palmer amaranth cover were not affected by steaming or mulching (Figure 5).

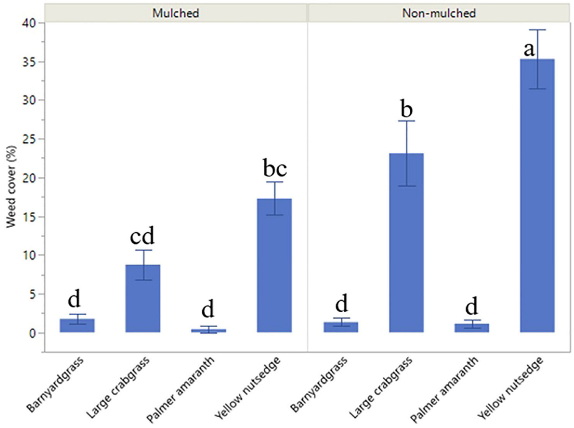

FIGURE 3
Mean cumulative 2-year weed cover (%) by weed species in mulched and non-mulched treatment collected based on the overall plot cover of each weed species 10 weeks after transplanting (WAT) and rated using the scale of 0 to 100%, where 0% is no weed present, and 100% is complete coverage. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. Means followed by the same letter are not significantly different (*P* < 0.05).

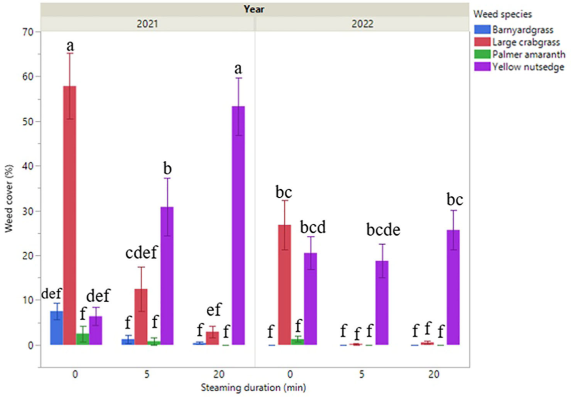

FIGURE 4
Mean weed cover (%) by weed species under soil steaming treatments of 0, 5, and 20 minutes among the year 2021 and 2022 collected based on the overall plot coverage by each weed species 10 weeks after transplanting (WAT) and rated using the scale 0 to 100%, where 0% is no weed present, and 100% is complete coverage. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. Means followed by the same letter are not significantly different (*P* < 0.05).

Tomato plant height

None of the parameters and their interactions were significantly different (P < 0.05) with respect to the heights of the tomato plants, except for steaming (Table 2). There was a considerable height increase (*P* < 0.05) in tomato plants under different steaming

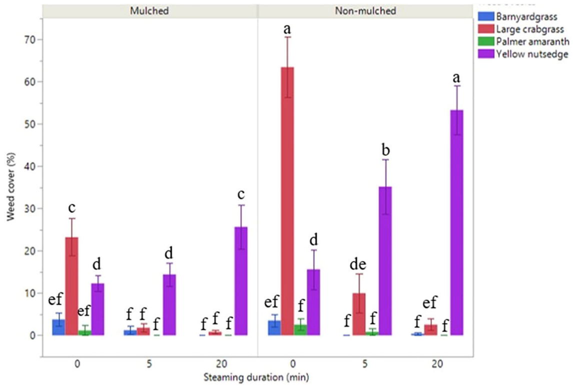

FIGURE 5
Mean cumulative 2-year weed cover (%) at different combinations of mulching and soil steaming treatment durations (0, 5, and 20 min) collected based on the overall plot coverage by each weed species 10 weeks after transplanting (WAT) and rated using the scale 0 to 100%, where 0% is no weed present, and 100% is complete coverage. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. Means followed by the same letter are not significantly different (*P* < 0.05).

TABLE 2 Analysis of variance for all the study parameters with respect to tomato plant height (t/ha).

Parameter	Year	Degree of freedom		Effects Tests		
		Treatment	Year x Treatment	Mean Squares	F-Ratio	Treatment p-values
Year	2	1	2	3101.7800	18.9300	0.0035
Steaming	2	2	4	2528.8900	15.4300	< 0.0001*
Year*Steaming	2	2	4	1214.4200	7.4100	0.0090
Cover crop	2	4	8	67.7900	0.3700	0.6890
Year*Cover crop	2	4	8	322.1800	1.7700	0.1750
Steaming*Cover crop	2	8	16	19.0300	0.1000	0.9800
Year*Steaming*Cover crop	2	8	16	25.8800	0.1423	0.9660
Mulching	2	1	2	114.6860	0.7000	0.4043
Year*Mulching	2	1	2	95.8200	0.5849	0.4458
Steaming*Mulching	2	2	4	15.0360	0.0918	0.9124
Year*Steaming*Mulching	2	2	4	105.3100	0.6428	0.5274
Cover crop*Mulching	2	4	8	202.4500	1.1134	0.3320
Year*Cover crop*Mulching	2	4	8	157.2600	0.8649	0.4240
Steaming*Cover crop*Mulching	2	8	16	57.4100	0.3157	0.8660
Year*Steaming*Cover crop*Mulching	2	8	16	62.5600	0.3441	0.8470

Parameters or interactions indicated by an asterisk (*) are significantly different ($P < 0.05$).

durations. At 10 WAT, plants were 12 and 13 cm taller in 5 and 20 min of steaming treatments, respectively, than plants that did not receive any soil steaming (Figure 6).

Tomato yield

The cover crop treatments did not result in yield differences from fallow treatment in both years. However, the interaction between steaming and year was significant (Table 3). The yield was similar regardless of mulching treatment in both years; however, on average, mulched plots produced 10 and 8 t/ha greater yield than non-mulched plots in 2021 and 2022 (Figure 7). Among the mulched and non-mulched treatments in 2021, longer soil steaming duration increased the total yield ($P < 0.05$). In mulched plots, 5 and 20 min of soil steaming produced 101 and 103 t/ha, respectively, while at 0 min, the yield was only 39 t/ha. Similar results were found in non-mulched plots where 5- and 20-min soil steaming produced 94 and 108 t/ha tomato fruits, which were higher than 0 min of soil steaming (4 t/ha; Figure 8). In 2022, plastic mulching resulted in a significantly higher yield when 5 and 20 min of soil steaming were applied; the yield increased by 58 and 64 t/ha, respectively, compared with 0 min of soil steaming. Additionally, yields in the absence of mulching were significantly higher at 5 and 20 min of soil steaming (54 and 60 t/ha, respectively) than at 0 min of steaming (7 t/ha; Figure 8).

Marketable fruit yields were significantly greater at 5 and 20 min (30 and 37 t/ha, respectively) than at 0 min (10 t/ha) of soil steaming in mulched treatment. The yield was higher in mulched treatments and was 0.37, 47, and 53 t/ha at 0, 5, and 20 min, respectively, compared to non-mulched (Figure 9). These results are consistent with the total number of cull fruits reported in this experiment. The total number of cull fruits at 5

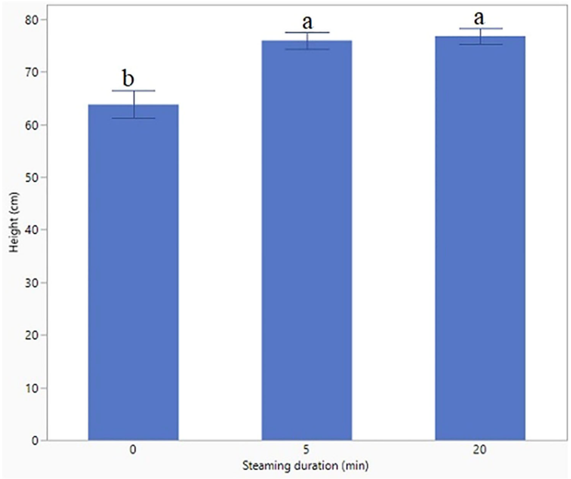

FIGURE 6
Mean cumulative 2-year height (cm) of tomato plants at 0, 5, and 20 min of soil steaming. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of $\leq$ 0.05. Means followed by the same letter are not significantly different ($P < 0.05$).

TABLE 3 Analysis of variance for all the study parameters with respect to total yield (t/ha).

Parameter	Year	Degree of freedom		Effects Tests		
		Treatment	Year x Treatment	Mean Squares	F-Ratio	Treatment p-values
Year	2	1	2	28818.9000	82.3400	< 0.001*
Steaming	2	2	4	62448.2800	178.4400	< 0.001*
Year*Steaming	2	2	4	5150.7500	14.7100	< 0.001*
Cover crop	3	3	9	556.9300	1.5900	0.1965
Year*Cover crop	3	3	9	406.4600	1.1600	0.3286
Steaming*Cover crop	6	6	36	607.9400	0.2800	0.9406
Year*Steaming*Cover crop	6	6	36	350.0700	1.0000	0.4298
Mulching	2	1	2	4154.1000	11.8600	0.0008*
Year*Mulching	2	1	2	159.1200	0.4547	0.5017
Steaming*Mulching	2	2	4	2719.8400	7.7700	0.0007*
Year*Steaming*Mulching	2	2	4	346.2300	0.9800	0.3756
Cover crop*Mulching	3	3	9	200.5500	0.5731	0.6341
Year*Cover crop*Mulching	3	3	9	873.0500	2.4900	0.0600
Steaming*Cover crop*Mulching	6	6	36	252.2900	0.7200	0.6330
Year*Steaming*Cover crop*Mulching	6	6	36	226.6500	0.6400	0.5919

Parameters or interactions indicated by an asterisk (*) are significantly different ($P < 0.05$).

and 20 min soil steaming was higher than 0 min for both mulched and non-mulched treatment.

Statistical analysis identified significant differences in the marketable, cull, and total tomato yield ($P < 0.05$). For all treatments that included 5 or 20 min of soil steaming, the marketable, cull, and total yield were significantly greater than the non-steamed treatments (Figure 9).

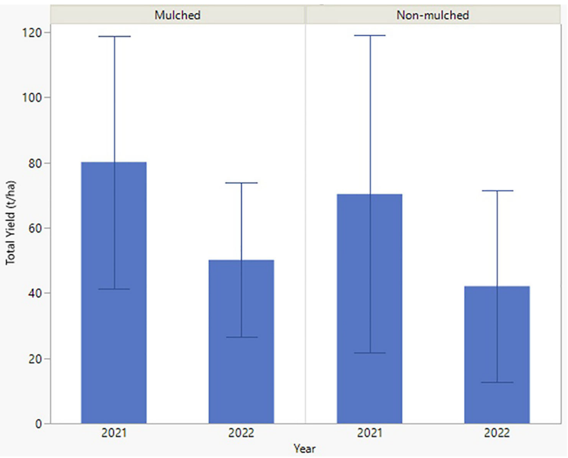

FIGURE 7
Total yield (t/ha) of marketable and cull fresh fruits in response to plastic mulching and absence of plastic mulch. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. In 2021 and 2022, mean totals were not significantly different (P = 0.35 and 0.20, respectively).

Discussion

Some weed species are impacted more than others by cover crops. From the present research, the effect of the winter cover crop did not significantly reduce weed populations. Similar results

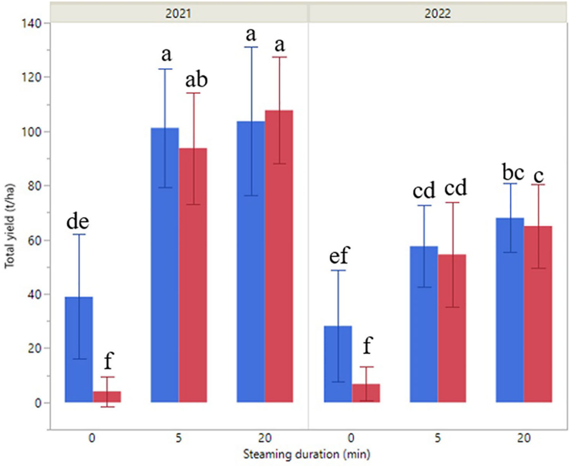

FIGURE 8
Total tomato yield (t/ha) in response to plastic mulching and different durations of soil steaming. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.05. In 2021 and 2022, mean totals were significantly different ($P < 0.05$) compared to 0 min of soil steaming. Means followed by the same letter are not significantly different (P < 0.05).

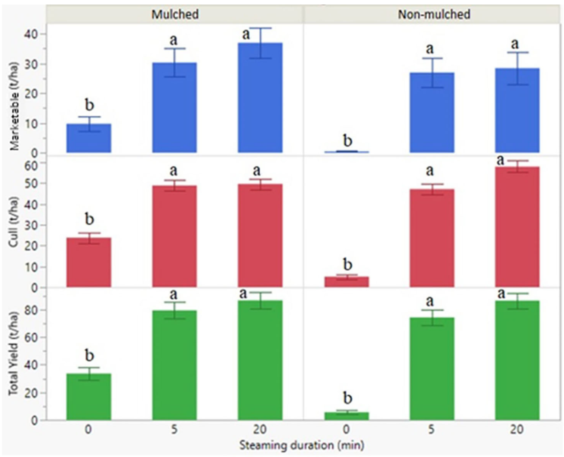

FIGURE 9
Cumulative marketable, cull, and total fruit yields (t/ha) in response to plastic mulching and different durations of soil steaming. The error bars indicate the standard error of three measurements. All results were run through LS-method in JMP Pro 16.1 (SAS Institute Inc., Cary, NC, USA). For data that met the assumptions for ANOVA, treatment means were separated using the Tukey's test at an alpha level of ≤ 0.001. Means followed by the same letter are not significantly different (*P* < 0.05).

were observed by Mennan et al. (2009), where winter cover crops did not considerably reduce weed cover over the years. Some cover crop species have a lower potential to control weeds due to properties such as seed size, vigor, time of establishment, and weed species characteristics (Lee, 1985; Mennan et al., 2009). On the contrary, some winter cover crops such as wheat and sorghum have been associated with weed suppression, especially in fallow season. Additionally, cover crop biomass is often correlated with weed biomass reduction (Mennan et al., 2009). The lack of weed control in our study may be attributed to cover crop biomass production being inadequate to affect the weeds adversely. The unavailability of cover crop and weed biomass data could be a possible limitation in our study. As such, we cannot confidently attribute insufficient weed control to cover crop biomass. Weed control is one indirect benefit within the many benefits to improve crop production, such as soil and water conservation and nutrient cycling that, in the long term, enhances the ecosystem dynamics (Ghimire et al., 2018).

Plastic mulch to manage weeds positively correlated with effective weed suppression. Mulching resulted in a more than 40% reduction in weed cover. Rajablariani et al. (2012) similarly reported that blue plastic mulch provides a 40% reduction in weed cover. Anzalone et al. (2010) found barley straw, considered a biodegradable mulch, reduced the cover of weeds by up to 40% in tomato. Mulch treatment promoted a significant reduction in yellow nutsedge and large crabgrass cover. Although not significant, mulching was able to reduce the cover of Palmer amaranth. Plastic mulching reduces the cover of most weed species. It is a common production practice in intensive vegetable production systems because it increases soil temperature, improves water management, decreases the growth of different weed species, and improves the use of nutrients in plants (Bond and Grundy, 2001; El-Beltagi et al., 2022).

In most cases, weed control in combination with soil steaming and mulching was better than in the absence of these treatments (Figure 5). Although not statistically different, barnyardgrass and Palmer amaranth showed lower weed cover in the presence of mulch at 5 and 20 minutes of steaming; however, steaming for 20 min resulted in complete weed suppression (100% for barnyardgrass and Palmer amaranth). Data presented in Figure 5 revealed that steaming the soil for 20 min significantly decreased the large crabgrass population for mulched and non-mulched treatment (100 and 96% weed suppression, respectively). Thi Melander and Jorgensen (2005) observed a 90% reduction in weed emergence when the soil temperature reached 61°C. Loenen et al., 2003 showed that steaming treatment at around 60°C for 3 min, followed by 8 min resulted in 100% control of common lambsquarters (*Chenopodium album* L.), an annual species. Our study also included three annual species: barnyardgrass, Palmer amaranth, and large crabgrass. The combination of mulching and steaming was ineffective against yellow nutsedge at 5 and 20 min. Since the yellow nutsedge plants can easily puncture plastic mulch, this control strategy is not useful in nutsedge control (Brandenberger et al., 2005). Nutsedge tubers cannot survive temperatures of 60°C (Rubin and Benjamin, 1984). Yet, nutsedge has been reported to survive soil temperatures of 80°C for 30 min. A likely explanation is that while shallower tubers cannot survive these temperatures, deeper rhizomes can escape fatal temperatures found in the upper layers of soils being steamed.

The success of the soil steaming usually depends on the temperature, duration of steaming, and the soil depth reached (Gelsomino et al., 2010). Here, the target temperature of 61°C was achieved and maintained for 5 and 20 min in separate treatments at a 4-in depth. Fennimore et al. (2014) found that the profile of weeds susceptible to steam corresponded to their seed coat permeability, morphology, and status of weed seeds (dormancy strategies) which would decrease the germination activity. According to Ascard (1995), the ability to produce new growth from vegetative and reproductive structures could explain the higher tolerance attributed to steaming. Gourd et al. (2002), in his research, found that soil steaming provided poor control (50%) of dandelion (*Taraxacum officinale* L.) species, which is also a perennial species like yellow nutsedge. In contrast, he showed that steaming effectively controlled (85%) downy brome (*Bromus tectorum* L.), an annual weed like barnyardgrass, large crabgrass, and Palmer amaranth. He also reported in his research that drought conditions could impact the steaming effectiveness since the steam was applied at three different times under different humidity conditions of 13, 28, and 62%, respectively. The low humidity may have decreased the benefit of steam applications. In general, steaming can effectively suppress numerous annual weed species; but perennials, some large weeds, and a few grass species may be tolerant to this method (Banks and Sandral, 2007). For some weed species, a higher soil temperature reduces seed viability more than the extended duration of steaming (Thompson et al., 1997). This is likely because the deeper roots can escape the hottest steam temperatures. For example, nutsedge tubers can grow several inches below the soil's surface, likely making it resistant to this method of soil steaming. The results found in the second year of the present experiment showed a higher yellow nutsedge population even in plots that received 20 min of steaming.

The use of mulching had no significant effect on tomato height. However, a significant enhancement was noted at 5 and 20 min of steaming, which resulted in taller plants than plots that were not steamed. The increase in plant height under steaming may be due to a lower weed cover in the plots, reducing resource competition between the crop and weeds, and conserving soil moisture (Hussain et al., 2022). Additionally, soil treated with steaming enhanced nutrient supplementation and nutrient delivery to the crop. For instance, exchangeable and soluble manganese, nitrite, and ammonia were released in the strawberry experiment after treatment with steam above 65°C (Fennimore et al., 2014).

The highest yield of fresh tomato weight per unit area was obtained from 5 and 20 min of soil steaming in both non-mulched and mulched treatments, while the lowest was observed in control plots. Soil steaming, therefore, had the most substantial influence on tomato yield. Zangoueinejad et al., 2018, found that the highest yield per unit area was obtained from a plastic mulch (20.93 kg m-2), while the lowest (1.24 kg m-2) was observed in the control plots without plastic mulch. In onions, yield increased by up to 300% using plastic mulch (Barla and Upasani, 2019). Our results show that the combination of steaming and plastic mulching severely impaired weed competition and increased tomato yield by 136 and 158% at 5 and 20 min, respectively, than 0 min of steaming. The severity of weed competition depends on weed cover, growing conditions, and agricultural practices employed (Zimdahl, 1980; Qasem, 1992). The critical period for weed suppression begins at the flowering stage and goes through fruit ripening (Rahimian and Shahriay, 2002). The presence of weeds in these periods can affect the yield parameters and fruit quality of the crop. Steaming has been reported to increase yield in processing tomato and effectively control weeds such as common lambsquarters (*Chenopodium album* L.) (Loenen et al., 2003).

A major concern of soil steaming for weed control is the cost. According to Luvisi et al. (2008), applying steaming averages $1,975 per hectare, which can be expensive when the entire field is treated. Although steaming is costly because of its high fuel and labor requirement (Peruzzi et al., 2017), recent studies have explored ways to improve soil steaming methods to reduce costs, such as the band steaming prototype created at the University of Pisa, Italy (Raffaelli et al., 2016; Guerra, 2022). The maximum use of steam in this study resulted in reduced weed biomass (up to 74%) and increased carrot yield compared to untreated plots (Raffaelli et al., 2016). Chemicals to control weeds are generally more cost-effective than non-chemical control methods. Several herbicides are labeled in tomato, such as S-metolachlor, fomesafen, metribuzin (Met), and halosulfuron that are applied as pre-and post-transplant in tomato production. However tomato is sensitive to herbicides such as metribuzin, and may cause severe injury resulting in the fruits becoming unfit for consumption (Kemble 2014; Mohseni-Moghadam and Doohan, 2017). Additionally, improper herbicide applications may result in drift causing environmental concerns and human health risks or destroy the entire crop production with invaluable losses (Hart and Pimentel, 2002; Papadakis et al., 2015; Mac Loughlin et al., 2022).

Conclusion

The integrated use of mulch and steam effectively decreased the weed cover, which resulted in higher tomato yield in these treatments. In particular, the combination of 5 and 20 minutes with plastic mulching was more effective in increasing tomato yield than in the absence of mulching and soil steaming. The use of plastic mulching was effective in reducing most weed species in this study. Additionally, the use of mulch did not show any adverse effect on tomato yield components, while soil steaming improved the tomato yield by up to four times. On the other hand, cover crops did not reduce weed cover compared to the fallow system. Therefore, an integrated approach to mulching combined with soil steaming is recommended for effective weed control.

Although steaming may not be a cost-effective strategy for controlling weeds, it may be more sustainable in the long run. Incorporating soil steaming, cover crops, and mulching may result in the application of lower herbicide rates, which is good for the environment. Lower rates of herbicides will also discourage the selection of herbicide-resistant weed populations, thus decreasing the risk of herbicide-resistance evolution. For future research, it will be crucial to measure additional parameters such as weed biomass, cover crop biomass, weed and tomato photosynthetic efficiencies, and soil properties to further assess the association of weed cover reduction with these parameters, if any.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

TT and SB designed the experiment. TO, BB, and AS conducted the experiment. AM, KB, VS, WS, JA, assisted with taking plant measurements; TO, SB, and TT conducted the data analysis; TO and TT wrote the original version of this paper. TT, SB, and TO provided technical expertise. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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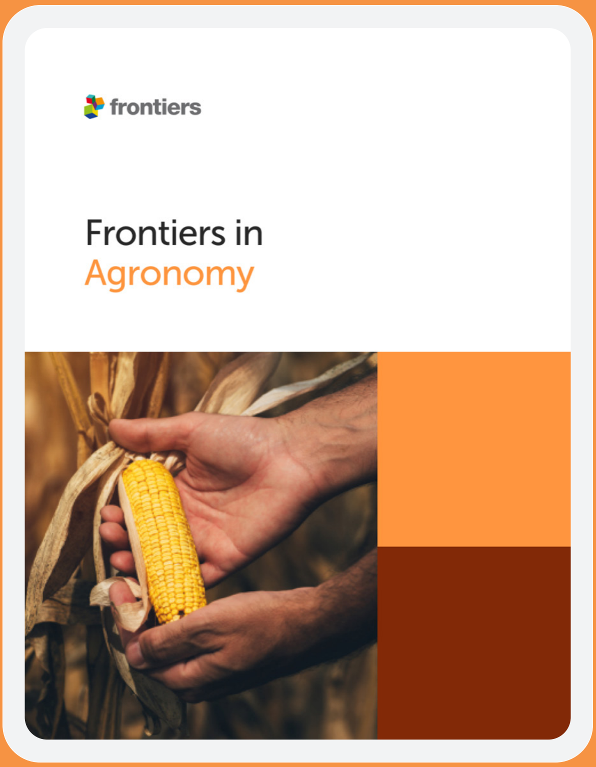

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