Weed community composition in simple and more diverse cropping systems

Abstract

Weed communities in three cropping systems suitable for the Midwestern USA were studied from 2017 through 2020 to examine how diversified cropping systems 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.) was diversified with cool-season crops, namely oat (*Avena sativa* L.), red clover (*Trifolium pratense* L.), and alfalfa (*Medicago sativa* L.) in 3-year and 4-year systems. Herbicide was not applied in oat, red clover, and alfalfa. The reduction in the mass of herbicide active ingredients applied in the 3-year and 4-year systems was associated with increased weed stand density, aboveground mass, and community diversity, but did not cause crop yield loss. The addition of the cool-season crops into the cropping system did not affect densities of emerged weeds but did affect weed growth. 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, diversity, evenness, richness, cropping system diversification, Midwestern USA

Introduction

The composition of weed communities found in agricultural fields is strongly affected by the types of crop grown and their attendant management practices (Mohler, 2001). The U.S. 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 woolly cupgrass (*Eriochloa villosa* (Thunb) Kunth) (Owen, 2008; Kruger et al., 2009; Reddy and Norsworthy, 2010). Aboveground composition of weed communities is the response of the seedbank to weather conditions, crops, and crop management practices (Légère et al., 2005; Culpepper, 2006; Smith and Gross, 2007). Improved understanding of how management practices influences weed community composition can inform weed managers whether crop losses to weed competition are likely to occur and whether the weed community is shifting toward dominance by species that are more or less 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; Tamburini et al., 2020; Bowles 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). 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-year 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.

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 (Storkey and Neve, 2018). Liebman et al. (2021) provided empirical evidence to support the hypothesis that seedbank diversity could be used as an indicator of cropping system sustainability as proposed by Storkey and Neve (2018). 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).

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 (2-year rotation with broadcast herbicide for weed control). 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

«««< HEAD 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 were provided in Nguyen and Liebman (in review). Briefly, a randomized complete block, split-plot design with four replications were used to study three different crop rotation systems (2-year, 3-year, or 4-year). The crop sequence in each rotation was presented in Nguyen and Liebman (in review). The main-plot factor ('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 effect, i.e., weed management regime applied in the corn phase (corn weed management), was represented by herbicide level (conventional - broadcast over the whole corn area, or low - banded 38 cm wide on top of corn rows). Details concerning crop genotypes and weed management regimes are provided in Table 1.

======= 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 were provided in Nguyen and Liebman (in review). Briefly, a randomized complete block, split-plot design with four replications were used to study three different crop rotation systems (2-year, 3-year, or 4-year). The crop sequence in each rotation was presented in Nguyen and Liebman (in review). The main-plot factor ('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, O4: oat in the 4-year rotation, and A4: alfalfa in the 4-year rotation). The split-plot effect, i.e., weed management regime applied in the corn phase (corn weed management), was represented by herbicide level (conventional - broadcast over the whole corn area, or low - banded 38 cm wide on top of corn rows). Details concerning crop genotypes and weed management regimes are provided in Table 1. »»»> fd81336defee112302aadeef9eb15831252ae278

Table 1: Crop variety or hybrid and management from 2017 to 2020

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	May 9	May 9	May 16	
	Interrow cultivation date	Jun. 7	Jun. 7	none	none
	Harvest date	Oct. 19	Oct. 19	Oct. 19	
	Herbicides applied (kg ai./ha)	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	May 8	May 8	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**
	Harvest date	Nov. 6	Nov. 6	Oct. 18	Oct. 18
	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	May 13	May 13
	Interrow cultivation date	Jun.8	none	none	none
	Harvest date	Oct. 2	Oct. 2	Sep. 23	Sep. 23
	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
	Weed sampling date	Sep. 14 and 15	Sep. 14 and 15	Sep. 16	Sep. 16

Weeds were not sown experimentally. 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 excluded from the assessment of weed community composition. Data were collected for individual species aboveground mass and density, total weed biomass and density, and crop yield. Weeds were surveyed four to six weeks before corn and soybean harvests, two to three 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 per an identification guide developed by Uva et al. (1997).

Weed total density and aboveground mass Weed aboveground samples were collected from eight quadrats arranged in a 4x2 grid throughout each experimental unit (eu). The total surveyed area was 18.5 m^2/eu in corn and soybean (8 x 3.05 m^2) and 2.2 m^2/eu (8 x 0.25m^2 or 8 x 0.28m^2) in oat and alfalfa. Plants were identified to species.

Weed individual species relative and absolute abundance All the same-species plants from an eu were enumerated, dried, and weighed together to make single data points per eu. Individual species density and aboveground mass were presented for each crop identity to illustrate the community composition in each crop phase.

Ecological indices Aboveground weed mass reflects species competitiveness and density represents population size. Both species aboveground mass and density were used to calculate species diversity, evenness, and richness in each eu. Simpson's diversity, evenness, and richness indices were calculated in terms of stand density and aboveground mass. 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 ith species (plants m^{-2}),
N represent density of all presented species (plants m^{-2}),
b_i represent aboveground mass of the ith species (kg m^{-2}),
B represent aboveground mass of all species, kg m^{-2}, and
p_{i_d} and p_{i_b} represent the proportional of density or aboveground biomass of the ith 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, for oat and alfalfa, whole plots were harvested (two adjacent subplots combined, 1530 m^2). Yields were adjusted to moisture concentrations of 155 g H_2O kg^{-1} for corn, at 130 g H_2O kg^{-1} for soybean, at 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 the 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 meet homogeneity of variance requirement. Type III sums of squared error were calculated with the `emmeans` package's `joint_tests` function to accommodate unbalanced data with interaction (version 1.7.1-1, Lenth et al., 2021). The data were ln-transformed by adding the minimum non-zero value for each response to all values before analysis, but 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, total aboveground density, total aboveground mass, single species density, and single 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., 2021) 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 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_{ijklm} 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-year, 3-year, and 4-year) and herbicide regime in corn (low or conventional). With this model, we tested the following sets of hypotheses:

1) Weed stand diversity, stand evenness, stand richness, aboveground mass diversity, aboveground mass evenness, aboveground mass richness, total aboveground density, and total aboveground mass increased as cropping system diversity increased.

2) Weed stand diversity, stand evenness, stand richness, aboveground mass diversity, aboveground mass evenness, aboveground mass richness, total aboveground density, and total aboveground mass in the same crop species differed between cropping system .

3) Weed stand diversity, stand evenness, stand richness, aboveground mass diversity, aboveground mass evenness, aboveground mass richness, total aboveground density, and total aboveground mass in the same crop species differed between different crop types within the a given cropping system.

4) Weed single species density and aboveground mass of the most abundant species differed between rotations in the same crop species, differed across rotations, and differed between crop type within a given cropping system. 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.

The first set of hypotheses was tested by contrasting the responses in the 2-year rotation with those in the average of the 3-year 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 across rotations, in the cool season crops across rotations, in the warm season versus 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, total weed aboveground mass, and total weed stand density were applied to data concerning the seven most abundant weed species. The fourth set of hypotheses was tested by contrasting individual weed species density and aboveground mass a) in the 2-year rotation versus the average of 3-year and 4-year rotations and in the 3-year versus 4-year rotation, b) in the same crop species or type across rotations, c) in different crop types within the same rotation, and d) in different crop types averaged over rotations.

A different set of linear mixed-effects model was used to analyze corn, soybean, and oat yields (`lme4` version 1.1-27.1, Bates et al., 2021):

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

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 hypotheses 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 versus 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 the Data Repository.

How did rotation system and corn weed management affect crop yields? Results of the experiment supported the first hypothesis that "diverse cropping systems, with reduced use of chemical herbicides, would provide weed control equal in effectiveness to the conventional approach in the 2-year rotation". Averaged over four years, soybean was the only crop whose yield was significantly affected by rotation (p-value = 0.0185, Table 2). Soybean yield was 16% higher in the 4-year rotation than in the 2-year rotation (p-value = 0.018). Crop yields in the experiment relative to averages for the state of Iowa and Boone County, where the experiment occurred, are presented in Figure 1. Corn weed management regime did not significantly affect crop yields (Table 2).

How did rotation system, crop species, and corn weed management affect community ecological indices? Crop identity (i.e., rotation system x crop phase combination) significantly affected weed aboveground mass diversity (p-value = 0.0007, Table 3A), evenness (p-value = 0.0003, Table 3B), and richness (p-values = 0.013); and stand density evenness (p-value = 0.0064) and richness (p-value = 0.0123, Table 3C). The ecological indices in each crop phrase, averaged over blocks, years and corn weed management are shown in Figure 2. The results of contrasts for the effects of rotation systems, rotation system within individual crops, and crop types on community ecological indices are shown in Tables 4 and 5. For all the significantly differences in ecological indices, crop types were more influential than rotations, with larger differences found across crop types than across rotations.

Table 2: Contrasts of rotation effect (expressed by Crop identity) on crop yields. 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 occured.

ANOVA					Comparison		
Source of variation	df1	df2	F.value	p.value	contrast	ratio	p.value
(A) - Corn							
Crop ID	2	6	3.190	0.1138	C2 vs C3	0.938	0.1882
Corn weed management	1	3	0.324	0.6088	C2 vs C4	0.929	0.1278
Crop ID x Corn weed management	2	6	2.205	0.1914	C3 vs C4	0.990	0.9507
(B) - Soybean							
Crop ID	2	6	8.224	0.0191	S2 vs S3	0.959	0.5499
Corn weed management	1	3	0.178	0.7018	S2 vs S4	0.862	0.0181
Crop ID x Corn weed management	2	6	0.623	0.5677	S3 vs S4	0.898	0.0670
(C) - Oat							
Crop ID	1	2	1.138	0.3979	O3 vs O4	0.913	0.3979

Note: Corn weed management: low herbicide or conventional. Crop ID: crop species and the cropping system to which each belong: 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.

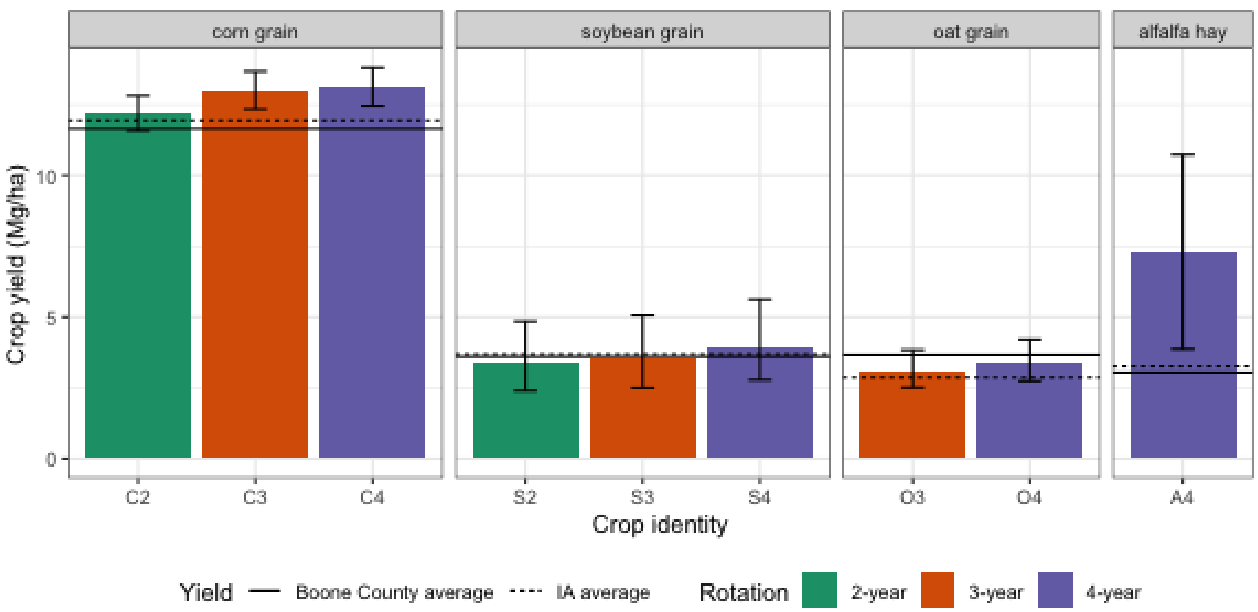

Figure 1: Mean crop yields by rotation from 2017 to 2020. The color-coded bars show crop yields (Mg ha^{-}1) in the experiment plots. The error bars show the 95% confidence intervals. The solid horizontal lines show mean yields for Iowa and dashed lines show mean yields for Boone County. Corn, soybean, and alfalfa yields in the experiment were averaged over four 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.

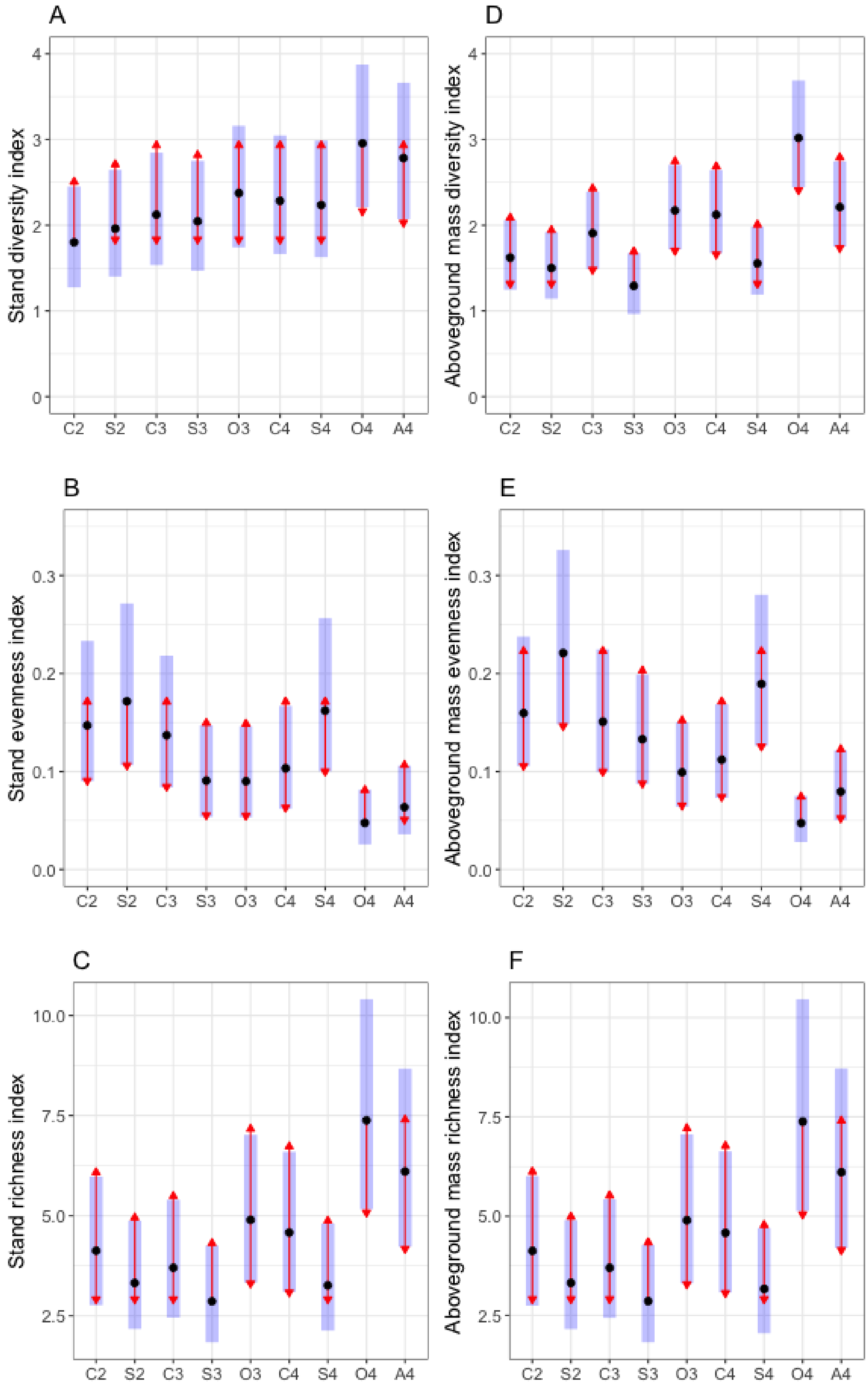

Figure 2: Weed community stand diversity (A), evenness (B), and richness (C) and community aboveground diversity (D), evenness (E), and richness (F). The abbreviations on the x-axis are crop identities, which are the combinations of the first letter in crop species names and the rotation in which it occured (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

Table 3: ANOVAs of crop identity, corn weed management, and their interactive effects on weed community ecological indices

			Stand density		Aboveground mass	
Source of variation	df1	df2	F.value	p.value	F.value	p.value
(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

Note: Corn weed management: low herbicide or conventional. Crop ID: crop species and the cropping system in which it occured: 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.

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 stand diversity index for the 3-year and 4-year rotation systems was comparable with that in the 2-year rotation (p-values = 0.0535 and 0.1575). For the individual crops (Table 4B), the weed stand density diversity index was comparable among rotations (p-values > 0.05). For different crop types (Table 4C), the weed stand density diversity index was significantly different between the average for the cool season crops (O3, O4, and A4) and the average for the warm season crops (C2, S2, C3, S3, C4, and S4) (p-value = 0.0145), but similar between the warm season and cool season crops in the same rotations (p-values = 0.4666 and 0.0987). The weed stand density diversity index was similar between oat and alfalfa (p-value = 0.7762).

Averaged over crop phases within the same rotation (Table 5A), the weed aboveground mass diversity index was significantly different between the 2-year rotation and the average of the 3-year and 4-year rotations (p-value = 0.0148), and between the 3-year and 4-year rotations (p-value = 0.0209). Averaged over the corn and soybean phases within the same rotation (Table 5A), the weed aboveground mass diversity index was similar between rotations (p-values = 0.4217 and 0.2426). For the individual crops (Table 4B), the weed aboveground mass diversity index was comparable across rotations, except for oat (p-value = 0.0351). For different crop types (Table 4C), the weed aboveground mass diversity index was significantly different between the cool season crops and warm season crops averages (p-values < 0.0001) and between the cool season and warm season crops within the same rotation (p-values = 0.034 and 0.0037). The weed aboveground mass diversity index was comparable between oat and alfalfa (p-value = 0.2583).

The hypothesis that "weed communities in the more diverse cropping systems are more even" was partially supported (Figure 2B and 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 (Figure 3C and D).

Averaged over crop phases within the same rotation (Table 4A), the weed stand density evenness index was significantly different between the 2-year rotation and the average of the 3-year and 4-year rotations (p-value

= 0.006), but comparable between the 3-year and 4-year rotations (p-value = 0.2802). Averaged over the corn and soybean phases within the same rotation (Table 4A), the weed stand density evenness index was comparable between rotations (p-values = 0.1539 and 0.5031). For the individual crops (Table 4B), the weed stand density evenness index was comparable between rotations (p-values > 0.05). For different crop types (Table 4C), the weed stand density evenness index was significantly different between the cool season crops average and the warm season crops average (p-value = 0.0002) and between the cool season and warm season crop in the 4-year rotation (p-value = 0.0012), but similar between the warm season and cool season crops in the 3-year rotation (p-values = 0.4418). The weed stand density evenness index was comparable between oat and alfalfa (p-value = 0.8986).

Averaged over crop phases within the same rotation (Table 5A), the weed aboveground mass evenness index was significantly different between the 2-year rotation and the average of 3-year and 4-year rotations (p-value = 0.0012), but similar between the 3-year and 4-year rotations (p-value = 0.0802). Averaged over the corn and soybean phases within the same rotation (Table 5A), weed aboveground mass evenness index was comparable between rotations (p-values = 0.1081 and 0.8682). For the individual crops (Table 4B), the weed aboveground mass evenness index was comparable across rotations (p-values > 0.05), except for oat (p-value = 0.0189). For different crop types (Table 5C), the weed aboveground mass evenness index was significantly different between the cool season crops average and the warm season crops average (p-value < 0.0001) and between the cool season and warm season crops in the 4-year rotation (p-value = 0.0002), but comparable between the warm season and cool season crops in the 3-year rotation (p-values = 0.141). The weed aboveground mass evenness index was comparable between oat and alfalfa (p-value = 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 stand density richness index was comparable in the 2-year rotation and in the average of the 3-year and 4-year rotations (p-values = 0.1819), but significantly different between the 3-year and 4-year rotation (p-value = 0.0257). Averaged over the corn and soybean phases within the same rotation (Table 4A), weed aboveground mass richness index was comparable between the 2-year rotation and the 3-year and 4-year rotations average (p-value = 0.7996) and between the 3-year and 4-year rotations (p-value = 0.3469). For individual crops (Table 4B), the weed stand density richness index was comparable between rotations (p-values > 0.05). For different crop types (Table 4C), the weed stand density richness index was significantly different between the cool season crops average and the warm season crops average (p-value = 0.0003) and between the cool season and warm season crops in the 4-year rotation (p-value = 0.0034), but comparable between the warm season and cool season crops in the 3-year rotation (p-values = 0.0725). The weed stand density richness index was comparable between oat and alfalfa (p-value = 0.9499).

Averaged over crop phases within the same rotation (Table 5A), the weed aboveground mass richness index was comparable in the 2-year rotation and in the average of the 3-year and 4-year rotations (p-values = 0.1967), but significantly different between the 3-year and 4-year rotations (p-value = 0.0309). Averaged over the corn and soybean phases within the same rotation (Table 5A), the weed aboveground mass richness index was comparable between the 2-year rotation and the 3-year and 4-year rotations average (p-value = 0.7694) and between the 3-year and 4-year rotations (p-value = 0.393). For the same crop types, (Table 5B), the weed aboveground mass richness index was comparable across rotations (p-values > 0.05). For different crop types (Table 5C), the weed aboveground richness index was significantly different between the cool season and warm season crop averages (p-value = 0.0003) and between the cool season and warm season crops in the 4-year rotation (p-value = 0.0766), but comparable between the cool season and warm season crops in the 3-year rotation (p-value = 0.0766). The weed aboveground mass richness index was comparable between oat and alfalfa (p-value = 0.9506).

General description of the weed flora Overall, 34 weed species were identified during the four years of data collection (Table 6). Combined over four years of data, 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

Table 4: Weed stand density ecological indices contrast significance. The abbreviations on the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged.

	Diversity index		Evenness index		Richness index	
Contrast	ratio	p.value	ratio	p.value	ratio	p.value
(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

Note: 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

Table 5: Weed aboveground mass ecological indices contrast significance. The abbreviations on the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged.

	Diversity index		Evenness index		Richness index	
Contrast	ratio	p.value	ratio	p.value	ratio	p.value
(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	<.0001	0.51	<.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

Note: 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 total weed density and 94.0% of the total weed biomass (Figure 3C and D).

How did rotation, crop species, and corn weed management affect weed community density and growth? Crop identity had a significant effect on weed community stand density (p-value < 0.0001) and weed aboveground mass (p-value $= 0.0057$), but corn weed management and its interaction with crop identity did not have a significant effect on weed community stand density or biomass (p-values > 0.05) (Table 4 and 5). Weed total stand density and aboveground mass in each crop identity category, averaged over blocks, years, and corn weed management regimes, are presented in Figure 3A and B. Contribution by the dominant species are presented in Figure 3C and 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-year and 4-year systems averages were comparable to those of the 2-year system (p-values = 0.058 and 0.9451, Table 7B1). The weed density in the 4-year rotation was 2.5 fold greater than in the 3-year rotation (p-value = 0.0368), but the aboveground mass was comparable between the 3-year and 4-year rotations.

For the individual crops (Table 7B2), increased rotation diversity tended to decrease weed abundance in corn and soybean and increase weed abundance in oat, but these trends were not significant (p-values = 0.6354 and 0.4041 for corn, 0.1834 and 0.0739 for soybean, and 0.3955 and 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 (Table 7B3), weed community density and aboveground mass were comparable between the warm season crops (corn and soybean, p-values = 0.2032, 0.3426, 0.065, and 0.1274) and between the cool season crops (oat and alfalfa, p-values = 0.774 and 0.687). Overall, the average weed community density in the cool season crops was 26-fold greater than that in the warm season crops (p-value < 0.0001), and the average weed aboveground mass in cool season crops was 16-fold greater than that in warm season crops (p-value = 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-value = 0.0012), but the weed community total aboveground mass was comparable between O3 and the average of the C3 and S3 phases (p-value = 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-value < 0.0001), and the average weed biomass for the O4 and A4 phases was 29-fold greater than for the C4 and S4 phases (p-value < 0.0001).

How did rotation, crop species, and corn weed management affect individual weed species abundance? The stand density and aboveground mass of the seven most abundant weed species are shown in Figure 3. The effects of crop identity (i.e., rotation system crossed with crop species), corn weed management, and their interaction on the seven most abundant weeds in the present study are shown in Table 8. No interactive effects between crop identity and corn weed management were seen in any of those seven species' density or aboveground mass. The main effects of crop identity and corn weed management on stand density and aboveground mass differed by species.

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. Among the seven most abundant weed species, the stand densities were all affected by crop identity, but the aboveground mass was affected by crop identity for only four out of seven species (Table 8).

Since increased weed stand density and aboveground mass were not correlated with increased crop yield loss, the magnitude of differences in individual weed density and aboveground mass are not presented here. Significance of differences in individual species density and aboveground mass (p-values) are presented to illustrate community composition shift. Individual species stand density and aboveground mass data were combined over four years and four blocks.

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
POLPY	*Polygonum pensylvanicum* L.	ascending much-branched summer annual
POPDE	*Polygonum perfoliatum* L.	spiny summer annual vine
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.	chump-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

Table 7: Community density and aboveground mass ANOVA and contrasts. The abbreviations in the contrast column are crop identities, which are the combinations of the first letter in crop species names and the rotation to which the crops belonged.

			Stand density		Aboveground mass	
Source of variation	df1	df2	F.value	p.value	F.value	p.value
(A) - ANOVA						
Crop ID	8	24	12.22	<.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
Contrasts ratio p.value ratio p.value						
(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	<.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	<.0001	28.70	0.0003
[(O3+O4)/2] vs A4			0.80	0.7440	1.49	0.6870

Note: 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.

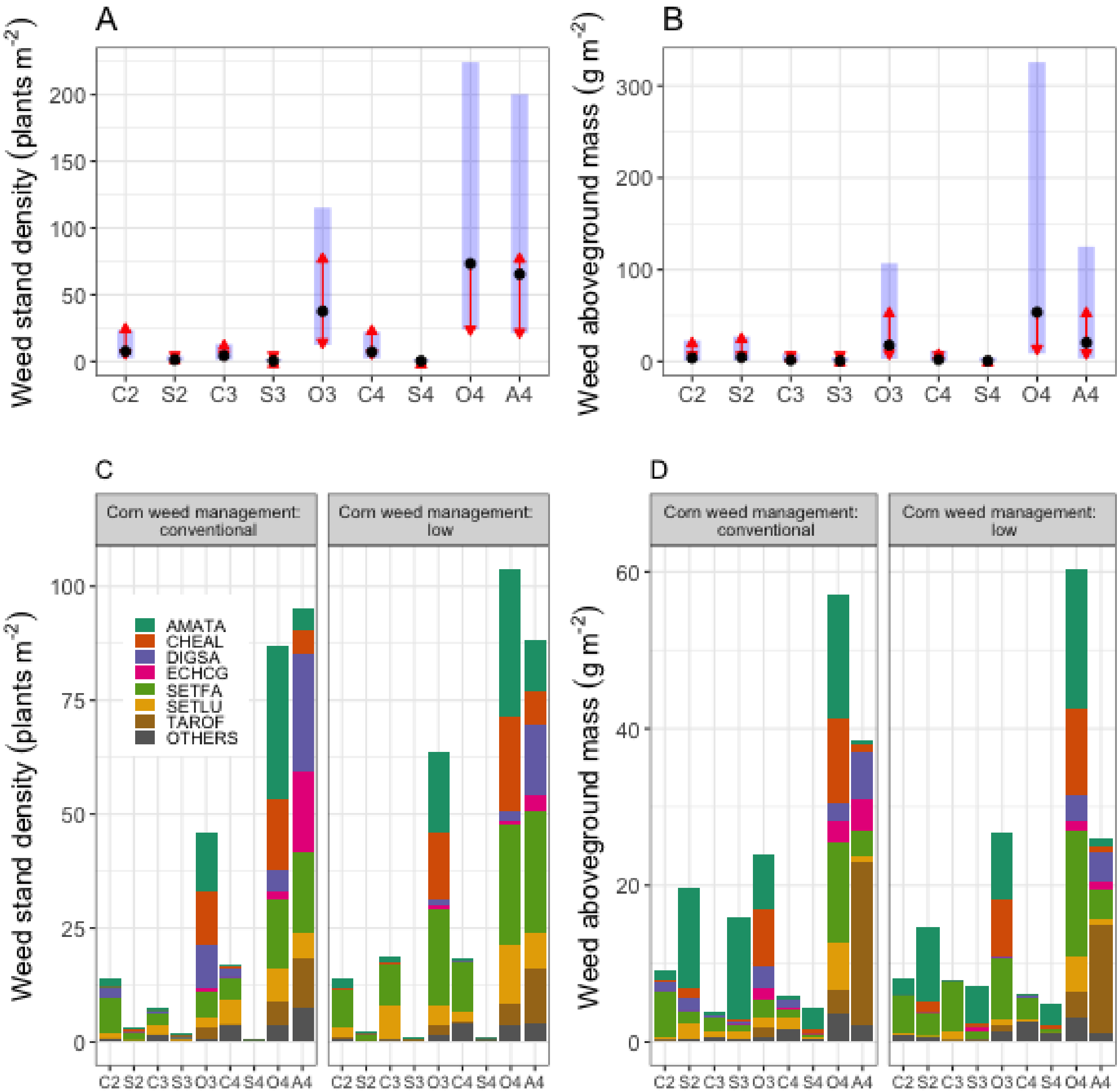

Figure 3: In panels A and B: weed community stand density and aboveground mass were averaged over four blocks, four years, and two corn weed management regimes; the black dots are estimated marginal means; the blue bars are 95% confidence intervals; the red arrows reflect the comparisons among means; overlapping arrows indicate non-significant differences. In panels C and 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 four 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 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 less abundant weed species which made up 6% of the whole community are grouped in OTHERS. The means displayed on panels A and B were estimated marginal means, calculated based on the analysis model (with `emmip` function) but the means displayed on panels C and D were arithmetic means, calculated from the data so they are slightly different.

Table 8: Treatment effects on the abundance of the most population and vigorous weed species, listed alphabetically. All the other weeds species were grouped into OTHERS

			Stand density		Aboveground mass	
Source of variation	df1	df2	F.value	p.value	F.value	p.value
(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	<.0001	15.53	<.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	<.0001	8.14	<.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	<.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	<.0001	35.81	<.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

Note: 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, and A4 - alfalfa in the 4-year rotation.

Averaged over crop identity, DIGSA and SETFA stand density and aboveground mass were affected by corn weed management (p-values = 0.0189 and 0.0196, Table 8). Averaged over corn weed management regimes, the differences in weed species stand density and aboveground mass were observed more often between crop types (Tables 9B and C, and Tables @ref(tab:10B and C)) than for individual crops across rotations (Tables 9A and 10A). 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 aboveground mass were comparable among all rotation systems averaged over crop phases (p-values > 0.05), among rotations for the same crop species (p-values > 0.05), and within the same crop type across rotations (p-values > 0.05). Averaged over the same crop types (warm season or cool season), AMATA stand density was significantly different in cool season versus warm season crops (p-value = 0.0001), but AMATA aboveground mass was comparable (p-value = 0.0906) in cool season and warm season crops. Within the same rotation, AMATA stand density was greater in the cool season than in the warm season crops (p-values 0.0143, and 0.0003), but AMATA aboveground mass was comparable in these crop environments (p-values = 0.2355, and 0.0493).

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 11-fold (p-value = 0.0001) and 96-fold (p-value = 0.0001) greater in oat than in alfalfa. CHEAL stand density and aboveground mass were significantly different between the 2-year rotation and the average of the 3-year and 4-year rotations, but comparable between the 3-year and 4-year rotations (p-values = 0.9195 and 0.6114). CHEAL stand density and aboveground mass were comparable across rotations for the same crop species (p-values > 0.05) and within the warm season crops (p-values > 0.05), but significantly different across crop types overall (p-values < 0.0001), between the warm season and cool season crops of the same rotation (p-values = 0.0001), and within the cool season crops (oat versus alfalfa).

The cool season crops, especially alfalfa were responsible for DIGSA stand density and aboveground mass differences between rotation averages. DIGSA stand density and aboveground mass were 14-fold (p-value = 0.0001) and 33-fold (p-value = 0.0001) greater in alfalfa than in oat. DIGSA stand density significantly was different between the 2-year rotation and the average of the 3-year and 4-year rotations (p-value = 0.0072) and between the 3-year and 4-year rotation (p-value < 0.0001). DIGSA aboveground mass was comparable between the 2-year and the average of the 3-year and 4-year rotations (p-value = 0.1098), but significantly different between the 3-year and 4-year rotations (p-value = 0.0001). DIGSA stand density and aboveground mass were comparable across rotations for the same crop species (p-values > 0.05), except for oat (p-values = 0.0062 and 0.0032). Within the 3-year rotation, DIGSA stand density was comparable among crop phases (p-value = 0.0603), but DIGSA aboveground mass was significantly different between oat and the average of corn and soybean phases (p-value < 0.0001). DIGSA stand density and aboveground mass were significantly different across crop types overall, between the warm season and cool season crops of the 4-year rotation (p-values = 0.0001), and within the cool season crops (oat versus alfalfa) (p-values < 0.0001).

ECHCG responses generally were similar to those of AMATA. ECHCG stand density and aboveground mass were comparable between all rotation averages (p-values > 0.05), across rotations for the same crop species (p-values > 0.05), within the same crop type across rotations (p-values > 0.05), and within the 3-year rotation (p-values > 0.05). Averaged over the same crop types, ECHCG stand density and aboveground mass were significantly different in cool season versus warm season crops (p-value = 0.0003 and 0.0012). Within the 4-year rotation, ECHCG stand density and aboveground mass were greater in the cool season than in the warm season crops (p-values 0.0014, and 0.0031).

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 across all rotation averages (p-values > 0.05), across rotations for the same crop species (p-values > 0.05), within the warm season crops across rotations (p-values > 0.05), and within the cool season crops (p-values > 0.05). Averaged over the same crop types, SETFA stand density and aboveground mass were significantly different in cool season versus warm season crops (p-value < 0.0001 and p-value = 0.0008). Within the same rotation, SETFA stand density and aboveground mass were greater in the cool season than in the warm season crops (p-values = 0.001, 0.018, 0.0001, and 0.0045).

SETLU stand density and aboveground mass were comparable in most pairs of comparison (p-values > 0.05), with the exception in the warm season versus cool season density (p-value = 0.0404).

The cool season crops, especially oat were responsible for TAROF stand density and aboveground mass differences across rotation averages. TAROF stand density and aboveground mass were 6-fold (p-value < 0.0001) and 20-fold (p-value = 0.0001) greater in oat than in alfalfa. TAROF stand density and aboveground mass were significantly different in the 2-year versus the average of the 3-year and 4-year rotations, and between the 3-year and 4-year rotations (p-values < 0.0001). TAROF stand density and aboveground mass were comparable among the warm season crops across rotations and within the same crops across rotations (p-values > 0.05), except in oat (p-values < 0.0001). TAROF stand density and aboveground mass were significantly different across crop types overall (p-values < 0.0001), across crop types within the same rotations (p-values = 0.0001, 0.0002 and < 0.0001), and between oat versus alfalfa (p-values ≤ 0.0001).

Table 9: Significance of difference in abundance of the top seven weed species. 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 occured.

	p-values													
	Stand density							Aboveground mass						
Contrast of the main-plot effect	AMATA	CHEAL	DIGSA	ECHCG	SETFA	SETLU	TAROF	AMATA	CHEAL	DIGSA	ECHCG	SETFA	SETLU	TAROF
(A) - Rotation system effects														
[(C2+S2)/2] vs [(C3+S3+O3+C4+S4+O4+A4)/7]	0.6105	0.0008	0.0072	0.1170	0.3011	0.1569	<.0001	0.3402	0.0199	0.1098	0.1417	0.9245	0.3588	<.0001
[(C3+S3+O3)/3] vs [(C4+S4+O4+A4)/4]	0.7077	0.9195	<.0001	0.0834	0.0927	0.0827	<.0001	0.8168	0.6414	0.0001	0.1040	0.4497	0.2420	<.0001
[(C2+S2)/2] vs [(C3+S3+C4+S4)/4]	0.1746	0.3889	0.6798	0.9584	0.1906	0.4944	0.8129	0.0893	0.2315	0.4852	0.8841	0.1566	0.5502	0.7608
[(C3+S3)/2] vs [(C4+S4)/2]	0.4533	0.3823	0.3213	0.9384	0.5877	0.6234	0.5105	0.4799	0.2676	0.4264	0.9958	0.9537	0.9148	0.4810
(B) - Rotation system effects within individual crops														
C2 vs [(C3+C4)/2]	0.3598	0.4995	0.8818	0.9497	0.5010	0.4277	0.9547	0.2696	0.4167	0.9499	0.9882	0.4070	0.5668	0.9237
C3 vs C4	0.6368	0.6510	0.2466	0.8579	0.3501	0.3990	0.6923	0.7802	0.6372	0.3994	0.7630	0.5131	0.6404	0.8309
S2 vs [(S3+S4)/2]	0.3065	0.5837	0.4658	0.9915	0.2337	0.8628	0.6958	0.1821	0.3720	0.3571	0.8252	0.2329	0.7847	0.7378
S3 vs S4	0.5543	0.4312	0.8088	0.9444	0.8620	0.8780	0.5914	0.4709	0.2708	0.7772	0.7687	0.5667	0.7516	0.4336
O3 vs O4	0.2890	0.6212	0.0062	0.2130	0.4848	0.2006	<.0001	0.3486	0.5666	0.0032	0.0768	0.3941	0.1539	<.0001
(C) - Crop type effects														
[(O3+O4+A4)/3] vs [(C2+S2+C3+S3+C4+S4)/6]	0.0001	<.0001	<.0001	0.0003	<.0001	0.0404	<.0001	0.0906	<.0001	<.0001	0.0012	0.0008	0.3316	<.0001
O3 vs [(C3+S3)/2]	0.0143	<.0001	0.0630	0.2248	0.0010	0.9435	0.0001	0.2355	<.0001	0.3924	0.3920	0.0180	0.5554	0.0002
[(O4+A4)/2] vs [(C4+S4)/2]	0.0003	<.0001	<.0001	0.0014	0.0001	0.0798	<.0001	0.0493	<.0001	<.0001	0.0031	0.0045	0.2706	<.0001
[(O3+O4)/2] vs A4	0.1606	0.0001	<.0001	0.1954	0.8068	0.1812	<.0001	0.0724	0.0001	0.0008	0.6762	0.1818	0.5132	0.0001

Note: 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.

Table 10: Means of difference in abundance of the top seven weed species. 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 occured.

	Contrast ratio														
	Stand density							Aboveground mass							
Contrast of the main-plot effect	AMATA	CHEAL	DIGSA	ECHCG	SETFA	SETLU	TAROF	AMATA	CHEAL	DIGSA	ECHCG	SETFA	SETLU	TAROF
(A) - Rotation system effects														
[(C2+S2)/2] vs [(C3+S3+O3+C4+S4+O4+A4)/7]	0.74	0.28	0.42	0.57	0.64	0.50	0.24	3.10	0.21	0.36	0.35	0.93	0.46	0.07
[(C3+S3+O3)/3] vs [(C4+S4+O4+A4)/4]	0.81	0.97	0.21	0.55	0.49	0.44	0.19	1.30	1.33	0.07	0.32	0.56	0.39	0.05
[(C2+S2)/2] vs [(C3+S3+C4+S4)/4]	2.45	1.37	1.14	0.98	1.86	0.70	0.95	9.26	2.30	1.60	0.89	3.54	0.58	0.86
[(C3+S3)/2] vs [(C4+S4)/2]	1.76	1.45	0.69	0.97	0.75	0.74	0.84	2.83	2.43	0.54	1.00	0.94	0.89	0.67
(B) - Rotation system effects within individual crops														
C2 vs [(C3+C4)/2]	2.33	1.42	0.93	0.97	1.56	0.56	1.02	7.45	2.21	1.06	1.02	2.81	0.48	0.94
C3 vs C4	1.65	1.31	0.54	0.89	0.49	0.49	0.87	1.78	1.70	0.40	0.69	0.39	0.50	0.85
S2 vs [(S3+S4)/2]	2.58	1.33	1.40	0.99	2.21	0.88	0.88	11.50	2.39	2.40	0.79	4.47	0.71	0.80
S3 vs S4	1.87	1.60	0.88	1.04	1.14	1.14	0.82	4.50	3.49	0.73	1.44	2.27	1.59	0.54
O3 vs O4	0.32	0.74	0.21	0.46	0.59	0.33	0.09	0.14	0.53	0.03	0.10	0.29	0.12	0.01
(C) - Crop type effects														
[(O3+O4+A4)/3] vs [(C2+S2+C3+S3+C4+S4)/6]	12.25	38.15	10.11	3.60	9.85	2.48	24.33	6.11	204.44	27.29	9.56	15.00	2.05	389.81
O3 vs [(C3+S3)/2]	10.94	67.07	2.43	1.94	11.32	1.05	4.33	8.70	571.14	2.26	2.54	22.34	0.47	19.10
[(O4+A4)/2] vs [(C4+S4)/2]	23.36	36.99	20.08	4.82	11.63	2.96	53.81	20.20	231.64	102.80	17.54	22.79	3.18	1482.81
[(O3+O4)/2] vs A4	3.71	10.75	0.07	0.49	1.17	0.37	0.17	28.24	94.46	0.03	0.64	5.38	0.43	0.05

Note: 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.

Discussion

Diversification of cropping systems led to increased weed community aboveground mass and stand density, increased weed community diversity and species richness, and decreased evenness, but did not reduce crop yield. Crop identity was the factor that 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 (Brookes and Barfoot, 2013; Stewart et al., 2011), 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 due to 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 individual 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 three 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 severity and incidence frequency 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 play more important roles than weeds in SDS outbreaks and 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 and 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 contribution to reductions of weed density.

We considered the weed management programs in the 3-year and 4-year rotations effective because the crop yields at our experiment site were comparable across rotations (Table 2) and to those of the state of Iowa and Boone County averages (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-year 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 four 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 units, 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 seedbank. The reproductive potential of AMATA was reduced substantially in cool season crops as compared to warm season crops Nguyen and Liebman (in review). 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 the 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).

Acknowledgements

The authors thank Matt Woods, Mike Fiscus, and the Iowa State University's Agronomy Research Farm crew for field management; Wendy Borja-Diaz, Lydia English, Jessica Juarez-Morales, Samantha Kanselaar, Jessica Nelson, Elizabeth Oys, Ana Poznanski, Andrew Riehl, Angela Soto-Saenz, Mickala Stallman, David Weisberger, and Wyatt Westfall for field and laboratory assistance; Katherine Goode, Audrey McCombs, Philip Dixon and ISU's statistical consulting group for data analysis assistance; Russ Lenth and other Stackoverflow community members for answering HTXN's coding questions; Micheal Owen for reviewing the manuscript; and Overleaf staff for LaTeX assistance in compiling the manuscript.

References

Guillaume Adeux, Eric Vieren, Stefano Carlesi, Paolo Bàrberi, Nicolas Munier-Jolain, and Stéphane Cordeau. Mitigating crop yield losses through weed diversity. *Nat Sustain*, 2(11):1018–1026, November 2019. ISSN 2398-9629. doi: 10/gmw5sz.

Rauno V. Alatalo. Problems in the measurement of evenness in ecology. *Oikos*, 37(2):199–204, 1981. ISSN 0030-1299. doi: 10/drcgjd.

Douglas Bates, Martin Maechler, Ben Bolker, Steven Walker, Rune Haubo Bojesen Christensen, Henrik Singmann, Bin Dai, Fabian Scheipl, Gabor Grothendieck, Peter Green, John Fox, Alexander Bauer, and Pavel N. Krivitsky. Lme4: Linear mixed-effects models using 'eigen' and S4, June 2021.

Damien Beillouin, Tamara Ben-Ari, Eric Malézieux, Verena Seufert, and David Makowski. Positive but variable effects of crop diversification on biodiversity and ecosystem services. *Glob Change Biol*, 27(19): 4697–4710, October 2021. ISSN 1354-1013, 1365-2486. doi: 10/gkkbqm.

Timothy M. Bowles, Maria Mooshammer, Yvonne Socolar, Francisco Calderón, Michel A. Cavigelli, Steve W. Culman, William Deen, Craig F. Drury, Axel Garcia y Garcia, Amélie C.M. Gaudin, W. Scott Harkcom, R. Michael Lehman, Shannon L. Osborne, G. Philip Robertson, Jonathan Salerno, Marty R. Schmer, Jeffrey Strock, and A. Stuart Grandy. Long-term evidence shows that crop-rotation diversification increases agricultural resilience to adverse growing conditions in North America. *One Earth*, 2(3):284–293, March 2020. ISSN 25903322. doi: 10/gjj4j5.

Graham Brookes and Peter Barfoot. Key environmental impacts of global genetically modified (GM) crop use 1996–2011. *GM Crops & Food*, 4(2):109–119, April 2013. ISSN 2164-5698. doi: 10/gddc8b.

Douglas D. Buhler and Robert G. Hartzler. Emergence and persistence of seed of velvetleaf, common waterhemp, woolly cupgrass, and giant foxtail. *Weed Science*, 49(2):230–235, April 2001. ISSN 0043-1745, 1550-2759. doi: 10/dmnt6f.

John Cardina, Gregg A. Johnson, and Denise H. Sparrow. The nature and consequence of weed spatial distribution. *Weed Science*, 45(3):364–373, 1997. ISSN 0043-1745. doi: 10/ghkhdv.

Center for Spatial Information Science and Systems. CropScape - Cropland Data Layer, 2021.

Xianni Chen, Xudong Wang, Matt Liebman, Michel Cavigelli, and Michelle Wander. Influence of residue and nitrogen fertilizer additions on carbon mineralization in soils with different texture and cropping histories. *PLoS One*, 9(7):e103720, July 2014. ISSN 1932-6203. doi: 10/f6ntt7.

Stéphane Cordeau, Richard G. Smith, Eric R. Gallandt, Bryan Brown, Paul Salon, Antonio DiTommaso, and Matthew R. Ryan. How do weeds differ in their response to the timing of tillage? A study of 61 species across the Northeastern United States: Weed response to timing of tillage. *Ann Appl Biol*, 171(3):340–352, November 2017. ISSN 00034746. doi: 10/gcgzch.

A. Stanley Culpepper. Glyphosate-induced weed shifts. *Weed Technology*, 20(2):277–281, June 2006. ISSN 0890-037X, 1550-2740. doi: 10/c9rw9b.

Adam S. Davis, Philip M. Dixon, and Matt Liebman. Cropping system effects on giant foxtail (*Setaria faberi*) demography: II. Retrospective perturbation analysis. *Weed Science*, 51(6):930–939, December 2003. ISSN 0043-1745, 1550-2759. doi: 10/btzt2v.

Adam S. Davis, John Cardina, Frank Forcella, Gregg A. Johnson, George Kegode, John L. Lindquist, Edward C. Luschei, Karen A. Renner, Christy L. Sprague, and Martin M. Williams. Environmental factors affecting seed persistence of annual weeds across the U.S. Corn Belt. *Weed Science*, 53(6):860–868, December 2005a. ISSN 0043-1745, 1550-2759. doi: 10/dmvcdf.

Adam S. Davis, Karen A. Renner, and Katherine L. Gross. Weed seedbank and community shifts in a long-term cropping systems experiment. *Weed Science*, 53(3):296–306, June 2005b. ISSN 0043-1745, 1550-2759. doi: 10/fkd3dj.

Adam S. Davis, Jason D. Hill, Craig A. Chase, Ann M. Johanns, and Matt Liebman. Increasing cropping system diversity balances productivity, profitability and environmental health. *PLOS ONE*, 7(10):e47149, October 2012. ISSN 1932-6203. doi: 10/f39nr2.

Philip Dixon. Should blocks be fixed or random? *Conference on Applied Statistics in Agriculture*, May 2016. ISSN 2475-7772. doi: 10/gjggh8.

Frank Forcella. Debiting the seedbank: Priorities and predictions. *Aspects of Applied Biology*, 69:151–162, 2003.

Frank Forcella, Robert G. Wilson, Karen A. Renner, Jack Dekker, Robert G. Harvey, David A. Alm, Douglas D. Buhler, and John Cardina. Weed seedbanks of the U.S. Corn Belt: Magnitude, variation, emergence, and application. *Weed Science*, 40(4):636–644, 1992. ISSN 0043-1745.

Guillaume Fried, Elena Kazakou, and Sabrina Gaba. Trajectories of weed communities explained by traits associated with species' response to management practices. *Agriculture, Ecosystems & Environment*, 158: 147–155, September 2012. ISSN 0167-8809. doi: 10/f36znx.

Sabrina Gaba, Guillaume Fried, Elena Kazakou, Bruno Chauvel, and Marie-Laure Navas. Agroecological weed control using a functional approach: A review of cropping systems diversity. *Agron. Sustain. Dev.*, 34 (1):103–119, January 2014. ISSN 1773-0155. doi: 10/gkmznh.

Katherine Goode and Kathleen Rey. ggResidpanel: Panels and interactive versions of diagnostic plots using 'ggplot2', May 2019.

Glen L. Hartman, Hao-Xun Chang, and Leonor F. S. Leandro. Research advances and management of soybean sudden death syndrome. *Crop Protection*, 73:60–66, July 2015. ISSN 0261-2194. doi: 10/gmw5d7.

Natalie D. Hunt, Jason D. Hill, and Matt Liebman. Reducing freshwater toxicity while maintaining weed control, profits, and productivity: Effects of increased crop rotation diversity and reduced herbicide usage. *Environ. Sci. Technol.*, 51(3):1707–1717, February 2017. ISSN 0013-936X. doi: 10/f9nrv2.

Natalie D. Hunt, Jason D. Hill, and Matt Liebman. Cropping system diversity effects on nutrient discharge, soil erosion, and agronomic performance. *Environ. Sci. Technol.*, 53(3):1344–1352, February 2019. ISSN 0013-936X. doi: 10/gfw9q2.

Natalie D. Hunt, Matt Liebman, Sumil K. Thakrar, and Jason D. Hill. Fossil energy use, climate change impacts, and air quality-related human health damages of conventional and diversified cropping systems in Iowa, USA. *Environ. Sci. Technol.*, 54(18):11002–11014, September 2020. ISSN 0013-936X. doi: 10/gkc28b.

Stevan Z. Knezevic, Sean P. Evans, Erin E. Blankenship, Rene C. Van Acker, and John L. Lindquist. Critical period for weed control: The concept and data analysis. *Weed Science*, 50(6):773–786, 2002. ISSN 0043-1745. doi: 10/b4bc8z.

Tammy M. Kolander, John C. Bienapfl, James Edward Kurle, and Dean K. Malvick. Symptomatic and asymptomatic host range of *Fusarium virguliforme*, the causal agent of soybean sudden death syndrome. *Plant Disease*, 96(8):1148–1153, August 2012. ISSN 0191-2917. doi: 10/gmw5ff.

Greg R. Kruger, William G. Johnson, Stephen C. Weller, Micheal D. K. Owen, David R. Shaw, John W. Wilcut, David L. Jordan, Robert G. Wilson, Mark L. Bernards, and Bryan G. Young. U.S. grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. *Weed Technology*, 23(1):162–166, March 2009. ISSN 0890-037X, 1550-2740. doi: 10/fjs6sc.

Leonor F. S. Leandro, Sharon Eggenberger, Chiliang Chen, Jeffrey Williams, Gwyn A. Beattie, and Matt Liebman. Cropping system diversification reduces severity and incidence of soybean sudden death syndrome caused by *Fusarium virguliforme*. *Plant Disease*, 102(9):1748–1758, September 2018. ISSN 0191-2917. doi: 10/gd439q.

Anne Légère, F. Craig Stevenson, and Diane Lyse Benoit. Diversity and assembly of weed communities: Contrasting responses across cropping systems. *Weed Research*, 45(4):303–315, August 2005. ISSN 1365-3180. doi: 10/dp4rnv.

Russell V. Lenth, Paul Buerkner, Maxime Herve, Jonathon Love, Hannes Riebl, and Henrik Singmann. Emmeans: Estimated marginal means, aka least-squares means, November 2021.

Matt Liebman. Weed management: A need for ecological approaches. In Charles L. Mohler, Charles P. Staver, and Matt Liebman, editors, *Ecological Management of Agricultural Weeds*, pages 1–39. Cambridge University Press, Cambridge, 2001. ISBN 978-0-521-56068-9. doi: 10.1017/CBO9780511541810.002.

Matt Liebman and Eric R. Gallandt. Many little hammers: Ecological management of crop-weed interactions. In Louise E. Jackson, editor, *Ecology in Agriculture*, Physiological Ecology, pages 291–343. Academic Press, 1997. ISBN 978-0-12-378260-1. doi: 10.1016/B978-012378260-1/50010-5.

Matt Liebman and Charles P. Staver. Crop diversification for weed management. In Charles L. Mohler, Charles P. Staver, and Matt Liebman, editors, *Ecological Management of Agricultural Weeds*, pages 322–374. Cambridge University Press, Cambridge, 2001. ISBN 978-0-521-56068-9. doi: 10.1017/CBO9780511541810.008.

Matt Liebman, Huong T. X. Nguyen, Matthew M. Woods, Natalie D. Hunt, and Jason D. Hill. Weed seedbank diversity and sustainability indicators for simple and more diverse cropping systems. *Weed Res*, 61(3):164–177, June 2021. ISSN 0043-1737, 1365-3180. doi: 10/ghz5bj.

Fabián D. Menalled, Katherine L. Gross, and Mark Hammond. Weed aboveground and seedbank community responses to agricultural management systems. *Ecological Applications*, 11(6):1586–1601, 2001. ISSN 1939-5582. doi: 10/dpj63j.

Charles L. Mohler. Weed evolution and community structure. In Charles L. Mohler, Charles P. Staver, and Matt Liebman, editors, *Ecological Management of Agricultural Weeds*, pages 444–493. Cambridge University Press, Cambridge, 2001. ISBN 978-0-521-56068-9. doi: 10.1017/CBO9780511541810.011.

Charles L. Mohler and Sue Ellen Johnson. Crop disease pathogens hosted by common agricultural weeds. In *Crop Rotation on Organic Farms: A Planning Manual*, number 177 in NRAES, pages 143–147. Natural

Resource, Agriculture, and Engineering Service (NRAES) Cooperative Extension, Ithaca, NY, 2009. ISBN 978-1-933395-21-0.

E. Kathryn Morris, Tancredi Caruso, François Buscot, Markus Fischer, Christine Hancock, Tanja S. Maier, Torsten Meiners, Caroline Müller, Elisabeth Obermaier, Daniel Prati, Stephanie A. Socher, Ilja Sonnemann, Nicole Wäschke, Tesfaye Wubet, Susanne Wurst, and Matthias C. Rillig. Choosing and using diversity indices: Insights for ecological applications from the German Biodiversity Exploratories. *Ecol Evol*, 4(18): 3514–3524, September 2014. ISSN 2045-7758, 2045-7758. doi: 10/f6kkwf.

Huong T. X. Nguyen and Matt Liebman. Impact of cropping system diversification on vegetative and reproductive characteristics of waterhemp *Amaranthus tuberculatus*). *Frontiers in Agronomy*, in review.

Roger Nkoa, Micheal D. K. Owen, and Clarence J. Swanton. Weed abundance, distribution, diversity, and community analyses. *Weed Science*, 63(SP1):64–90, February 2015. ISSN 0043-1745, 1550-2759. doi: 10/gddj7f.

Micheal D. K. Owen. Weed species shifts in glyphosate-resistant crops. *Pest Management Science*, 64(4): 377–387, April 2008. ISSN 1526-4998. doi: 10/ck4hjf.

Roger William Payne. The design and analysis of long-term rotation experiments. *Agronomy Journal*, 107(2): 772–785, March 2015. ISSN 00021962. doi: 10/f673ct.

Evelyn Chrystalla Pielou. *The Interpretation of Ecological Data: A Primer on Classification and Ordination*. John Wiley & Sons, September 1984. ISBN 978-0-471-88950-2.

Joby M. Prince, David R. Shaw, Wade A. Givens, Micheal D. K. Owen, Stephen C. Weller, Bryan G. Young, Robert G. Wilson, and David L. Jordan. Benchmark study: I. Introduction, weed population, and management trends from the benchmark survey 2010. *Weed Technology*, 26(3):525–530, September 2012. ISSN 0890-037X, 1550-2740. doi: 10/gdf4rc.

R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, November 2021.

Krishna N. Reddy and Jason K. Norsworthy. Glyphosate-resistant crop production systems: Impact on weed species shifts. In *Glyphosate Resistance in Crops and Weeds*, pages 165–184. Wiley-Blackwell, 2010. ISBN 978-0-470-63439-4. doi: 10.1002/9780470634394.ch9.

Matthew R. Ryan, Richard G. Smith, Steven B. Mirsky, David A. Mortensen, and Rita Seidel. Management filters and species traits: Weed community assembly in long-term organic and conventional systems. *Weed Science*, 58(3):265–277, 2010. ISSN 0043-1745. doi: 10/cxhk78.

Richard G. Smith and Katherine L. Gross. Assembly of weed communities along a crop diversity gradient. *Journal of Applied Ecology*, 44(5):1046–1056, 2007. ISSN 1365-2664. doi: 10/d8dvks.

Christie L. Stewart, Robert E. Nurse, Laura L. Van Eerd, Richard J. Vyn, and Peter H. Sikkema. Weed control, environmental impact, and economics of weed management strategies in glyphosate-resistant soybean. *Weed Technology*, 25(4):535–541, December 2011. ISSN 0890-037X, 1550-2740. doi: 10/dkxnsg.

Gray Stirling and Brian Wilsey. Empirical relationships between species richness, evenness, and proportional diversity. *The American Naturalist*, 158(3):286–299, September 2001. ISSN 0003-0147. doi: 10/dxpbpj.

Jonathan Storkey and Paul Neve. What good is weed diversity? *Weed Res*, 58(4):239–243, August 2018. ISSN 0043-1737. doi: 10/gdwv5r.

Giovanni Tamburini, Riccardo Bommarco, Thomas Cherico Wanger, Claire Kremen, Marcel G. A. van der Heijden, Matt Liebman, and Sara Hallin. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Sci. Adv.*, 6(45), November 2020. ISSN 2375-2548. doi: 10/gjcwv8.

Richard H. Uva, Joseph C. Neal, and Joseph M. Ditomaso. *Weeds of the Northeast*. Cornell University Press, Ithaca, NY, May 1997. ISBN 978-0-8014-8334-9.

Rocio van der Laat, Micheal D. K. Owen, Matt Liebman, and Ramon G. Leon. Postdispersal weed seed predation and invertebrate activity density in three tillage regimes. *Weed Science*, 63(4):828–838, December 2015. ISSN 0043-1745, 1550-2759. doi: 10/gddc93.

David Weisberger, Virginia Nichols, and Matt Liebman. Does diversifying crop rotations suppress weeds? A meta-analysis. *PLoS ONE*, 14(7):e0219847, July 2019. ISSN 1932-6203. doi: 10/gh28qh.

Paula R. Westerman, Matt Liebman, Fabián D. Menalled, Andrew H. Heggenstaller, Robert G. Hartzler, and Philip M. Dixon. Are many little hammers effective? Velvetleaf (*Abutilon theophrasti*) population dynamics in two- and four-year crop rotation systems. *Weed Science*, 53(3):382–392, June 2005. ISSN 0043-1745, 1550-2759. doi: 10/bgc2x2.

Gail C. Wisler and Robert F. Norris. Interactions between weeds and cultivated plants as related to management of plant pathogens. *Weed Science*, 53(6):914–917, 2005. ISSN 0043-1745. doi: 10/b8v78r.

Robert L. Zimdahl. The need for historical perspective. In Robert L. Zimdahl, editor, *Weed Science - A Plea for Thought - Revisited*, SpringerBriefs in Agriculture, pages 1–23. Springer Netherlands, Dordrecht, 2012. ISBN 978-94-007-2088-6. doi: 10.1007/978-94-007-2088-6_1.