Environmental Science & Technology

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Reducing Freshwater Toxicity while Maintaining Weed Control, Profits, And Productivity: Effects of Increased Crop Rotation Diversity and Reduced Herbicide Usage

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Supporting Information

ABSTRACT: Increasing crop rotation diversity while reducing herbicide applications may maintain effective weed control while reducing freshwater toxicity. To test this hypothesis, we applied the model USEtox 2.0 to data from a long-term Iowa field experiment that included three crop rotation systems: a 2-year corn-soybean sequence, a 3-year corn-soybean-oat/red clover sequence, and 4-year corn-soybean-oat/alfalfa−alfalfa sequence. Corn and soybean in each rotation were managed with conventional or low-herbicide regimes. Oat, red clover, and alfalfa were not treated with herbicides. Data from 2008−2015 showed that use of the low-herbicide regime reduced freshwater toxicity loads by 81−96%, and that use of the more diverse rotations reduced toxicity and system dependence on herbicides by 25−51%. Mean weed biomass in corn and soybean was <25 kg ha^{-1} in all rotation × herbicide combinations except the low-herbicide 3-year rotation, which contained ∼110 kg ha^{-1} of weed biomass. Corn and soybean yields and net returns were as high or higher for the 3- and 4-year rotations managed with the low-herbicide regime as for the conventional-herbicide 2-year rotation. These results indicate that certain forms of cropping system diversification and alternative weed management strategies can maintain yield, profit, and weed suppression while delivering enhanced environmental performance.

INTRODUCTION

Heavy reliance on chemical inputs has dominated weed management strategies in U.S. agriculture for the past half century. Prior to this, farmers relied on cultivation, hand pulling, and crop rotation as weed management strategies, but following the advent and increased availability of herbicides, farmers were swift to adopt these efficient and low cost means to suppress weed populations in lieu of the more labor intensive counterparts.[2] Within the last 10 years annual herbicide usage for corn and soybean production in states representing more than 90% of national production was 160 000 and 110 000 Mg of active ingredients (a.i.), respectively.[1]

Increasing intensive herbicide use has been linked to numerous environmental and human health impacts, including contamination of surface and groundwater bodies, damage to nontarget plant and animal species such as insect pollinators and amphibians, greater frequency of non-Hodgkin's lymphoma in the agricultural workforce, and increased rates of birth defects.[3−9] Additionally, because of the intense selection pressures provided by herbicides, weed populations are evolving resistance to popular herbicides, rendering them ineffective.[10−12] There are at least 155 documented cases of populations of weed species having evolved herbicide resistances in the U.S., with many of these cases occurring in the central region used for corn and soybean production.[13,14] Recent strategies for mitigating the risk of herbicide resistance in weeds have included increasing the quantity and diversity of herbicides applied, but this is not always effective due to the evolution of multiple herbicide resistances and enhanced detoxification pathways within weed populations.[15]

All these reasons, coupled with the goal of maintaining and improving crop yields, point to the need to incorporate alternative weed management strategies that protect agroecosystem productivity and health. Previous research has shown the agronomic, financial, and environmental benefits of integrated weed management strategies that make use of crop rotation diversity, cover cropping, intercropping, and combinations of mechanical and cultural weed control tactics to prevent weed population increases and limit weed competition against crops.[16,17] Additional weed management tactics, such as the use of crop genotypes with improved competitive and allelopathic abilities, may also become more common in the future.[18,19] In addition to sustained and increased grain yields, harvested biomass, and farmer profits, Davis et al., observed

Received: August 12, 2016
Revised: November 16, 2016
Accepted: December 23, 2016

DOI: 10.1021/acs.est.6b04086
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

effective weed suppression under treatments of increased crop rotation diversity and reduced herbicide inputs coupled with mechanical weed control methods.[17] Deytieux et al. found that implementing an integrated weed management system that incorporates crop rotation diversity, mechanical weeding, and other cultural weed practices delivered reduced eutrophication potential, lower global warming potentials, reduced greenhouse gas emissions, and lowered aquatic and human toxicity, relative to the more conventional standard.[20] In a study of multiple farms across France, Lechenet et al. found that when organic farms were excluded from analyses, there was no positive correlation between pesticide usage and either crop productivity or profitability.[21] In European corn-based systems, Vasileiadis et al. found no significant differences in income margins when comparing an integrated weed management system to a conventional, more herbicide-intensive system.[22] These empirical findings provide support for the incorporation of a more diverse suite of tools for effective weed management.

In the present study, we extended the work of Davis et al. which examined an integrated weed management strategy that incorporated both reduced chemical inputs with mechanical weed suppression and increased crop rotation diversity.[17] Their study consisted of three treatments: a 2-year rotation managed with a conventional herbicide regime, and more diverse 3-year and 4-year rotations managed with a low herbicide regime.[17] Thus, the effects of cropping system diversity and herbicide regime were confounded. The work presented here used the full factorial set of cropping systems × herbicide regimes to examine main and interactive effects of both factors. We made use of long-term, large-scale plots at Iowa State University's Marsden Farm, which have been the subject of other reports.[17,23–25]

We compared management strategies that comprised suites of practices and weed suppression tactics that changed dynamically over years in response to cropping system needs, rather than a static set of treatments applied invariably over multiple years. We evaluated freshwater toxicity loads through the combined use of field collected data and process toxicity models. We used the treatment frequency index (TFI) to describe the level of dependence on external herbicide inputs, as described by Deytieux et al. and Lechenet et al.[20,21] TFI is a function of the herbicide application rate, lowest registered dose, and treated and plot surface area. A greater TFI value reflects a greater level of dependence on herbicide inputs. Toxicity performance metrics were described by freshwater toxicity loads as expressed by Comparative Toxic Units (CTUe) on per-area (CTUe ha^{-1}), per yield of biomass (CTUe Mg^{-1}), and per-net income (CTUe $\$^{-1}$) bases. We also measured weed biomass in corn and soybean crops (kg ha^{-1}).

We expected that the freshwater toxicity of a low herbicide regime achieved through banded chemical applications combined with interrow cultivation would be lower than that of the conventional broadcast herbicide regime across all performance metrics, but we were specifically interested in the magnitude of the differences. We also predicted that freshwater toxicity load would decrease across all performance metrics as crop rotation diversity increased. These metrics are relevant to the agronomic and sustainability communities, as they encompass key economic, agronomic, and environmental characteristics of farming systems.

■ MATERIALS AND METHODS

Geographic and System Scope. The Iowa State University Marsden Farm is in Boone County, in central Iowa (42°01′N, 93° 47′W), an area dominated by conventional corn and soybean production. The experimental plots have been maintained since 2002. Soils at the site are all fertile Mollisols. Data were collected during 2008–2015 from an experiment arranged as a randomized complete block split-plot design, with blocks representing four replicates of each crop phase for each rotation system. Each crop phase of each rotation system was present during each year of the experiment. Each rotation crop main plot was 18 × 85 m, and each herbicide treatment subplot was 9 × 85 m. All crops were managed with standard farm machinery, and all farm chemicals used were common commercial products. More detailed information concerning the experiment site and its management can be obtained from Davis et al., Gómez et al., and in the Supporting Information (Table S1).[17,23]

Weed Management Regimes. Corn and soybean crop phases of all rotations were subjected to two herbicide treatment regimes: conventional (CONV) and low (LOW). CONV treatments received broadcast spray applications of pre- and postemergence herbicides to corn and soybean, while LOW treatments received a postemergence banded spray application followed by one or two between-row cultivations in corn and soybean. Banded spray applications were 38 cm wide and centered over the corn and soybean rows. Herbicide selection was driven by the identity, size, and density of observed weed species. Consequently, herbicide products applied differed among years and herbicide regimes (Table S1, Supporting Information). Oat, red clover, and alfalfa crops received no herbicide treatments throughout the study, as weeds were suppressed by mowing stubble and removing hay. Results of this 8-year study capture variations in weather, weed species shifts within the community, and other factors that required shifts in herbicide use among years, while still maintaining the strategies of the CONV and LOW treatments.

Crop Rotation Diversity. There were three levels of crop rotation diversity. The 2-year rotation consisted of a rotation of corn and soybean in alternate years, the 3-year rotation consisted of corn-soybean-oat + red clover, and the 4-year rotation consisted of corn-soybean-oat + alfalfa–alfalfa crop phases. During the 4-year rotation, alfalfa was maintained for hay production for the year after establishment. Composted cattle manure was applied to the 3-year and 4-year rotations preceding corn production.

USEtox Model and Data. Data used to populate the USEtox toxicity model were derived from 2008–2015 operations logs for the field experiment. These logs included names of the specific active ingredient (a.i.) applied, application rate (kg a.i. ha^{-1}), herbicide treatment type (banded or broadcast), and commercial product name. Freshwater toxicity loads were calculated using the USEtox 2.0 model.[26] USEtox generates midpoint characterization factors (CF) to estimate potential freshwater ecotoxicity impacts per mass of herbicide a.i. applied, expressed in CTUe of potentially affected fraction of species integrated over time and volume (PAF m^3 $kg_{emitted}^{-1}$). The function of a CF is to bridge the mass of applied herbicide to the freshwater ecosystem response by accounting for its persistence in the environment, and its transport via air, soil, and freshwater pathways.[27] USEtox CFs are a function of (1) the fraction of pesticide a.i. transferred via

DOI: 10.1021/acs.est.6b04086
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

air, soil, or water compartments (i) to freshwater ($f_{i,w}$); (2) the environmental fate factor ($FF_{w,w}$), that is, how the a.i. persists in the environment; and (3) the effect factor (EF_w), which describes the freshwater aquatic species response to an increase of a toxic substance in the freshwater environment:

$$CF = f_{i,w} \times FF_{w,w} \times EF_w$$

The fraction of pesticide transferred ($f_{i,w}$) from air or soil to freshwater is driven by the physiochemical properties of each pesticide a.i., particularly the partition coefficient. The $FF_{w,w}$ describes the active ingredient persistence in the environmental compartment (e.g., air, soil, water), and is measured in $(kg_{in\ water}/(kg_{emitted}/day))$ = day.[27] EF_w values represent the compiled toxicity effect concentrations ($HC50_{EC50}$) from laboratory data and were used to estimate the following freshwater species response:

$$EF_w = 0.5/HC50_{EC50}$$

where $HC50_{EC50}$ is the average hazardous concentration of pesticide a.i. at which 50% of exposed test populations are chronically impacted in laboratory tests. The species-specific concentration at which 50% of exposed test populations are affected is expressed by EC50.[28] Chronic toxicity lab data for freshwater populations (HC50) are comprised of EC50 data from at least three phyla, where EC50 are data points representing the concentration at which 50% of test populations are affected.[28] EF units are the number of potentially affected species (PAF) in a cubic meter of freshwater per kilogram of a.i. emitted ($PAF\ m^3\ kg_{emitted}{}^{-1}$).[27] The 0.5 factor represents the point value along the chronic PAF curve at which there is 50% species lethality. $HC50_{EC50}$ values are found in the Aquatic Impact Indicators Database (AiiDA) and are comprised of three taxonomic tiers of toxicity calculations:[28]

- Substance-species: all available species-level lab toxicity data. If there are multiple EC50 data points for one species, then the geometric mean is calculated and used as the species-representative toxicity value ($EC50_s$).
- Substance-phylum: all available phylum-level lab toxicity data. If there are multiple $EC50_s$ data points for one phylum, then the geometric mean is calculated and used as the phylum-representative toxicity value ($EC50_p$).
- Substance-impact: this is the geometric mean of the $EC50_p$ from all available phyla (there must be at least 3 distinct phyla represented for use in the toxicity database), and is used as the substance toxicity value ($HC50_{EC50}$) that goes into the EF calculation.

However, this method provided only potential freshwater toxicity, assuming homogeneous transport of each a.i. through air, soil, and water, and did not include postfield application fate.[17,29] Due to the unique physiochemical properties of each a.i., emission factors were applied to more accurately capture the emission fate following in-field application.[29] We combined methods of vapor pressure-derived air emissions factors and the following assumptions from soil and water emissions models to calculate emissions factors to rural air, freshwater, and agricultural soil compartments (Table 1).[30,31]

- Air emissions fraction dependent on a.i. vapor pressure (Table 1)[30]
- Maximum water emissions fraction set at 5%[31]
- Remaining fraction up to 85% emitted to soil[31]

Table 1. Vapor Pressure Derived Pesticide Emissions Factors

vapor pressure (mPa)	air emission factor
$p > 10$	0.95
$1 < p < 10$	0.50
$0.1 < p < 1$	0.15
$0.01 < p < 0.1$	0.05
$p < 0.01$	0.01

Herbicide a.i.-specific freshwater toxicity ($T_{ecosystem}$) was reported in CTUe units and calculated using the following equation:[30]

$$T_{ecosystem} = M_t \times \sum_i (EF_{ti} \times CF_{ti})$$

where M_t is the mass of the applied herbicide a.i.(t), EF_{ti} is the herbicide- and emissions compartment-specific (i) vapor pressure-derived emissions fraction, and CF_{ti} is the herbicide- and compartment-specific toxicity characterization factor. Herbicide regime and crop specific $T_{ecosystem}$ were averaged over the study time period, then averaged over rotation system to produce annual average toxicity loads by herbicide treatment within rotation system. The toxicity of each herbicide used in the field experiment was compared against the most commonly used herbicides in Iowa for both corn and soybean to determine whether results found in the experiment were comparable to commercial farm systems in Iowa.[32,33]

Performance Metric Calculations. Performance metrics for the different rotation and herbicide treatments in 2008–2015 included level of dependence of each system on herbicides as described by treatment frequency index (TFI), weed biomass in corn and soybean crops, and freshwater toxicity load per 1) hectare, 2) net returns to land and management, and 3) Mg of corn grain, soybean grain, and total harvested biomass.

Treatment frequency index is a metric of herbicide use and reliance and was calculated according to the following equation:[21]

$$TFI = (\text{Herbicide application rate} \times \text{Treated surface area}) / (\text{Lowest registered dose} \times \text{Plot surface area})$$

Toxicity load describes the impact of herbicide application on the land, and TFI describes the level of dependence on herbicides of a particular weed management system. Herbicide application rates came from the experiment management logs and the lowest registered dose was determined from commercial herbicide product labels. Treated and plot surface areas were described by the treatment area of the broadcast and banded herbicide regimes, and the experimental plot dimensions. We calculated the TFI for each commercial herbicide, regardless of how many a.i. were included within each product. When multiple herbicides were used within a single herbicide regime, we summed the TFI for each herbicide applied. Like toxicity load, crop specific TFI was averaged over the study time period, then averaged over rotation system to produce annual average TFI by herbicide treatment within rotation system.

Net returns to land and management were estimated using field operations logs for labor demands, input costs, crop yields, and agricultural economics databases. All costs were calculated using year- and product-specific information. All costs

DOI: 10.1021/acs.est.6b04086
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

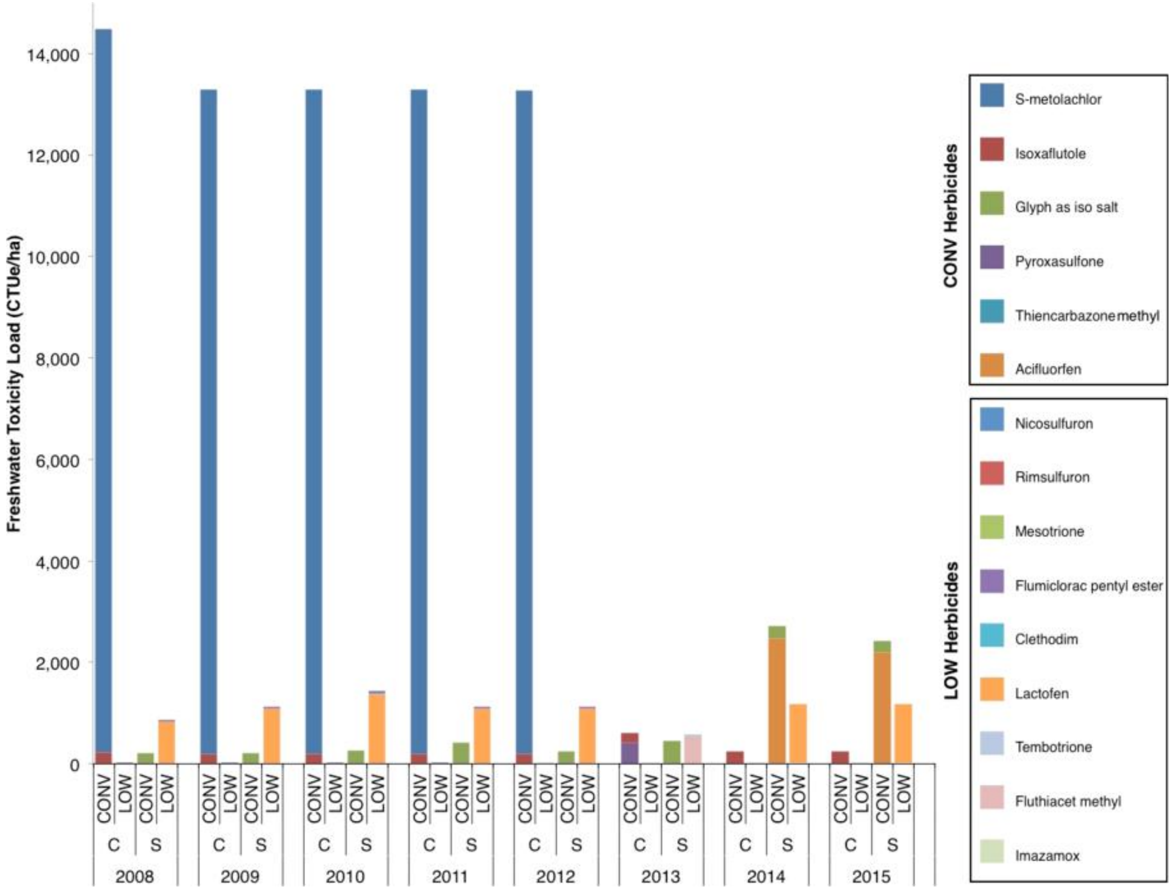

Figure 1. Temporal profile of freshwater toxicity loads of herbicides used for corn (C) and soybean (S) crops at the Marsden Farm experiment plots by active ingredient and herbicide regime, where CONV and LOW represent the conventional and low herbicide regimes, respectively. "Glyph as iso salt" represents the isopropylamine salt of glyphosate.

associated with machinery operations, seeds, labor, and crop insurance were Iowa-based.[34−41] Costs associated with herbicides were from Midwest annual reports (Table S2, Figure S3, Table S4, Supporting Information).[42−49] Costs associated with amending soil nutrients through manure application in the 3- and 4-year rotation systems assumed that manure was produced by on-farm or neighboring-farm livestock with the costs of labor and machinery required for application; no cost was assigned to the manure itself, which was assumed to be a waste product from the livestock enterprise. This approach is consistent with that used by Liebman et al. (2008), Cruse et al. (2010), and Davis et al. (2012), and is appropriate with the caveat that if farmers purchased manure, net returns would be reduced.[17,24,50] Values of crop products were drawn from market-year averages published by the National Agricultural Statistics Service and represent Iowa prices.[51−58] All cost categories and budgets for each rotation system × herbicide regime combination are shown in Table S2 in Supporting Information.

Aboveground weed biomass from corn and soybean plots was determined in each herbicide regime subplot each year prior to crop harvest based on eight randomly selected 3.05 m × 0.76 m sampling areas. Weed biomass samples were placed in forced air ovens for drying, then weighed at ∼0% moisture content.

Harvested crop biomass consisted of corn grain yields, soybean yields, oat grain and straw yields, and alfalfa hay yields. Six rows of each corn and soybean subplot were harvested using a combine equipped with a yield monitor. Oat grain was also harvested by a combine with yield monitor, though measurements were taken over the whole plot area (1530 m^2). Oat straw and alfalfa were each mowed and baled from the 1530 m^2 oat and alfalfa plots; alfalfa was harvested three to five times per year following establishment with oat. All biomass values represented the dry weight of oat straw, alfalfa hay, and oat, soybean, and corn grain.

Statistical Analyses. Linear mixed effect models were used to test the effects of rotation system, herbicide regime, and their interaction on the performance metrics. For metrics including variation across blocks (CTUe $\$^{-1}$, weed biomass kg ha^{-1}, and CTUe Mg corn $yield^{-1}$, CTUe Mg soybean $yield^{-1}$, and CTUe Mg total $yield^{-1}$), we treated block and year as random factors and rotation and herbicide regime as fixed factors. For metrics with no variation across blocks (CTUe ha^{-1} and TFI), we treated year as a random factor, and rotation and herbicide regime as fixed factors. Residuals were examined after plotting as a function of predicted values, and response variables were natural log or rank transformed if they did not meet criteria of normality and homogeneous variance. Pairwise comparisons of means were conducted using Tukey's HSD multiple comparison tests ($\alpha = 0.05$). All analyses were conducted using JMP Pro 12.0.1 (JMP Software, SAS Institute, Inc.).

RESULTS

Corn herbicides applied to the experimental plots were 14 times less toxic than those applied to soybean ($mean_{corn}$ = 1694

DOI: 10.1021/acs.est.6b04086
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

CTUe kg a.i.$^{-1}$, mean$_{soy}$ = 24,504 CTUe kg a.i.$^{-1}$), on an average per-mass basis (Table S1, Supporting Information). However, the magnitude of these differences varied between the herbicide regimes. Herbicides applied under the CONV corn regime were 29% less toxic than those applied to soybean under the same regime (mean$_{corn\ conv}$ = 3006 CTUe kg a.i.$^{-1}$, mean$_{soy\ conv}$ = 4262 CTUe kg a.i.$^{-1}$), whereas under the LOW regime, soybean herbicides were approximately 85 times more toxic on a per-mass basis than those applied to corn (mean$_{corn\ low}$ = 382 CTUe kg a.i.$^{-1}$, mean$_{soy\ low}$ = 32,601 CTUe kg a.i.$^{-1}$). By the experimental design of the broadcast and banded application techniques, as well as due to the particular products applied, application rates were 21 times and 13 times greater under the CONV regime than the LOW regime in corn and soybean, respectively (mean$_{corn\ conv}$ = 1.27 kg a.i. ha^{-1}, mean$_{corn\ low}$ = 0.06 kg a.i. ha^{-1}, mean$_{soy\ conv}$ = 1.60 kg a.i. ha^{-1}, mean$_{soy\ low}$ = 0.12 kg a.i. ha^{-1}).

Freshwater toxicity loads (CTUe ha^{-1}) were the product of specific herbicide a.i. toxicities (CTUe kg^{-1}) and application rates (kg a.i. ha^{-1}). From 2008 to 2015, the annual average freshwater toxicity load of the CONV regime was approximately nine times greater than that of the LOW regime (mean$_{conv}$ = 9454 CTUe ha^{-1}, mean$_{low}$ = 1087 CTUe ha^{-1})(Figure 1). The comparison of toxicity loads between 2008–2012 and 2013–2015 shows substantial decreases in toxicity load within the CONV regime between the two time periods (Figure 2). This decrease can be attributed to the

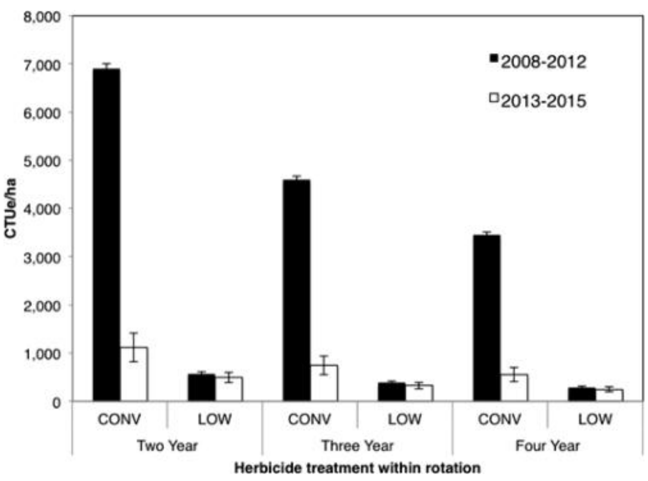

Figure 2. Comparison of freshwater toxicity loads from 2008–2012 and 2013–2015. Error bars represent one standard error of the mean.

retirement of S-metolachlor in 2012, which had a specific toxicity load of 7196.9 CTUe kg a.i.$^{-1}$ (Table S1, Supporting Information). Following this, acifluorfen dominated the profile after its introduction in 2014. Similarly, across the LOW regime, lactofen dominated the toxicity load profile in all years except 2013, when it was replaced by fluthiacet methyl (Figure 1).

System dependence on herbicides was represented by the Treatment Frequency Index, for which a higher TFI value reflected greater herbicide dependence. Rotation system, herbicide regime, and the interaction between them had significant ($p < 0.05$) effects on TFI (Table 2). The CONV regime used within the 2-year rotation had the greatest dependence on herbicides; its TFI value was 33% and 50% greater than for its 3-year and 4-year counterparts, respectively.

Table 2. Results of Statistical Tests for Main and Interactive Effects of Rotation System and Herbicide Regime on CTUe ha^{-1} and Rotational TFI

	$P > F$	
source of variation	CTUe ha^{-1}	rotational TFI
rotation system (ROT)	<0.0001	<0.0001
herbicide regime (HERB)	0.0002	<0.0001
ROT × HERB	0.074	0.040

This was expected, as herbicides were applied for only two years in the 3- and 4-year rotation systems. The greatest reductions were observed between CONV and LOW regimes with an average 81% reduction in herbicide dependence across rotation systems (Figure 3).

Rotation system and herbicide regime each had a significant ($p < 0.05$) influence on freshwater toxicity loads on a per-hectare basis (F_{Rot} = 436.8, F_{Herb} = 23.8) (Table 2). Any departure from the 2-year rotation system resulted in a decrease in freshwater toxicity loads ($p < 0.05$) (Table 2). Adding two crop phases with no herbicide applications resulted in a 50% reduction in toxicity loads (Figure 3).

Comparison with Iowa. Table S1 in Supporting Information describes all herbicide a.i. inputs used in the Marsden Farm experiment, their respective freshwater toxicity values, and the overall freshwater toxicity loads of each crop and herbicide regime. The average toxicities of the commercial corn and soybean herbicides most commonly used in Iowa are comparable to those of corn and soybean herbicides applied in our experiment (Table 3).[32,33] Herbicides applied to Iowa soybeans were, on average, more toxic per kg a.i. purchased than those applied to corn and the same pattern was observed at Marsden Farm (Table S1, Supporting Information, Table 3).

The interaction between rotation system and herbicide treatment had a significant ($p < 0.05$) effect on toxicity load per dollar of net returns to land and management (Table 4), for which we observed 38% and 51% reductions in toxicity loads within the CONV regime when adding one and two additional years of nonchemically treated crops to the corn-soybean rotations, respectively (Figure 4). Shifting from the CONV to LOW herbicide regime significantly reduced toxicity loads per dollar of net returns between 84% and 87% across all rotation systems (Figure 4).

We detected no significant differences in net returns to land and management (Figure 4) across all rotation systems and herbicide regimes, highlighting the potential to significantly reduce freshwater toxicity while maintaining income.

Weed suppression was evaluated in terms of weed biomass production (kg ha^{-1}) in corn and soybean plots within the contrasting rotation systems and herbicide regimes. Rotation system and herbicide regime each had a significant effect ($p < 0.05$) on weed biomass (Table 4). There was no significant difference ($p < 0.05$) in weed biomass between CONV and LOW herbicide regimes in the 2- and 4-year rotations, whereas there was a difference in the 3-year rotation (Figure 5). Thus, within the LOW herbicide regime, the addition of both oat and alfalfa crops to a corn-soybean rotation (i.e., the 4-year rotation system) was necessary to maintain weed control consistent with that of the conventional 2-year rotation system. However, in all rotation system × herbicide regime combinations, mean weed biomass in corn and soybean was <110 kg ha^{-1}, which was a small fraction of the harvested mass of corn and soybean grain (Figures 6 and 7).

DOI: 10.1021/acs.est.6b04086
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

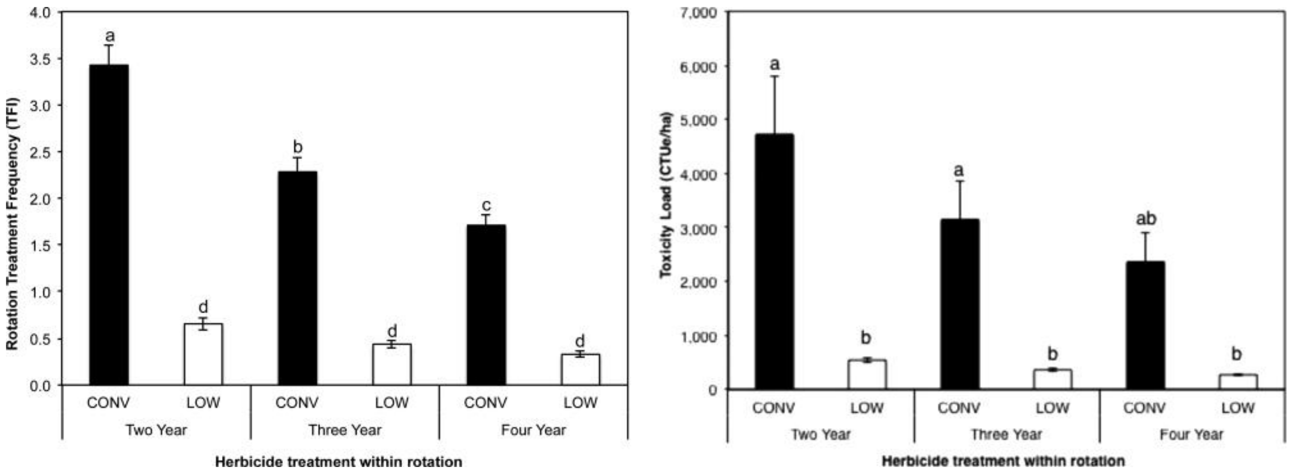

Figure 3. Mean annual treatment frequency index (L) and mean annual freshwater toxicity load per hectare (R) by rotation system and herbicide regime. Error bars represent one standard error of the mean, and mean values topped with different letters indicate significant differences ($p < 0.05$) between treatments.

Table 3. Average Freshwater Toxicity of Herbicides Used at the Marsden Farm Experiment Site (2008−2015) Compared with the Toxicity of Iowa's Most Commonly Used Commercial Herbicides for Corn (2014) and Soybean (2012)[32,33]

	Marsden Farm (CTUe kg a.i. $^{-1}$)			Iowa (CTUe kg a.i. $^{-1}$)		
crop type	max	mean	min	max	mean	min
corn	7197	1694	20	8454	2274	20
soybean	144 215	24 504	183	144 215	16 087	33

Rotation system, herbicide regime, and the interaction of these factors had significant effects on toxicity loads per Mg harvested corn grain (Table 4). Across all rotation systems, shifting from a CONV to LOW herbicide regime resulted in nearly 100% reductions in toxicity load per Mg corn harvested (Figure 6). Annual harvested corn yields in the experiment plots during 2008−2015 were unaffected by both herbicide regime and rotation system (Figure 6), and were slightly greater than those reported for Boone County for the same time period.[59]

Toxicity loads per Mg harvested soybean were affected by an interaction between rotation system and herbicide ($p < 0.05$) (Table 4). Toxicity decreased when shifting from a LOW to a CONV herbicide regime, specifically by 51% and 47% from the 2-year rotation LOW treatment to the 3-year and 4-year rotation CONV treatments, respectively (Figure 7). We also observed significant gains in soybean yields when adding one or more crops to the 2-year CONV (15−21%) and LOW (26−34%) systems (Figure 7). For soybeans, the 3-year and 4-year CONV systems produced the highest grain yields while carrying the lowest toxicity loads. The mean soybean yield of 3.3 Mg ha^{-1} obtained in our experiment plots was similar to the Boone County average of 2.8 Mg ha^{-1} between 2008 and 2015.[59]

Within the CONV regime, toxicity loads per average annual total harvested crop biomass decreased by 30% as oat and alfalfa were added to the 2-year corn-soybean rotation (Figure 8). Annual harvested biomass within the 2-year system was on average 27% higher than yields in the 3-year and 4-year rotations, due to the lower yields of the oat + clover mixture and alfalfa crop phases (Figure 8). Toxicity loads decreased by 88% between CONV and LOW herbicide regimes across all rotation systems, while total harvested biomass remained unaffected by herbicide regime.

DISCUSSION

From 2008 to 2015, in response to the use of crop diversification strategies and integrated mechanical-chemical weed control tactics, we observed grain yields, net returns to land and management, and weed suppression similar to a conventionally managed less diverse system, while also achieving substantial reductions in freshwater toxicity loads and system dependence on herbicide inputs. These results reveal potential pathways away from conventionally managed systems toward more environmentally sustainable production systems. These results also extend those reported by Davis et al. by examining in greater detail the relationships between herbicide treatment strategies, crop rotation diversity, and agronomic, economic, and environmental performance.[17]

Davis et al. found that in 2003−2011, 3-year and 4-year rotations managed with low herbicide inputs not only maintained weed suppression, and maintained or enhanced

Table 4. Results of Statistical Tests for Main and Interactive Effects of Rotation System and Herbicide Regime on CTUe Evaluated Per Dollar Net Return to Land and Management, Mg of Weed Biomass in Corn and Soybean Crops, CTUe per Mg of Soybean and Corn Grain Yields, and CTUe Per Mg of Total Harvested Crop Mass

	$P > F$				
source of variation	CTUe \$$^{-1}$	corn and soybean weeds (kg ha $^{-1}$)	CTUe Mg soybean $^{-1}$	CTUe Mg corn^{-1}	CTUe Mg crops^{-1}
rotation system (ROT)	<0.0001	0.022	0.0004	0.005	<0.0001
herbicide regime (HERB)	0.001	0.019	0.091	<0.0001	<0.0001
ROT × HERB	0.003	0.719	0.004	0.031	0.382

DOI: 10.1021/acs.est.6b04086
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

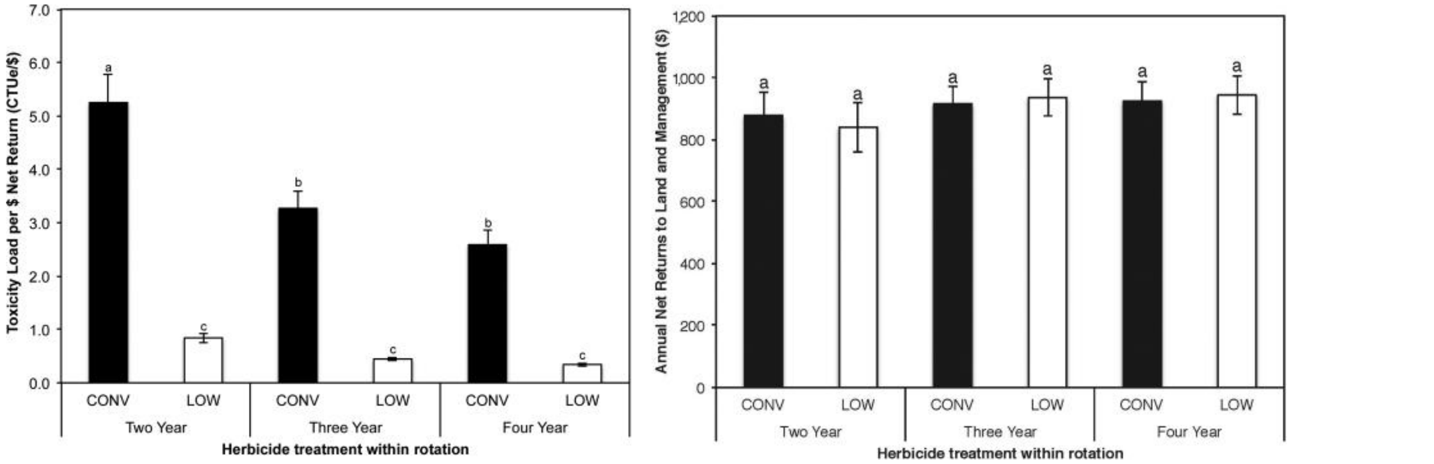

Figure 4. Mean annual freshwater toxicity per annual returns to land and management (L), and mean annual net returns to land and management (R). Error bars represent one standard error from the mean, and mean values with different letters indicate significant differences ($p < 0.05$) across treatments.

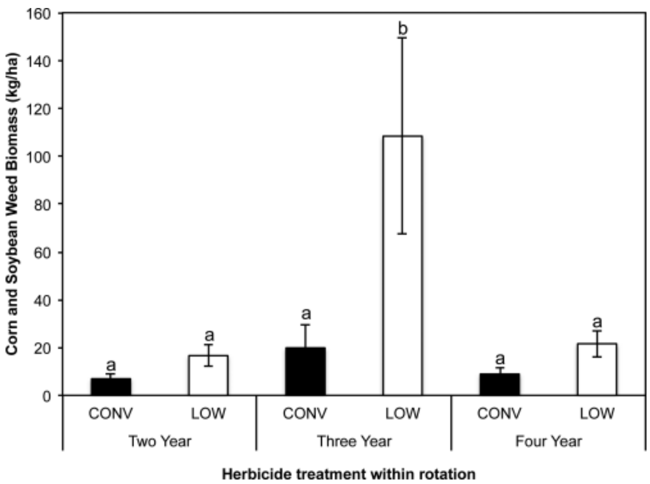

Figure 5. Mean annual biomass of weeds found in corn and soybean. Error bars represent one standard error from the mean, and mean values with different letters indicate significant differences ($p < 0.05$) across treatments.

corn and soybean yields relative to a 2-year rotation managed with conventional herbicide inputs, but also reduced freshwater toxicity loads by 200-fold.[17] Our research shows similar benefits in terms of yield, net returns, and reduced reliance on herbicide inputs, though in this study we isolated the effects of rotation, herbicide regime, and the interaction between them. For the period of 2008–2015 covered in the present study, reductions in freshwater toxicity were less than those reported by Davis et al. for 2003–2011 due to a number of factors.[17] These include the use of different herbicide a.i. between 2003–2011 and 2008–2015, and changes in a.i. characterization factors in USEtox 2.0 due to improvements in the freshwater toxicity indicators database. The release of USEtox 2.0 included the exclusive use of the AiiDA aquatic indicators toxicity database, which was not present in previous versions of USEtox and brings greater transparency and consistency to the use of toxicity data in our study.[60]

Increasing cropping system diversity and altering herbicide regime led to both reduced dependence on chemical herbicides and reduced freshwater toxicity loads, adding more supporting evidence of the benefits of integrated weed management

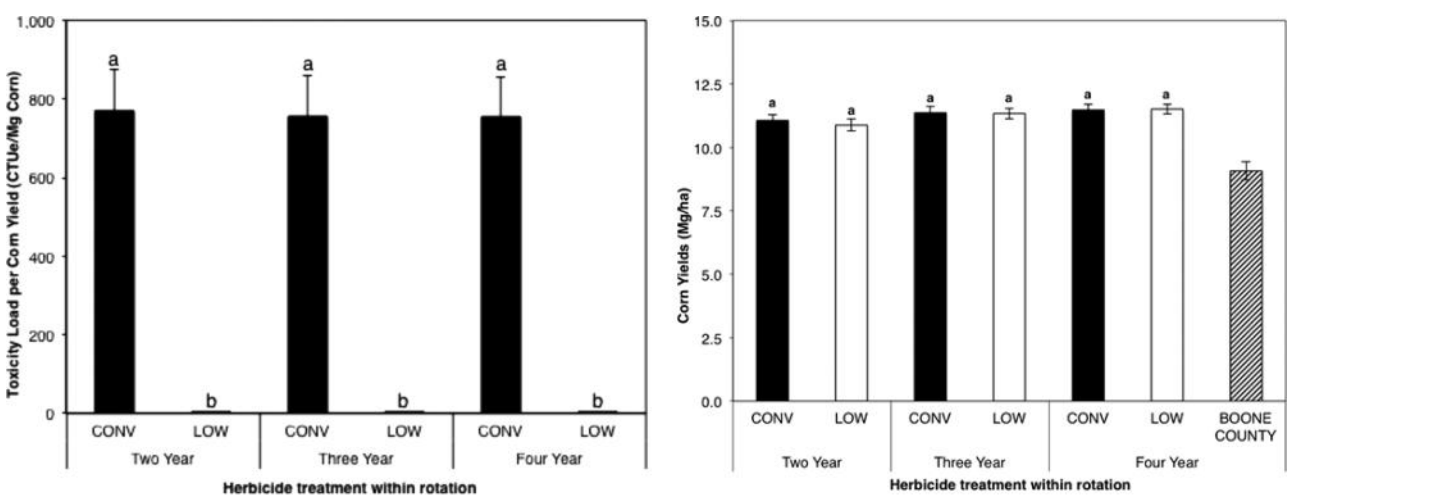

Figure 6. Mean annual freshwater toxicity per Mg of harvested corn (L) and mean annual harvested corn for Marsden Farm and Boone County (R). Error bars represent one standard error from the mean, and mean values with different letters indicate significant differences ($p < 0.05$) across treatments.

DOI: 10.1021/acs.est.6b04086
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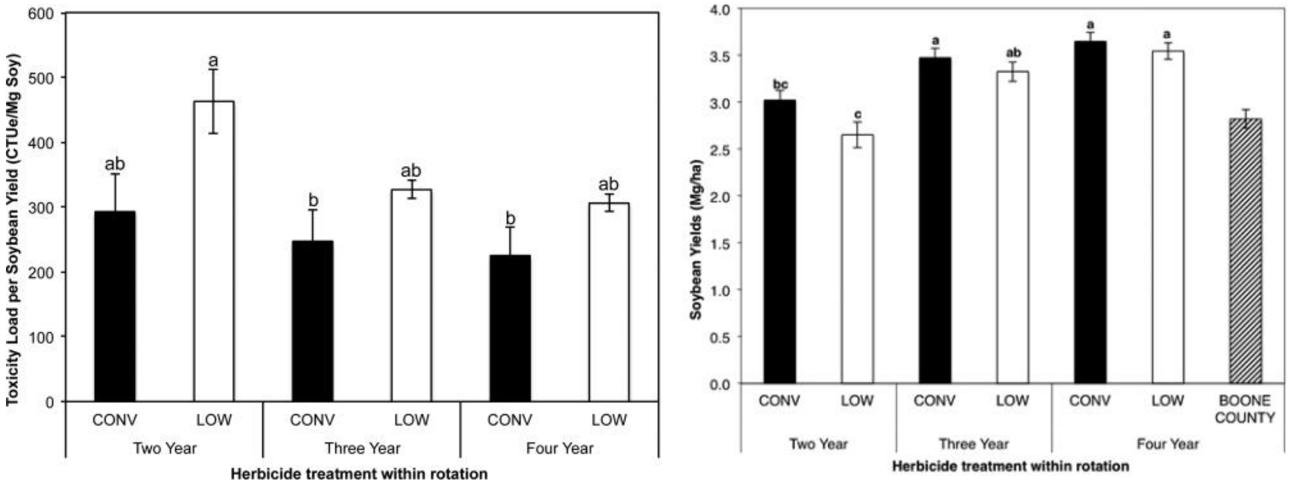

Figure 7. Mean annual freshwater toxicity per ton of harvested soybeans (L) and mean annual harvested soybeans for Marsden Farm and Boone County (R). Error bars represent one standard error from the mean, and mean values with different letters indicate significant differences ($p < 0.05$) across rotation systems.

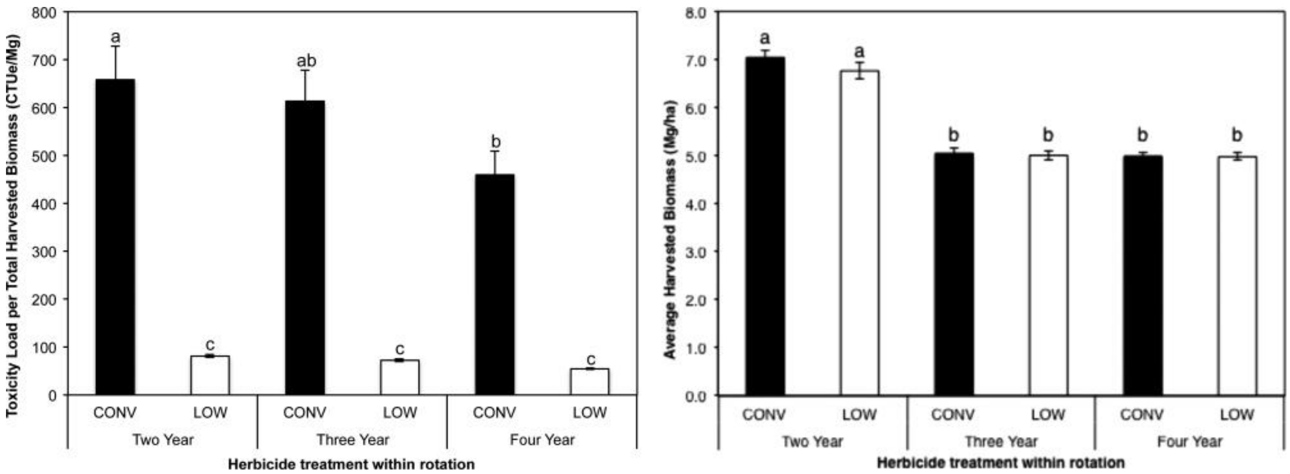

Figure 8. Mean annual freshwater toxicity per ton of harvested biomass, including corn grain, soybeans, oat grains, oat straw, and alfalfa (L) and mean annual harvested biomass (R). Error bars represent one standard error from the mean, and mean values with different letters indicate significant differences ($p < 0.05$) across treatments.

systems.[10,20] Used together, these strategies can lead to additional benefits including lower reliance on purchased external inputs, greater fossil energy use efficiency, and deployment of a broader suite of tools to apply in future pest management programs.[21] Much of the rotation system effect was due to the incorporation of crops requiring no herbicide inputs (Figure S5, Supporting Information). However, the shift to a LOW herbicide regime reduced toxicity loads per unit of land area by an average 89%, regardless of the level of rotation diversity. Such a substantial reduction in emissions to freshwater systems could improve ground and surface water quality and reduce loss of freshwater biota diversity.[4,61]

Herbicide selection was a large driver of freshwater toxicity loads, as we saw substantial decreases as one herbicide (S-metolachlor) was retired. Substituting lower toxicity herbicides for higher toxicity herbicides can potentially reduce overall toxic emissions to the environment. However, farmers do not often have access to this information when making herbicide selections, or the consideration of toxicity is outweighed by other factors such as availability, cost, or weed presence.[62] Framing weed management strategies around reducing system herbicide dependence through increased rotation diversity and reduced herbicide application techniques rather than around specific a.i. toxicity could offer alternatives for farmers lacking access to toxicity information.

Net Returns. We detected no significant differences in net returns to land and management across herbicide regimes or rotation systems, indicating that each alternative weed management strategy produced significant freshwater toxicity reductions without generating a negative economic impact. In addition, the joint implementation of a more diverse rotation system and a reduced herbicide regime delivered significant reductions in toxicity per net returns (Table 4), the greatest of which was 85% between CONV within the 2-year rotation and the LOW within the 4-year rotation system (Figure 4). Perceived economic risk is often a barrier to adopting more environmentally positive management practices, and our results add to a growing body of research that demonstrates that a farmer can reduce freshwater toxicity loads without having to take on significant economic losses.[11,16,17]

Weed Control in Corn and Soybean. Our findings indicate that weed suppression was largely maintained across herbicide treatments, while significantly reducing the negative impacts to freshwater systems, with the exception of the low-

DOI: 10.1021/acs.est.6b04086
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herbicide regime within the 3-year rotation; in which case, mean weed biomass in corn and soybean was 86% greater than the average of all other treatments, but still <110 kg ha^{-1}. In an experiment with corn, soybean, and wheat, Doucet et al. reported that combined use of crop rotations and herbicide management resulted in more effective weed control than either rotation or herbicide treatment alone.[63] Similarly, Chikowo et al. showed that integrated weed management approaches effectively suppressed the densities of both broad-leaved and grassy weed species over the long-term while reducing negative environmental impacts to water, soil, and wildlife biodiversity.[64] Practices such as diversified crop sequencing, mechanical weed destruction, and cover cropping may all serve as important parts of a feasible spectrum of weed management tools.[64,65]

Toxicity Loads per Corn, Soybean, and Total Biomass Harvest. The effects of herbicide regime on toxicity load per mass of crop yield emerged most dramatically in corn plots, where a shift from CONV to LOW herbicide regimes resulted in substantial (>90%) decreases in freshwater toxicity loads per Mg of harvested corn grain, while maintaining yields across rotation system and herbicide regime. Similar results of a combined reduced-chemical and mechanical weed suppression program were found in a 3-year study of applying banded spray treatments with mechanical cultivation.[66] This has important implications, as over 95% of planted corn acres in 2014 were treated with herbicides in the most productive corn states, including Iowa.[33] Because of the long-term nature of our experiment, consistent management practices, and stabilization of soil conditions in each rotation system, we observed relatively stable corn and soybean yields over the 8-year study, and those yields were similar to those reported for the surrounding county.[59] Consistent with what Davis et al. (2012) found, corn yields in our study were slightly higher in the longer rotations than the 2-year system.[17] The mean a.i. toxicity of corn herbicides used in the LOW regime was one-eighth that of the CONV regime, indicating that careful selection of herbicide a.i. can result in significant reductions to freshwater toxicity loads (Table S1, Supporting Information). These results suggest that broad-scale implementation of a low-herbicide regime for corn in the U.S. Corn Belt could translate into considerable amounts of avoided herbicide emissions into surface and groundwater bodies, while maintaining crop yields.

The pattern within soybean plots of our experiment was more complex. We found that a shift from CONV to LOW herbicide regimes resulted in an average 42% increase in freshwater toxicity loads per Mg of soybean harvested across all rotations. This was largely driven by herbicide selection, as the herbicides applied in the LOW soybean regimes were, on average, eight times more toxic than those applied in the CONV regime ($mean_{SoyLow}$ = 32 601 CTUe kg^{-1}, $mean_{SoyConv}$ = 4262 CTUe kg^{-1}) (Table S1, Supporting Information). The two most toxic herbicides applied in this study were used in the LOW regimes, fluthiacet methyl (144 215 CTUe kg^{-1}) and lactofen (15 546 CTUe kg^{-1}) (Table S1, Supporting Information). Replacing the most toxic herbicides used in the LOW soybean regime with the herbicides applied in the CONV regime and banding them would result in a 50% reduction in toxicity loads across the board.

Soybean yields increased as rotation diversity increased because of a reduced incidence and severity of soybean sudden death syndrome, caused by the fungal pathogen *Fusarium virguliforme* in those systems.[67] Herbicide products differed between herbicide regimes used for soybean due to differences in crop genetics. In 2008−2013, a transgenic, glyphosate-tolerant soybean cultivar was used in the CONV herbicide treatment, but a nontransgenic, glyphosate-susceptible cultivar was used in the LOW herbicide treatment. Thus, glyphosate could not be applied to the LOW treatment in those years without killing the crop. We did use the same glyphosate-tolerant soybean cultivar in both herbicide regimes in 2014−2015, though we did not use glyphosate in the LOW treatment in those years, consistent with what had been done in 2008−2013. The reduction in soybean sudden death syndrome due to increased rotation diversity and concomitant increases in soybean yield were consistent among years.

This study provides evidence that contradicts the long-held perception that a system without heavy reliance on chemical inputs is not productive.[11,20] Shifting from a CONV to a LOW treatment system consistently reduced toxicity loads per mass of total harvested biomass by an average of 88%, while the amount of harvested biomass remained unchanged. Herbicide treatments were much more effective at reducing toxicity loads than rotation system, supporting the idea that alterations in weed management can generate effective reductions in environmental degradation while maintaining essential agronomic functions.[17,68]

The management practices and herbicide inputs applied in this experiment were largely representative of those applied in Iowa and across the top producing corn and soybean states in the U.S.[32,33] Given the significance of both rotation diversity and herbicide treatment effects on freshwater toxicity loads, producers now have multiple points of intervention for weed management, each having its unique risks and benefits. Our results support the idea that integrated systems may not require making trade-offs between productivity and environmental sustainability.[21] The necessary functions of an agroecosystem such as productivity, profitability, and weed suppression were maintained while freshwater toxicity loads were significantly reduced under more diverse rotation systems and reduced herbicide regimes. Alone, each strategy was an effective yet low-risk option, and together they could provide opportunities whereby farmers could modify their current weed management strategies and reap the benefits of reduced system dependence on herbicides, reduced toxicity loads per dollar return, and per corn and soybean grain yields. A "mix and match" approach could provide starting points for farmers to adopt management strategies that aid in transitioning toward largely more sustainable agricultural systems.[69]

■ ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org/. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b04086.

Detailed information on herbicide a.i. content, application rates, and net economic return calculations as described in the text (PDF)

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DOI: 10.1021/acs.est.6b04086
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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We express our thanks to Craig Chase, Ann Johanns, Michael Fiscus, David Sundberg, and Matthew Woods for assistance with field work and economic analyses. This research was supported by research grants from the US Department of Agriculture National Research Initiative (Projects 2006-35320-16548 and 2014-67013-21712), the Leopold Center for Sustainable Agriculture (Projects 2007-E09, 2010-E02, 2013-XP01, and 2014-XP01), and the Iowa Soybean Association.

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DOI: 10.1021/acs.est.6b04086
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Supporting Information for

Reducing freshwater toxicity while maintaining weed control, profits, and productivity: effects of increased crop rotation diversity and reduced herbicide usage

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Table S1. Summary of all herbicide inputs and their specific a.i. toxicity loads by corn and soybean crops

	Corn Herbicide Inputs (kg a.i./ha)		Corn Herbicide Toxicity (CTUe/kg a.i.)		Corn Herbicide Toxicity Load (CTUe/ha)	
Year	Low herbicide[a]	Conv herbicide[b]	Low herbicide[a]	Conv herbicide[b]	Low herbicide[a]	Conv herbicide[b]
2008	mesotrione[#] (0.053)	S-metolachlor[†] (1.981)	mesotrione (20.1)	S-metolachlor (7196.9)	mesotrione (1.07)	S-metolachlor (14257.10)
	nicosulfuron[§] (0.013)	isoxaflutole‡ (0.088)	nicosulfuron (87.8)	isoxaflutole (2649.9)	nicosulfuron (1.14)	isoxaflutole (233.20)
	rimsulfuron[¶] (0.007)		rimsulfuron (1234.0)		rimsulfuron (8.64)	
Total	**0.073**	**2.069**	**1,341.9**	**9,846.8**	**10.85**	**14,490.30**
2009	mesotrione (0.053)	S-metolachlor (1.820)	mesotrione (20.1)	S-metolachlor (7196.9)	mesotrione (1.07)	S-metolachlor (13098.40)
	nicosulfuron (0.013)	isoxaflutole (0.070)	nicosulfuron (87.8)	isoxaflutole (2649.9)	nicosulfuron (1.14)	isoxaflutole (185.50)
	rimsulfuron (0.007)		rimsulfuron (1234.0)		rimsulfuron (8.64)	
Total	**0.073**	**1.890**	**1,341.9**	**9,846.8**	**10.85**	**13,283.90**
2010	mesotrione (0.053)	S-metolachlor (1.820)	mesotrione (20.1)	S-metolachlor (7196.9)	mesotrione (1.07)	S-metolachlor (13098.40)
	nicosulfuron (0.013)	isoxaflutole (0.070)	nicosulfuron (87.8)	isoxaflutole (2649.9)	nicosulfuron (1.14)	isoxaflutole (185.50)
	rimsulfuron (0.007)		rimsulfuron (1234.0)		rimsulfuron (8.64)	
Total	**0.073**	**1.890**	**1,341.9**	**9,846.8**	**10.85**	**13,283.90**
2011	mesotrione (0.053)	S-metolachlor (1.820)	mesotrione (20.1)	S-metolachlor (7196.9)	mesotrione (1.07)	S-metolachlor (13098.40)
	nicosulfuron (0.013)	isoxaflutole (0.070)	nicosulfuron (87.8)	isoxaflutole (2649.9)	nicosulfuron (1.14)	isoxaflutole (185.50)
	rimsulfuron (0.007)		rimsulfuron (1234.0)		rimsulfuron (8.64)	
Total	**0.073**	**1.890**	**1,341.9**	**9,846.8**	**10.85**	**13,283.90**

2012	tembotrione° (0.046)	S-metolachlor (1.820)	tembotrione (186.2)	S-metolachlor (7196.9)	tembotrione (8.57)	S-metolachlor (13098.40)
		isoxaflutole (0.070)		isoxaflutole (2649.9)		isoxaflutole (185.50)
Total	**0.046**	**1.890**	**186.2**	**9,846.8**	**8.57**	**13,283.90**
2013	tembotrione (0.049)	pyroxasulfone†† (0.210)	tembotrione (186.2)	pyroxasulfone (1997.2)	tembotrione (9.12)	pyroxasulfone (419.40)
		isoxaflutole (0.070)		isoxaflutole (2649.9)		isoxaflutole (185.5)
Total	**0.049**	**0.280**	**186.2**	**4,647.1**	**9.12**	**604.90**
2014	tembotrione (0.049)	thiencarbazone methyl√ (0.037)	tembotrione (186.2)	thiencarbazone methyl (178.1)	tembotrione (9.12)	thiencarbazone methyl (6.60)
		isoxaflutole (0.092)		isoxaflutole (2649.9)		isoxaflutole (243.80)
Total	**0.049**	**0.129**	**186.2**	**2,828.0**	**9.12**	**250.40**
2015	tembotrione (0.049)	thiencarbazone methyl (0.037)	tembotrione (186.2)	thiencarbazone methyl (178.1)	tembotrione (9.12)	thiencarbazone methyl (6.60)
		isoxaflutole (0.092)		isoxaflutole (2649.9)		isoxaflutole (243.80)
Total	**0.049**	**0.129**	**186.2**	**2,828.0**	**9.12**	**250.40**

	Soybean Herbicide Inputs (kg a.i./ha)		Soybean Herbicide Toxicity (CTUe/kg a.i.)		Soybean Herbicide Toxicity Load (CTUe/ha)	
Year	Low herbicide	Conv herbicide	Low herbicide	Conv herbicide	Low herbicide	Conv herbicide
2008	flumiclorac pentyl ester[‡‡] (0.015)	glyphosate as iso salt[¶¶] (1.120)	flumiclorac pentyl ester (2342.3)	glyphosate as iso salt (182.9)	flumiclorac pentyl ester (35.13)	glyphosate as iso salt (204.80)
	clethodim[§§] (0.051)		clethodim (185.5)		clethodim (9.46)	
	lactofen[##] (0.053)		lactofen (15545.8)		lactofen (823.93)	
Total	**0.119**	**1.120**	**18,073.6**	**182.9**	**868.52**	**204.80**
2009	flumiclorac pentyl ester (0.015)	glyphosate as iso salt (1.120)	flumiclorac pentyl ester (2342.3)	glyphosate as iso salt (182.9)	flumiclorac pentyl ester (35.13)	glyphosate as iso salt (204.8)
	clethodim (0.051)		clethodim (185.5)		clethodim (9.46)	
	lactofen (0.070)		lactofen (15545.8)		lactofen 1088.21)	
Total	**0.136**	**1.120**	**18,073.6**	**182.9**	**1,132.80**	**204.80**
2010	flumiclorac pentyl ester (0.023)	glyphosate as isopropylamine salt (1.400)	flumiclorac pentyl ester (2342.3)	glyphosate as iso salt (182.9)	flumiclorac pentyl ester (53.87)	glyphosate as iso salt (256.10)
	clethodim (0.051)		clethodim (185.5)		clethodim (9.46)	
	lactofen (0.088)		lactofen (15545.8)		lactofen (1368.03)	
Total	**0.162**	**1.400**	**18,073.6**	**182.9**	**1,431.36**	**256.10**
2011	flumiclorac pentyl ester (0.015)	glyphosate as iso salt (1.050)	flumiclorac pentyl ester (2342.3)	glyphosate as iso salt (182.9)	flumiclorac pentyl ester (35.13)	glyphosate as iso salt (192.00)
	clethodim (0.051)	glyphosate as potassium salt (1.203)	clethodim (185.5)	glyphosate as potassium salt (182.9)	clethodim (9.46)	glyphosate as potassium salt (220.0)

		lactofen (0.070)		lactofen (15545.8)		lactofen (1088.21)	
	Total	**0.136**	**2.253**	**18,073.6**	**365.8**	**1,132.8**	**412.00**
2012		flumiclorac pentyl ester (0.015)	glyphosate as iso salt (1.347)	flumiclorac pentyl ester (2342.3)	glyphosate as iso salt (182.9)	flumiclorac pentyl ester (35.13)	glyphosate as iso salt (246.40)
		clethodim (0.051)		clethodim (185.5)		clethodim (9.46)	
		lactofen (0.070)		lactofen (15545.8)		lactofen (1088.21)	
	Total	**0.136**	**1.347**	**18,073.6**	**182.9**	**1,132.8**	**246.40**
2013		fluthiacet methyl[⌡] (0.004)	glyphosate as iso salt (2.471)	fluthiacet methyl (144214.8)	glyphosate as iso salt (182.9)	fluthiacet methyl (576.86)	glyphosate as iso salt (451.90)
		clethodim (0.054)		clethodim (185.5)		clethodim (10.02)	
	Total	**0.058**	**2.471**	**144,400.3**	**182.9**	**586.88**	**451.90**
2014		imazamox[≈] (0.023)	glyphosate as iso salt (1.326)	imazamox (717.5)	glyphosate as iso salt (182.9)	imazamox (16.50)	glyphosate as iso salt (242.50)
		lactofen (0.075)	acifluorfen[£] (0.297)	lactofen (15545.8)	acifluorfen (8341.9)	lactofen (1165.94)	acifluorfen (2477.50)
	Total	**0.098**	**1.623**	**16,263.3**	**8,524.8**	**1,182.44**	**2,720.00**
2015		imazamox (0.023)	glyphosate as iso salt (1.203)	imazamox (717.5)	glyphosate as iso salt (182.9)	imazamox (16.50)	glyphosate as iso salt (220.00)
		lactofen (0.075)	acifluorfen (0.263)	lactofen (15545.8)	acifluorfen (8341.9)	lactofen (1165.94)	acifluorfen (2193.90)
	Total	**0.098**	**1.466**	**16,263.3**	**8,524.8**	**1,182.44**	**2,413.90**

[a] Low herbicide treatments involved a banded herbicide spray

[b] Conventional herbicide treatments involved broadcast herbicide spray

† S-metolachlor: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl]acetamide

‡ Isoxaflutole: (5-cyclopropyl-1,2-oxazol-4-yl)-[2-methylsulfonyl-4-(trifluoromethyl)phenyl]methanone

§ Nicosulfuron: 2-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-N,N-dimethylpyridine-3-carboxamide

¶ Rimsulfuron: 1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-ethylsulfonylpyridin-2-yl)sulfonylurea

Mesotrione: 2-(4-methylsulfonyl-2-nitrobenzoyl)cyclohexane-1,3-dione

‡‡ Flumiclorac pentyl ester: pentyl [2-chloro-4-fluoro-5-(1,3,4,5,6,7-hexahydro-1,3-dioxo-2H-isoindol-2 yl)phenoxy]acetate

§§ Clethodim: 2-[1-[[(E)-3-chloroprop-2-enoxy]amino]propylidene]-5-(2-ethylsulfanylpropyl)cyclohexane-1,3-dione

¶¶ Glyphosate: N-(phosphonomethyl) glycine in the form of its isopropylamine or potassium salt

Lactofen: 2-ethoxy-1-methyl-2-oxoethyl-5-[2-chloro-4-(trifluoromethyl)-phenoxy]-2-nitrobenzoate

[°] Tembotrione: 2-[2-chloro-4-methylsulfonyl-3-(2,2,2-trifluoroethoxymethyl)benzoyl]cyclohexane-1,3-dione

†† Pyroxasulfone: 3-[[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)pyrazol-4-yl]methylsulfonyl]-5,5-dimethyl-4H-1,2-oxazole

[∫]Fluthiacet methyl: methyl 2-[2-chloro-4-fluoro-5-[(3-oxo-5,6,7,8-tetrahydro-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfanylacetate

[√]Thiencarbazone methyl: methyl 4-[(3-methoxy-4-methyl-5-oxo-1,2,4-triazole-1-carbonyl)sulfamoyl]-5-methylthiophene-3-carboxylate

[£]Sodium salt of Acifluorfen: sodium;5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate

[≈] Imazamox: 5-(methoxymethyl)-2-(4-methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl)pyridine-3-carboxylic acid

Table S2. Mean annual non-land production costs for Marsden Experimental Farm, 2008 – 2015 with their standard errors.

Total Non-Land Costs ($/ha)						
2008-2015	Two Year		Three Year		Four Year	
Cost category	CONV	LOW	CONV	LOW	CONV	LOW
Pre-harvest machinery	84.62	100.96	84.10	95.81	66.61	75.39
Seeds	184.91	134.02	165.10	131.18	149.43	123.99
Fertilizers	250.55	250.55	98.48	98.48	143.42	143.42
Herbicides	66.17	36.35	44.11	24.23	33.08	18.17
Other pesticides	4.42	4.42	2.95	2.95	2.21	2.21
Insurance, miscellaneous	89.25	86.31	66.74	64.80	59.95	58.47
Harvest machinery, grain drying, hauling	160.08	158.76	151.09	152.13	206.67	207.50
Labor	20.91	27.35	28.90	33.37	37.79	41.14
Total non-land cost	860.92	798.74	641.47	602.94	699.17	670.29
Total non-land cost SE	29.47	30.56	28.34	29.40	29.25	29.74

All costs, including herbicides, were calculated with year-specific and product-specific information. Variation in herbicide expenditures among years reflects changes in herbicide products used, variation in doses applied, and changes in product prices (Table S2, Figure S3).[1–16] 'Miscellaneous' refers to a loss contingency for planning errors and inefficiencies, as well as training costs, planning time, and membership fees.

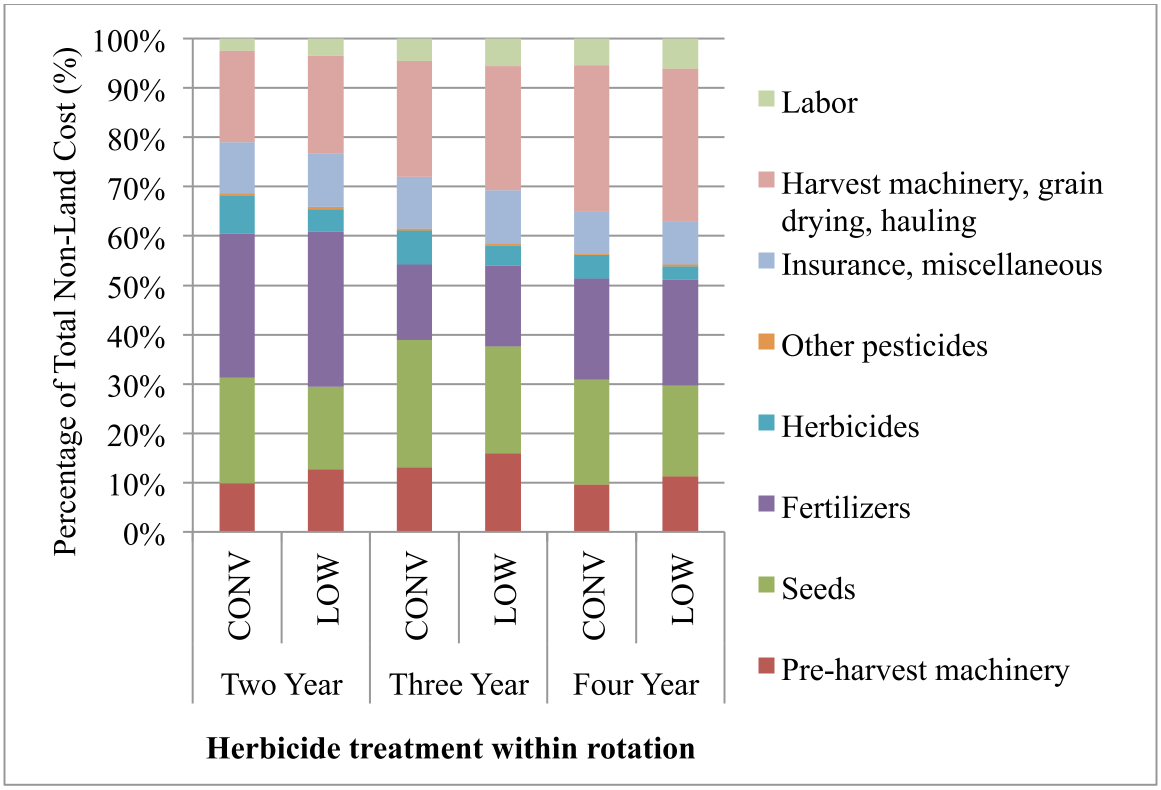

Figure S3. Budgetary composition of non-land costs by rotation system and herbicide regime.

Table S4. Summary of herbicide costs by rotation, herbicide regime, and crop[1–16]

2-year rotation, CONV herbicide costs ($/ha)

	2008	2009	2010	2011	2012	2013	2014	2015
Corn	108.26	100.11	111.77	106.85	107.52	96.50	97.52	96.82
Soybean	9.91	16.10	19.59	22.63	15.51	18.77	72.72	58.12
Rotation average	59.08	58.11	65.68	64.74	61.52	57.64	85.12	77.47

2-year rotation, LOW herbicide costs ($/ha)

	2008	2009	2010	2011	2012	2013	2014	2015
Corn	42.16	44.34	48.81	45.97	25.02	26.48	27.99	28.55
Soybean	22.87	28.90	48.98	38.48	38.83	16.20	46.41	51.59
Rotation average	32.52	36.62	48.89	42.22	31.92	21.34	37.20	40.07

3-year rotation, CONV herbicide costs ($/ha)

	2008	2009	2010	2011	2012	2013	2014	2015
Corn	108.26	100.11	111.77	106.85	107.52	96.50	97.52	96.82
Soybean	9.91	16.10	19.59	22.63	15.51	18.77	72.72	58.12
Oat/clover	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rotation average	39.39	38.74	43.78	43.16	41.01	38.43	56.74	51.65

3-year rotation, LOW herbicide costs ($/ha)

	2008	2009	2010	2011	2012	2013	2014	2015
Corn	42.16	44.34	48.81	45.97	25.02	26.48	27.99	28.55
Soybean	22.87	28.90	48.98	38.48	38.83	16.20	46.41	51.59
Oat/clover	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rotation average	21.68	24.41	32.60	28.15	21.28	14.23	24.80	26.71

4-year rotation, CONV herbicide costs ($/ha)

	2008	2009	2010	2011	2012	2013	2014	2015
Corn	108.26	100.11	111.77	106.85	107.52	96.50	97.52	96.82
Soybean	9.91	16.10	19.59	22.63	15.51	18.77	72.72	58.12
Oat/alfalfa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alfalfa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rotation average	29.54	29.05	32.84	32.37	30.76	28.82	42.56	38.74

4-year rotation, LOW herbicide costs ($/ha)

	2008	2009	2010	2011	2012	2013	2014	2015
Corn	42.16	44.34	48.81	45.97	25.02	26.48	27.99	28.55
Soybean	22.87	28.90	48.98	38.48	38.83	16.20	46.41	51.59
Oat/alfalfa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alfalfa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rotation average	16.26	18.31	24.45	21.11	15.96	10.67	18.60	20.03

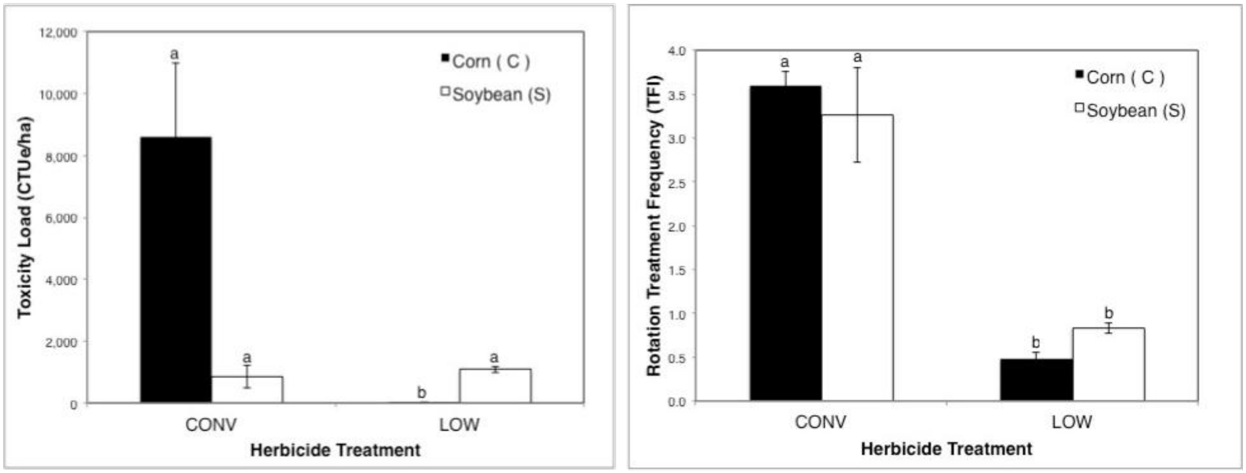

Figure S5. Mean annual toxicity load (L) and TFI (R) per crop and herbicide regime for corn and soybean. Error bars represent one standard error of the mean, and mean values topped with contrasting lower-case letters indicate significant differences ($p < 0.05$) within the crop and between herbicide treatments.

Herbicide Selection Impact Analysis

Within the corn system, CONV toxicity loads were significantly reduced ($p < 0.05$) when shifting to a LOW herbicide regime (Figure S5). However, within the soybean system, there was no significant difference ($p > 0.05$) between herbicide regimes (Figure S5). This suggests that within a soybean cropping system, herbicide a.i. toxicity emerges as a greater driver of toxicity loads than herbicide treatment. We observed significant differences in mean annual TFI between herbicide treatments for both corn and soybean with significant decreases when shifting from the CONV to the LOW herbicide regime (Figure S5). All herbicide treatments are the same across rotation system.

Table S6. Means and standard errors (SE) for CTUe per hectare and Rotation TFI, as affected by rotation system and herbicide regime

Performance Metric	Two Year		Three Year		Four Year		SE
	Means						
	CONV	LOW	CONV	LOW	CONV	LOW	
CTUe ha^{-1}	4,727 (39.5)	543 (21.5)	3,151 (34.8)	362 (13.9)	2,363 (30.0)	272 (7.4)	566 (3.0)
Rotation TFI	3.4 (44.1)	0.7 (18.8)	2.3 (35.9)	0.4 (11.9)	1.7 (29.5)	0.3 (6.9)	0.1 (1.6)

*CTUe ha^{-1} and TFI were both rank transformed before analysis of variance. Values in parentheses represent the transformed means and standard errors.

Table S7. Means and standard errors (SE) for CTUe evaluated per unit of net return to land and management, per unit of weed biomass in corn and soybean crops, per unit of soybean and corn grain yields, and per unit of total harvested crop mass, as affected by rotation system and herbicide regime

Performance Metric	Two Year Means		Three Year Means		Four Year Means		SE
	CONV	LOW	CONV	LOW	CONV	LOW	
CTUe $\$^{-1}$	5.3 (157.2)	0.8 (79.2)	3.3 (137.9)	0.5 (48.1)	2.6 (122.4)	0.3 (34.2)	0.3 (5.6)
Corn & Soybean Weed kg ha^{-1}	6.9 (0.8)	16.6 (2.0)	19.9 (1.3)	108.4 (2.8)	8.9 (0.7)	21.6 (2.0)	17.4 (0.3)
CTUe Mg Soybean $^{-1}$	292.8 (78.7)	463.1 (139.5)	247.1 (69.9)	326.9 (115.3)	225.3 (67.8)	306.1 (107.7)	42.1 (8.8)
CTUe Mg $Corn^{-1}$	770.3 (148.8)	0.9 (56.6)	755.9 (141.9)	0.9 (46.3)	755.0 (142.7)	0.9 (42.6)	72.5 (4.9)
CTUe Mg $Crops^{-1}$	658.0 (155.4)	81.0 (70.8)	613.0 (146.6)	72.0 (57.9)	460.0 (121.5)	54.0 (26.8)	44.0 (5.1)

* CTUe $\$^{-1}$, CTUe Mg $Soybean^{-1}$, CTUe Mg $Corn^{-1}$, and CTUe Mg $Crops^{-1}$ were rank transformed and Corn & Soybean Weeds kg ha^{-1} was natural log transformed before analysis of variance. Values in parentheses represent the transformed means and standard errors.

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(14) *2013 North Dakota herbicide compendium*; North Dakota State University: Fargo, ND, 2013.

(15) *2014 North Dakota herbicide compendium*; North Dakota State University: Fargo, ND, 2014.

(16) *2015 North Dakota herbicide compendium*; North Dakota State University: Fargo, ND, 2015.