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Agroecological management of weeds – minimizing chemical herbicides in arable cropping

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Agroecological management of weeds – minimizing chemical herbicides in arable cropping

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How farmers perceive perennial weeds in Northern France and Eastern Germany

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European farmers are required to follow the transition towards sustainable agriculture and food systems. Perennial weed management without chemical herbicides and inversion tillage is challenging farmers. Questions arise to cope with these spreading weeds. Our study focuses on farmers' perceptions and experiences of perennial weeds and their control in Northern France and Eastern Germany. A survey was developed to explore the situation regarding present concerns and future problems for perennial weed control. The survey conducted from winter 2020/21 to spring 2021 targeted conventional, conservation and organic farms. We found a high level of awareness for perennial weeds. On average, 80.0% of Northern French farmers and 65.9% of Eastern German farmers revealed present concerns about perennial weeds. Both, Northern French and Eastern German farmers perceived perennial weeds are more damaging to crop production than other pests. In both regions, the farmers considered *Cirsium arvense* (L.) Scop. as the most important perennial weed. While the majority of the Eastern German farmers observed field infestations of *Elymus repens* (L.) Gould, Northern French farmers more often reported *Sonchus arvensis* L. infestations. More than 50% of the farmers stated *Rumex* spp. infestations in Northern France and Eastern Germany. Interestingly, Eastern German farmers are more concerned about future perennial weed problems than Northern French farmers. The reasons for farmer's future concerns are probably connected to the farming system. In both regions, conservation and conventional farmers heavily rely on herbicides for perennial weed control, however, more farms used the active ingredient glyphosate in Eastern Germany. Nonetheless, perennial weed control is a major concern for organic farmers in both regions. We conclude that optimizing and integrating non-chemical alternatives is promising in all farming systems. Research activities are required to provide farmers and extension services with novel and profitable perennial weed management practices.

KEYWORDS

farmers' perceptions, perennial weed management, survey, weed control, farmer questionnaires

1 Introduction

Perennial weeds reproduce both sexually, by setting seeds, and by clonal propagules (Håkansson, 2003). Creeping perennials ensure their lifeforms by subterranean storage organs, like roots, rhizomes or stolons (Anderson, 1999). Their clonal systems facilitate survival and spatial spread in arable fields by sprouting from these vegetative propagules (Harper, 1979; Navas and Goulard, 1991). Classified as geophytes that regenerate their above-ground plant biomass from subterranean sources, creeping perennial weeds may in general occur in different agroecosystems. Farming systems suffer from perennial weed infestations (Turner et al., 2007; Riemens et al., 2010; DeDecker et al., 2014). Some species are strongly adapted to arable land frequently disturbed (Tørresen et al., 2003; Bergkvist et al., 2017; Brandsæter et al., 2017).

The weediness and persistence of perennial weeds mainly depend on their vegetative growth and creeping root system (Håkansson, 1982) which allow the species to store nutritive elements and remain alive for several years (Buhler, 1994). For this reason, perennial weed management strategies require a multi-year approach, including specific preventive measures (e.g., crop rotation), cultural methods (e.g., competitive crops and varieties), and direct weed control tactics (e.g., use of herbicides, stubble management, mowing), which depend on the farm type and farm equipment (Mohler et al., 2021). Perennial weed control is one of the main challenges in organic farming (Bond and Grundy, 2001; Turner et al., 2007), more than in conventional farming. In organic farming systems, preventing perennial weeds by tillage practices, precisely displayed at the adequate moment, is important as synthetic chemical herbicides for weed control are excluded (Gruber et al., 2012). Cultural methods are specially appropriated to minimize gaps in which perennials may proliferate uncontrollably (Melander et al., 2012). Closing gaps in competition by subsidiary crops, e.g., cover crops, catch crops, either under-sown in the main crop or established after harvest, both for the purpose to perform competition in the period between main crops, is an important strategy to manage creeping perennial weeds (Vanhala et al., 2006; Bergkvist et al., 2010; Ringselle et al., 2015; Thomsen et al., 2015; Kolberg et al., 2018).

Such a systemic approach is different to the common trend in conventional farming where perennial weed control commonly includes direct control tactics (Harker and O'Donovan, 2013; Favrelière et al., 2020). Herbicides are central in the conventional approach to manage perennial weeds (McErlich and Boydston, 2014), while inversion tillage by a mouldboard plough and stubble cultivation in the intercropping period are crucial for non-chemical weed control (Brandsæter et al., 2017).

In conservation agriculture, the farmers rely on the same weed management practices as in conventional tillage systems but eliminate most or all of the tillage practices. Those inverting the soil are completely omitted. By reducing tillage kind, depth and frequency in conservation farming, perennial weeds became more prevalent than under conventional tillage systems (Pekrun and Claupein, 2004). Likely, conservation farming is depending much more on cultural (e.g., crop rotation, competitive cultivars, cover crops) and chemical control options (Soane et al., 2012). Indeed, conservation farmers design their cropping systems around the use of the non-selective active ingredient glyphosate (Andert et al., 2018; Pardo and Martínez, 2019; Beckie et al., 2020). Across European countries, one third of the acreage of annual cropping systems and half of the acreage of perennial tree crops are annually sprayed with glyphosate (data from 2013-2017, Antier et al., 2020). In Germany, detailed analyses of on-farm application patterns revealed that glyphosate was used for stubble and pre-sowing application on 34.0% of all fields (Andert et al., 2018). Among the French DEPHY farms (network, which represents more than 3,000 farms) with arable crops, 59% used glyphosate regularly or occasionally (Lapierre et al., 2019).

While the use of glyphosate remains by far the most effective practice for controlling perennial weeds in conventional and conservation farming, reduction strategies and acceptable alternatives are urgently required as glyphosate is expected to be increasingly restricted or even banned in Europe (Fogliatto et al., 2020; Kudsk and Mathiassen, 2020; Tataridas et al., 2022; Triantafyllidis et al., 2023). More and better agro-ecological weed management was likewise the specific goal of the European Directive 2009/128/EC on sustainable use of pesticides. Experimental results prove the efficacy of non-chemical control of perennial weeds, e.g., mechanical cutting (Bond and Grundy, 2001; Tiley, 2010), repeated mowing and hoeing (Graglia et al., 2006; Brandsæter et al., 2012; Bergkvist et al., 2017), stubble cultivation (Pekrun and Claupein, 2004), inversion tillage (Thomsen et al., 2015; Brandsæter et al., 2017), competition by cover crops (Vanhala et al., 2006; Kolberg et al., 2018) and vertical and horizontal cutting with minimum soil disturbance (Ringselle et al., 2018; Brandsæter et al., 2020).

Here, we present a survey to gain knowledge about farmers' perennial weed management on-farm. We analyzed data from a survey among farmers in Northern France and Eastern Germany. We chose these study regions because the restriction or outright ban of the common active ingredient glyphosate is planned or occurring in France and Germany (Tosun et al., 2019; Beckie et al., 2020; Leonelli, 2023).

The 2017 EU-wide renewal of approval of glyphosate (currently approved until 15 December 2023) has caused considerable discontent among Member States, triggering the enactment of several national or regional measures. Despite Glyphosate was the most widely used herbicide active substance (9,700 tonnes in 2018 according to the Ministry of Ecological Transition and Solidarity 2019), France was the first country to announce an intention to ban glyphosate within three years (2017-20) (Kinniburgh, 2023). Nevertheless, instead of instituting a full ban on glyphosate in 2020, France merely announced new regulations which further restrict use authorizations for products containing glyphosate. In general though, France was the first European country with an overall pesticide reduction target of 50% (Ecophyto plan for 2018, proposed by the Grenelle Environment Forum in 2007), equivalent to the EU's 2030 goal under the Farm to Fork Strategy (European Commission, 2020). Even France has failed to reduce pesticide use (Hossard et al., 2017), the policy adopted by the country (Ecophyto II and II+ plans for 2025) promotes the agro-ecological transition of its farms (Chauvel et al., 2022). Likewise, the German government's Arable Farming Strategy 2035 sets out the clear direction for the reduction of pesticides (Bundesministerium für Ernährung und Landwirtschaft, 2021a). Starting 2020, the German government has also implemented a glyphosate reduction strategy and proposed to ban the use of plant protection products containing glyphosate in Germany after December 31, 2023 (Bundesministerium für Ernährung und Landwirtschaft, 2021b).

At the same time, many European farmers and farmers' unions are vigorously opposed to the ban of glyphosate (Bjørnåvold et al., 2023). It is specially challenging for no-till agriculture because these systems can be difficult to set up and lack sustainable solutions for weed management without such type of herbicides (Kassam, 2019). Without glyphosate, fundamental changes in farming practices, and perennial weeds in particular are expected (Kudsk and Mathiassen, 2020) and a systemic approach is needed to design no herbicides systems (Chikowo et al., 2009; Reboud et al., 2019).

The objective of this study was to explore the practical experience of farmers. We wanted to answer the following questions:

1. Which practices do farmers apply to control perennial weeds?
2. Are farmers concerned or worried about perennial weeds?
3. How do farmers in Northern France and Eastern Germany perceive perennial weeds currently and in the future?

The analyses focus the regional and farm type level to see whether differences in production conditions and systems have an influence on farmers' perceptions and experiences of perennial weeds and their control. Both regions chosen are characterized by arable cropping and include farms of different types.

2 Materials and methods

2.1 Study regions

Surveys were carried out among farmers in Northern France and Eastern Germany (Figure 1). In France, farmers in four regions surrounding Paris were surveyed: Normandy, Centre-Val de Loire, Ile de France and Hauts-de-France. In Germany, the survey was conducted in five federal states in eastern Germany: Saxony-Anhalt, Saxony, Thuringia, Brandenburg and Mecklenburg-Western Pomerania. The landscape of both regions is homogeneous and mainly characterized by large areas of cropland managed under conventional farming focused on winter wheat (*Triticum æstivum* L.), winter barley (*Hordeum vulgare* L.), and winter oilseed rapeseed (*Brassica napus* L.) (Andert et al., 2015; Ayerdi Gotor et al., 2020). The mean annual temperature between the years 1991 and 2020 was 10.8°C with mean annual precipitation of 712 liters per square meter in the region of Northern France (Météo France, SAFRAN, 2023), 9.1°C and 635 liters per square meter in the region of Eastern Germany (Deutscher Wetterdienst, 2022), respectively.

2.2 Surveys

We developed a questionnaire-based survey (see the Supporting Information) to elicit details about perennial weeds and their control practices in Northern France (15 farms) and Eastern Germany (41 farms). The surveys were conducted from winter 2020/21 to spring 2021 and were targeted at conventional, conservation and organic farms (Table 1). There were no

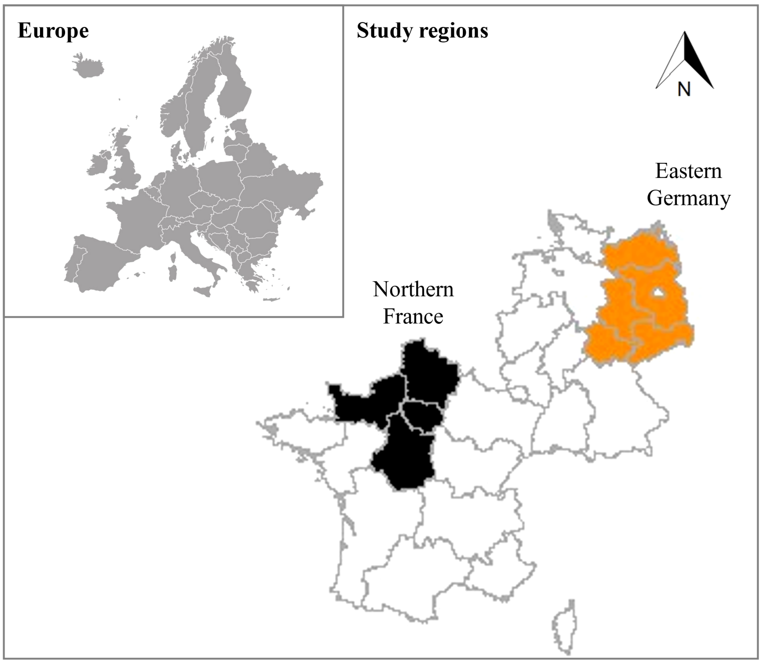

FIGURE 1
Location of the study regions Northern France and Eastern Germany.

incentives for farmers to participate in the survey. Participation was on a voluntary basis for both countries.

With a mixture of closed (a question that presents respondents with pre-populated answer choices) and open (allow respondents to answer in open text format) question techniques, general and detailed questions regarding farm demographics, farmers' perceptions of perennial weeds in arable crops and management of perennial weeds in arable crops. We widely used 'Likert Scale Questions' which offer a set of answer options that cover a range of opinions (Joshi et al., 2015).

In Northern France, 15 farmers who had to manage perennial weeds on their fields and who used cover crops (even if perennial management was not the main purpose of their cover crops) were surveyed. The Northern French online questionnaire consisted of questions on the role of cover crops, the ranking of perennial control methods, the presence and the evolution of perennial pressure over the years and the place of perennials in the ranking of the most problematic bio-aggressors. The Northern French farmers surveyed were pre-selected to have as many farmers in organic farming as in conventional farming and conventional farming with low or no tillage. Thus, among the 15 French farmers, there were: five in organic agriculture (no glyphosate, with tillage), five in soil conservation agriculture (two farmers) or in simplified cultural working (three farmers) (with glyphosate use, with low or no tillage) and five in conventional farming (with glyphosate use and tillage). The contact details of the French farmers were given with their agreement by a chamber of agriculture advisers, they were contacted by telephone to explain the objectives of the survey and to invite them to participate. We contacted 51 farmers and selected 15 of them, on several criteria: questions and problems with perennial weeds, presence of cover crops in their succession and balance between organic, no-till and conventional farmers. Those who accepted received the online questionnaire by email. These farmers were then interviewed at their homes or on their farms. During the French in-person interviews, the answers to the previous online questionnaire were reviewed with the farmers, to validate them, to answer the questions they had no time to answer and to clarify some questions they were not sure about. The personal interview also allowed us to characterize their farm (e.g., size of the farm, type of soil, description of the rotation). The Northern French farmers have not received any incentives for their voluntary participation.

In Eastern Germany, the anonymous questionnaire-based survey was published online, using the survey software EvaSys provided by Electric Paper Evaluationssysteme GmbH. The survey contained three main question categories, partially with sub-questions (a total of 25 questions). The web link to the survey was published in regional farming magazines. Participation in the survey was voluntary. The completed questionnaires were then returned to the University of Rostock and checked manually for non-response, incomplete and inconsistent responses (the values/answers entered for the questions must be consistent with the options). A total of 41 farmers clicked on the survey link. Information about response rates, e.g., how many farmer viewed the survey link and how many of them did actually respond are not available. The completion rate was 100%. We verified returned questionnaires by connecting the values/answers entered for the questions to make sure that a farmer did not fill out the questionnaire twice, the so-called 'survey fraud' (Singh and Sagar, 2021).

TABLE 1 Number of participants in the survey for the individual farm types organic, conservation and conventional.

Farm type/region	Northern France	Eastern Germany
Number (n)		
Organic	5	7
Conservation	5	13
Conventional	5	21
Total	15	41

The data originate from two surveys, conducted separately in Northern France and Eastern Germany.

2.3 Data handling and analyses

The two data sets needed some adjustments to ensure comparability, while several questions of the Northern French and Eastern German surveys were similar, some parts were different. From the Northern French survey, only the ranking of perennial control methods, the presence and the evolution of perennial pressure over the years and the place of perennials in the ranking of the most problematic bio-aggressors were taken into account. The same restriction was applied to the Eastern German survey data.

A set of four questions were aimed at characterizing the demographic characteristics of participating farmers (Table 2). The 'Likert Scale Questions' focused on farmers' perceptions of perennial weed infestations including 'Ranking of perennial weeds in comparison to the three pests', 'Infestation of perennial weeds', 'Effectiveness of five methods to control perennial weeds', 'Concerns about perennial weeds', 'Estimation of future problems with perennial weeds' (Table 3). Furthermore, farmers were asked to name 'Most problematic perennial weeds' as the open question.

TABLE 2 Farm and management variables surveyed.

Variable	Level
Region	Northern France
	Eastern Germany
Farm type	Organic
	Conservation
	Conventional
Soil type	Sand
	Loam/loss
	Clay
Main crop	Winter cereal
	Summer crop
	Winter oilseed rape

The data originate from two surveys in Northern France and Eastern Germany.

Statistical analyses and scientific graphics were done in R, version 4.0.2 (R Core Team, 2022). The following packages were included: 'agricolae' (univariate analyses, de Mendiburu and Yaseen, 2020), vegan (multivariate analyses, Oksanen et al., 2014) and ggplot (graphs, Wickham, 2016).

Mean differences in farm and production characteristics, farmers′ perception of the perennial weed infestation (%) and damage potential of perennial weeds compared to other pests in crop production of Northern French and Eastern German farmers were compared by using Welch two sample t-test.

Differences in farm and production between the three farm types 'conventional', 'conservation' and 'organic' were tested with the non-parametric Kruskal-Wallis test.

Variation partitioning based on adjusted R^2 in redundancy analysis (RDA) divided the variation of the ordinal response variables 'Concerns about perennial weeds' (Figure 2) and 'Perceptions about perennial weeds' (Figure 3) among the explanatory variables 'farm size', 'farm type', 'region', 'soil type' and 'rotation length'. These variables were chosen to be influencing factors of a range of possible drivers for perennial weed infestation.

3 Results

3.1 Farm characteristics

Farms were significantly smaller in Northern France (184 ha) than in Eastern Germany (966 ha) (Table 4). We found a strong correlation (ρ=0.5) between farm size and region (Figure A1).

TABLE 3 Farmers' perception variables with their respective levels, which were presented to the farmers within the survey.

Variable	Level
Ranking of perennial weeds in comparison to the three pests: annual weeds, pathogens, animal pests	Perennials are most difficult Perennials are more difficult Perennials are less difficult Perennials are least difficult
Infestation of perennial species	*Cirsium arvense* *Sonchus arvensis* *Elymus repens* *Rumex*
Most problematic perennial species	Open question
Effectiveness of five methods to control perennial weeds: Crop rotation Herbicides Glyphosate Inversion tillage Non-inversion tillage Cover crops	Not effective Somewhat effective Very effective
Concerns about perennial weeds	Not concerned Somewhat concerned Very concerned
Estimation of future problems with perennial weeds	Perennials less problematic Perennials more problematic

The data originate from two surveys, conducted separately in Northern France and Eastern Germany.

Significantly longer rotations were cropped on Northern French farms (seven years) than on Eastern German farms (four years). Winter cereals were the main crop in both regions. In Northern France, the mean on-farm soil type was clay and loam/loss, while in Eastern Germany it was sand and loam/loss.

On average, farms size of conservation (963 ha) and conventional (759 ha) was bigger than that of organic farms (444 ha) (Table 5). Crop rotations were longer on organic farms (six years) than on conservation (five years) and conventional (four years) farms. Winter cereals dominated the crop rotations of all farm types, the proportion of summer cereals was highest for organic farms (25%). The main soil type for the conservation and the conventional farm was loam/loss, and for organic farms sand, respectively.

3.2 How farmers control perennial weeds

In both regions, all conservation and conventional farmers used herbicides to control perennial weeds (Figure 4). More farms applicated the active ingredient glyphosate in Eastern Germany than in Northern France. No herbicides, including glyphosate, were applied in organic farms. Among the tillage practices, inversion tillage is common on conventional and organic farms in Northern France. Regardless of the farm type, cover crops were widely used in Northern France to control perennial weeds (100% of farmers) and, to a lesser extent, in Eastern Germany (65%).

3.3 How farmers perceive perennial weeds and their management

In both regions, the farmers considered *C. arvense* as the most important perennial weed (Figure 5). More than 80% of the Eastern German farmers observed field infestations of *E. repens*, while only 20% of Northern French farmers confirmed the species. In contrast, Northern French farmers more often reported *S. arvensis* infestations. More than 50% of the farmers stated *Rumex* spp. infestations in Northern France and Eastern Germany.

Farmers perceived perennial weeds as more damaging for crop production than other pests (e.g., annual weeds, plagues, diseases) (Figure 6). Particularly, Northern French farmers (67%) rated perennial weeds as more damaging. 25% and 13% of Eastern German and Northern French farmers, respectively, ranked perennial weeds as the most damaging pest group. Among the three farm types, farmers anticipated the potential damage of perennial weeds similarly (Figure 6).

Generally, farmers experienced crop rotation, inversion tillage and herbicide use as effective practices to control perennial weeds (Figure 7). Particularly, conventional and conservation farmers perceived the use of herbicides as very effective. In contrast, cover crops were mentioned as only somewhat effective. We found significant differences between Northern France and Eastern Germany in farmers' perceptions of how effective non-inversion tillage is to control perennials. Non-inversion tillage is expected to be not effective (French farmers).

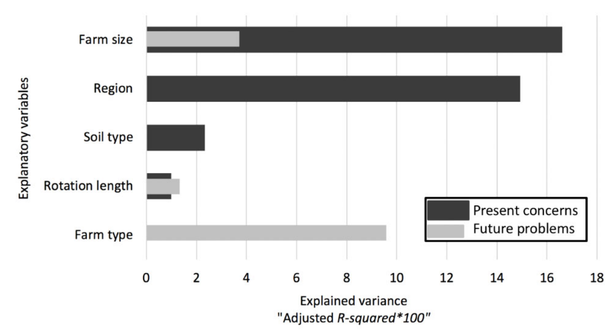

FIGURE 2
Explanatory variables (part of variance) that explain how farmers are concerned presently (grey bars) and in future (black bars) about perennial weeds: farm size, farm type, region, soil type, and rotation length given.

3.4 Explaining how farmers perceive perennial weeds with farm and management characteristics

Overall, explanatory variables explained a total of 34.9% of the variance in farmers' present concerns, and 12% for future problems respectively (Figure 2). Regarding present concerns, the variable farm size explained most of the variance (16.6%). Region explained an additional 14.9% in the farmer perceptions and another 2.3% of the variance resulted from the factor soil type, for rotation length 1% respectively. On average, 80% of Northern French farmers and 65.9% of Eastern German farmers revealed present concerns about perennial weeds (Table 6).

The variable farm type had the largest effect (8.1%) on farmers' perceptions of future perennial weed problems. In Northern France, more organic and conservation than conventional farmers fear future perennial weed problems (Table 6). In general, Eastern German farmers are more afraid about future perennial weed problems than Northern French farmers. Future concerns about perennial weeds in Eastern Germany were not significantly depending on the farm types (Table 6).

The farm size and rotation length only explained 3.2% and 1.2% respectively (Figure 2).

As each of the French farmers confirmed *C. arvense* infestations, relevant factors for *C. arvense* field infestation were only analysed for German farmer participants. The rotation length had the largest effect on the *C. arvense* infestation (16.1%). Farm size affected *C. arvense* field infestations equally strongly as the soil type (13.5% and 12.8% respectively). There was no effect by farm type.

The other weed species (*S. arvensis, E. repens* and *Rumex* spp.) responded differently to explanatory variables (Figure 3). For *E. repens*, almost 60% of the variance was pure region effects (36.5% net effect). Soil type explained an additional 11.9% in the *E. repens* infestation and another 10.8% of the variance resulted from the factor rotation length. The rotation length explained most of the variance for *S. arvensis* infestation (9.5%). Farm size showed a stronger effect on *S. arvensis* variation than soil type, farm type and region. The variables soil type (3.3%) and farm type (2.2%) explained most of the *Rumex* spp. field infestation. The other variables only explained 0.8% (farm size), 1.1% (region) and 1.5% (rotation length).

4 Discussion

This study analysed farmers' perceptions and experiences on perennial weeds and their control in Northern France and Eastern Germany. Opinion surveys offer comprehensive pictures of farmers' appraisements (Ulber and Rissel, 2018; Andert et al., 2019; Lanker et al., 2020; Matousek et al., 2022).

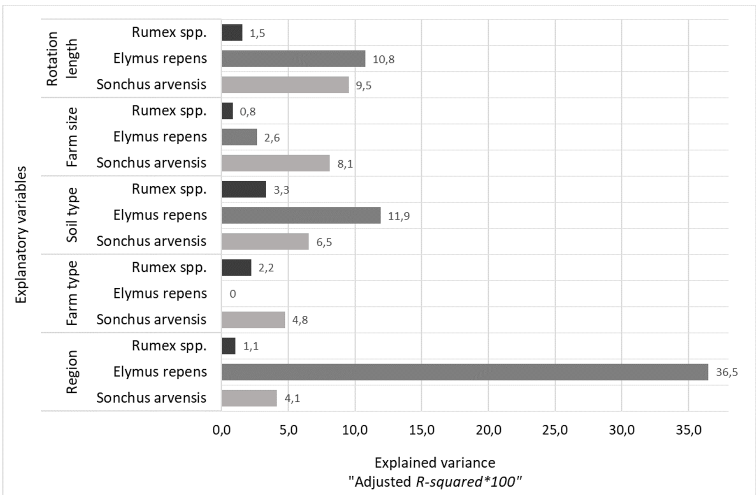

FIGURE 3
How farmers perceive *Rumex* spp., *Elymus repens* and *Sonchus arvensis* in fields explained by the variables farm size, farm type, region, soil type, and rotation length given as part of variance (%).

TABLE 4 Variables describing farm production at Northern French and Eastern German farms.

Variable	Northern France	Eastern Germany	*P* value
Average farm size (min-max)	184 (90-350)	966 (40-5000)	<0.0001
Average rotation length (min-max)	7 (4-11)	4 (3-7)	<0.001
Main crop			
Percentage of farms (%)			
Winter cereal	93	82	0.28
Summer crop	7	15	0.09
Oilseed rape	0	3	0.39
Main soil type			
Percentage of farms (%)			
Clay	53	17	<0.01
Loam/loss	40	39	0.40
Sand	7	44	<0.001

Test method for each of the variables: Welch two sample t-test.

TABLE 5 Variables describing farm production situations of organic, conservation and conventional farmers participating in the survey.

	Organic	Conservation	Conventional
Average farm size (ha) (min-max)	444^{c} (79-2700)	963^{a} (40-5000)	759^{b} (60-2100)
Average rotation length (years) (min-max)	6^{a} (3-11)	5^{ab} (4-10)	4^{b} (3-9)
Main crop			
Percentage of farms (%)			
Winter cereal	75^{b}	83^{b}	92^{a}
Summer crop	25^{a}	11^{b}	8^{b}
Oilseed rape	0^{a}	6^{a}	0^{a}
Main soil type			
Percentage of farms (%)			
Clay	25^{a}	28^{a}	27^{a}
Loam/loss	33^{a}	39^{a}	42^{a}
Sand	42^{a}	33^{b}	31^{b}

Different letters (a, b) in a line represent significant differences ($p < 0.05$) between three farm types. Test methods: non-parametric Kruskal-Wallis test.

We aimed to include organic, conservation and conventional farmers from two European countries into our study in order to reveal the range of variations in perennial weed management.

We found a high level of awareness for perennial weeds. On average, 80.0% of Northern French farmers and 65.9% of Eastern German farmers revealed present concerns about perennial weeds (Table 6). Both, Northern French and Eastern German farmers perceived perennial weeds as more damaging to crop production than other pests.

Sample sizes in our study differed between the study regions Northern France and Eastern Germany. The questionnaire-based survey among Eastern German farmers allowed the acquisition of a larger sample size with lower effort compared to the French survey. A potential limitation of the present study is the sample size of participants (Andrade, 2020); increasing the number of respondents to consolidate our findings would further support the study results. Matousek et al. (2022), describe that the recruitment of participants is effortful because many farmers are expected to be blamed for using glyphosate-based herbicides, and thus, were probably sceptical about the related perennial weed control topic. Therefore, our results provide unique indications for future perennial weed management in Europe. Another limitation of our study is that voluntary recruiting participant in the study region Eastern Germany can cause an unwanted pre-selection of participants. This is a general weakness in questionnaire-based samples (Wu et al., 2022). Pre-selecting the Northern French farmers' by the criterion that they had to manage perennial weeds on their fields, on the other hand may have overestimated the creeping perennial weeds and their need to control. Nevertheless, the findings of this study offer valuable new insight how farmers perceive and control perennial weeds currently and in the future. Moreover, because of their concern about the perennial weeds, farmers could more easily provide a sound advice and share their expertise on solutions to manage the weeds, by soil disturbance or cover crops.

The ability to reproduce vegetatively is a unique characteristic among arable weeds that promotes the survival of perennial species over winters, dry seasons or other unfavorable periods to growth (Håkansson, 1982). Farmers' awareness of perennial weeds and their suitable control tactics are especially important because the infestations are likely to spread rapidly, and have negative impact lasting several years if effective management is not undertaken. Overall, 100% of the surveyed farmers listed *C. arvense* as the most important perennial weed species, and more than 50% of farmer participants stated *Rumex* spp. infestations (Figure 4). The two species are troublesome weeds in both arable lands and grasslands (mainly pastures), but *Rumex* spp. are also early colonizers of many disturbed areas (Zaller, 2004; Favrelière et al., 2020). Interestingly, field infestations of *E. repens* are most prominent among farmers in Eastern Germany. Probably, *E. repens* infestations are favored by common reduced tillage, cereal-dominated crop rotations and high-intensive nitrogen fertilization in this region (Andreasen and Skovgaard, 2009; Andert et al., 2016; Ringselle et al., 2020). Northern French farmers more often reported *S. arvensis* infestations. This species is especially known and problematic in Nordic countries in arable crops and, to some extent, in grasslands

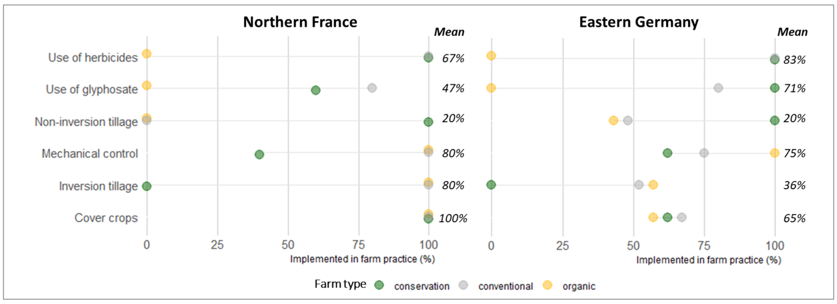

FIGURE 4
Implemented farm practices for control of perennial weeds of Northern French farmers and Eastern German farmers (Figure 1B) participating in the survey. Results were derived from a survey of 15 farmers in Northern France and 41 farmers in Eastern Germany. Farmers' farm practices were grouped per region and farm type.

(Vanhala et al., 2006; Tørresen et al., 2010; Andersson et al., 2013). Whilst, temperature and concentration of CO_2 are increasing globally, *S. arvensis* reacts with higher biomass and reproduction (Tørresen et al., 2019). For this reason, this species is a candidate to profit from climate change (Tørresen et al., 2019), and thus, might potentially spread to other regions.

One of the most interesting results of our study is that farmers in Eastern Germany are more concerned about future perennial

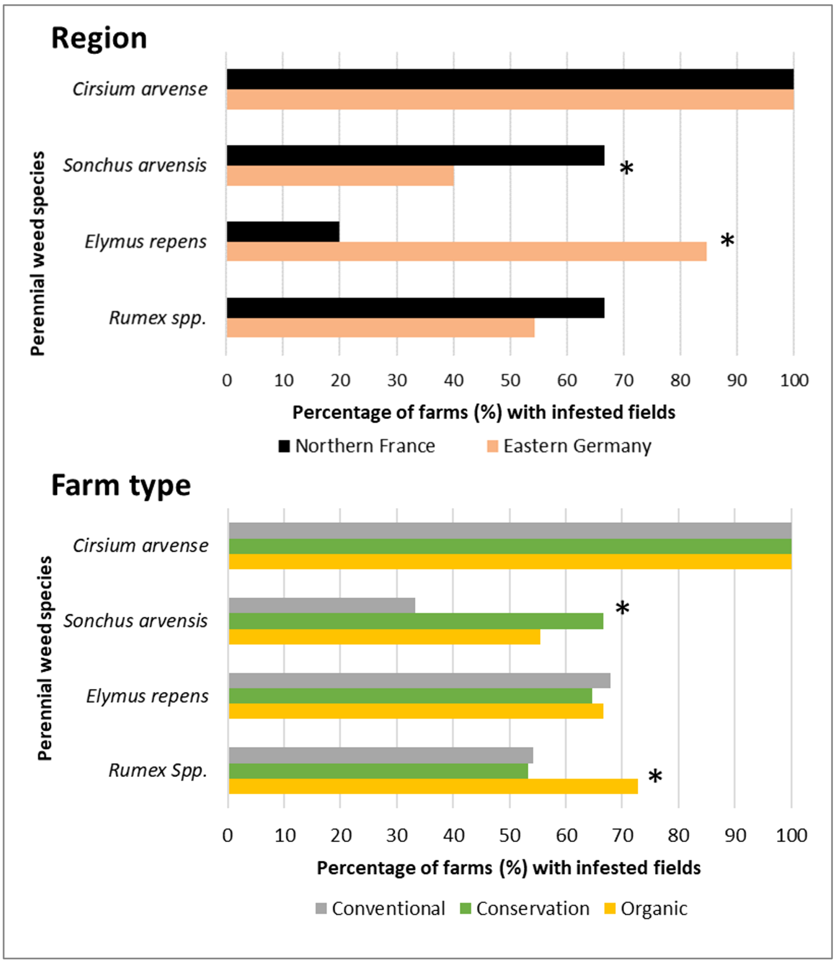

FIGURE 5
How farmers perceive creeping perennial weeds infestation. * represents significant differences ($p < 0.05$) between the regions (Northern France, Eastern Germany) and farm types (conventional, conservation, organic).

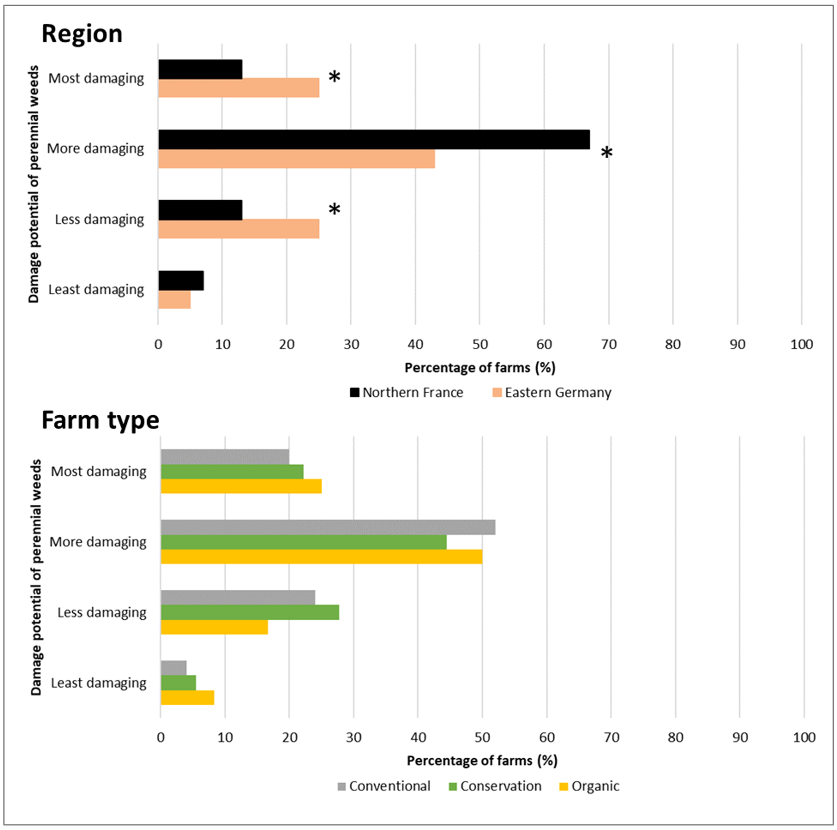

FIGURE 6
How farmers perceive the damage potential of creeping perennial weeds compared to other pests in crop production (e.g., annual weeds, animal pests, diseases). * represents significant differences ($p < 0.05$) between the regions (Northern France, Eastern Germany) and farm types (conventional, conservation, organic).

weed problems than those in Northern France. We attribute the differences in farmers' concerns to regional production differences in our study. The farms were significantly smaller and crop rotations were longer in Northern France than in Eastern Germany (Table 4). Perennial weed control of Eastern German farmers is mainly based on chemical herbicide use, while inversion soil disturbance and competition by cover crops is widely used by the surveyed Northern French farmers (Figure 4). As introduced, disturbance and competition are two important processes which are used to manage creeping perennials weeds non-chemically. Obviously, farmers in Northern France are aware of these agro-ecological weed control tools, designing their cropping systems to be less dependent on herbicides. These cropping systems are expected to be more resilient for future farming.

Farmers in the two regions are differently concerned about perennial weeds in the future. In each region, however, farmers'

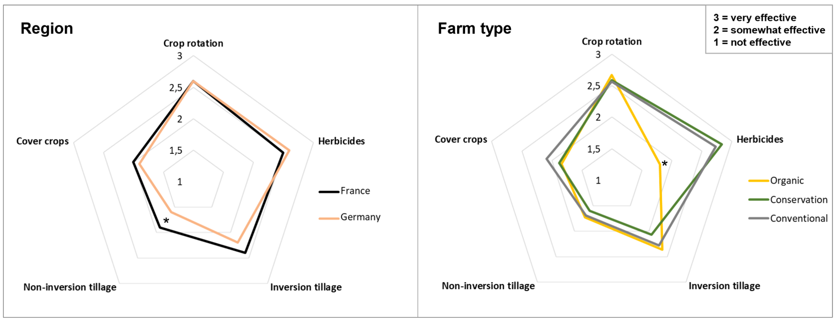

FIGURE 7
Farmers' perceptions of the effectiveness ('not effective', 'somewhat effective' or 'very effective') of different management methods (crop rotation, herbicides, inversion tillage, non-inversion tillage and cover crops) for the control of perennial weeds. Farmers' perceptions were grouped per region and farm type. * represents significant differences ($p < 0.05$) between farm types.

TABLE 6 How farmers are concerned presently and in future about perennial weeds per region and farm type.

	Northern France			Eastern Germany		
	Organic	Conservation	Conventional	Organic	Conservation	Conventional
	Percentage of farmers (%)					
Present concerns	80.0	100.0	60.0	71.4	53.8	71.4
Future problems	80.0	80.0	60.0	95.2	92.3	95.2

perceptions of future perennial weed problems were mainly explained by differences between the farm types (Figure 2). The reasons for farmers' future concerns are fairly obvious and connected to the farming system. In both regions, conservation and conventional farmers heavily rely on herbicides for perennial weed control (Figure 1). Indeed, Andert et al. (2022) observed that *C. arvense* was more common in fields less frequently treated with glyphosate. Currently, there is a lack of effective alternatives to glyphosate to manage conservation fields without disturbing the soil in the long term (Nichols et al., 2015), because even direct perennial weed control by selective herbicides might be less effective than in the past decades. Tavaziva et al. (2019) stated that 100% of the recommended MCPA (2-methyl-4-chlorophenoxyacetic acid) dosage is required to obtain the strongest control of *C. arvense*. This finding is in accordance with results from a study of *C. arvense* and *S. arvensis* where less reduced herbicide doses of MCPA gave an increase in above-ground biomass as compared with recommended dose (Fogelfors and Lundkvist, 2008).

The demand for reducing both selective and non-selective herbicides will increase the complexity of farm management and decision-making (Rossi et al., 2012; Jussaume et al., 2022) for perennial weed management in conservation and conventional farming. However, as expected and already described by other authors, perennial weed control is a major concern for organic farmers (Turner et al., 2007; Melander et al., 2012; Brandsæter et al., 2020). Likewise, in Northern France and Eastern Germany, organic farmers are concerned about future perennial weed problems (Table 6), as direct physical weeding techniques, like harrowing, inter-row hoeing, brushing and even flaming the crops, have not shown to be effective to control perennials (Melander et al., 2005). Indeed, creeping perennial weeds might threaten the future of organic cereal production (Salonen et al., 2013; McErlich and Boydston, 2014), especially under reduced-tillage (Armengot et al., 2015). Thus, controlling perennial weed is a continuous challenge for farms of all types.

While inversion tillage is of paramount importance to reduce perennial weed infestations, many farmers want to reduce the intensive soil tillage as it consumes much energy and labor costs, compacts the ground and diminishes soil biological activities (Cooper et al., 2016; Zikeli and Gruber, 2017). New ways of applying belowground disturbance without turning the soil include mechanical tools which cut roots/rhizomes horizontally (linked to weed species with deep root systems) or vertically (linked to weed species with shallow root systems) (Thomsen et al., 2015; Brandsæter et al., 2017; Ringselle et al., 2018; Brandsæter et al., 2020; Weigel and Gerowitt, 2022). These techniques may assist to overcome the trade-off between perennial weed control and reduced tillage. As cover crops are already established in farming systems, it will be promising to further develop control tactics which combine non-inversion tillage practices and cover crops. Developing agro-ecological weed management techniques and successfully implementing these in farming systems for consistent perennial weed control will be important for long-term viability of agricultural systems. Organic farmers could help in the way to design new systems. Long-term experiments on weed management demonstrate that it is possible to reduce herbicides dependencies, if a systemic and agroecological approach is engaged (Deytieux et al., 2012; Lechenet et al., 2017). Indeed, the unique characteristics of perennial weeds complicate the task of reducing or even eliminating the use of herbicides. However, the implementation of non-chemical perennial weed control might become an example to transform agriculture, shaping the approach to ensuring food security and fostering sustainable methods of production (Vanbergen et al., 2020).

5 Conclusions

Farmers in Northern France and Eastern Germany are concerned about perennial weeds in the future. The demand for reducing herbicides will increase the complexity of perennial weed control for conservation and conventional farming. The outright ban of glyphosate could completely challenge the development of conservation agriculture in which the management of perennial weeds highly depends on this active ingredient. Up to now, perennial weed control was especially a major obstacle for organic farming. However, this is expected to change considerably in the future, because perennial weed control might become a challenge to all farmers.

The ability to reproduce vegetatively of those weeds promotes them an uncredible capability to survive over winters, dry seasons or other unfavorable periods and therefore they are a sort of key indicators to assess the successfulness of "no-less herbicides systems". These specific weeds may give the chance to farmers-advisers to re-design their crop management in a systemic way, all the more as "easy herbicides solutions" do not exist anymore. Therefore, integrating and optimizing non-chemical weed control alternatives is required in all farming systems. We conclude, that perennial weeds as 'difficult-to-control weeds' should take a special position in National Action Plans of the EU Member States. Research activities should provide all farmers and extension services with novel and profitable perennial weed management practices. Moreover, the EU countries should long-term monitor perennial weeds and the consequences of their control.

Data availability statement

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

Author contributions

Conceptualization, SA, MV-M, and BG; Data curation, MH and JG; Formal analysis, SA, MH, and JG; Funding acquisition, SA, MV-M, and BG; Supervision, MV-M and BG; Validation, SA and JG; Visualization, SA and MH; Writing—original draft, SA; Writing—review and editing, SA, JG, MV-M, and BG. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2023.1247277/full#supplementary-material

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Side-effects of laser weeding: quantifying off-target risks to earthworms (Enchytraeids) and insects (*Tenebrio molitor* and *Adalia bipunctata*)

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With challenges posed by chemical and mechanical weed control, there are now several research and commercial projects underway to develop autonomous vehicles equipped with lasers to control weeds in field crops. Recognition systems based on artificial intelligence have been developed to locate and identify small weed seedlings, and mirrors can direct a laser beam towards the target to kill the weed with heat. Unlike chemical and mechanical weed control, laser weeding only exposes a small area of the field for the treatment. Laser weeding leaves no chemicals in the field after the treatment or does not move the soil which may harm crop roots and non-target organisms. Yet, it is well-known that laser beams can harm living organisms; the effect on the environment and fauna should be studied before laser weeding becomes a common practice. This project aimed to study the effect of laser on some living non-target organisms. We investigated the effect of laser treatment on the mortality of two species of earthworms (*Enchytraeus albidus* and *Enchytraeus crypticus*), larvae, pupas, and beetles of yellow mealworm beetles (*Tenebrio molitor*) and the two-spotted lady beetle (*Adalia bipunctata*) for increasing dosages of laser energy. In all earthworms experiments except one, the mortality rates of the worms living in the uppermost soil layer of clay, sandy, and organic soil exposed to laser heating were not significantly different from the controls even with laser dosages up to 23.8 J mm^{-2}. Laser doses sufficient to kill plants were lethal to the insects, and lower doses that did not kill plants, killed or harmed the insects across all life stages tested. The larger beetles survived higher doses than smaller. Laser weeding is a relatively new technology and not yet widely practiced or commercialized. Therefore, we do not discuss and compare the costs of the different weeding methods at this early stage of the development of the technology.

KEYWORDS

integrated weed management, laser non-target, laser eco-toxicology, non-chemical weed control, site-specific weed management, thermal weed control

1 Introduction

Weed control with laser beams has achieved increasing attention as the fast development in artificial intelligence has enabled recognition of the location and identification of plant species precisely and rapidly (Rakhmatulin et al., 2021). Furthermore, laser can be guided by mirrors to target weeds (Rakhmatulin and Andreasen, 2020). When small weeds are hit correctly in the meristem, the heat from the laser can kill the plants (Heisel et al., 2001; Coleman et al., 2021). Lasers are powered by electricity, which can be supplied by batteries, charged from renewable (non-fossil) energy sources reducing CO_2 emission compared to commonly used weed control methods. Suppose a laser beam has a diameter of 2 mm and there are 150 weeds m^{-2}, then only 0.5% of the total area will be exposed to the treatment. A common practice of herbicide application and mechanical weed control exposes most of the field or the whole area to the treatments, and chemicals often stay on the soil surface or in the soil matrix for a while with the risk of affecting non-target organisms and the environment negatively (Thiour-Mauprivez et al., 2019; Mehdizadeh et al., 2021). Consequently, replacing herbicide application and mechanical weed control with laser weeding seems to be a method to reducing some of the negative environmental impact of weed control.

If the laser beam hits non-target organisms, they are likely harmed or killed. That may happen, for example, if insects or other organisms (1) are on the target plant at time of exposure, (2) move into the laser beam, or (3) if unintended platform movement results in the inaccurate position of the laser energy.

The effect of the laser beam on the weed plants depends on physical parameters (*e.g.*, laser wavelength, beam diameter, and dose (J)) (Rakhmatulin and Andreasen, 2020) and biological factors (*e.g.*, plant species, plant size, and developmental stage) (Heisel et al., 2001; Andreasen et al., 2022). These factors may also be crucial for the effect of laser beams on non-target organisms and should be investigated.

It is not economically feasible to study the effect of laser on all potentially exposed organisms, and therefore model organisms are often used (Eggen et al., 2004). We examined how laser treatment affected the survival rate of two earthworms and two insects.

Enchytraeids (class Oligochaeta, family Enchytraeidae) are ecologically important soil worms due to their activity in bioturbation and decomposition of organic matter in many soil types (Didden, 1993; Castro-Ferreira et al., 2012). Enchytraeids are widespread on moist soil types from the arctic to the tropics, occurring in quantities from 100s to more than 100,000 individuals m^{-2}. Generally, they thrive within a temperature range of 8 °C–25 °C. Enchytraeids are often used as model organisms in toxicological laboratory tests (*e.g.*, Cedergreen et al., 2013; Gomes et al., 2013; He and van Gestel, 2013). *Enchytraeus albidus* and *E. crypticus* are white worms with a long thin body with a soft skin and a fast reproduction rate. They can grow up to 3 cm long. They have been considered suitable to assess ecotoxicity in many different soil types due to their larger tolerance range to pH (4.4–8.2), clay (1–29%) and content of organic matter in the soil (1.2–42%) (Kuperman et al., 2006; Castro-Ferreira et al., 2012).

Tenebrio molitor (Coleoptera: Tenebrionidae) is a holometabolous insect (complete life cycle with egg, larva, pupa, and adult stages) and is considered a pest due to its ability to consume stored flour, grains, or animal feeds. The larvae are called mealworms. The larva is white and reaches 2–2.5 cm in length. They gradually become yellow and then darker brown. They have three pairs of legs and are active crawlers. The *T. molitor* beetle reach 25 mm in length and are the largest insects infesting stored products (Davidson and Lyon, 1979). *Tenebrio molitor* has often been used as a test insect in ecotoxicological studies as it is easy to propagate, feed, and keep indoors (*e.g.*, McCallum et al., 2013; Lv et al. 2014; Bednarska and Świątek, 2016; Fei et al., 2022).

Adalia bipunctata (Coleoptera: Coccinellidae) is an aphidophagous beetle also called two- spotted ladybug/two-spotted ladybird/two-spotted lady beetle. It is native to North America and Western and Central Europe (Hodek et al., 2012). The larvae and adults feed on aphids and other small insects, so *A. bipunctata* is therefore considered a beneficial insect. It has been commercialized for aphid pest control in protected environments (Wyss et al., 1999; Khan et al., 2016). Adults reach a length of 3.5 –5.2 mm (Gordon, 1985).

The aim of this study was to investigate how a fiber laser with a wavelength of 2 µm and a 2 mm diameter affected the mortality of two species of soil worms (*E. albidus* and *E. crypticus*) when the soil surface was exposed to increasing doses of laser energy. The 2 µm wavelength from the fiber laser is mainly absorbed by the water inside the target and is more beneficial for weed control than a CO_2 laser, which energy is primarily absorbed on the surface of the plant (Wieliczka et al., 1989). Therefore, a thulium-doped 2 µm fiber laser has been installed in the autonomous vehicle for laser weeding developed in the EU project WeLASER (https://welaser-project.eu/). A laser energy dose of 15 J mm^{-2} may be used to control seedlings of weeds in agricultural and horticultural fields. Weed plants on the cotyledons and two permanent leaf stages are usually killed when they are exposed to 10 J mm^{-2} (Heisel et al., 2002; Andreasen et al., 2022). We also exposed larvae, pupae, and beetles of *T. molitor* and the *A. bipunctata* beetle to increasing doses of laser energy. We hypothesized that all organisms would be negatively affected if they were exposed to an energy level of 8–24 J mm^{-2}) which may be used to control dicotyledon and monocotyledon weeds at the early stages of development (Coleman et al., 2021; Andreasen et al., 2022).

2 Materials and methods

2.1 Laser equipment

We used a thulium-doped 50 W fiber laser with a wavelength of 2 µm with a collimated beam (Ø: 2 mm) manufactured by Futonics Laser GmbH, Katlenburg-Lindau, Germany. The laser was placed within a steel box (68 cm × 68 cm × 68 cm) with a door with a metal interlock (Figure 1). On laser activation, the door locks automatically to avoid risk of laser exposure.

The target organisms were placed approximately 40 cm below the laser head and exposed to increasing dosages of laser energy (from 0 to 23.9 J mm^{-2})

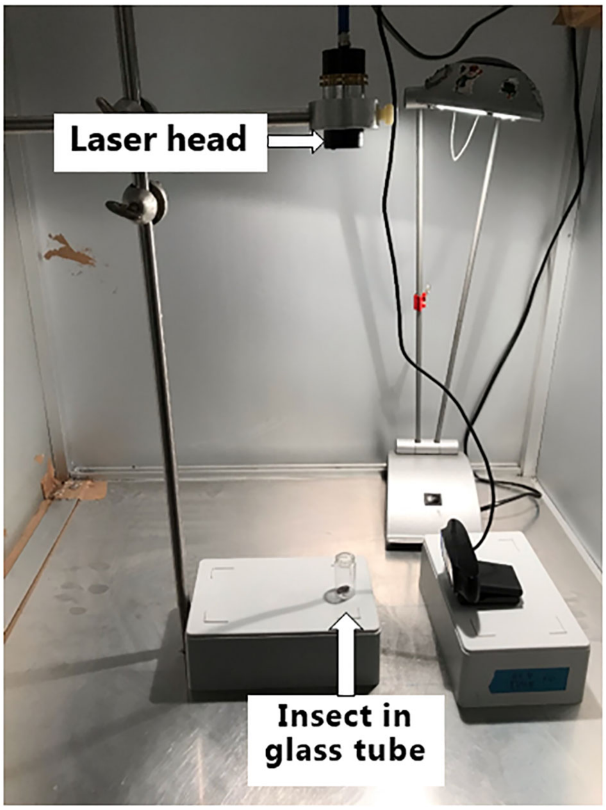

FIGURE 1
The laser was placed within a steel box (68 cm × 68 cm × 68 cm) with a door with a metal interlock. The organisms were placed below the laser head.

2.2 Laser experiments with Enchytraeus spp.

2.2.1 Culturing conditions of Enchytraeus spp.

Enchytraeus albidus and *E. crypticus* were cultured in soil in plastic buckets with lids (length: 13.5 cm; Ø: 13 cm). Three soil types were used: a) sandy soil containing about 9% clay, 10% silt, 32% fine sand, 47% coarse sand, and 2% organic matter, b) clay soil containing about 28% clay, 23% silt, 10% fine sand, 36% coarse sand, and 3% organic matter, and c) an organic soil based on sphagnum (Pindstrup mixture 2 (https://www.pindstrup.dk/professionel/product-details/pindstrup-f%C3%A6rdigblanding-2), Pindstrup mosebrug a/s, Ryomgaard, Denmark). There was one bucket for each soil type and worm species (n=6). The buckets were weighed after adding soil and water, and afterward once a week to check the soil moisture. Water loss was replenished by adding an appropriate amount of deionized water. The buckets were placed in the dark in a climate cabinet at 20°C ± 2°C. The soil was kept moist but not wet corresponding to 40–60% of the water-holding capacity. Four holes (2 mm) were made in the lids of the buckets to allow adequate gaseous exchange with the atmosphere. The soil was cautiously broken up with a spatula each week to facilitate aeration. The worms were fed with rolled oats weekly. The rolled oats were ground and autoclaved (121°C, 105 min, 1200 mbar) before use to avoid infestation with flour mites (*e.g.*, *Glyzyphagus* sp. and *Astigmata, Acarina*) or predacious mites [*e.g.*, *Hypoaspis scimitus* (Cosmolaelaps) and *Gamasida* (Acarina)]. If the oat became contaminated with fungi, it was removed and replaced.

2.2.2 Experiments with *Enchytraeus albidus* and *E. crypticus*

To study the effect of the laser on the earthworms, 10 g of dry soil was moistened with demineralized water to achieve approximately 50% of the water-holding capacity. The moist soil was placed in a 50 ml plastic tube (length: 11.2 cm; Ø: 30 mm), with 10 holes ((Ø: 1 mm) in the lid to ensure gas exchange.

After the soil was placed in the tubes three worms were transfer to each tube and kept for one day in a climate cabinet in darkness at 20°C ± 2°C before laser treatment. For each soil type and laser doses (0 (control), 0.5, 1, and 1.5 seconds corresponding to 0, 8.0, 15.9, and 23.9 J m^{-2}), 10 tubes with three *E. albidus* worms and 10 tubes with three *E. crypticus* worms were used. Three soil types were chosen because the heat transfer from the laser depends on the soil textures. The lid of the tubes was moved before the soil surface in the tubes was exposed to the laser beam from above. After the treatment, the tubes were placed with the lids in a climate cabinet in darkness at 20°C ± 2°C. The number of immobile, live, dead, or missing enchytraeids in all treatments were recorded by empty the tubes and searching through the soil with a spatula 7 days after the treatment. Worms could be missing because they were dead, decomposed and dissolved during the period. The experiment was repeated four times with *E. albidus*, using the same worms, as they were not affected by the treatment, but only done once with *E. crypticus* due the lack of worms.

2.3 Experiments with *Tenebrio molitor*

Tenebrio molitor were bought from the company InsektOrama A/S, Herning, Denmark. Adults and half of the delivered larvae were used for the first experiment and lasered two days after the delivery. The rest of the larvae were reared to produce an adequate number of new adults, larvae (to repeat the experiment) and pupae (for the first experiment and its repetition). When larvae were developed, they were moved to a round open plastic tray (10 cm high; Ø: 29 cm) and placed in a climate cabinet (at 20°C ± 2°C in darkness). The bottom of the tray was covered with rolled oats (3 cm high) necessary for the larvae's rearing. Additional oats were added when necessary to ensure a continuous supply of feed. Four to five slices of fresh potato (Ø: 3–4 cm) were added to the tray every second day to ensure water supply. The tray was checked daily, and as soon as a pupa was observed, it was immediately separated from the larvae and placed in a new, similar tray. Adults were isolated from pupae in the same way to avoid cannibalism. Adults were producing eggs, creating a new generation of larvae required to repeat the experiment. All insect stages were kept and reared under the same conditions until they were lasered.

Ten individuals each of larvae, pupae, and adult *T. molitor* were exposed to the laser beam for 0, 1, 10, 20, 50, 100, and 500 ms corresponding to 0, 0.016, 0.16, 0.32, 0.80, 1.59, and 7.95 J mm^{-2}. Ten individuals of each developmental stage of *Tenebrio molitor* were treated with each dose with 4 replicates (10 × 4 individuals). The larvae were retained in a groove in a small piece of wood during the treatment. The pupae were placed on a piece of paper, and the adults were placed in a glass tube (length: 40 mm; Ø: 18 mm) to prevent them escaping the treatment (Figure 1). The laser was aimed at the dorsal surface, approximately halfway along the length of the body, but because they were alive and able to move some were hit in other locations. Afterwards, the insects were carefully moved to four plastic transparent boxes with lids (12 cm × 12 cm × 4 cm) (10 individuals per box) immediately after lasering. The lids were partially (1/3 of the lid) covered by a net (holes: 1 mm × 1 mm) ensuring gas exchanges.

The bottom of the boxes was covered with approximately 1 cm of rolled oats and slices of fresh potato (Ø: 3–4 cm) was added as feed and water supply. Additional rolled oats and fresh potato was added when necessary to ensure a continuous supply of feed and water. The number of live and dead individuals were counted 8 and 15 days after the laser treatment. Damages and deformities were noted.

2.4 Experiments with *Adalia bipunctata*

Adalia bipunctata, produced by EWH BioProduction ApS, Tappernøje, Denmark was bought via Horticoop Scandinavia A/S, Hinnerup, 143 Denmark. The beetles were kept in transparent plastic containers (28 cm × 20 cm × 22 cm) with lids, partially (2/3) covered by a net (holes: 1 mm × 1mm) allowing gas exchange, and kept in a climate cabinet at 21°C with 12 hours light from 8 a.m. to 8 p.m. The beetles were fed with honey diluted with water (ratio 1:10) during the experimental periods. The diluted honey was supplied via pieces of filter papers (3 cm × 3 cm). Four to five new papers were placed in the box with the beetles every day. The beetles were kept and reared in the same conditions until being lasered.

For each laser treatment, ten beetles were exposed to a laser dose. Each beetle was placed in a plastic tube (length: 50 mm; Ø: 5 mm) during the irradiation preventing the beetle from escaping. We aspired to hit the beetle in the center of their dorsal surface, but they were able to move a little and therefore some were hit in other places. The laser dosages were 0, 1, 10, 20, 50, 100, and 500 ms corresponding to 0, 0.016, 0.16, 0.32, 0.80, 1.59, and 7.95 J mm^{-2}. After exposure, each beetle was moved to a plastic container together with the nine other beetles receiving the same dose and kept exactly the same way as the pretreated adults. The mortality rate was recorded over 15 days. There were four replicates for each dose (total 4 ×10 beetles). Two independent experiments were done.

2.5 Statistical analyses

Statistical analyses were done using the statistical program R´ (R Core Team, 2021). All data sets were initially analyzed using a dose-response model. If no dose-response trend was present in the data, the lowest observed adverse effect level (LOAEL) and the no observed adverse effect level (NOAEL) were found as the lowest dose showing a significant effect compared to the control group and the highest dose with a non-significant effect compared to the control group, respectively.

2.5.1 *Enchytraeus albidus* and *E. crypticus*

Since no clear dose-response trend were observed for mortality of *Enchytraeus albidus* and *E. crypticus*, data were analyzed with a logistic regression model with laser dose as factorial explanatory variable. For *E. albidus* exposed in clay and sandy soil with four repetitions, a logistic mixed model was used instead. For these models, laser dose was included as a factorial fixed effect and repetition was included as random effect. Pairwise comparisons to the control group to find NOAEL and LOAEL were based on the fitted models (Hothorn et al., 2008).

2.5.2 *Tenebrio molitor*

A non-linear dose-response model for binary data was used to describe the association between laser dose and mortality of the individual life stages. A three-parameter log-logistic model assuming an upper limit of 100% mortality was fitted to the data from each repetition and for 8 and 15 days after treatment, individually. The effective dose (ED_x) killing a percentage, x, of the individuals remaining after adjustment for background mortality was estimated from each individual model and combined in a meta-analytic linear mixed model for each ED_{20} and ED_{80}, separately. Each model included the day of observation as fixed effect and repetition as random effect. For plotting, parameters from each model fit were combined in a second step using a meta-analytic linear mixed model (Jensen et al., 2020). The model included the two-way interaction of day of observation and model parameters as fixed effect, repetition as random effect with corresponding standard deviations that were assumed different for each of the three model parameters, and an unstructured variance-covariance matrix. Pairwise comparisons of parameters and ED-values between days of observation were based on the estimated meta-analytic models as *post hoc* pairwise comparisons (Hothorn et al., 2008).

2.5.3 *Adalia bipunctata*

Data for *Adalia bipunctata* were analyzed in a similar way as data for *Tenebrio molitor* but for five different time points. For day 1 and day 4, there were no background mortality and accordingly a two-parameter log-logistic model was used. For day 8, 11, and 15, a three-parameter log-logistic model was used.

3 Results

3.1 *Enchytraeus albidus* and *E. crypticus*

In all earthworms experiments except one, the mortality rates of the worms living in clay, sandy, and organic soil exposed to laser heating were not significantly different from the controls (Supplementary Table S1). Consequently, the NOAEL was the highest dose of 23.9 J mm^{-2} and the LOAEL could not be estimated. For *E. crypticus*, the highest laser dose of 23.9 J mm^{-2}

was the only dose significantly higher than the control (p=0.0185), and accordingly the LOAEL, making 15.9 J mm^{-2} the NOEAL (Supplementary Table S1).

3.2 *Tenebrio molitor*

3.2.1 Larvae

In both experiments, the dose influenced the mortality of the larvae. A non-linear function fitted the data well (Figure 2). Model parameters and ED_{20}, ED_{50}, and ED_{80} values are shown in Supplementary Table S2.

The mortality after 8 days did not change significantly. The larvae from the control group (0 J mm^{-2}) developed into normal pupae and beetles. At the smallest dose (0.016 J mm^{-2}) some larvae and pupae developed normally, but some developed into beetles with wing deformities. When the dose was increased to 0.32 J mm^{-2}, most of the insects (all stages) were living, but the living larvae received a spot burn from the laser treatment while living adults had deformed wings. The dead insects became brown, dark or with a big dark spot from the laser. The living larvae developed into deformed beetles that almost immediately died. At a dose of 0.80 J mm^{-2}, more than half of the larvae died. Most of the dead insects were at the larva stage and very few could complete metamorphosis resulting in severely deformed insects, and in some cases, they became half pupa and half beetle. Most of the dead larvae were dark and brown, and the few living ones received a dark spot burn from the laser (Figure 3).

Very few larvae survived a dose of 1.59 J mm^{-2}. Most larvae died having a dark dehydrated and burned appearance. At a dose of 7.95 J mm^{-2}, almost all the larvae died the first week after application at larva stage. Only one transformed into a pupa. The dead larvae became completely black or brown with the hemolymph running out from the burned hole in their body just after treatment (Figure 3).

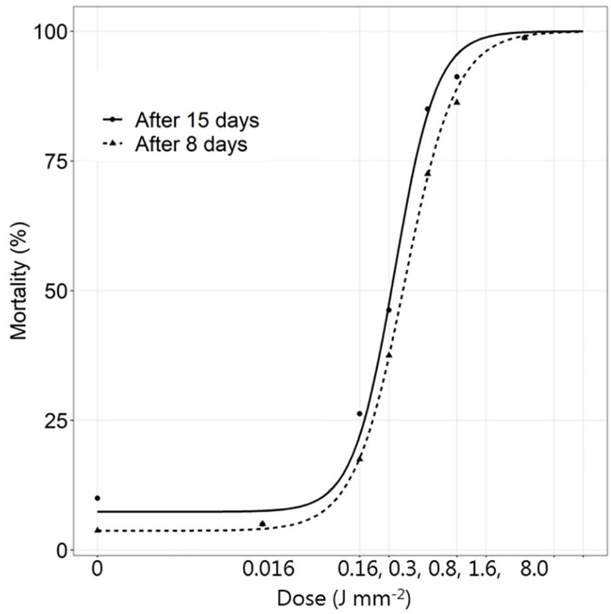

FIGURE 2
The mortality (%) of *Tenebrio molitor* larvae 8 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam with a diameter of 2 mm. Points show mean values (n=20).

3.2.2 Pupae

In contrast to larvae and adults, the mortality of the pupae increased between 8 and 15 days (Figure 4 and Supplementary Table S3). Fifteen days after the laser application, all pupae in the control groups developed into beetles with normal appearance and high survival rate (90%) (Figure 5A). When a dose of 0.016 J mm^{-2} was applied, all pupae developed into beetles with the same survival rate as the control group. However, one third developed deformed wings and/or body (Figure 5B). When the dose was increased to 0.16 J mm^{-2}, the survival rate declined approximately 68%. The living pupae all developed into adults, which, however, had some wing and body deformities. Most of the non-living pupae and beetles were discoloured and broke into small pieces (Figure 5C). When the dose was doubled (0.32 J mm^{-2}), the survival rate decreased even more, and the mortality rate rose to 62%. The appearance of non-living adults varied from injured (Figure 5D), cut into pieces or dead bodies without any indication of abnormal appearance. A dose of 0.80 J mm^{-2} increased the mortality to approximately 85%. Many pupae did not develop into adults as a high number of dead pupae had a brown dark color or completely dark and dehydrated body. A dose of 1.59 J mm^{-2} almost killed all insects after 15 days (mortality ~ 97.5%). Many pupae did not develop into adults due to high mortality. Dead pupae had a brown dark color or a completely dark and dehydrated body. At the highest dose (7.95 J mm^{-2}), all pupae died within the first week after the irradiation with a burned-like appearance (Figure 5E). None of them managed to develop further to the adult stage indicating that the death happened a few days after the laser treatment.

3.2.3 Adults

At the lowest dose (0.016 J mm^{-2}), the mortality did not differentiate from the controls (Figures 6, 7A and Supplementary Table 4). Treatment with 0.16 J mm^{-2} increased mortality. After applying a dose of 0.32 J mm^{-2}, the mortality rate increased to ca 2.5%. Most of the living adults had a spot derived from the laser beam while most of the non-living adults had a small hole from the laser (Figure 7B). More than half of the adults died at a dose of 0.80 J mm^{-2} with a hole from the laser. At the highest dose, the mortality was about 92.5% and most beetles were killed immediately with hole in the body (Figure 7C).

3.3 *Adalia bipunctata*

In general, the non-linear model fitted the data well (Figure 8). Model parameters are shown in Supplementary Table S5. During the 15 days, some of the non-exposed beetles died. The mortality increased over time and with increasing dosages. Even 0.016 J mm^{-2} affected the shape of the dose-response curve, and the beetle's elytron became brownish in color (Figure 9). A dose of 0.80 J mm^{-2} severely harmed the beetles, and almost all beetles died during the 15 days. A dose of 7.95 J mm^{-2} immediately killed all beetles burning significant holes in the beetles (Figure 9).

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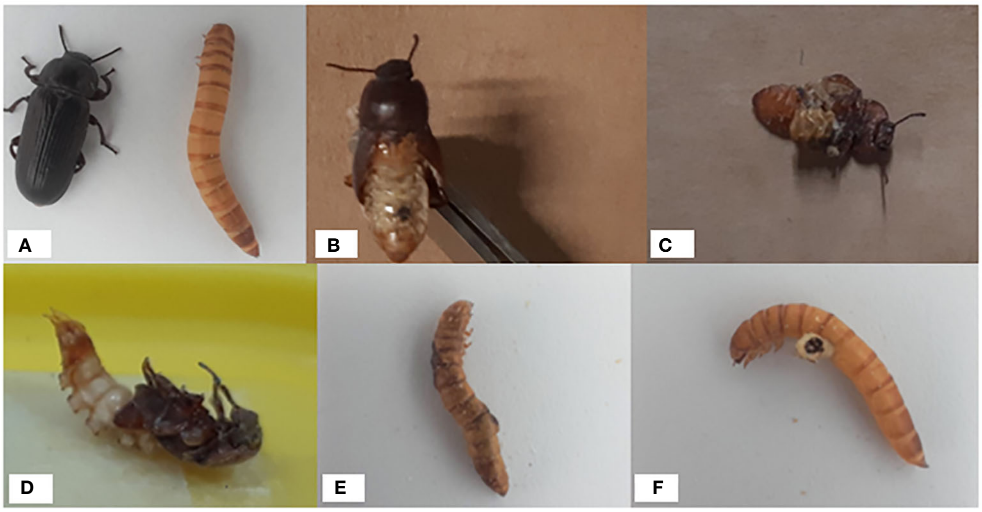

FIGURE 3
Deformities observed during the study of *T. molitor* (larvae experiment): **(A)** *T. molitor* control adult and larva. **(B)** 0.016 J m^{-2}: Larva becomes a deformed alive adult. **(C)** 0.32 J m^{-2}: Larva developed into a deformed adult and died. **(D)** 0.80 J m^{-2}: Larva did not complete metamorphosis (severely deformed insect: half pupa, half adult). **(E)** 1.59 J m^{-2}: Burned (brownish-dark) larva. **(F)** 7.95 J m^{-2}: Larva were rapidly killed after exposure, and there was significant damage caused to the insects, e.g., hemolymph flowed out of the laser hole in the insects' body.

4 Discussion

4.1 Earthworms (*Enchytraeus albidus* and *E. crypticus*)

There were no differences in mortality between the control group and worms exposed to all laser doses, with a single exception with *E. crypticus* in sandy soil at a dose of 15.9 J mm^{-2}. The spread of the heat in the soil from the laser beam depends on the water content and soil structure and composition. We cannot exclude that other conditions like a higher water content or other soil types would result in other mortalities. We consider a soil water content of 50% of the water capacity to be realistic in the early spring when weed control usually is conducted, but it depends on many factors in the field (e.g., variation in soil composition, precipitation, and evaporation). The earthworms in the tubes were living in a very small soil volume (<15 g) during the experiment mimicking the uppermost part of the soil profile. Although heat corresponding to 23.9 J mm^{-2} was executed on a spot with a 2 mm diameter, which easily kills weed seedling, the exposure did not warm up the soil sufficiently to affect the mortality of the worms living lose to the soil surface within the seven days.

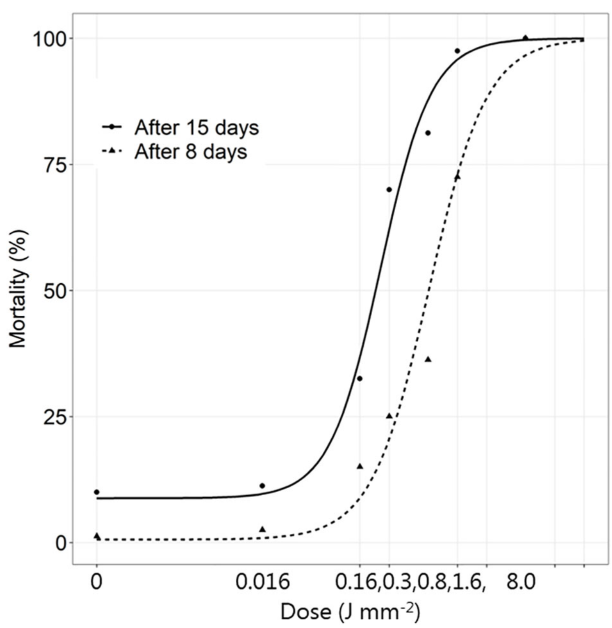

FIGURE 4
The mortality (%) of *Tenebrio molitor* pupae 8 and 15 days after exposure to increasing dosages of laser energy (J m^{-2}) from a thulium-doped 2 μm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

4.2 Insects

Tenebrio molitor is a large model insect. Larger insects, such as *T. molitor*, appear to be more resistant to the laser than smaller insects such as *A. bipunctata*. In general, the insects were all killed immediately at a laser dose corresponding to what would be appropriate for killing small weed seedlings (50 J mm^{-2}). We aimed to focus the laser on the middle of the body of the insects, but because the insects were able to move a little, there would be variations in where the laser hit. If we had focused the laser on the head or the rear part, the mortality might have been different.

4.3 Laser safety

We used a collimated beam in the experiments to precisely give the wanted dose independent of the precise distance to the target.

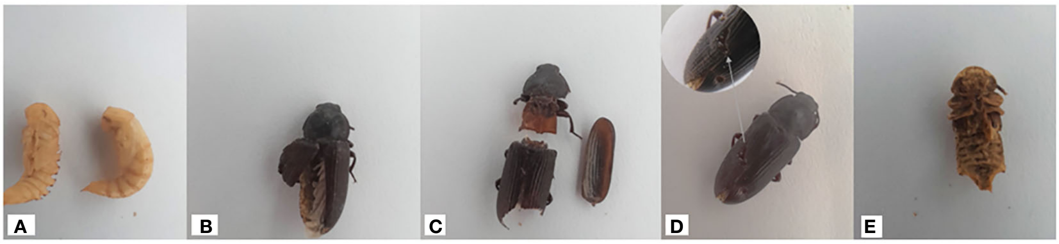

FIGURE 5
Typical deformities that were observed on *Tenebrio molitor* after laser application (pupae experiment): **(A)** *T. molitor* control pupa. **(B)** 0.016 J m^{-2} (1 ms): Pupa developed into adult with deformed wings. **(C)** 0.16 J m^{-2}: Non-living adult and adults broken into pieces. **(D)** 0.32 J m^{-2}: Pupa transformed to an injured adult. **(E)** Pupa died few days after laser application having a brownburned appearance.

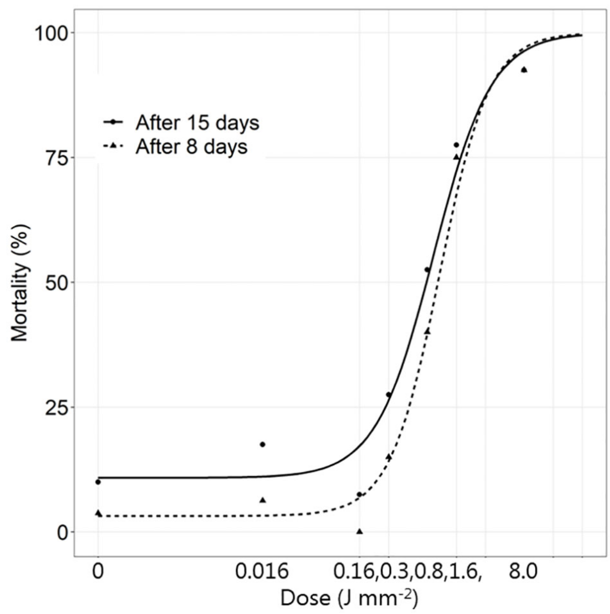

FIGURE 6
The mortality (%) of *Tenebrio molitor* beetles 8 and 15 days after exposure to increasing dosages of laser energy **(J)** from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

However, if stones or other reflecting materials are hit with a collimated laser beam, the reflected beam may escape the target area, and humans or larger animals (dogs, hares, etc.) may be exposed, burned, or blinded. Therefore, the laser beam should not be collimated in laser weeding robots, but only be focused and concentrated on the meristem of the weed seedlings. If the laser then hits a reflecting material, the beam will be spread in a cone and the risk of harming the humans, animals and other plants would be significantly reduced due to the lower dose per area. That means that only insects or other organisms placed exactly in the focus point would receive the dose determined for the target plant. The further away from the focus point the lower the dose an organism would receive and the less harmful the exposure.

Some insects benefit the crop like ladybugs, spiders, and predatory beetles, as they can reduce the number of harmful insects (*e.g.*, aphids (Aphididae) and rape beetles (*Meligethes aeneus*)). Some beneficial insects like ladybugs have characteristic colors and can easily be identified with recognition tools (Rakhmatulin et al., 2021). In principle, a laser-weeding robot could be programmed to recognize the different between beneficial and pest arthropods and kill the latter.

On the other hand, small dosages of laser energy could be considered to control harmful arthropods. Rakhmatulin (2021) used machine vision and showed that a low-cost device could be used to kill mosquitoes with a laser. The company Photonic Sentry

FIGURE 7
Typical deformities that were observed on *Tenebrio molitor* adults after laser application (adults' experiment): **(A)** 0.016 J m^{-2}: T. molitor adults were alive with normal appearance. **(B)** 0.32 J m^{-2}-: a living adult with a spot from the laser. **(C)** 7.95 J m^{-2}-: T. molitor adult died immediately with a large hole in its body.

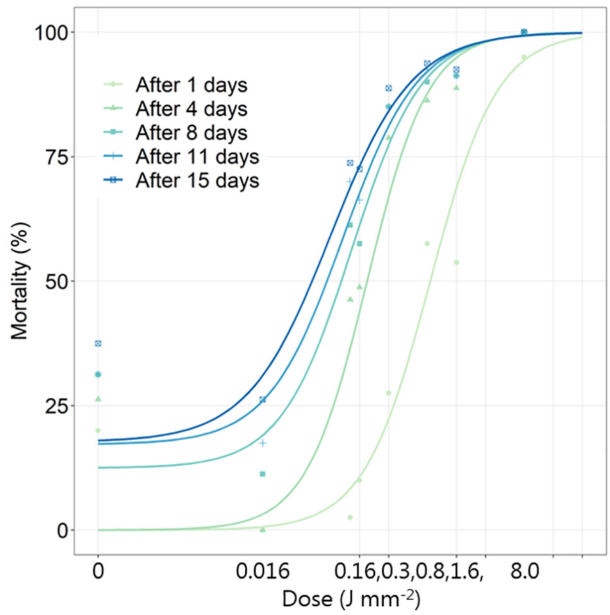

FIGURE 8
The mortality (%) of *Adalia bipunctata* beetles 1, 4, 8, 11 and 15 days after exposure to increasing dosages of laser energy **(J)** from a thulium-doped 2 μm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

(https://photonicsentry.com/) has introduced a laser mosquito neutralization technology to combating malaria. Flying insects are common vectors for the transmission of pathogens between crop plants (Heck, 2018). Mullen et al. (2016) presented proof of principle for an optical system capable of highly specific vector control using a combination of optical sources, detectors, and sophisticated software to search, detect, and identify flying insects in real-time, with the capability of eradication using a lethal laser pulse. They focused on two insect species: *Diaphorina citri*, a vector of the causal agent of citrus greening disease, and *Anopheles stephensi*, a malaria vector. There seems to be a great potential to use laser technology to protect people and crops from pestilent flying insects (Keller et al., 2020). Our experiments showed that laser dosages, which will not harm the plants, could significantly damage harmful insects.

4.4 Laser weeding compared to herbicide application and mechanical weed control

Laser weeding seems to be a promising tool to replace or supplement herbicides and mechanical weed control, with only a small treatment area in contrast to other weed control measures. Unlike herbicide spraying, laser weeding leaves no chemicals in the field that may harm non-target organisms after the treatment. Herbicides may evaporate or leach to surface and groundwater and may expose the environment to short or long-term unwanted side-effects. Laser weeding only leaves the ash from the burned weed meristem in the field after the treatment, which may be taken up by the crop plants as fertilizer.

In contrast to laser weeding, mechanical weeding impact shallow living worms negatively (and potentially other soil organisms), as the weeding implements are passing through the top layer of the soil (Doran and Zeiss, 2021). Mechanical weeding also harms beneficial organisms on the soil surface, like spiders and predatory beetles (Michalko et al., 2019; Symondson et al., 2022). Therefore, laser weeding seems to have less negative impact on the environment that other weed control measures.

5 Conclusion

The earthworms were mostly unharmed when the soil surface was exposed to laser dosages up to 23.9 J mm^{-2} as the soil protected

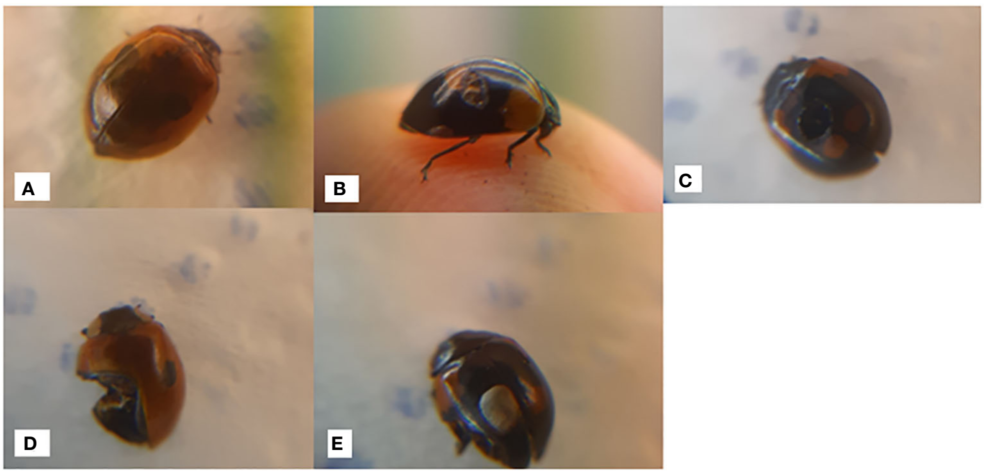

FIGURE 9
Malformations on the body of the adults *Adalia bipunctata* observed in the study. **(A)** 0.05 J (0.16 J mm^{-2}): Brownish color on the outer shell. **(B)** 0.1 J (78 J mm^{-2}) Small damage on an alive adult. **(C)** 2.5 J (50 ms J mm^{-2}) severe damage, dead adult **(D)** 25 J (500 ms J mm^{-2}) Acute damage: dead adult one day after lasering. **(E)** 78.57 J mm^{-2}), fungal infection.

them. Laser doses sufficient to kill plants were lethal to the insects, and lower doses that did not kill plants, killed or harmed the insects across all life stages tested. The larger *T. monitor* beetle survived higher doses than the smaller *A. bipunctata* beetle. The results indicate that pest control might be a possibility using laser dosages which do not harm plants. In general, the probability of harming insects with dosages used for laser-weeding is small, as only a tiny proportion of the area will be exposed for the treatment, even with a high weed density. Using another type of laser may give other results. Developing a recognition tool using artificial intelligence to differentiate between pests and beneficial arthropods would make pest control possible without harming beneficial organisms at the same time as the laser weeding takes place.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements.

Author contributions

CA was responsible for funding acquisition and the design of the experiments. EV and KJ conducted the experiment with insects. SJ made the statistical analyses. CA wrote the first draft of the article, and all authors added to the manuscript, reviewed, edited, and accepted the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2023.1198840/full#supplementary-material

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Corrigendum: Side-effects of laser weeding: quantifying off-target risks to earthworms (Enchytraeids) and insects (*Tenebrio molitor* and *Adalia bipunctata*)

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KEYWORDS

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A Corrigendum on

Side-effects of laser weeding: quantifying off-target risks to earthworms (Enchytraeids) and insects (*Tenebrio molitor* and *Adalia bipunctata*)

by Andreasen C, Vlassi E, Johannsen KS and Jensen SM (2023). *Front. Agron.* 5:1198840. doi: 10.3389/fagro.2023.1198840

In the published article, the dosages were calculated incorrectly. We have therefore made a number of corrections to the text.

In the **Abstract**, the sentence "In all earthworms experiments except one, the mortality rates of the worms living in the uppermost soil layer of clay, sandy, and organic soil exposed to laser heating were not significantly different from the controls even with laser dosages up to 236 J mm^{-2}." should read "In all earthworms experiments except one, the mortality rates of the worms living in the uppermost soil layer of clay, sandy, and organic soil exposed to laser heating were not significantly different from the controls even with laser dosages up to 23.8 J mm^{-2}."

In the **Introduction**, paragraph 8, the sentences "A laser energy dose of 236 J mm^{-2} may be used to control seedlings of weeds in agricultural and horticultural fields. Weed plants on the cotyledons and two permanent leaf stages are usually killed when they are exposed to 157 J mm^{-2} (Heisel et al., 2002; Andreasen et al., 2022). We also exposed larvae, pupae, and beetles of *T. molitor* and the *A. bipunctata* beetle to increasing doses of laser energy. We hypothesized that all organisms would be negatively affected if they were exposed to an energy level of 79 –236 J mm^{-2}) which may be used to control dicotyledon and monocotyledon weeds at the early stages of development (Coleman et al., 2021; Andreasen et al., 2022)." should read "A laser energy dose of 15 J mm^{-2} may be used to control seedlings of weeds in agricultural and horticultural fields. Weed plants on the cotyledons and two permanent leaf stages are usually killed when they are exposed to 10 J mm^{-2} (Heisel et al., 2002; Andreasen et al., 2022). We also exposed larvae, pupae, and beetles of *T. molitor* and the *A. bipunctata* beetle to increasing doses of laser energy. We hypothesized that all organisms would be negatively affected if they were exposed to an energy level of 8–24 J mm^{-2}) which may be used to control dicotyledon and monocotyledon weeds at the early stages of development (Coleman et al., 2021; Andreasen et al., 2022)."

In **Section 2.1**, paragraph 2, the sentence "The target organisms were placed approximately 40 cm below the laser head and exposed to increasing dosages of laser energy (from 0 to 235.71 J mm^{-2})" should read "The target organisms were placed approximately 40 cm below the laser head and exposed to increasing dosages of laser energy (from 0 to 23.9 J mm^{-2})."

In **Section 2.2.2**, paragraph 2, the sentences "After the soil was placed in the tubes three worms were transfer to each tube and kept for one day in a climate cabinet in darkness at 20°C ± 2°C before laser treatment. For each soil type and laser doses (0 (control), 0.5, 1, and 1.5 seconds corresponding to 0, 78.6, 157.1, and 235.7 J m^{-2}), 10 tubes with three *E. albidus* worms and 10 tubes with three *E. crypticus* worms were used." should read "After the soil was placed in the tubes three worms were transferred to each tube and kept for one day in a climate cabinet in darkness at 20°C ± 2°C before laser treatment. For each soil type and laser dose (0 (control), 0.5, 1, and 1.5 seconds corresponding to 0, 8.0, 15.9, and 23.9 J m^{-2}), 10 tubes with three *E. albidus* worms and 10 tubes with three *E. crypticus* worms were used."

In **Section 2.3**, paragraph 2, the sentence "Ten individuals each of larvae, pupae, and adult *T. molitor* were exposed to the laser beam for 0, 1, 10, 20, 50, 131 100, and 500 ms corresponding 0, 0.15, 1.57, 3.14, 7.86, 15.71, and 78.57 J mm^{-2}." should read "Ten individuals each of larvae, pupae, and adult *T. molitor* were exposed to the laser beam for 0, 1, 10, 20, 50, 100, and 500 ms corresponding to 0, 0.016, 0.16, 0.32, 0.80, 1.59, and 7.95 J mm^{-2}."

In **Section 2.4**, paragraph 2, the sentence "The laser dosages were 0, 1, 10, 20, 50, 100, and 500 ms corresponding to 0, 0.15, 1.57, 3.14, 7.86, 15.71, and 78,57 J mm^{-2}." should read "The laser dosages were 0, 1, 10, 20, 50, 100, and 500 ms corresponding to 0, 0.016, 0.16, 0.32, 0.80, 1.59, and 7.95 J mm^{-2}."

In **Section 3.1**, the sentences "Consequently, the NOAEL was the highest dose of 235.71 J mm^{-2} and the LOAEL could not be estimated. For *E. crypticus*, the highest laser dose of 235.71 J mm^{-2} was the only dose significantly higher than the control (p=0.0185), and accordingly the LOAEL, making 157.14 J mm^{-2} the NOEAL (Supplementary Table 1)." should read "Consequently, the NOAEL was the highest dose of 23.9 J mm^{-2} and the LOAEL could not be estimated. For *E. crypticus*, the highest laser dose of 23.9 J mm^{-2} was the only dose significantly higher than the control (p=0.0185), and accordingly the LOAEL, making 15.9 J mm^{-2} the NOEAL (Supplementary Table 1)."

In **Section 3.2.1**, paragraph 2, the sentences "At the smallest dose (0.16 J mm^{-2}) some larvae and pupae developed normally, but some developed into beetles with wing deformities. When the dose was increased to 3.14 J mm^{-2}, most of the insects (all stages) were living, but the living larvae received a spot burn from the laser treatment while living adults had deformed wings. The dead insects became brown, dark or with a big dark spot from the laser. The living larvae developed into deformed beetles that almost immediately died. At a dose of 7.86 J mm^{-2}, more than half of the larvae died." should read "At the smallest dose (0.016 J mm^{-2}) some larvae and pupae developed normally, but some developed into beetles with wing deformities. When the dose was increased to 0.32 J mm^{-2}, most of the insects (all stages) were living, but the living larvae received a spot burn from the laser treatment while living adults had deformed wings. The dead insects became brown, dark or with a big dark spot from the laser. The living larvae developed into deformed beetles that almost immediately died. At a dose of 0.80 J mm^{-2}, more than half of the larvae died."

In **Section 3.2.1**, paragraph 3, the sentences "Very few larvae survived a dose of 15.71 J mm^{-2}. Most larvae died having a dark dehydrated and burned appearance. At a dose of 78.57 J mm^{-2}, almost all the larvae died the first week after application at larva stage." should read "Very few larvae survived a dose of 1.59 J mm^{-2}. Most larvae died having a dark dehydrated and burned appearance. At a dose of 7.95 J mm^{-2}, almost all the larvae died the first week after application at larva stage."

In **Section 3.2.2**, the sentences "When a dose of 0.16 J mm^{-2} was applied, all pupae developed into beetles with the same survival rate as the control group. However, one third developed deformed wings and/or body (Figure 5B). When the dose was increased to 1.57 J mm^{-2}, the survival rate declined approximately 68%. The living pupae all developed into adults, which, however, had some wing and body deformities. Most of the non-living pupae and beetles were discoloured and broke into small pieces (Figure 5C). When the dose was doubled (3.14 J mm^{-2}), the survival rate decreased even more, and the mortality rate rose to 62%. The appearance of non-living adults varied from injured (Figure 5D), cut into pieces or dead bodies without any indication of abnormal appearance. A dose of 7.85 J mm^{-2} increased the mortality to approximately 85%. Many pupae did not develop into adults as a high number of dead pupae had a brown dark color or completely dark and dehydrated body. A dose of 15.71 J mm^{-2} almost killed all insects after 15 days (mortality ~ 97.5%). Many pupae did not develop into adults due to high mortality. Dead pupae had a brown dark color or a completely dark and dehydrated body. At the highest dose (78.57 J mm^{-2}), all pupae died within the first week after the irradiation with a burned-like appearance (Figure 5E)." should read "When a dose of 0.016 J mm^{-2} was applied, all pupae developed into beetles with the same survival rate as the control group. However, one third developed deformed wings and/or body (Figure 5B). When the dose was increased to 0.16 J mm^{-2}, the survival rate declined approximately 68%. The living pupae all developed into adults, which, however, had some wing and body deformities. Most of the non-living pupae and beetles were discoloured and broke into small pieces (Figure 5C). When the dose was doubled (0.32 J mm^{-2}), the survival rate decreased even more, and the mortality rate rose to 62%. The appearance of non-living adults varied from injured (Figure 5D), cut into pieces or dead bodies without any indication of abnormal appearance. A dose of 0.80 J mm^{-2} increased the mortality to approximately 85%. Many pupae did not develop into adults as a high number of dead pupae had a brown dark color or completely dark and dehydrated body. A dose of 1.59 J mm^{-2} almost killed all insects after 15 days (mortality ~ 97.5%). Many pupae did not develop into adults due to high mortality. Dead pupae had a brown dark color or a completely dark and dehydrated body. At the highest dose (7.95 J mm^{-2}), all pupae died within the first week after the irradiation with a burned-like appearance (Figure 5E)."

In **Section 3.2.3**, the sentences "At the lowest dose (0.15 J mm^{-2}), the mortality did not differentiate from the controls (Figures 6, 7A and Supplementary Table 4). Treatment with 1.57 J mm^{-2} increased mortality. After applying a dose of 3.14 J mm^{-2}, the mortality rate increased to ca 2.5%.Most of the living adults had a spot derived fromthe laser beam while most of the non-living adults had a small hole from the laser (Figure 7B). More than half of the adults died at a dose of 7.86 J mm^{-2} with a hole from the laser." should read "At the lowest dose (0.016 J mm^{-2}), the mortality did not differentiate from the controls (Figures 6, 7A and Supplementary Table 4). Treatment with 0.16 J mm^{-2} increased mortality. After applying a dose of 0.32 J mm^{-2}, the mortality rate increased to ca 2.5%. Most of the living adults had a spot derived fromthe laser beam while most of the non-living adults had a small hole from the laser (Figure 7B). More than half of the adults died at a dose of 0.80 J mm^{-2} with a hole from the laser."

In **Section 3.3**, the sentences "Even 0.15 J mm^{-2} affected the shape of the dose-response curve, and the beetle's elytron became brownish in color (Figure 9). A dose of 7.86 J mm^{-2} severely harmed the beetles, and almost all beetles died during the 15 days. A dose of 78.57 J mm^{-2} immediately killed all beetles burning significant holes in the beetles (Figure 9)." should read "Even 0.016 J mm^{-2} affected the shape of the dose-response curve, and the beetle's elytron became brownish in color (Figure 9). A dose of 0.80 J mm^{-2} severely harmed the beetles, and almost all beetles died during the 15 days. A dose of 7.95 J mm^{-2} immediately killed all beetles burning significant holes in the beetles (Figure 9)."

In **Section 4.1**, the sentences "There were no differences in mortality between the control group and worms exposed to all laser doses, with a single exception with *E. crypticus* in sandy soil at a dose of 157 J mm^{-2}. The spread of the heat in the soil from the laser beam depends on the water content and soil structure and composition. We cannot exclude that other conditions like a higher water content or other soil types would result in other mortalities. We consider a soil water content of 50% of the water capacity to be realistic in the early spring when weed control usually is conducted, but it depends on many factors in the field (e.g., variation in soil composition, precipitation, and evaporation). The earthworms in the tubes were living in a very small soil volume (<15 g) during the experiment mimicking the uppermost part of the soil profile. Although heat corresponding to 235,7 J mm^{-2} was executed on a spot with a 2 mm diameter, which easily kills weed seedling, the exposure did not warm up the soil sufficiently to affect the mortality of the worms living close to the soil surface within the seven days." should read "There were no differences in mortality between the control group and worms exposed to all laser doses, with a single exception with *E. crypticus* in sandy soil at a dose of 15.9 J mm^{-2}. The spread of the heat in the soil from the laser beam depends on the water content and soil structure and composition. We cannot exclude that other conditions like a higher water content or other soil types would result in other mortalities. We consider a soil water content of 50% of the water capacity to be realistic in the early spring when weed control usually is conducted, but it depends on many factors in the field (e.g., variation in soil composition, precipitation, and evaporation). The earthworms in the tubes were living in a very small soil volume (<15 g) during the experiment mimicking the uppermost part of the soil profile. Although heat corresponding to 23.9 J mm^{-2} was executed on a spot with a 2 mm diameter, which easily kills weed seedling, the exposure did not warm up the soil sufficiently to affect the mortality of the worms living close to the soil surface within the seven days."

In **Section 4.2**, the sentence "In general, the insects were all killed immediately at a laser dose corresponding to what would be appropriate for killing small weed seedlings (78.57–157.14 J mm^{-2})." should read "In general, the insects were all killed immediately at a laser dose corresponding to what would be appropriate for killing small weed seedlings (50 J mm^{-2})."

In **Section 5**, the sentence "The earthworms were mostly unharmed when the soil surface was exposed to laser dosages up to 236 J mm^{-2} as the soil protected them." should read "The earthworms were mostly unharmed when the soil surface was exposed to laser dosages up to 23.9 J mm^{-2} as the soil protected them."

The dosages given in Figure 2, Figure 4, Figure 6 and Figure 8 were also incorrect. The corrected figures appear below.

There was an error in the caption for Figure 3. The corrected caption appears below.

Figure 3 Deformities observed during the study of *T. molitor* (larvae experiment): **(A)** *T. molitor* control adult and larva. **(B)** 0.016 J m^{-2}: Larva becomes a deformed alive adult. **(C)** 0.32 J m^{-2}: Larva developed into a deformed adult and died. **(D)** 0.80 J m^{-2}: Larva did not complete metamorphosis (severely deformed insect: half pupa, half adult). **(E)** 1.59 J m^{-2}: Burned (brownish-dark) larva. **(F)** 7.95 J m^{-2}: Larva were rapidly killed after exposure, and there

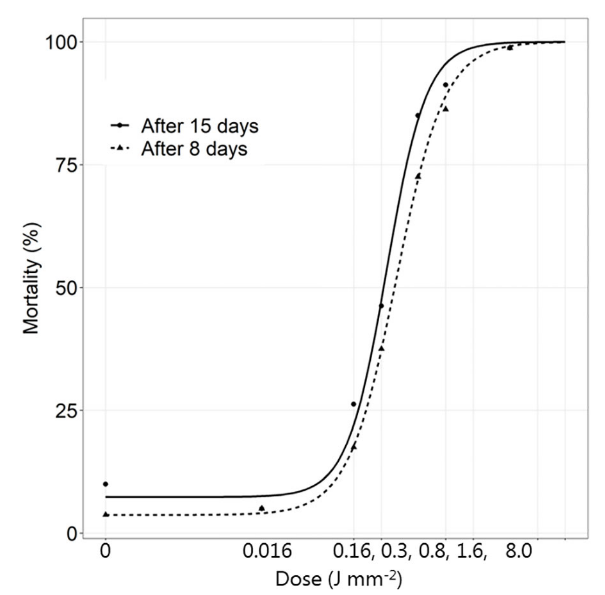

FIGURE 2
The mortality (%) of *Tenebrio molitor* larvae 8 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam with a diameter of 2 mm. Points show mean values (n=20).

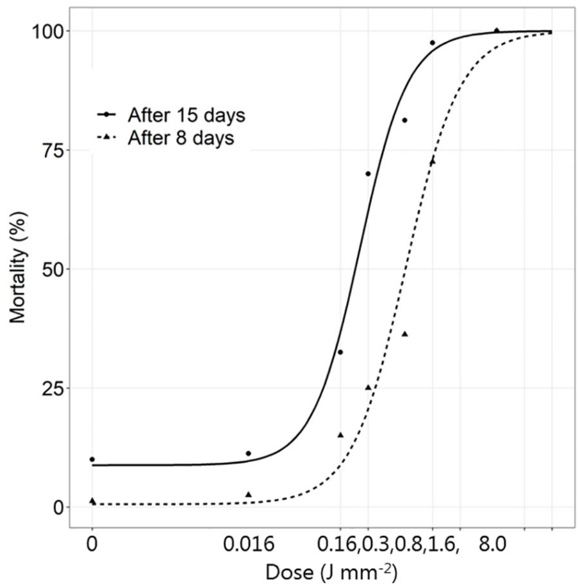

FIGURE 4
The mortality (%) of *Tenebrio molitor* pupae 8 and 15 days after exposure to increasing dosages of laser energy (J m^{-2}) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

was significant damage caused to the insects, e.g., hemolymph flowed out of the laser hole in the insects' body.

There was an error in the caption for Figure 5. The corrected caption appears below.

Figure 5 Typical deformities that were observed on *Tenebrio molitor* after laser application (pupae experiment): **(A)** *T. molitor* control pupa. **(B)** 0.016 J m^{-2} (1 ms): Pupa developed into adult with deformed wings. **(C)** 0.16 J m^{-2}: Non-living adult and adults broken into pieces. **(D)** 0.32 J m^{-2}: Pupa transformed to an injured adult. **(E)** Pupa died few days after laser application having a brown-burned appearance.

There was an error in the caption for Figure 7. The corrected caption appears below.

Figure 7 Typical deformities that were observed on *Tenebrio molitor* adults after laser application (adults' experiment): **(A)** 0.016 J m^{-2}: *T. molitor* adults were alive with normal appearance.

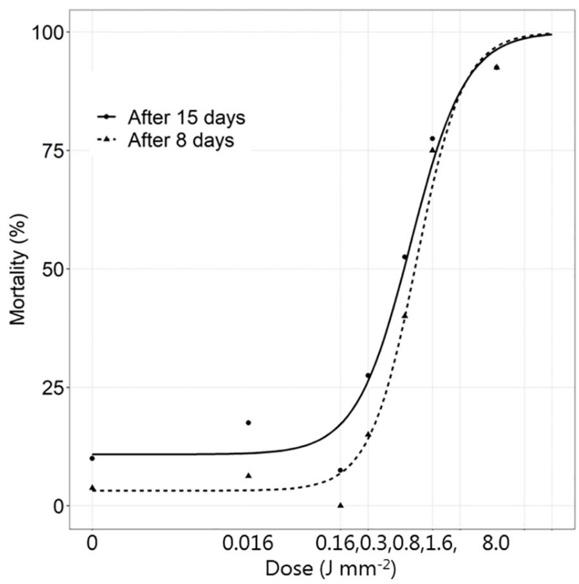

FIGURE 6
The mortality (%) of *Tenebrio molitor* beetles 8 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

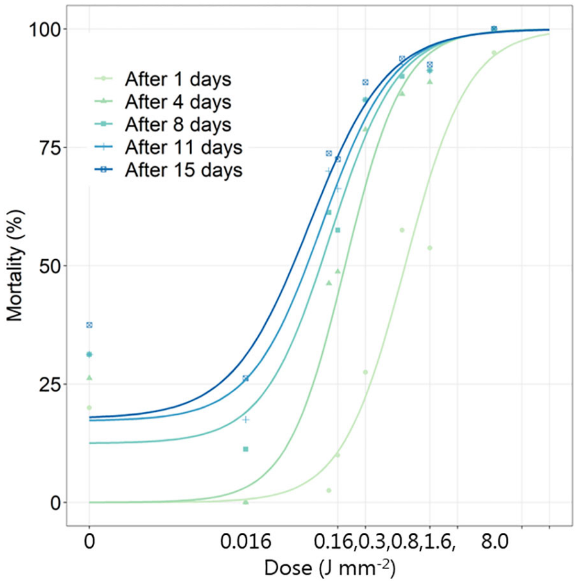

FIGURE 8
The mortality (%) of *Adalia bipunctata* beetles 1, 4, 8, 11 and 15 days after exposure to increasing dosages of laser energy (J) from a thulium-doped 2 µm 50 W fiber laser with a collimated beam (Ø = 2 mm). Points show mean values (n=20).

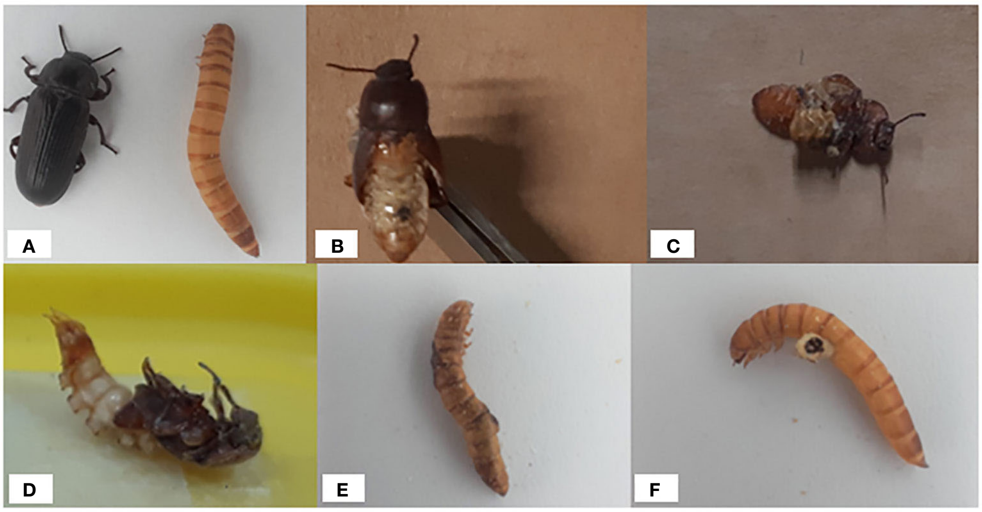

FIGURE 3
Deformities observed during the study of *T. molitor* (larvae experiment): **(A)** *T. molitor* control adult and larva. **(B)** 0.016 J m^{-2}: Larva becomes a deformed alive adult. **(C)** 0.32 J m^{-2}: Larva developed into a deformed adult and died. **(D)** 0.80 J m^{-2}: Larva did not complete metamorphosis (severely deformed insect: half pupa, half adult). **(E)** 1.59 J m^{-2}: Burned (brownish-dark) larva. **(F)** 7.95 J m^{-2}: Larva were rapidly killed after exposure, and there was significant damage caused to the insects, e.g., hemolymph flowed out of the laser hole in the insects' body.

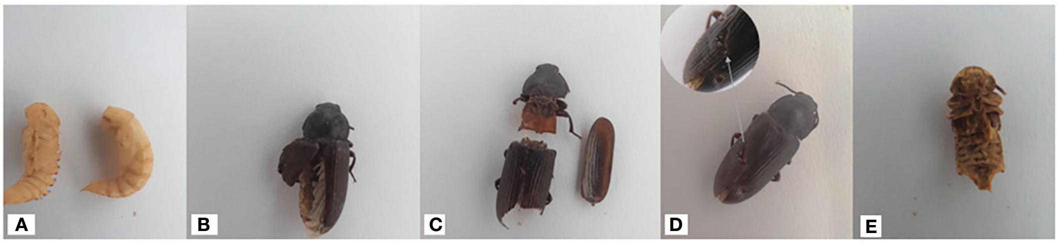

FIGURE 5
Typical deformities that were observed on *Tenebrio molitor* after laser application (pupae experiment): **(A)** *T. molitor* control pupa. **(B)** 0.016 J m^{-2} (1 ms): Pupa developed into adult with deformed wings. **(C)** 0.16 J m^{-2}: Non-living adult and adults broken into pieces. **(D)** 0.32 J m^{-2}: Pupa transformed to an injured adult. **(E)** Pupa died few days after laser application having a brown-burned appearance.

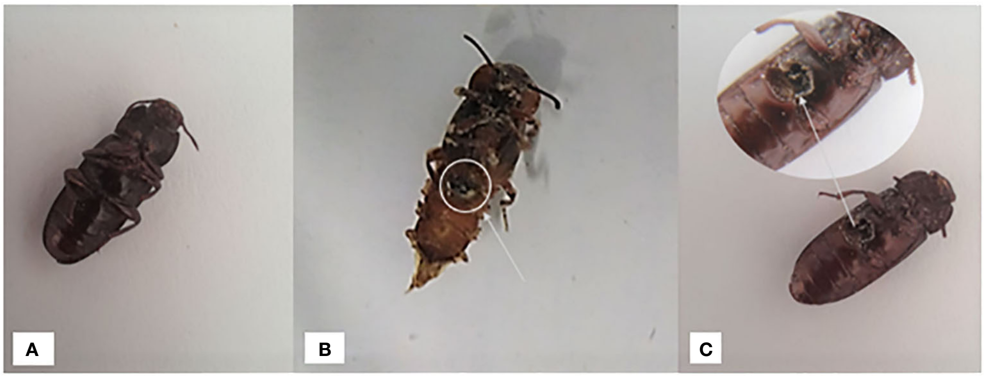

FIGURE 7
Typical deformities that were observed on *Tenebrio molitor* adults after laser application (adults' experiment): **(A)** 0.016 J m^{-2}: *T. molitor* adults were alive with normal appearance. **(B)** 0.32 J m^{-2}: a living adult with a spot from the laser. **(C)** 7.95 J m^{-2}: *T. molitor* adult died immediately with a large hole in its body.

(B) 0.32 J m^{-2}: a living adult with a spot from the laser. **(C)** 7.95 J m^{-2}: *T. molitor* adult died immediately with a large hole in its body.

There was also an error in Supplementary Table 1. The dosages mentioned were not correct. This material updated in the original article.

The authors apologize for these errors and state that they do not change the scientific conclusions of the article in any way. The original article has been updated.

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Weed control with saturated steam in organic highbush blueberry

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Weed management is often a predominant and costly problem in the production of organic blueberries. Geotextile weed fabrics of woven polyethylene are widely used in organic blueberry fields to suppress weeds growing within the rows. Weeds, such as *Convolvulus arvensis* L., grow at the base of the blueberry plants or through openings and around the edges of the weed fabric, thus requiring hand weeding. This study evaluates the integration of saturated steam (SS), a rotary brush (RB), and organic herbicides for weed control in blueberries. Dose–response studies indicated that SS applied at 121°C and at 7.4 m^3 ha^{-1} of steam (3,655 MJ ha^{-1}) resulted in over 90% control and a reduction in the dry weights of *C. arvensis*. When treatments were directed to the base of the blueberry plants, SS at 7.4 m^3 ha^{-1} provided 80% control of *C. arvensis* 28 days after treatment (DAT) and was comparable to hand weeding. Both of these treatments outperformed capric plus caprylic acid (CC) (33.2 kg ai ha^{-1}) or ammonium nonanoate (AN) (24.3 kg ai ha^{-1}) applications, despite *C. arversis* regrowth being observed. Four repetitive basal applications of SS of up to 29.6 m^3 ha^{-1} over two consecutive years caused minimal and transient damage to new basal shoots of 'Elliot' and 'Duke' blueberries; basal shoot cross-sectional area compared with the non-treated was unaffected. In contrast, basal application of AN treatments damaged or killed basal shoots. When treatments were applied to the edge of the weed fabric, SS (7.4 m^3 ha^{-1}) reduced weed biomass by 42% to 93% at 28 DAT compared with the non-treated. The RB treatment reduced weed biomass from 72% to 99% in all experiments, while CC and AN reduced biomass by 18% to 54%. A partial budget analysis indicated that SS and the RB were 3- and 6.5-fold less expensive than organic herbicides, respectively. Integrating physical (SS) and mechanical (RB) treatments improved weed control. The latter, however, damaged the weed-suppressing fabric where preexisting holes were present, generated dust, and increased the chance of fruit contamination. The SS was safe for the weed-suppressing fabric and the blueberry, but weed regrowth following treatment and copious water requirements hindered its feasibility.

KEYWORDS

non-chemical, *Vaccinium corymbosum* L., *Convolvulus arvensis* L., no-till, organic herbicides, mechanical weed control, synthetic mulch, thermal weed control

1 Introduction

Highbush blueberry is economically important in the United States, with the production being valued at over US$986 million in 2022 (USDA, 2023). The hectarage in the USA has increased continuously over the past decades, rising by almost 250%, increasing from 16,320 ha in 2000 to nearly 40,000 ha in 2021 (USDA, 2023). The western United States is an important region for the production of blueberries; together Oregon and Washington account for roughly 35% of domestic hectarage. The Pacific Northwest is the world's largest producer of organic highbush blueberries. Proper weed management is one of the most challenging aspects of organic production, as producer options are limited (Strik and Vance, 2016). Weed management is often identified as a major, costly production problem, hindering the expansion of organic adoption (Strik, 2016). Weed competition can make highbush blueberry economically inviable, as it has been shown to reduce blueberry growth by 38% and yield by up to 92% (Burkhard et al., 2009).

Highbush blueberry plants have a shallow root system located in the top 0.5 m of soil (Valenzuela-Estrada et al., 2008) and, consequently, are sensitive to weed competition. Soil tillage is detrimental to blueberry plants. Blueberries are often grown in raised beds with sawdust mulch to improve soil drainage and plant growth (Strik, 2016). Synthetic geotextile fabrics, commonly called weed mats or synthetic mulches, have become increasingly commonplace in organic blueberry fields in the past decade as they effectively suppress weed growth within the planting rows (Strik, 2016). Synthetic mulches are placed over the sawdust mulch for optimal results (Strik and Davis, 2021). These synthetic mulches require a significant initial investment but are cost-effective as they can last for many years, reduce labor in weeding, and improve crop growth. However, creeping and climbing weed species, such as *Convolvulus arvensis* L., can grow through the openings at the base of the blueberry plants and around or over the weed fabric's edges, evading proper control (Figure 1). For this reason, organic producers often rely on labor-intensive hand weeding in those areas to prevent yield losses and interference during crop harvest. The increasing costs and labor scarcity require new weed management approaches in organic blueberry fields (Strik and Vance, 2016).

Sustainable organic blueberry production requires cost-effective weed control methods compatible with blueberry and synthetic mulches. Organic herbicides are compatible with the production systems, but often do not have the efficacy required (Strik and Vance, 2016) and are costly (Dayan and Duke, 2010). Flaming has proven incompatible with synthetic mulches, although weed control with steam and hot water has been successful in other systems (Hansson, 2002; Kristoffersen et al., 2008). Steam transfers thermal energy to the targeted plants, raising tissue temperature and causing cell damage (Bauer et al., 2020). Another approach for thermal weed control is hot foam, which utilizes a biodegradable foaming agent that will trap the heat and improve weed control (Antonopoulos et al., 2023) Rotary brushes are a mechanical weed control option; these brushes rotate along vertical or horizontal axes, uprooting weeds in vegetable systems (Melander, 1997). The literature lacks data about the efficacy of physical and chemical options for weed control in organic blueberries, whether applied alone or in combination, as well as energy demands and associated costs. In this study, we aimed to assess the crop safety and efficacy of organic weed control by saturated steam (SS), a rotary brush (RB), and organic herbicides in blueberries.

FIGURE 1
Convolvulus arvensis in organic highbush blueberry **(A)**. The shoots emerge in the spring and can climb the plants when growing at the base of the plant or in adjacent areas **(B)**. The *C. arvensis* shoots will hinder the mechanical harvest and will set seeds in the fall **(C, D)**.

2 Materials and methods

Two study protocols were developed to evaluate organic weed control tools in northern highbush blueberry. The first study goal was to assess the impact of steam temperature and the speed of operation on weed control. The second study goal was to compare the efficacy of different weed control tools and their combinations for organic blueberry production.

2.1 The saturated steam equipment

A commercial unit generated saturated steam (SS) (Satusteam™ SW900; Weedtechnics, Terrey Hills, NSW, Australia) for this study (Figure 2). The unit has a diesel-powered boiler operating at 6,205 kPa and generating 0.6 m^3 h^{-1} of SS. The boiler consumes 2 L of diesel per hour. The SS temperature can be regulated from ambient to 121°C, with the highest temperature being recommended by the manufacturer. A gasoline-powered pump moves the water from the reservoir (0.4 m^3) using 0.5 L h^{-1}. A circular applicator, with a 0.55-m radius, or a hand-held device 0.5 m wide delivers SS at ambient pressure. A metal nozzle is mounted inside the circular applicator. The equipment is mounted on a 1.2-m trailer and towed by a tractor. The steamer was pulled by a 14-kW tractor. The published information on SS performance on weed control was limited. The first study goal was to assess the impact of steam temperature and application volume on weed control.

2.2 Saturated steam boiler temperature and application volume

Two field experiments were performed to compare boiler temperatures ranging from ambient to 121°C. The sites were infested with *C. arvensis* (Table 1). Treatments included ambient temperature (~ 26°C ± 6°C), and 65°C, 79°C, 93°C, 107°C, and 121°C boiler temperatures. All treatments were applied by the tractor with the circulator applicator at a constant steam volume of 7.4 m^3 ha^{-1}. The experimental plots were 0.5 m wide by 3 m long; treatments were applied with the hand-held unit to ensure a constant boiler temperature.

Six additional studies were conducted to evaluate application volume in different weed species (Table 1). The dosage level was varied by adjusting the travel speed from 0.4 km h^{-1}, 0.8 km h^{-1}, 1.6 km h^{-1}, 2 km h^{-1}, and 4 km h^{-1}. A non-treated control was included as a reference. The experimental plots were 0.5 m by 5 m in the application volume studies. All studies were designed as randomized complete blocks, with four replicates per treatment level. The treatment consisted of a single application of SS. The above-ground biomass was sampled 14 days to 18 days after treatment (DAT). A single quadrat (0.5 m by 0.5 m) per plot was placed in the plot's center, and the biomass was hand-harvested and dried in an oven at 65.6°C until it reached a constant weight and then it was weighed.

2.3 Basal application of saturated steam in blueberry

2.3.1 Blueberry tolerance

The blueberry shrub consists of several shoots originating from buds at the plant base; the shoots continue to grow for many years. In their second year, shoots, called canes, are surrounded by the periderm, or bark, while the younger shoots are surrounded by a cuticle (Gough, 1993). These 1-year-old shoots are essential to defining blueberry bush architecture and productivity (Strik et al., 2014). Any damage to the young basal shoots can have detrimental long-term effects on blueberry bushes, as new shoots are trained to replace older, less unproductive canes (Strik et al., 2003). Blueberry cultivars can differ in basal shoot number, length, and growth vigor,

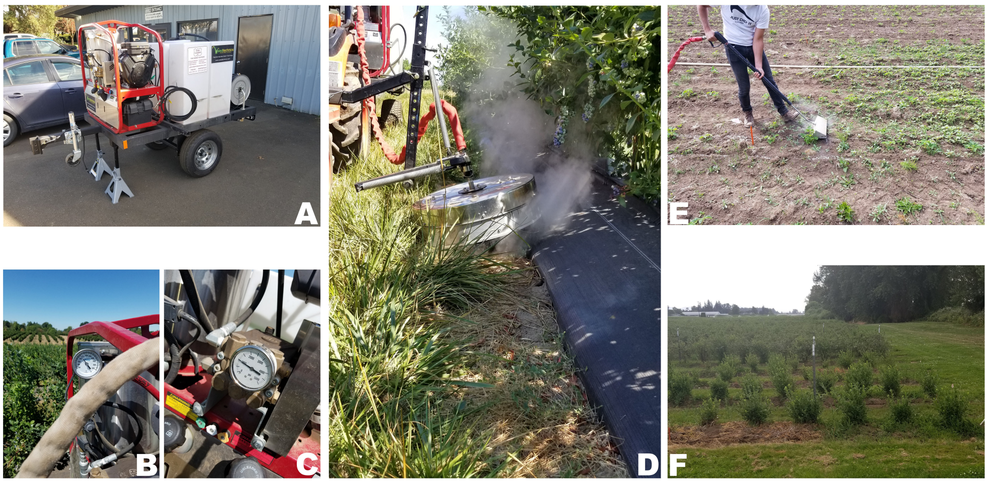

FIGURE 2
The steamer SW900 (Weedtechnics) was modified to fit a 1.2-m-wide trailer **(A)**. It was operating at 121°C **(B)** and at 6,205 kPa producing 0.6 m^3 h^{-1} of saturated steam (SS) **(C)**. The SS was delivered by a 0.5-m circular tractor-mounted applicator **(D)** or a hand-held unit **(E)**. The effects on the weeds included wilting, which was noticeable immediately, with foliage death observed in a few days **(F)**.

TABLE 1 Summary of field studies conducted for each research objective by trial location (Corvallis and Independence, OR, USA) and start date.

Location	Start date	Duration	Weed species	Treatments	*n*	Steam(m^3 ha^{-1})
Study 1: determine boiler temperature (T) requirements (0°C to 121°C)						
Independence	28 June 2018	14 days	*Convolvulus arvensis*	7	4	7.4
Corvallis	4 August 2020	14 days	*Convolvulus arvensis*	7	4	7.4
Study 2: determine steam output requirements (dose response)						
Independence	31 July 2018	14 days	*Kickxia elatine* (L.) Dumort.	6	4	0–14.8
Corvallis	6 June 2018	14 days	*Convolvulus arvensis*	8	4	0–14.8
Corvallis	3 October 2018	14 days	*Convolvulus arvensis*	8	4	0–14.8
Independence	3 July 2019	14 days	*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot	6	4	0–14.8
Independence	3 August 2020	14 days	*Convolvulus arvensis*	4	6	0–14.8
Study 3: steam basal application						
Independence	1 August 2019	28 days	*Convolvulus arvensis*	8		
Study 4: blueberry tolerance						
Corvallis	6 June 2019	2 years	–	7	10	
Study 5: integrated weed control						
Independence	17 July 2018	84 days	*Polygonum aviculare* L. *Kickxia elatine*, *Sonchus oleraceus* L.	25	4	
Independence	9 May 2019	84 days	*Epilobium septentrionale* (D.D. Keck) R.N. Bowman & Hoch *Polygonum aviculare*	25	6	
Independence	1 August 2019	84 days	*Polygonum aviculare* L. *Kickxia elatine* *Sonchus oleraceus* L.	225	6	

n, number of replicates.

so the impact of weed management may be cultivar dependent (Strik et al., 2014).

The basal application of SS was evaluated to control weeds growing at the plant's base; this application was compared with an organic herbicide applied as a spray or with a sponge wiper. Both management strategies were compared for efficacy and crop tolerance. A 2-year study was conducted on the Corvallis, OR OSU Lewis Brown Research farm in a mature highbush blueberry field. The blueberry plants were 3.35 m tall, spaced 0.9 m apart and supported on a "T" trellis system; the raised berms were 0.3 m in height and 1.2 m in width. The cultivars 'Duke' and 'Elliot' were planted in adjacent field sections. Surface drip irrigation was installed on both sides of the planting row, and the field was mulched with sawdust beneath the synthetic mulch.

The experiments consisted of 10 treatments, which were organized as a randomized complete block replicated four times. The experimental plots included three plants. Each cultivar was an independent study. The treatments were applied to the base of the blueberry plants targeting the lower 0.5 m of the plant, and a non-treated control was included as a reference. The SS was applied at 7.4 m^3 ha^{-1}, 14.8 m^3 ha^{-1}, and 29.6 m^3 ha^{-1} using the hand-held applicator. The herbicide ammonium nonanoate (AN) (AXXE®; BioSafe Systems, LLC, East Hartford, CT, USA) was applied as a basal-directed spray at 24.3 kg ai ha^{-1}, 48.6 kg ai ha^{-1}, and 97.2 kg ai ha^{-1}, or the equivalent of a field rate, two, and four times the field rate. The approved field rate for blueberry is up to 13% vol/vol of the commercial product in 0.74 m^3 ha^{-1} of carrier volume. The AN treatment used a CO_2-pressurized backpack sprayer with three AI 11008 (TeeJet®) nozzles at 275 kPa. The AN was also tested as a sponge-wiper application at one, two, and four times the field rate. The sponge-wiper application was by hand-held sponger wiper (Drift Free Green Sponge Gun Weed Wiper; Smucker, Harrisburg, OR), connected to a pressurized backpack sprayer, and saturated before each application. The sponge-wiper treatments included AN at 13% vol/vol applied for one, two, or four passes; manipulating the spray concentration could affect solution viscosity and delivery. The treatments were applied on 17 June 2019, and reapplied on 29 July 2019, 42 days after the initial treatment (DAIT). The study was repeated in 2020, with the treatments applied on 19 June 2020 (368 DAIT), and reapplied on 30 July 2020 (409 DAIT).

The visual estimates of basal shoot and canopy injury were assessed on a 0% to 100% scale, with 0% being no effect and 100% being plant death. Injury was recorded monthly throughout the experiment. No canopy injury was observed during the study. At the end of the experiment, the diameter of three basal shots per plot was recorded at 0.2 m above the ground and converted to a cross-sectional area. The length of the shoot was also recorded at that time.

2.3.2 Efficacy of *C. arvensis* control

A commercial organic blueberry field near Independence, OR, USA, planted with ′Last Call′ blueberries and infested with *C. arvensis* was used for the study. The blueberry plantings were 3.35 m × 0.90 m apart. The plants were grown on berms, 0.3 m high by 1.2 m wide. The berms were mulched with Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] sawdust covering black geotextile polyethylene landscape fabric. The blueberry bushes were trellised in a two-wire "T" trellis system, with a drip irrigation line placed on both sides of the bushes. The experiment was initiated in May 2019 when the *C. arvensis* shoots were between 10 cm and 15 cm in length. The plants with *C. arvensis* were selected for the study, which consisted of seven treatments organized in a randomized complete block design with four replicates. Each experimental unit consisted of three blueberry plants. The treatments included SS, the organic herbicide capric plus caprylic acid (CC) (SUPRRESS®; Westbridge Agricultural Products, Vista, CA, USA), and AN applied as spray or by sponge wiper. Hand weeding and a non-treated control were included as references. The SS treatments were applied using by hand-held application directed to the lower 50 cm of the plant as a single pass delivering 7.4 m^3 ha^{-1} at 121°C and at 6,205 kPa. The spray application of CC and AN were made at 19 kg ai ha^{-1} and 24.2 kg ai ha^{-1}, the equivalent of 9% vol/vol and 13% vol/vol in 748 L ha^{-1} of carrier volume, respectively. The sponge wiper applications of CC and AN were made at 9% vol/vol and 13% vol/vol, respectively. The assessments included visual estimates of crop injury and *C. arvensis* control, made at 7 DAT and 28 DAT on a scale of 0 to 100 representing no control to complete control, respectively. Crop injury was similarly estimated as indicated in the previous section. At 28 DAT, *C. arvensis* shoots and leaves were collected, dried, and weighed.

2.3.3 Integrated weed control

Three field trials were conducted in 2018 and 2019 in commercial organic highbush blueberry, as described in section 2.2.2. These trials will be referred to as experiment 1, initiated in the summer of 2018; experiment 2, initiated in the spring of 2019; and experiment 3, initiated in the summer of 2019. The cultivars in experiments 1, 2, and 3 were 'Last Call', 'Mega Blue', and 'Aurora', respectively.

The experiments were organized as a 5 × 5 factorial in a randomized complete block design with four replications in experiment 1 and six in experiments 2 and 3. The experimental units were 3.35 m by 25 m and included 26 blueberry plants. Factor A was the first application of SS (7.4 m^3 ha^{-1}), a RB, AN (24.2 kg ai ha^{-1}), or CC (19.2 kg ai ha^{-1}), and a non-treated control as reference. Factor B was a second treatment applied 28 days later, resulting in 25 combinations. The retreatment interval was selected based on the optimum for hot water retreatment interval (Hansson and Ascard, 2002). All treatments were applied to a 0.5-m strip of ground running parallel to the weed mat on both sides of the row. The SS treatments were applied by a tractor-mounted circular applicator. The brush weeding was performed by an in-row weeder with a rotary brush attachment (ID David, Murcia, Spain). The brush consists of nylon brushes rotating at 4,000 revolutions per minute (rpm) to 5,000 rpm in the vertical plane in the direction of tractor movement at 1.6 km h^{-1}. The brush was positioned to remove weeds at the soil surface while having minimal contact with the plastic mulch. The AN and CC were applied by CO_2-pressurized backpack sprayer using AI11008 nozzles, which were calibrated to deliver 0.74 m^3 ha^{-1}.

Weed control and green weed coverage were recorded at 28 DAT and 56 DAT. Weed control was assessed on a scale from 0% to 100%, where 0% represents no effect or control, and 100% was death of all target plants. The green weed coverage was assessed using the online image analysis tool Canopeo (Patrignani and Ochsner, 2015), with red-to-green and the blue-to-green ratios set at 0.95, and excess green set at 20. The pictures were taken at 1.5 m above the soil level using a point-and-shoot digital camera (Nikon Coolpix W300). The above-ground weed biomass was recorded at 28 DAT and 56 DAT in different locations within the experimental unit. Biomass was dried, and weight was recorded.

2.4 Statistical analysis

The SS output per time was converted into volume per area by *Equation 1*:

$$\text{SS dose} = \left(\frac{\text{SS flow}}{\text{s} \times \text{w}}\right) \times 10,000, \tag{1}$$

where the SS dose is saturated steam in m^3 of SS ha^{-1}, the SS flow is the flow rate of SS in m^3 SS h^{-1}, s is the operational speed in m h^{-1} and w is the width of the treated area in m. The result is multiplied by a factor to convert the rate to a per ha basis.

The SS physical properties under isobaric conditions of 6,205 kPa were used to calculate the energy density in MJ ha^{-1} (NIST, 2023), using *Equation 2*:

$$\rho_{energy} = \text{Vol} \times \text{density} \times \text{H}, \tag{2}$$

where ρ_{energy} is the energy density applied (MJ ha^{-1}), Vol is the SS volume (m^3 ha^{-1}), density of saturated steam (kg m^{-3}), and H is the enthalpy in MJ kg^{-1}. A similar calculation was performed to calculate the energy under different SS temperature conditions.

The recorded above-ground dry weights were fitted to a three-parameter non-linear regression using the DRC package v 3.0.1 (Ritz et al., 2015) of R software v 4.3.0 (R Core Team, 2023), as seen in *Equation 3*:

$$y = d/(1 + \exp(b(\log x) - \log(e))), \tag{3}$$

where d is the upper limit of the variable, b is the slope of the curve around the inflection point, x is the energy rate and e is the inflection point for 50% reduction in biomass. The Akaike information criterion was used to select the best model. The ED procedure in DRC package was used to estimate the effective doses causing a 90% and 99% reduction in biomass. The experiments were analyzed separately because of the differences in the weed species present.

Blueberry basal shoot length, shoot caliper, and weed biomass were fitted to a generalized linear mixed model using package lme4 version 1.1–3.4 and the "lmer" procedure. The assumptions of normality and homogeneity of variance were tested by Shapiro–Wilk's and Levene's tests, respectively. A log + 1 transformation was applied to the weed biomass data to meet these assumptions; back-transformed data are presented. The weed control and injury data were fitted to a generalized linear mixed model with a logit link distribution using the glmmTMB package version 1.1.7. ANOVA was performed using the glmmTMB function and the means were separated by the Dunnett's test when compared with the non-treated or Sidak's test when making multiple comparisons using the emmeans (estimated marginal means) package v 1.8.7.

2.5 Partial budget analysis

A partial budget analysis compared application costs for the different weed control treatments. For the steamer and brush, variable costs were calculated by adding the equipment costs, fuel usage, and labor. For the organic herbicides, variable costs were calculated by adding the herbicide costs, fuel usage, and labor. Diesel charges of US\$1.09 L^{-1} and gasoline charges of US\$1.23 L^{-1} were used to calculate the cost of fuel. Labor was charged at US\$19 h^{-1}. Tractor hourly costs were based on methods proposed by Edwards (Edwards, 2023). The fixed costs included machinery and spray equipment. The analysis did not include the purchase prices of the tractor or the sprayer. The final cost was calculated for the treatment of one-fifth of the total field, or the equivalent area adjacent to the geotextile weed fabric.

3 Results

3.1 Saturated steam boiler temperature and application volume

Weed control with SS relies on the transference of thermal energy stored in the SS to the weed foliage. Heat transfer increases with the magnitude of the temperature difference (Ascard et al., 2007). The energy level applied when changing the boiler temperature from 38°C to 121°C was 1,214 MJ ha^{-1} to 3,586 MJ ha^{-1} at 7.4 m^3 ha^{-1} of SS (Figure 3). Control and biomass reduction of *C. arvensis* was only observed at SS temperatures above 65°C (2,018 KJ ha^{-1}). The energy required to provide 50% control or biomass reduction (ED_{50}) was 2,386 MJ ha^{-1} and 1,563 MJ ha^{-1}, respectively, and 1.6- to 2.3-fold more energy was required to provide 90% control or biomass reduction (ED_{90}), respectively (Table 2).

The energy applied was also manipulated by altering the application volume; however, as SS volume increases, the cost of treatment increases because of the greater consumption of water, fuel, and labor. The efficacy of the SS was weed species dependent (Table 3, Figure 4), when increasing energy (and volumes) from 0 MJ ha^{-1} to 7,173 MJ ha^{-1} (0 m^3 ha^{-1} to 14.8 m^3 ha^{-1}) at a constant temperature of 121°C. The ED_{50} values of dicotyledonous species *Kickxia elatine* (L.) Dumort., *C arvensis*, and *Amaranthus retroflexus* L. were 827 MJ ha^{-1}, 1,563 MJ ha^{-1}, and 1,767 MJ ha^{-1}, respectively. The ED_{90} values for the same species ranged from 1,853 M J ha^{-1} to 3,643 M J ha^{-1}, which were below or similar to the

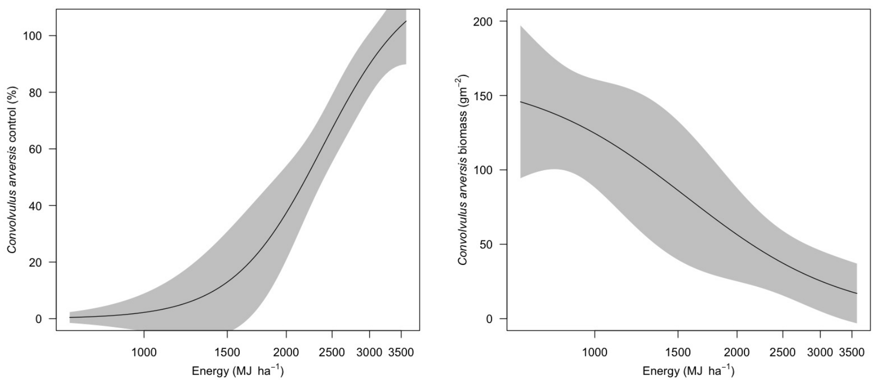

FIGURE 3
Convolvulus arvensis control (%) and above-ground dry biomass response to the energy level applied by manipulating the saturated steam boiler temperature. The shaded area indicates a 95% confidence interval. The saturated steam was applied at 7.4 m^3 ha^{-1}, and data were recorded 28 days after treatment.

TABLE 2 *Convolvulus arvensis* dry weights in response to increasing temperatures of saturated stem.

	Slope	Max	Effective energy (MJ ha^{-1})		
	b	D	50%	90%	99%
Control	–4.3 (1.8)	120.1 (23.8)	2,386 (269)	3,854 (1,146)	6,506 (3,351)
Biomass	2.5 (1.5)	163.4 (64.9)	1,563 (753)	3,643 (770)	9,171 (5,814)

Log-logistic regression parameters and standard errors are presented.
Observation error (±) is the standard error of the mean.
The effective temperature was calculated for reduction of variable by 50%, 90%, and 99%.

TABLE 3 Dry weight in response to saturated stem temperature.

Convolvulus arvensis	Slope	Max	Effective energy (MJ ha^{-1})		
	b	D (g m^2)	50%	90%	99%
Convolvulus arvensis	2.6 (1.5)	163.5 (64.9)	1,563 (753)	3,643 (± 770)	> 7,500
Lolium multiflorum (small)	1.1 (0.5)	66.5 (10.2)	2,429 (1,057)	15,640 (± 14,483)	NC
Lolium multiflorum (large)	1.6 (1.8)	125 (7.7)	> 20,670	NC	NC
Kichia elatine	2.3 (0.8)	53.5 (6.4)	827 (178)	2,152 (660)	6,107 (3,977)
Festuca rubra	1.1 (0.9)	20.6 (5.4)	1,769 (1,280)	> 5,908	NC
Amaranthus retroflexus	45.8 (1.2)	13.8 (2.9)	1,767 (212)	1,853 (494)	1,953 (1,324)

The log-logistic regression parameters and standard errors are presented.
NC, not calculated.
Observation error (±) is the standard error of the mean.
The effective energy was calculated for the reduction of variables by 50%, 90%, and 99%.

energy delivered when applying 7.4 m^3 ha^{-1} of SS. By contrast, the ED_{50} for the monocots *Festuca rubra* L. and *Lolium perenne* ssp. *multiflorum* (syn. *Lolium multiflorum* Lam.) ranged from 1,769 MJ ha^{-1} to 2,429 MJ ha^{-1}. The ED_{50} could not be calculated for *L. multiflorum* because the energy required to impact it was > 7,173 MJ ha^{-1}. Similarly, the ED_{90} and ED_{99} for the monocot species were beyond the tested rates, and thus not calculated. These results confirmed that operating the boiler at the maximum temperature more effectively controlled *C. arvensis* and applying SS at 7.4 m^3 ha^{-1} controlled most dicotyledonous plants for 14 days.

3.2 Basal application of saturated steam in blueberry

3.2.1 Blueberry tolerance

The basal applications of SS at 7.4 m^3 ha^{-1} and 14.8 m^3 ha^{-1} resulted in 10% to 15% injury to cultivar 'Duke' at 14 DAIT, while nearly 50% injury was observed with SS at 29.6 m^3 ha^{-1} (Figure 5). At all the tested rates, the injury due to SS was limited to the shoot's lower foliage, and SS injury to basal shoots increased after SS but later diminished regardless of the SS rate. The spray treatment of AN at 24.3 kg ai ha^{-1} caused 63% injury at 14 DAIT and reduced to 50% at 28 DAIT, but injury rates of 30% remained by the end of the study. The injury rate increased with AN rate and remained at 50% to 75% at 480 DAIT with the higher rates. The AN applications with the sponger wiper were as injurious to 1-year-old shoots as was an AN spray application. The 'Elliot' blueberry response to the treatments was comparable to 'Duke', with AN treatments causing marginally higher injury levels in 'Elliot'.

Cross-sectional area and length of non-treated blueberry averaged 70 mm^2 and 71.8 cm in 'Duke' and 72.5 mm^2 and 85.4 cm in 'Elliot' (Figure 6), respectively. The treatment with SS up to 29.3 m^3 ha^{-1} did not affect basal shoot cross-sectional area or length compared with non-treated based on Dunnett's test regardless of cultivar. AN sprayed at 97.2 kg ai ha^{-1} reduced the cross-sectional areas and lengths of basal shoots of 'Duke' plants by 57% and 62%, respectively, compared with non-treated plants. In the cultivar 'Elliot', AN reduced cross-sectional area and length by 45% and 52%, respectively. The detrimental effects of AN applied

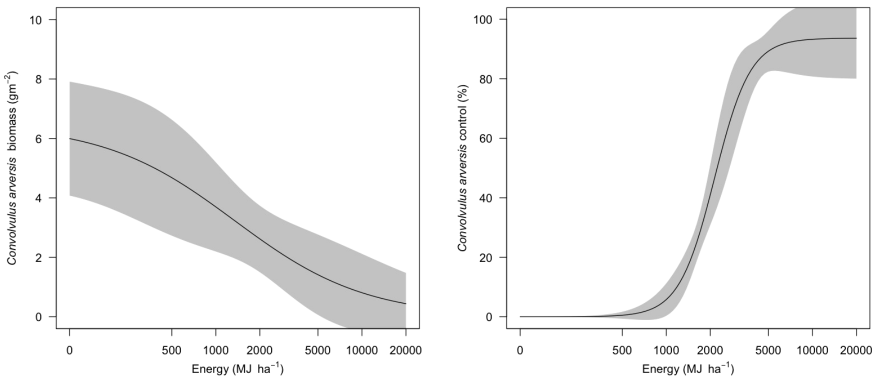

FIGURE 4
Convolvulus arvensis above-ground dry biomass and the control in response to increasing energy levels applied with saturated steam. The energy levels were applied by changing the amount of saturated steam applied per area at 121°C and at 6,205 kPa. The shaded area indicates a 95% confidence interval. The data were recorded 28 days after treatment.

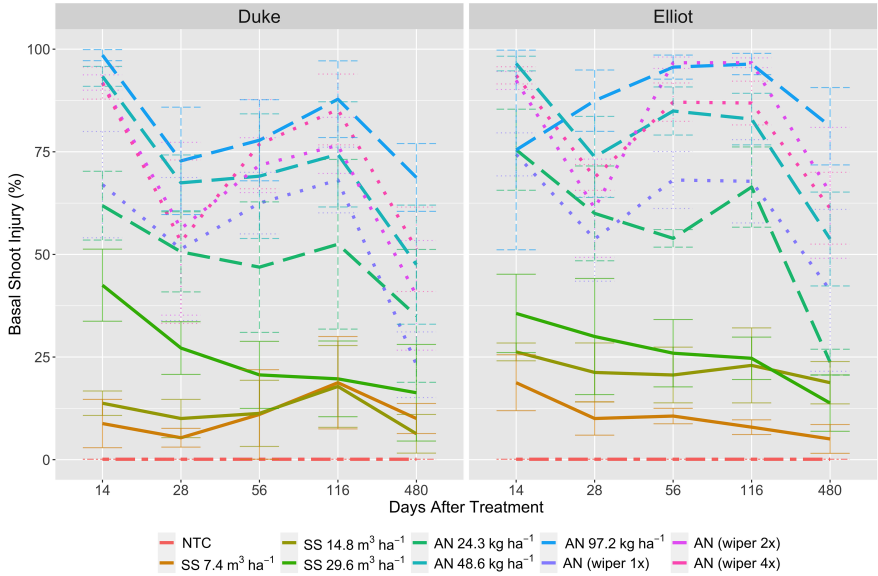

FIGURE 5
Highbush blueberry basal shoot injury response to increasing levels of saturated steam or ammonium nonanoate. The mature plants of cultivars 'Duke' and 'Elliot' were used in this study. The treatments were applied to the lower 50 cm of the blueberry bush. The treatments presented on the graph are non-treated (NTC) (short–long–dashed line), saturated steam (SS; solid line), and ammonium nonanoate, which was applied as a spray (long dashed line) or as a wiper treatment (dotted line). The treatments were applied in June 2019 and reapplied 42 days later. The study was repeated in 2020 with treatments applied 368 days and 409 days after the initial treatment. The means and standard errors are presented (*n* = 4).

with a sponge-wiper were noticed in the 'Elliot' two- and fourfold treatments.

3.2.2 Efficacy of *C. arvensis* control

Manual removal controlled 93% of *C. arvensis* at 7 DAT, and was no different than SS 77% (Table 4). Both treatments performed better than AN (45%–48%) or CC spray, or with the wiper (6%). *C. arvensis* regrew in all treatments; however, manual removal and SS provided better control of *C. arvensis* with 83% and 60% control at 28 DAT, respectively. *C. arvensis* dry weight was lowest with SS, with 6.4 g plant^{-1}, or 56% less biomass than the control (14.7 g plant^{-1}). CC reduced *C. arvensis* biomass compared with the control, whereas AN did not affect *C. arvensis* biomass. The AN spray caused 47% injury to young blueberry shoots at 7 DAT, which lessened to 20% at 28 DAT. However, AN applied by the wiper caused significantly less injury, with 11% and 3% injury observed at 7 DAT and 28 DAT, respectively. The SS caused 29% injury at 7 DAT, although shoots had recovered by 28 DAT (2%). *C. arvensis* regrowth, likely from root buds, occurred even when the entire above-ground part of *C. arvensis* was treated with SS, AN, or CC, or then it was manually removed.

3.3 Integrated weed control

Because a significant effect of assessment timing was observed, each assessment was analyzed separately. At 28 DAT, only the effect of factor A was present for weed control, coverage, and biomass, as treatment B was imposed subsequently (Table 5). There was a significant effect of factors A, B and their interactions for weed control in all experiments, and in experiments 1 and 3 for coverage was observed at 56 DAT. There were no interactions of factors in weed biomass. Data were analyzed by interacting factors A and B at 56 DAT for consistency.

The SS significantly affected weed control, coverage, and weed biomass in all experiments, but the effect varied among experiments and with evaluation timing. The primary weed species in experiment 1 were *Polygonum aviculare* L., *K. elatine*, and *Sonchus oleraceus* L. The average air temperature was 29°C, and no rainfall was recorded during the study. The SS treatment provided 48% weed control at 28 DAT in experiment 1, and was not different from the RB (61%) (Figure 7A). Both treatments provided better control than organic herbicides AN and CC, which provided 33% and 24% control, respectively. All treatments halved weed coverage compared with the control in experiment 1 (Figure 7B); the differences among the treatments were not significant. The SS, RB, and AN treatments reduced weed biomass significantly in experiment 1 (by 82%–93%), and CC reduced biomass by 45% compared with the control. Experiment 2 was initiated in the spring season; *Epilobium ciliatum* Raf., *S. oleraceus*, and *P. aviculare* were the dominant weed species. The average air temperature was 23°C and 68 mm of rainfall was recorded during the study. None of the treatments attained a high weed control in experiment 2, with SS controlling 15% of weeds at 28 DAT, while other treatments resulted in 6% to 9% control. The SS and CC provided the lowest coverage levels, while the RB and AN treatments did not differ from the non-treated control. Weed biomass reduction was evident in SS and the RB at 56 DAT in experiment 2, whereas AN and CC did not reduce biomass compared with non-treated. In experiment 3, the average air

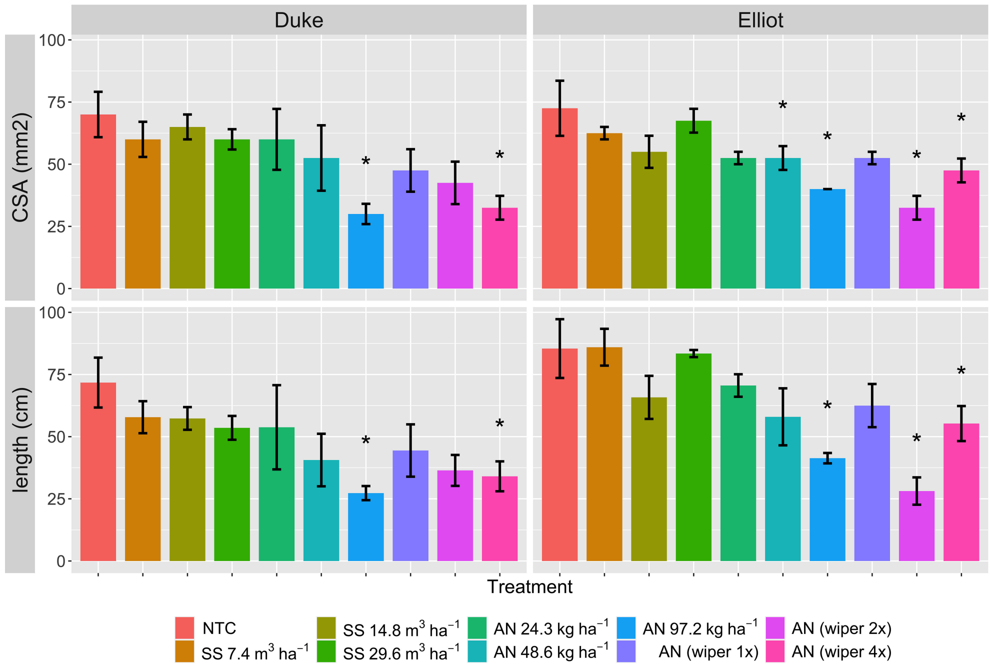

FIGURE 6
Highbush blueberry basal shoot cross-sectional area (CSA) (mm^2) and length (cm) in response to increasing levels of saturated steam (SS) or ammonium nonanoate (AN) and nontreated control (NTC). The mature plants of cultivars 'Duke' and 'Elliot' were used in this study. The SS was applied to the lower 50 cm of the blueberry bush at 7,407 L ha^{-1}, 14,814 L ha^{-1}, or 29,628 L ha^{-1}. The AN was applied as a spray or as a sponge wiper treatment. The spray treatments were delivered at 24.3 kg ai ha^{-1}, 48.6 kg ai ha^{-1}, or 97.2 kg ai ha^{-1}. The wiper treatments were applied at 13% vol/vol at one, two, or four passes for each application to achieve the targeted rate. The treatments were first applied in June 2019 and reapplied 6 weeks later. The study was repeated in 2020. The means and standard errors are presented (n = 4). An asterisk (*) indicates that the means are significantly different from the non-treated controls based on the Dunnett's test ($p \leq 0.05$) within a cultivar and measurement type.

temperature was 28°C for the first month, and 22°C for the second month of the study. A total of 82 mm of rainfall was recorded in the final 15 days of the study. The RB provided the greatest level of control (62%), followed by SS (44%). AN provided 40% control and was not different from SS or CC (29%). Weed coverage ranged from 13% to 18%, compared with 26% coverage in the control. The SS reduced weed biomass by 68% in experiment 3, and the RB, AN, and CC reduced it by 38%–44%, compared with the control.

Weed control efficacy provided by SS improved with retreatment. At 56 DAT, the SS treatments provided 81% to 93% control in experiment 1, the highest control regardless of treatment (Figure 8A). The RB provided control from 58% to 88%, with the highest control when following brush in factor A. Weed control with organic herbicides at factor B was dependent on factor A; lower control was observed in treatments that included CC in factor A. Weed coverage following treatment with SS (7%–19%) was lowest

TABLE 4 ***Convolvulus arvensis*** **control, biomass, and highbush blueberry response to saturated steam and organic herbicides applied as a spray or with a sponge wiper in a certified organic field near Independence, OR, USA in the summer of 2019.**

Treatment	Rate	Control		Biomass	Crop injury	
		7 DAT	28 DAT	28 DAT	7 DAT	28 DAT
		%		g $plant^{-1}$	%	
NTC	–	–	–	14.7 ab	0 c	0 b
MR	–	93 a	83 a	8.0 ab	0 c	0 b
SS	7.4 m^3	77 ab	60 ab	6.4 b	29 ab	2 b
CC spray	19 kg ai	6 c	27 b	10.4 ab	5 c	0 b
CC wiper	9%	6 c	26 b	8.0 ab	8 bc	0 b
AN spray	24.3 kg ai	45 b	28 b	19.1 a	47 a	20 a
AN wiper	13%	48 b	11 b	16.3 ab	11 bc	3 b

NTC, non-treated control; MR, manual removal; SS, saturated steam; CC, capric plus caprylic acid; AN, ammonium nonanoate; DAT, days after treatment.
The means followed by the same letter within a column were not significantly different based on Sidak's test (p > 0.05).

TABLE 5 Summary of the main effects and interactions for weed control, ground coverage, and weed biomass at three studies in commercial organic highbush blueberry fields near Independence, OR, USA, between 2018 and 2019.

Study		4 WAT			8 WAT		
		A	B	A*B	A	B	A*B
1	Weed Control	< 0.0001*	0.2830	0.5652	0.0005*	< 0.0001*	< 0.0001*
	Coverage	< 0.0001*	0.1416	0.6817	0.1611*	< 0.0001*	0.0126*
	Biomass	< 0.0001*	0.9392	0.5972	0.0005*	< 0.0001*	0.4763
2	Weed Control	< 0.0001*	0.8950	0.9592	0.02*	< 0.0001*	< 0.0001*
	Coverage	< 0.0001*	0.9097	0.9831	0.0016*	< 0.0001*	0.3939
	Biomass	< 0.0001*	0.7808	0.5115	0.0209*	< 0.0001*	0.4916
3	Weed Control	< 0.0001*	0.6579	0.5871	0.0272*	< 0.0001*	< 0.0001*
	Coverage	< 0.0001*	0.8924	0.1624	< 0.0001*	< 0.0001*	0.038*
	Biomass	< 0.0001*	0.9240	0.8107	0.023*	< 0.0001*	0.4986

Observation error (±) is the standard error of the mean.
The effective energy was calculated for the reduction of variables by 50%, 90%, and 99%. WAT, weeks after treatment.
A, factor A was the initial application; B, factor B was the second application made 4 weeks after the A application. * designates statistical significance.

in most cases (Figure 8B), and highest following treatment with CC. The weed biomass differed significantly among treatments, with the resultant biomass following SS in factor B ranging from 0 g m^{-2} to 8 g m^{-2}, following the RB ranging from 3 g m^{-2} to 18 g m^{-2}, and following CC ranging from 0 g m^{-2} to 102 g m^{-2} (Figure 8C). Weed control levels were lower in experiment 2 relative to experiment 1. The SS controlled 40% to 70% of the weeds in experiment 2, and the brush weeder controlled 21% to 48%. However, the RB in factor B resulted in lower weed coverage and biomass in experiment 2. The results in experiment 3 indicate that SS and the RB provided the greatest weed control, that is, 82%–95%, and 74%–94% control, respectively. Similarly, the weed biomass was lowest in factor B following SS and RB treatments.

The non-chemical treatments SS and the RB improved weed control by 22%, reduced weed cover by 15%, and biomass by 48.3 g m^{-2}, compared with AN and CC on 28 DAT (Table 6). Treatments including SS also performed better than treatments without SS for weed control (+12.3%), weed coverage (−7.9%), and weed biomass (−32.4 g m^{-2}). However, SS was not different from the RB at 28 DAT. Similar responses were observed at 56 DAT. There was no difference between treatments integrating SS and the RB compared with those only treated with SS or the RB.

3.4 Partial budget analysis

The costs for treating one-fifth of the field were calculated for all treatments; the RB (US$86.61) and SS (US$135 ha^{-1}) provided an economic advantage (Table 7). AN and CC were 2.0 to 3.5 times costlier than SS. The RB costs are likely overestimated in this study because we used the same operational capacity for SS and the RB in the calculations. However, the RB can operate at greater speeds than the SS, and operates in the field without large quantities of water, which must be transported. The RB was the least costly treatment in the study.

4 Discussion

This study evaluated how SS temperature and application volume affected weed control. SS efficacy against *C. arvensis* increased with boiler temperature (Figure 2). This was expected because SS energy density increases with temperature, reaching 0.512 MJ kg^{-1} at 121°C and 6,205 kPa (NIST, 2023). Furthermore, the magnitude of heat transfer is directly proportional to the difference in temperature, thus improving energy transfer from the SS to plant leaves. In this study, SS effects were only observed at temperatures of 65°C or greater, which is similar to the previously reported temperature (Hansson and Mattsson, 2003; de Cauwer et al., 2015). A non-linear relationship between the SS application energy (volume) and biomass reduction was confirmed, with the ED_{90} for dicotyledonous weeds at 3,643 MJ ha^{-1} (7.4 m^3 ha^{-1}) or lower, whereas the ED_{90} for monocotyledonous weeds was greater than 7,400 MJ ha^{-1} (Table 3). Although the 7.4 m^3 ha^{-1} volume of SS required to achieve adequate weed control is high, it is significantly lower than the volume required for hot foam ranging from 87 m^3 ha^{-1} to 133.3 m^3 ha^{-1} (Martelloni et al., 2019; Antonopoulos et al., 2023). The greater tolerance of monocots is attributed to protected growing points at or below the soil surface making these plants less fragile (Bauer et al., 2020), and their upright leaf architecture likely reduces heat transfer efficiency (de Cauwer et al., 2015). In *L. multiflorum*, the SS efficacy was reduced by 8.5-fold in 25-cm-tall plants, compared with 7-cm-tall plants, based on a ED_{50} of 2,429 MJ ha^{-1} (Table 3). The *L. multiflorum* ED_{50} is lower than the value reported for *L. perenne* (7,500 MJ ha^{-1}) with hot water at 98°C (de Cauwer et al., 2016). Previous studies reported that across species, smaller plants need three- to fivefold less energy than older ones (Hansson and Ascard, 2002; de Cauwer et al., 2015); a similar response is observed following flame weeding (Ascard, 1994; Ascard, 1995).

This study confirms that SS can be safely applied to blueberry plants. The SS did not affect the growth of young blueberry shoots

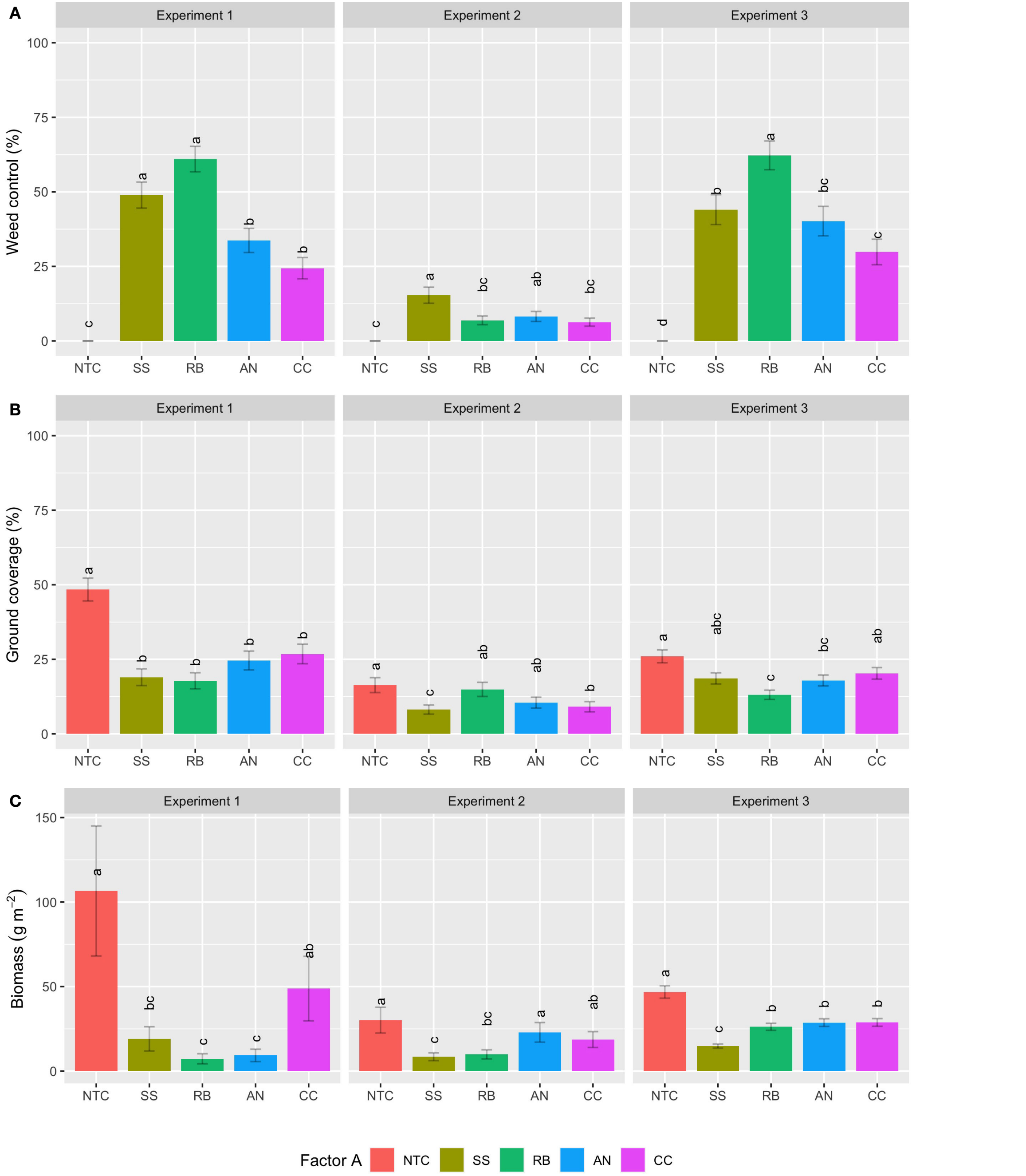

FIGURE 7
Weed control **(A)**, weed coverage **(B)**, and weed biomass **(C)** 4 weeks after treatment A application in three organic highbush blueberry field studies near Independence, OR, USA, in 2018 and 2019. The treatments included a non-treated control (NTC), saturated steam (SS) applied at 7,407 l ha^{-1}, a rotary brush weeder (RB), ammonium nonanoate (AN) applied at 24.3 kg ai ha^{-1}, and capric plus caprylic acid (CC) applied at 19.3 kg ai ha^{-1}. The bars labeled with different letters within each study and panel are significantly different based on Sidak's test ($p \leq 0.05$).

after multiple applications at rates of up to 29.3 m^3 ha^{-1}, with damage being restricted to shoot foliage (Figures 5 and 6). The greater tolerance of shoots to SS than foliage can be attributed to reduced heat transfer due to the vertical position of the shoots, their uneven bark surface, and xylem water flux dissipating heat (Chatziefstratiou et al., 2013). Conversely, damage was observed with AN applied as a spray or sponge wiper. AN is a non-selective foliar herbicide (Dayan and Duke, 2010; Webber et al., 2010), so directed spray treatment was expected to damage young shoots. The wiper was in contact with the young shoots to simulate an application targeting *C. arvensis* climbing in the plant. The AN damage with sponge application damage was probably due to young shoots not having bark; AN moved through the shoot's cuticle, much as if it moved through the leaf cuticle. The injury levels were much lower (i.e.,< 11%) when the treatments were to plants infested with *C. arvensis* (Table 4). Although no report is available in blueberry, non-selective

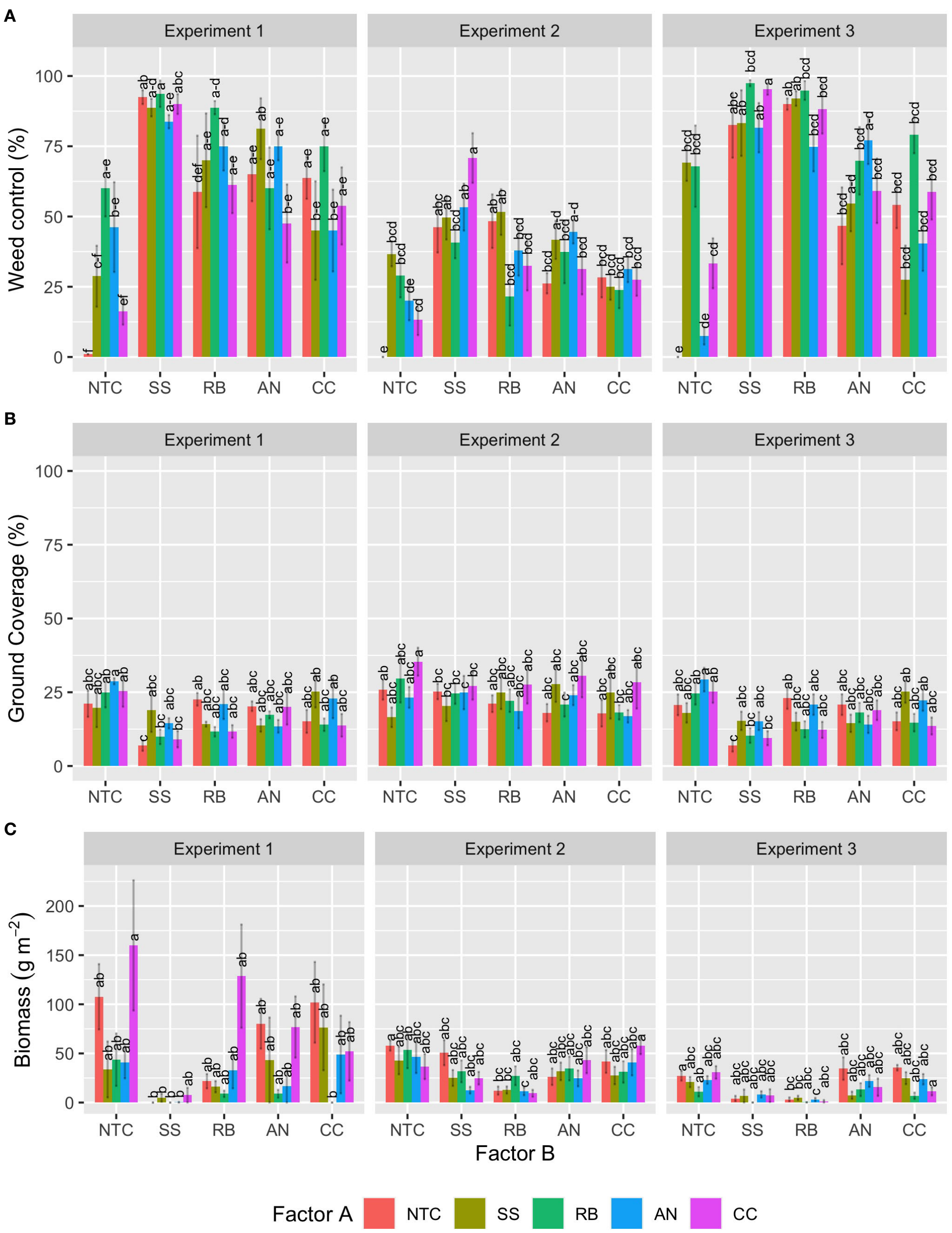

FIGURE 8
Weed control **(A)**, weed coverage **(B)**, and weed biomass **(C)** 8 weeks after treatment A application and 4 weeks after treatment B application in three organic highbush blueberry field studies near Independence, OR, USA, in 2018 and 2019. Factor B in the horizontal axis includes the non-treated control (NTC), saturated steam (SS) applied at 7,407 L ha^{-1}, a rotary brush weeder (RB), ammonium nonanoate (AN) applied at 24.3 kg ai ha^{-1}, and capric plus caprylic acid (CC) applied at 19.3 kg ai ha^{-1}. The bars labeled with different letters within each study and panel are significantly different based on Sidak's test ($p \leq 0.05$).

herbicides with sponge wipers have damaged other crops (Harrington and Ghanizadeh, 2017; Moyo et al., 2022).

The SS at 7.4 m^3 ha^{-1} consistently controlled weeds in organic blueberry fields, for up to 28 days. The optimum results were observed with the SS applied twice, as SS weed control was 50% or less 28 days after a single application (Figure 8), but increased up to 93% control after a second application (Figure 8). The SS improved weed control 12%–43% compared with treatments without SS (Table 6). SS efficacy was reduced in experiment 2 compared with experiments 1 and 3. This is likely a result of continuous weed emergence in response to rainfall observed during experiment 2 (spring), and not in experiments 1 and 3 (summer). The drought-stressed plants in experiments 1 and 3 may be more sensitive to SS. Previous studies documented that hot water better controlled

TABLE 6 Summary of selected contrasts to examine the effects of treatments for weed control, ground coverage, and weed biomass in a combined analysis for three studies in commercial organic highbush blueberry fields near Independence, OR, USA, between 2018 and 2019.

Contrasts	Weed control (%)				Cover (%)				Biomass (g m^{-2})			
	Diff.	(SE)	*t*-ratio	*p*-value	Diff.	(SE)	*t*-ratio	*p*-value	Diff.	(SE)	*t*-ratio	*p*-value
28 days												
No chem vs chem	22.1	(3.3)	6.68	*	−15.4	(3.8)	−4.01	*	−48.3	(9.4)	−5.11	*
SS vs no SS	12.3	(5.5)	2.243	*	−7.9	(6.6)	−1.20	NS	−32.5	(16)	−2.03	*
SS vs RB	−4.9	(2.2)	−2.186	*	3.7	(2.7)	1.34	NS	7.9	(6.6)	1.19	NS
56 days												
No chem vs chem	5.6	(1.6)	4.11	*	−5.18	(1.3)	−3.99	*	−88.9	(26.7)	−3.34	*
SS vs no SS	43.7	(15.3)	2.86	*	−38.1	(14.5)	−2.63	*	−572	(300)	−1.90	NS
SS vs RB	0.02	(0.6)	0.03	NS	0.0	(0.7)	−0.02	NS	0.04	13.4	0.00	NS
Integrated vs. Single	0.27	(0.9)	0.28	NS	0.24	0.95	0.25	NS	−3	(19)	−0.15	NS

The comparisons were made for data collected a 28 days and 56 days.
Diff, difference; NS, non-significant.
SE standard error (±) the mean.
T-ratio, significant effects ($p < 0.05$) are followed by *.
No chem vs chem, no chemical weed control (saturated steam and rotary brush weeder) versus organic herbicides (ammonium nonanoate and capric plus caprylic acid).
SS vs no SS, treatments including saturated steam (SS) versus treatments without SS.
SS vs RB, saturated steam versus a rotary brush weeder.

drought-stressed plants, while air temperature had little effect on hot water efficacy (Hansson and Mattsson, 2003). The SS treatment costs were significantly lower than organic herbicides (Table 6). It is important to consider that the costs assumed that one-fifth of the field was treated and does not include the cost of transporting water and the expected reduction in operational capacity resulting from refilling the water tank. SS consumes a significant amount of water (7.4 m^3 ha^{-1}), has a low speed of operation (≤ 1.6 km h^{-1}), and coupled with the short-lived weed control effect (< 28 days), would create a logistic challenge to ensuring prompt treatment of large areas. SS was a viable control only if used with the synthetic mulch, greatly reducing the size of the treated area. An important detail was that SS did not damage the weed mat (Moretti, personal observation). In our studies, we observed frequent adjustments and maintenance needed to maintain the operation of the steamer. Adjustments included regulating fuel pressure to control boiler temperature and steam flow, and maintenance done to the water tank, boiler, and thermostat, which were damaged primarily by vibration, dust, and moisture, respectively. The current steamer design is not adequate for commercial farm use under inclement weather conditions.

Weed control efficacy achieved by the RB was comparable to that of the SS in this study (Table 6), with the highest weed control in experiments 1 and 3 (up to 93%), and the lowest in experiment 2 (20%–50%). Similar to SS, the RB control was short-lived. The nylon brushes remove only the above-ground portion of the plants, so regrowth was observed shortly after treatment under moist conditions. The efficacy of the RB is improved with reduced operation speed; the nylon brushes provide greater rotation per unit area and deliver increased work intensity (Bond and Grundy, 2001). In this study, we operated the RB at 1.6 km h^{-1}, to be consistent with SS and to minimize damage to the synthetic mulch. Lower speeds immediately tore the synthetic mulch. At the speed adopted, damage to the synthetic mulch only occurred where preexisting damage was present. Proper equipment positioning and an experienced tractor operator would likely eliminate this damage. In dry conditions, the RB was the most effective and least costly option (US\$86.61 ha^{-1}). However, the RB produced significant dust. The dust can disperse pathogenic microorganisms to humans and onto the fruit (Kumar et al., 2018), and can create a significant source of contamination. The RB would not be compatible in a system without synthetic mulch because the nylon brushes would damage the shallow blueberry roots and scatter the organic mulches.

The organic herbicides AN and CC did not provide consistent weed control in this study (Figures 7, 8, Table 6), and were the most expensive treatments (Table 7). The erratic performance of AN and CC observed in this study is consistent with previous studies reporting poor performance in cool-season vegetables (Johnson and Davis, 2014; Johnson and Luo, 2018) and cover crops (Lewis et al., 2020). It is unlikely that the assessment interval (28 days) contributed to the poor performance of these herbicides. Previous studies have reported 70%–75% control with pelargonic acid, a similar fatty acid herbicides, when assessed 7–14 DAT (Travlos et al., 2020). Others reported excellent control with AN, with 91% control at 33 DAT (Parkash et al., 2022). The erratic performance of fatty acid herbicides has been documented previously (Johnson and Luo, 2018), and their performance is affected by the weed species in

TABLE 7 Partial budget for a weed control treatment in organic highbush blueberry production.

Equipment	SS	RB	AN	CC
Fixed costs				
Purchase price	US$15,000.00	US$10,000.00	–	–
Estimated annual use (h)	200.00	200.00	–	–
Annual ha	16.20	16.20	–	–
Ownership costs			–	–
Ownership length (years)	10	10	–	–
Capital recovery (5%)	US$7,500.00	US$5,000.00	–	–
Taxes, insurances (1.5%)	US$225.00	US$150.00	–	–
Ownership per hour	US$38.60	US$25.70	–	–
Variable costs			–	–
Repairs (15%)	US$2,250.00	US$1,500.00	–	–
Fuel cost (diesel)/hour	US$9.34	–	–	–
Fuel costs (gasoline)/hour	US$2.07	–	–	–
Labor (US$/H)	US$19.50	US$19.50	US$13.50	US$20.90
Pesticide sprayer (US$/ha)	0	0	US$76.60	US$76.60
Material cost (US$/L)			US$13.50	US$19.40
Material per ha (L 0.2 ha^{-1})			US$25.70	US$17.80
Cost of material per treated hectare			US$181.40	US$406.90
Cost per hour	US$42.17	US$27.00		
Operational capacity (ha h^{-1})	0.31	0.31		
Cost per banded acre (20%)	US$135.20	US$86.61	US$257.90	US$483.50

The treatments were calculated assuming a banded application over one-fifth of a hectare.
The treatments included saturated steam (SS), a rotary brush weeder (RB), ammonium nonanoate (AN), and capric plus caprylic acid (CC).

the sites, as the efficacy of fatty acid herbicides depends on weed species, density, and developmental stage (Loddo et al., 2023). The lower efficacy and higher costs suggest that organic herbicides are not cost-effective in organic highbush blueberries at this time.

5 Conclusion

This study is the first to evaluate the use of SS for weed control in organic highbush blueberries. When SS was applied at 121°C and at 7.4 m^3 ha^{-1} of steam, or the equivalent of 3,655 MJ ha^{-1}, it controlled and reduced dry weights of *C. arvensis* by 90%. SS can be safely used when applied to the base of highbush blueberry up to 29.3 m^3 ha^{-1}. Importantly, SS is compatible with systems using synthetic mulches. Despite SS efficacy in controlling both dicotyledonous and monocotyledonous weed species at 7.4 m^3 ha^{-1} practical challenges surfaced. Weed regrowth post treatment and the demand for copious amounts of water constrained the feasibility of employing SS in commercial blueberry production. Overcoming these challenges demands developing strategies to curtail water use and extend the duration of weed control. This is crucial for making SS a viable weed management option in commercial highbush blueberry.

Data availability statement

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

Author contributions

MM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RP: Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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

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Using integrated weed management systems to manage herbicide-resistant weeds in the Canadian Prairies

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Although herbicides have been a dominant and effective weed control strategy for decades in Western Canada, herbicide resistance and the lack of new modes of action have resulted in weed management challenges. Integrated weed management strategies have been shown to be successful in controlling certain weed species that are problematic in cropping systems. The objective of this study was to investigate integrated weed management strategies that have been successful on individual species to determine their applicability to a multiple weed species that may coexist in a field. In addition, harvest weed seed control was incorporated into these integrated weed management strategies to determine its impact in western Canadian cropping systems. A 5-year rotational study was conducted from 2016 to 2020 at Beaverlodge, Lacombe, and Lethbridge, AB; Scott and Saskatoon, SK; and Carman, MB, that incorporated integrated weed management strategies such as rotational crop diversity (including winter annuals and perennials), increased seeding rates, crop silaging, chaff collection, and with or without in-crop herbicides. This research confirmed success in managing some species of weeds such as wild oat when increased seeding rates, 2 years of early cut silage barley, and competitive winter cereals were incorporated into a cropping system, even when no in-crop herbicides were applied. However, some weed growth morphologies (e.g., twining weeds) or life cycles (e.g., facultative winter annuals) were not managed successfully with this combination of strategies. Chaff collection provided incremental weed control benefits but did not serve as a replacement for herbicidal weed control. Weed densities had an apparent impact on the success of these integrated weed management strategies, suggesting that the sooner they are adopted, the more likely they are to be successful at maintaining or reducing weed densities. This study not only showed the ability to reduce reliance on herbicides with strategies that can be effective in Western Canada but also highlighted the need for further understanding of different weed species and their responses to integrated weed management strategies, as well as the complexity of managing a weed community with integrated weed management.

KEYWORDS

integrated weed management, crop rotation, cultural control, harvest weed seed control, weed community dynamics

1 Introduction

Weeds cause substantial crop yield losses in western Canadian cropping systems. These losses reduce the net returns that farmers receive. Herbicide-resistant weeds can be difficult to control, resulting in increased costs due to reduced yields, ineffective management, and the need for additional control strategies. From 2014 to 2017, it was estimated that 9.6 million ha in a total area of 16.2 million ha in Western Canada was occupied by herbicide-resistant weeds (Beckie et al., 2020). The perceived cost of herbicide resistance for producers in that time frame averaged $33/ha or an annual cost of $530 million dollars (Beckie et al., 2020). Preliminary results of surveys conducted more recently indicate that the frequency and extent of infestation by herbicide-resistant weeds has increased, as has the annual cost to producers (C. Geddes, unpublished data).

Integrated weed management (IWM) strategies, or systems that combine chemical, physical, cultural, and/or biological control methods, can be effective at managing weeds, including those with herbicide resistance. However, weed management plans that integrate herbicide management tactics, including rotation of herbicide mode of action, tank mixing, and layering pre-seeding and post-emergence herbicides are often mistaken for IWM (Harker and O'Donovan, 2013). True IWM incorporates not only herbicide-based management but also cultural, physical, or biological control strategies. Successful IWM strategies incorporate life-cycle diversity, rotation design, competitive crop canopies, no or reduced tillage cropping systems, and maintenance of crop residues on the soil to help disrupt weed population dynamics (Anderson, 2005). In Western Canada, cultural practices including diverse crop rotations, utilizing competitive crop cultivars, incorporating silage production into the rotation, and increasing crop seeding rates have been shown to effectively suppress wild oat (*Avena fatua* L.) (O'Donovan et al., 1999; O'Donovan et al., 2000; Harker et al., 2003; Harker et al., 2009; Harker et al., 2016). Incorporating those strategies into a defined IWM system can result in synergistic improvements to weed management (O'Donovan et al., 2007; Blackshaw et al., 2008; Harker et al., 2009; Harker et al., 2016). A study combining many of those strategies with competitive winter annuals or perennials, rotations that included early cut silage crops and excluded wild oat herbicides for 3 years, still maintained similar wild oat density and biomass as a canola–wheat rotation that incorporated a full herbicide regime annually (Harker et al., 2016).

Although there are combinations of IWM strategies that can be effective, it is important to also look at the integration of novel cultural strategies into these IWM systems to increase their robustness and likelihood of long-term success. There has been a recent upsurge of interest in harvest weed seed control (HWSC) strategies and their potential to be incorporated into global cropping systems (Walsh et al., 2018; Shergill et al., 2020; Akhter et al., 2023). Although many of the previously mentioned strategies directly focus on limiting disturbance and increased crop competition with emerged weeds, HWSC focuses on preventing seed-bank input from weeds that have survived to crop maturity (Walsh et al., 2018). Incorporation of silaging strategies targets a different weed life-cycle stage (seed production) with a similar end goal of reducing the weed seed bank (Harker et al., 2003), but the marketable commodity from that rotational cycle is silage. If a producer is not operating a mixed operation (livestock and grain production), then they may have limited opportunity to market silage. Land trading between adjacent grain and mixed operations may provide a solution on a local scale; however, this is rare. Incorporation of HWSC technologies allows for prevention of weed seed-bank inputs while still allowing production of a marketable grain commodity. In addition, these strategies could both be incorporated into a rotation, in different rotational years/crops in the overall system. Studies on HWSC in Canada have demonstrated that efficacy was species-dependent based on seed retention levels (Burton et al., 2016; Burton et al., 2017; Beckie et al., 2018), but no studies have looked at benefits or risks of incorporating HWSC into a crop rotation or IWM program in Western Canada. Available HWSC methods include chaff collection, impact mills, narrow windrow burning, chaff lining or tramlining, and bale-direct systems (Walsh et al., 2018)

Many of the IWM cropping system studies in Western Canada have focused on management of wild oat (Harker et al., 2003; Harker et al., 2009; O'Donovan et al., 2013; Harker et al., 2016). A recent study found that rotations that included a perennial crop, or with two winter cereal crops in rotation or with incorporation of silage crops in a flax (*Linum usitatissimum* L.) rotation, were successful in managing wild oat and false cleavers (*Galium spurium* L.) with reduced herbicide applications (Benaragama et al., 2022). Although true IWM strategies can increase costs and complexity of managing weeds (Norsworthy et al., 2012; Ervin and Frisvold, 2016; Owen, 2016), these strategies are needed to provide growers options and strategies to reduce their reliance on herbicides, particularly in the face of continuously increasing herbicide-resistance pressures (Heap, 2023).

It must be recognized that weeds do not appear in isolation in producer fields but in weed communities where multiple species are coexisting simultaneously in a field. Therefore, the objective of this study was to investigate impacts of effective IWM strategies that have been studied for a single species for their efficacy levels on a weed community. The weed community included a common broadleaf and a common grass weed species across locations, in combination with other locally dominant weed species. In addition, a secondary objective was to evaluate the efficacy chaff collection as a HWSC strategy impact on weed community management in Western Canada. IWM strategy rotations were compared on weed and crop variables to the most common spring annual crop rotation on the Canadian Prairies: a repeated canola–wheat rotation at recommended seeding rates with a full herbicide regime (Beckie and Harker, 2017).

2 Materials and methods

The experiments were conducted at six locations across Western Canada from 2016 to 2020 [Beaverlodge, AB (55.2°N, 119°W); Lacombe, AB (52.5°N, 113.7°W); Lethbridge, AB (49.7°N, 112.8°W); Scott, SK (52.4°N, 108.8°W); Saskatoon, SK (52.5°N, 106.5°W); and Carman, MB (49.5°N, 98°W)]. In 2016, plot areas at all locations were treated pre-seeding with glyphosate (900 g ae ha^{-1}) and bromoxynil (290 g ai ha^{-1}) to manage early emerging weeds. After the pre-seeding applications, the plot areas were supplemented with 100 seeds m^{-2} of wild oat and 250 seeds m^{-2} of wild buckwheat (*Fallopia convolvulus* (L.) Á Löve) to ensure consistent grass and broadleaf species for comparison among the locations. In addition, location-specific weed species were also supplemented on the basis of local weed flora for development of a weed community (Table 1). All plots were direct seeded in long-term no-till or reduced tillage field areas.

Soil samples were collected at each location each year before seeding and analyzed for soil nutrients at commercial laboratories. Fertilizer additions and blends were made to achieve the recommendations resulting from the soil tests for macronutrients. Fertilizer was primarily side-banded with some seed-placed starter fertilizer; however, this practice did vary slightly on the basis of seeding equipment at each location. Seeding equipment was primarily knife opener drills or air seeders with 25.4 cm (Saskatoon and Scott) or 30.5 cm (Beaverlodge, Lethbridge, and Lacombe) row spacing. Carman seeding equipment was a disc opener seeder with 19-cm row spacing. Fungicides and insecticides were applied as needed according to local disease and pest insect infestations. Pre-seed herbicides after the establishment year were location-specific and based on weed species and density. Plot dimensions were 3 m × 10 m at Scott, 2.3 m × 8 m in Saskatoon, 4 m × 8 m at Carman, 4.3 m × 12 m at Lethbridge, and 3.7 m × 15 m in Lacombe and Beaverlodge. Additional location characteristics including soil organic matter, soil type, and soil pH are described in Table 1.

At each location, 14 treatments were arranged in a randomized complete block design with four replications (Table 2). Crop seeding rates were at 1× (typical recommended seeding rate), 1.5× or 2× (increased rates as an IWM strategy) as follows: spring cereals, 200 seeds m^{-2} (1×) and 400 seeds m^{-2} (2×); canola (*Brassica napus* L.), 100 seeds m^{-2} (1×) and 150 seeds m^{-2} (1.5×); field pea (*Pisum sativum* L.), 80 seeds m^{-2} (1×) and 120 seeds m^{-2} (1.5×); fababeans (*Vicia faba* L.), 40 seeds m^{-2} (1×) and 60 seeds m^{-2} (1.5×); winter cereals, 300 seeds m^{-2} (1×) and 600 seeds m^{-2} (2×); and alfalfa (*Medicago sativa* L.), 9 kg ha^{-1} (1×). Although doubling seeding rates in all crops would have been ideal from an IWM strategy perspective, the large seed size of fababean and pea can cause logistical challenges at high seeding rates, and the high seed cost of canola seed renders this an economically irrelevant strategy from a production perspective. Incorporation of 1.5× rates allowed us to continue to incorporate increased seeding rates as an IWM strategy while balancing logistic and practical considerations. In-crop herbicide product selections, where applications were required in the treatment regime, were location-specific based on the weed community present (Table 1). Early-cut barley (*Hordeum vulgare* L.) silage was cut 1 week after head emergence (Zadoks 65) (Zadoks et al., 1974) to leverage as much weed control out of this technique as possible (Harker et al., 2003). Each treatment was initiated (2016) with the same crop and IWM strategies across all treatments. The trial was initially seeded at a 2× seeding rate of spring wheat (*Triticum aestivum* L.) and no in-crop herbicide applications in 2016 to ensure establishment and naturalization of targeted weed species. The treatments then integrated different factors including crop species in rotation, crop life cycles (incorporation of winter annuals and perennials), herbicide regime (no in-crop or conventional practice which includes one to two in-crop herbicide applications per season), incorporation of silage harvest, and chaff collection (yes or no) (Table 2) over three growing seasons (2017 to 2019). The only exception to the no in-crop herbicide regime was the initial establishment year of alfalfa where a single in-crop herbicide treatment was allowed to assist in establishment, with no herbicides applied thereafter.

Chaff collection was chosen as the HWSC strategy in this trial. Ideally, a physical impact mill would have been incorporated as they are currently of most interest to Western Canada producers, but they are not currently available for plot sized combines. By collecting and removing the chaff from the plot area, we simulated the effect of an impact mill on the weed populations; although, residue removal is unique to chaff collection (Walsh et al., 2017). Chaff collection is a less common HWSC technique (Walsh et al., 2018) but still has the potential to be equally effective in terms of weed management, and it is far simpler to implement in plot scale research. Chaff collection equipment was designed to match the harvesting equipment available at each location. Examples of some of the chaff collection systems are shown in Figure 1.

The cumulative effects of the treatments were determined after the 3 years of differentiated treatments. In 2020, the final year of the study, all treatments were seeded to a 2× seeding rate of spring wheat, and no herbicides were applied. This strategy allowed

TABLE 1 Location characteristics where the rotational study was conducted between 2016 and 2020, including target weeds that were included in the study at each location, soil characteristics, and growing season precipitation for each year as a percent of the long-term average.

Location	Weeds[δ]	Soil organic matter	Soil pH	Soil texture	Growing season precipitation				
		%			2016	2017	2018	2019	2020
					% Long-term average*				
Beaverlodge	Volunteer canola Lambsquarters Cleavers[β]	8.8	7.9	Clay loam	145	120	125	104	74
Lethbridge	Kochia[β] Wild mustard Lambsquarters Redroot pigweed Roundleaf mallow	3.6	7.8	Sandy clay loam	98 (125)	60 (96)	60 (78)	72 (81)	91 (109)
Lacombe	Cleavers Hempnettle Lambsquarters Volunteer canola Henbit[β]	8.8	7.3	Clay	105	73	83	80	99
Saskatoon	Wild mustard Cleavers	4.4	7.3	Clay	86	51	62	72	94
Scott	Wild mustard Cleavers[β] Kochia Shepherd's Purse[β] Cleavers Lambsquarters[β] Volunteer Canola[β] Narrowleaf Hawksbeard[β]	2.7	6.2	Loam	89	90	71	103	117
Carman	Volunteer Canola Redroot pigweed Foxtail spp.	2.7	5.6	Sandy loam	116	58	67	91	53

*Long-term average, measured in mm, from the Canadian Climate Normals 1981–2010 from https://climate.weather.gc.ca/climate_normals/index_e.html.
[β]Weed was not supplemented or seeded, it was naturally present at the study location.
[δ]Volunteer canola, *Brassica napus* L.; Lambsquarters, *Chenopodium album* L.; cleavers, *Galium spurium* L.; kochia, *Bassia scoparia* (L.) A.J. Scott; wild mustard, *Sinapis arvensis* L.; redroot pigweed, *Amaranthus retroflexus* L.; roundleaf mallow, *Malva rotundifolia* L.; hempnettle, *Galeopsis tetrahit* L.; henbit, *Lamium amplexicaule* L.; Shepherd's purse, *Capsella bursa-pastoris* (L.) Medik.; narrow-leaved hawksbeard, *Crepis tectorum* L.; Foxtail spp., *Setaria viridis* (L.) P. Beauv and *Setaria pumila* (Poir.) Roem. & Schult.
Precipitation values in parentheses are the percent of the long-term.

examination of the weed communities with no confounding effects of crop types or severely reduced weed populations from in-crop herbicide applications. Treatment 2, a canola–wheat rotation with full in-crop herbicide application regime represents a common crop rotation sequence on the Canadian Prairies. As such, the other treatments were compared with this treatment as a "standard" of many farmers would be doing and the level of weed control that would be expected or desired by producers.

Weed densities were determined approximately 2–3 weeks after crop emergence and prior to in-crop herbicide application, each year in two 0.5-m^2 quadrats in each plot. Weeds were counted and identified to species, where possible. Prior to early-cut silage barley harvest, crop and weed biomass (separated by broadleaf and grass weeds) were determined from the same quadrats. For each of the study years, the location of the quadrat was shifted to ensure no confounding effects of biomass removal in the prior year. The biomass samples were dried at 60°C until moisture content stabilized for a dry weight measurement. For the silage barley, the plants were swathed, and the material was removed from the plot at the appropriate time. Grain plots were swathed or left standing until maturity and harvested with plot combines, at which time chaff was collected by treatment when indicated. Seed was cleaned and dockage recorded for each plot. For the initial year of alfalfa growth, it was cut only once; subsequently two to three cuts were harvested each year depending on location and the respective growing conditions, and total dry weight biomass was determined. Alfalfa was terminated at the end of the growing season in 2019 using a mixture of clopyralid (166 g ai ha^{-1}) and glyphosate (445 g ae ha^{-1}).

Weed seed-bank samples were collected in the fall of 2020, after grain harvest. A "W" pattern was utilized to take 12 soil samples per plot to a depth of 8 cm using a circular core sampler with a diameter of 10 cm. Subsamples were bulked into a single sample. Soil was dried at 30°C, sieved, and washed with a 250-µm screen. Large-seeded weeds like wild oat were removed and counted by hand. The remaining sample was mixed with approximately 5 L of potting soil (JiffyMix, Professional Gardener, Calgary, AB) with fertilizer (Harrell's ProFertilizer [14-14-14: N-P_2O_5-K_2O], Lakeland, FL), placed in trays of 55 cm × 28 cm × 1.3 cm, under light emitting diode (LED) germination lights (Monios-L T5 Grow Lights 120W,

TABLE 2 Treatment list for the 5-year rotational study.

Trt	2017				2018				2019			
	Crop	SR	Herbicide	HWSC	Crop	SR	Herbicide	HWSC	Crop	SR	Herbicide	HWSC
1	Alfalfa	1×	No	N/A	Alfalfa	1×	No	N/A	Alfalfa	1×	No	N/A
2	Canola	1×	Yes	No	Wheat	1×	Yes	No	Canola	1×	Yes	No
3	Canola	1×	Yes	Yes	Wheat	1×	Yes	Yes	Canola	1×	Yes	Yes
4	Canola	1×	No	Yes	Wheat	1×	No	No	Canola	1×	No	Yes
5	Fababean	1×	Yes	No	Barley	1×	Yes	No	Canola	1×	Yes	No
6	Fababean	1×	No	Yes	Barley	1×	No	Yes	Canola	1×	No	Yes
7	Fababean	1.5×	No	Yes	Barley	2×	No	Yes	Canola	1.5×	No	Yes
8	Pea	1×	Yes	No	Winter Wheat	1×	Yes	No	Canola	1×	Yes	No
9	Pea	1×	No	Yes	Winter Wheat	1×	No	Yes	Canola	1×	No	Yes
10	Pea	1.5×	No	Yes	Winter Wheat	2×	No	Yes	Canola	1.5×	No	Yes
11	Silage Barley	2×	No	N/A	Winter Triticale	2×	No	No	Silage Barley	2×	No	N/A
12	Silage Barley	2×	No	N/A	Winter Triticale	2×	No	Yes	Silage Barley	2×	No	N/A
13	Silage Barley	2×	Yes	N/A	Fall Rye	2×	Yes	Yes	Canola	1.5×	Yes	Yes
14	Silage Barley	2×	No	N/A	Fall Rye	2×	No	Yes	Canola	1.5×	No	Yes

[1]All treatments were seeded to wheat, seeding rate 2×, 0 herbicide, and no harvest weed seed control in 2016 and 2020.
Each year[1], the treatments are described by the crop grown, the seeding rate of the crop, whether or not herbicides were used, and whether or not harvest weed seed control was incorporated through use of chaff collection. The 3 years where the rotations are differentiated are presented here. SR, seeding rate; HWSC, harvest weed seed control. average when including supplemental irrigation. N/A = Not applicable.

Monios-L, online) with a 16-h/8-h light:dark period in a temperature-controlled room at ~25°C. Weed seedlings were identified, counted, and removed. After 3 weeks of growth, the tray was placed into a −18° C freezer for 3 weeks to promote breaking of dormancy through cold stratification. After 3-week cold treatment, samples were removed, mixed by hand, and placed back under the LED germination lights. Recruited seedlings were again identified and removed. Trays were exposed to a final cold treatment for a minimum of 6 weeks, and the growth was repeated once more. The data were converted to seeds m^{-2} for analysis (O'Donovan et al., 2013).

FIGURE 1
Chaff collection systems used in applicable treatments at **(A)** Beaverlodge, **(B)** Lacombe, and **(C)** Lethbridge. Systems were designed to fit the harvesting equipment available for the study at each location.

2.1 Treatment modifications

Treatment modifications because of logistical challenges became necessary at specific locations and years, as were pesticide applications for additional pests. Insecticidal controls were applied in Beaverlodge in 2017 and 2019 for flea beetles (*Phyllotreta cruciferae* Goeze and *Phyllotreta striolata* F.), in Lacombe in 2017 and 2019 for flea beetles, and in Lacombe and Lethbridge in 2017 for pea leaf weevil (*Sitona lineatus* L.). These applications generally allowed us to maintain treatments, but, in some cases, abiotic and biotic stresses required treatment changes. Decisions were made to maintain the integrity of the treatment as much as possible. In Lacombe in 2017, an infestation of pea leaf weevil resulted in a complete decimation of emerging alfalfa seedlings at approximately the second trifoliate stage. In order to not cause further disturbance and stimulation of the seed bank and based on the weather forecast, a 2× seeding rate of alfalfa (18 kg ha^{-1}) was broadcast on the plots prior to a day of rain. It was anticipated that, without incorporation, the alfalfa germination/emergence would be reduced compared with seeded alfalfa, hence the doubling of the seeding rate. This seeding rate also allowed for some additional predation of the alfalfa by remaining pea leaf weevils while allowing the alfalfa to establish. Drought issues resulted in a similarly poor alfalfa stand at Saskatoon and poor establishment for unknown reasons were also observed in Scott, so the same reseeding strategy was utilized. At Lethbridge, a late season drought in 2017 resulted in supplemental seed being broadcast (regular seeding rate) later in the summer as the first cut of alfalfa resulted in unusually high plant mortality. At Saskatoon in 2018, the winter wheat in treatments 8–10 and the winter triticale (× *Triticosecale* Wittmack) in treatments 11 and 12 suffered a high level of winter kill (Table 2). In treatments 8–10, spring barley grown for grain was substituted to simulate an earlier harvest than typical spring cereals. In treatments 11 and 12, the rotational sequence was switched to silage barley in 2018 and winter triticale being the primary crop in 2019. However, winter kill again compromised the treatment and spring barley for grain was grown in 2019 instead. In Scott in 2018, the winter wheat and winter triticale also showed high levels of winter kill; however, the densities met the industry-recommended plant stand to not reseed and were left to grow for the growing season. Fababean in Carman in 2017 was sprayed out and reseeded later than is typical (June 12) due to incorrect seeding rates at initial seeding. Weed densities in Carman were not assessed in 2020 due to COVID-19 restrictions, and so weed densities in 2020, including wild oat and wild buckwheat, were analyzed across the five other locations.

2.2 Statistical analysis

Data, including wild oat and wild buckwheat densities, grass weed biomass, broadleaf weed biomass, crop biomass, wheat yield, and dockage, were analyzed with the PROC GLIMMIX procedure of SAS, version 9.4 (Littell et al., 2006; SAS Institute, 2013). Treatment was considered a fixed effect, and location and replicate nested in location were considered random effects. Because the locations covered the broad geography of the Canadian Prairies and the desire was to make treatment inferences beyond the study location, it was appropriate to consider location effects and their interactions with the treatments as random (Yang, 2010). Experimental treatment effects were considered fixed. A log-normal distribution was utilized on the basis of Akaike's corrected information criterion (AICc) (Hurvich and Tsai, 1989) and fit of residuals. A comparison of means was conducted utilizing a Dunnett's test with the canola–wheat full herbicide regime (treatment 2) used as the control or comparison treatment and a p-value $\alpha = 0.05$. Data and standard errors were back-transformed for presentation in the original scale.

In consideration of the fact that weed community composition differed among locations,and to determine consistency of treatment efficacy, data were also analyzed for each variable within each location. The same log-normal distribution was utilized, treatments were fixed effects, and replication was a random effect. A comparison of means was conducted as above. A "site compliance" comparison was done by summarizing the number of mean comparisons from the by-location analysis that agreed or disagreed with the same comparison from the combined location analysis mean comparisons to provide descriptive information on the consistency of treatment differences among locations (Harker et al., 2016).

Analysis of wild oat and wild buckwheat densities in the seed bank followed the same procedure as above; however, a negative binomial distribution was utilized to improve the fit of the residuals. Other weed species' seed-bank densities were analyzed by location as at each location the weed community composition differed. Similarly, analysis of seedling weed densities aside from wild buckwheat and wild oat were conducted by location due to differences in weed community composition between locations. On a few occasions [wild mustard (*Sinapis arvensis* L.) and false cleavers (hereafter referred to as cleavers) in Saskatoon, cleavers in Scott], normal distributions were used for individual seedling densities, and, on one occasion (henbit (*Lamium amplexicaule* L.) in Lacombe), a lognormal distribution provided the best model fit, based on AICc and residuals. Similarly, seed-bank densities of most weed species best fit a negative binomial error distribution; however, a log-normal error distribution was used for hempnettle (*Galeopsis tetrahit* L.) in Lacombe, wild mustard in Lethbridge, cleavers in Saskatoon and Scott, and kochia [*Bassia scoparia* (L.) A.J. Scott.] in Scott, as well as a normal distribution for wild mustard in Saskatoon.

3 Results

3.1 Notable weather anomalies

Notable divergences in precipitation occurred at Beaverlodge in 2016 and 2020; Lethbridge, Lacombe, and Saskatoon in 2017–2019 (somewhat mitigated in Lethbridge through the use of supplemental irrigation); Scott in 2018; and Carman in 2017, 2018, and 2020 (Table 1). These deviations from the normal may have played a role in densities of various weed species. For example, at Lacombe and the surrounding area in 2016, it was noted as a year that seemed to encourage the growth of wild oat. At Lacombe and Beaverlodge in 2018, it snowed in the second week of September, and, at Lacombe, snow continued until early October. This delayed harvest and reduced the crop quality in those environments (data not shown).

3.2 Weed densities

Wild oat was the common grass weed across locations. Densities in the "standard" comparison treatment averaged 46 m^{-2}. In general, the treatments investigated resulted in either static wild oat densities in comparison with treatment 2 or increases where in-crop herbicides were removed. Wild oat densities in the final year of the rotational study were affected by treatment (p < 0.0001). Densities increased in the canola–wheat rotation with chaff collection at a 1× seeding rate (treatment 4), in the diversified spring annual rotation with chaff collection at a 1× seeding rate (treatment 6), in the diversified spring annual rotation with increased seeding rates (treatment 7), in the diversified life cycle (incorporating winter cereals) combined with chaff collection at a 1× seeding rate (treatment 9), and in the diversified life-cycle treatment describe previously but incorporating chaff collection (treatment 10) (Figure 2). All the listed treatments included no in-crop herbicides. However, at three out of the five locations, in treatments 9 and 10, which included no in-crop herbicide and winter wheat in rotation, densities were not greater than the standard comparison (treatment 2). Interestingly, treatments without in-crop herbicides that included winter triticale or fall rye (*Secale cereale* L.) in combination with silage barley (treatment 11: silage barley twice plus winter triticale at increased seeding rates; treatment 12: silage barley twice, winter triticale, chaff collection, and increased seeding rate; and treatment 14: silage barley, fall rye, and canola; chaff collection; increased seeding rate) had wild oat densities that were statistically similar to the "standard" treatment where a full in-crop herbicide regime was used. These winter cereals had higher winter survival than winter wheat across locations and were therefore more competitive. At three locations in the diversified spring annual treatment with chaff collection and increased seeding rate (treatment 7), densities were similar compared with that in treatment 2. The alfalfa treatment showed some variability with similar wild oat densities across locations, and, at three individual locations, lower wild oat density at one location and greater wild oat density at one location. Across locations, no treatment was successful in reducing wild oat densities below those observed in treatment 2.

Wild buckwheat was the common broadleaf weed across locations, and densities in the standard comparison treatment (treatment 2) averaged 6 m^{-2}. Wild buckwheat showed little response to the integrated weed management strategies chosen. Although wild buckwheat was affected by treatment (p = 0.0483), no treatments differed in wild buckwheat densities compared with that in treatment 2 (Figure 3). At one out of the five locations, the diversified life cycle rotation with chaff collection, no herbicides, and a baseline seeding rate (treatment 9); the diversified life cycle rotation as described previously but with an increased seeding rate (treatment 10); and the rotation incorporating silage barley, fall rye, and canola with an increased seeding rate, no in-crop herbicide, and chaff collection (treatment 14) all resulted in increases in wild buckwheat densities.

Weed species chosen as targets in many cases had similar densities among treatments (p > 0.05), or none of the treatments

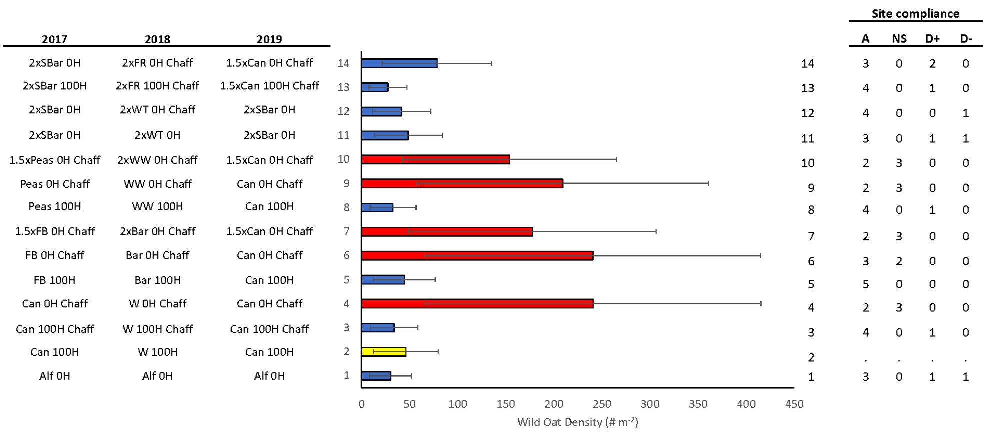

FIGURE 2
Wild oat density across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant increases in wild oat density are red, whereas significant reductions in densities are green, based on a Dunnett's comparison of means using an α = 0.05. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates that chaff was collected.

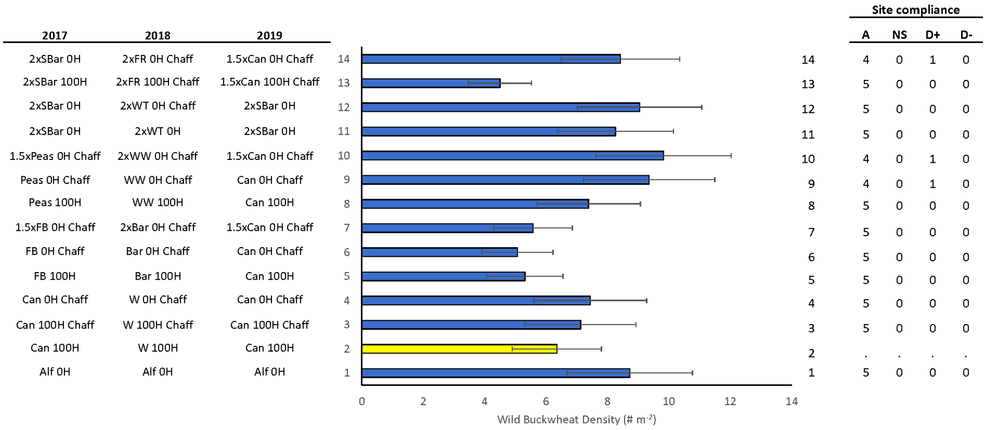

FIGURE 3
Wild buckwheat density across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Increased densities are highlighted in red, whereas decreased densities treatment are highlighted in green, based on a Dunnett's comparison of means using an α = 0.05. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates that chaff was collected.

differed in comparison with the standard treatment 2. Only those weed species that were affected by treatment and showed significant differences are presented here. Cleaver density at Beaverlodge was reduced in the diversified life-cycle rotation with a baseline seeding rate and full in-crop herbicides (treatment 8); in the rotation with silage barley twice, in combination with winter triticale, increased seeding rate, and no in-crop herbicide (treatment 11); in the rotation that included silage barley, fall rye, and canola at an increased seeding rate, with full in-crop herbicides and chaff collection (treatment 13) (Supplementary Figure 1A), at Lacombe in the alfalfa treatment (treatment 1); in the standard rotation with no in-crop herbicides but with chaff collection added (treatment 4); in the diversified spring annual rotation with no in-crop herbicides but with chaff collection added (treatment 6); in the diversified spring annual rotation as previously described but with an increased seeding rate (treatment 7); in the diversified life-cycle treatment with full in-crop herbicides (treatment 8); in the rotation with silage barley twice in addition to winter triticale, no in-crop herbicides, and increased seeding rates (treatment 11); in the silage barley and triticale rotation as previously described but including chaff collection (treatment 12); and in the silage barley, fall rye, and canola rotation with full in-crop herbicides, chaff collection, and increased seeding rate (treatment 13) (Supplementary Figure 1B). In Lacombe, cleavers densities increased in the diversified spring annual rotation with no in-crop herbicide, chaff collection, and a baseline seeding rate (treatment 6), and, in Saskatoon, densities increased in the diversified life-cycle rotation with no in-crop herbicides, chaff collection, and an increased seeding rate (treatment 10) (Supplementary Figure 1C) when compared with the standard treatment densities at the respective locations. Many of the treatments showing reduced cleaver densities include full in-crop herbicide regime, chaff collection, increased seeding rates, winter cereals, and silage barley in combination. Increased densities at Saskatoon were observed in treatments with no in-crop herbicide, increased seeding rates, and chaff collection. The perennial alfalfa treatment was variable in terms of efficacy on cleaver density with no difference to the standard at Beaverlodge (although numerically lower), significantly lower density compared with that in the standard at Lacombe and statistically similar although numerically higher density at Saskatoon. Volunteer canola was lower in treatments that did not include canola in rotation the year previous, as well as in some canola-containing rotations where chaff collection was included [treatment 6 (diversified spring annual, no in-crop herbicides, and chaff collection) at all three locations, treatment 9 (diversified life cycle, no in-crop herbicide, and chaff collection) at Lacombe and Scott, and numerous treatments at Scott] (Supplementary Figure 2). Kochia densities at Lethbridge were only reduced compared with that in the standard in the diversified life-cycle treatment with no in-crop herbicides, chaff collection, and an increased seeding rate (treatment 10), although no other treatments increased densities, even in the absence of herbicides (Supplementary Figure 3A). Wild mustard at Saskatoon was reduced in the perennial alfalfa (treatment 1), as well as the diversified spring annual treatment with no in-crop herbicide, chaff collection, and increased seeding rates (treatment 7), and the diversified life-cycle treatment with no in-crop herbicide, chaff collection, and increased seeding rates (treatment 10), whereas densities increased in the standard canola–wheat rotation with full in-crop herbicide but chaff collection added (treatment 3) (Supplementary Figure 3B). Shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.] densities at Scott were greater in the standard canola–wheat rotation with no in-crop herbicide and with chaff collection (treatment 4); in the diversified life cycle with no in-crop herbicide, chaff collection, and an increased seeding rate (treatment 10); and in the silage barley and triticale rotation with no in-crop herbicide but an increased seeding rate (treatment 11) (Supplementary Figure 3C).

3.3 Weed and crop biomass

Weed biomass was separated by grass and broadleaf weeds, and, in both categories, weed biomass was affected by the cropping system treatments. Grass weed biomass was influenced by the treatment ($p < 0.001$) and, in comparison with the canola–wheat standard treatment, increased in treatment 6, a diversified spring annual treatment with no in-crop herbicides, baseline seeding rates, and chaff collection (Figure 4). In two locations, the alfalfa treatments reduced grass weed biomass, as did treatment 8, a more diversified annual cropping rotation. There were single locations where the winter cereals and silage barley in rotation reduced grass weed biomass. Across locations, differences from the standard canola–wheat rotation were limited; most treatments did not differ in grass weed biomass.

Broadleaf weed biomass, while affected by treatment (p = 0.0203), showed no differences from the standard canola–wheat treatment when analyzed across locations (Figure 5). Within locations, however, biomass was reduced in treatment 4 (canola–wheat, baseline seeding rate, no herbicide, chaff collection) at three locations and reduced in treatment 6 (diversified spring annual, no in-crop herbicide, chaff collection), treatment 9 (diversified life cycle, no in-crop herbicide, chaff collection), and treatment 10 (same as previous but with increased seeding rate) at one location. At one location, biomass was also reduced in the perennial alfalfa treatment.

Wheat biomass in 2020 was affected by cropping system treatment ($p < 0.0001$). When compared with the standard treatment among locations, wheat biomass was reduced in treatment 4 (canola wheat, no in-crop herbicides, chaff collection), treatment 6 (diversified spring annual, no in-crop herbicide, chaff collection), treatment 7 (same as previous but with increased seeding rate), treatment 9 (diversified life cycle, no in-crop herbicide, chaff collection), and treatment 10 (same as previous but with increased seeding rate) (Figure 6). However, treatment 4 was similar to the standard treatment at 50% of the locations when analyzed separately, and treatment 6 was not different at 33% of the locations, treatment 7 at 50% of the locations, treatment 9 at 66% of the locations, and treatment 10 at 83% of the locations. At a single location, a greater crop biomass was observed in the perennial alfalfa treatment; in treatment 3, which was the standard system plus chaff collection; and in treatment 8, which was a diversified annual crop rotation with full herbicide applications.

3.4 Crop yield and quality

Wheat yield in the final year of the experiment was affected by treatment ($p < 0.0001$). When compared with the standard canola–wheat rotation, a final-year yield was reduced in treatment 4 (canola wheat, no in-crop herbicides, chaff collection), treatment 6 (diversified spring annual, no in-crop herbicide, chaff collection),

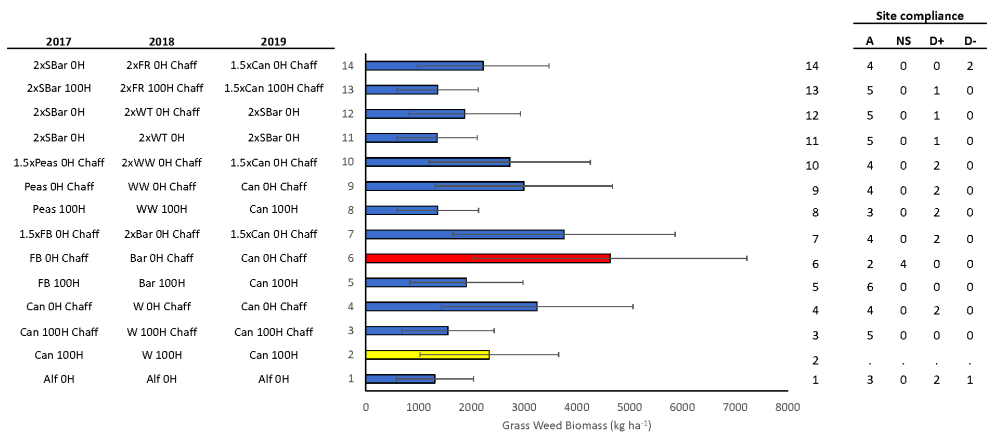

FIGURE 4
Grass weed biomass across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant increases in grass weed biomass are highlighted in red, whereas significant reductions in grass weed biomass are highlighted in green, based on a Dunnett's comparison of means using an $\alpha = 0.05$. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates that chaff was collected.

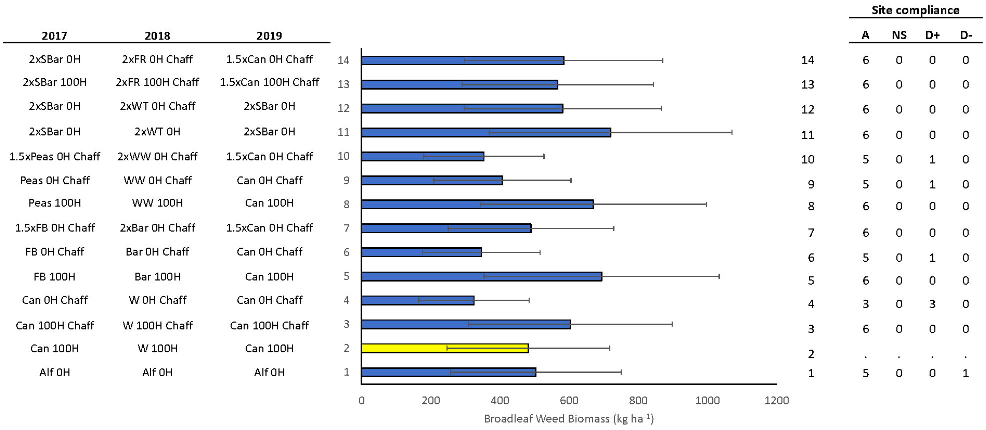

FIGURE 5
Broadleaf weed biomass across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant increases in broadleaf weed biomass are red, whereas significant reductions in biomass are green, based on a Dunnett's comparison of means using an α = 0.05. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates that chaff was collected.

treatment 7 (same as previous but with increased seeding rate), treatment 9 (diversified life cycle, no in-crop herbicide, chaff collection), and treatment 10 (same as previous but with increased seeding rate) (Figure 7), all of which did not receive an in-crop herbicide. Again, with each of these treatments, there were individual locations where there were no differences in wheat yield. Yield was greater at one location in the standard canola–wheat rotation where chaff collection was added (treatment 3). Treatments with no in-crop herbicide that included silage barley and a competitive winter cereal produced yields similar to the standard canola–wheat rotation.

Dockage was also affected by cropping system treatment ($p <$ 0.0001). In comparison with the standard canola–wheat rotation, dockage was reduced among locations in the perennial alfalfa

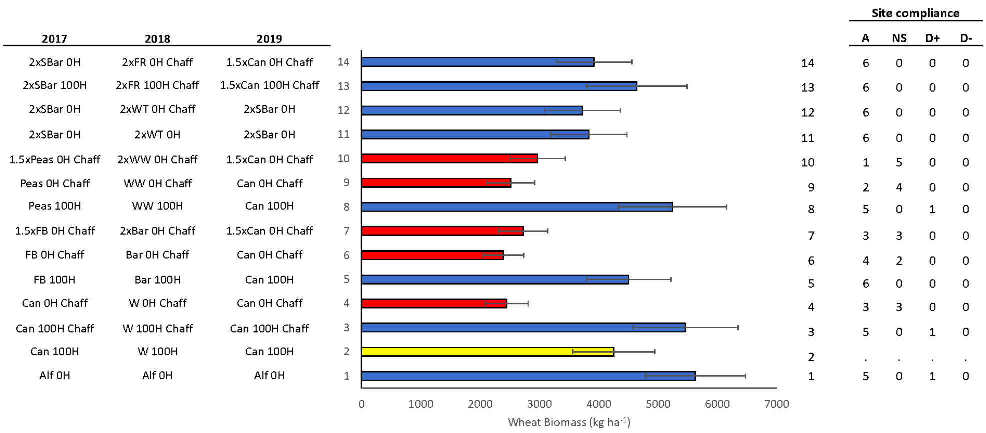

FIGURE 6
Wheat crop biomass across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant biomass reductions are in red, whereas significant biomass increases are in green, based on a Dunnett's comparison of means using an α = 0.05. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates chaff was collected.

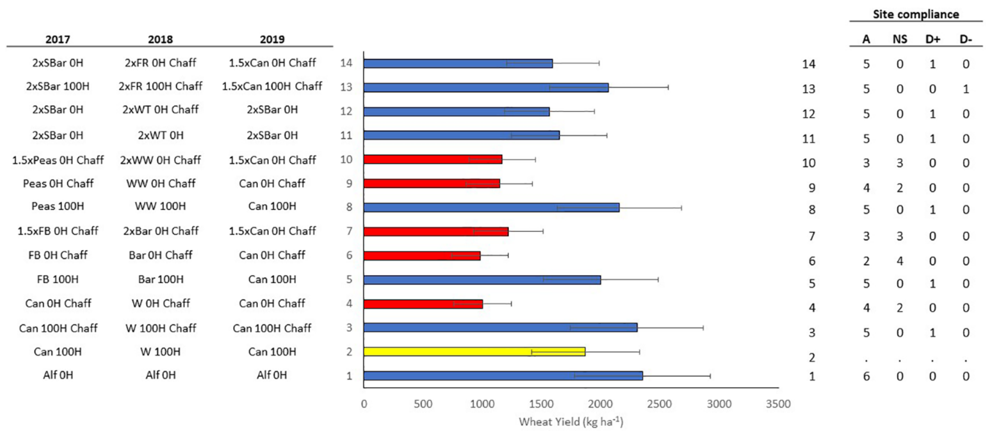

FIGURE 7
Wheat yield across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant yield reductions are in red, whereas significant yield increases are in green, based on a Dunnett's comparison of means using an $\alpha = 0.05$. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, it indicates that chaff was collected.

treatment and in treatment 3, which has the same treatments as the standard rotation but includes chaff collection (Figure 8). Density differences in these treatments were significant at half and one-third of locations, respectively, in the by-location analyses. Treatment 13, which included chaff collection in 2 years, also had lower dockage in 33% of locations, although the across-location comparison was not significant.

3.5 Weed seed-bank densities

Several IWM treatments reduced wild oat seed-bank densities compared with the standard canola–wheat rotation ($p < 0.0001$) (Figure 9). Across locations, reductions in the wild oat seed-bank density were observed in treatment 3 (chaff collection added to the standard treatment), treatment 5 (diversified spring annual rotation

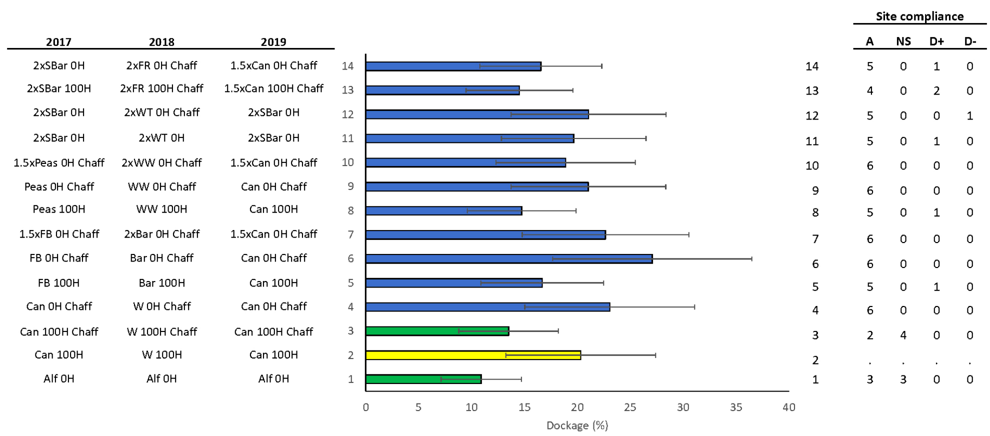

FIGURE 8
Dockage at wheat harvest across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant increases in dockage are red, whereas significant decreases in dockage are green, based on a Dunnett's comparison of means using an $\alpha = 0.05$. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, it indicates that chaff was collected.

with full herbicide regime), treatment 8 (addition of winter wheat to spring annual rotation with full herbicide regime), and treatments 11 and 12 (2 years of 2× silage barley plus 2× winter triticale both with and without chaff collection) (Figure 9). Density differences in these treatments were significant across locations, but not at the majority of individual locations when analyzed separately. Conversely, wild oat seed-bank densities were greater than in the standard in treatment 4 (canola–wheat standard with no in-crop herbicide but with chaff collection), treatments 6 and 7 (diversified spring annual rotation with no in-crop herbicide regardless of seeding rate), and treatments 9 and 10 (addition of winter wheat to a diversified annual crop rotation with no in-crop herbicide regardless of seeding rate) (Figure 9). The perennial alfalfa rotation and the silage barley, fall rye, and canola rotations, both with and without in-crop herbicide, had similar wild oat seed-bank densities to the standard treatment.

Wild buckwheat seed-bank densities were also affected by treatment (p = 0.0006) but differed from the standard canola–wheat rotation only in the perennial alfalfa treatment where wild buckwheat densities were higher (Figure 10). However, this was only the case at one-third of the locations when analyzed separately. Other locations showed no differences compared with that in the standard treatment.

At individual locations, weed seed-bank density was often similar among treatments or compared with the standard treatment. However, a few individual species and location combinations did have effects that warrant discussion. Lambsquarters (*Chenopodium album* L.) seed-bank densities at Beaverlodge were reduced in treatment 8 (winter wheat in a diversified annual system with full herbicide regime) and increased in treatments 12 and 14 (silage barley and winter triticale, and silage barley, fall rye, and canola at increased seeding rates with no in-crop herbicide and chaff collection where possible) compared with the standard (Supplementary Figure 4A). Redroot pigweed (*Amaranthus retroflexus* L.) seed-bank densities in Carman were reduced in treatment 1 (alfalfa), treatment 4 (no in-crop herbicide but chaff collection in canola–wheat), treatment 5 (diversified spring annual, full herbicide regime), treatment 6 (diversified spring annual, no herbicide regime), treatment 7 (diversified spring annual, increased seeding rate, no in-crop herbicide), treatment 9 (winter wheat with spring annuals, no in-crop herbicide, chaff collection), and treatment 10 (winter wheat rotation with increased seeding rates, no in-crop herbicide, chaff collection) (Supplementary Figure 4B). Green and yellow foxtail (*Setaria viridis* (L.) P. Beauv and *Setaria pumila* (Poir.) Roem. & Schult, respectively) seed-bank densities at Carman were combined due to challenges differentiating the species at the one-leaf stage and analyzed as foxtail species. Foxtail species densities were reduced in treatment 1 (alfalfa), treatment 4 (no in-crop herbicide but chaff collection in canola–wheat), treatment 5 (diversified spring annual, full herbicide regime), treatment 6 (diversified spring annual, no herbicide regime), treatment 7 (diversified spring annual, increased seeding rate no in-crop herbicide), treatment 9 (winter wheat with spring annuals, no in-crop herbicide, chaff collection), treatment 10 (winter wheat rotation with increased seeding rates, no in-crop herbicide, chaff collection), and treatment 11 (increased seeding rates of silage barley and winter triticale with no in-crop herbicide applications) (Supplementary Figure 4C) compared with the standard. Cleaver seed-bank density was affected by the treatments at both Lacombe (Supplementary Figure 5A) and Saskatoon (Supplementary Figure 5B). In Lacombe, densities were reduced compared with that in the standard in the alfalfa rotation,

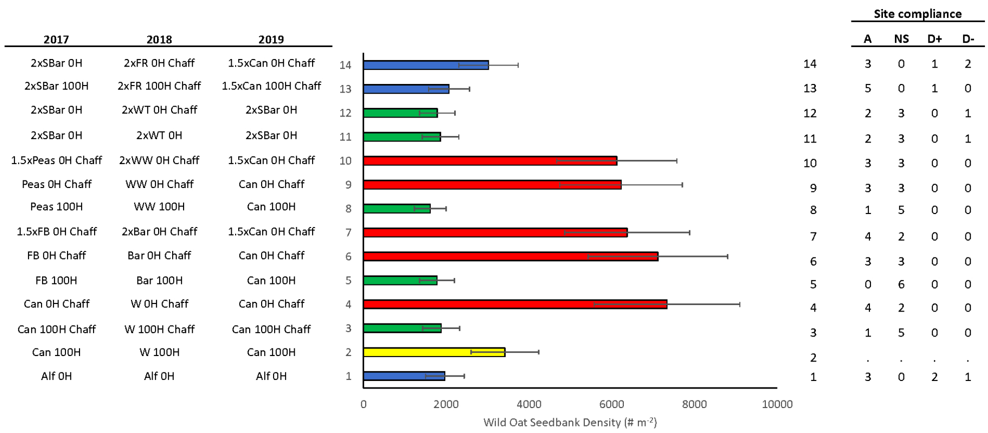

FIGURE 9
Wild oat seed-bank density across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant increases in the wild oat seed bank are red, whereas significant reductions in the wild oat seed bank are green, based on a Dunnett's comparison of means using an α = 0.05. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates that chaff was collected.

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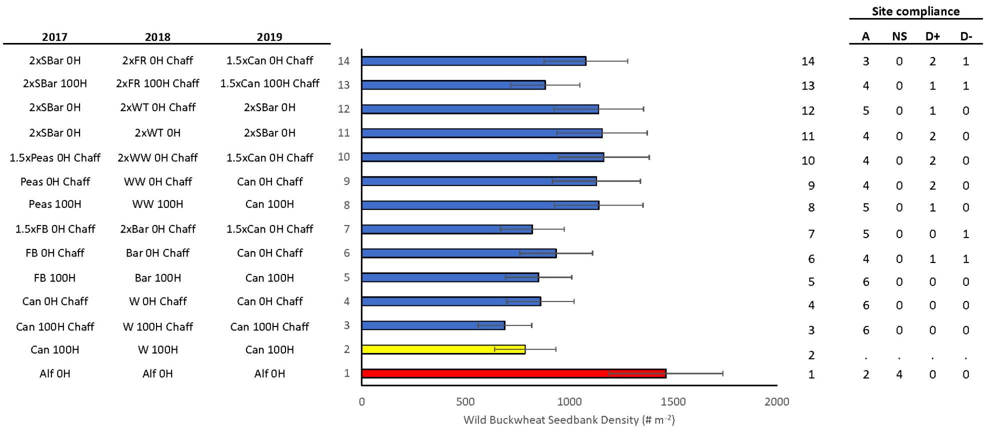

FIGURE 10
Wild buckwheat seed-bank density across locations by treatment in the final year of the rotational study. The yellow-highlighted treatment is the canola–wheat rotation with standard seeding rate, full herbicide, and no harvest weed seed control, to which all the other treatments are contrasted. Significant increases in wild buckwheat seed-bank densities are red, whereas significant decreases in seed-bank densities are green, based on a Dunnett's comparison of means using an $\alpha = 0.05$. Error bars indicate standard errors. Site compliance indicates the number of individual locations with Dunnett's comparison of means that are in agreement with across-location comparisons to the yellow-highlighted treatment: A, agreement; NS, not significant when across-location comparison is significant; D+, significant difference (same pattern) when across-location comparison is not significant; D–, significant difference (opposite pattern) when across-location comparison is not significant. Alf, alfalfa; Can, canola; W, wheat; FB, fababean; Bar, barley (grain); WW, winter wheat; SBar, silage barley; WT, winter triticale; FR, fall rye. If the numbers are present in front of the crop, then they indicate an increased seeding rate. 0H and 100H indicate no or full herbicide regimes, respectively, and if the word "chaff" is present, then it indicates chaff was collected.

in the diversified spring annual rotation with no herbicide but with chaff collection (treatment 6), in the winter wheat treatment with full herbicide application (treatment 8), and in the silage barley-fall rye-canola rotation with full in-crop herbicide regime (treatment 13). In Saskatoon, cleaver seed-bank densities were greater than the standard in the diversified spring annual rotation with no in-crop herbicide and chaff collection (treatment 6). Seed-bank densities were substantially higher in Lacombe than in Saskatoon. Finally, wild mustard seed-bank densities were over double the standard in Saskatoon in treatment 13, which is the silage barley-fall rye-canola rotation with full in-crop herbicide regime (Supplementary Figure 5C).

4 Discussion

4.1 Wild oat and grassy weeds

Management of wild oat densities was possible, even without in-crop herbicides, but it was generally less effective than in previous studies on the Canadian prairies (Harker et al., 2016). In previous studies, treatments of 2 years of increased seeding rate and early cut silage in rotation with a winter cereal or through the use of a spring annual crop with full in-crop herbicide application, regardless of which winter cereal was utilized, maintained relatively low wild oat densities (Harker et al., 2016). In the current study, however, wild oat densities were maintained at an acceptable level when a competitive winter cereal was utilized or when herbicides were used in-crop in all 3 years. Previous research also showed that diversifying rotations and utilizing increased crop seeding rates were beneficial for managing wild oat (O'Donovan et al., 2000; Harker et al., 2009). In this study, diversifying rotations to spring annuals or including a winter cereal was not enough to forego in-crop herbicides while maintaining or reducing wild oat seed densities. This result agrees with recent results from Benaragama et al. (2022), where crop diversification strategies in which spring annual crops were included in rotation did not improve management of wild oat.

Treatments were compared with the standard canola–wheat rotation, but not to all other treatments. Treatments 6 and 7 and treatments 9 and 10 differed between each other by an increased seeding rate. The higher seeding rate treatments had numerically fewer wild oat; however, all of these treatments still had greater densities than the standard treatment. Similarly, using Lacombe as an example, while wild oat densities were maintained compared with that in the standard rotation, densities around 200 wild oat m^{-2} (160 m^{-2} in the standard treatment) (data not shown) would not be considered a successfully managed population by most producers. Densities were lower in the Harker et al. (2016) study than in the current study; differences between the studies include the inclusion of a weed establishment year with no herbicides in the current study and the utilization of canola in the final year with a full herbicide regime. The herbicide application in canola in 2014 in the study by Harker et al. (2016), in combination with the competitiveness of the canola crop itself, likely restricted wild oat growth in the final year of their study. In addition, the establishment year allowed nearly full seed production for the wild oat in the current study, increasing the densities in comparison with that in the study by Harker et al. (2016). It is worth noting, however, the greater success of the IWM strategies at lower wild oat densities reported by Harker et al. (2016). Most farmers are likely to begin incorporating IWM strategies when they are out of other options;

this is typically when weed densities have already risen, in some cases, due to resistance or due to other failures in previous control strategies. Our study results and on-location observations suggests that the adoption of IWM strategies at that time may reduce the impact or reliability of those strategies in reducing the population; farmers are more likely to find success with these strategies if they start incorporating them when weed densities are lower. In the Canadian Prairies, a substantial rise in herbicide-resistant wild oat to the herbicide groups available for selective in-crop applications in many crops has been observed (Beckie et al., 2020). This study contrasts with canola–wheat rotations with full in-crop herbicides in regions where populations can be found where the in-crop herbicides would have limited to no efficacy (Beckie et al., 2020). This result may improve the perception and acceptability of some of the less effective IWM strategies.

It is known that winter cereals generally suppress wild oat better than spring cereals (Brown, 1953; Thurston, 1962; Beres et al., 2010); however, our results suggest the importance of successful crop establishment. Establishment has also been shown to impact competitiveness in spring cereal crops (O'Donovan et al., 2005). Poor stand establishment of winter wheat has been one of the impediments to the increased adoption of the crop in Western Canada (Beres et al., 2016) and, when poorly established, also allows weeds the opportunity to flourish. More winter hardy (and therefore more competitive) winter crops such as fall rye or winter triticale provide alternatives; however, their acreage and market demand are lower (Statistics Canada, 2023).

Grass weed biomass was composed predominantly of wild oat when averaged across locations, based on weed densities and researcher observations. Foxtail species made up a substantial proportion of grass weed biomass at the Manitoba location. Grass weed biomass was notably higher in this study compared with recent IWM studies on wild oat (Harker et al., 2016). Fewer treatments in the current study showed an increase or decrease in wild oat biomass compared with that in the standard canola–wheat rotation; however, biomass and variability in biomass was also larger, possibly impacting the number of significant comparisons. Grass weed biomass across locations increased when diversification of the spring annual crop rotation was utilized alone; however, the combination of increased seeding rates and diversification of rotation did help. It was possible to maintain grass weed biomass with 3 years of no herbicides, by utilizing winter cereals, early cut silage barley chaff collection, and increased seeding rates, similar to other recent studies (Harker et al., 2016). This provides opportunity to reduce reliance on in-crop grass herbicides, of which there are few options for rotating herbicide modes of action. This, in turn, reduces selection for herbicide resistance (Powles and Yu, 2010) to those in-crop herbicides as they are being utilized less. However, the level of observed control at some locations in these treatments that would allow elimination of in-crop herbicides may not have been considered sufficient by producers.

Several IWM strategies in the current study maintained the wild oat seed bank at similar levels to the standard canola–wheat rotation with full in-crop herbicides. A reduction in the seed bank was observed through chaff collection alone, which is unexpected as wild oat was identified as a poor target for HWSC due to low levels of seed retention at harvest (Tidemann et al., 2016; Tidemann et al., 2017). In the seed bank, however, in contrast to biomass and seed densities, we observed the benefits of rotation diversification when herbicides were applied, similar to that reported by O'Donovan et al. (2013) and Harker et al. (2009), although the latter reported the effects on weed seed production rather than the seed bank. We also observed a benefit of 2 years of early cut silage barley similar to what has been previously reported (Harker et al., 2003), particularly in combination with increased seeding rates of a competitive winter cereal (Harker et al., 2016). Interestingly, the wild oat seed-bank densities were only maintained at the same level as the standard in the treatments where there was 1 year of silage barley, compared with 2 years of silage barley (treatments 13 and 14 vs. treatments 11 and 12; Figure 9). This is consistent with Benaragama et al. (2022) where, in a flax-based rotation where a single year of silage barley was employed, there was no obvious benefit when compared with that in the spring annual grain crop. The alfalfa treatment also provided 3 years of no grass herbicides while maintaining the wild oat seed bank, even reducing the seed bank at two of the five locations. The wild oat seed-bank results are in agreement with those of Harker et al. (2016): diverse crop life cycles, and strategic employment of early-cut silage barley, increased seeding rates, and, in our case, incorporation of HWSC, can reduce wild oat growth and seed production enough to effectively manage wild oat seed banks. Seed dormancy and the persistent nature of wild oat seed banks mean that effects of this management may not be immediately apparent in seedling densities, even when the treatment effect is present (Harker et al., 2016; Selig et al., 2022). Long-term studies are needed to fully elucidate the long-term impact of IWM strategies.

4.2 Wild buckwheat

Wild buckwheat was selected as a common broadleaf weed due to its prevalence across the Canadian Prairies (Leeson, 2016; Leeson et al., 2017; Leeson et al., 2019). However, across locations, populations were low. With none of the treatments showing a density difference to the standard canola–wheat rotation, it becomes a question of whether all the treatments are equally effective or equally ineffective for wild buckwheat management. Blackshaw and Lindwall (1995) showed that, in a fallow system, tillage could be effective at managing wild buckwheat, but herbicides alone often did not, and that control was typically optimized in systems where tillage and herbicides were combined. This agrees with the idea of "many little hammers" being incorporated into an IWM system (Liebman and Gallandt, 1997). However, in Western Canada, there has been a substantial shift to no-till or minimum tillage production systems. Some of the strategies that we employed including rotational diversity, silage barley, increased seeding rates, and HWSC did not appear to be overly effective additions to wild buckwheat management. Previous research has shown benefits of increased seeding rates and narrowing row spacing on weed communities that included wild buckwheat in dry bean (Blackshaw et al., 2000), but the benefit of increased planting density was not apparent in the crops utilized in our study.

Burton et al. (2017) also identified wild buckwheat as a good target for HWSC, yet clear benefits of chaff collection were not apparent in the current study. Low population densities may have reduced our ability to measure differences between treatments. From the seed-bank perspective, wild buckwheat densities were increased in the perennial alfalfa treatment. This was unexpected as the repeated cuts of alfalfa were expected to reduce seed production in the species. Why this treatment showed increased seed-bank densities is unclear. As only two of the locations had higher wild buckwheat seed-bank densities and the four others had similar densities to the comparison treatment, perhaps the two locations had weaker alfalfa stands that did not compete or establish as well. This is an area warranting further investigation.

4.3 Broadleaf weeds

Few treatment effects were observed for broadleaf weed biomass. However, in some locations, broadleaf weed biomass was reduced in the treatment where no herbicides were applied, contrary to what would be expected (Figure 5). However, in those locations, grass weed biomass was often quite high. The authors expect that the extreme competition from the grass weeds, and particularly wild oat, resulted in decreased broadleaf weed biomass. Therefore, the reduction in broadleaf weed biomass was likely due to the ineffectiveness of the treatment at managing grass weeds, rather than the effectiveness of the treatment on broadleaf weed management. The broadleaf weed community was generally maintained compared with that in the standard canola–wheat treatment; however, high levels of variability raise questions on success of the treatments on different broadleaf weed growth habits. For example, there were substantial differences in the response of cleaver density at the three locations where the species was present (Supplementary Figure 1). Differences in the overall density of cleavers between locations at the start of treatment differentiation may have played a role; however, the species also seems less responsive to strategies such as increased seeding rates. We hypothesize that twining–growth-habit species like wild buckwheat and cleavers may not be as responsive to increased crop competition as other non-twining species like wild oat, due to their ability to climb into the crop canopy to acquire light. This is an area that requires further investigation. Benaragama et al. (2022) noted benefits from winter cereals when two consecutive winter crops were incorporated into a rotation, a cropping system rotation that was not included in the current study. However, this only occurred at locations with good winter crop establishment (Benaragama et al., 2022) as successful establishment and overwinter survival of winter-annual crops were required for competition with weeds, similar to the observations in this study. Perennial alfalfa has been shown to successfully manage cleavers (Benaragama et al., 2022); however, this benefit was not apparent in the current study for seedling densities although seed-bank densities were reduced in Lacombe.

Volunteer canola densities were greater in wheat crops preceded by canola in rotation. This highlights not only the importance of crop sequencing to allow for management of preceding crop volunteers but also the need for optimizing harvest settings and minimizing harvest losses of preceding crops. Canola harvest losses of up to 6,100 seeds m^{-2} have been recorded on commercial farms in the Prairies (Cavalieri et al., 2016); thus, harvest management can have a clear effect on the weed densities and the weed seed bank. Early cut silage was an effective addition to managing volunteer canola, as the silage process would occur before seed set and seed loss (Supplementary Figure 2) (Harker et al., 2003; Tidemann et al., 2017). It is likely that low canola densities in some of the treatments (i.e., treatments 4 and 6) are a result of the ineffectiveness of the treatment on wild oat management.

Kochia, while one of the predominant weed issues in the southern Prairies, particularly due to rapid emergence of herbicide resistance to multiple herbicide groups (Geddes et al., 2021a; Geddes et al., 2021b; Geddes et al., 2022; Sharpe et al., 2023) did not show obvious impacts of increased seeding rate, crop rotation, or early cut silage. In Lethbridge, only the rotation with increased seeding rates of peas, winter wheat and canola in rotation, with chaff collection reduced kochia densities (Supplementary Figure 3A), which may have been a result of their lack of efficacy on grass weed management, rather than efficacy on kochia. However, winter wheat in crop rotations or other IWM strategies including increased seeding rate, crop rotation, and narrow row spacing have been effective in other studies for kochia management (Geddes, unpublished data).

Wild mustard densities were reduced in the alfalfa treatments, as well as in treatments that tended to correspond with high grass weed biomass (Supplementary Figure 3B). The seed-bank densities of wild mustard were quite variable; however, treatment 13 showed an increase in seed-bank densities (Supplementary Figure 5C). This was intriguing as this treatment utilized full herbicide rates in comparison with treatment 14, which was the same treatment but without herbicides, where seed-bank densities were lower. Another western Canadian study has shown improvement in management of wild mustard with narrower row spacing and increased crop densities (Kirkland, 1993). Our study did not show as much responsiveness of wild mustard to seeding rate; however, the authors of the previous study measured wild mustard biomass specifically, whereas we focused on density. Wild mustard may warrant additional studies to determine effects of strategies such as winter cereals and early cut silage on management.

Shepherd's purse densities were quite variable and were greater in some of the winter cereal treatments in Scott (Supplementary Figure 3C), particularly those where no herbicides were utilized. Shepherd's purse as a facultative winter annual species is expected to be less affected by incorporation of winter cereals in the rotation for early competition. This species has not previously been the focus of many IWM studies as it is relatively easy to control with available herbicides. However, it is possible that facultative winter annual broadleaves such as shepherd's purse may require additional research to determine how they may be affected by recommended IWM strategies for other weeds. Their ability to emerge in the fall alongside the fall seeded crop may eliminate the competitive advantage of diversifying crop rotations with winter cereals.

Lambsquarters' seed-bank density also responded poorly to the winter cereal treatments where no herbicides were used

(Supplementary Figure 4A). Lambsquarters is not a facultative winter annual so the reason for the lack of control in these treatments is unclear and perhaps warrants additional study. The exception is the winter wheat treatment where herbicides were applied and densities were reduced. The overall impact of winter cereals in rotation, without herbicide application, needs to be dissected further. In contrast, redroot pigweed seed-bank density (Supplementary Figure 4B) was reduced by a number of treatments including perennial alfalfa and a number of other treatments where herbicides were not applied. The reductions in the treatments without herbicides are likely where grass weed competition became dominant.

Overall, it is clear that broadleaf weed species do not all respond the same to IWM strategies (Table 3). In particular, research should investigate further the impact of IWM strategies on twining–growth-habit weeds such as cleavers and wild buckwheat, and facultative winter annuals, as their biology gives them opportunity to avoid the impact of these competition-based strategies. In addition, it is important to identify those species that are not affected by strategies being recommended for a dominant problem weed such as wild oat (Table 3). It is not ideal to recommend a weed management strategy for one species that results in another becoming more abundant or problematic if those strategies allow that species to flourish.

4.4 Crop biomass, yield, and dockage

Crop biomass and yield consistently showed similar treatment effects. Treatments with no herbicides that did not include a competitive winter cereal and/or 2 years of silage barley resulted in decreased final-year wheat crop yields (Figures 6, 7). Although not different in the across location analysis, one location showed increases in crop biomass in the perennial alfalfa treatment and in the canola–wheat rotation where chaff collection was added and an increase in yield where the chaff collection was added. Similarly, reductions in dockage were observed at three locations for the alfalfa treatment and two locations for the canola wheat rotation with chaff collection (Figure 8). The benefit of alfalfa could result from weed control (Benaragama et al., 2022) or from nitrogen fixation; however, our weed control results were not as consistent as that in the work by Benaragama et al. (2022), meaning the benefit did not carry through to the wheat yield. It was interesting to see the benefit of chaff collection at one location and a numerically higher albeit statistically similar yield across locations. Although incorporation of HWSC into cropping systems has been shown to reduce weed populations (Walsh et al., 2018; Shergill et al., 2020; Akhter et al., 2023), its benefit in this study was unexpected, given that weed populations were dominated by wild oat that has been reported as a poor target for HWSC (Burton et al., 2016; Burton et al., 2017; Tidemann et al., 2017). This study also demonstrates that HWSC is not a replacement for herbicides but is intended and is most effective as an additional, incremental tool for weed management strategies (Walsh and Powles, 2014). Longer-term studies, where the seed banks are impacted over a longer period, particularly for weeds such as wild oat with a dormant seed bank, may show additional benefits of HWSC to weed densities and, as a result, to crop biomass and yield.

TABLE 3 A summary of effective and ineffective management strategies on the various weed species considered in the study.

Weed	Summary of effective management strategies	Summary of ineffective management strategies
Volunteer canola	- Reduced canola in rotation - Chaff collection - Early cut silage	
Lambsquarters	- Diversified life cycle with herbicides (SB)	- Barley silage (SB) - Winter cereals with no herbicides
Cleavers	- chaff collection (primarily Lacombe) - Silage barley twice with a competitive winter cereal - Full herbicide rates with diversified life cycles	- Increased seeding rates - Diversified life cycle without herbicides - Alfalfa efficacy highly variable by location
Kochia	- Diversified life cycle, no in-crop herbicide, chaff collection, increased seeding rate	
Wild mustard	- Alfalfa - Diversified rotation (spring annual and life cycle), increased seeding rate, chaff collection	- Chaff collection alone - Increased seeding rate alone
Redroot pigweed	- Alfalfa (SB) - Diversified rotations without herbicides	N/A
Roundleaf mallow	Treatment not significant	Treatment not significant
Hempnettle	Treatment not significant	Treatment not significant
Henbit	Treatment not significant	Treatment not significant
Shepherd's Purse	N/A	- No in-crop herbicide - Diversified life cycle - Winter cereals
Narrowleaf Hawksbeard	Treatment not significant	Treatment not significant
Foxtail species	Similar to wild oat	Similar to wild oat
Wild oat	- Competitive winter cereals with 2 years of silage barley - Alfalfa (SB) -Chaff collection with herbicides (SB) -Increased seeding rates in combination with other tactics -Diversified crop rotations and chaff collection (SB)	- Winter cereals with poor survival - Increased seeding rate alone - Diversified spring annuals
Wild buckwheat	N/A	- Alfalfa (SB) - Primarily no differences by treatment

This is generalized across the study locations. SB indicates effectiveness on seed-bank densities in particular. Treatment not significant indicates no significant treatment effect in the analysis. N/A = Not applicable.

4.5 Practical implications

This study concurs with previous research showing the potential to manage wild oat without herbicides for 3 years (Harker et al., 2016; Benaragama et al., 2022). However, it also highlights challenges associated with managing entire weed communities utilizing the same IWM tactics for each weed species and reducing or removing herbicide applications for 3 years. There were some tactics that can be beneficial for the majority of the weed community, whereas others may not be effective based on weed life cycles (facultative winter annuals) or growth habit (twining weeds) (Table 3). However, although tactics such as silage barley and winter cereals show efficacy in managing weed communities or species, it will be important to investigate the economics and marketability of these crops. Silage barley can effectively reduce wild oat (Harker et al., 2003; Harker et al., 2016), but, if a farmer does not have livestock or neighbors in need of livestock feed, then their product does not have a market. This highlights the need for continued investigation into IWM strategies such as HWSC that can impact weed management without changing the product or marketability of the producer's rotation. However, as shown in this study, HWSC is not an effective replacement for herbicides or as a stand-alone weed management strategy. Developing IWM strategies that provide the desired level of weed management and economic and environmental sustainability and that can be practically incorporated into farming operations is an on-going challenge, made even more difficult by regionality and differences in farm values, equipment, and specific problem pests. In addition, higher weed densities can limit the ability of IWM strategies to successfully manage weeds, emphasizing that success will be achieved most easily by early adoption of the strategies. This contrasts with the typical contemporary approach where new tactics are adopted only when current strategies are no longer effective. This study not only shows the ability to reduce reliance on herbicides with strategies that can be effective in Western Canada but also highlights the need for further understanding of our different weed species and their responses to IWM strategies, as well as the complexity of managing weed communities with IWM.

Data availability statement

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

Author contributions

BT: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. KH: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. SS: Conceptualization, Investigation, Project administration, Supervision, Validation, Writing – review & editing. CW: Conceptualization, Project administration, Supervision, Writing – review & editing. EJ: Conceptualization, Investigation, Writing – review & editing. RG: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. NL: Conceptualization, Investigation, Writing – review & editing. TT: Conceptualization, Investigation, Writing – review & editing. ES: Conceptualization, Investigation, Writing – review & editing. RB: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. CG: Conceptualization, Formal Analysis, Investigation, Project administration, Supervision, Writing – review & editing. HK: Formal Analysis, Investigation, Project administration, Supervision, Writing – review & editing. GS: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. AM: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. CG: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. LM: Data curation, Investigation, Methodology, Writing – review & editing. PR: Data curation, Investigation, Methodology, Writing – review & editing. ES: Data curation, Investigation, Methodology, Writing – review & editing. JZ: Data curation, Investigation, Methodology, Writing – review & editing.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2023.1304741/full#supplementary-material

SUPPLEMENTARY FIGURE 1

Cleavers seedling density at **(A)** Beaverlodge; **(B)** Lacombe and **(C)** Saskatoon in the final year of the rotational study. The yellow highlighted treatment is the standard seeding rate, full herbicide, no harvest weed seed control canola-wheat rotation to which all the other treatments are contrasted. Significant increases in density are red, while significant decreases in density are green, based on a Dunnett's comparison of means using an α=0.05. Error bars indicate standard errors.

SUPPLEMENTARY FIGURE 2

Canola seedling density at **(A)** Beaverlodge; **(B)** Lacombe and **(C)** Scott in the final year of the rotational study. The yellow highlighted treatment is the standard seeding rate, full herbicide, no harvest weed seed control canola-wheat rotation to which all the other treatments are contrasted. Significant increases in density are red, while significant decreases in density are green, based on a Dunnett's comparison of means using an α=0.05. Error bars indicate standard errors.

SUPPLEMENTARY FIGURE 3

Seedling densities for **(A)** Lethbridge kochia; **(B)** Saskatoon wild mustard and **(C)** Scott shepherd's purse in the final year of the rotational study. The yellow highlighted treatment is the standard seeding rate, full herbicide, no harvest weed seed control canola-wheat rotation to which all the other treatments are contrasted. Significant increases in density are red, while significant decreases in density are green, based on a Dunnett's comparison of means using an α=0.05. Error bars indicate standard errors.

SUPPLEMENTARY FIGURE 4

Seedbank densities for **(A)** Beaverlodge lambsquarters; **(B)** Carman redroot pigweed and **(C)** Carman foxtail species in the final year of the rotational study. The yellow highlighted treatment is the standard seeding rate, full herbicide, no harvest weed seed control canola-wheat rotation to which all the other treatments are contrasted. Significant increases in seedbank density are red, while significant decreases in seedbank density are green, based on a Dunnett's comparison of means using an α=0.05. Error bars indicate standard errors.

SUPPLEMENTARY FIGURE 5

Seedbank densities for **(A)** Lacombe cleavers **(B)** Saskatoon cleavers and **(C)** Saskatoon wild mustard in the final year of the rotational study. The yellow highlighted treatment is the standard seeding rate, full herbicide, no harvest weed seed control canola-wheat rotation to which all the other treatments are contrasted. Significant increases in seedbank density are red, while significant decreases in seedbank density are green, based on a Dunnett's comparison of means using an α=0.05. Error bars indicate standard errors.

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Toxicological risk assessment of mechanical-chemical vs. chemical weed control techniques in sugar beet in Germany using SYNOPS-GIS

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Introduction: The EU Farm to Fork strategy aims to reduce the use of pesticides and associated toxicological risks. However, the risks coming along with currently available alternatives to chemical weed control in sugar beet have not yet been evaluated. Therefore, the aim of this study was to determine the toxicological risks to arthropods, aquatic and soil organisms caused by mechanical-chemical in comparison to conventional chemical weed control in sugar beet.

Materials and methods: The risk assessment was performed using SYNOPS-GIS, a process-based model calculating the environmental fate of pesticides and the exposure risk to arthropods, aquatic and soil organisms.

Results and discussion: Overall, broadcast spraying of conventional herbicides caused low to very low toxicological risks in most regions and years in Germany. Nevertheless, there were considerably higher risks to aquatic and soil organisms from conventional broadcast spraying in northern Germany than in other regions of Germany. With conventional herbicides, mechanical-chemical weed control reduced toxicological risks proportionally to the reduction in application amount. In contrast, band spraying of the new herbicide with the active ingredients foramsulfuron and thiencarbazone-methyl caused an aquatic risk as broadcast spraying with conventional herbicides, although the application rate was 120 times lower. This was due to high toxicity of both active ingredients of the new herbicide to water plants.

Conclusions: Not only the application amount of herbicides but also environmental toxicity should be included in assessment approaches such as the EU "Harmonized Risk Indicator".

KEYWORDS

toxicological risk, weed control, sugar beet, SYNOPS, exposure toxicity ratio, CONVISO

1 Introduction

Weed control in sugar beet is currently carried out on over 90% of all sugar beet fields in Germany by broadcast herbicide spraying (Roß et al., 2018). However, the use of herbicides is criticized due to their adverse effects to human health and the environment (Geiger et al., 2010; Torretta et al., 2018). According to EU's Farm to Fork strategy, the use of pesticides and the associated toxicological risk must be reduced by 50% by 2030 (European Commission, 2020). To achieve this goal, the European Commission wants to promote alternative weed control techniques like mechanical and mechanical-chemical weeding. In sugar beet, solely mechanical weed control is typically done with tractor hoes between the rows and with hand hoes within the rows. However, hand hoeing is very time consuming and expensive (Fishkis et al., 2024) and is only worthwhile for organic farmers because the selling price of organic sugar beet is three times higher than that of conventional sugar beet (Bundesanstalt für Landwirtschaft und Ernährung (BLE), 2023). For conventional farmers, the hand hoe is therefore not affordable. Solar-driven FarmDroid hoe robots, capable of in-row and interrow hoeing are since recently commercially available and provide a cheaper and efficient alternative to conventional mechanical weed control; however, since weeds in close vicinity of sugar beet must be removed by hand hoeing, this technique is still too expensive for conventional farmers (Kopfinger and Vinzent, 2021; Starck et al., 2021). Combined mechanical-chemical weed control with a tractor hoe between rows and band application of herbicides within rows is financially viable for conventional farmers (Schröllkamp et al., 2015) but has hardly been used in practice in Germany (in 1% of fields in 2020 and 2021, n = 637; Farm survey on sugar beet cultivation, data not published). The reason for this is a low area performance of both tractor hoe and conventional band sprayer (maximal working width of 6 m) compared to the broadcast sprayer (30 m working width). Recently, however, broadcast sprayers capable of in-row herbicide band spraying were launched on the market, making in-row weed control better practicable in future. Additional weed control between rows with a tractor hoe is still required, but the additional labor and machinery costs are more than offset by the lower cost of herbicides, so that the total costs of modern combined mechanical-chemical weed control is about 25% lower than that of chemical weed control (Fishkis et al., 2024). The reduced use of herbicides by combined mechanical-chemical methods suggests a lower toxicological risk of herbicide exposure. However, a quantitative assessment of the toxicological risk associated with different mechanical-chemical weed control methods has not yet been conducted.

Several risk indicators of pesticides use exist at the European level (Reus et al., 2002; Bockstaller et al., 2009). An overview of these indicators, which differ in their objectives, temporal and spatial scales, and evaluation methods, is provided by a survey conducted as part of the OECD "Expert Group on Pesticide Risk Indicators" (OECD, 2016; Pierlot et al., 2017). SYNOPS is a pesticide risk indicator developed in Germany to assess the terrestrial and aquatic environmental risks of pesticide use. It has been used since 2005 as a part of the "National Action Plan for the Sustainable Use of Pesticides (NAP)" for annual reporting on the development of the risk associated with the use of pesticides in Germany (NAP, 2017). The SYNOPS model calculates predicted environmental concentrations (PEC) of each active ingredient (AI) of agrochemicals in soil, surface water and field margin, based on weather, soil, topography, and agronomic data and relates them to toxicity endpoints of various non-target organisms, which are summarized in the pesticide property database (Lewis et al., 2016). The resulting Exposure Toxicity Ratio (ETR) is used as toxicity risk indicator (Gutsche and Strassemeyer, 2007). Environmental risk assessment using the SYNOPS model has been repeatedly applied for pesticide use data of cereals, rapeseed, and sugar beet (Gutsche et al., 2012; Strassemeyer and Golla, 2018). The regional approach of SYNOPS-GIS is to apply the field specific assessments of SYNOPS on all fields in a considered region and aggregate them on regional level. Nause et al. (2021) assessed the risks of broadcast applications of different herbicides using SYNOPS-GIS based on herbicide application data from 2314 randomly selected sugar beet fields. They showed that some combinations of active ingredients, application dates and field-specific environmental conditions provoked higher risks, although in most cases the risks were below the "elevated risk level" of ETR=1. Strassemeyer and Golla (2018) found that among pesticides applied in cereals and rapeseed, herbicides had the highest contribution to the aquatic risk. Gutsche et al. (2012) reported a strong reduction of toxicological risks if using a "Minimal dosage strategy" with a high number of herbicides with reduced application rates against a "Common practice strategy" with a smaller number of herbicides applied at usual application rates. However, the risks associated with the currently available combined mechanical-chemical weed control methods in sugar beet have not yet been assessed.

It remains an open question whether the toxicological risks of techniques with reduced herbicide application (such as band or spot spraying) decrease linearly with the decrease in the amount of herbicide applied. Therefore, the objectives of this study were to calculate the acute and chronic risks for arthropods, soil and aquatic non-target organisms associated with different conventional and new-coming mechanical-chemical and chemical weed control techniques in sugar beets in different soil-climate regions in Germany, using SYNOPS-GIS model. In addition, the exposure risks of herbicides frequently used by German sugar beet farmers in 2011-2018 is compared with the risks of a new herbicide technology, which has been recently registered in Germany.

2 Materials and methods

2.1 Method of spatial risk analysis

The spatial data basis for the risk analyses carried out with the SYNOPS-GIS model were the field blocks with arable crops from ATKIS (AdV, 2008). The geometries of the ATKIS field blocks were intersected with further digital data sets such as the digital soil map BÜK1000N (Richter et al., 2007) and the digital elevation model

DGM-10 (DGM, 2016). As a result, field-related soil parameters and the slope gradients of the individual areas were derived. By intersecting the field geometries with ATKIS water bodies, the distance to one or more surface water bodies, and the water body type and width class of the relevant water body was determined. The allocation of the sugar beet crop (as well as other crops) to the ATKIS field blocks was based on data on the cultivation statistics at municipality level (Gocht and Röder, 2014). For each field the suitability for the cultivation of sugar beet and other crops was derived from a combination of field size and Soil Quality Rating (Mueller et al., 2007). The objective function for the distribution of crop types maximizes the sum of the suitability for cultivation across all crops within a municipality, i.e. the crops are preferentially distributed to the fields that have the highest specific suitability for cultivation. The different crops are distributed to the agricultural fields in such a way that the sum of the cultivated areas corresponds to the crops' area according to the Thünen Agricultural Atlas (Gocht and Röder, 2014).

In addition, spatial allocations to districts, water catchment areas or soil-climate regions (Roßberg et al., 2007) are available to enable later aggregation of the predicted risk indices at field-level for different spatial areas. Daily values for temperature, precipitation, global radiation, and wind from about 280 weather stations and 2800 precipitation stations of the German Weather Service (DWD, 2016) were used as climatic model input variables. These data were interpolated to a 1 km x 1 km grid and then assigned to the individual fields. The information on the active ingredient content and the application conditions of the plant protection products (PPP) used was obtained by linking to the online database of approved plant protection products of the BVL (BVL, 2019). The application requirements with respect to (i) the minimum distances to be maintained from surface water bodies, and (ii) the runoff reduction on areas with slopes > 2% and > 4% were taken from the database and integrated in the risk analysis. The toxicological and physical chemical properties of PPP active ingredients were taken from the online Pesticide Property Database (PPDB) (Lewis et al., 2016). Both databases are updated every three months.

2.2 Derivation of generic application patterns of herbicides used in sugar beet

For the risk assessment performed in this study, generic application patterns of herbicides used in sugar beet were generated based on data from annual farm surveys on sugar beet cultivation in Germany, carried out by the sugar companies, the sugar beet grower associations, and the Institute of Sugar Beet Research (Stockfisch et al., 2013). From this survey, applied PPPs are ascertained and transmitted to Julius Kühn-Institute (JKI), where the data are included in the PAPA dataset (Roßberg et al., 2017). For privacy reasons, it was not possible to precisely assign each collected application pattern to the field to which it was applied. In our study, in order to analyze regional differences in the risk of PPP applications, all survey data were assigned to one of six regions which had been previously identified using a cluster analysis of 50 soil-climate regions in Germany and are referred to as CEPI regions (Clusters for Evaluation of PSM use Intensity, Dachbrodt-Saaydeh et al., 2019). The soil-climate regions were in turn formed by a cluster analysis of municipalities with similar soil properties, temperatures and precipitation (Dachbrodt-Saaydeh et al., 2019). From the survey data, three herbicide application patterns were identified for each CEPI region for each year in the period 2011-2018 using the procedure described below. These three application patterns correspond to three spraying sequences with a high, medium and low herbicide intensity, reflecting the range of chemical weed control practice in sugar beet representative for each CEPI region and year.

The herbicide use intensity was derived from the treatment index (TI), which summarizes the number of PPP applications over the course of a cropping period standardized to the maximum permitted application rate per application pattern (Roßberg, 2013). According to its TI, each herbicide application pattern, recorded in the survey from one specific CEPI region and year, was assigned to one of the three groups: (1) low intensity: TI ≤ 33-percentile, (2) medium intensity 66-percentile > TI > 33-percentile; (3) high intensity TI ≥ 66-percentile. Note that TI is not a toxicological index and was used to reflect the differences in agronomic application intensity caused by differing weed infestation and not the differences in potential ecotoxicological risks.

Further, for each CEPI region, year and intensity level, the herbicide applications in each month were counted and the months with the most frequent applications were defined as application periods. Next, the most frequently applied herbicides or tank mixtures in each CEPI region and intensity level were selected. A similar procedure was used to determine the application dates. First, the most frequent treatment date per year was selected. The next most frequent treatment date with a minimum interval of 7 days was then taken as the subsequent treatment date. The modal value of the application rates of the selected herbicides was used as the most frequent application rate (or the respective application rates of the tank-mix partners). Finally, the TIs of generated herbicide sequences were checked for compliance with the mean TI values of the intensity levels for the CEPI region. If the difference between the mean and the generated TIs was greater than 0.5, then other or additional herbicide applications were selected, and the subsequent steps were run again. The generation of generic application patterns was (semi-)automated by using a JKI-internal web application, thereby increasing the degree of reproducibility. In addition, an integrated plausibility test provided information on overdosage (application rate > 100% of the approved application rate) and too low application rates (< 5% of the approved application rate). Finally, the generic application patterns were checked for plausibility by experts from the Institute of Sugar Beet Research: two experts responsible for the evaluation of data from the Germany-wide farm survey on sugar beet cropping and another one responsible for the analysis of national sugar beet herbicide trials, and further by eight experts from regional sugar beet growers associations and consultants from the sugar industry both responsible for advising beet farmers in their area. Table 1 shows an example of generic application pattern of herbicides for CEPI Region D in 2018.

TABLE 1 Generic application pattern of herbicides for three intensity levels for CEPI region D in 2018.

Date	Herbicide	Application rate (l ha^{-1})	Active Ingredient (Application rate, g/ha)
Intensity level 1[1]			
2018-04-24	Betanal MAXXPRO	0,8	Phenmedipham (48), Lenacil (21.6), Ethofumesat (60), Desmedipham (37.6)
	DEBUT	0,015	Triflusulfuron (7.29)
	GOLTIX TITAN	1,7	Quinmerac (68), Metamitron (892.5)
2018-05-09	Betanal MAXXPRO	0,8	Phenmedipham (48), Lenacil (21.6), Ethofumesat (60), Desmedipham (37.6)
	DEBUT	0,015	Triflusulfuron (7.29)
	GOLTIX TITAN	1	Quinmerac (40), Metamitron (525)
2018-05-24	Betanal MAXXPRO	0,8	Phenmedipham (48), Lenacil (21.6), Ethofumesat (60), Desmedipham (37.6)
	Metafol SC	2,3	Metamitron (1600.8)
Intensity level 2			
2018-04-16	Betanal MAXXPRO	0,8	Phenmedipham (48), Lenacil (21.6), Ethofumesat (60), Desmedipham (37.6)
	Betasana SC	1,25	Phenmedipham (200)
	Metafol SC	1,25	Metamitron (870)
2018-04-27	Betanal MAXXPRO	0,8	Phenmedipham (48), Lenacil (21.6), Ethofumesat (60), Desmedipham (37.6)
	DEBUT	0,02	Triflusulfuron (9,72)
2018-05-15	Betanal MAXXPRO	0,8	Phenmedipham (48), Lenacil (21.6), Ethofumesat (60), Desmedipham (37.6)
	DEBUT	0,02	Triflusulfuron (9,72)
	GOLTIX TITAN	1	Quinmerac (40), Metamitron (525)
2018-05-25	DEBUT	0,02	Triflusulfuron (9,72)
	Goltix Gold	1,5	Metamitron (1050)
	GOLTIX TITAN	1	Quinmerac (40), Metamitron (525)
Intensity level 3			
2018-04-23	Belvedere Extra	1,25	Phenmedipham (187.5), Ethofumesat (250), Desmedipham (62.5)
	GOLTIX TITAN	2	Quinmerac (80), Metamitron (1050)
2018-05-06	Betanal MAXXPRO	2	Phenmedipham (120), Lenacil (54), Ethofumesat (150), Desmedipham (94)
	GOLTIX TITAN	2	Quinmerac (80), Metamitron (1050)
2018-05-18	Belvedere Extra	1,25	Phenmedipham (187.5), Ethofumesat (250), Desmedipham (62.5)
	Betanal MAXXPRO	2	Phenmedipham (120), Lenacil (54), Ethofumesat (150), Desmedipham (94)
	DEBUT	0,02	Triflusulfuron (9,72)
	Goltix Gold	2	Metamitron (1400)
	GOLTIX TITAN	2	Quinmerac (80), Metamitron (1050)
	LONTREL 600	0,05	Clopyralid (30)
	STEMAT	0,5	Ethofumesat (250)

[1]Three intensity levels of generic application patterns for a single CEPI region reflect differences in agronomic application intensity caused by differing weed infestation and not the differences in potential ecotoxicological risk.

2.3 Model scenarios for risk assessment with SYNOPS

Risk assessment in SYNOPS-GIS was carried out for conventional broadcast herbicide application and for four combined mechanical-chemical weed control methods (Table 2). Only post-emergent weed control strategies were considered. To simulate conventional herbicide (CH) broadcast spraying, the generic application patterns of herbicides (see 2.2) were applied to 100% area of a sugar beet field (Table 2, No. 1). To evaluate the toxicological risks associated with band spraying (CH-broadcast-band spraying, CH-band spraying; Table 2, No. 2, 3) the application rate of the herbicides listed in the generic application patterns was set at 44%, assuming a row spacing of 45 cm and a width of the sprayed band of 20 cm. The smallest spatial unit in SYNOPS-GIS is a single field, so heterogeneity of input data within the field could not be accounted for. Therefore, application rates had to be adjusted to correspond to the cumulative amount of herbicides applied in a field. In CH-broadcast-band spraying (Table 2; No. 2), since the first application was made to the entire field and subsequent band applications were 44% compared to CH-broadcast spraying, the total amount of herbicide applied over the growing season was equivalent to 63% of the amount by CH-broadcast spraying. For the evaluation of the toxicological risk of CH-spot-spraying, the application rate of the herbicides listed in the generic application patterns was set at 12.5%, assuming that the distance between rows was 45 cm and the spot size was 10 cm x 10 cm (18 cm in-row

TABLE 2 Weed control techniques in sugar beet used in SYNOPS GIS to calculate the toxicological risk for soil organisms, arthropods and aquatic organisms.

	Weed control techniques (CH-conventional herbicides; NHT-new herbicide technology)	
No.	Name	Description of post-emergent weed control techniques
1	CH-broadcast spraying	Two to four full-area herbicide applications 10 to 14 days apart, depending on weed infestation pressure and weather conditions. Active ingredients and application rates are listed in Table 1.
2	CH-broadcast-band spraying	The first herbicide application to the entire area, while the remaining applications as band applications to the row. The weeds between the rows are controlled by hoeing.
3	CH-band spraying	All two to four herbicide applications applied as band-spraying in the row. The weeds between the rows are controlled by hoeing.
4	CH-spot spraying[1]	Spot-spraying over the sugar beet plants with spot size of 0.1 m x 0.1 m, to control weeds in close vicinity to the crop, whereas the weeds on the remaining area are controlled by hoe robot.
5	NHT-band spraying	Two applications of the new herbicide CONVISO ONE with 0.5 l/ha (AI: Foramsulfuron,Thiencarbazone-methyl) in the rows of ALS-tolerant sugar beet variety. The weeds between the rows are controlled by hoeing.

[1]This technique is not yet available on the market, but the first test results are promising (Starck et al., 2021), and it is expected that the method will be available soon.

distance of beet plants). For NHT band spraying (Table 2; No. 5), two applications of 0.011 kg/ha Foramsulfuron and 0.0066 kg/ha Thiencarbazone-methyl were used as model input, which corresponds to 44% of the maximum quantity per application permitted for this herbicide in Germany for broadcast spraying on undrained fields (Oct. 2022). The total amount of applied active ingredients for NHT-band spraying corresponds to 1% from the total sum of active ingredient rates in kg/ha applied in generic application pattern of medium intensity (Table 1). The new herbicide acts both via the leaves and the soil, belongs to ALS-inhibitor mode of action and is applicable in combination with ALS-tolerant sugar beet varieties solely. Only one of the conventional herbicides included in generic application patterns comprises an active ingredient, namely Triflusulfuron-methyl, with the same mode of action as the new one. Other conventional herbicides include active ingredients with other modes of action: lipid biosynthesis inhibitors, plant growth regulators, photosynthesis inhibitors and shoot-growth inhibitors.

2.4 Methodology of risk assessment at site level and spatial aggregation of risk indices

In the SYNOPS model, the toxicological risk for non-target arthropods, and aquatic and soil organisms used as reference organisms was expressed by the Exposure Toxicity Ratio (ETR), i.e. the ratio of the exposure of reference organisms to PPP active substances under worst-case conditions and the toxicity of these active substances to these organisms (Equation 1).

$$\mathrm{ETR} = \frac{Exposure}{Toxicity} \tag{1}$$

The methodology for calculating the toxicological risk was presented in detail by Strassemeyer et al., 2017. Briefly, the predicted environmental concentration (PEC) or the estimated PPP exposure in surface waters is calculated for PPP inputs by considering spray drift, surface runoff and erosion. The concentrations of active ingredients in field margins are calculated via field applications and spray drift, whereas the concentration of an active ingredient in soil is calculated by considering field applications and crop interception. First order degradation of the substances in soil, water and on plants is considered (Strassemeyer et al., 2017).

The half-maximal lethal concentration (LC50), effect concentration (EC50) or lethal rate (LR50), and the no-effect concentration (NOEC) of the individual active ingredients is used to quantify toxicity. In sugar beet, several combinations of active ingredients are typically used on several dates to control weeds with herbicides (Table 1). Active ingredients with identical or different modes of action can interact additively or produce antagonistic or even synergistic effects that are greater than the sum of the individual effects (Knillmann et al., 2021; Dietrich et al., 2022). However, a sufficiently good performance of the additive approach by prediction of mixture toxicity of pesticides has been confirmed for aquatic organisms (Belden et al., 2007). For terrestrial organisms, the possible synergistic or antagonistic effects of most active ingredient combinations are unknown. Therefore, in the present study, the ETRs of the individual active ingredients or applications were aggregated according to the concept of concentration addition (Verro et al., 2009; Vaj et al., 2011; Zhan and Zhang, 2012) to capture the risk across the entire weed control strategy (multiple active ingredients and/or multiple applications) for a specific reference organism. The risk aggregation of an application pattern was performed in three steps. First, for each day, the acute ($\mathrm{ETR}_{A(RO)}$)and chronic risk ($\mathrm{ETR}_{C(RO)}$) of a given active ingredient (AI) was calculated for a given reference organism (RO). Second, for each day, the ETR values of multiple AIs were summed up to derive the daily risk caused by all AIs for a given reference organism. Third, the 90th percentile of 365 daily ETRs was derived, representing the acute (Equation 2) and chronic (Equation 3) risk of the entire herbicide application strategy for a given reference organism and field.

$$\mathbf{ETR}_{A(RO)} = \underset{1\leq t\leq 365}{\mathbf{P\,90}} \sum\nolimits_{1\leq i\leq n} \frac{\boldsymbol{PEC(t,\mathrm{AI}_i)}}{\mathbf{0.1}*\boldsymbol{LC50/LR50/EC50(RO,\mathrm{AI}_i)}} \tag{2}$$

$$\mathbf{ETR}_{C(RO)} = \underset{1\leq t\leq 365}{\mathbf{P\,90}} \sum\nolimits_{1\leq i\leq n} \frac{\boldsymbol{PEC_{TWA}(t,\mathrm{AI}_i)}}{\boldsymbol{NOEC(RO,\mathrm{AI}_i)}} \tag{3}$$

where PEC(t, AI_i) is the predicted environmental concentration for t-th day and i-th AI. $\mathrm{PEC}_{\mathrm{TWA}}$ is the time weighted average concentration of 7 days. LC50, LR50, LD50 and NOEC refer to specific RO to i-th AI. n represents the number of AIs with additive effect. Acute endpoints are multiplied by a factor of 0.1, resulting in a safety factor of 10 for acute risks, because the acute endpoints represent the higher hazard than the chronic endpoints.

To assess the risk of PPP exposure to surface waters, SYNOPS considers the following five reference organisms: algae (al), aquatic invertebrates (e.g., Daphnia sp., da), fish (fi), aquatic plants (Lemna sp., le), and sediment organisms (Chironomus sp., ch). Risk indices were first calculated separately for each of the reference organisms. Then, the acute (Equation 4) and chronic (Equation 5) aquatic risk was each calculated as the maximum of the risk indices of the aquatic reference organisms.

$$ETR_{A(aq)} = \mathrm{MAX}\left(ETR_{A(al)}, ETR_{A(da)}, ETR_{A(fi)}, ETR_{A(le)}, ETR_{A(ch)}\right) \quad (4)$$

$$ETR_{C(aq)} = \mathrm{MAX}\left(ETR_{C(ch)}, ETR_{C(da)}, ETR_{C(fi)}\right) \quad (5)$$

$ETR_{A(aq)}$ and $ETR_{C(aq)}$ represent acute and chronic risk for aquatic organisms.

For the soil concentration, it was assumed that the AI is distributed in the upper 2.5 cm soil layer. Toxicity data of the reference organisms in soil were available for earthworms (ew) and springtails (Collembolae; co) (Equation 6).

$$ETR_{C(soil)} = \mathrm{MAX}(ETR_{C(ew)}, ETR_{C(co)}) \quad (6)$$

$ETR_{C(soil)}$ represents chronic risk for soil organisms.

For the three reference organisms in field margin (FM) biotopes, honeybees (bi), predatory mites (Typhlodromus pyri; tp), and braconid wasps (Aphidius rhopalosiphi; ar), only acute toxicity values are included under regulatory approval. Therefore, only acute risks were calculated as the maximum of the indices of the three ROs in FM biotopes (Equation 7).

$$ETR_{A(FM)} = \mathrm{MAX}(ETR_{A(bi)}; ETR_{A(ar)}; ETR_{A(tp)}) \quad (7)$$

$ETR_{A(FM)}$ represents the risk for organisms in FM. All calculations were performed assuming that the distance requirements to water bodies and terrestrial small structures as well as the runoff requirements were met. The calculated ETR for organisms in surface waters, non-target arthropods (NTA) in field margins, and for soil organisms were assigned to five risk classes:< 0.01 no risk; 0.01 ≤ ETR< 0.1 very low risk; 0.1 ≤ ETR< 1 low risk; 1 ≤ ETR< 10 elevated risk; ETR ≥ 10 high risk (Strassemeyer et al., 2017).

The calculated field- and year-specific risk indices $ETR_{A(aq)}$, $ETR_{C(aq)}$, $ETR_{A(FM)}$ and $ETR_{C(soil)}$ were stored in a database. From this database, the risk indices for different spatial units could be aggregated, analyzed, and presented. In this study, for assessing regional differences in toxicological risk in Germany, the data were aggregated on soil-climate region (SCR) level (Roßberg et al., 2007), as the aggregation of data at the CEPI-region leveled spatial differences and was therefore less informative. For each of the 36 sugar beet growing SCR, the 90th percentile of each risk index from all sugar beet fields within a given SCR in each year was calculated. For visualization, the SCR-specific risk indices were clustered within 6 CEPI-regions, whereby the variance between SCRs within individual CEPI-regions and the variance between CEPI-regions can be illustrated. Since the same herbicide application pattern was used for all SCR within individual CEPI regions, the variance in risk index between SCR within a single CEPI-region is caused solely by differences in environmental characteristics. In addition, the 90th percentile of risk indices from all sugar beet fields in Germany was also calculated to obtain aggregated risk indices for Germany as a whole.

2.5 Statistical evaluation

Statistical analyses were performed with R version 4.1.1 (R Core Team, 2020). Generalized linear mixed models (glmm) with SCR as a fixed effect and year as a random effect, followed by the Sidak method of multiple mean comparison ($p<0.05$), were applied to assess regional differences in SCR-aggregated ETRs associated with CH-broadcast spraying. The SCRs were further subdivided in two groups with high and low risk respectively. The difference between the two groups with respect to different environmental parameters was tested using Welch`s t-test. Furthermore, the glmm was applied to estimate effects of different environmental parameters on ETR associated with CH-broadcast spraying at the field level. The gamma-distribution of model residuals with log link was considered both for modeling field and SCR-aggregated ETRs. Residual diagnostics was calculated using DHARMA package. The models were build using glmmTMB package.

The ETRs associated with five weed control methods (Table 2) in six selected SCR were statistically evaluated by mixed linear models (nlme package), considering weed control method, SCR and their interaction as fixed effects and the effect of year as a random effect. The response variable was log-transformed to fulfil the model requirements. Residuals of the final models were checked for homoscedasticity by Levene's test as well as graphically and for normal distribution by the Shapiro–Wilk test as well as graphically. Since F test for fixed effects showed that the interaction between weed control method and SCR was not significant ($p>0.05$) it was excluded from the final model. Marginal means for each method were then calculated using package emmeans. Sidák's method was further used to determine confidence intervals for each group mean and statistical significance ($p<0.05$) of difference. Additionally, the acute aquatic risk, $ETR_{A(aq)}$ associated with NHT-band spraying and CH-broadcast spraying was analyzed in all sugar beet growing SCR using generalized linear mixed models. Weed control method, SCR and their interaction were incorporated as fixed effects and the effect of year as a random effect. The gamma-distribution of model residuals with log link was considered. T-test was applied to evaluate the difference in toxicological risk between both weed control methods within each SCR ($p<0.05$). Residual diagnostics was calculated using DHARMA package.

3 Results

Acute aquatic ($ETR_{A(aq)}$) and chronic soil organism ($ETR_{C(soil)}$) risk from CH-broadcast spraying varied widely among SCR, but also among the 8 study years (2011-2018). In most SCR and years, the 90th percentile of acute aquatic risk was very low at ≤ 0.1, but in SCR 101, 152, 154, 157, and 158 it was 0.2-0.8 in all 8 study years, which was still considered low risk but significantly higher than in most other regions (Figure 1). SCRs with elevated risks had a significantly lower minimal distance to water bodies and a significantly higher share of standing water than the other SCRs (Table 3). The greater the distance to water bodies and the higher the proportion of flowing waters, the lower the log-transformed aquatic risk, as indicated by the negative estimates of the corresponding predictors in the glmm analyses (Table 4). The slope of the field was also significantly lower in the SCRs with elevated aquatic risk, although it had a positive effect on aquatic risk, but the effect of a lower slope was more than offset by the stronger effect of a high proportion of standing water (Tables 3, 4). 90th percentile of chronic risk for soil organisms remained low at 0.1–0.2 in most SCR but significantly (p<0.05) increased to 1.0–1.5 (elevated risk) in individual SCR (105, 151, 153, 156; Figure 1). The SCRs with high risk for soil organisms had a significantly higher organic C content in topsoil compared to other SCRs (Table 3). According glmm, this parameter was one of the strongest positive predictors of $ETR_{C(soil)}$ (Table 4).

The chronic aquatic risk ($ETR_{C(aq)}$) and the acute risk for non-target arthropods ($ETR_{A(FN)}$) were very low in all SCRs and years and thus, are not shown here. Figure 2A shows the 90th percentile of acute aquatic and chronic risks for soil organisms for combined mechanical-chemical weed control techniques in comparison to CH-broadcast spraying in 6 SCR. For this comparison, one SCR per CEPI region was selected, where the toxicological risk by broadcast spraying was higher than that of the other SCR in the same CEPI region. Both weed control method (n=5) and SCR (n=6) were significant for acute aquatic (F=168, p<0.001; F=33, p<0.001) and chronic risk for soil organisms (F=2552, p<0.001; F=1182, p<0.001) as evaluated by mixed model analysis. For all methods except NTH-band spraying, there was a nearly linear relationship between herbicide application amount and associated toxicological risk (Table 5). However, weed control techniques with reduced herbicide use of 63% and 44% of the full herbicide application amount did not differ significantly in acute aquatic risk and, in some regions, in chronic risk to soil organisms as indicated by pairwise comparison of means (Figures 2A, B). The NTH-band spraying caused as much acute aquatic risk as the CH-broadcast spraying in six selected SCRs (Figure 2A). In contrast, in all regions and years, the chronic risk to soil organisms was much lower with NTH-band spraying compared with CH-broadcast spraying (Figure 2A). The chronic risk to aquatic organisms and acute risk to field margin organisms associated with NHT-band spraying remained negligible (not shown).

Additionally the acute aquatic risk in all sugar beet growing SCRs was compared between CH-broadcast spraying and NHT-band spraying. GLMM analysis showed that the interaction between SCR and weed control strategy was significant ($\chi 2$ = 289, p<0.001). The t-test indicated that in most SCRs the acute aquatic risk did not differ between two methods but was significantly higher (p<0.05) at 10 out of 35 SCRs and significantly lower for 4 out 35 SCRs for NTH-band spraying compared to CH-broadcast spraying (Figure 3). In SCR 152 and 158, the NHT-band spraying caused even an elevated acute aquatic risk (ETR > 1) in some years (Figure 3).

In the JKI geoportal, the ecotoxicological risks calculated with SYNOPS-GIS are presented in the form of interactive maps (https://sf.julius-kuehn.de/mapviewer/evaherb). This allows the comparison of the toxicological risk for different regions, aggregation levels and

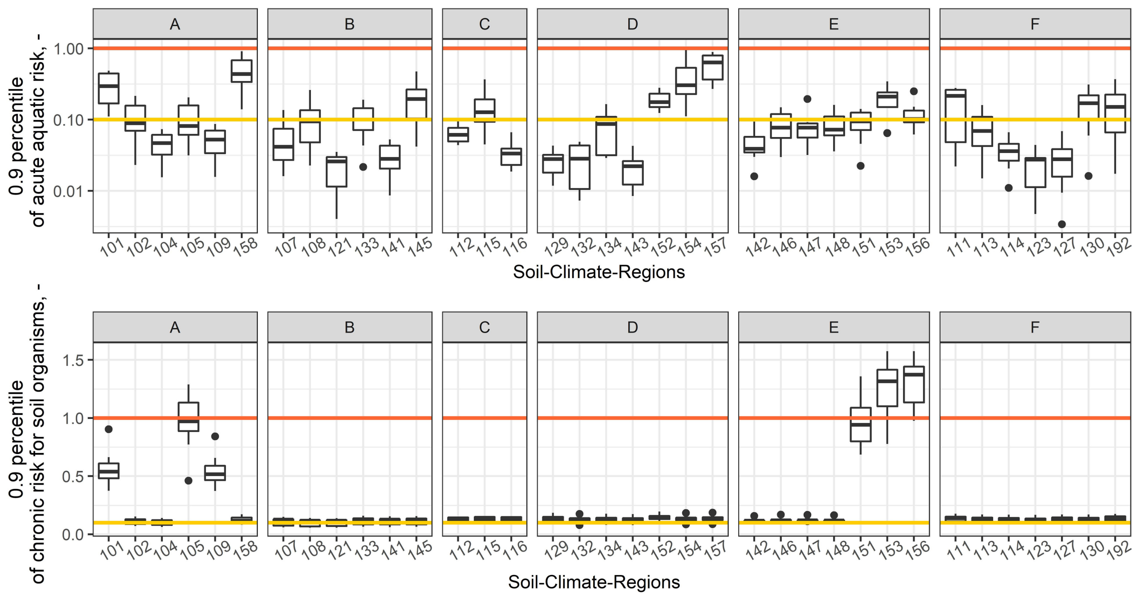

FIGURE 1
90th percentiles of acute aquatic ($ETR_{A(aq)}$) and chronic risk for soil organisms($ETR_{C(soil)}$) in different soil-climate-regions in 6 CEPI regions **(A–F)**. The location of CEPI regions and soil-climate-regions in Germany are given in Figure 6. Each boxplot includes 8 data points (8 years, 2011-2018). The yellow line shows the transition from very low to low risk, and the orange line from low to elevated risk.

TABLE 3 Mean environmental parameters in soil-climate regions with elevated and low toxicological risks.

	Acute aquatic risks		Chronic risks for soil organisms	
	Group with elevated risk (n=5) SCR: 101,152,154,157,158	Group with low risk (n=30) SCRs which are not in Group 1	Group with elevated risks (n=6) SCR: 101,105, 109,151,153,156	Group with low risks (n=29) (SCR which are not in Group 1)
Minimal distance to water a body (m)	30.2*	100.9	58.1*	97.3
Share of standing water bodies[1]	0.9*	0.3	0.7*	0.4
C_{org} in 0-20 cm (%)	1.5^{ns}	1.9	4.0*	1.5
Sand content in 0-20 cm (%)	57.8^{ns}	42.0	63.5*	41.0
Field slope (%)	3.0*	5.2	2.1*	5.3

[1]Proportion of standing water bodies in the total number of standing and flowing water bodies.
Significant differences (p<0.05) between groups are labelled with asterisk.
The difference between two groups was tested for significance using Welch's t-test.

TABLE 4 Environmental parameters impact on acute aquatic risks ($ETR_{A(aq)}$) and chronic risk for soil organisms ($ETR_{C(soil)}$) as analyzed with general linear mixed models.

Acute aquatic risk				
$ETR_{A(aq)}$~MinDist+Width+Flowing_water(yes/no)+Slope+Sand+C_content + (1 | Year), family=Gamma(link="log") Random effects: Groups Name Std.Dev. Year (Intercept) 0.577 Number of obs: 255196, groups: Year, 8 Dispersion estimate for Gamma family (sigma^2): 0.973				
	Estimate	Std. Error	Z value	p-value
Intercept	-1.69	0.20	-8.3	$<2*10^{-16}$
Minimal distance to water	-0.004	$2*10^{-5}$	-156.8	$<2*10^{-16}$
Water body width	-0.30	$5*10^{-4}$	-605.1	$<2*10^{-16}$
Flowing water-1 (0 standing; 1 flowing)	-3.69	$4*10^{-3}$	-828.6	$<2*10^{-16}$
Slope	0.20	$6*10^{-4}$	361.1	$<2*10^{-16}$
Sand %	-0.001	$1*10^{-4}$	-11.5	$<2*10^{-16}$
Corg %	-0.11	$8*10^{-4}$	-141.5	$<2*10^{-16}$
Chronic risk for soil organisms				
$ETR_{C(soil)}$~ Flowing_water(yes/no)+Slope+Sand+C_content + (1 |Year), family=Gamma(link="log") Random effects: Groups Name Std.Dev. Year (Intercept) 0.15 Number of obs: 317440, groups: JAHR, 8 Dispersion estimate for Gamma family (sigma^2): 0.153				
Intercept	-2.71	0.05	-51.2	$<2*10^{-16}$
Flowing water_1 (0 standing; 1 flowing)	-0.02	$2*10^{-3}$	-12.6	$<2*10^{-16}$
Slope	0.001	$2*10^{-4}$	6.7	$<3*10^{-11}$
Sand %	$8*10^{-4}$	$3*10^{-5}$	24.1	$<2*10^{-16}$
Corg %	0.15	$3*10^{-4}$	509.0	$<2*10^{-16}$

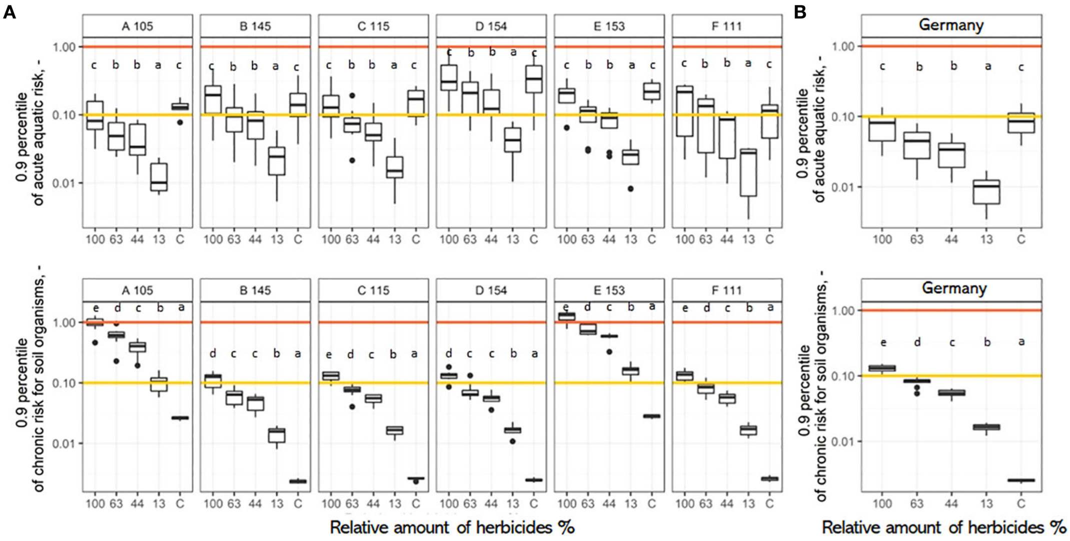

FIGURE 2
Toxicological risk ($ETR_{A(aq)}$; $ETR_{C(soil)}$) of reduced-herbicide weed control techniques compared with the risk of conventional broadcast spraying aggregated over selected soil-climate-regions (105, 145, 115, 154, 153, 111) in CEPI regions A-F **(A)** and aggregated over Germany **(B)**. The location of the soil-climate-regions and CEPI regions is given in Figure 6. Each boxplot includes 8 data points for 8 years (2011-2018). "C" denotes CONVISO ONE Band-spraying method. Significant differences between weed control methods are indicated with different lowercase letters.

TABLE 5 Toxicological risks for aquatic ($ETR_{A(aq)}$) and soil organisms ($ETR_{C(soil)}$) of mechanical-chemical compared to chemical weed control.

Weed control technique	Relative herbicide amount based on total g AI ha^{-1}) compared to the amount applied for CH-broadcast spraying (see 2.3)	Acute aquatic risk	Chronic risk for soil organisms
	%	in % of risk by CH-broadcast spraying	
		Mean (± SD)	
CH-broadcast spraying	100	100,0 (0,0)	100,0 (0,0)
CH-broadcast-band spraying	63	54,3 (3,8)	58,7 (3,3)
CH-band spraying	44	41,8 (0,8)	42,2 (2,1)
CH-spot spraying	13	12,5 (0,6)	12,2 (0,9)
NHT-band spraying	1	105,0 (27,3)	2,2 (0,3)

Toxicological risks (Figure 2A) calculated with SYNOPS were aggregated across six soil climate regions for a specific weed control method and the risk indices were presented as a percentage of risk caused by broadcast spraying.

weed control techniques for individual years from 2011-2018. For example, Figure 4 shows the 90th percentile of the acute aquatic risk $ETR_{A(aq)}$ maps for CH-broadcast spraying and NHT-band-spraying based on weather data in 2015 at the county level in Germany. In addition, the web application offers the possibility to set the area ratio between CH-broadcast spraying and another weed control method (e.g., mechanical weed control) in Germany (Figure 5). Thus, the risks for different combinations of techniques can be evaluated.

4 Discussion

4.1 Regional variability of ecotoxicological risks associated with broadcast spraying of conventional herbicides

Risk to aquatic and soil organisms associated with conventional broadcast herbicide spraying varied significantly between the SCR within a single CEPI region (Figure 1). Since the AI, application rates and the sequence of applied herbicides were identical within a CEPI region (section 2.2), the differences in toxicological risk across SCRs within a CEPI-region could not be referred to the variability in application data but must be due to variability of environmental conditions between the SCR. The SCR with permanently higher acute aquatic risk (> 0.1) or chronic risk for soil organisms (> 0.5) risk were all located in northern Germany (Figure 6). The higher aquatic risk in the northern areas is mainly due to the higher density of water bodies and the higher proportion of standing waters. In standing waters, AI content decreases only due to degradation,

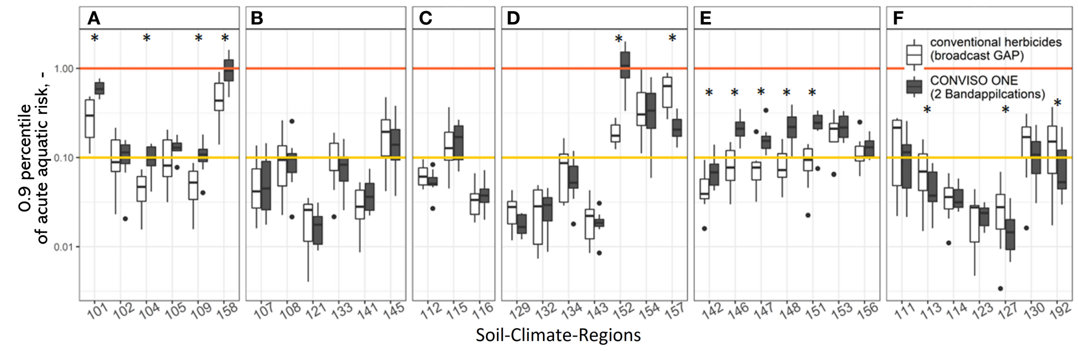

FIGURE 3
Regional variability in acute aquatic risk ($ETR_{A(aq)}$) for twofold band application of CONVISO ONE in comparison to broadcast spraying of conventional herbicides in CEPI regions **A-F**. Each boxplot includes 8 data points for 8 years (2011-2018). Significant differences between weed control methods are indicated with an asterisk.

whereas in flowing waters, removal by water flow is additionally accounted for by SYNOPS. The high risk to soil organisms is mainly due to the higher Corg content in northern SCRs compared to other SCRs (Tables 3, 4). SYNOPS considers the Corg-dependent distribution coefficient to estimate the fraction of AI that remains in the soil and is not exposed to surface runoff (Strassemeyer et al., 2017). Nause et al. (2021) also reported that sugar beet fields with elevated aquatic risk had shorter distance to water bodies compared to those with lower aquatic risk indices.

The 90th percentile of chronic aquatic risk ($ETR_{C(aq)}$) and the 90th percentile of acute risk for non-target organisms in field margins ($ETR_{A(FM)}$) remained very low in all SCRs and are not shown here. Since the chronic aquatic risk only considers aquatic invertebrates, fish, and sediment organisms (Equation 5) it is expected to give lower values than the acute risk, which also includes algae and higher water plants as reference organisms (Equation 4). The reason for this is that for most AI no chronic endpoints were available. The actual version of PPDB includes also chronic toxicity endpoints ($NOEC_{96\ hours}$) for algae, which could be used in future studies for assessment of chronic aquatic risk.

Nause et al. (2021) calculated toxicological risk indices associated with 2314 pesticide application records from sugar beet farms using SYNOPS. They also found the risk for field margin organisms to be very low even considering insecticide and fungicide applications. The acute aquatic risk tended to be higher in their study, most probably due to the inclusion of insecticides, while the chronic risk to soil organisms was similar to our study. However, in contrast to our study, the 90th percentile of chronic risk to aquatic organisms was as high as the acute aquatic risk. This was most likely because their study included fungicides and insecticides, which have

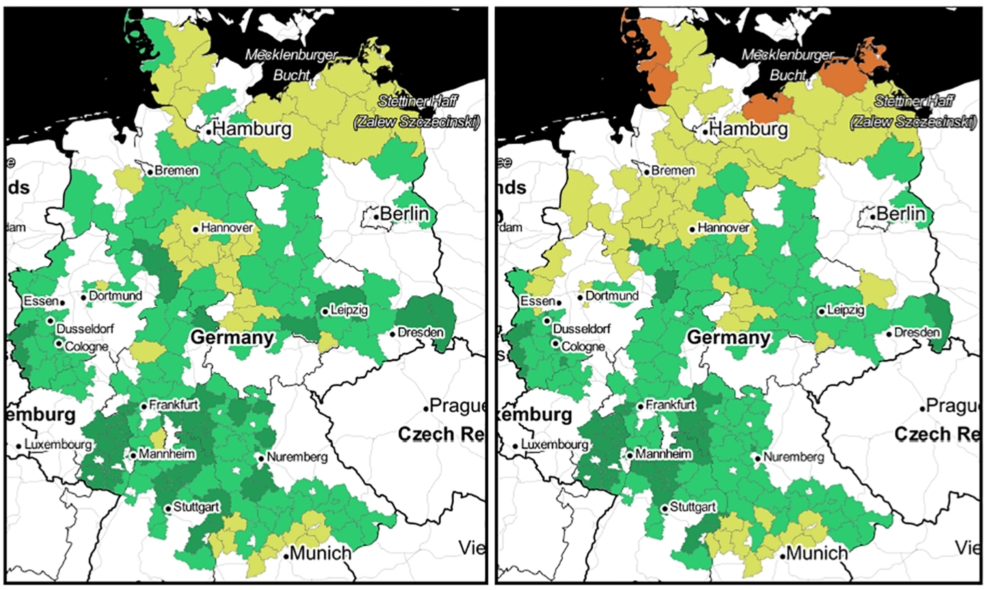

FIGURE 4
90th percentile of acute aquatic risk $ETR_{A(aq)}$ for conventional broadcast herbicide application (left) and for Conviso-One band spraying (right) in 2015 at county level in Germany. Orange shows elevated risk (1.0-10.0), Yellow - "low risk" (0.1-1), green - "very low risk" (0.01-0.1), dark green - "no risk" (<0.01).

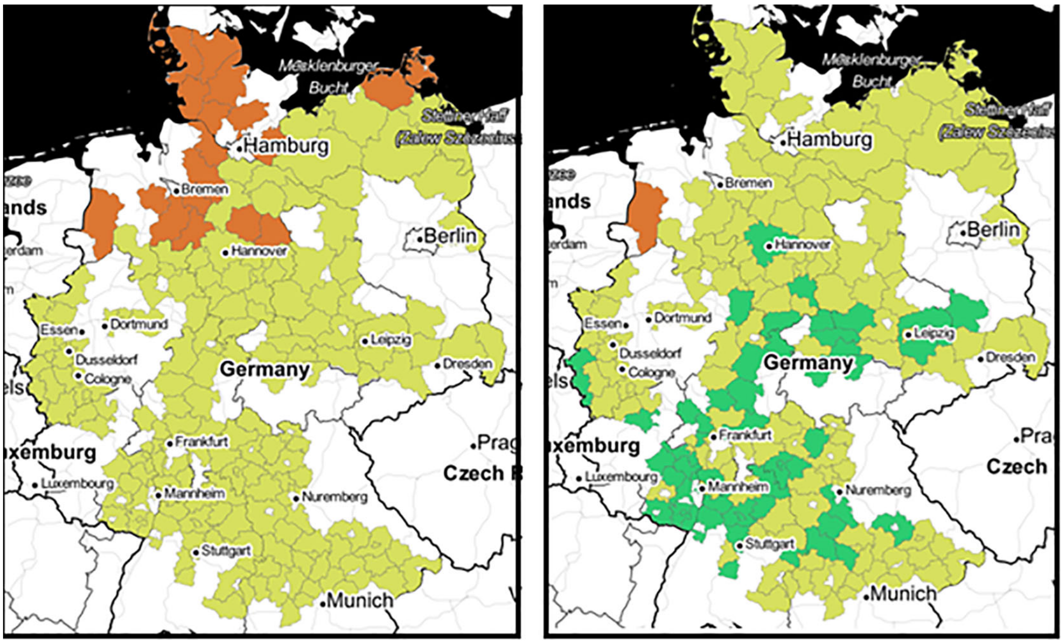

FIGURE 5
90th percentile of chronic risk for soil organisms $ETR_{C(soil)}$ for conventional broadcast herbicide application (left) and for a combination of 70% fields with mechanical weed control (with a toxicity of zero) and 30% fields with chemical weed control (right) in 2017 at the county level in Germany. Orange shows elevated risk (1.0-10.0), Yellow - "low risk" (0.1-1), green - "very low risk" (0.01-0.1).

a greater impact on the three chronic reference organisms: fish, aquatic invertebrates, and sediment organisms.

Overall, it is important to note that using the identical application patterns of herbicides within CEPI-regions in our study is a simplification and thus, has limitations. The application pattern of PPPs in sugar beet cultivation is specifically adapted to the conditions of the respective field (farm survey sugar beet cultivation 2010 - 2021). The generalized application pattern of a CEPI region, however, considers only an average of used applications, while the strategies used especially in challenging fields with water proximity or erosion risk are not taken into account. The spectrum and frequency of active ingredients used for weed control in sugar beet was reviewed by Roßberg et al. (2017) and Nause et al. (2021). Nause et al. (2021) quantified the aquatic risk associated with the application of individual active ingredients and found that increased aquatic risk was not due to application of individual active ingredients or their application rate, but rather due to combinations of active ingredients, application dates and field-specific environmental conditions. Our study showed significant differences between the different soil-climate regions in toxicological risk to both aquatic and soil organisms. Additionally, our results indicate that the variance in aquatic toxicological risk between CEPI-regions is not as high as the variance within some CEPI-regions, thus either the aquatic toxicological risk is not affected by application patterns or CEPI regions do not capture the difference in herbicide application patterns for sugar beet. Thus, the aggregation of toxicological risks associated with herbicide applications by CEPI-region does not appear to be meaningful for sugar beet.

4.2 Risks of weed control techniques with reduced vs. full herbicide input

While reduced input of conventional herbicides resulted in a linear decrease in risk values, NHT-band spraying caused the same acute aquatic risk as the CH-broadcast spraying (Figure 2; Table 5). The reason for the higher aquatic toxicity of NHT-band spraying was a strong sensitivity of duckweed (*Lemna minor*) to the two active ingredients of a new herbicide (EC50< 0.001 mg L-1 for Thiencarbazone-methyl and Foramsulfuron). The highest acute aquatic risk of NHT-band spraying was observed in the same SCRs as by broadcast spraying (101,158, 152,154, Figure 3) and was mainly due to the low average distance to surface waters and the higher proportion of standing waters in these SCRs (Tables 3, 4). The chronic toxicity to aquatic organisms remained low, however, as no NOEC values (chronic toxicity endpoints) were available for duckweed and algae and these species were therefore excluded from the chronic toxicity calculation (Eq.5). The chronic toxicity endpoints of Thiencarbazone-methyl with NOEC= 3.54mg L-1 for aquatic invertebrates and NOEC=4.8 mg L-1 for fish (Lewis et al., 2016) are much higher compared with EC50 for duckweed (see above), resulting in low chronic aquatic risk from NHT-band spraying (data not shown). In this study, the SYNOPS GIS risk assessment was not performed for NHT-broadcast spraying. However, since there is a linear relationship between application rate and risk scores for conventional herbicides (Table 5), it can be assumed that an increase in application rate by a factor of 2.3 in the case of broadcast application of the same herbicide will increase the risk score accordingly. It could be seen from Figure 3 that the acute

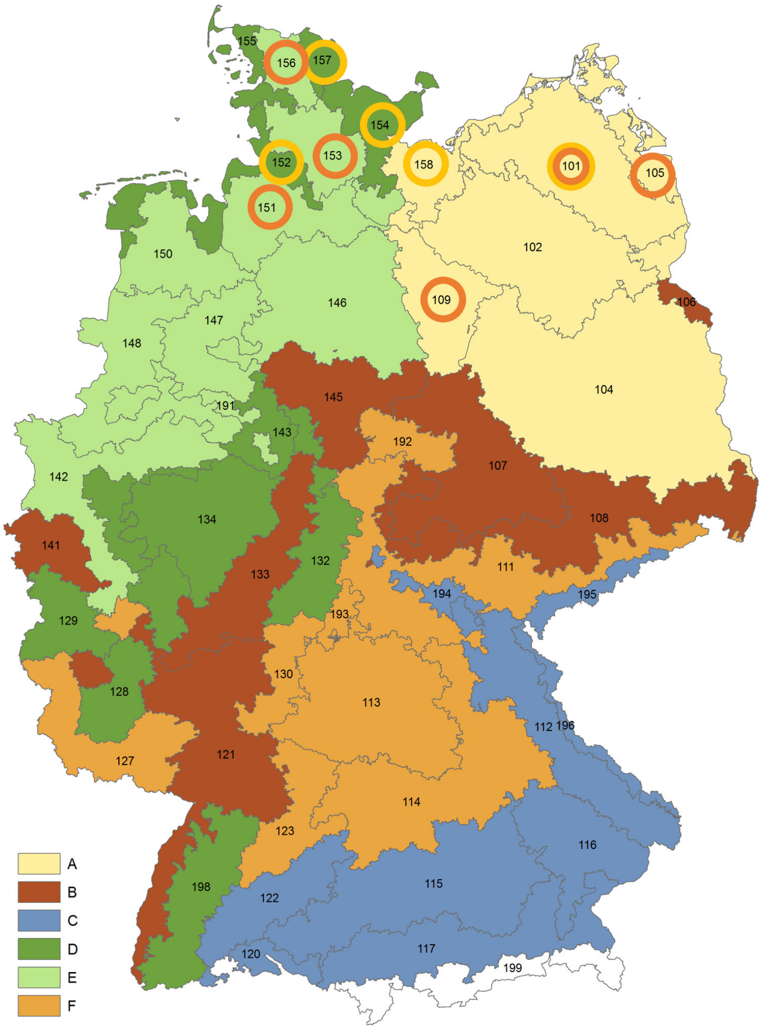

FIGURE 6
CEPI regions **(A–F)** and soil-climate-regions (101-198) (Dachbrodt-Saaydeh et al., 2019). Soil climate regions with elevated 90th percentile acute aquatic (> 0.1) and chronic risk for soil organisms (> 0.5) (see Figure 1) were indicated by yellow and orange circles, respectively.

aquatic risk in this case would increase to ≥ 1 for some SCR and would have to be classified as an elevated risk for the respective SCR.

4.3 Data interpretation and limitations of the study

It is important to note that the magnitude of the calculated risk depends on the level of aggregation. For example, the 90th percentile acute aquatic risk of broadcast spraying was< 1 in all SCR (Figure 1) but was > 1 for 1759 fields in Germany (0.5% of all fields, data not shown). The calculation of the 90th percentile of the field-level ETR for Germany (Figure 2B) yields lower values than the calculation at the SCR level (Figure 2A). However, although the risk is low at the SCR level and very low for Germany it may be elevated at individual fields. This must be considered to avoid misinterpretation of our results.

In the present study random distribution of sugar beet on arable fields conditioned by field size and soil quality is a source of high uncertainty. In future assessment crop categorizations from satellite data could be used (Tetteh et al., 2021) to reduce the uncertainty of random crop distributions as conducted in this study.

The results presented here are pure model calculations. The validation of SYNOPS model has been previously conducted based on measured concentrations of selected pesticides in surface water in Mexico on papaya plantations (Hernández-Hernández et al., 2007), for one catchment in Germany (Strassemeyer et al., 2017), and for several sites in France (Pierlot et al., 2017) and showed in general satisfactory results. However, it is important to note that the environmental conditions at these monitoring sites and the measured substances only represent a small excerpt of the potential model parameterizations (De Baan, 2020) and, therefore, are only a first step in the model evaluation process. Currently, there has not been a comparison between the predicted and measured concentrations in soil and on non-target plants. Thus, further studies are needed to validate the exposure and potential effects on soil organisms and non-target arthropods. In addition, this study did not report risks for non-target plants, because at the time when the assessments were conducted the toxicity endpoints for non-target plants and degradation rates were available (in the pesticide property database) only for 44% and 6% of all herbicides respectively. Thus, toxicological risks for non-target plants associated with different weed control techniques must be determined in future.

5 Conclusions

In this study, toxicological risks of mechanical-chemical and chemical weed control techniques were calculated for 360,848 beet fields over 8 years (2011 - 2018) in Germany using SYNOPS-GIS. The acute and chronic risks for aquatic organisms, chronic risks for soil organisms and acute risks for non-target arthropods were aggregated as 90th percentiles at the level of soil-climate regions and for whole Germany. Additional information is provided by interactive maps available on JKI geoportal for open access. Using this web-application the toxicological risks for different weeding strategies, different regions, aggregation area and aggregation levels can be compared.

Our results indicated that the toxicological risk of conventional broadcast spraying was low for most soil-climate regions and was mostly due to risk for soil and aquatic organisms, whereas the risk for non-target arthropods was negligible. The elevated aquatic risk was mostly caused on fields with close distance to standing water bodies, whereas elevated chronic risk for soil organisms was mostly due to elevated organic carbon content in topsoil, promoting retention of AI in upper soil. The reduced application amounts of conventional herbicides due to mechanical-chemical weed control caused a linear reduction in toxicological risk for aquatic and soil organisms. Since elevated risks for soil and aquatic organisms by broadcast spraying were estimated for northern Germany, the advancement of mechanical-chemical and mechanical weed control methods appears to be especially important in northern Germany but also for the fields with short distance to water bodies or with elevated organic carbon content. However, the mechanical-chemical weed control with a new herbicide, containing two ALS-inhibiting active ingredients (Thiencarbazone-methyl and Foramsulfuron) did not reduce the risk to aquatic organisms compared to the broadcast application of conventional herbicides with other modes of action. This was due to the high toxicity of the two active ingredients in the new herbicide to common duckweed (*Lemna minor*). Therefore, the application of combination of Thiencarbazone-methyl and Foramsulfuron must be limited to fields that are at a sufficient distance from water bodies. Since ALS-inhibiting herbicides are needed in much lower quantities compared to conventional herbicides with other modes of action, their use has a strong reducing effect on the quantity-based EU Harmonized Risk Indicator. This was introduced to measure success in reducing the environmental risks associated with pesticide use. Thus, the results of the present study highlight the limitations of the EU Harmonized Risk Indicator and the need to consider not only quantity but also toxicological risk in future risk assessment approaches.

Data availability statement

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

Author contributions

OF: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing. JS: Conceptualization, Data curation, Methodology, Software, Writing – review & editing. FP: Software, Visualization, Investigation, Writing – review & editing. CR: Data curation, Resources, Writing – review & editing. H-JK: Supervision, Writing – review & editing.

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Conflict of interest

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

Publisher's note

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Combining disturbance and competition to control creeping perennial weeds in a field study on three northern European sites

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Controlling creeping perennial weeds is challenging throughout all farming systems. The present study distinguished and explored three different methods to control them non-chemically: disturbance with inversion, disturbance without inversion, and competition. Focusing on *Cirsium arvense*, *Elymus repens*, and *Sonchus arvensis*, we conducted a field study (2019–2021) at three northern European sites in Germany, Finland, and Norway. We investigated the effects of the control methods ploughing (inversion disturbance), root cutting (non-inversion disturbance), and cover crops (competition) alone. Root cutting was conducted using a prototype machine developed by "Kverneland". Eight treatments were tested in factorial designs adapted for each site. Control methods were applied solely and combined. Response variables after treatments were aboveground weed biomass and grain yield of spring cereals. The control method of ploughing was most effective in reducing weed biomass compared to root cutting or cover crops. However, compared to the untreated control, a pronounced additive effect of root cutting and cover crops occurred, reducing weed biomass (–57.5%) similar to ploughing (–66%). Pooled over sites, the response was species-specific, with each species showing a distinct reaction to both control methods. *C. arvense* was most susceptible to root cutting, followed by *E. repens*, while *S. arvensis* showed no susceptibility. Crop yield losses were prevented compared to untreated plots by ploughing (+60.57%) and root cutting (+30%), but not by cover crops. We conclude that the combination of non-inversion disturbance and competition is a promising strategy to reduce the reliance on herbicides or inversion tillage in the management of perennial weeds.

KEYWORDS

root cutting, ploughing, cover crops, *Cirsium arvense*, *Elymus repens*, *Sonchus arvensis*, sustainable agriculture

1 Introduction

The perennial weed species *Cirsium arvense* (L.) Scop., *Elymus repens* (L.) Gould, and *Sonchus arvensis* L. are widespread in organic and conventional farming systems (Melander et al., 2013; Verwijst et al., 2018; Salonen et al., 2023). All three species thrive through vegetative propagation by either belowground creeping roots (*C. arvense*, *S. arvensis*) or rhizomes (*E. repens*). In this article, the term "roots" is used as a general term for both creeping roots and rhizomes, except when "rhizomes" are addressed explicitly.

In arable farming, creeping perennial weeds require control because crop yield losses occur in all production systems (Buhler et al., 2000; Weber and Gut, 2005). Yield losses are related to perennial weed infestations in general (Hartl, 1989; Brandsæter et al., 2012), or more specifically, with dense stands of *C. arvense*, *E. repens*, and *S. arvensis* reducing crop yield (Behrens and Elakkad, 1981; Melander, 1995; Vanhala et al., 2006).

Creeping perennials are mainly controlled by harrowing and ploughing, or in conventional farming, by applying glyphosate pre-plant as the dominating active ingredient (Håkansson, 2003; Brandsæter et al., 2017). These prevalent control measures are considered to have questionable aspects related to sustainability (Brandsæter et al., 2017; Tavaziva, 2017; Ringselle et al., 2020; Andert et al., 2023). Frequent inversion tillage through ploughing increases the risk of soil erosion and nutrient leaching (Aronsson et al., 2015). Regarding herbicides, it is a general goal to reduce the use not only because of potential environmental and health concerns, but also because of an increasing prevalence of herbicide resistance (Gunnarsson et al., 2017; Chauhan, 2020). Furthermore, glyphosate might face restrictions in the near future (Fogliatto et al., 2020; Tataridas et al., 2022; Triantafyllidis et al., 2023). Hence, controlling creeping perennial weeds without herbicides and intensive tillage would serve both pesticide regulations and environmental concerns.

Non-chemical management of creeping perennials in arable farming follows two general principles: Either disturbing plants or suppressing growth by competition (Weigel et al., 2023). Both disturbance and competition aim to weaken the plants and to reduce the overall infestation level (Håkansson, 2003). However, the methods differ. Disturbance reaches into the soil, affecting creeping roots directly, while competition suppresses creeping roots indirectly. When targeting perennial weeds, cover crops must have the ability to be strong competitors above ground (Bicksler and Masiunas, 2009; Wedryk and Cardina, 2012; Ringselle et al., 2015). Additionally, root competition between crops and cover crops is also important, as evidence suggests that belowground competition is a significant factor in various types of vegetation (Kroon et al., 2003). Disturbance can involve either soil inversion or no soil inversion, leading to the distinction of three different methods: disturbance with inversion, disturbance without inversion, and competition. Any effect of these three control methods will become evident in biannual or longer time periods through changes in the aboveground biomass production of perennial weeds.

Disturbance with inversion (ploughing) fragments creeping roots and buries the fragments to deeper soil layers (Håkansson, 2003). These fragments vary in size from short to long, approximately between 5 and 50 cm (Håkansson, 2003). Fragmentation leads to the depletion of root reserves as it induces root sprouting (Weigel and Gerowitt, 2022). Shoots emerging from buried fragments demand more energy to reach the soil surface (Dock Gustavsson, 1997; Håkansson, 2003). By combining fragmentation and burial, ploughing is an effective and reliable method for controlling perennial weeds (Brandsæter et al., 2011; Melander et al., 2012; Brandsæter et al., 2017).

Disturbance without soil inversion also fragments roots but does not bury them. It is referred to as "root cutting". Ringselle et al. (2018) used a tool vertically slitting the soil and showed that *E. repens* shoot numbers decreased by approximately 30%. In a 2-year field study without a crop, Weigel and Gerowitt (2022) demonstrated that root cutting horizontally slitting the soil six times per year reduced shoot numbers of *C. arvense* by 75%. Although different tools have been used, these two studies indicate that *C. arvense* is more susceptible to cutting roots than *E. repens*. Results on *S. arvensis* are so far missing, but might be similar to *C. arvense* as both species propagate through creeping adventitious roots, while *E. repens* creeps through rhizomes (Lalonde and Roitberg, 1994; McClay and Peschken, 2002; Boström et al., 2013). In addition, the depth of the roots differs. Roots of *S. arvensis* and especially *E. repens* run shallow at depths of less than 10 cm, while the majority of *C. arvense* runs considerably deeper. In conjunction with the choice of the applied root cutting depth, the depth of the roots could be crucial. Root fragments of *C. arvense* resulting from tillage had only a minor role in new shoot development, when these fragments were located above the deeper running and intact root system (Thomsen et al., 2013). Species with a shallow root system (*E. repens* and *S. arvensis*) might be less affected by (deep) root cutting than those with a deeper root system (*C. arvense*).

Competition between weeds and crops is intensified when cover crops close the gap of open soil in the period between two main crops. Included in agronomical concepts, cover crops aim, in addition to other ecosystem services, to reduce perennial weeds (Bakker, 1960; Brandsæter et al., 2012; Brandsæter et al., 2017). Under-sown cover crops alone have been often unable to adequately suppress perennial weeds (Brandsæter et al., 2012; Ringselle et al., 2015; Reimer et al., 2019), except when the cover crops produced a large amount of biomass (Bergkvist et al., 2010).

So far, the combination of disturbance by root cutting with competition by cover crops, as a reasonable concept to work without ploughing, has not been investigated. While ploughing is an established, well-known method inverting the soil, root cutting without inverting the soil is an innovative technology. Field experiments were established on three sites in Northern Europe, in which the factors ploughing, root cutting, and cover crops were combined in eight treatments. The experiments exclusively focused non-chemical weeding. Targeted perennial weed species were *C. arvense*, *E. repens*, and *S. arvensis*. The experiments lasted for two subsequent years to account for biannual effects. We hypothesize that:

1. Root cutting and ploughing reduce weed biomass equally, but reductions vary among *S. arvensis*, *C. arvense*, and *E. repens* (H1).
2. Root cutting controls *C. arvense* more effectively than *S. arvensis* and *E. repens* (H2).

3. Adding cover crops to root cutting or ploughing increases the effectiveness of perennial weed control (H3).
4. All three methods of control by inversion tillage (ploughing), non-inversion tillage (root cutting), or competition effects (cover crops) prevent crop yield losses (H4).

2 Materials and methods

2.1 Study sites

In summer 2019, three field experiments were established, which ran for 2 years until the crop harvest in summer 2021. The experiments were located in Germany (Rostock, 54°01′N 12°14′E, 37 m.a.s.l.), Finland (Ruukki, 64°37′N 25°09′E, 47 m.a.s.l.), and Norway (Ås, 59°40′N 10°47′E, 75 m.a.s.l.). The experiments in Rostock and Ruukki were carried out on a conventional and an organic farm, respectively. The Ås experimental area was certified as organic prior to the experiment but taken out of the certification when starting the experiment. The soil types of the sites were sandy loam (pH = 6.8) in Rostock, fine sand with high soil organic matter (pH = 6.6) in Ruukki and silty clay loam with poor natural drainage in Ås (pH = 5.8), (Table A1). The crop rotations had been dominated by cereal crops (Rostock = Spring wheat, Ruukki = Spring oats, Ås = Spring wheat, spring barley, and spring oat) in the past at all sites. Details on weather conditions can be found in Table A1.

2.2 Site cultivation

All sites featured spring cereals in both consecutive experimental years. These consisted of spring wheat cv. KWS Mistral in 2020 (400 seeds m^{-2}) and Servus in 2021 (410 seeds m^{-2}), both (180 kg ha^{-1}) in Rostock (Seed drill type Rapid, 4 m, Väderstad, Sweden), spring oats cv. Niklas (215 kg ha^{-1}, 550 seeds m^{-2}) in Ruukki (Seed drill type Junkkari 2.5 m, Junkkari Oy, Finland), and spring barley cv. Brage (200 kg ha^{-1}, 500 seeds m^{-2}) in 2020 and spring wheat cv. Mirakel (225 kg ha^{-1}, 600 seeds m^{-2}) in 2021 in Ås (Seed drill type Nordsten 2.5 m, Nordsten, Denmark).

Seedbed preparation differed among sites; before sowing a field cultivator (Cruiser XL, Horsch, Germany), run a single pass (10 cm depth) in Rostock, run a single pass in Ruukki (power harrow, Kuhn, Germany), and there were two passes (6 cm depth) by a rotary harrow (Kuhn, Germany) in Ås. For each site, the fertilization was as follows: Rostock: cattle manure with 75 kg total N ha^{-1}, Ruukki: meat-bone meal (Ecolan Agra 8-4-8 with 40 kg total N ha^{-1}), and Ås: dried chicken manure, added bone meal ("Marihøne Pluss"), [N (8%)–P (4%)–K (5%), respectively], and pelletized fertiliser, with application corresponding to 100 kg total N ha^{-1} in the spring of 2019 and 2020, and 150 kg N ha^{-1} in 2021.

2.3 Treatments

All sites carried the same factors of PL (ploughing), RC (root cutting), and CC (cover crop). A factorial combination of cover crop (with/without), root cutting in spring and autumn (with/without), and ploughing in spring (with/without) resulted in a total of eight treatments:

- Untreated control (UC)
- Cover crop (CC)
- Root cutter (RC)
- Plough (PL)
- Root cutter + cover crop (RC+CC)
- Plough + cover crop (PL+CC)
- Plough + root cutter (PL+RC)
- Plough + root cutter + cover crop (PL+RC+CC)

The treatment operations were repeated annually on the same plots in the period autumn 2019 to crop harvest 2021. Root cutting was done twice and ploughing was done once per year. Table 1 gives details when, how, and where each measure was carried out. Root cutting was done by the "Kverneland horizontal root cutter" (Figure 1). This root cutter is a prototype machine, horizontally cutting and fragmenting belowground root and shoot parts without inverting the soil by using five very flat, wide, and inflexible goosefoot shares (54 cm wide). Depth of root cutting was 20–25 cm in spring and 10 cm in autumn. Ploughing was conducted in spring at 20 cm (Rostock), 23 cm (Ruukki), and 25 cm depth (Ås). During the periods between the cultivation of spring cereals, cover crops of *Sinapis alba* L. (25 kg ha^{-1} both years) in Rostock and a ryegrass + clover mixture in Ruukki (15 kg ha^{-1} in Ruukki both years) and Ås (20 kg and 11 kg ha^{-1} in 2020 and 2021, respectively) were grown (Ruukki: *Lolium multiflorum* var. *italicum* Lam. + *Trifolium repens*; Ås: *Lolium perenne* + *Trifolium repens*).

2.4 Experimental design

The trial in Rostock had a complete randomized block design, while the trials in Ruukki and Ås had a split-block design with ploughing (with/without) on main plots.

In Ruukki, individual plot size (subplots, gross) was 5.0 by 7.5 m. The main plot size (gross) was 30 by 15 m (300 m^2). In Ås, individual plot size (subplots, gross) was 5.0 by 10 m, while the net size was 3.5 by 7.75 m. The main plot size (gross) was 35 by 10 m (350 m^2). In Rostock, plot size varied between 9.56 m^2 and 99.42 m^2. The plot size differed because whole thistle patches were taken as individual plots in Rostock. The whole experimental area covered 50 by 400 m (20,000 m^2).

2.5 Weed assessments

The species *C. arvense*, *S. arvensis*, and *E. repens* were in focus. *C. arvense* was present in Rostock and Ås, and *E. repens* and *S. arvensis* were present in Ruukki and Ås. In Ås, the perennial species *Stachys palustris* and *Vicia cracca* also occurred. The biomasses of all mentioned species added together resulted in the variable *CRPW* (creeping perennial weeds).

TABLE 1 Dates for management and assessment operations in in Rostock, Ruukki, and Ås.

Management and assessment	Rostock			Ruukki			Ås		
	2019	2020	2021	2019	2020	2021	2019	2020	2021
Root cutting (spring, deep)		27 Jan	31 Mar		20 May	18 May		20 Apr	25 Apr
Ploughing[1]		28 Jan	31 Mar		28 May	1 Jun	2 May	20 Apr	28 Apr
Seedbed preparation, ploughed					2 Jun	3 Jun	3 May	22 Apr	29 Apr
Seedbed preparation, no plough.					2 Jun	3 Jun		22 Apr	29 Apr
Sowing cereals		18 Mar	31 Mar		5 Jun	8 Jun	3 May	24 Apr	29 Apr
Sowing cover crop (Ruukki and Ås)					5 Jun	7 Jun	8 Aug	24 Apr	30 Apr
Grain harvesting		20 Aug	31 Aug		9 Sep	9 Sep	6 Aug	20 Aug	26 Aug
Soil cultivation (Rostock)	24 Aug	22 Aug							
Sowing cover crops (Rostock)	5 Sep	15 Sep							
Root cutting (autumn, shallow)	5 Sep	15 Sep		7 Oct	1 Oct		23 Sep	31 Aug	
Grain yield		20 Aug	31 Aug		9 Sep	9 Sep		20 Aug	26 Aug
Weed biomass		19 Aug	30 Aug		15 Sep	13 Sep		13–23 Aug	10–30 Aug
Weed number	8 Jul	8 Jul	8 Jul	29 Jun			2–5 Aug	13–23 Aug	10–30 Aug

At all sites, weed biomass was assessed before crop harvest in 2020 and 2021. In Rostock, weed biomass and shoot densities were assessed in 10 survey areas of 1 m^2 each per plot. In Ruukki, two survey areas of 0.5 m^2 each per plot and in Ås four survey areas of 0.5 m^2 per plot were evaluated. The survey areas were in the same position in both years. Biomass sampling simulated cutting at crop harvest; hence, plants were cut 5 cm above the soil surface. The biomass samples were dried at 70°C for 72 h to determine the dry weight in Ås and Rostock. In Ruukki, air-dry weight was evaluated. Samples were dried in an air flow dryer at 40°C for several days. In the statistical analysis, biomass is always given as dry matter (DM) in g m^{-2}.

2.6 Crop assessments

Plots were combine harvested. In Ruukki and Ås, plot parts just outside the sample areas for weeds were harvested; in Ruukki, these were 1.5 m wide and 7.5 m long (11.25 m^2), while they were 1.5 m wide and 7.75 m long (11.63 m^2) in Ås. In Rostock, a plot harvester (1.5 m width) combined through the center of each patch.

In all three countries, grain samples were dried before storage. In Rostock and Ruukki, grain samples were dried in sacks with warm air. After drying, the grain samples were screened by experimental screening machine. In Ås, the grain yield of the plots was weighed at harvest and dried for storage. Grain

FIGURE 1
The "Kverneland horizontal root cutter"; technical drawing by Kverneland Group Norway (left), picture by Marian Malte Weigel (right).

moisture at harvest, grain weight per hectoliter, and screening percentage were determined. Samples were screened and moisture was measured. The cleaned grain yield was adjusted to 85% dry matter kg ha^{-1}, before statistical analysis.

2.7 Statistical analyses

Data analysis (ANOVA) was conducted using the GLIMMIX package in SAS version 9.4 (SAS Institute Inc.), enabling mixed-effects modeling to accommodate both fixed and random sources of variation. Response variables were perennial aboveground weed biomass (*CRPW*) or crop grain yield. The Tukey–Kramer pairwise comparison was used to determine differences between treatments.

The initial weed densities assessed before onset of the experiments (end of June to early August 2019 depending on site, cf. Table 1) were always tested as a covariate in the analysis of weed biomass as this was a likely source of variation. Response variables were transformed with either ln(w + 1) or square root (w), where ln (·) is the natural logarithm function, to achieve the response variable analyzed being nearly normally distributed with approximately homogeneous variance, and in order to tone down the influence of certain deviant values.

The following mixed linear models were used. The models assumed that all random effects were independent, normally distributed random variables with an expected value of zero, and their respective variances were estimated from the data.

2.7.1 Model 1

This model was used to analyze the response variables perennial weed biomass and grain yield (w) when two (Ruukki and Ås) or all (Rostock, Ruukki, and Ås) sites were combined, and assumed a split-block design. The model included a general mean (μ), main effects of ploughing i, root cutting j, cover crop k, year l and site m, their two-, three-, four-, and five-factor interactions, a linear covariate (x), random effects of block n and plot o, their interactions, and an error term (ε). The covariate was only considered when analyzing weed biomass.

$$
\begin{aligned}
w_{ijklmno} = {} & \mu + \pi_i + \tau_j + \nu_k + \gamma_l + \sigma_m + (\pi\,\tau)_{ij} + (\pi\,\nu)_{ik} + (\pi\,\gamma)_{il} \\
& + (\pi\,\sigma)_{im} + (\tau\,\nu)_{jk} + (\tau\,\gamma)_{jl} + (\tau\,\sigma)_{jm} + (\nu\,\gamma)_{kl} \\
& + (\nu\,\sigma)_{km} + (\gamma\,\sigma)_{lm} + (\pi\,\tau\,\nu)_{ijk} + (\pi\,\tau\,\gamma)_{ijl} \\
& + (\pi\,\tau\,\sigma)_{ijm} + (\pi\,\nu\,\gamma)_{ikl} + (\pi\,\nu\,\sigma)_{ikm} + (\pi\,\gamma\,\sigma)_{ilm} \\
& + (\tau\,\nu\,\gamma)_{jkl} + (\tau\,\nu\,\sigma)_{jkm} + (\tau\,\gamma\,\sigma)_{jlm} + (\nu\,\gamma\,\sigma)_{klm} \\
& + (\pi\,\tau\,\nu\,\gamma)_{ijkl} + (\pi\,\tau\,\nu\,\sigma)_{ijkm} + (\pi\,\tau\,\gamma\,\sigma)_{ijlm} \\
& + (\pi\,\nu\,\gamma\,\sigma)_{iklm} + (\tau\,\nu\,\gamma\,\sigma)_{jklm} + (\pi\,\tau\,\nu\,\gamma\,\sigma)ijklm + \beta \\
& \cdot x + Bn(m) + (\pi B)in(m) + (\tau B)jn(m) + (\nu B)kn(m) \\
& + Po(m) + \varepsilon ijklmno
\end{aligned}
$$

The terms $B_{n(m)}$, $(\pi B)_{in(m)}$, $(\tau B)_{jn(m)}$, and $(\nu B)_{kn(m)}$ are random effects of block n, and its interaction with ploughing i, root cutting j, and cover crop k, respectively. In this model, block n and plot o were nested within site m. $P_{o(m)}$were included to account for the two observations from the 2 years, and the same plot may be correlated.

2.7.2 Model 2

This model analyzed the response variables weed biomass or grain yield in Ruukki or Ås separately and assumed a split-block design. The covariate was only considered when analyzing weed biomass.

$$
\begin{aligned}
w_{ijklno} = {} & \mu + \pi_i + \tau_j + \nu_k + \gamma_l + (\pi\,\tau)_{ij} + (\pi\,\nu)_{ik} + (\pi\,\gamma)_{il} \\
& + (\tau\,\nu)_{jk} + (\tau\,\gamma)_{jl} + (\nu\,\gamma)_{kl} + (\pi\,\tau\,\nu)_{ijk} + (\pi\,\tau\,\gamma)_{ijl} \\
& + (\pi\,\nu\,\gamma)_{ikl} + (\tau\,\nu\,\gamma)_{jkl} + (\pi\,\tau\,\nu\,\gamma)_{ijkl} + \beta \cdot x + B_n \\
& + (\pi B)_{in} + (\tau B)_{jn} + (\nu B)_{kn} + P_o + \varepsilon_{ijklno}
\end{aligned}
$$

The terms B_n, $(\pi B)_{in}$, $(\tau B)_{jn}$, and $(\nu B)_{kn}$ are random effects of block n, and its interaction with ploughing i, root cutting j, and cover crop k, respectively. P_o were included to account for the two observations from the 2 years, and the same plot may be correlated.

2.7.3 Model 3

This model was used to analyze the response variables weed biomass or grain yield in Rostock separately and assumed a randomized complete block design. The covariate was only considered when analyzing weed biomass.

$$
\begin{aligned}
w_{ijklno} = {} & \mu + \pi_i + \tau_j + \nu_k + \gamma_l + (\pi\,\tau)_{ij} + (\pi\,\nu)_{ik} + (\pi\,\gamma)_{il} \\
& + (\tau\,\nu)_{jk} + (\tau\,\gamma)_{jl} + (\nu\,\gamma)_{kl} + (\pi\,\tau\,\nu)_{ijk} + (\pi\,\tau\,\gamma)_{ijl} \\
& + (\pi\,\nu\,\gamma)_{ikl} + (\tau\,\nu\,\gamma)_{jkl} + (\pi\,\tau\,\nu\,\gamma)_{ijkl} + \beta \cdot x + B_n + P_o \\
& + \varepsilon_{ijklno}
\end{aligned}
$$

3 Results

According to our hypotheses, control effects on total aboveground weed biomass were elaborated first. Results of *C. arvense*, *E. repens*, *S. arvensis*, and creeping perennial weeds (*CRPW*) are presented separately, across sites and per site. Yield effects are analyzed for each site. Factorial analyses unravelled the effects of PL, RC, and CC. These results are referred to in those tables and figures, addressing the factors and their interactions, hence the combined effects. Additive effects of the control methods on the response variables are analyzed comparing all designed experimental treatments. These results are referred to in those tables and figures addressing the full treatments. Important percentages (%) of an increase or decrease in the given response variable for a factor (with/without) or a treatment [compared to untreated (UC)] are provided in the text.

3.1 Biomass of Cirsium arvense

Although factor site was not significant (Table 2), differences were still observed in the analysis of the control methods for each site (Table 3). In Rostock, biomass reductions were significant for factor PL (−74.5%), RC (−44.3%), and CC (−35.7%) (Table 3; Figures 2A, B). Disturbance treatments, specifically when including PL, were more effective (−77.9%) in reducing biomass than competition by treatment CC (Figure 3A). By adding methods of competition to disturbance in the treatments, biomass was reduced. In particular, the RC+CC treatment exhibited additive effects, resulting in a −72% reduction (RC = −46.38%, CC = −26.2%). Treatment RC+CC achieved the same effects as treatment PL.

In Ås, the factor PL reduced *C. arvense* biomass (−79.4%) but not significantly, while factor RC (−73.5%) was significant (Table 3; Figure 2C). Factor CC (−18%) significantly reduced biomass. PL*RC interacted negatively; therefore, no additive effect in the treatment PL+RC occurred (Table 3; Figure 2C). In general, all treatments including PL, RC, or CC reduced biomass with no differences between these treatments (Figure 3A). *C. arvense* biomass of PL and RC was lower in 2021 than in 2020 with a significant effect of the year (Table 3).

The effects of factor PL and RC on biomass were more pronounced in Ås than in Rostock (Table 2), underscored by the almost significant interactions Site*RC and Site*PL. A significant three-way interaction of Site*Year*PL is caused as PL was more effective in 2021 compared to 2020 in Ås.

3.2 Biomass of Elymus repens

The effect of factor PL differed strongly between the years. In 2020, PL increased biomass of *E. repens* by +34.3% (Table 3; Figure 2E), while in 2021, PL had a reversed effect, reducing biomass by −23.8%. Notably, across both years, the effect of factor RC was not different to that of PL (Figure 2D). In Ruukki, biomass of *E. repens* was reduced in factor RC by −19%. RC effect was strongest in 2021 with a decrease of −31.7% (Figure 2F) showing a positive interaction Year*RC.

In Ås, factor PL reduced *E. repens* biomass by −82.1%. In 2021, factor CC increased biomass of *E. repens* by +220% (Table 3; Figure 2G). However, compared to no CC, this increase was not significant. The mean biomass of *E. repens* by CC was significantly lower in 2021 than in 2020.

Across Ruukki and Ås, the factor PL reduced biomass by −38.8%. A difference between the two sites is indicated by the Site*PL interaction (Table 2). Effect of factor RC was significant,

TABLE 2 Effects on the aboveground weed biomass (g m^{-2}) of factors PL (yes/no), RC (yes/no), CC (yes/no), site (Rostock, Ruukki, and Ås), year (2020/2021), and their interactions for *C. arvense* (Rostock, Ås), *E. repens* (Ruukki, Ås), *S. arvensis* (Ruukki, Ås), and the sum of all creeping perennial weeds (*CRPW*, including *S. palustris* and *V. cracca* in Ås) (Model 1), ANOVA table, shoot density assessed before crop harvest in 2019 was used as a covariate (cir19/son19/ely19).

Factors (fixed)	*C. arvense*		*E. repens*		*S. arvensis*		*CRPW*	
	Rostock + Ås		Ruukki + Ås		Ruukki + Ås		Rostock + Ruukki + Ås	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Plough (PL)	47.4	**0.001**	19.86	**0.0006**	0.03	0.8648	235.4	**<0.0001**
Root cutter (RC)	13	**0.0021**	9.37	**0.0194**	0.18	0.6788	19	**<0.0001**
PL*RC	22.96	**0.0002**	0.24	0.6334	1.07	0.3139	40.79	**<0.0001**
Cover crop (CC)	3.59	0.0744	1.52	0.2853	1.02	0.3254	7.68	**0.01**
PL*CC	0.16	0.6985	2.86	0.1121	0.45	0.5094	0.51	0.4814
RC*CC	0.13	0.7207	1.85	0.2172	0.83	0.3753	1.2	0.2822
PL*RC*CC	0.08	0.7785	0	0.9557	1.73	0.205	0.99	0.3276
Year	54.49	**<0.0001**	10.37	**0.0023**	11.82	**0.0012**	94.36	**<0.0001**
Year*PL	36.44	**<0.0001**	30.38	**<0.0001**	3.5	0.0676	68.82	**<0.0001**
Year*RC	0.26	0.6098	4.85	**0.0325**	2.34	0.1324	4.8	**0.0318**
Year*PL*RC	3.56	0.0651	0.88	0.3532	0.52	0.4727	2.47	0.1208
Year*CC	0.62	0.434	4.87	**0.0321**	0.74	0.3939	0.29	0.5929
Year*PL*CC	0	0.9598	2.89	0.0954	0.61	0.4377	0.66	0.4176
Year*RC*CC	1.19	0.2812	0.26	0.6095	1.57	0.2156	0.09	0.7676
Year*PL*RC*CC	0.52	0.4733	0	0.9946	1.25	0.2696	0.88	0.3517
Site	3.6	0.0684	63.38	**<0.0001**	1.34	0.2862	40.56	**<0.0001**
Site*PL	1.49	0.2715	33.23	**<0.0001**	3.67	0.1041	109.01	**<0.0001**

(Continued)

TABLE 2 Continued

Factors (fixed)	*C. arvense*		*E. repens*		*S. arvensis*		*CRPW*	
	Rostock + Ås		Ruukki + Ås		Ruukki + Ås		Rostock + Ruukki + Ås	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Site*RC	4.21	0.0551	0.34	0.5816	0	0.9676	1.94	0.1632
Site*PL*RC	0.37	0.5506	6.37	**0.0231**	0.72	0.4056	16.58	**<0.0001**
Site*CC	0.98	0.3366	0.3	0.6141	0	0.9567	6.8	**0.004**
Site*PL*CC	0.69	0.416	1.03	0.3248	0.55	0.47	1.34	0.2738
Site*RC*CC	1.69	0.2105	0.74	0.4179	3.68	0.0711	1.28	0.2949
Site*PL*RC*CC	0.01	0.9439	5.06	**0.0412**	0.1	0.7519	0.5	0.612
Site*Year	4.31	**0.0433**	129.45	**<0.0001**	12.08	**0.0011**	24.02	**<0.0001**
Site*Year*PL	8.4	**0.0056**	1.35	0.2514	0.27	0.6055	5.78	**0.0047**
Site*Year*RC	2.81	0.1003	0.92	0.3427	0.79	0.378	0.7	0.4996
Site*Year*PL*RC	0.09	0.7596	3.38	0.0721	10.09	**0.0026**	0.93	0.399
Site*Year*CC	0.97	0.3304	5.77	**0.0203**	0.01	0.9132	2.21	0.1175
Site*Year*PL*CC	1.46	0.2335	2.62	0.1118	0.16	0.6869	0.75	0.4763
Site*Year*RC*CC	0.7	0.408	0.04	0.8403	0.82	0.3689	0.81	0.448
Site*Year*PL*RC*CC	1.2	0.2785	0.89	0.3495	0.27	0.6049	0.57	0.5699
cir19/son19/ely19/crp19	4.28	**0.0449**	3.83	0.0565	0.75	0.3925	1.78	0.1865
Transformation:	ln (*x*+1)		sqr (*x*)		ln (*x*+1)		sqr (*x*)	

Bold values designate p-values ≤ 0.05.

TABLE 3 Effects on the aboveground weed biomass (g m^{-2}) of factors PL (yes/no), RC (yes/no), CC (yes/no), year (2020/2021), and their interactions for *C. arvense*, *E. repens*, and *S. arvensis* in Rostock (Model 3), Ruukki, or Ås (Model 2), ANOVA table, shoot density assessed before crop harvest in 2019 was used as a covariate (cir19/son19/ely19).

Factors (fixed)	*C. arvense*				*E. repens*				*S. arvensis*			
	Rostock (DE)		Ås (NO)		Ruukki (FI)		Ås (NO)		Ruukki (FI)		Ås (NO)	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Plough (PL)	92.98	**<0.0001**	11.08	0.0798	0.04	0.8462	26.57	**0.0182**	1.45	0.3147	2.6	0.1781
Root cutter (RC)	22.18	**0.0001**	11.53	**0.0075**	11.19	**0.0017**	0.92	0.3805	0.2	0.6664	0.15	0.7063
PL*RC	21.49	**0.0001**	12.2	**0.0083**	3.41	0.0719	0.27	0.6056	0.06	0.812	0.34	0.575
Cover crop (CC)	12.47	**0.002**	0.3	0.599	1.01	0.3897	1.68	0.3099	0.47	0.5116	0.77	0.3972
PL*CC	2.99	0.0983	0.24	0.6343	0.19	0.6666	0.9	0.3504	1.67	0.2301	0	0.9961
RC*CC	0	0.9654	0.27	0.6155	0	0.9691	1.35	0.2952	0	0.9701	5.37	**0.0395**
PL*RC*CC	0.01	0.9168	0	0.9706	4.13	**0.0485**	0.32	0.575	0.41	0.5384	0.77	0.4042
Year	22.22	**<0.0001**	28.31	**<0.0001**	94.11	**<0.0001**	28.61	**<0.0001**	0	0.9822	51.16	**<0.0001**
Year*PL	18.76	**0.0002**	9.9	**0.0044**	20.71	**<0.0001**	2.36	0.135	2.04	0.1662	0.74	0.3975
Year*RC	1.55	0.2254	4.82	**0.038**	7.57	**0.0087**	0.03	0.8612	0.15	0.7052	4.61	**0.042**
Year*PL*RC	3.21	0.0856	2.51	0.1262	0.79	0.3792	2.01	0.1665	5.43	**0.0285**	4.88	**0.0369**
Year*CC	0.25	0.6203	0.04	0.8471	0	0.9615	8.15	**0.0077**	0.2	0.6578	0	0.947

(Continued)

TABLE 3 Continued

Factors (fixed)	*C. arvense*				*E. repens*				*S. arvensis*			
	Rostock (DE)		Ås (NO)		Ruukki (FI)		Ås (NO)		Ruukki (FI)		Ås (NO)	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Year*PL*CC	1.43	0.243	1.34	0.2579	0.02	0.8832	0.87	0.3574	0.5	0.4846	0.02	0.8878
Year*RC*CC	0.14	0.7109	0.07	0.8001	0.15	0.6982	0.45	0.5077	1.67	0.2088	2.27	0.1449
Year*PL*RC*CC	0.58	0.4539	0.14	0.7115	1.03	0.3171	0.02	0.8836	0.13	0.7248	1.61	0.2162
cir19/son19/ely19			4.39	**0.0498**			10.89	**0.0034**	12.73	**0.0021**		
Transformation	No		ln (*x*+1)		ln (*x*+1)		ln (*x*+1)		ln (*x*+1)		sqr (*x*)	

Bold values designate p-values ≤ 0.05.

reducing biomass by −19.2%. CC reduced biomass neither as an individual treatment nor when added to the control methods ploughing or root cutting. In contrast to *C. arvense* biomass, only disturbance reduced biomass of *E. repens*. Both disturbance factors, RC and PL, resulted in lower biomass in 2021 than in 2020, highlighted by Year*RC and Year*PL interactions. Analyzed separately, factor PL had no effect in Ruukki (Table 3).

3.3 Biomass of Sonchus arvensis

In Ås, the interaction RC*CC was significant (Table 3). Treatments PL+RC, RC+CC, and PL+RC+CC reduced biomass between 2020 and 2021 significantly, but not across both years (Table 3; Figure 3C). The interaction Year*RC was significant, because RC increased biomass in 2020 but decreased it in 2021 (Table 3). Additionally, Year*PL*RC significantly interacted, resulting in lower biomass values in 2021 than in 2020 for the combined factors PL and RC (Figure 2H; Table 3).

Analyzing Ruukki and Ås together, no significant effects of factors or any treatments occurred (Table 2). None of the results allow for a conclusive, statistical-based evaluation for *S. arvensis*. An interaction between Site*Year resulted from similar biomass values across the 2 years in Ruukki, unlike the decline observed in Ås in 2021 compared to 2020. Treatment PL+RC+CC was most effective in reducing biomass compared to UC, leading to a non-significant decrease of −55.2%.

3.4 Biomass of CRPW (creeping perennial weeds)

When analyzing *CRPW* data across all three sites, factors PL, RC, and CC, and the interaction PL*RC were significant (Table 2). PL, RC, and CC reduced biomass by −54.4%, −34.5%, and −19.2%, respectively. Owing to the negative interaction of PL*RC, no additive effect occurred for treatment PL+RC (Figure 4). All disturbance treatments reduced *CRPW* biomass compared to UC (Figure 4). In contrast, treatment CC did not reduce *CRPW* biomass compared to UC. Nevertheless, by adding the control methods of RC (−43.6%) and CC (−14.4%), treatment RC+CC resulted in a −57.5% reduction, which was only exceeded by PL+RC+CC (−76.1%). No additive effects occurred for the methods of PL and CC.

In Ås and Rostock, factors relying on disturbance (PL and RC) clearly affected the weed biomass, whereas the effects of CC were most notable in Rostock (Site*CC) (Table 2). In Ruukki, results varied depending on species and year. Solely, RC reduced biomass across all sites. In Ruukki, PL even increased weed biomass in 2020 (Site*PL), while PL across both sites reduced *CRPW* biomass. The year effect was significant with biomass values being lower (−35.2%) in 2021 compared to 2020.

3.5 Grain yield

Considering a significant site effect (ANOVA results not shown; Figure 5, see the scale of the ordinate axis), yield was analyzed separately for each site.

Yields in Rostock were higher in 2020 than in 2021 (Figure 5A; Table 4). Across both years, only factor PL (+28.72%) significantly increased yield. RC and CC did not prevent yield losses. No differences between treatments could be verified. Nevertheless, in 2021, after 2 years of experimental time, treatments including PL (PL+RC, PL+RC+CC) had the highest yield levels.

In Ruukki, yields were lower in both years than on the other sites (Figure 5B). Yields were higher for factors PL (+35.5%) and also RC (+21.5%). In 2021, yields in all disturbance treatments delivered higher yields than in 2020. Treatments including disturbance did not differ from each other.

The overall yield level in Ås was the highest (Figure 5C). In both years, disturbance increased yield levels by factors PL (+59.5%) and RC (+15%) (Table 4). All treatments including PL resulted in higher yields compared to UC (Figure 5C). Treatment CC had the lowest yield in both years, which were even lower than in UC. The highest yields were measured in PL (2020) and PL+CC (2021) treatments (Figure 5).

When comparing sites, only factor PL significantly affected grain yields at all three sites. While RC also increased yields, the effect varied between sites, having no effect in Rostock but in Ruukki and Ås. CC alone did not increase yields at any site but reduced yields in Ås.

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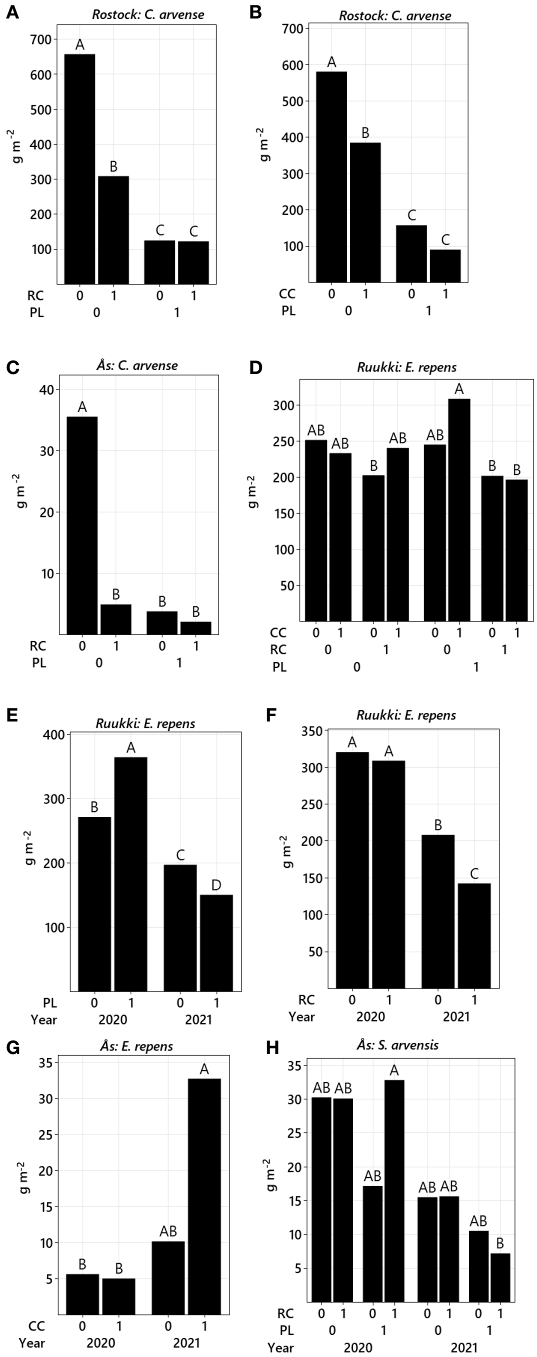

FIGURE 2
Total aboveground biomass (back-transformed LS means) for factors ploughing (PL), root cutting (RC), cover crops (CC), and year. *C. arvense* **(A–C)**, *E. repens* **(D–G)**, *S. arvensis* **(H)**, in Rostock **(A, B)**, Ruukki **(D–F)**, and Ås **(C, G, H)**. Treatments not sharing the same letter are significantly different (p-value $\leq$ 0.05).

4 Discussion

Controlling creeping perennial weeds is challenging throughout all farming systems. Our study explored three different methods to control them non-chemically: disturbance with inversion, disturbance without inversion, and competition. We analyzed perennial weed control effects of these methods: ploughing as inversion disturbance, root cutting as non-inversion disturbance, and cover crops to perform competition. Three species were investigated, being different in their aboveground growth and belowground clonal system. We discuss the results along the hypotheses stated in the introduction.

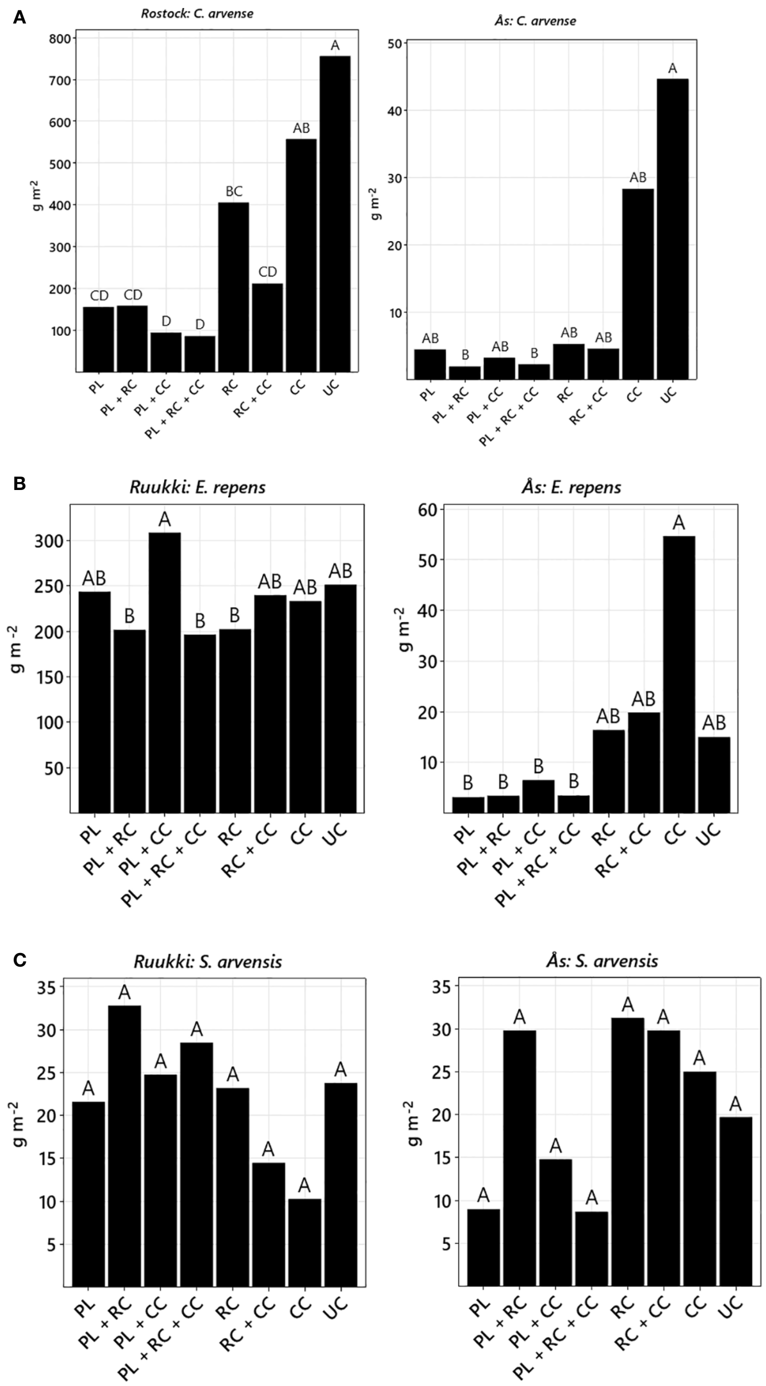

FIGURE 3
Total aboveground biomass (back-transformed LS means) of treatment ploughing (PL), root cutting (RC), cover crop (CC), root cutter + cover crop (RC+CC), plough + cover crop (PL+CC), plough + root cutter (PL+RC), plough + root cutter + cover crop (PL+RC+CC), and untreated control (UC). **(A)** *C. arvense*, **(B)** *E. repens*, and **(C)** *S. arvensis*, mean of experimental years, each site separately. Treatments not sharing the same letter are significantly different (Tukey–Kramer, *p*-value ≤ 0.05).

4.1 Root cutting and ploughing reduce weed biomass equally, but reductions vary among, *C. arvense*, *E. repens*, and *S. arvensis* (H1)

Our experimental setup allows one to directly compare the effects of factors PL and RC on perennial weeds (Tables 2, 3; Figure 2). Pooled over sites, statistical analysis revealed no significant differences in the responses of all three species to ploughing or root cutting, although the reaction varied among species. When separated by sites, PL (79.45%) in Rostock reduced *C. arvense* biomass more than RC (46.38%). In contrast, in Ås, PL did not reduce biomass more than RC. For *E. repens*, RC gave better effects than PL in Ruukki, while the opposite was the case in Ås. For

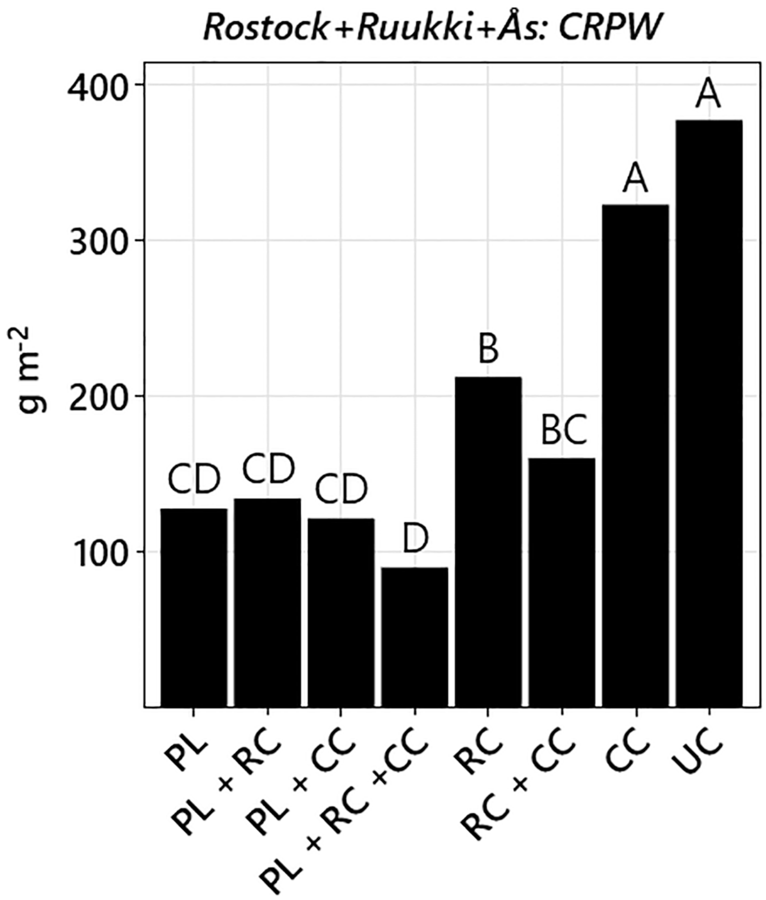

FIGURE 4
Total aboveground biomass (back-transformed LS means across both years and all three sites) of all creeping perennial weeds (*CRPW)* in the treatments ploughing (PL), root cutting (RC), cover crop (CC), root cutter + cover crop (RC+CC), plough + cover crop (PL+CC), plough + root cutter (PL+RC), plough + root cutter + cover crop (PL+RC+CC), and untreated control (UC). Treatments not sharing the same letter are significantly different (Tukey–Kramer, *p*-value ≤ 0.05).

S. arvensis, both factors, PL and RC, achieved poor and similar effects in Ruukki and Ås. Thus, hypothesis 1 is partly accepted, as the results depended on whether the sites are pooled or not. The variation among the species is evidently supported by the results.

To our knowledge, this study stands out as the first to directly compare the common method of ploughing with the innovative root cutting method for perennial weed control. Ploughing has been the standard for managing perennial weeds through tillage. Other non-inversion cultivation techniques, like different harrows or cultivators, did not achieve comparable results unless they are used in higher frequency—at least three times per year (Verschwele and Häusler, 2004; Lukashyk et al., 2008; Brandsæter et al., 2012). The fact that the non-inversion disturbance tool "Root cutter" provided results almost equal to ploughing is remarkable. We value the potential of the "Root cutter" in reducing perennial weed biomass with non-inversion disturbance as highly promising.

Moreover, root cutting offers potential environmental advantages by not inverting the soil. One environmental benefit is that weed control can commence in autumn using RC, thereby postponing PL until the following spring. Other important aspects such as impacts on soil structure, erosion, and energy consumption are yet to be answered in future studies.

4.2 Root cutting controls *C. arvense* more effectively than *S. arvensis* and *E. repens* (H2)

Factor RC reduced biomass of *C. arvense* (−53.8%) and *E. repens* (−19.1%) but not *S. arvensis*. Therefore, hypothesis 2 is accepted. Root fragmentation induces re-sprouting, leading to root reserve depletion (Håkansson, 2003). Different species exhibit varying abilities to re-sprout after fragmentation: Re-sprouting of *S. arvensis* might have been less vigorous compared to *C. arvense* and *E. repens*, and thus, less reserves were depleted especially in autumn. This reluctance to re-sprout, termed bud dormancy, is in contrast between *S. arvensis* and the other two species, with several studies pointing out this difference (Brandsæter et al., 2010; Tørresen and Gerowitt, 2022). Tørresen and Gerowitt (2022) explored the sprouting ability under conditions resembling the Nordic autumn climate, finding that while *C. arvense* needed warmer conditions and *E. repens* sprouted under all/wider conditions, *S. arvensis* did not sprout at all. To some extent, this explains why *S. arvensis*, unlike *C. arvense*, could not be controlled by treatment RC+CC (Figure 3). In contrast to *S. arvense*, *C. arvense* exhibits stronger activity and vegetative growth during late summer

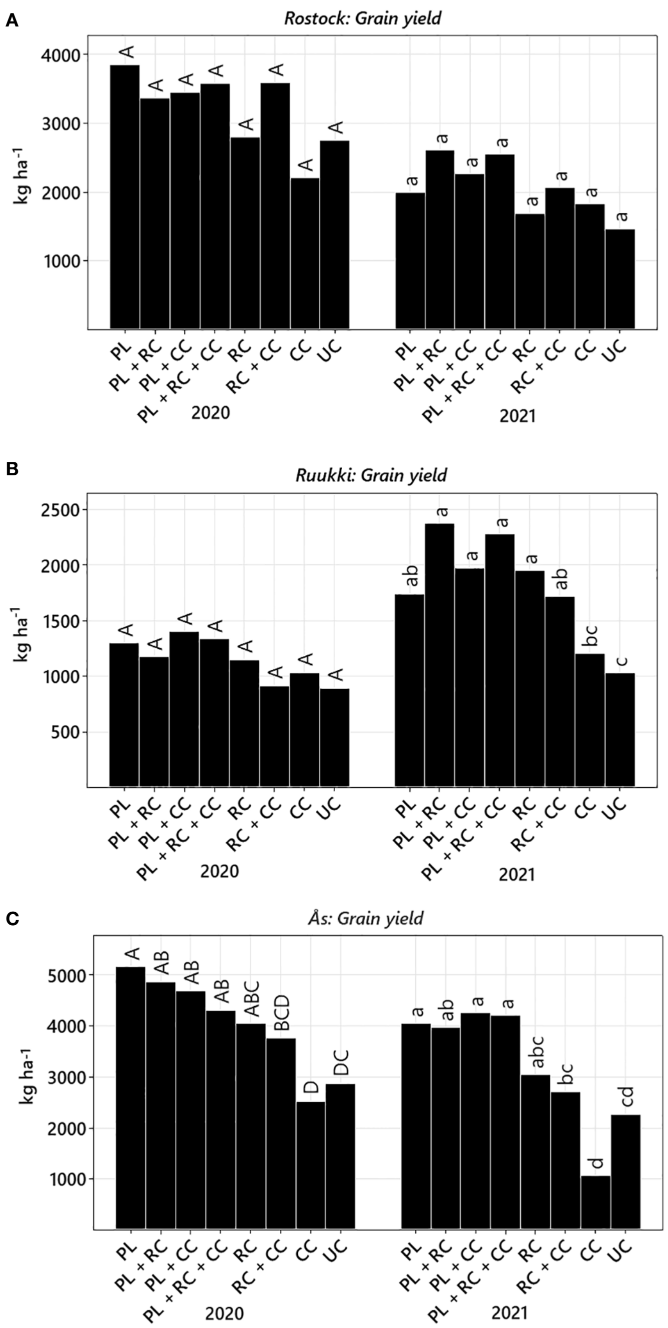

FIGURE 5
Grain yield per year (back-transformed LS means) of treatment ploughing (PL), root cutting (RC), cover crop (CC), root cutter + cover crop (RC+CC), plough + cover crop (PL+CC), plough + root cutter (PL+RC), plough + root cutter + cover crop (PL+RC+CC), and untreated control (UC) in Rostock **(A)**, Ruukki **(B)**, and Ås **(C)**. Treatments not sharing the same letter are significantly different (Tukey–Kramer, p-value ≤ 0.05). Please note the different scale of the ordinate axis.

and autumn. Treatment RC+CC adding competition to disturbance obviously controlled this active growth pattern better than the lagged one of *S. arvensis*. These results emphasize that timing of disturbance should be in accordance with species specific periods of vigorous re-sprouting in future applications of the root cutter.

Typically, spring ploughing is recognized as an effective tillage method for managing *S. arvensis* (Brandsæter et al., 2011). Consistent with our findings, the low efficacy of both disturbance and competition on *S. arvensis* was also observed by Brandsæter et al. (2012).

C. arvense with deep creeping roots and *E. repens* with shallow rhizomes were susceptible to disturbance, while *S. arvensis* with shallow and deep creeping roots was not susceptible. As a result, the sensitivity to disturbance could not be clearly attributed to the type of creeping organ (creeping roots, rhizomes) or to the occurrence of deep or shallow roots.

When explaining the better control of root cutting on *C. arvense* compared to *S. arvensis*, and also to *E. repens*, another factor is probably more important. The depth of root cutting in spring was

TABLE 4 Effects on grain yield (kg ha^{-1}) of factors PL (yes/no), RC (yes/no), CC (yes/no), year (2020/2021), and their interactions for site Rostock (Model 3), Ruukki, and Ås (Model 2), ANOVA table.

Factors (fixed)	Grain yield					
	Rostock (DE)		Ruukki (FI)		Ås (NO)	
	F	*p*	*F*	*p*	*F*	*p*
Plough (PL)	13.57	**0.0014**	25.92	**0.0065**	96.25	**0.0023**
Root cutter (RC)	2.89	0.1038	12.11	**0.0034**	9.9	**0.0118**
PL*RC	0.86	0.3641	1.71	0.2112	33.2	**0.0003**
Cover crop (CC)	0.49	0.4932	0.24	0.6429	4.71	0.0582
PL*CC	0.44	0.5157	0.42	0.5253	2.48	0.1495
RC*CC	1.31	0.2648	2.73	0.1193	0.5	0.4964
PL*RC*CC	0.54	0.4688	0.65	0.4341	1.05	0.3321
Year	43.13	**<0.0001**	163	**<0.0001**	123.07	**<0.0001**
Year*PL	0.15	0.7000	9.72	**0.0047**	6.76	**0.0157**
Year*RC	0.04	0.8513	35.97	**<0.0001**	0.83	0.3723
Year*PL*RC	2.55	0.1235	0.25	0.6230	0.82	0.3753
Year*CC	0.42	0.5214	0.11	0.7386	0.9	0.3527
Year*PL*CC	0	0.9566	0.24	0.6304	15.42	**0.0006**
Year*RC*CC	2.71	0.1125	1.3	0.2662	2.27	0.1448
Year*PL*RC*CC	0.07	0.7888	0.55	0.4674	1.36	0.2551
Transformation	No		ln (*x*)		ln (*x*)	

Bold values designate p-values ≤ 0.05.

20–25 cm in our experiments. Research by Thomsen et al. (2013) demonstrated that due to shoots emerging from the intact root system below normal tillage depth, *C. arvense* was very susceptible to disturbance of its root system caused by deep tilling in the spring or early summer. This is also the reason why deep ploughing (e.g., to 25 cm) in spring controlled *C. arvense* (Brandsæter et al., 2011).

4.3 Adding cover crops to root cutting or ploughing increases the effectiveness of perennial weed control (H3)

All three methods of control significantly reduced *CRPW* biomass in the factorial analysis (Table 2). Most effective in reducing *CRPW* biomass was treatment PL+RC+CC. Among the different control principles, disturbance proved to be more effective than competition in reducing *CRPW* biomass. This observation is consistent with the findings of previous studies in which disturbance tended to be more effective in reducing biomass of perennial weeds than competition (Håkansson, 2003; Brandsæter et al., 2012; Reimer et al., 2019; Salonen and Ketoja, 2020).

Treatments PL and RC both reduced *CRPW* biomass, with PL reducing *CRPW* biomass more than RC. However, when adding CC to RC (treatment RC+CC) the difference to PL became non-significant (Figure 4). Thus, adding competition through CC to RC amplified the control, resulting in pronounced additive effects. In contrast, adding CC to PL did not result in a comparable additive effect (treatment PL+CC, Figure 4). Therefore, hypothesis 3 can be accepted for root cutting and cover crops (RC+CC) but not for ploughing and cover crops (PL+CC).

The evaluated additive effects of the treatment RC+CC might result from the strategy of shallow root cutting (10 cm depth) in autumn and deep cutting in spring (20–25 cm depth). In contrast, treatments with PL were only ploughed in spring. Shallow cutting, which fragments the underground root and shoot parts, induces intensive re-sprouting of *C. arvense* (Weigel and Gerowitt, 2022). Frequently employed, such cutting gradually reduces *C. arvense* infestations. However, the immediate response is a burst of re-sprouting and the emergence of new shoots (Weigel and Gerowitt, 2022). This sprouting depletes root reserves needed to fuel the growth of the new shoots (Håkansson, 2003). While the reserves are depleted, they are accompanied by the emergence of new shoots. These shoots can be then suppressed by cover crops. A dynamic of re-sprouting depleting the reserves and a subsequent control of the emerged shoots can be initiated. As perennial weeds are vulnerable to light competition (Bakker, 1960; Edwards et al., 2000), well-established cover crops post-re-sprouting (via treatment RC+CC) allow to benefit from this dynamic. Unlike RC, adding CC to PL (PL+CC) did not result in enhanced control. Ploughing was conducted in spring, but not in autumn. The dynamic of inducing re-sprouting through disturbance in autumn followed by competition through cover crops was probably precluded through this timing.

4.4 All three methods of control by inversion tillage (ploughing), non-inversion tillage (root cutting), or competition effects (cover crops) prevent crop yield losses (H4)

Only factor PL resulted in higher yields across all sites; thus, disturbance by ploughing ensured the most reliable yields. Factor RC also increased yields; however, the magnitude varied among sites, having no effect in Rostock but having an effect in Ruukki and Ås. Comparing yields between untreated and control treatments indicates that the methods ploughing or root cutting or both prevented yield losses, but not cover crops alone (Figure 5). Therefore, hypothesis 4 is accepted for PL and RC, but rejected for CC. In Ås, even lower yields were measured in treatment CC than in UC. Notably and in contrast to Ruukki, a winter-hardy ryegrass species was used as cover crop. Its ability to survive winters was crucial. On plots without ploughing, the rotary harrow in spring only partially terminated the ryegrass, which survived the winter. Subsequently, the persistent cover crop competed with both perennial weeds and the new cash crop. Brandsæter et al. (2012) investigated the repeated undersowing of clover in spring cereals and showed that the presence of clover in the cereal crop reduced yield by competing with the crop.

5 Conclusions

In general, disturbance proved to be the more effective perennial weed control principle compared to competition, with inversion disturbance by ploughing being the most reliable. With respect to perennial weed control, farmers could simply carry on with ploughing. However, with respect to the undesired effects of ploughing on soil health and energy demand, feasible alternatives to ploughing are available for the management of perennial weeds. In our study, the combination of root cutting and cover crops had strong additive effects controlling perennials as reliable as ploughing. The extent of this dynamic varied slightly between species. We conclude that combining non-inversion disturbance with root cutting and competition can become an effective approach to control perennial weeds without inversion tillage.

Our study directly compared the perennial weed control effects of the traditional method of ploughing and the novel method of root cutting with a pilot prototype machine. Although root cutting showed great potential as an alternative method to ploughing in terms of perennial control, further research regarding important aspects like soil structure, erosion, and energy consumption is required to support its widespread use with facts and data about these crucial issues. The advantages of root cutting compared to ploughing are likely also in these aspects and will further fuel practical implementation. The commercial supply of root cutters is a pre-requisite for this. The widespread use of root cutters in practical farming will then ensure that suitable combinations with cover cropping will be on-farm evaluated and improved.

Data availability statement

Generated datasets are available by request to the corresponding author with a situation-dependent restriction on who can access them. Requests to access the datasets should be directed to marian.weigel@uni-rostock.de.

Author contributions

MW: Writing – original draft. TB: Data curation, Visualization, Writing – review & editing, Formal Analysis. JS: Methodology, Validation, Writing – review & editing. TL: Methodology, Writing – review & editing, Validation. BG: Conceptualization, Formal Analysis, Methodology, Supervision, Validation, Writing – review & editing. LB: Conceptualization, Formal Analysis, Methodology, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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

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Appendix

TABLE A1. Mean monthly temperature and precipitation for Rostock, Ruukki, and Ås during cultivation periods in 2021 and 2021.

	Year	Month	Mean Temperature (°C)	Precipitation (mm)
Rostock	2020	Mar	4.4	24.6
Rostock	2020	Apr	8.8	9
Rostock	2020	May	11.5	27
Rostock	2020	Jun	17.2	100
Rostock	2020	Jul	15.9	48.4
Rostock	2020	Aug	19.5	12.8
Rostock	2020	Sep	13.9	67.2
Rostock	2021	Mar	3.6	55
Rostock	2021	Apr	5	28.6
Rostock	2021	May	10.4	98.4
Rostock	2021	Jun	18.5	33.2
Rostock	2021	Jul	18.3	61
Rostock	2021	Aug	15.8	4.6
Rostock	2021	Sep	14.6	84.4
Ruukki	2020	Mar	-1.6	23.2
Ruukki	2020	Apr	0.5	13.7
Ruukki	2020	May	6.5	31.4
Ruukki	2020	Jun	16.9	36.6
Ruukki	2020	Jul	14.8	165.1
Ruukki	2020	Aug	14	30.2
Ruukki	2020	Sep	10.2	94.9
Ruukki	2021	Mar	-3.3	31.2
Ruukki	2021	Apr	2.1	53
Ruukki	2021	May	7.7	56.5
Ruukki	2021	Jun	16.3	44.6
Ruukki	2021	Jul	18.3	32.3
Ruukki	2021	Aug	13.3	127.2
Ruukki	2021	Sep	7.3	45
Ås	2020	Mar	2.2	n.a.
Ås	2020	Apr	6.4	30.2
Ås	2020	May	9.4	47.2
Ås	2020	Jun	17.6	115.4
Ås	2020	Jul	14.3	127.9
Ås	2020	Aug	16.2	50.6
Ås	2020	Sep	12	81
Ås	2021	Mar	2.3	n.a.
Ås	2021	Apr	4.7	18.2

(Continued)

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TABLE A1. Continued

	Year	Month	Mean Temperature (°C)	Precipitation (mm)
Ås	2021	May	9.6	72.2
Ås	2021	Jun	16.1	34.6
Ås	2021	Jul	18.9	95.4
Ås	2021	Aug	15.3	7.8
Ås	2021	Sep	12.5	75.2

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Effect of cover crops mixtures on weed suppression capacity in a dry sub-humid environment of Argentina

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Cover crops (CC) are increasingly used worldwide within integrated weed management scenarios. They are usually established between two commercial crops and are not harvested, grazed, or incorporated into the soil, but remain on the surface during their growth cycle. The aim of this work was to determine the performance of different CC mixtures and their effects on weed suppression in the south-central region of Buenos Aires province, Argentina. Field experiments were carried out in 2019 and 2020 at the CEI Barrow (MDA-INTA, Tres Arroyos), where both binary and ternary CC mixtures were evaluated. Binary mixtures consisted of winter cereals (*Avena sativa*, *Secale cereale*) and legumes (*Vicia villosa*, *Vicia sativa*) while ternary mixtures were obtained by combining binary mixtures with canola (*Brassica napus*). Weed emergence counting was performed on a 14- day basis to determine weed total density. Vegetation cover of the CC mixtures as well as biomass production from crops and weeds were estimated. The specific composition of the mixtures showed a greater influence on vegetation cover than on biomass production, which would depend mainly on the prevailing environmental conditions. The type of vetch used, the addition of canola, as well as, the proportion of cereals in the mixture determined the CC effect on weeds. CC were consistently more effective in suppressing weed biomass than seedling emergence density. However, weed seedling suppression by CC was similar to or even greater than the chemical-based control ($p<0.0001$). The average biomass of weeds in all CC (pooled data) was highly reduced when compared to the weedy control (12 vs 259 g m^{-2}, $p<0.001$), and similar responses were obtained when compared to the chemical fallow (8 g m^{-2}). The mixture *S. cereal*+ *V. villosa*+ canola stood out for presenting the highest values of productivity and vegetation cover and high weed suppression. These results provide support for the choice of CC mixtures in a dry sub-humid area of Argentina, aimed at maximizing interference with weeds and also to demonstrate their benefits in short- and long-term management. Therefore, CC implementation within crop sequences should be considered as a complementary tool contributing to the development of more sustainable management strategies.

KEYWORDS

service crops, integrated management, weed suppression, emergence density, weed biomass, biomass production, vegetation cover

1 Introduction

Given the need to comprehensively address the problem of weeds in agricultural systems, cover crops (CC) are a useful tool that could be included within integrated weed management (IWM) program. CC are usually established between two commercial crops (Reeves, 1994) and non-harvested, grazed, or incorporated into the soil, but remain on the surface during their growth cycle to improve soil fertility and enhance yields (Ruffo and Parsons, 2004; Scavo et al., 2022). There is a growing interest by farmers and researchers in the adoption of diverse CC mixtures (Groff, 2008; Wortman et al., 2013) considering their ability to offer multiple ecosystem services within cropping systems (Brainard et al., 2011). CC diversification has potential to improve weed management (MacLaren et al., 2019), resulting in a key mechanism to facilitate the transition to the "Agroecological Crop Protection" approach, which promotes the reduction of pest impacts through crop management practices compatible with healthy agricultural and food systems, agroecological principles, and the "one health" concept (Deguine et al., 2023). However, their potential suppressive effect on weeds depends both on the number of species as well as their combination within the mixture (Finney and Kaye, 2017; Suter et al., 2017; Baraibar et al., 2018). So far the use of diverse CC mixtures is relatively incipient and the experimental results are both scarce and inconsistent (Davis et al., 2016; Holmes et al., 2017). Therefore, it is necessary to develop empirical evidence to understand how CC interferes with weeds (Florence et al., 2019). Binary mixtures (BM) (i.e., those formed by two botanical families mainly legumes and grasses) are commonly used as CC due to their high resource efficiency compared to other species or functional groups combinations (Dhima et al., 2007; Hayden et al., 2014). Their suppressive effect on weeds was reported by Akemo et al. (2000) and Hayden et al. (2012). Ternary mixtures (TM) (i.e., formed by three botanical families) may confer additional benefits associated with each component, where the most common include grasses, legumes, and cruciferous. The latter has been less explored concerning weed control (Haramoto and Gallandt, 2004; Björkman et al., 2015; Lorin et al., 2015). Holmes et al. (2017) determined that the exclusion of cruciferous in TM generated an increase in weed biomass, given mainly by their high productivity under the prevailing conditions of interspecific competition. Conversely, Mesbah et al. (2019) observed no differences in weed biomass between both types of mixtures. Therefore, benefits offered by multi-species conjugation versus BM are often considered inconsistent or eventually site-specific regarding weed suppression (Schonbeck et al., 2017).

CC suppress weeds by competition (Ngouajio and Mennan, 2005; Holmes et al., 2017), selective allelopathic activity (Weston, 1996), and physical interference (den Hollander et al., 2007). The allelopathic effect can be species-specific (Norsworthy et al., 2007) and have been reported mainly in grasses such as rye and oats (Kato-Noguchi et al., 1994; Schulz et al., 2013). Therefore, a combination of allelopathic CC might be more effective for a wide range of weeds (Creamer and Stinner, 1997; Wortman et al., 2013). Moreover, since the suppressive effect exerted by CC involves a combination of mechanisms, their suppressive effect on weed would likely depend on the CC specific composition, as well as on the site-specific environmental conditions, cultural practices, and the weed community present (Liebman and Dyck, 1993; Hayden et al., 2012; Baraibar et al., 2018). Therefore, species mixtures are expected to exhibit diverse and complementary suppression mechanisms (physical and chemical) (Baraibar et al., 2017; Schappert et al., 2019), which would increase the suppression capacity (Brainard et al., 2011; Schipanski et al., 2014; Finney et al., 2016).

CC biomass production is often used as an indicator of the capacity to suppress weeds (Brennan and Smith, 2005; Wayman et al., 2015) due to competition for resources (Finney et al., 2016). Also, successful and highly productive CC need a fast initial growth to reach the highest leaf area index to maximize solar radiation interception (Elhakeem et al., 2021). This is why the vegetation cover is used as a parameter to evaluate weed suppression as it correlates negatively with the dry weight of the weeds (Kruidhof et al., 2008; Uchino et al., 2011). The information about the quantity of biomass produced by a species mixture, specifically about to the contribution of each species to the total biomass is scarce (Davis et al., 2016), since many species have been evaluated in monocultures (Holmes et al., 2017). This is why the development of mixtures that favor beneficial interactions for the control of spontaneous vegetation is of great relevance for the design and reproduction of sustainable cropping systems (Brooker et al., 2021). It is also unclear whether the high CC productivity of grasses and legumes can be further improved by including additional functional groups based on eco-physiological traits that show another pattern of productivity in time and space, such as cruciferous (Cong et al., 2018). In addition, studies on species interactions can be useful for a better understanding of the process and consequently improve biomass production (Wendling et al., 2017). In CC mixtures it would be expected that the diversity in the form of growth would allow to creation of a more complete canopy cover to restrict the availability of light for weeds: grasses and cruciferous grow upright, while legumes make them prostrate or extended (MacLaren et al., 2019). The spatial and temporal complementarity of biomass production between CC components can be a useful tool to increase efficiency in the capture of resources to the detriment of weeds (Döring et al., 2012; Finn et al., 2013). Grasses and cruciferous tend to suppress effectively through rapid growth and high biomass production (Brennan and Smith, 2005; Brainard et al., 2011; Hayden et al., 2012; Dorn et al., 2015; Finney et al., 2016), while legumes grow more slowly and are less competitive (Hayden et al., 2012; Lawson et al., 2015).

In Argentina, the lines of research related to the effects of CC on the physicochemical properties of the soil focused mainly in the availability of nitrogen and/or water (Capurro et al., 2012; Restovich et al., 2012; Cazorla et al., 2012; Vanzolini et al., 2013). Whereas, its effect as part of the IWM has been addressed more recently and to a lesser extent, addressing weed communities present at a specific time of the cycle or in the residues mainly during the onset of commercial crops (Baigorria et al., 2013; Miranda et al., 2014; Acciaresi et al., 2016; Kahl et al., 2016; Lobos et al., 2019). Few approaches characterize weed emergence dynamics throughout the CC cycle to evaluate the suppressive effect in different stages (Buratovich and Acciaresi, 2019). It is essential to determine the dynamics of weeding through the diversity of species and their abundance for a better understanding of the processes that regulate crop-weed interactions, thus facilitating the incorporation of IWM-based practices (Buratovich and Acciaresi, 2017). Likewise, the latest ReTTA (ReTTA Relevamiento de Tecnología Aplicada, 2021) determined that the use of CC in Argentina quintupled in the last 5 years and, that this greater implementation was mainly based on the search for a solution against weeds that are difficult to control.

Based on the previous statements, it is necessary to develop further studies to understand how CC influences weed suppression. Novel information is required to support the choice of the best CC species for weed management and to increase knowledge of the behavior of mixtures under variable environmental conditions.

The objective of this contribution was to study the performance of different CC mixtures (productivity and vegetation cover) on weed suppression during two successive periods in the south-central region of Buenos Aires, Argentina. The hypothesis were that (1) the specific composition of the CC mixtures affects the productivity and vegetation cover; (2) binary and ternary mixtures studied as CC interfere with both seedling emergence dynamics and growth of autumn-winter-spring (A-W-S) weeds; (3) the level of weed suppression by CC mixtures is influenced by the level of biomass production and plant cover generated.

2 Materials and methods

2.1 Experimental design

Field experiments were carried out during 2019 and 2020 at the Chacra Experimental Integrada (CEI) Barrow (Tres Arroyos, Buenos Aires, Argentina; 38° 20” S; 60° 13” W).

CC binary mixtures (BM) consisted of winter cereals (*Avena sativa* or *Secale cereale*) with legumes (*Vicia villosa* or *Vicia sativa*), while ternary mixtures (TM) combined BM with canola (*Brassica napus*) (Table 1). Sowing rates were determined based on previous works (see Hayden et al., 2014; Baraibar et al., 2017; Finney and Kaye, 2017; Holmes et al., 2017), along with the contributions and recommendations of seed suppliers, producers, and researchers of the region. Since early sowing is pointed out by other authors (Baigorria et al., 2011; Baraibar et al., 2018) as the most recommended practice to maximize biomass production, the CC were seeded on March 21th (2019) and March 16th (2020). Before planting, all legumes were treated with *Rhizobium leguminosarum biovar viciae* at a dose of 200 cm^3 50 kg^{-1}. Two control treatments were used as reference: weedy plots (W, without control) and chemical fallow (CF, with non-selective herbicide). In the latter, two glyphosate LS 60% (1.8 L ha^{-1}) applications were performed at different times depending on the composition of the weed community and the relative abundance of each species.

In the entire trial area for the two study years, the predecessor crop was wheat (*Triticum aestivum*), which was harvested in December to simulate a sequence of crops typical of the region under study. In 2019, crop sowing was carried out under conventional tillage using a disc harrow and a field cultivator. In 2020 a non-tillage system was applied and fallow consisted of an application of glyphosate LS 60% (1.8 L ha^{-1}) days before the sowing. The sowing depth was calibrated to 1-2 cm, as it is a recommended value for both large and small seeds (Murrell et al., 2017). The distance between furrows was 20 cm and the planting density was variable depending on the type of mixtures (Table 1). A completely randomized block design with four replicates was used. The experimental units (EU) consisted of 3 m wide by 6 m long plots (18 m^{-2}). The evaluated treatments consisted of eight CC mixtures and two controls. Conforming a total of 40 with a 720 m^{-2} net experimental area (Figure 1).

According to Soil Taxonomy (USDA, 1975), soil belongs to a “Tres Arroyos” series, with original material based on loess sediments and classified as Paleudol petrocalcic. These soils are characterized by having a horizon profile: Ap/A (0-22cm) and BA (22-32 cm) loam-clay-sandy with subangular block structure, Btn (32-75 cm) with a clayey texture and coarse prism structure. At 75 cm is the petrocalcic horizon (INTA, 2014). Chemical soil analyses at a depth of 0-20 cm, for both years at the study site before sowing, indicated adequate conditions for the correct development of the crops: acidic pH, medium to high values of organic matter and phosphorus, although with a low nitrate content (Table 2).

TABLE 1 Seeding rate of each species used for mixtures of binary or ternary cover.

Botanical family	Latin name	Common name	Cultivar	Seeding rate	
				kg ha^{-1}	pl m^{-2}
Poaceae	*Avena sativa*	Oats	Sureña	30	105
	Secale cereale	Rye	Ricardo INTA	20	64
Fabaceae	*Vicia villosa*	Hairy vetch	Ascasubi INTA	20	49
	Vicia sativa	Common vetch	Hilario INTA	40	52
Brassicaceae	*Brassica napus*	Canola	Hyola 830 CC	3	71
2-species: **binary mixtures**	Oats+ Hairy vetch **(OHV)**				
	Oats+ Common vetch **(OCV)**				
	Rye+ Hairy vetch **(RHV)**				
	Rye+ Common vetch **(RCV)**				
3-species: **ternary mixtures**	Oats+ Hairy vetch+ Canola **(OHVC)**				
	Oats+ Common vetch+ Canola **(OCVC)**				
	Rye+ Hairy vetch+ Canola **(RHVC)**				
	Rye+ Common vetch+ Canola **(RCVC)**				

2.2 Meteorological data

The study area is characterized by a dry sub-humid water regime with an average annual total rainfall of 757.8 millimeters (1938-2014 series), being spring and part of autumn the most rainy seasons, while winter the driest. The average annual temperature is 14.9°C, with the warmest month being January and the coldest July (Borda, 2016). Weather data in the study were recorded at the meteorological station of CEI Barrow located in the same experimental site (38° 20” S; 60° 13” W). In 2020, the total

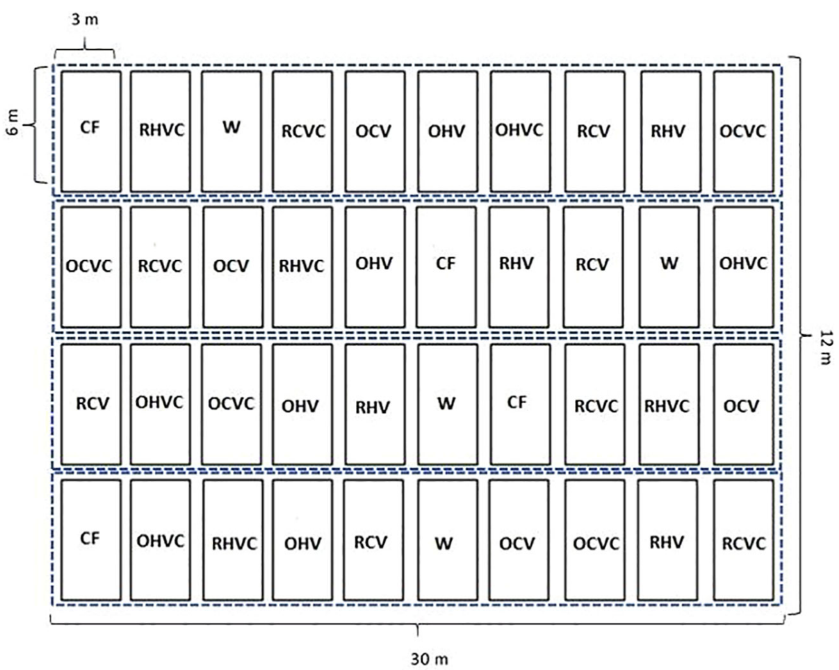

FIGURE 1
Experimental design in complete randomized blocks with four replicates (indicated by the blue dotted line) with the measurements of each experimental units and approximate measurements of the entire test (not counting borders and paths). The experimental units are represented and the acronyms indicate randomly assigned treatments in each block: CF (Chemical fallow), W (Weedy fallow), OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola).

TABLE 2 Results of the chemical analysis of soil (pH, organic matter, nitrates and phosphorus) for the year 2019 and 2020.

Parameter/ Year	2019	2020
pH	6.26	6.4
Organic matter (%)	4.07	3.64
Nitrate (ppm)	7.4	9.6
Phosphorus (ppm)	20.46	25.13

rainfall recorded during the CC cycle was 54% higher than in 2019, the latter showing a deficit of 109 mm compared to historical values. Likewise, the latter showed a longer period of minimum temperatures below the historical value (March, July, and September) and earlier occurrence of frosts in the cycle, compared to the year 2020 (Table 3).

2.3 Data collection

To evaluate the first hypothesis, in the middle of October, vegetation cover percentages (% VC) of CC mixtures were estimated by taking three digital photographs (0.25 m^2 each) per EU. The total number of photographs taken was 120, further processed with the CobCal v 2.1 software. Biomass of both CC mixtures and weeds was determined by harvesting the aerial vegetation during mid-spring present in 0.5 m^2, resulting from the sum of the biomass contained in 2 quadrats of 0.25 m^2 randomly distributed in each EU. Aerial dry biomass values of the different components of the mixtures were obtained after oven drying at 65°C for a week.

To test the second hypothesis, both biomass and density of weed individuals were evaluated. In both years, weekly destructive seedling counts of autumn-winter-spring (A-W-S) species were performed in randomly distributed 0.25 m^{-2} frames (n=4). Total density (pl m^{-2}) was determined as the sum of seedlings that emerged throughout the CC cycle. In order to characterize the weeds surveyed, the abundance (%) and average frequency of each weed species were assessed. The total number of frames evaluated was 40, one for each EU. Data were represented for those species with abundance or frequency ≥10%.

2.4 Statistical analysis

An analysis of variance (ANOVA) was performed to evaluate the effect of the CC treatments on each study variable. Data were transformed to improve homoscedasticity if necessary. Fisher's least significant difference test ($p \leq 0.05$) was used for mean comparison. Statistical analysis was performed using Infostat® software (Di Rienzo et al., 2014).

To evaluate the third hypothesis, a linear regression analysis was performed between each variable measured on the CC (vegetation cover and dry biomass) and the variables of the weed (dry biomass and density).

For all the studied parameters, scatter plots of the observed vs predicted residues were analyzed to assess compliance with the model's assumptions (normality, homoscedasticity, and independence). Residual plots indicated that the variances were normally distributed and homogenous. At this point, the total emergence density (pl m^{-2}) and the emergence 85 days after planting (DAP) in 2020 of A-W-S and dicotyledons weed species data, were transformed into a log(x) and square root (x+1) to comply with homoscedasticity of variance and normality of data.

3 Results

3.1 Biomass production and vegetation cover of CC mixtures

The average biomass production of the CC was 39% higher in 2020 compared to 2019 (9360 vs. 5678 kg ha^{-1}) (Figure 2). In 2019, aboveground biomass was 54% higher in the TM formed by oats or

TABLE 3 Record of average maximum and minimum temperature (T°), days with frost and precipitation (mm) during the CC cycle for the years 2019, 2020 and the historical average of the area (1939-2019 series).

Month	Maximum T°			Minimum T°			Days with frost			Precipitation (mm)		
	2019	2020	Historical	2019	2020	Historical	2019	2020	Historical	2019	2020	Historical
May	24.4	29.6	24.9	10.9	14.7	11.3	0	0	0.1	51.4	81.2	82
April	23.8	21.0	20.6	8.4	8.5	7.7	1	0	1.3	27.2	109.5	67
May	17.7	16.9	16.4	5.5	7.9	5.2	6	2	4.2	61.2	45.2	54
June	15.2	14.1	12.9	5.3	3.8	2.7	4	5	8.5	50.5	153	42
July	13.9	12.0	12.5	1.3	2.3	2.1	15	13	10.1	12.4	62.9	41
August	16.5	16.4	14.5	2.4	3.0	2.6	14	13	8.9	11.1	20.6	42
September	18.2	18.1	17	3.5	3.4	4.2	7	10	5.9	42.2	37.1	53
October	19.0	20.5	19.8	6.1	6.2	6.7	1	5	2.2	88.8	83.8	71
Total										234	505	452

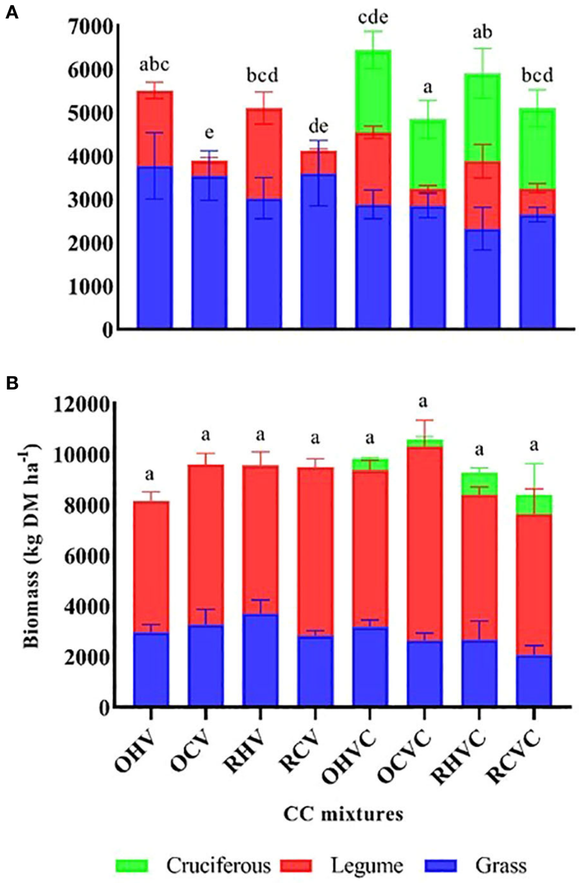

FIGURE 2
Production of total aerial biomass (kg DM ha^{-1}) of the different CC mixtures and each component: grass (in blue), legume (in red) and cruciferous (in green), for the years 2019 **(A)** and 2020 **(B)** in different treatments: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola). The bars represent average values and different letters indicate significant differences among treatments in Fisher's LSD test (p<0.05).

rye + hairy vetch compared to rye or oats + common vetch (p=0.0006) (Figure 2A). Likewise, hairy vetch showed higher productivity compared to common vetch in all mixtures (p=0.0114). In 2020, no differences were observed in biomass production between CC treatments (Figure 2B). However, it is important to note that rye or oats + hairy vetch were among the mixtures with the highest biomass production in both years, with average values of 6168 and 9551 kg ha^{-1}.

Regarding the composition of the mixtures, a negative relationship (p<0.0001) was found between the percentage of legume and grass (r= -0.71) of the mixture (Figures 3A, C). The contribution of each component to the total biomass varied between years. In 2019, the proportion of grasses was higher, but mixtures with hairy vetch were balanced, while in 2020 legumes were dominant. In 2019, the incorporation of canola in the BM negatively affected the biomass of both grasses and legumes (Figures 2A, B), although in the joint balance, the contributions of aerial biomass of canola exceeded this depression. A negative relationship (p=0.005) was established between the % of grass and the % of canola (r=-0.48) in the mixtures (Figure 3B). In contrast, canola contributed very little biomass to the TM in 2020, which could be due to establishment failures caused by hare damage that reduced the plant stand at the beginning of the cycle.

Regarding VC, in 2019 the TM based on rye + hairy vetch presented the highest values (p<0.0001), followed by the BM oats or rye + hairy vetch and the TM oats + common or hairy vetch. While the BM made up of hairy vetch presented the lowest VC percentage with higher values for the rye-based mixture (Figure 4A). Hairy vetch presented higher (p<0.0001) VC than common vetch in all the mixtures evaluated and the addition of canola (p=0.0001) increased the VC of the BM. In 2020, the TM formed by rye + common or hairy vetch presented higher VC (p=0.0006) compared to all the evaluated mixtures, except for the BM rye + common vetch which did not present significant differences (Figure 4B). An effect of grass

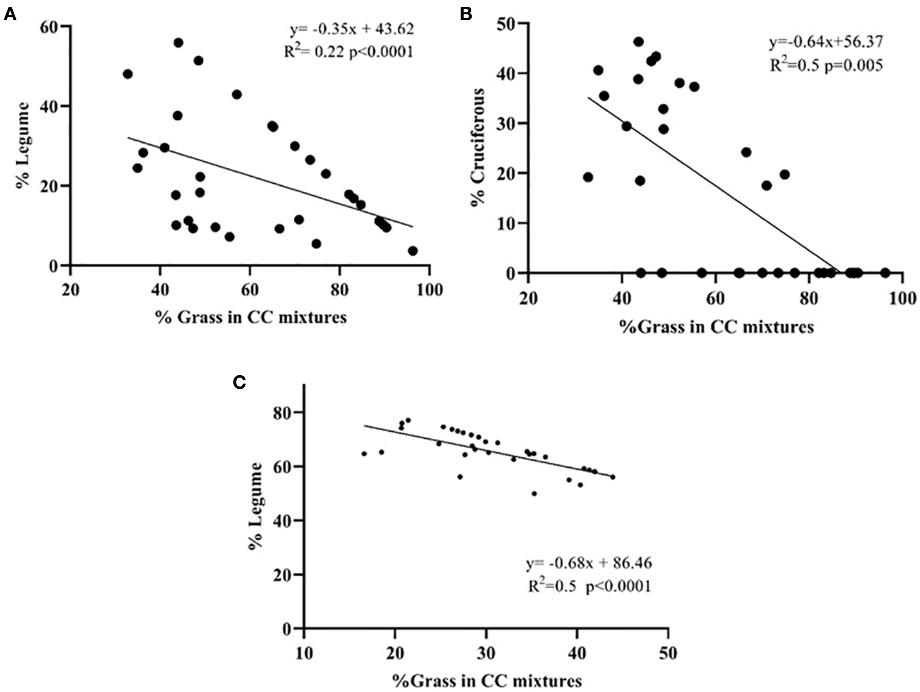

FIGURE 3
Linear relationships between the percentage (%) of grass in the CC mixtures and the resulting legume and cruciferous percentage for the years 2019 **(A, B)** and 2020 **(C)**. The points indicate the biomass production of the groups of botanical families for the different CC evaluated.

was also observed (p=0.0001) and, as in the first year of study, of the canola aggregate in SM (p=0.01). At this point, rye generated higher VC than oats and TM had higher VC than BM (Figure 4B).

The average VC was higher in 2020 than in 2019, highlighting that, in the second year, all mixtures achieved VC > 90%. While, in 2019, the maximum value of VC reached 63%, thus suggesting that the low rainfall regime was a limiting environmental factor.

For both years negative relationships were found between VC and the % of grasses in the CC (r=-0.44 and -0.36). VC decreased (p=0.01 and p= 0.04) when the proportion of grasses in the mixtures increased (Figures 5A, B). This variable also showed a positive correlation with the % of legumes (r=0.45) and Canola (r=0.62), for the years 2019 (p=0.01) and 2020 (p=0.009) respectively. This would determine that the proportion of legumes and cruciferous plants was important (p=0.01 and 0.009) to increase VC (Figures 5C, D).

3.2 Characterization of the weed species surveyed

The A-W-S weed surveyed during 2019 and 2020 consisted predominantly of annual dicotyledonous species. Under conventional tillage (2019), *Anagallis arvensis* presented the highest relative abundance and average frequency of occurrence in all CC and sampling dates, followed by *Conyza sumatrensis* and *Polygonum aviculare* (Table 4). Under no-tillage (2020), *P. aviculare* showed the highest relative abundance followed by *C. sumatrensis*. In turn, these had the highest average frequency of occurrence while *A. arvensis, G. spicata, Cirsium vulgare* and *Lolium* spp. showed the lowest values.

P. aviculare and *C. sumatrensis* were among the most abundant weeds regardless of the period considered. The variation found in the relative abundance of weed species surveyed between study years could be due to the differences between tillage systems and/or the contrasting rainfall regime between years.

3.3 Weed emergence density

In 2019, no significant differences were observed between CC and the control treatments in A-W-S weed emergence density. Also, no significant differences between the different types of CC mixtures (Figure 6A). In part, this could be due to the limiting water conditions prevailing this year (Table 3).In contrast, in 2020, all the CC mixtures suppressed weed emergence by 88-98% (p<0.0001) compared to chemical fallow and weedy plots, respectively (Figure 6B). Also, it is important to note that in both years, the CC generated an early suppression of weed emergence. As showed in Figure 7A, in 2019, at the beginning of the cycle (53 DAP), a greater emergence of A-W-S weeds (p=0.009) was observed in weedy plots compared to CC (polled data) (48 vs 17 pl m^{-2}). In addition, no significant differences were observed between most CC mixtures and the chemical fallow, with the exception of BM oat +

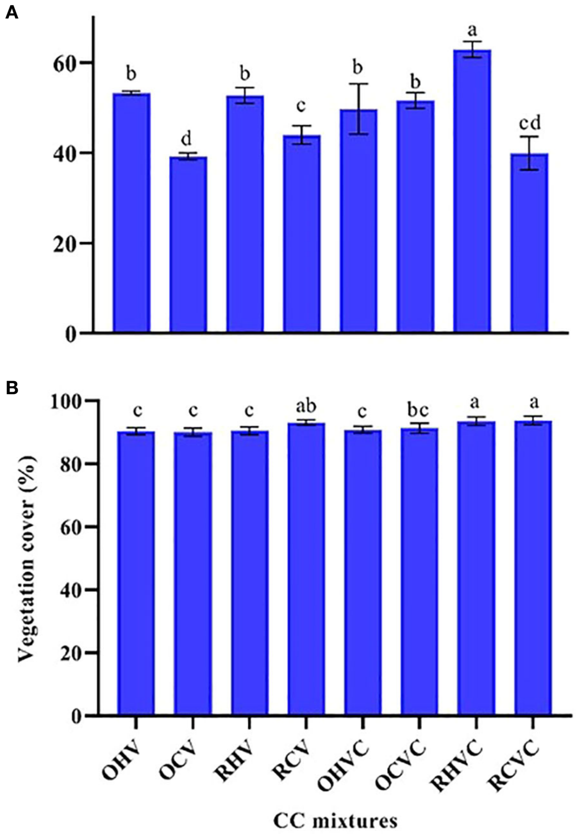

FIGURE 4
Vegetation cover (%) of the different CC mixtures: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), for the years 2019 **(A)** and 2020 **(B)**. The bars represent mean values and the same letters indicate non-significant differences among treatments determined by Fisher's LSD test (p<0.05).

hairy vetch which presented the greatest emergence (p=0.01), comparable to weedy plots (Figure 7A). In 2020 (85 DAP), both controls showed a greater emergence of A-W-S weeds (p=0.0007), which were mainly dicotyledonous species, compared to the CC (polled data) (30 vs 9 pl m^{-2}) and the BM rye + hairy vetch was the mixture with the lowest emergence (p=0.04) of weeds (Figure 7B). No significant relationship was observed between CC biomass production (or vegetation cover) and weed emergence density (p=0.6).

3.4 Weed biomass

CC mixtures reduced weed biomass by 94.5 and 98% compared to the weedy plots for 2019 and 2020, respectively (Figure 8). These values were comparable to those obtained under chemical fallow and, in general, all mixtures showed low biomass levels except oats-hairy vetch and rye-common vetch BM in 2019. In terms of differences between CC mixtures, for the first year, the oats-hairy vetch BM presented higher biomass (p<0.0001) compared to the rye-common vetch TM, the BM, and TM based on rye-hairy vetch and the TM made up of oats-common vetch. Likewise, BM showed higher biomass (p=0.0468) compared to TM (Figure 8A). In 2020, the TM based on rye-hairy vetch and the BM of the same species with common vetch showed a higher biomass of spontaneous plants (p<0.0001) compared to the oats- common vetch BM (Figure 8B). Rye had a higher biomass (p=0.0325) compared to oats (Figure 8B). No significant relationship was observed between CC biomass production (or vegetation cover) and weed biomass (p= 0.67 and 0.15).

4 Discussion

Results obtained in this contribution suggest that the composition of the CC mixtures would have a greater influence on total biomass production in years with a limited rainfall regime. Under such environmental conditions, the addition of canola to the CC mixture showed a tendency to increase productivity, while *V. villosa* showed greater stability compared to *V. sativa*. This could be explained by the differential behavior of both types of vetch, since *V. villosa* is more tolerant to prolonged periods of water deficit and low temperatures (Renzi, 2013; Renzi et al., 2019). For both years of study, mainly the type of vetch used, the addition of canola in BM, and the proportion of grasses affected the vegetation cover. This reveals the strong competitive capacity of grass species which must

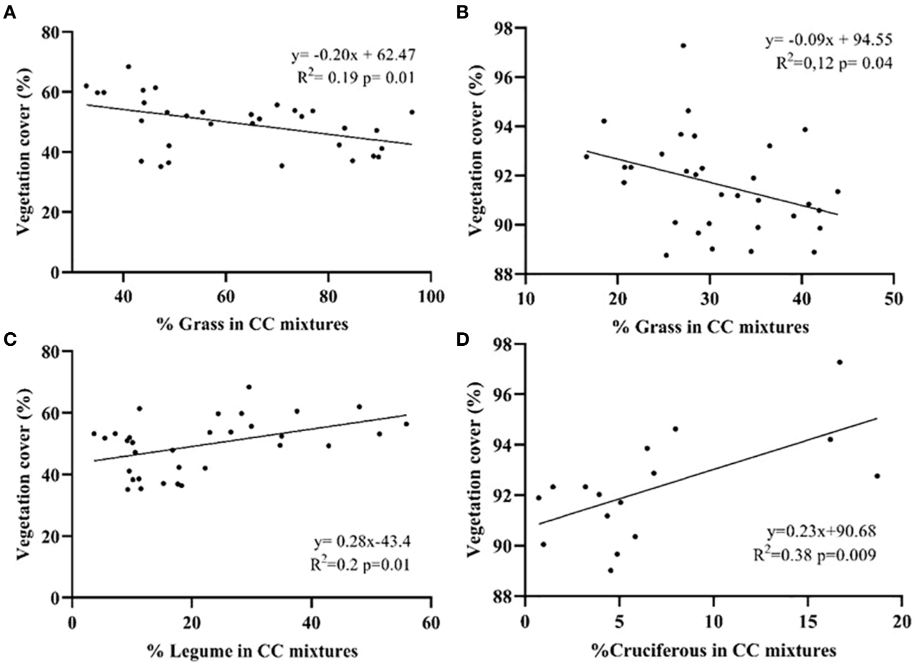

FIGURE 5
Linear relationships between vegetation cover (%) at the end of the cycle based on the % of grass for the years 2019 **(A)** and 2020 **(B)**, legume and cruciferous in the mixtures for the years 2019 **(C)** and 2020 **(D)**. The points indicate the vegetation cover generated for the % of grass, legume, or cruciferous in the different CC evaluated.

be taken into account when designing CC mixtures if the goal is to achieve a good diversity of its components. Grass seedling rates should be reduced by half to a quarter in a mixture (compared to monocultures) to achieve a balanced ratio with legumes, which tend to be weak competitors and must be planted at the same densities as monocultures to ensure establishment (White et al., 2016). In addition, the vertical orientation of the grass leaves would allow the passage of light through the upper strata (Elhakeem et al., 2021). Therefore, it is important to add other species to the mix with different strategies for using space, mainly those species with horizontal canopy architecture (MacLaren et al., 2019). Thus, the specific composition of the CC mixtures would have a greater influence on the vegetation cover than on the total biomass production, which would depend mainly on the prevailing environmental conditions, confirming the first hypothesis. The environmental conditions played a pivotal role in the results as indicated by the differences observed between years.

In this contribution we found evidence of an early suppressive effect of the CC mixtures on the emergence dynamics and growth of A-W-S weeds (Figures 6–8). Weed suppression values (66 and 94%) were similar to those provided by chemical methods (see Teasdale and Mohler, 1992; Osipitan et al., 2018) and are considered sufficiently high to prevent seed bank replenishment (Liebman and Nichols, 2020). In addition, an early weed emergence reduction would clearly decrease weed-crop competition (Hock et al., 2006). Based on these results, we decided to accept the second

TABLE 4 Average frequency of occurrence (%) and abundance (%) of the different autumn-winter-spring (A-W-S) and autumn-winter (A-W) weed species: surveyed in all mixtures of CC and controls for 2019 (conventional tillage) and 2020 (non-tillage).

Species	Botanical family	Lifecycle	Abundance (%)		Frequency (%)	
			2019	2020	2019	2020
Anagallis arvensis	Primulaceae	Annual (A-W)	19±9	5±3.5	45.5±12	14±7
Conyza sumatrensis	Asteraceae	Annual (A-W-S)	12±4	13±3	28±7	29±4
Polygonum aviculare	Polygonaceae	Annual (A-W-S)	11±5	21±9	20±8	29±15
Gamochaeta spicata	Asteraceae	Perennial	6±3	4±2	15±5	12.5±3
***Lolium* spp.**	Poaceae	Annual (A-W-S)	5±4	1±3	14±8	6±7
Cyclospermum leptophyllum	Apiaceae	Annual (A-W)	7±5	–	15±7	–
Cirsium vulgare	Asteraceae	Annual (A-W-S)	–	6.5±5	–	12±6

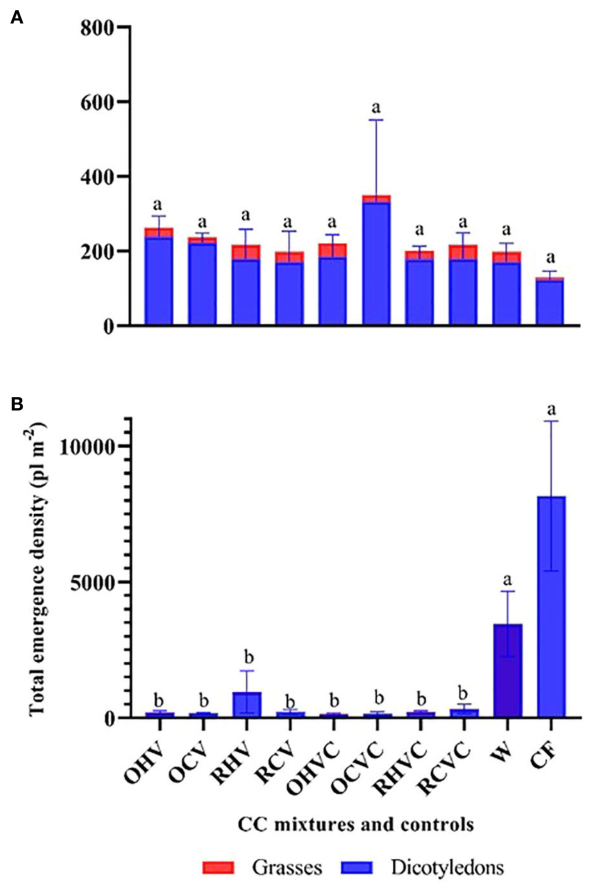

FIGURE 6
Total emergence density (pl m^{-2}) of A-W-S weeds: dicotyledons (in blue) and grasses (in red) for the different mixtures and controls: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), CF (Chemical fallow), W (Weedy fallow), for the years 2019 **(A)** and 2020 **(B)**. A-W-S and dicot weed data were transformed into a log(x) and square root (x+1). Mean values of untransformed data are shown in bars, significant differences among treatments are indicated by different letters) using Fisher's LSD test (p<0.05).

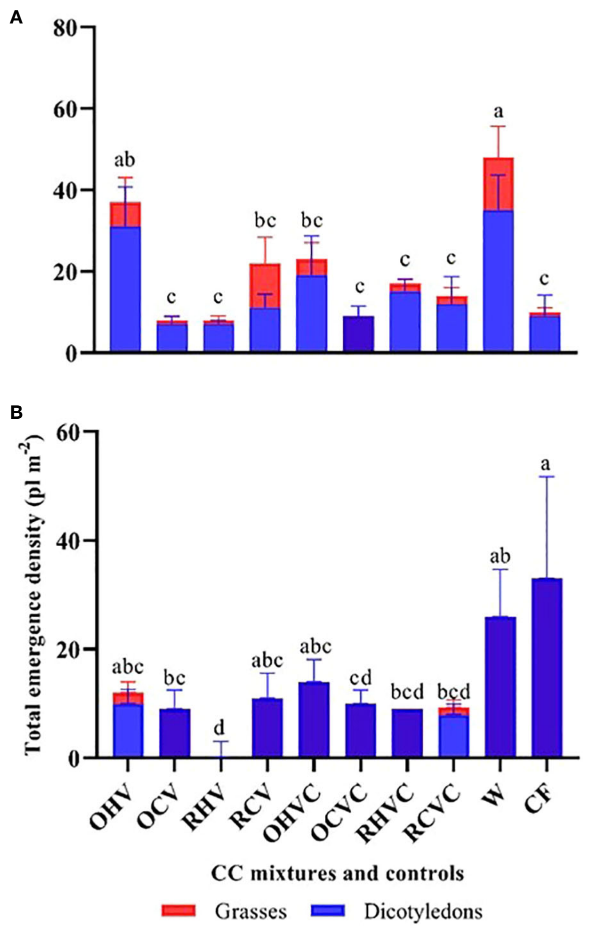

FIGURE 7
Emergence density (pl m^{-2}) of A-W-S weeds: dicotyledons (in blue) and grasses (in red) for the different mixtures and controls: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), CF (Chemical fallow), W (Weedy fallow), at the beginning of the CC cycle for the years 2019 **(A)** and 2020 **(B)**. 2020 A-W-S and dicot weed data were transformed into a square root (x+1). Mean values of untransformed data are shown in bars, significant differences among treatments are indicated by different letters) using Fisher's LSD test (p<0.05).

hypothesis. However, the study demonstrates that for both years of the study, CC mixtures were consistently more effective to suppress weed biomass compared to weed density. The latter was only lower than the control treatments in 2020. Despite this fact, both weed biomass production and final density levels were similar to those obtained by chemical fallow. These results are comparable to those reported by Piñeiro et al. (2019) for different types of CC and sites in Argentina. Conversely, Buratovich and Acciaresi (2019) observed a greater reduction in weed biomass in CC compared to the use of herbicides. The benefits of CC mixtures should be addressed within integrated weed management scenarios, considering the potential impact of these results on favoring seedbank depletion (Liebman and Nichols, 2020; Tiwari et al., 2021).

Finally, although many contributions have cited a positive relationship between biomass production (Finney et al., 2016; Florence et al., 2019; MacLaren et al., 2019) or vegetation cover (Kruidhof et al., 2008; Uchino et al., 2011; Dorn et al., 2015; Elhakeem et al., 2021) and weed suppression, no such relationships were observed in this work. Therefore, we decided to reject the third hypothesis. However, evidence was found that the composition of the mixtures affected the performance of CC. Therefore, these results could indicate that resources complementarity among CC species would influence on the weed suppressive capacity of the mixture. However, more studies should be conducted to comprehensively address the effect of CC mixtures on the weed dynamics.

5 Conclusions

The suppression of weed emergence and biomass exerted by CC mixtures were similar to (or even greater than) the chemical-based

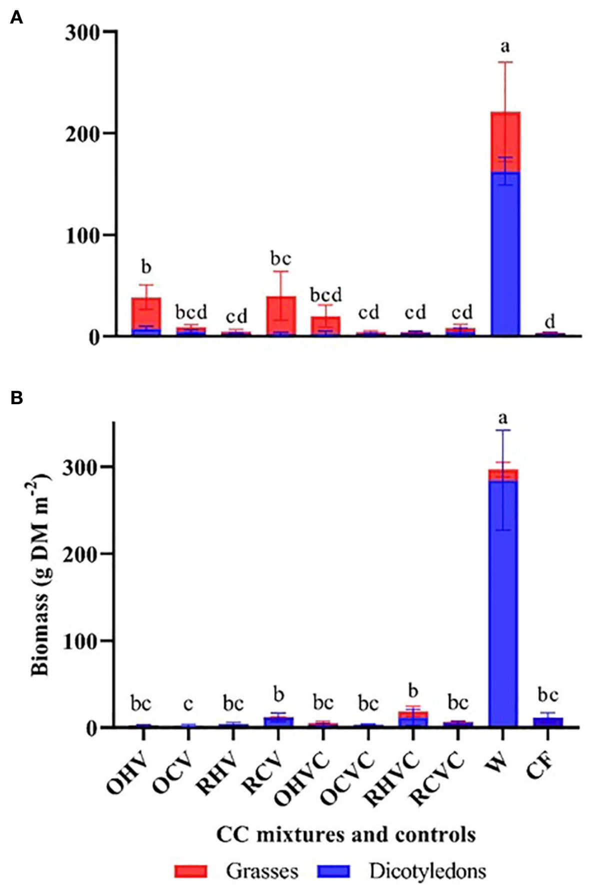

FIGURE 8
Aerial biomass (g m^{-2}) of A-W-S weeds: dicotyledons (in blue) and grasses (in red) at the end of the cycle for the different mixtures and controls: OHV (Oats+ Hairy vetch), OCV (Oats+ Common vetch), RHV (Rye+ Hairy vetch), RCV (Rye+ Common vetch), OHVC (Oats+ Hairy vetch+ Canola), OCVC (Oats+ Common vetch+ Canola), RHVC (Rye+ Hairy vetch+ Canola), RCVC (Rye+ Common vetch+ Canola), CF (Chemical fallow), W (Weedy fallow), for the years 2019 **(A)** and 2020 **(B)**. Average values of untransformed data are presented in the bars, and differences among treatments are indicated by different letters using Fisher's LSD test ($p<0.05$).

control. Average weed biomass figures for all CC mixtures were highly reduced when compared to the weedy control. In this sense, the mixture *S. cereale* + *V. villosa* + canola stood out for presenting the highest performance (productivity and vegetation cover) and high weed suppression. Obtained results support the idea that the use of species with functional differences is a practical recommendation criterion when designing better mixtures. Therefore, the implementation of CC within cropping sequences should be considered as an efficient and complementary tool, to promote IWM tactics and the design of more sustainable agricultural practices in the south-central region of Buenos Aires province, Argentina. From the authors perspective, this contribution provides novel results showing for the first time the effect of different CC mixtures on weed suppression at a community level in a dry subhumid environment of Argentina. It also provides valuable information in a poorly studied field,as weed emergence dynamics in CC. Future research should incorporate new variables in order to understand the relationship between the CC mixtures and weed suppression, such as: (i) the biomass production of both weeds and CC in the initial stages of the crop, (ii) the photosynthetic activity index active radiation as a complement to the vegetal cover, and (iii) the level of the weed seed bank before and after the implementation of the practice.

Data availability statement

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

Author contributions

MM: Writing – original draft. GC: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing. MY: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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

Publisher's note

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Soil solarization as a non-chemical weed control method in tree nursery production systems of the Pacific Northwest, USA

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Introduction: Herbicide application in tree nurseries is limited because of the potential for chemical injury to the large diversity of trees species grown, the lack of registered products, and increasing restrictions on herbicide use, necessitating the costly practice of hand weeding. Soil solarization can reduce the weed seedbank by trapping solar energy under clear plastic film, resulting in high soil temperatures lethal to imbibed weed seeds and seedlings. The objective of this study was to determine if soil solarization would be an effective weed management strategy in Pacific Northwest, USA, tree production systems.

Methods: Field studies were conducted at three commercial tree nurseries in Oregon and Washington over two years to test soil solarization in reducing the naturally occurring weed seedbank and the time required to hand weed fields. Further field and laboratory tests were conducted with five weed species: *Poa annua, Polygonum pensylvanicum, Amaranthus retroflexus, Portulaca oleracea*, and *Cyperus esculentus*. Weed seeds and tubers were buried in packets at 5 and 10 cm to determine their viability after 6 weeks of solarization. A laboratory study was conducted with all but *C. esculentus* to quantify the exposure time at 45, 50, and 55°C required for 90% death (T_{90}).

Results: Soil solarization was particularly effective in reducing the emergence of naturally occurring weeds in the fall and winter, when weed emergence was reduced by 94-96%. Emergence was reduced 67-81% during the subsequent spring and early summer. Nine to ten months after solarization, solarized areas had a 52 – 69% reduction in hand weeding time compared to non-solarized areas. In field trials with buried seed and tuber packets, mortality differed by location and depth, with *P. annua* and *P. pensylvanicum* having the greatest percent seed mortality followed by *A. retroflexus* and variable results for *P. oleracea* and *C. esculentus*. In lab studies, seed mortality differed depending on species and temperature; however, at 55°C, there was a relatively rapid drop in seed viability for all species, and T_{90} values ranged from 1.2 to 41 h whereas at 45° C the range was 47 to > 3000 h. Similar to the field studies, *P. annua* and *P. pensylvancium* were more sensitive to heat, followed by *A. retroflexus* and *P. oleracea*.

Conclusion: Soil solarization can be an effective weed management tool in reducing the weed seedbank in Pacific Northwest tree nurseries and other fall-sown crops but may not work for certain, thermotolerant weed species such as *C. esculentus*.

KEYWORDS

seed viability, weed seedbank, heat unit accumulation, hand weeding reduction, integrated weed management

1 Introduction

Production of tree seedlings is an integral part of the agricultural and forest products economy in the Pacific Northwest (PNW), USA (USDA, 2014). Over $1.1 billion in nursery and greenhouse products were sold in 2020 with approximately one-third of the crop value from field-grown trees (ODA, 2022). PNW nurseries also produce bareroot conifer and hardwood seedlings for reforestation; in 2022, 79 million seedlings were produced and 185,350 hectares were planted in Oregon and Washington (Pike et al., 2023). Because of the increased restrictions for use of certain soil fumigants (EPA, 2012), the limited number of herbicide options, and the potential for crop injury, tree seedling nurseries rely on hand weeding after crop establishment which is labor intensive and costly. For these reasons, there is a strong demand to find an alternative to soil fumigation.

Soil solarization is a non-chemical pre-planting practice which has been found to be comparable to other methods to manage soilborne pathogens and weeds in regions with high solar radiation (Stapleton and DeVay, 1986; Gullino et al., 2022). Soil solarization creates conditions lethal to many mesophilic weed species that grow between 20 and 45°C by heating soil under a clear film applied over the soil surface during the summer months. To ensure good contact of the film with the soil surface, soil solarization is applied in the following steps: soil is cultivated, smoothed, irrigated, and the film is laid (Elmore et al., 1997; Wilen and Elmore, 2007). The film is sealed by burying the edges to reduce heat escape. The treatment durations differ depending on local conditions, but there is general agreement that 6 weeks of solarization is effective against many pests (Stapleton et al., 2005). The Pacific Northwest was previously considered to be a marginally suitable area for soil solarization because of its short, mild summers. However, Parke (2016) found that clear plastic film with anti-condensation (AC) and infra-red retaining (IR) properties increased the maximum soil temperature achieved during soil solarization compared to the previous studies conducted in Oregon (Pinkerton et al., 2000; Peachey et al., 2001). These new types of plastics improved energy capture and heat retention and made soil solarization a more feasible practice comparable to locations, for example California, where soil solarization has been utilized previously with success (Stapleton et al., 2005).

The effect of soil solarization is greatest near the soil surface and decreases with depth (Stapleton, 2000). Because most of the viable seeds in the soil seedbank are concentrated in the top 5 cm, soil solarization works best when it is followed by planting practices with little to no disturbance of the soil (Akinola et al., 1998). Reduced survival of weed seeds in soil ultimately reduces the weed population density, thus, reducing the cost of weed control. However, the efficacy of soil solarization can vary based on the weed species and environmental conditions (Stapleton et al., 2005).

The main mode of action of soil solarization is hydrothermal killing of seeds and seedlings (Katan and DeVay, 1991; Stapleton, 2000). Annual weeds, such as *Sonchus oleraceus* (L.), *Poa annua* (L.), and *Polygonum equisetiforme* (S.), are more effectively controlled by soil solarization than are perennial species (Rubin and Benjamin, 1984; Peachey et al., 2001). Among the annual species, winter annuals have lower thermotolerance than summer annuals because they are better adapted to germinate at lower soil temperatures (Rubin and Benjamin, 1984; Egley, 1990; Elmore, 1991; Hoyle and McElroy, 2009). Winter annual species, compared to summer annual species, germinate in shorter day conditions, are more temperature-sensitive and require smaller temperature increases to be effectively controlled (Egley, 1990). A 1-week solarization period was enough to control susceptible winter annuals such as *Poa annua*, *Montia perfoiata* (Donn ex Willd.) Howell and *Senecio vulgaris L.* (Katan and DeVay, 1991), whereas summer annual species required higher solarization temperatures and/or a longer duration (Egley, 1990). Hard seeded species, regardless of the life cycle, are generally not controlled by soil solarization (Elmore, 1991).

A wide range of weed species have been reported to be susceptible to soil solarization (Cohen and Rubin, 2007), but results can be inconsistent due to the variation in environmental conditions, soil type, plastic type used, evaluation methods, weed seed source, and seed position in the soil profile (Standifer et al., 1984; Al-Hammadi, 2006). Imbibed seeds become more vulnerable to high temperatures due to increased metabolic activity (Egley, 1990). In addition, for some weed species, soil solarization can promote germination by creating higher temperatures in the soil profile (Londale, 1993) and increased CO_2 concentrations (Rubin and Benjamin, 1984; Baskin and Baskin, 1998). Both maximum soil temperature and accumulated soil temperature determine the hydrothermal effect on weed seeds (Stapleton et al., 2005). Soil moisture is needed for seed imbibition but also improves the temperature conductivity so that heat reaches deeper depths within the soil profile (Maher et al., 1986).

Although hydrothermal killing is the main mode of action in solarization, there are other factors that can influence the state of seeds or soilborne pathogens such as fluctuating daily temperatures, soil moisture, nutrient composition, and microbial community shifts (DeVay and Katan, 1991; Funahashi and Parke, 2016; Funahashi and Parke, 2018; Funahashi and Parke, 2020; Funahashi et al., 2021). These changes in environmental conditions created by soil solarization can induce or release seed dormancy and affect the sensitivity of seeds depending on species. Induced seed dormancy caused by soil solarization does not reduce the seedbank. However, dormancy could still reduce the weed infestation in the following seasons by increasing crop competition, reducing control costs, and decreasing seed viability due to microbial decay or predation. Soil solarization that does not increase temperatures enough to cause seed mortality may still reduce seed vigor of sensitive species which survive the treatment (Stapleton, 1990).

When soil solarization releases seeds from dormancy, fatal germination may occur at depths greater than a seedling can emerge. In addition, seedlings are typically more sensitive to heat than seeds. Thus, if seeds germinate, seedlings could be killed before they reach the soil surface by soil temperatures created by soil solarization. Fatal germination can contribute to the long-term effect of soil solarization by diminishing the number of viable seeds in the soil profile.

The objective of the study was to determine if soil solarization would be an effective non-chemical weed management strategy in PNW commercial tree nurseries. Studies were conducted to evaluate the use of soil solarization to reduce naturally occurring weed seedbank populations, reduce the time required for hand weeding in planted tree seedling fields and to predict the response of selected weed species to soil solarization by determining thermal death curves under controlled laboratory conditions.

2 Materials and methods

2.1 Field studies

2.1.1 Site description

Field experiments were conducted at three tree seedling nursery sites in the Pacific Northwest: Clackamas Co., Oregon; Yamhill Co., Oregon; and Thurston Co., Washington during the summers of 2016 and 2017 (Table 1). Seed bed preparation, fertilization, seeding, and hand weeding were conducted by the nursery staff.

2.1.2 Plot establishment

For both naturally occurring and buried seed packets, plots were established on three raised beds with three replications of two treatments (solarized or non-solarized). The treatment duration was 6 weeks during July and August. Individual plots were 1.2 m by 30.5 m with a 4.7 m buffer between the treatments.

2.1.3 Naturally occurring weed emergence

In order to assess soil solarization efficacy on naturally occurring weed populations, non-solarized plots were sprayed with glyphosate at 0.75 kg a.e. ha^{-1} to control weeds that emerged during the 6-week solarization period. Three to 11 months after soil solarization and film removal, naturally-occurring weeds were identified and counted in the Clackamas and Yamhill trials before weed control measures were taken. Quadrats (50 cm x 50 cm) were placed at 1, 8, 15, and 22 m along the center of each plot to avoid edge effects and disturbed areas caused by seed packet and instrumentation removal.

2.1.4 Hand weeding time in solarized and non-solarized treatments

Standard weeding protocols were used at each site by nursery staff. The time required to hand weed solarized and non-solarized plots (1.2 m by 30.5 m) was recorded following emergence counts of naturally occurring weeds in the trials at the Clackamas and Yamhill locations during fall/winter and spring/summer months.

2.1.5 Seed packet preparation

Five weed species were tested: *Poa annua* (L.), *Polygonum pensylvanicum* (L.), *Amaranthus retroflexus* (L.), *Portulaca oleracea* (L.) and at one site, *Cyperus esculentus* (L.). *Portulaca oleracea* and *P. pensylvanicum* seeds were planted, and plants were maintained in a greenhouse located in Corvallis, OR, to produce seeds for the study. Populations of the other species were obtained through seed collection from fields in Benton or Yamhill Co, OR. Seeds were stored at room temperature (21°C) in dry, dark conditions until use. Germination of the stored seeds was > 95% for each species (data not shown). Fifty seeds of each species were sealed in a packet of water permeable nylon mesh (105-µm, 4 cm by

TABLE 1 Site descriptions and trial information for field studies.

Trial Year	Study Site	Latitude	Longitude	Solarization Treatment Period	Soil Type
2016	Yamhill Co., OR	45.319278	-123.177444	7/6/2016 - 8/17/2016	Silty clay loam (20% sand, 29% clay, 51% silt)
2016	Clackamas Co., OR	45.426392	-122.325208	7/7/2016 - 8/18/2016	Silty clay loam (16% sand, 33% clay, 51% silt)
2016	Thurston Co., WA	46.872513	-123.056537	6/29/2016 - 8/10/2016	Loamy fine sand (86% sand, 9% clay, 6% silt)
2017	Yamhill Co., OR	45.316243	-123.176952	7/14/2017 - 8/25/2017	Silt loam (1% sand, 14% clay, 85% silt)
2017	Clackamas Co., OR	45.427177	-122.331686	7/19/2017 - 8/30/2017	Silt loam (15% sand, 18% clay, 66% silt)
2017	Thurston Co., WA	46.869609	-123.068862	7/13/2017 - 8/24/2017	Loamy fine sand (77% sand, 0% clay, 23% silt)

4 cm; Pentair Aquatic Eco-Systems, Inc., Apopka, FL). A packet of each species was placed randomly inside a larger flat bag made of window screen and sealed (26 cm by 10 cm). Nylon mesh packets and window screen bags were sealed using an impulse sealer (AIE-305; American International Electric Inc., City of Industry, CA).

Cyperus esculentus tubers were collected at the experimental site in Thurston Co, WA. Tuber size ranged between 0.8 cm to 1.5 cm. *Cyperus esculentus* was included only in the Thurston trials to avoid the introduction of the species to the other locations. Fifty tubers of *C. esculentus* were sealed in a window screen bag (12 cm by 10 cm).

2.1.6 Buried packet placement

Weed packets were buried at 5 and 10 cm depths at the center of each plot. Soil temperature was monitored at 5 cm and 15 cm with CS655 sensors attached to a CR-1000 datalogger (Campbell Scientific, Logan, UT) (Hill, 2019) and at 10 cm using iButtons (Wada, 2019) (Thermochron DS1922L, OnSolution Pty. Ltd., Castle Hill, NSW, Australia). Measurements were taken every 30 min throughout the duration of the trial. Sensors were installed at the center of each plot near where packets were buried. Total accumulated temperature hours during the 6-week studies were grouped into 5°C ranges and classified by solarized and non-solarized plots, depth, and location. An HMP60 Campbell SCI weather station was used at each location to monitor air temperature.

The plots were irrigated to field capacity at the beginning of the trials using overhead irrigation the night before the plastic application at the Yamhill and Thurston locations. At the Clackamas location, three lines of drip irrigation tape were installed on top of the beds, and plots were irrigated after the plastic was applied. Non-solarized plots were irrigated the same as the solarized treatment at each site.

Solarized plots were covered with clear plastic film 'C790-IR-AC low tunnel' (1.4 mil; Ginegar Plastic Products, Ltd, Santa Maria, CA). The edges of the film were held in place by covering the edge with a 30-cm wide band of soil along the raised beds. No film was applied to the non-solarized treatment.

2.1.7 Seed viability assessment

Seed packets were removed after 6 weeks and seed viability was assessed in the laboratory. Any seeds in the packets that germinated pre-removal were counted as dead (fatal germination). Intact seeds were placed in a Petri dish containing a blotter paper moistened with 10 mL deionized water. Seeds were incubated in a dark growth chamber set to 12 h alternating temperatures of 15/20°C for *P. annua* and 20/26°C for the other species. Germinated seeds were counted after 14 days. Seeds were considered germinated when the emerged radicals were greater than 3 mm long. Seeds that did not germinate during this period were assessed using the tetrazolium (TZ) staining method to confirm whether seeds were dormant or dead (Patil and Dadlani, 2009). Seed coats were partially removed or pierced with a fine needle and soaked in 1% triphenyl TZ chloride solution (Sigma-Aldrich, St. Louis, MO). Seeds were incubated at 30°C for 6 h in the dark for *P. annua* and 10 h for other species. The embryos were exposed under a dissection microscope and counted as viable when the embryo stained red and had no deformation or fungal infection. Percent total seed viability (TV) was calculated as the sum of non-dormant and dormant seed (% positive TZ test).

2.1.8 Tuber viability

Cyperus esculentus seedlings that emerged during the trial were uprooted and transplanted individually in a pot (3 × 3 × 6 cm, Growers Nursery Supply, Inc. Salem, OR) and placed in a greenhouse after the trial to determine viability. The greenhouse environment was 27/20°C day/night with 14 h of light in addition to ambient sunlight. Survival counts were taken after 2 weeks. The number of tubers recovered from the packets was recorded, and tubers were planted in a plastic tray (25 × 25 × 6 cm) filled with potting mix (Sunshine Mix 1 Potting Mix, Sun Gro Horticulture, Bellevue, WA) and grown under the same greenhouse conditions as the transplanted seedlings. Trays were watered as necessary, and sprouting was assessed after 4 wk. Tuber viability based on tuber sprouting and survival rate of transplanted seedlings was compared between non-solarized and solarized samples.

2.2 Statistical analysis

2.2.1 Field studies

The buried seed packet, weed emergence, and hand weeding time data were subjected to Welch's t-test to compare the mean of the response variables to solarized and non-solarized treatments from the same trial year and site. The buried seed packet trial also included burial depth as a response variable. The location and timing data for weed emergence were not pooled because of sample variance (Levene's test, $P< 0.05$), and differences in mean and interactions tested by ANOVA ($P< 0.05$). R (version 3.5.2) and the Agricolae package were utilized for each analysis.

2.2.2 Laboratory studies

The experiment was conducted in growth chambers utilizing a completely randomized design with both temperature and duration as independent variables. Seed viability was a dependent variable to the treatment. The study was repeated. Based on Levene's test for homogeneity of variance, there were no differences in the variability of seed viability in the two trials for each species of the same treatment and duration ($P > 0.05$). Therefore, data from the two trials were pooled and analyzed as 6 replications. Seed mortality data were analyzed using the DRC package on R (version 3.5.2) and fitted to the 2-parameter Weibull model defined as follows:

$$v = 100e^{-e^{b[\log(d)-a]}} \quad (1)$$

where v is percent viability of seeds, b is the slope of the curve, the parameter d is a duration of the treatment in hours, and the parameter a is a duration of the treatment in hours at the inflection point of the viability curve. The upper limit of seed viability was fixed to 100%, and the lower limit to 0%. For each temperature treatment, parameter estimates, the time required to kill 90% of seeds tested (T_{90}), and 95% confidence intervals were determined using the summary and estimated effective temperature and time for seed mortality.

2.3 Laboratory study

2.3.1 Seed preparation

Four weed species were tested: *P. annua*, *P. pensylvanicum*, *A. retroflexus, and P. oleracea*. The same weed seed collections were used in the laboratory study as the field study. Twenty-five seeds of each species were placed on moist blotter paper (Steel Blue Blotter; Anchor Paper Corporation, St. Paul, MN) and sealed in a capsule (Meter Group, Pullman, WA; 3.9 x 1.1 cm diameter) before being placed in germination chambers.

2.3.2 Heat treatments

Three temperature treatments, 45, 50, 55°C, were chosen from the range of reported temperatures in the top 5 cm of the soil profile during the soil solarization field trials (Table 2). The capsules enclosing the imbibed seeds were incubated at a constant temperature. The study included three replications for each treatment and species. The study was repeated. The sampling time interval varied from 0.5 h to 24 h based on the sensitivity of a species to each temperature treatment. The incubation trial was continued until a species reached 100% seed mortality for each temperature or for 336 h. Deionized water was added as necessary to maintain similar moisture levels within the capsules for incubation trials that lasted more than 7 d.

2.3.3 Seed viability assessment

After the heat treatment, seed viability was assessed using the TZ method described for the field study (Patil and Dadlani, 2009).

3 Results

3.1 Field studies

3.1.1 Soil temperature

At the three site locations during both years, temperatures ranged from 14 to 58°C in solarized plots and 11 to 44°C in non-solarized plots at the 5 cm depth (Table 2; Supplementary Figures 1–6). At 10 cm depths, temperature ranged from 16 to 51°C in solarized plots and 13 to 36°C in non-solarized plots (Table 3; Supplementary Figures 1–6).

The major difference in accumulated soil temperature hours between soil solarization treatments was in the 40 to 45°C range. At this temperature range, the maxium accumulated hours in any non-solarized soil treatment was 16. There were no temperature readings above 40°C at the 10 cm depth at any site or year in non-solarzed soil plots. In contrast, accumulated hours between 40 to 45°C in solarized treatments ranged from 80 to 144 and 43 to 167 at 5 and 10 cm, respectively, over both years and across all sites. Solarized plots accumulated between 13 and 79 hr above 50°C at the 5 cm depth and only 1 hr at 10 cm.

The Washington site had fewer soil temperature accumulated hours above 40°C than the two Oregon sites in both years. The maximum soil temperatures were similar in solarized treatments during both years at the different locations; however, the maximum air temperature averaged between 0.5-3°C warmer at both Oregon locations depending on the year (Figures 1, 2). In 2017, the average maximum air temperature was ≥ 2°C warmer at each location and would account for the greater accumulated thermal hours ≥40°C than in 2016 at both the 5 and 10 cm depths (Figure 3).

3.2 Emergence of naturally occurring weed species

Naturally occurring weed species varied by location and date of emergence. Each weed species used in the burial packets, other than *P. pensylvanicum*, was also found at one of the sites. The other primary weeds accounting for >50% of emergence were *Cardamine oligosperma* (L.), *Cerastium vulgatum* (L.), *Draba verna* (L.), *Capsella bursa-pastoris* (L.), and *Lamium amplexiaule* (L.) (Supplementary Table 1). At each location and date, weed seedling emergence was significantly reduced in the solarized treatment compared to the non-solarized treatment. At the Clackamas site in 2016, fall weed emergence counts were 11 and 273 weeds m^{-2} in solarized and non-solarized plots, respectively (Figure 4). Spring weed emergence counts were 8 and 40 weeds m^{-2} in solarized and non-solarized plots, respectively. The winter weed emergence counts in 2017 at the Clackamas site were 7 and 115 weeds m^{-2} in solarized and non-solarized plots, respectively. Spring weed emergence counts from the 2017 plots were 7 and 37 weeds m^{-2} in solarized and non-solarized plots, respectively.

Spring weed emergence counts on the 2016 plots at the Yamhill site were 8 and 24 weeds m^{-2} in solarized and non-solarized plots, respectively (Figure 5). Spring weed emergence counts in 2017 plots were 4 and 21 weeds m^{-2} in solarized and non-solarized plots, respectively.

3.3 Time required to hand weed

Soil solarization reduced weed emergence after the nursery tree seeds were planted in the fall and the effect of solarization persisted to the following early summer when hand weeding was conducted. The decrease in weed emergence resulted in a reduction in hand weeding time, translating to savings in labor costs. In 2016 and 2017, the hand weeding time was reduced in solarized beds by 69 and 63% at Clackamas, and by 56 and 52% at Yamhill, respectively (Table 4).

3.4 Fate of weed seed in buried packets

3.4.1 Fate of *Poa annua* weed seed in buried packets

In 2016, at the 5 cm depth, 97, 83, and 93% of *P. annua* seed were dead after the 6-week study in solarized plots compared to 20, 5, and 3% in non-solarized plots at the Yamhill, Clackamas, and Thurston study sites, respectively (Table 5). In solarized plots at 10 cm, 73, 64, and 57% of *P. annua* seed were dead compared to 7, 6, and 3% dead seed in non-solarized plots at the Yamhill, Clackamas and Thurston sites, respectively.

In 2017, at the 5 cm depth, there were 0 viable seed in solarized plots at both locations, as compared to 100% or 80% viable seed in

TABLE 2 Season-long accumulated thermal hours summary at the 5 cm depth for non-solarized (NONSOL) and solarized (SOL) treatments.

	Year	2016						2017					
	Location	Yamhill		Clackamas		Thurston		Yamhill		Clackamas		Thurston	
	Treatment	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL
	Temp Range (°C)	(accumulated hours)											
Soil Temperature (°C)	10 to < 15	0	0	1	0	1	0	0	0	0	0	11	0
	15 to < 20	212	10	275	0	391	16	75	0	41	1	172	16
	20 to < 25	473	68	411	106	443	160	370	45	385	8	392	106
	25 to < 30	269	279	206	330	153	407	332	277	402	189	284	332
	30 to < 35	55	294	114	260	21	248	219	267	177	361	149	228
	35 to < 40	0	186	2	148	0	132	13	212	3	260	0	190
	40 to < 45	0	136	0	124	0	43	0	167	0	145	0	126
	45 to < 50	0	35	0	39	0	2	0	41	0	45	0	12
	50 to < 55	0	0	0	1	0	0	0	0	0	0	0	0

non-solarized plots at Clackamas and Yamhill, respectively. *Poa annua* viability at the 10 cm depth was more variable in 2017, with 69, 90, and 95% dead seed in solarized plots and 5, 94, and 37% viable seed in non-solarized plots and at the Yamhill, Clackamas, and Thurston sites, respectively.

3.4.2 Fate of *Polygonum pensylvanicum* weed seed

Solarization killed *P. pensylvanicum* primarily by fatal germination. In the solarized plots, fatal germination ranged from 89 to 100% at the 5 cm depths in 2016 (Table 6). In 2017, solarization killed 87% of *P. pensylvanicum* by fatal germination at the Yamhill site and 100% of seeds were dead at the Clackamas and Yamhill sites at the 5 cm depth. In solarized plots at the 10 cm depth, seed mortality was greater than 90% at Yamhill and Thurston during both years and 91 and 65% in Clackamas in 2016 and 2017, respectively. In 2016, the non-solarized plot seed viability was 62 to 78% in the Thurston trial at 5 and 10 cm, respectively. Seed viability was more than 90% at both depths in non-solarized plots at the Yamhill and Clackamas sites.

TABLE 3 Season-long accumulated thermal hours summary at the 10 cm depth for non-solarized (NONSOL) and solarized (SOL) treatments.

	Year	2016						2017					
	Location	Yamhill		Clackamas		Thurston		Yamhill		Clackamas		Thurston	
	Treatment	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL	NON SOL	SOL
	Temp Range (C°)	(accumulated hours)											
Soil Temperature (°C)	10 to < 15	0	0	1	0	1	0	0	0	0	0	11	0
	15 to < 20	212	10	275	0	391	16	75	0	41	1	172	16
	20 to < 25	473	68	411	106	443	160	370	45	385	8	392	106
	25 to < 30	269	279	206	330	153	407	332	277	402	189	284	332
	30 to < 35	55	294	114	260	21	248	219	267	177	361	149	228
	35 to < 40	0	186	2	148	0	132	13	212	3	260	0	190
	40 to < 45	0	136	0	124	0	43	0	167	0	145	0	126
	45 to < 50	0	35	0	39	0	2	0	41	0	45	0	12
	50 to < 55	0	0	0	1	0	0	0	0	0	0	0	0

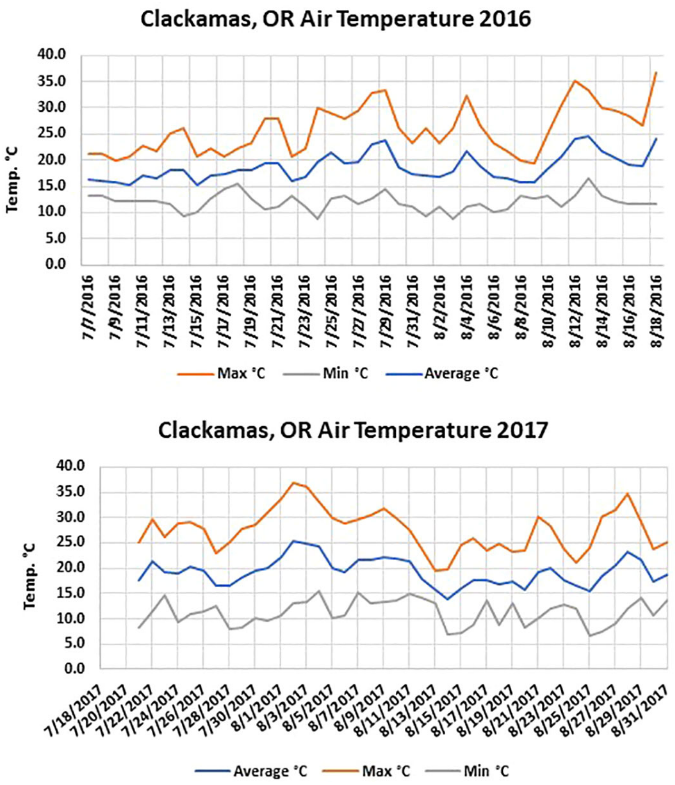

FIGURE 1
Air temperature at the Clackamas, OR location during the 2016 and 2017 solarization studies.

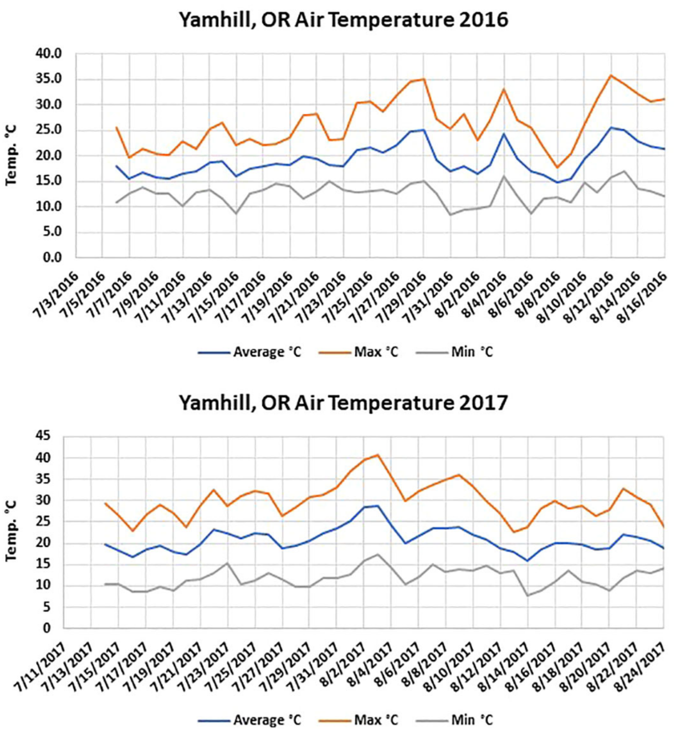

FIGURE 2
Air temperature at the Yamhill, OR location during the 2016 and 2017 solarization studies.

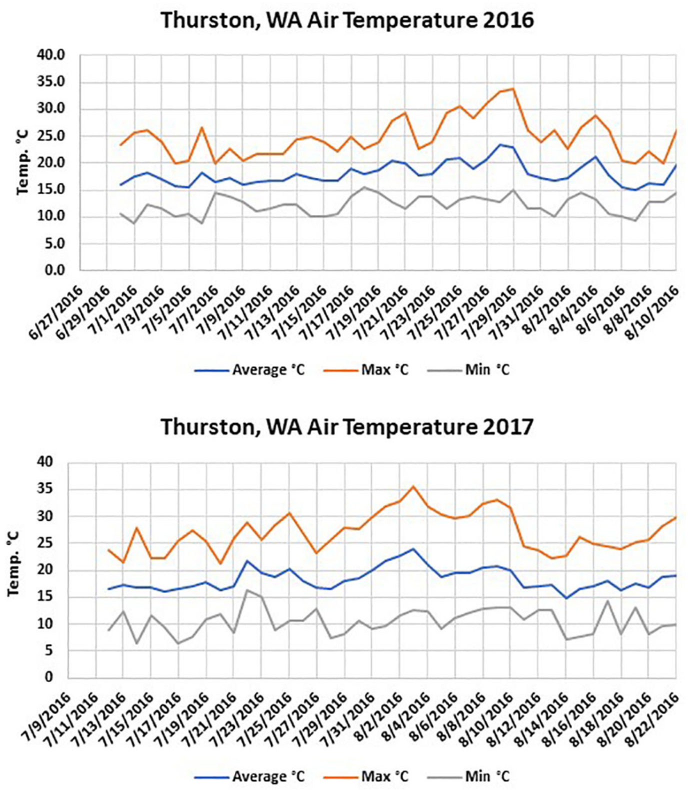

FIGURE 3
Air temperature at the Thurston, WA location during the 2016 and 2017 solarization studies.

3.4.3 Fate of *Amaranthus retroflexus* weed seed

Seed mortality of *A. retroflexus* was 2 to 84% at the 5 cm depth in solarized plots for both years and locations (Table 7). Seed mortality in solarized plots at the 10 cm depth ranged between 0 to 40% depending on the site and year. Seed mortality in non-solarized plots ranged between 0 to 1% at both 5 and 10 cm depths depending on the location and year.

In most cases the percentage of dormant seeds in solarized plots was greater than in non-solarized plots. This was true for both depths but especially at the 10 cm depth (Supplementary Table 5).

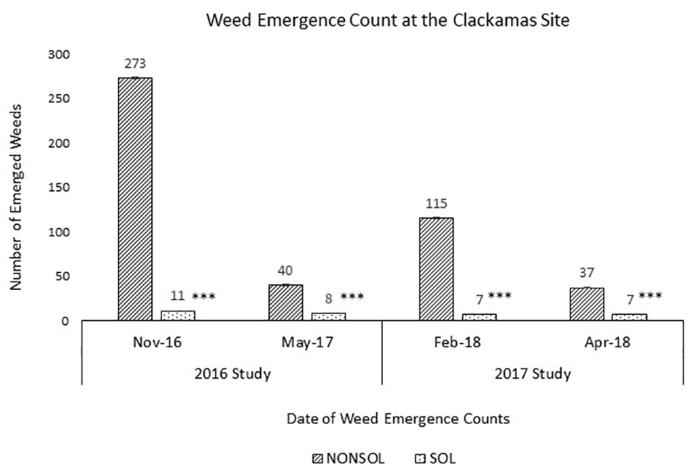

FIGURE 4
Weed emergence counts at the Clackamas site after solarization in 2016 and 2017 studies. Four m^2 counts were taken per individual plot across three replications and averaged for non-solarized (NONSOL) and solarized (SOL) treatments. Welch's two sample t-test were carried out to compare the emergence counts between non-solarized and solarized treatments. Asterisks indicate the statistical significance with the p-value <0.05(*), 0.01 (**) or 0.001(***). Values are mean of seedling count per $1m^2$ area.

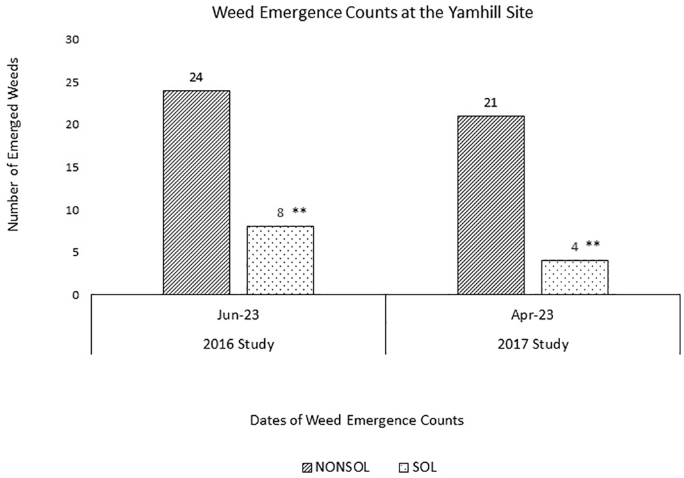

FIGURE 5
Weed emergence counts at the Yamhill site after solarization in 2016 and 2017 studies. Four m^2 counts were taken per individual plot across three replications and averaged for non-solarized (NONSOL) and solarized (SOL) treatments. Welch's two sample t-test were carried out to compare the emergence counts between non-solarized and solarized treatments. Asterisks indicate the statistical significance with the p-value <0.05(*), 0.01(**) or 0.001(***). Values are mean of seedling count per $1m^2$ area.

In 2016, *A. retroflexus* seed at the 10 cm depth in solarized plots was 52, 62, and 30% dormant and seed in non-solarized plots was 16, 0, and 6% dormant at Yamhill, Clackamas, and Thurston sites, respectively. In 2017, seed at the 10 cm depth in solarized plots was 0, 92, and 50% dormant and seed in non-solarized plots was 0, 28, and 32% dormant at Yamhill, Clackamas, and Thurston sites, respectively. These results suggest *A. retroflexus* may be sensitive to other changes caused by the solarization, such as an imbalance of gaseous compounds (Horowitz et al., 1983), rather than the hydrothermal process alone. As a summer annual, *A. retroflexus*, is less sensitive to greater heat variation. Soil solarization did reduce viable seed in some years and even with increasing the proportion of dormant seed, minimal disturbance during nursery planting can reduce the number of emerged weeds.

TABLE 4 Time (minutes) required for one person to hand weed a non-solarized (NONSOL) verses a solarized (SOL) 1.2 m x 30.5 m plot.

Trial	Weeding Date	NONSOL	SOL	Reduction
		min	min	%
2016 Yamhill	6/8/2017	22.6	9.9 *	56.2
2017 Yamhill	6/2/2018	7.9	3.8 *	51.9
2016 Clackamas	5/22/2017	25.4	7.9 *	68.9
2017 Clackamas	5/6/2018	36.6	13.6 *	62.8

Welch's two sample t-test were conducted to compare the time required for one person to hand weed a plot. Values are time in minutes. Asterisks indicate the statistical significance (p-value < 0.05). SOL = solarized and NONSOL = non-solarized plots.

3.4.4 Fate of *Portulaca oleracea* weed seed

Portulaca oleracea had the most heat-tolerant seed. In 2016, at both depths and all sites, viable seed was 63 to 85% in solarized plots and 80 to 91% viable seed in non-solarized plots (Table 8). In 2017, viable seed was 89 to 100% in solarized plots and 98 to 100% in non-solarized. The greatest seed mortality in solarized plots was 37% at 10 cm in Thurston in 2016.

3.4.5 Fate of *Cyperus esculentus* tubers

Solarization suppressed the sprouting of tubers during the treatment period, however, it did not reduce the frequency of sprouting of recovered tubers in comparison to the non-solarized treatment (Table 9). Chase et al., 1999, found similar results where oscillating temperatures of 45 and 26°C (day and night, respectively) slowed sprouting of *C. esculentus* but did not cause mortality. In a controlled study, 100% mortality of *C. esculentus* was achieved after 16, 8, and 2 h for 50, 55, and 60°C constant temperatures (Webster, 2003). However, a 6-week soil solarization field study was conducted where temperatures were >60°C for 49% of the study and *Cyperus* spp. tubers were still viable (Chase, 1999). Variation in nutsedge mortality likely reflects its different response to oscillating vs. constant high temperature (Miles et al., 1996). As this northernmost location was the only field site where *C. esculentus* was studied, it is not known if soil solarization at other, more southerly locations in the PNW would be effective.

4 Results – controlled study

The thermal death curve of each species is presented in Figure 6. The parameter estimates are summarized in Table 10.

TABLE 5 Fate of *Poa annua* seeds from weed seed packets buried in field trials.

Location	Year	Depth	Total Viability (%)			Dead Seed (%)			Fatal Germination (%)		
			NONSOL	SOL		NONSOL	SOL		NONSOL	SOL	
Yamhill	2016	5 cm	14	2.7	*	20	97.3	*	66	0.1	**
		10 cm	18	26		6.7	73.3	*	75.3	0.7	**
	2017	5 cm	80.7	0	**	4.7	100	***	14.6	0	
		10 cm	5.3	31		6	69	***	88.7	0	**
Clackamas	2016	5 cm	14	17.3		4.7	82.7	*	81.3	0	**
		10 cm	25.3	34		1.3	64	**	73.4	2	***
	2017	5 cm	100	0	***	0	100	***	0	0	
		10 cm	94	10	***	0	90	***	6	0	*
Thurston	2016	5 cm	7.3	7.3		3.3	8.7		89.3	84	*
		10 cm	17.3	37.3	*	2.7	6		80	56.7	***
	2017	5 cm	42	1.3	*	0	98.7	***	58	0	*
		10 cm	37.3	4.7		0	95.3	***	62.7	0	

Total viability is the sum of non-dormant seeds (%) and dormant seeds (%). Non-dormant seeds germinated during the germination test. Dormant seeds tested positive to tetrazolium (TZ) staining. Dead seeds tested negative to TZ staining. Fatal germination accounts for germination which occurred pre-removal of seed packets from the field trial.
Welch's two sample tests were conducted to compare non-solarized (NONSOL) and solarized (SOL) seed samples from the same location, year and the depth. Values are response variables in percentage. Asterisks indicate the statistical significance with the p-value < 0.05 (*), 0.01 (**) or 0.001 (***).

The T_{90} differed among species for each temperature treatment. For all species, 95% confidence intervals were smaller at higher temperature treatments. Among the four species, *P. annua* was most sensitive with a T_{90} under 10 h at 50°C. *Polygonum pensylvanicum* and *A. retroflexus* required 10 h at 55°C for a similar response. *Portulaca oleracea* was the only species that did not reach 100% mortality in the 45 and 50°C treatments.

The susceptibility of the different weed species varied depending on accumulated thermal hours; however, the rate of mortality for all species increased rapidly at 55°C. The lower the temperature and the greater the thermotolerance of a species, the more variable the effects on seed viability, resulting in wide confidence intervals and large standard errors for the parameter estimates. The thermal dose-response curves described in this study are a simple and

TABLE 6 Fate of *Polygonum pensylvanicum* seeds from weed seed packets buried in field trials.

Location	Year	Depth	Total Viability (%)			Dead Seed (%)			Fatal Germination (%)		
			NONSOL	SOL		NON SOL	SOL		NON SOL	SOL	
Yamhill	2016	5 cm	94.7	0	***	0	0		5.3	100	***
		10 cm	96.7	4.0	***	0	0		3.3	96	***
	2017	5 cm	99.3	8.7	**	0	4.7		2	86.7	*
		10 cm	91.3	2.7	***	0	2		8.7	95.3	***
Clackamas	2016	5 cm	97.3	8.7	***	0	2		2.7	89.3	***
		10 cm	98.7	10.7	***	0	0		1.3	89.3	***
	2017	5 cm	94.0	0.0	**	0	100	***	6	0	
		10 cm	98.7	2.0	***	0	32		1.3	63.3	*
Thurston	2016	5 cm	76.7	10.0	**	11.3	0.7		12	89.3	***
		10 cm	62.0	38.7		2	0		36	61.3	
	2017	5 cm	98.7	0.7	***	0	99.3	***	1.3	0	
		10 cm	97.3	0	***	0	38.7	*	2.7	61.3	**

Total viability is the sum of non-dormant seeds (%) and dormant seeds (%). Non-dormant seeds germinated during the germination test. Dormant seeds tested positive to tetrazolium (TZ) staining. Dead seeds tested negative to TZ staining. Fatal germination accounts for germination which occurred pre-removal of seed packets from the field trial.
Welch's two sample tests were conducted to compare non-solarized (NONSOL) and solarized (SOL) seed samples from the same location, year and the depth. Values are response variables in percentage. Asterisks indicate the statistical significance with the p-value < 0.05 (*), 0.01 (**) or 0.001 (***).

TABLE 7 Fate of *Amaranthus retroflexus* seeds from weed seed packets buried in field trials.

Location	Year	Depth	Total Viability (%)			Dead Seed (%)			Fatal Germination (%)		
			NONSOL	SOL		NONSOL	SOL		NONSOL	SOL	
Yamhill	2016	5 cm	95.3	52		1.3	6.7		3.3	41.3	
		10 cm	99.3	74		0.7	0		5.3	26	
	2017	5 cm	100	46.7		0	53.3		0	0	
		10 cm	100	100		0	0		0	0	
Clackamas	2016	5 cm	96	72.7		0.7	2		3.3	25.3	
		10 cm	95.3	73.3	*	0	1.3		4.7	25.3	
	2017	5 cm	100	80		0	19.3		0	2	
		10 cm	99	96		1	0		0	4	
Thurston	2016	5 cm	94	58.7	*	0	2		6	39.3	
		10 cm	96.7	41.3	**	0	0		3.3	58.7	
	2017	5 cm	99.3	16	***	0.7	84	**	0	0	
		10 cm	100	52.7		0	40	*	0	4	

Total viability is the sum of non-dormant seeds (%) and dormant seeds (%). Non-dormant seeds germinated during the germination test. Dormant seeds tested positive to tetrazolium (TZ) staining. Dead seeds tested negative to TZ staining. Fatal germination accounts for germination which occurred pre-removal of seed packets from the field trial.
Welch's two sample tests were conducted to compare non-solarized (NONSOL) and solarized (SOL) seed samples from the same location, year and the depth. Values are response variables in percentage. Asterisks indicate the statistical significance with the p-value < 0.05 (*), 0.01 (**) or 0.001 (***).

conservative form of predicting the susceptibility of weed seeds, which can be applied as fundamental information to develop models to predict effectiveness of soil solarization.

5 Discussion

Solarized soils accumulated 140 hours of temperatures >45°C at the 5 cm depth across all locations and both years. Based on laboratory results, only *P. annua* and *P. pensylvanicum* had estimated alpha values below 140 hours for 90% mortality at 45°C. *Amaranthus retroflexus* and *P. oleracea* had an estimated accumulation of 218 and >3000 hours at 45°C for 90% mortality, respectively. The effect of soil solarization on weed seeds was species dependent and the field studies were consistent with the laboratory studies with *P. annua* and *P. pensylvanicum* having the greatest percent seed mortality followed by *A. retroflexus* and variable results for *P. oleracea* and *C. esculentus*.

Naturally occurring populations of *P. annua* emergence were reduced by 97 – 100% and buried packets had >90% seed mortality after soil solarization. Similar results were achieved with *P. annua* in other soil solarization studies (Chase et al., 1999; Peachey et al., 2001; Benlioğlu et al., 2005).

Polygonum pensylvanicum was not present in naturally occurring populations at the different locations, however *Polygonum persicaria* (L.) was present and soil solarization reduced natural population emergence by 97%. Similar results were achieved in buried packets of *P. pensylvanicum* where 89 – 100% seed mortality occurred from soil solarization. *Polygonum pensylvanicum* and *P. persicaria* are summer annuals; however, both species were sensitive to soil solarization.

Naturally occurring *A. retroflexus* was present at one location with 100% reduction in weed emergence in solarized soil compared to non-solarized. Soil solarization decreased *A. retroflexus* seed viability in both years and depths in buried seed packets; however, results were inconsistent ranging from 0 to 84% seed mortality. In buried seed packets, there was an increase in percent dormant seed of *A. retroflexus* compared with the non-solarized treatment. Dormancy increased at both the 5 cm and 10 cm depths with ≥50% of the seeds dormant after solarization at each location. Most viable seeds in the soil seedbank are concentrated in the top 5 cm (Akinola et al., 1998), and for many species, emergence is reduced significantly as the depth increases. The ability to emerge from deeper depths is related to the size of the seeds (Benvenuti et al., 2001; Grundy et al., 2003). Benvenuti et al. (2001) reported 50% of *A. retroflexus* germinated at 5.4 cm and no seeds germinated deeper than 8 cm. Our results demonstrate a potential long-term soil seed bank survival mechanism for *A. retroflexus*, even under the higher temperatures that soil solarization produces, and which killed other weed species. Therefore, this study demonstrated that seed viability must be tested with a TZ test to confirm seed mortality.

Portulaca oleracea seed remained viable at higher temperatures compared to the other species in the seed packets. There was a percent reduction in viable seeds in 2016 in solarized compared to non-solarized populations; however, accumulated thermal units were lower in 2016 compared to 2017. Benlioğlu et al. (2005) saw a >95% reduction of *P. oleracea* after 49 days of soil solarization in Turkey, which also has a Mediterranean climate. However, the average maximum temperature obtained in the study was 47°C, well above the average maximum temperature of 27°C at the three locations in the PNW. *Portulaca oleracea* weed seed have been

TABLE 8 Fate of *Portulaca oleracea* seeds from weed seed packets buried in field trials.

Location	Year	Depth	Total Viability (%)			Dead Seed (%)			Fatal Germination (%)		
			NONSOL	SOL		NONSOL	SOL		NONSOL	SOL	
Yamhill	2016	5 cm	86	71.3	*	0	0		14	28.7	
		10 cm	82	85		0	0		18	15	
	2017	5 cm	98	96		2	3.3		0	0	
		10 cm	99.3	89		0.7	2		0	0	
Clackamas	2016	5 cm	95.3	84.7		1.3	0		3.3	15.3	
		10 cm	90.7	85.3		0	0		9.3	14.7	*
	2017	5 cm	100	100		0	0		0	0	
		10 cm	100	100		0	0		0	0	
Thurston	2016	5 cm	80	64.7		0.7	8.7		19.3	26.7	
		10 cm	81.3	63.3		0	0		18.7	36.7	*
	2017	5 cm	100	100		0	0		0	0	
		10 cm	100	100		0	0		0	0	

Total viability is the sum of non-dormant seeds (%) and dormant seeds (%). Non-dormant seeds germinated during the germination test. Dormant seeds tested positive to tetrazolium (TZ) staining. Dead seeds tested negative to TZ staining. Fatal germination accounts for germination which occurred pre-removal of seed packets from the field trial.
Welch's two sample tests were conducted to compare non-solarized (NONSOL) and solarized (SOL) seed samples from the same location, year and the depth. Values are response variables in percentage. Asterisks indicate the statistical significance with the p-value < 0.05 (*), 0.01 (**) or 0.001 (***).

recorded to survive soil temperatures greater than 60°C depending on the amount of moisture present and the length of exposure (Egley, 1990).

The thermal dose-response curve estimated the T_{90} for *P. oleracea* to be > 3000 h at 45°C, 828 h at 50°C, and 40 h at 55°C. In a similar study, Dahlquist et al. (2007) using a non-linear regression model based on seed germination reported the T_{90} as 19 h at 50°C and 1 h at 60°C. However, they did not test viability of non-germinated seed. The differences in results suggest the previously reported mortality may include dormant seeds and *P. oleracea* may be more tolerant to constant temperature treatments than previously reported. High temperatures are one of the factors which can induce secondary dormancy in summer annual seeds (Forcella et al., 2000).

The most compelling evidence that soil solarization can be an effective weed management method in PNW tree nurseries comes from field studies on naturally occurring weed species carried out in two sites over two years. Solarization reduced naturally occurring weed emergence for several months after the solarization film was removed compared with non-solarized plots. There was a 94-96% decrease in weed emergence in solarized plots compared to non-solarized plots during the fall and winter counts at Clackamas in 2016 and 2017. The large effect on fall and winter weeds may reflect a greater number of winter annual weed species present compared to summer annuals and the lower thermotolerance of winter annuals previously discussed. While counts of naturally occurring weeds were overall lower in spring and early summer, weed emergence in solarized plots was still 67-81% lower than non-solarized plots even after 7-9 months. Even during the summer following solarization, solarized plots required less time to hand weed. Hand weeding is one of the major costs in nursery production, and the current shortage of nursery workers can

TABLE 9 Fate of *Cyperus esculentus* tubers from recovered buried packets.

			Seedlings Recovered			Live Seedlings			Tubers Recovered			Tubers Germinated		
Location	Year	Depth	NONSOL	SOL		NONSOL	SOL		NONSOL	SOL		NONSOL	SOL	
			count			%			count			%		
Thurston Co.	2016	5 cm	32.3	6.7		100	43.3		47.7	43.3		41.9	43.3	
		10 cm	2.0	0.0		33.3	na		51.3	53.3		50.0	50.0	
	2017	5 cm	12.3	6.3		100	15.7	*	51.7	43.7	*	71.0	57.8	
		10 cm	0.0	0.0		na	na		50.0	50.0		68.7	53.3	

The number of seedlings and tubers collected at the time of packet removal. Each packet initially contained 50 tubers. Live seedlings are the percentage of recovered seedlings which survived for two weeks after transplanting in the greenhouse. The nutlets germinated are a percent germination of recovered nutlets two weeks after transplanting in the green house.
Welch's two sample tests were conducted to compare non-solarized (NONSOL) and solarized (SOL) samples from the same year and the depth. Asterisks indicate the statistical significance (p-value < 0.05).

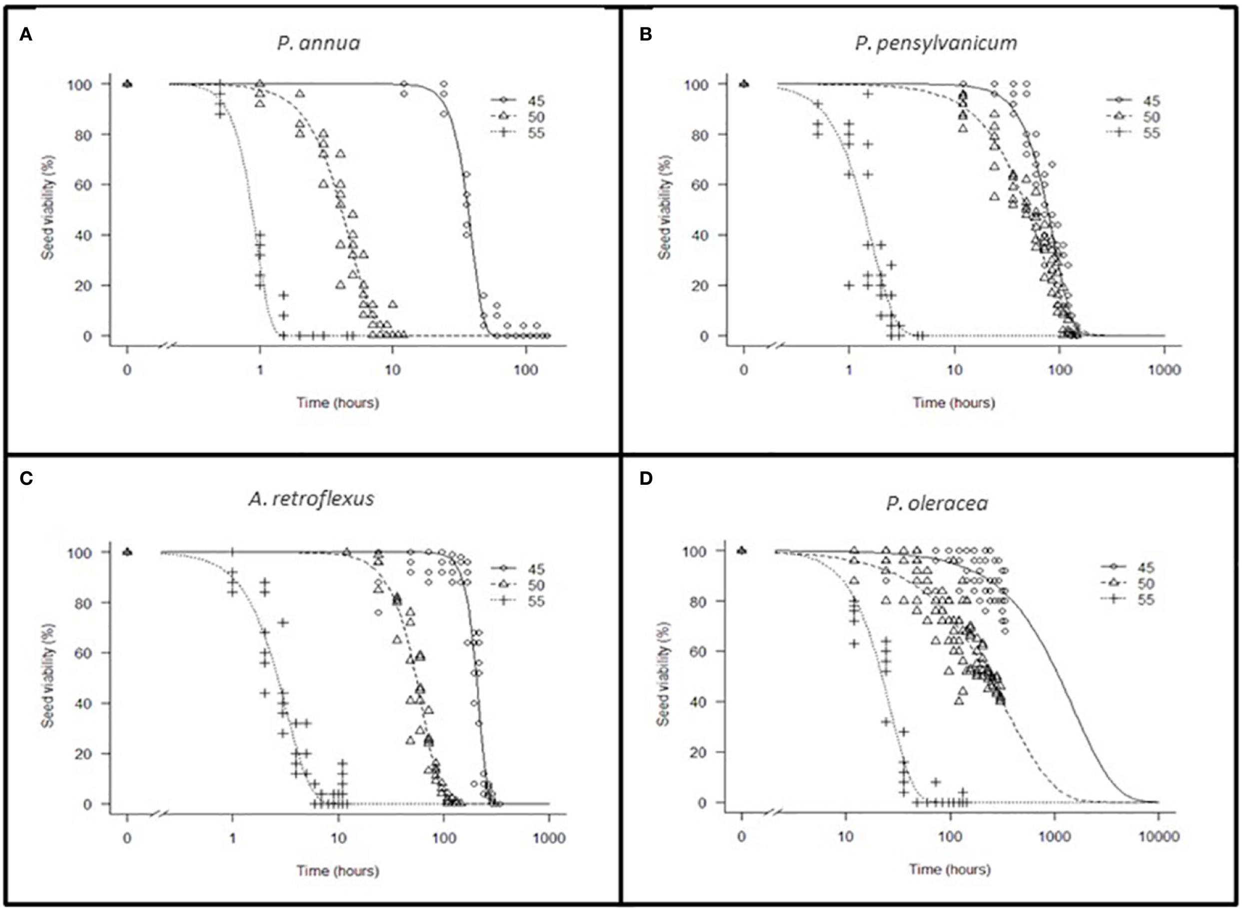

FIGURE 6
Thermal dose-response curve of *Poa annua* **(A)**, *Polygonum pensylvanicum* **(B)**, *Amaranthus retroflexus* **(C)**, *Portulaca oleracea* **(D)** percent viability vs. time at constant temperature treatments. Seed viability at each sampling time at 45 (o), 50 (Δ), and 55(+) °C. The x-axis of *P. oleracea* **(D)** is a factor of 10 greater than other axes.

further exacerbate timely weed management. Hand weeding time in these studies was reduced by 50 to 69% in solarized plots. The reduction in hand weeding enables nurseries to save labor costs and/or redirect workers to tasks that do not require as much physical labor (personal communication).

In addition, with the reduced need for hand weeding there is less soil disturbance that results in fewer viable weed seed being brought closer to the soil surface and subsequently reduces to weed emergence.

6 Conclusion

These studies demonstrate that soil solarization can be an effective nonchemical weed management technique for PNW tree nurseries, even though it is not lethal to all weed species. A reduction in naturally occurring weed emergence of > 94% rivals the effectiveness of chemical herbicides (Chauhan and Abugho, 2012) without the potential confounding result of herbicide damage, the development of resistance and chemical drift (Case et al., 2005). We observed more consistent results across years in the Oregon sites than the more northerly Washington site. Solarization appears to work best on weed species intolerant of high temperatures and on seeds incapable of emerging from deeper soil depths that are not sufficiently heated by solarization. Solarization was not effective in preventing emergence of *C. esculentus*, a species that emerges from deeply buried, hardy tubers. Although this species was found only at the Washington site, the presence of this pernicious weed in forest nurseries previously justified the use of methyl bromide fumigation to prevent its dispersal. Solarization is not reliable enough to manage this species, but our studies show that solarization is effective against many of the weed species encountered in commercial PNW nurseries, expanding the geographic zones previously considered suitable for soil solarization (Katan and Gamliel, 2009).

Soil solarization is not without limitations. It requires the use of plastic, which is costly and represents a potential disposal hazard. In Oregon, the solarization film has been picked up and recycled for manufacturing agricultural plastics, but the market for recycled plastic is unpredictable. Nurseries must also invest in specialized equipment for laying the plastic. The Clackamas nursery included in these studies continues to solarize their seedling fields both as a cost- and labor-saving strategy, but also to reduce their weed seedbank for the long term (grower personal communication).

TABLE 10 The sampling intervals and parameter estimates for each species and temperature in the controlled study.

Species	Temperature (°C)	Sampling Interval (h)	*b* estimate	*b* SE	*a* estimate (h)	*a* SE
Poa annua	45	12	5.1	13.9	39.5	85.9
	50	1	2.4	23.8	4.8	79.9
	55	0.5	4.3	8	0.9	72
Polygonum pensylvanicum	45	12	2.5	0.2	89	1.8
	50	12	1.4	0.1	63.9	2
	55	0.5	2	0.2	1.7	0.1
Amaranthus retroflexus	45	24	5.8	0.4	217.8	2.2
	50	12	2.6	0.2	63.5	1.3
	55	1	1.9	0.2	3.2	0.1
Portulaca oleracea	45	24	1.1	0.3	1527.7	723.8
	50	12	0.9	0.1	344.1	16.4
	55	12	2	0.2	26.7	0.8

Fall sown crops enable solarization to occur during warmer summer months for greater accumulated thermal hours and greater weed seed mortality. This timing restricts the use of soil solarization for many cropping systems; however, there are potential opportunities to utilize solarization to manage weeds in a fallow system or a transition from conventional to organic production with limited weed control options (Mallory-Smith et al, 2019; Parke et al., 2018). In addition to weed management, solarization has been shown to kill plant pathogens (Funahashi and Parke, 2016; Funahashi and Parke, 2018). Solarization could be used as part of a management program where soil fumigation is not feasible in managing both weeds and plant pathogens due to pesticide application buffer restrictions.

Soil solarization can be a viable option for managing weeds in PNW tree nurseries. A preliminary online soil solarization program (Online Soil Solarization Program, 2019) has been established to help PNW growers determine the feasibility of achieving soil temperatures sufficient to kill certain soilborne pests. Growers can select their target pathogen or weed species, nearest weather station, prior year to use for forecasting weather, soil solarization start and end date, and lower boundary soil temperature (temperature at 50 cm depth), then run the model to determine the soil depth at which a lethal temperature will be achieved, and how long it will take. Application of the predictive model will reveal new opportunities for solarization in horticultural and forest nurseries in the PNW but also point out limitations in applying solarization for managing certain heat-resistant weed species, especially at northerly locations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. The full data set for soil temperature is available at ScholarsArchive@OSU (https://ir.library.oregonstate.edu/?locale=en). Further inquiries can be directed to the corresponding author.

Author contributions

NW: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. PB: Data curation, Writing – review & editing. BH: Data curation, Investigation, Methodology, Writing – review & editing. CM-S: Conceptualization, Funding acquisition, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. JP: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2023.1321372/full#supplementary-material

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The effect of two-year application of pelargonic acid on the growth of *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L.

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Synthetic herbicides are used for perennial weed management, but owing to environmental and health concerns they face increasing regulatory restrictions. Consequently, there is growing interest in ecologically friendly alternatives including bio-herbicides based on natural compounds such as the active ingredient pelargonic acid (PA). PA acts as a broad-spectrum non-selective contact herbicide. However, when used as a contact herbicide, regrowth of the aboveground parts of plants still presents a challenge. The aim of this study was to investigate the control effect of a two-year application of PA on perennial weeds. The study was conducted between spring 2020 and autumn 2021 as a semi-field experiment. The factors were two levels of weed species (*Cirsium arvense* and *Sonchus arvensis*), three levels of herbicide treatment (untreated control, PA, and glyphosate), and three levels of initial ramet size (5, 10, and 15 cm). The results showed that a two-year application of PA increased its efficacy on *C. arvense* and *S. arvensis* when combined with the smaller initial ramet size (5 cm), but did not prevent regrowth in either species. PA efficacy was greater on *C. arvense* than on *S. arvensis*. The plant coverage decreased by 24 % when the initial ramet size was 5 cm for *C. arvense*, while for *S. arvensis* with the same initial ramet size it was reduced by just 4 %. For PA-treated *C. arvense* with an initial ramet size of 5 cm, aboveground biomass and belowground biomass were reduced by 43 % and 22 % respectively. In *S. arvensis*, the reductions in aboveground and belowground biomass for an initial ramet sizes of 5 cm were 13 % and 12 % respectively. In general, PA efficacy was not as high as glyphosate efficacy for both species. In conclusion, the results revealed that after PA application the regrowth of shoots from the creeping roots in *C. arvensis* and *S. arvensis* decreased when the initial ramet size was 5 cm. This reduction suggests that PA efficacy on these plants increases when it is applied repeatedly on the same patches with smaller initial root fragments.

KEYWORDS

perennial weeds, bio-herbicide, ramet, creeping thistle, sow-thistle

1 Introduction

As perennial weeds, *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L. represent significant threats to agricultural productivity due to their ability to persist and spread over time and compete with crops for resources (Ramesh et al., 2017). *C. arvense* (creeping thistle) and *S. arvensis* (sow thistle) are found in various crops and are considered highly problematic in temperate regions (Liew et al., 2013; Tørresen and Gerowitt, 2022; Andert et al., 2023). Both weeds form patches and mainly have rapid vegetative reproduction through their subterranean reproductive organs (horizontal creeping roots) and abundant seed production (Tørresen and Gerowitt, 2022). Their allelopathic effects on some crops, especially the prevention of seed germination or seedling growth have been reported in previous studies (Szabó and Halbritter, 2015; Bashir et al., 2018; Egushova and Anokhina, 2022).

There are various control methods for these weeds, including chemical, mechanical, and cultural methods (Melander et al., 2012). The common chemical control method for perennial weeds is the use of systemic herbicides, e.g. the non-selective active ingredient glyphosate in the intercropping period (Beckie et al., 2020). Furthermore, certain systemic herbicides are registered for application during the cropping period, e.g., the active ingredient metsulfuron-methyl can be used in cereals, effectively controlling a broad spectrum of weeds including *C. arvense* (Bhullar et al., 2013; Zargar et al., 2019). For decades, synthetic herbicides have been crucial for perennial weed control owing to their practical and financial advantages (Loddo et al., 2023). However, for herbicides such as glyphosate, stricter regulations concerning their registration and usage have been implemented in numerous countries due to concerns about herbicide resistance and their adverse effects on the environment and human health (Antier et al., 2020; Beckie et al., 2020). The 'Farm to Fork' strategy is one of the key components of the European Green Deal that reflects the growing interest in sustainable and ecologically friendly weed control solutions, including synthetic herbicide substitutions (European Commission, 2020; Radicetti and Mancinelli, 2021). Alternative approaches include mechanical methods, cultural tools, and alternative herbicides, which are non-chemical, natural, or less toxic (Synowiec et al., 2017; Ibáñez and Blázquez, 2018; Beckie et al., 2020).

Bio-herbicides are products of natural origin for weed control that are either microorganisms or products derived from living organisms, including the natural metabolites produced by these organisms (Cordeau et al., 2016). The herbicidal effects of natural substances, such as plant essential oils and organic acids, have been the subject of many studies in recent years. (Barton et al., 2014; Synowiec et al., 2017; Casella et al., 2023; Kouki et al., 2023). Among tested natural active ingredients, pelargonic acid (PA) is the sole ingredient available on the market (Loddo et al., 2023). PA is a naturally occurring fatty acid found in foods such as vegetables and fruits and has been approved as a safe food agent in numerous countries (Ciriminna et al., 2019). PA degrades quickly in the environment and does not cause long-term runoff contamination in rainy seasons (EFSA (European Food Safety Authority), 2021). Bio-herbicides that contain the active ingredient PA are known as burndown herbicides and are increasingly used for weed control, e.g. on gardens, lawns, and walkways (Ciriminna et al., 2019).

In light of PA's potential as a bio-herbicide and its ability to contribute to the reduction of synthetic herbicides, researchers have been exploring its efficacy, and have shown that PA is the most successful bio-herbicide available (Webber et al., 2014a; Carroll et al., 2022; Muñoz et al., 2022; Pannacci et al., 2022). However, its effectiveness varies between weed species (Webber et al., 2014a; Webber et al., 2014b). Monocotyledon weeds such as *Alopecurus myosuroides* Huds. and *Lolium rigidum* Gaud are less sensitive to PA, and may display reduced and transient symptoms at higher doses (Travlos et al., 2020; Loddo et al., 2023). Dicotyledon weeds also exhibit considerable differences in sensitivity to PA (Webber et al., 2014a; Webber et al., 2014b; Loddo et al., 2023). Furthermore, there is evidence of regrowth after the application of PA for most weeds (Ciriminna et al., 2019; Muñoz et al., 2020; Travlos et al., 2020; Muñoz et al., 2022; Loddo et al., 2023). Due to the occurrence of regrowth, previous studies have suggested sequential applications of PA with short intervals within a growing season (Barker and Prostak, 2009; Webber et al., 2014a; Webber et al., 2014b). Earlier investigations mainly concentrated on annual weeds, particularly examining the impact of PA with a focus on the aboveground parts of plants (Webber et al., 2014a; Webber et al., 2014b; Ciriminna et al., 2019; Muñoz et al., 2020; Travlos et al., 2020; Muñoz et al., 2022; Pannacci et al., 2022; Loddo et al., 2023).

The presence of creeping roots in *C. arvense* and *S. arvensis* negatively affects the success of control methods (Liew et al., 2013). Adventitious buds are formed on the horizontal creeping roots, from which new shoots can emerge (Brandsæter et al., 2010; Liew et al., 2013). The activities of adventitious buds and the emergence of shoots differ from species to species (Brandsæter et al., 2010). Furthermore, the ability of creeping roots to sprout varies significantly during the season (Brandsæter et al., 2010; Liew et al., 2013). For *S. arvensis*, the sprouting capacity appears to decrease in late summer to early autumn (Håkansson, 1969; Håkansson and Wallgren, 1972; Brandsæter et al., 2010), while sprouting for *C. arvense* does not decrease as long as environmental conditions allow it (Brandsæter et al., 2010). The root fragment of the creeping perennial is called a ramet, which is genetically identical to the mother plant (Tørresen and Gerowitt, 2022), and it is often induced by mechanical soil disturbance (Håkansson, 2003). Large ramets can rapidly produce new *C. arvense* plants, while smaller ramets often do not survive to produce vegetative offspring due to their low carbohydrate reserves (Hamdoun, 1972). Similar to *C. arvense*, the emergence and number of sprouts in *S. arvensis* depend on the dry matter content of the roots (Lemna and Messersmith, 1990; Vanhala et al., 2006). When the creeping roots are fragmented by tillage, the resulting smaller ramets are less viable and have less dry matter, making them more likely to die (Vanhala et al., 2006). Furthermore, there is a phenological stage at which belowground biomass reaches its minimum dry weight before it begins to increase again (Tavaziva, 2012). In both species, this stage occurs when they have between four and seven leaves (Håkansson, 2003). Depleting the belowground carbohydrate reserves through fragmentation of the regenerative structures and applying treatments to the lowest belowground biomass have been suggested by previous research as offering better control (Håkansson, 1969; Gustavsson, 1997; Brandsæter et al., 2010). To achieve successful control, it is essential to apply control methods to these perennial weeds when they are most sensitive to disturbance (Verwijst et al., 2018).

An earlier study investigated the effects of plant growth stage, application volume and the addition of adjuvant on PA efficacy to control *C. arvense*. The results demonstrated that PA efficacy is greater when applied on a 4-8 leaf-stage plant using an increased application volume, and also by adding an adjuvant (Ganji et al., 2022). The present study investigated the feasibility of repeated PA application over two consecutive years on the same spot (patch of *C. arvense* and *S. arvensis*) considering their initial ramet size. Its aim was to determine the PA herbicidal impact on the entire life cycle of the perennial weed species and their regrowth patterns that might not be identified in shorter-term studies. Moreover, the efficacy of PA as a potential herbicidal treatment was compared with that of the commonly used active ingredient glyphosate (GLY). This design facilitated a comprehensive comparison of the effects of PA treatment and two reference conditions: the untreated control (UC), representing the baseline or natural state, and GLY treatment, which serves as a standard for effective perennial weed control (Hudek et al., 2021; Kanatas et al., 2021).

The present study focused on perennial weeds with creeping roots and examined the influence of initial ramet size on PA efficacy after two applications within two consecutive years on the same spot (patch of *C. arvense* and *S. arvensis*), with the aim of improving knowledge about the control of perennial weeds with creeping roots using PA. Considering the regenerative capacity of the creeping roots, the objectives of this study were to determine (i) whether the initial ramet size influences PA efficacy and regrowth of *C. arvense* and *S. arvensis* after PA application and (ii) whether there are any differences between these perennial weeds in terms of PA efficacy and regrowth patterns after repeated application.

It was hypothesized that repeated application of PA over two consecutive years reduces growth parameters, aboveground and belowground biomass, and flower numbers in both species. It was expected that *S. arvensis* would be more susceptible to PA than *C. arvense* due to seasonal variations that impact the sprouting abilities of *S. arvensis* by demonstraiting reduced capacity in late summer to early autumn (Håkansson, 1969; Håkansson and Wallgren, 1972; Brandsæter et al., 2010). For this reason, it was assumed that regrowth after herbicide application would differ between these species. Finally, it was also assumed that smaller *C. arvense* and *S. arvensis* ramets have a lower regenerative capacity, which reduces regrowth and increases PA efficacy.

2 Materials and methods

A two-year pot experiment (from spring 2020 to autumn 2021) under semi-field conditions was conducted in the experimental field of Rostock University in northeast Germany (location Rostock: 54° 4' 6.726" N, 12° 4' 54.0876" E).

Figure 1 provides a concise overview of the experimental workflow used in this semi-field experiment. The process included (1) the preparation of the semi-field experiment by planting ramets in pots, (2) the application of the herbicide treatments in each experimental year, and (3) visual assessments to measure the efficacy of the herbicides compared with the untreated control.

The experiment was a factorial, completely randomized block design with four replications. The factors were plant species with two levels, treatments with three levels, and ramets with three levels of initial size: 5, 10, and 15 cm (Table 1).

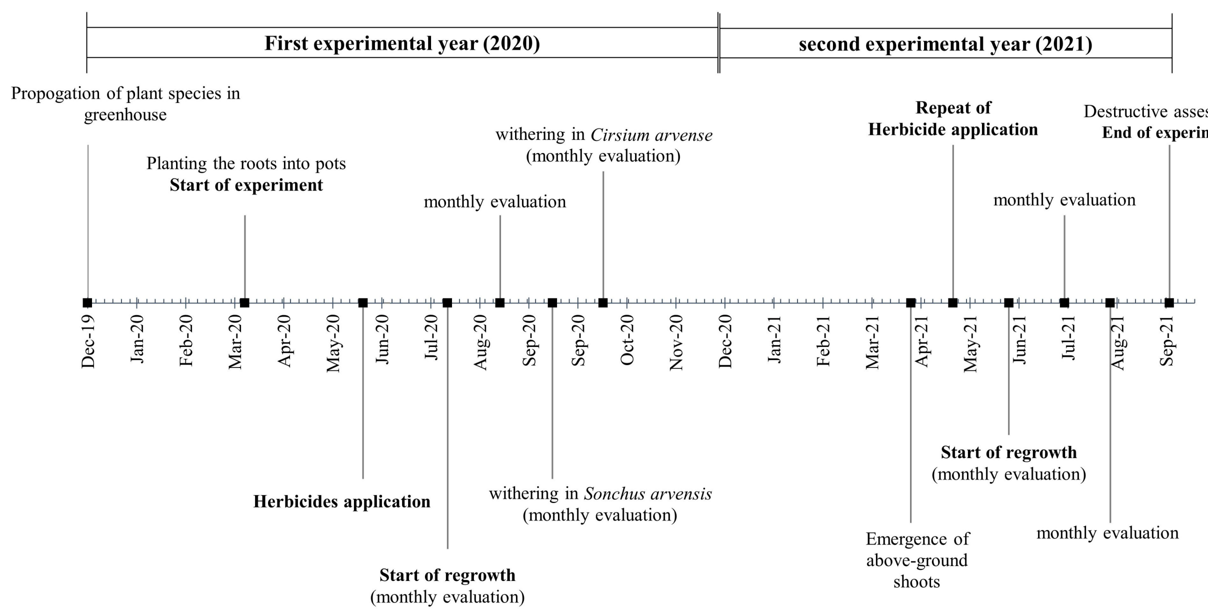

FIGURE 1
Experimental set up, assessments, and activities over the two years of the experiment.

TABLE 1 Overview of factorial experiment design with plant species, herbicide treatments, and initial ramet size as factors.

Factors	Levels of factor	Abb.[1]	Description of herbicides	
			Used amount	Active ingredient content (g/L)
Treatments (Herbicides)	Untreated control	UC	–	–
	Pelargonic acid	PA	16 L/ha	680 g/L pelargonic acid
	Glyphosate	GLY	3 L/ha	480 g/L glyphosate
Plant Species	*Cirsium arvense*			
	Sonchus arvensis			
Ramet initial size	5 cm			
	10 cm			
	15 cm			

[1]Abbreviation for treatment names.

For this research, Beloukha® (680 g/L pelargonic acid) and Roundup Powerflex® (480 g/L glyphosate and 393.6 g/L acid equivalent) were used as the PA and GLY treatments, respectively.

2.1 Ramet planting in pots

In December 2019, creeping roots of *C. arvense* were collected from the University of Rostock's experimental field and those of *S. arvensis* from a field near Güstrow (53°46'14.6"N, 12°09'59.3"E). The collected roots were stored by propagation in pots in the greenhouse. In February 2020, the pots were buried in the soil and filled with a mixture of ½ field soil, ¼ potting soil, and ¼ compost. The pot size was 200 L with a diameter of 80 cm (pot surface area 0.503 m^2). The distance between pots was one meter. The creeping roots obtained from propagated greenhouse pots (cleaned and washed to eliminate the remaining soil) were used for the semi-field experiment. Roots that were more than 3 mm in diameter were fragmented into ramets 5, 10, and 15 cm in length, and weighed. The term ramet is used in the text instead of root fragment because the root fragments used in this experiment were derived from one progenitor of each plant species, and shared the same genotypes as the parent plants (Tørresen and Gerowitt, 2022). Finally, one single ramet was planted directly at a 5 cm soil depth in each pot. The pots were irrigated immediately after the ramets were planted and again when there was a need for irrigation on warm days during the growing seasons in both experimental years. After the establishment of the pots in the soil and until the end of the experiment in the second year, other weeds were removed from the pots by hand. Before herbicide application in both experimental years, fertilization was undertaken to achieve nutrient conditions comparable with spring cereal fields. Hakaphos Blau® as an NPK fertilizer (15 % N, 10 % P, and 15 % K) was applied to the soil at the amount of 16.65 g per pot as a balanced nutrient solution. These rates correspond to 50 kg of nitrogen per hectare, which is less fertilization than regular spring cereal fields as there was no crop in the pots to compete for the nutrients.

2.2 Application of herbicide treatments

At the end of May 2020, herbicide treatments were applied to the *S. arvensis* pots. Owing to the late emergence of *C. arvense* sprouts, the herbicide application for this species was performed two weeks later. The plant growth stage at the time of herbicide applications for both plant species was the four-to-eight-leaf-stage considering the compensation point according to Håkansson (2003) and BBCH 14-18 according to Meier (2018), and each pot had one or two plants (shoots from the same ramet).

At the beginning of May 2021, the herbicide treatments were applied again. Each pot was treated with the same herbicide treatment as the previous year. The plant growth stage at herbicide application time was four-to-eight-leaf, but each pot had many shoots emerged from the same ramet which was recorded. In both years, a plot-spraying device with a pressure of 2.1 bar and a speed of 4 kilometers per hour was utilized for the herbicide applications. The application volume for the treatments was 200 L/ha. The operated flat jet nozzle was size 02.

2.3 Assessments

The herbicide treatments were assessed by visually estimating the percentage of necrotization. The assessments were conducted at 1, 7, 14, and 21 days after treatment (DAT) of the herbicides. A value of 0 % necrotization was equivalent to completely vital vegetation, while a value of 100 % represented completely dead vegetation. The level of necrotization was interpreted as herbicide efficacy. The pots were evaluated for regrowth from the ramets starting 28 days after herbicide treatment (28^{th}_DAT). To be able to monitor the regrowth pattern and effect of the treatments over the long-term experimental period and identify the possible variability in regrowth pattern, data on plant height, shoot density per m^2, and BBCH stage (Meier, 2018) were collected monthly until the end of the growing season. The monthly evaluations were performed from July to October 2020 and from April to September 2021. To obtain information on how the weed species population was established and regrew after herbicide application during the growing season, in August of each year before the withering of the plants, the shoot density per m^2, the percentage coverage of the soil surface by the plant, and the number of flowers were assessed. All emerged shoots were counted and then shoot density per m^2 was calculated according to a pot surface area of 0.503 m^2. Counting of the flowers indicates the reproductive capacity of the weed species, and helps to understand the potential for seed production after application of the treatments. By undertaking this assessment in August, the weed's reproductive success was estimated, before it completed its life cycle. Aboveground and belowground biomass was measured per pot using the destructive method at the end of the experiment in 2021, which determined the effectiveness of the herbicide treatment at controlling weed growth. For this, the collected plant materials were placed in an oven at 60 °C for at least 24 hours, and the dried biomass was then measured. As mentioned in the experimental setup, ramets were weighed before being planted in the pots, and this was used as a covariate in the statistical analysis. Figure 1 gives an overview of the above-mentioned experimental setup and assessments.

2.4 Weather conditions

Figure 2 shows the weather conditions for the entire experimental period. The average air temperature for the growing season (March to September) was 13.2 °C in 2020 and 13.1 °C in 2021. In the 2020 growing season, the minimum average soil temperatures were 5.9 °C and 5.6 °C respectively at 5 cm and 20 cm belowground level in March. In August, the maximum average temperatures at 5 cm and 20 cm belowground level were 19.6 °C and 18.8 °C respectively. In 2021, the minimum soil temperature at these belowground levels averaged 2.3 °C and 2.1 °C, respectively, and the maximum was 20.7 °C in 5 cm and 19.8 °C in 20 cm soil depth in July. The total amount of precipitation in 2020 was 428 mm. Of this amount, 321 mm occurred in the growing season. The precipitation amount from January until the end of September 2021 was 500 mm, of which 376 mm occured in the growing season (Figure 2).

2.5 Statistical analysis

Data on herbicide efficacy (necrotization percentage) were analyzed using linear mixed model analysis (LMM), with weed species, herbicide treatments, initial ramet size, DATs and their interaction as fixed effects, and replicates as random effects. In this analysis, the following model (Equation 1) was fitted for two consecutive years, 2020 and 2021. For each year, the same model structure with shared fixed effects was used, but allowed for separate random intercepts (b_m) to capture year-specific variability:

$$\text{Herbicide efficacy}(\%)_{\text{ijklm}} = \mu + S_i \times H_j \times RS_k \times D_l + b_m + \epsilon_{ijkl} \quad (1)$$

where μ is the overall mean; S_i, H_j, RS_k and D_l represent the fixed effects for "weed species", "herbicide treatment", "initial ramet size", and "DATs" respectively; b_m represents the random intercepts for the grouping variable "block", and ϵ_{ijkl} is the error term that accounts for unexplained variability in the model.

The long-term effectiveness of PA was revealed through a separate analysis at 21^{st}_DAT for each experimental year, providing information to understand the sustained impact of weed control over the course of the experiment. The same model

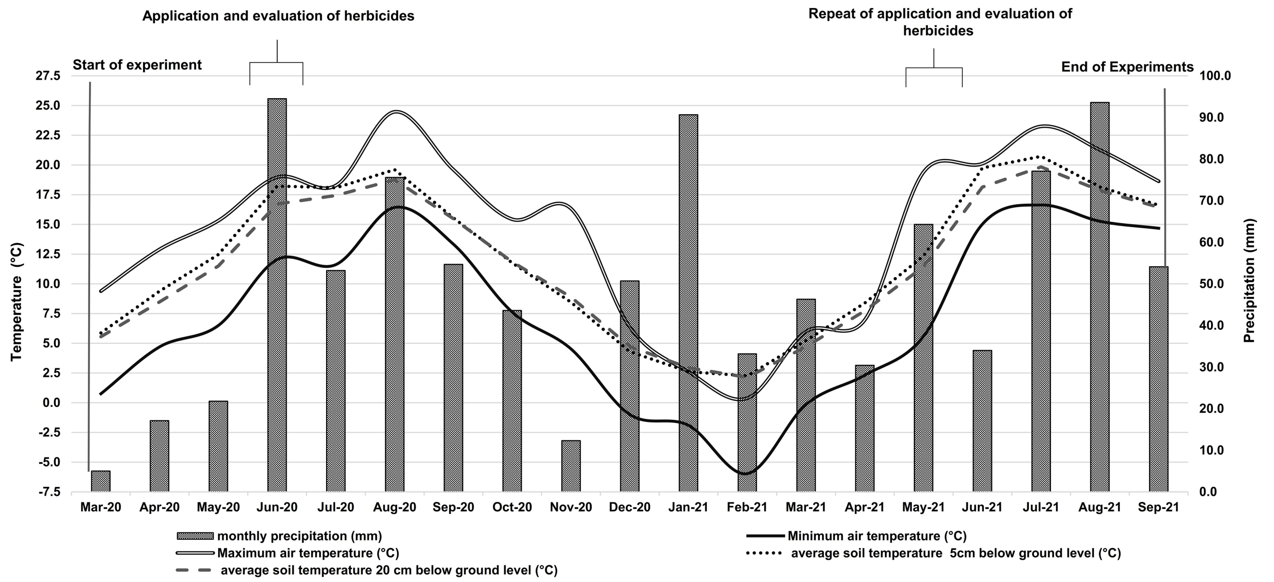

FIGURE 2
Air temperature (°C), soil temperatures at 5 cm and 20 cm below-ground level, and precipitation (mm) during the experimental period in 2020 and 2021. Information for both years was obtained from the research weather station of the University of Rostock's Department of Hydrology and Applied Meteorology.

structure (Equation 2) with shared fixed effects was utilized to analyze the herbicide efficacy in the final assessment at 21^{st}_DAT as follows:

$$\text{Herbicide efficacy at } 21^{st}\_\text{DAT}(\%)_{\text{ijkl}} = \mu + S_i \times H_j \times RS_k + b_l + \epsilon_{ijk} \tag{2}$$

where μ is the overall mean; S_i, H_j and RS_k are the fixed effects for "weed species", "herbicide treatment", "initial ramet size", and "DATs" respectively; b_l represents the random intercepts for the grouping variable "block", and ϵ_{ijkl} is the error term.

As the experimental years were not independent of each other, in order to examine the effect of factors on the measured variables after regrowth, which were shoot density per m^2, the percentage coverage of the soil surface by the plant, and the number of flowers, the data from the measurements at the end of the experiment were utilized for statistical analysis using LMM (Equations 3–7). Three factors (weed species, herbicide treatment, and initial ramet size) and their interaction were fixed effects in this analysis. The random effects were replicates (blocks) and the initial weight of ramets (as covariates). It was assumed that the association between the initial weight of ramets and measured variables depended on the magnitude of weight, thus the heterogeneity of weight was modeled as a random effect in the data analysis of this research. The model equations are as follows:

$$\text{Aboveground biomass}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \tag{3}$$

$$\text{Belowground biomass}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \tag{4}$$

$$\text{Shoot density}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \tag{5}$$

$$\text{Soil coverage }(\%)_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \tag{6}$$

$$\text{Flower head}_{ijk} = \mu + S_i \times H_j \times RS_{ij} + b_k + \epsilon_{ijk} \tag{7}$$

where μ is the overall mean; S_i is the effect of species; H_j is the effect of herbicide treatment; RS_{ij} is the effect of ramet size; b_k represents the random intercept for block and initial weight of ramets; ϵ_{ijk} is the random error term.

For the analysis of the monthly evaluation of plant height, in addition to the fixed effects of weed species, herbicide treatments, initial ramet size, and their interactions, the fixed effect of the experimental year and its interaction with the fixed effects of these three factors were included in the model (Equation 8). To account for variability within different levels of block and month, random intercepts were included. This allowed the model to capture random variations in plant height within these nested grouping variables. In addition, the model assumed a first-order autoregressive correlation structure (AR1) within each combination of block and month. This choice of correlation structure accounted for potential temporal autocorrelation in plant height measurements within the same block and month combinations. The model equation is as follows:

$$\text{Height}_{ijklm} = \mu + S_i \times H_j \times RS_k \times Y_l + b_m + \varepsilon_{ijkl} \tag{8}$$

where μ is the overall mean; S_i is the effect of species; H_j is the effect of herbicide treatment; RS_k is the effect of ramet size; Y_l is the effect of year; b_m represents the random intercept for block and month in which the subscript m represents different levels of the month nested within block, and ε_{ijkl} is the random error term.

All the models were fitted using the restricted maximum likelihood (REML) method. To evaluate the goodness of fit of the linear mixed models (LMMs), the variance decomposition method was employed to calculate conditional R-squared values for all models. The variance decomposition method was chosen because it has the ability to dissect the total variance in the response variable into components related to fixed effects, random effects, and residual error. The same method was used to calculate marginal R-squared values for all models except the model for plant height. The log-likelihood method was utilized due to the unique characteristics of this particular model, which required a different approach for evaluating goodness of fit. The log-likelihood method was more appropriate in this specific case, as it was able to capture the subtle details of the data better, resulting in a more precise evaluation of the model's performance.

For all the data, pairwise comparisons were conducted using Tukey's HSD tests on the results of LMMs to identify significant differences between treatments by including all the interactions of fixed effects. R version 4.3.0 (R Core Team, 2023) was used to conduct all statistical analyses, and the packages "nlme" (Pinheiro et al., 2023), "lme4" (Bates et al., 2015), "lmerTest" (Kuznetsova et al., 2017), and "emmeans" (Lenth et al., 2018) were used for the LMMs and pairwise comparisons.

3 Results

3.1 Herbicide efficacy

The results of herbicide efficacy at all DATs showed that the main effect of herbicide treatment on herbicide efficacy was statistically significant, which means that the relationship between herbicide treatment and herbicide efficacy varied at different DATs. The results did not express a significant difference between initial ramet sizes or between the weed species *C. arvense* and *S. arvensis* (Supplementary Table 1). However, ramet sizes and species will be presented separately in the text due to biological concerns.

Herbicide efficacy across multiple assessment days in both years showed that the PA treatment had highest efficacy on both weed species at 1^{st}_DAT in 2020, which decreased over the experimental period. PA treatment efficacy for both species was significantly higher than the GLY treatment and UC for all ramet sizes at 1^{st} and 7^{th}_DAT in 2020. The PA treatment showed a statistically significantly higher efficacy on the 5 cm ramet size in *C. arvense* compared with all UC treatments until 21^{st}_DAT in 2020 (Supplementary Table 1). The PA treatment efficacy between various ramet sizes was not significant. For both weed species in 2020, the differences between 1^{st}_DAT and $14^{th}/21^{st}$_DAT were significant for PA treatment efficacy on all ramet sizes. PA treatment efficacy at 7th_DAT was significantly different from 21st_DAT for *C. arvense* ramet sizes 10 cm and 15 cm and for all ramet sizes of *S. arvensis* (Supplementary Table 1 and Figure 3).

After repeated application in 2021, the effect of the PA treatment showed a similar trend and efficacy on both weed species. The GLY treatment in this experiment showed 90 -100 % efficacy for all ramet sizes in both species starting at 7th_DAT in 2020 and on a day between 7th and 14th_DAT in 2021. The plants with the smallest ramet size treated with GLY in 2020 did not regrow in 2021. The *C. arvense* plants with initial ramet sizes of 10 cm and 15 cm treated with GLY did not regrow in the second year of the experiment either. Therefore, GLY treatment efficacies for all ramet sizes of *C. arvense* and the 5 cm ramet size in *S. arvensis* are not displayed on the graph in the second year (Figure 3).

For the evaluation of herbicide efficacy at 21st_DAT in both 2020 and 2021, individual effects of weed species and ramet size were found to be non-significant (Supplementary Figure 1). At 21st_DAT in both years, the PA treatment exhibited an effect that was not statistically significant, whereas the GLY treatment demonstrated a statistically significant impact. In 2020, the interaction effects between *S. arvense* and the PA treatment as well as between the 5 cm ramet size and the PA treatment did not

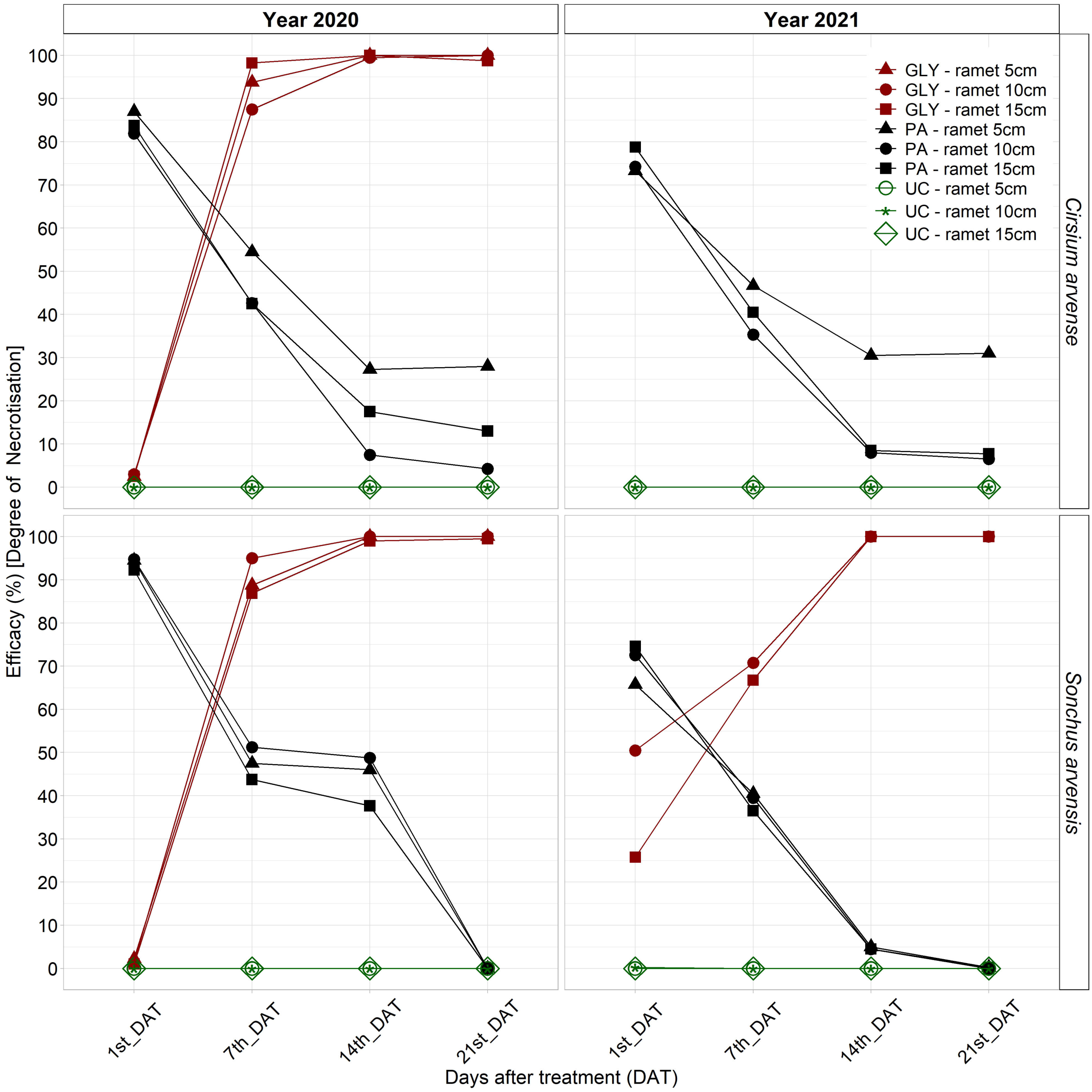

FIGURE 3
PA treatment efficacy (%) [degree of necrotization] compared with the untreated control and GLY treatment at 1st, 7th, 14th, and 21st_DAT after application on *C. arvense* and *S. arvensis* in the first year and repeated application in the second year of experiment. PA, Pelargonic acid; GLY, glyphosate; UC, untreated control.

exhibit a statistical significance. In 2021, the interaction effects between the GLY treatment and the initial ramet size of 10 cm showed a significant positive influence. There was a statistically significant positive association between the 5 cm ramet size and herbicide efficacy when the PA treatment was applied (Supplementary Figure 1 and Figure 3).

3.2 Regrowth after herbicide application

3.2.1 Nondestructive regrowth evaluation

For shoot density per m^2, the main effects of weed species and the interaction between weed species with the GLY treatment were significant, while other fixed effects and their interactions did not have a significant effect (Figure 4). According to these results, the difference between species was statistically significant. With the untreated control and considering the same initial ramet size, the shoot density of *S. arvensis* was on average 249.8 shoots per m^2 greater than *C. arvense*. With the PA treatment, after regrowth the shoot density of *C. arvense* for all initial ramet sizes was significantly lower than for *S. arvensis*. There was no statistically significant difference between the PA and UC treatments in all initial ramet sizes for both weed species. However, when not accounting for initial ramet size, application of PA compared with untreated control led to a maximum 9 % reduction in shoot density of *C. arvense* per m^2. Shoot density in the GLY treatments was significantly lower than in all the other treatments (Figure 4).

The results showed significant differences in soil coverage between the two plant species and between the three levels of herbicide (Figure 5). The average soil coverage of *S. arvensis* was 27.8% higher than *C. arvense* when *S. arvensis* with the same initial ramet size was exposed to the UC treatment. The average soil coverage by plants treated with GLY was 65.4 % lower than that of UC plants in the same weed species and with the same initial ramet size. This difference was also statistically significant. Although PA-treated *C. arvense* plants covered an average lower percentage of the soil surface on average than PA-treated *S. arvensis* plants, the difference between the two species was not significant for any ramet size. In *C. arvense*, the initial ramet size of 5 cm treated with PA covered around 50% of the soil surface, while for the same ramet size under UC around 70 % of the soil surface was covered. *S. arvensis* with an initial ramet size of 10 cm also showed the lowest soil coverage compared with UC with the same initial ramet size. However, the differences in both cases were not statistically significant (Figure 5).

The main effect of herbicide treatments on the number of flowers was significant (Figure 6). When comparing untreated plants with the same initial ramet size, *S. arvensis* produced approximately 10 flowers more than *C. arvense* on average. PA-treated plants of *S. arvensis* in 5 cm and 15 cm ramet sizes produced a smaller number of flowers compared with UC of the same ramet sizes, while a smaller number of flowers were produced by *C. arvense* with initial ramet sizes of 5 cm and 10 cm, but these differences were not statistically significant (Figure 6).

3.2.2 Destructive evaluation of biomass

The results for aboveground and belowground biomass demonstrated no evidence of differences between the two weed species after regrowth (Figure 7; Supplementary Tables 2, 3). The fixed effect of herbicide treatments was significant. No significant difference was found in aboveground or belowground biomass between the PA treatments and UC treatments. The effect of

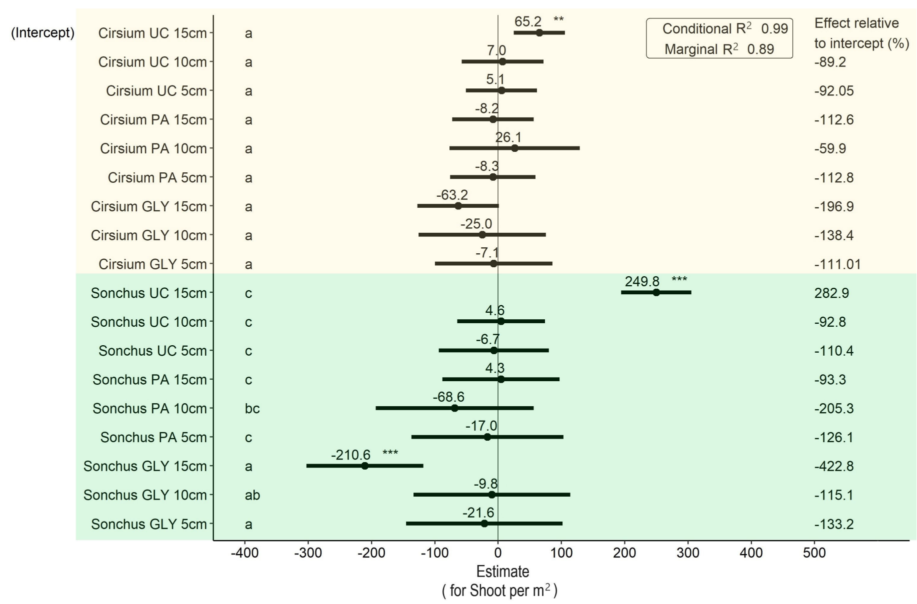

FIGURE 4
Results of the LMM analysis followed by Tukey's HSD on shoot density per m^2 at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * p< 0.05, ** p< 0.01, *** p< 0.001. Groups with different letters are significantly different at p< 0.05 level based on Tukey's HSD test. PA, Pelargonic acid; GLY, glyphosate; UC, untreated control; Cirsium, *Cirsium arvense*; Sonchus, *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

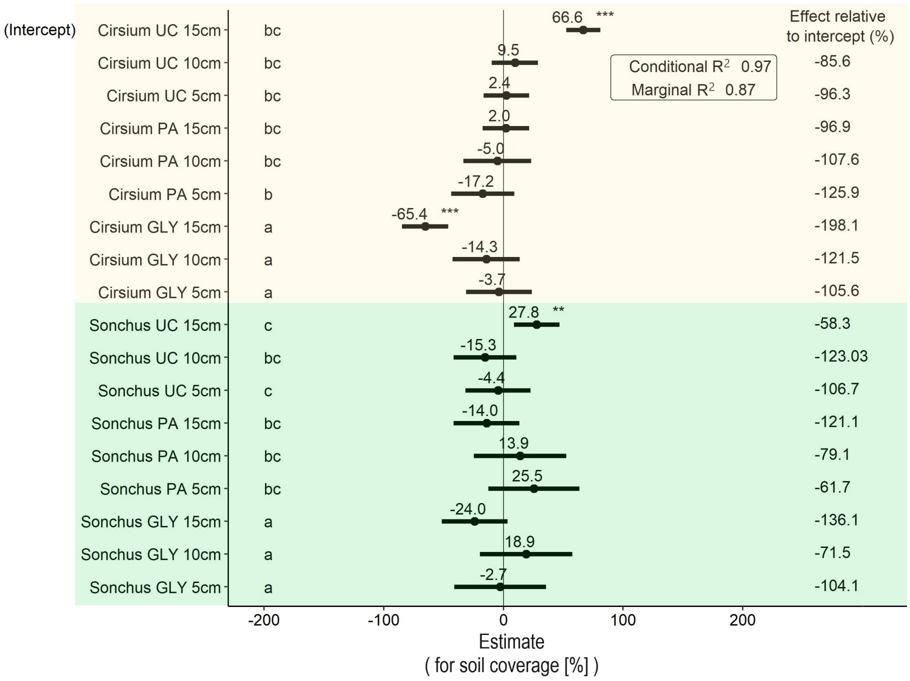

FIGURE 5
Results of the LMM analysis followed by Tukey's HSD on soil surface coverage (%) at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$. Groups with different letters are significantly different at $p< 0.05$ level based on Tukey's HSD test. PA, Pelargonic acid; GLY, glyphosate; UC, untreated control; Cirsium, *Cirsium arvense*; Sonchus, *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

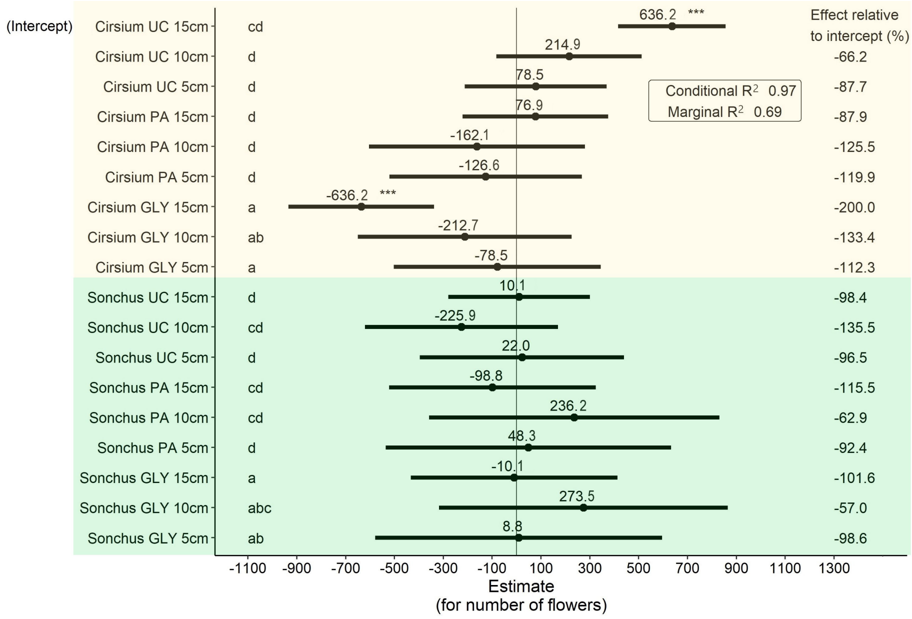

FIGURE 6
Results of the LMM analysis followed by Tukey's HSD on the number of produced flowers at the end of the experiment. Significance codes obtained from the LMM analysis for main effects shown in this graph are as follows: * $p< 0.05$, ** $p< 0.01$, *** $p< 0.001$. Groups with different letters are significantly different at $p< 0.05$ level based on Tukey's HSD test. PA, Pelargonic acid; GLY, glyphosate; UC, untreated control; Cirsium, *Cirsium arvense*; Sonchus, *Sonchus arvensis*. 5 cm, 10 cm and 15 cm represent the initial size of ramets.

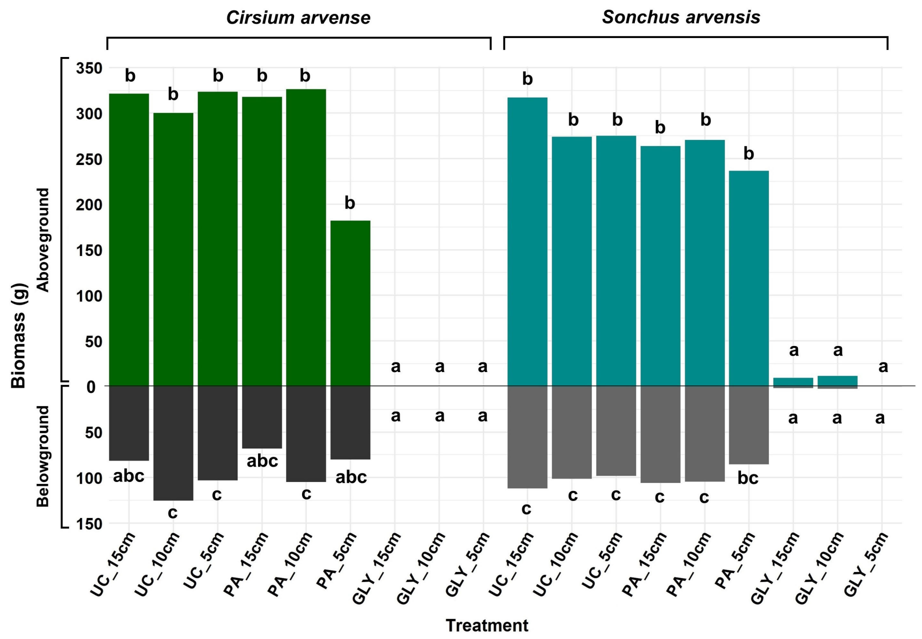

FIGURE 7
Aboveground and belowground biomass of *C. arvense* and *S. arvensis* after regrowth. Different letters show significant differences between treatments at p< 0.05 using Tukey's HSD test separately for aboveground biomass and belowground biomass. PA, Pelargonic acid; GLY, glyphosate; UC, untreated control; 5 cm, 10 cm and 15 cm represent the initial size of ramets.

initial ramet size on both biomass categories was not significant either. However, the results showed a significant interaction effect between PA treatment and ramet size of 5 cm in the case of aboveground biomass. The results also suggested that there might be an interaction effect on aboveground biomass between species, PA treatment, and ramet size of 5 cm, but this was not strong enough to be considered statistically significant. In the case of belowground biomass, there was a statistically significant interaction effect between species and ramet size of 10 cm (Supplementary Tables 2, 3).

The results showed no significant differences in aboveground and belowground biomass between PA-treated plants and UC treatments for any ramet size (Figure 7). Nevertheless, when comparing the UC treatments with PA treatments in *S. arvensis*, the lowest aboveground biomass of 236.7 g and belowground biomass of 85.4 g belonged to PA-treated plants with an initial ramet size of 5 cm, which corresponds to reduction of 13 % and 12 % in aboveground and belowground biomass respectively. The lowest aboveground biomass for *C. arvense* was 181.9 g obtained from PA-treated plants with an initial ramet size of 5 cm, but the lowest belowground dry biomass was 68.2 g obtained from an initial ramet size of 15 cm treated with PA. When comparing untreated control and PA-treated plants in *C. arvense*, the application of PA decreased the belowground biomass of *C. arvense* by 22 % with a 5 cm initial ramet size and by 16 % when the ramet sizes were larger. The aboveground biomass of *C. arvense* with a 5 cm initial ramet size was reduced by 43 %, while for the larger ramet sizes, it was just 2 % (Figure 7).

3.3 Plant height during the experimental period

The main effects of experimental year, weed species, and herbicide treatments on plant height were statistically significant (Figure 8; Supplementary Table 4). Other significant interaction effects on plant height were found between species and the year 2021, and between herbicide treatments and the year 2021. Specifically, *S. arvensis* had almost the same height in 2021 compared with 2020, while *C. arvense* was taller in 2021 than in 2020. Generally, *C. arvense* tended to be significantly taller than *S. arvensis*. In the case of herbicide treatments, the PA treatment had a more negative effect on plant height in 2020 than in 2021. The application of the PA and GLY treatments resulted in significantly shorter plants compared with the UC, while initial ramet size did not have a significant effect on height (Supplementary Table 4). In 2021, PA-treated plants of *C. arvense* with an initial ramet size of 10 cm were significantly shorter than *C. arvense* plants with the same initial ramet size in the UC treatment. For *S. arvensis*, there were no significant differences between the PA and UC treatments (Figure 8; Supplementary Table 4).

4 Discussion

The aim of this study was to investigate the effects of twice application of PA on *C. arvensis* and *S. arvensis* in two consecutive growing seasons. Their regrowth after PA applications was

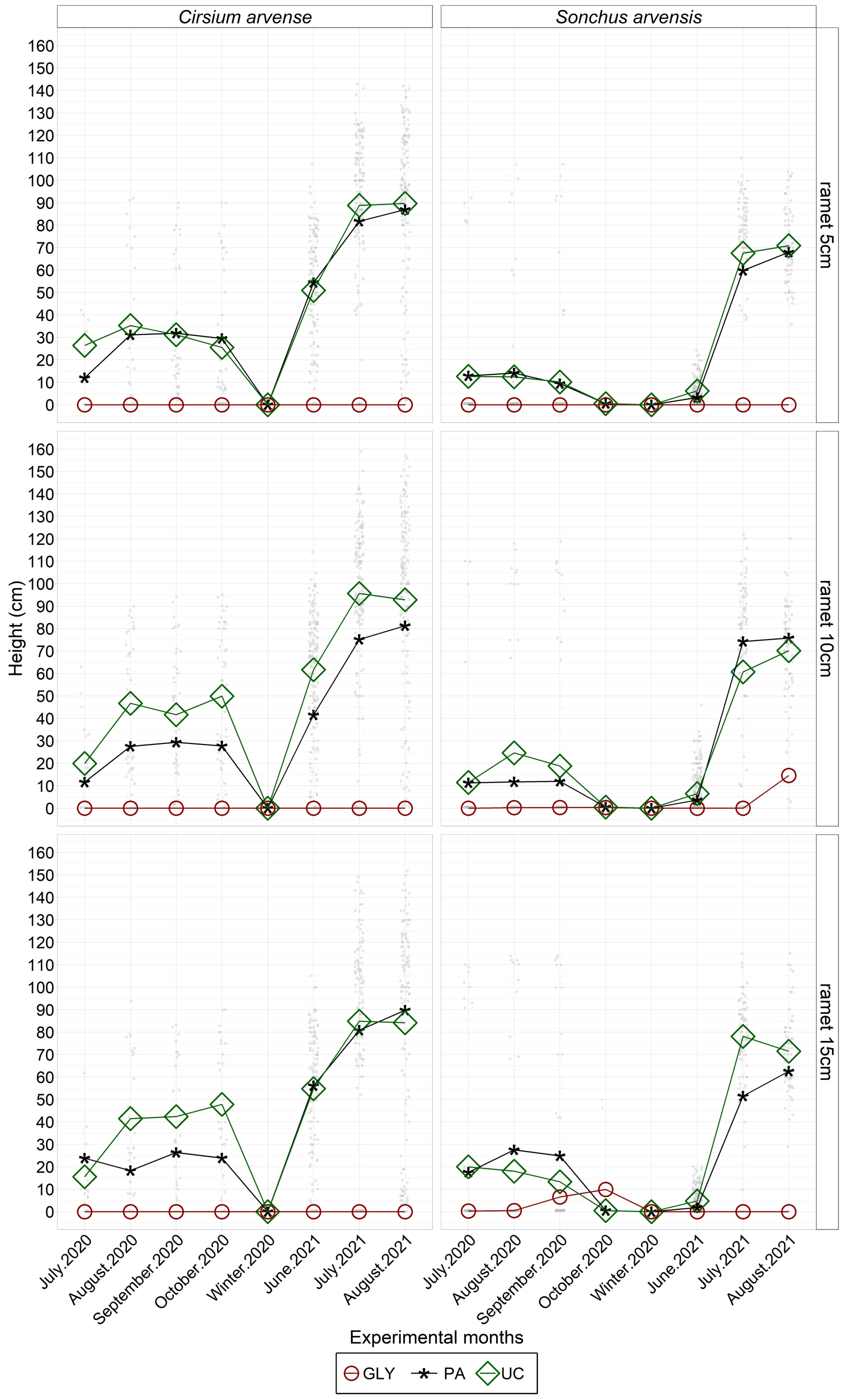

FIGURE 8
Monthly measured plant heights (cm) of regrown *C. arvense* and *S. arvensis* after herbicide applications during the experimental period. PA, Pelargonic acid; GLY, glyphosate; UC, untreated control.

evaluated by combining two approaches: targeting the aboveground parts of weed species with twice application of PA, and addressing the belowground root systems using three different initial ramet sizes. By adopting different initial ramet sizes, the objective was to obtain insights into the effects of fragmenting the creeping root system of perennials as it would be affected by mechanical disturbance (e.g. tillage practices) in real on-farm situations. However, the use of initial ramet sizes and their effect on PA efficacy did not represent mechanical control, rather, offered a valuable perspective and understanding of the fragmentation effects of creeping roots.

This study provided evidence that PA application reduces the aboveground and belowground biomass, as well as flower production in *C. arvensis* and *S. arvensis*. This was remarkable when the initial ramet size was smaller, suggesting that PA exhibits an enhanced efficacy on these plants when applied on the same patches repeatedly in combination with smaller initial root fragments. However, the quantity and persistence of these effects were lower than those produced by the GLY treatment. After application of GLY in the first experimental year, *C. arvense* did not regrow regardless of ramet size, and *S. arvense* demonstrated regrowth only for ramet sizes of 10 cm and 15 cm. In November 2023, the European Commission announced the extension of the glyphosate license for another decade after months of debate. However, this renewed license would be accompanied by new limitations and rules. The statement also highlighted that governments retained the authority to restrict the use of glyphosate in their own countries if they deemed the risks too high (Casassus, 2023), particularly concerning the preservation of biodiversity (Sullivan and Sullivan, 2003; Andert et al., 2022; El Jaouhari et al., 2023). Given the strong criticism regarding the use of glyphosate, as well as the new limitations and rules imposed by the commission, it is still crucial to find sustainable alternatives for it (Antier et al., 2020; Beckie et al., 2020; Casassus, 2023). Glyphosate is a non-selective systemic herbicide. Its effect is not visible in the early days after application because the plant takes some days to absorb and distribute glyphosate inside its tissues, which varies depending on the plant type (annual, perennial), growth stage, and environmental conditions (Sprankle et al., 1975; Satchivi et al., 2000; Fadin et al., 2018). PA causes necrotic lesions on plant aerial parts by attacking and destroying cell membranes of the plant epidermis, and causing rapid tissue dehydration (Ciriminna et al., 2019; Campos et al., 2022b). The PA mode of action implies that as a burndown herbicide, it could be a fast but temporary solution for controlling weeds, especially when there are work bottlenecks such as weather conditions or time constraints (Webber et al., 2014c; Campos et al., 2022a; Pannacci et al., 2022). Although PA may offer various advantages, it is occasionally misinterpreted as being similar to glyphosate and other pre-emergence herbicides, creating misleading expectations about its effectiveness and thus improper use (Campos et al., 2022b). A single application of PA does not provide lasting weed control (Patton et al., 2019; Loddo et al., 2023). Nevertheless, the present results show that it has the potential to be used in combination with other approaches, and thus offers an alternative to glyphosate against perennial weeds. Its combination with the smallest ramet size reduced growth parameters compared with the untreated control, even though there was no second fragmentation or soil mechanical treatments during the two-year experimental period. This finding is similar to the findings previously reported by Kanatas et al. (2020) that the use of a stale seedbed method integrated with the application of PA decreases perennial weeds. Other studies using PA as a weed control tool suggest the use of PA as a valuable tool for weed management approaches that use multiple tactics (Kanatas et al., 2022; Pannacci et al., 2022; Loddo et al., 2023). Due to its rapid burn-down effect, PA has a wide range of practical applications in weed management (Crmaric et al., 2018; Krauss et al., 2020), such as precision spot weeding (Webber and Webber, 2011; EFSA (European Food Safety Authority), 2021), crop desiccation, and sucker control in plants (Coleman and Penner, 2008; Short et al., 2020). Given its effectiveness on many annual herbaceous weeds, it could potentially be used to manage weed growth in stubble and for pre-sowing herbicide applications (Andert and Gerowitt, 2020). The use of soil cultivation tools on a farm infested with perennial weeds such as *S. arvensis* leads to root fragmentation and buries the fragmented roots deep in the soil (Brandsæter et al., 2017), or brings them to the soil surface and enhances the decay of root fragments (Vanhala et al., 2006). It is likely that small perennial weed plants remain in the field after harvest, and if stubble cultivation does not control these small plants, they will accumulate nutrient reserves in their creeping roots for the next growing season (Håkansson, 2003; Vanhala et al., 2006). During this period, PA application might help achieve successful perennial weed control because PA can be applied on the 4 8-leaf-stage plants (Ganji et al., 2022) that are not only at their sensitive aboveground stage (Håkansson, 2003; Tavaziva, 2012), but also face a lack of nutrient reserves due to fragmentation by soil cultivation tools (Brandsæter et al., 2010). In this study, enhancement in PA efficacy was observed when applied on plants at the 4-8-leaf-stage, that grew from initial smaller ramet sizes, proving the sensitivity of plants because of their smaller amount of nutrient reserves according to the abovementioned research findings.

It was anticipated that *S. arvensis* would probably be more susceptible to PA than *C. arvense* due to seasonal variations affecting its sprouting ability. As anticipated, the regrowth of weed species after herbicide did differ, but contrary to expectations, *C. arvense* seemed to be more susceptible to PA than *S. arvensis* (Figures 7, 8). The assessments of plant growth parameters after regrowth suggested that shoot density per m^2 and soil coverage varied based on the weed species (Figures 4, 5). When comparing weed species considering the same initial ramet size, *S. arvensis* has a higher shoot density per m^2. When comparing the effects of PA treatment on shoot density between the two species, *C. arvense* has a lower shoot density than *S. arvensis*. According to the investigation carried out by Liew et al. (2013), the higher shoot density in *S. arvensis* compared with *C. arvense* is due to the presence of a higher bud density on adventitious roots of *S. arvensis*. In *S. arvensis*, there was an observable effect of 5 cm ramet size on PA efficacy. There was a lower shoot density per m^2 in PA-treated *S. arvensis* with 5 cm initial ramet compared with the untreated control. Previous studies have confirmed that a longer root fragment of *S. arvensis* produces more shoots than a shorter one (Anbari et al., 2011). In the untreated conditions of the current study, *S. arvensis* generally exhibited greater soil coverage than *C. arvense*. PA application resulted in a lower percentage of coverage for both species, although it was lower for *C. arvense* plants, particularly with smaller initial ramets, than for *S. arvensis* plants. These results are in agreement with the findings of Ward and Mervosh (2012) whose application of a PA treatment for two consecutive years on the same plot effectively reduced the coverage of *Microstegium vimineum*.

In general, PA-treated plants produced a smaller number of flowers than untreated control plants (Figure 6). Among PA-treated plants in both species, the smaller ramet size exhibited a smaller number of flowers. In a semi-field experiment using boxes, Anbari et al. (2011) tested the sprouting and shoot development of *S. arvensis* in relation to initial ramet size. They reported a positive correlation between the flower number and ramet length in *S. arvensis* and proved that the fragmentation of creeping roots delayed growth and reduced flower production (Anbari et al., 2011). These results are in agreement with the results of the present study.

The 5 cm initial ramet size enhanced PA efficacy, and a reduction in the aboveground and belowground biomass of both species under the mentioned treatments was observed, although it was not statistically significant (Figure 7). In previous studies conducted by Gustavsson (1997) on *C. arvense* and by Anbari et al. (2011) on *S. arvensis*, a smaller aboveground biomass was produced by a shorter ramet size than by a longer ramet size. The results of the present study in relation to aboveground biomass are in agreement with these findings. For *C. arvense*, the lowest belowground biomass was found for the 15 cm initial ramet size when comparing PA treatments with each other. Additionally, among untreated *C. arvense* plants, the biomass for the initial ramet size of 15 cm was the lowest. One reason for this could be environmental conditions. *C. arvense* biomass increases when more water is available (Sciegienka et al., 2011). When the temperature is lower and the photoperiod is shorter, then the root biomass of *C. arvense* is higher than shoot biomass. With an increase in temperature, the shoot growth increases and results in taller and more robust plants, which rapidly form flower heads (Hunter and Smith, 1972). In the present study, the aboveground biomass produced by plants with the initial ramet size of 15 cm was high due to favorable temperatures and high precipitation in both experimental years (particularly the high precipitation one month before biomass measurements). Moreover, PA-treated *C. arvense* with an initial ramet size of 15 cm produced a larger number of flowers than untreated *C. arvense* with the same initial ramet size. Furthermore, the produced aboveground biomass was almost similar between these two treatments, while the belowground biomass in PA-treated *C. arvense* was smaller than that of the untreated plants. It can thus be inferred that PA-treated *C. arvense* with the initial ramet size of 15 cm attempted to ensure its survival by producing more flowers, which leads to more aboveground biomass production and more belowground depletion. Additionally, it should be considered that there can be an effect of root longevity, and the creeping roots of *C. arvense* cannot live longer than one to two years (Moore, 1975; Bourdôt et al., 2000; Leathwick and Bourdôt, 2012).

Plant height is a direct indicator of herbicide impact, providing detailed information on how the herbicide influences the physical structure of plants. Therefore, the dynamic changes in monthly plant height after herbicide application provided insights into the regrowth patterns of both weed species in both experimental years (Figure 8). The average height of *S. arvensis* was similar in both years, while the height of *C. arvense* was greater in 2021. PA application reduced plant height compared with untreated plants in both years. However, this plant height reduction in 2020 was greater than in 2021. The results showed that the effects of species and herbicides varied depending on the year. This could be due to the variations in weather conditions between 2020 and 2021, and unexpected environmental effects, as discussed by Hunter and Smith (1972) and Sciegienka et al. (2011). The effect of PA on the height of weed species in the present study is in line with earlier studies on *Lolium rigidum* Gaud and *Avena sterilis* L., which reported a lower height in PA-treated plants compared with untreated plants (Travlos et al., 2020). Overall, the ramet size of 5 cm produced shorter plants in all treatments. This supports the findings of Sciegienka et al. (2011) on *C. arvense* that a smaller root fragment size produces shorter plants.

The herbicide efficacy analysis determined the high efficacy of PA compared with the untreated control and revealed the negative relationship of PA efficacy with days after applications in both years (Figure 3). Due to its rapid effect, PA efficacy was higher compared with other treatments at the beginning and until 7th day after application, but then declined over time due to the occurrence of regrowth. The findings of previous research on both annual and perennial species have demonstrated that PA reaches its maximum efficacy within several hours of application and remains effective for up to one week, although the plant regrowth subsequently reduces its efficacy (Muñoz et al., 2020; Travlos et al., 2020; Ganji et al., 2022; Muñoz et al., 2022; Pannacci et al., 2022; Ganji et al., 2023; Loddo et al., 2023).

5 Conclusions

It is concluded that a two-year application of PA on the same specific spot in combination with a smaller ramet size facilitates the development of integrated weed management (IWM) strategies. To reduce the infestation level of perennial weeds, PA application could be combined with mechanical fragmentation of creeping roots in the intercropping period. From today's perspective, however, PA is registered on the European market for use as a plant desiccant in potatoes and to kill suckers in perennial crops, such as hops and grapevine. It is currently not registered for other applications in arable crops. Since current policies towards restricting the use of synthetic herbicides in arable farming enforce the use of alternatives such as bio-herbicides, efforts at economic and political levels are required. To ensure the proper use of this active substance, it is crucial to educate farmers about integrated weed management, conduct field applications at recommended times, and adhere to label instructions. This would help a suitable niche market for this active ingredient to be established.

On the market, bio-herbicides based on PA are costly, and their application rate per hectare is higher compared with synthetic herbicides, and may not be cost-effective for large-scale applications in arable farming. Therefore, expanding the application time from multiple repeated applications in one year to a two-year repeated applications on the same spot might assist with financially balancing PA application costs and achieving acceptable weed control. Further research studies should be undertaken to perform financial comparisons of PA applications and synthetic herbicides. Additional studies regarding enhancements in the technical aspects of PA application, such as the incorporation of adjuvants or the adjustment of water volume to achieve more comprehensive plant coverage, are essential for more successful weed control.

Data availability statement

The data presented in this study are available upon reasonable request from the corresponding author. The data are not publicly available due to project funding policy. Requests to access the datasets should be directed to Eliyeh Ganji, eliyeh.ganji@uni-rostock.de.

Author contributions

EG: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. SA: Conceptualization, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2024.1330199/full#supplementary-material

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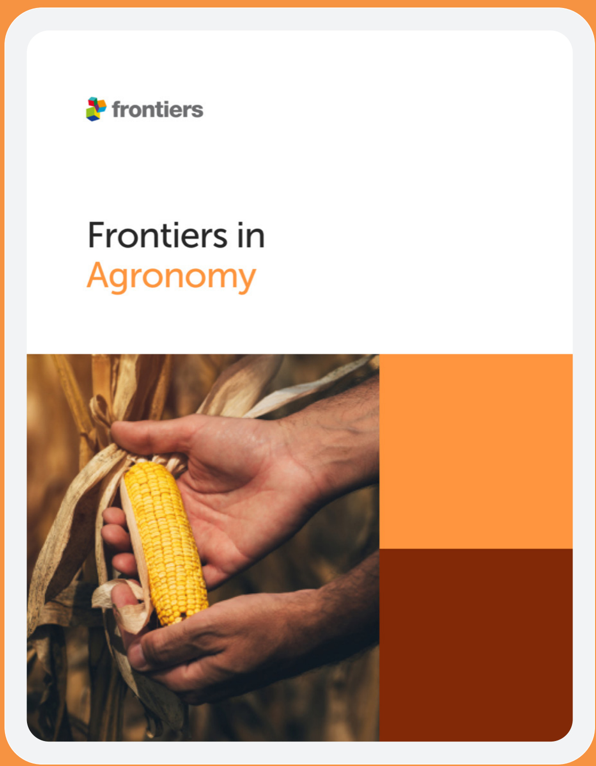

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