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Weed suppression and maize yield influenced by cover crop mixture diversity and tillage

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ABSTRACT

The integration of cover crops into cropping systems has demonstrated significant potential for enhancing weed suppression, improving soil health, and reducing dependence on chemical herbicides. A three-year field study conducted in Slovenia between 2020 and 2023 aimed to quantify the seasonal competitive relationships between cover crop and weeds while also evaluating potential yield benefits for the subsequent maize crop. A randomized complete block design accounted for variations in soil tillage (conventional and conservation) and cover crop type (single species: oilseed radish and berseem clover; a simple five-species cover crop mixture; and a functionally diverse seven-species cover crop mixture). Oilseed radish imposed the highest level of competition against weeds within the first four weeks post-sowing. The highest levels of weed suppression across years and tillage practices were observed with oilseed radish and the simple cover crop mixture (81–85 %). The functionally more diverse cover crop mixture was not more productive or weed suppressive than other cover crops neither did improve the level of weed control. The weed suppression level not closely linked to cover crop biomass production, suggests that rapid early growth may be more relevant functional trait for cover crop weed suppression capabilities. Maize yields were insignificantly improved with berseem clover and the most productive simple cover crop mixture by 0.7 t ha $^{-1}$. The present study confirms the importance of cover crops as a key non-chemical weed management strategy for diversification. However, it also indicates the need for developing trait-based cover crop mixtures to further enhance cover crop-weed competition and main crop yield outcomes under specific resource availability, management practices, and desired ecosystem services.

1. Introduction

Effective weed management is fundamental to agricultural productivity as weeds have the potential to cause major yield reductions across a variety of crops, posing a greater threat than other pests (Oerke, 2006). After the introduction of herbicides in the 1950s, chemical weed control became one of the most important management tools enabling rapid increase of agricultural production (Kudsk and Streibig, 2003). However, combined with other crop management simplifications, this has led to a high reliance on herbicides and increased environmental concerns related to water contamination and decreasing plant diversity (Riemens et al., 2008; Rosenbom et al., 2015). Weed management is increasingly important to preserve the high yield potential of modern crop genotypes (Storkey et al., 2021) but more sustainable weed management solutions are urgently needed due to climate change induced weed expansion (Peters et al., 2014), decreasing number of active substances (Chauvel et al., 2012), and increasing herbicide resistance (Neve et al., 2014; Hulme, 2022).

In recent decades, cover crops (CC) have increased in popularity and production area due to their multiple benefits in reducing soil erosion, improving soil fertility and providing weed suppression (Krutz et al., 2009; Brock et al., 2011; Cordeau et al., 2015). Cover cropping also provides opportunity for direct yield benefits from soil organic carbon increase (Vendig et al., 2023*)*. Whether CCs affect main crop yields positively or negatively remains under discussion, but multiple studies have highlighted highly CC specific magnitude of yield increase under various climatic conditions, soil properties and crop management (Fan et al., 2021; Chahal and Van Eerd., 2023).

Diversification of cropping systems with CC is an essential agro-ecological weed control strategy that promotes weed diversity, thereby reducing overall competitiveness and the dominance of herbicide-resistant species (Storkey and Neve, 2018; Hofmeijer et al.,

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2021).

Biomass accumulation and other management decisions related to species selection, establishment, and termination define the level of weed suppression provided by CC (Osipitan et al., 2019). Introduction of CC and combining them with tillage operations has been shown to improve weed control and contributes significantly to reducing herbicide use and tillage intensity (Teasdale, 1996; Mirsky et al., 2013; Wittwer and van der Heijden, 2020). However, when tillage is used to terminate CC their weed suppression effect is lower in comparison to CC residues left at the surface (Langeroodi et al., 2019) or in comparison to extended effects by employing CC as living or dead mulch in the succeeding crop (Bhaskar et al., 2021; Fernando and Shrestha, 2023). Recent suggestions for future integrated weed management (IWM) have highlighted the significance of effective soil management in relation to CC (Riemens et al., 2022). Yet, detailed seasonal side-by-side comparisons and assessments of CC effectiveness under various tillage systems remain largely unexplored. There are indications that the role of CC in weed suppression is more pronounced in conservation and no-tillage systems (Weber et al., 2017), while reports of inadequate weed control in reduced tillage systems can be found too (Wittwer and van der Heijden, 2020). Other factors such as CC functional traits, timing and management of sowing and termination, and management context (organic vs. conventional) may also influence weed biomass production (Björkman et al., 2015; Brainard et al., 2011). However, the interactions between these various factors across the CC growing period have not been adequately explored, in spite of their relevance.

CC multi-functionality, productivity, and yield stability can be increased using CC mixtures containing species with complementary traits (Blesh, 2018; Finney and Kaye, 2017; Florence et al., 2019; Franco et al., 2021). Although CC mixtures offer better usage of available resources than monocultures, recent studies show that CC biomass is not the only driver of weed suppression (Rouge et al., 2022; Smith et al., 2020). While the benefits of CC on limiting weed infestation have been confirmed in numerous reports, the advantages of multi-species CC over single species CC are not so evident (Weisberger et al., 2019; Florence at al., 2019). Furthermore, the underlying mechanisms of CC mixture weed suppression remain unclear (Nichols et al., 2020).

Although weed suppression using CC is a major topic of research, most studies have focused on the final outcome of weed suppression, leaving the overall seasonal quantification of the CC competitive effect on weed infestation largely unexamined. With a lack of studies exploring comprehensive seasonal competition outcomes between CC and weeds, the primary objective of the present study was to determine the dynamics and intensity of weed competition influenced by the CC selection (single CC species vs CC mixtures). Additional focus was put on elucidating how CC establishment, growth and competition are mediated by the impact of two CC tillage practices (conventional vs conservation tillage) and assessed their impact on the yield of the subsequent maize crop.

2. Material and methods

2.1. Study site and environmental conditions

In total, four field experiments have been conducted between 2020 and 2022 at the Agricultural Institute of Slovenia (Infrastructure Center Jablje pri Mengšu; 46°08'33.9'' N, 14°34'21.5'' E, 309 m a.s.l.). The study encompassed two primary cover crop (CC) trials initiated over two consecutive seasons, followed by two subsequent trials involving the main maize crop. The study site in the central region of Slovenia has a temperate continental climate, with a long-term (1980–2020) mean annual temperature of 9.2°C, and annual average precipitation of 1355 mm. During the CC growing season (August to December), the long-term mean monthly temperature is 8.6°C, accompanied by precipitation of 645 mm. Meteorological parameters during the growing season of CC were monitored with a weather station located approximately 1 km from the study site (Adcon, A753GSM, Adcon Telemetry GmbH, Austria).

Soil at the study site is classified as Eutric Cambisol on alluvial pebble and sand with silty-loam texture (25 % clay, 37 % silt and 38 % sand). The results of soil chemical analysis prior to planting in 2020 for the 0–25 cm soil layer yielded: pH (KCl), 6.9, bulk density, 1.39 g cm^{-3}; soil organic matter, 35.7 g kg^{-1}, total nitrogen, 2.1 g kg^{-1}, available phosphorus (P_2O_5), 18 mg kg^{-1}, available potassium (K_2O), 21.0 mg kg^{-1}. In 2021, the experiment was conducted on the adjacent field with a similar soil type and the results were: pH (KCl), 7.2; bulk density, 1.39 g cm^{-3}; soil organic matter, 35.0 g kg^{-1}; total nitrogen, 1.9 g kg^{-1}; available phosphorus (P_2O_5), 29 mg kg^{-1}; available potassium (K_2O), 33.0 mg kg^{-1}. For several years prior to the study, the fields used for the experiments were under conventional tillage with diverse crop rotation of maize, winter wheat, crimson clover, and soybean.

2.2. Experimental design and management practices

The experiment was designed as a split-plot in a randomized complete block design with four replications. The two experimental factors were tillage practice for CC establishment (main plots) and CC type (subplot). The main plots differed only in the two soil tillage practices used for cover crop (CC) establishment and termination: conventional tillage (CN) and conservation tillage (CS), while other crop and weed management practices remained consistent across all plots. Each of the two tillage practices for CC establishment and termination (CN and CS) had five sub-plot treatments (10 m x 6 m) corresponding to the two individual CC species, two CC mixtures and the unsown tilled control. Mulching of straw and plant residues occurred after the winter barley (*Hordeum vulgare* L.) harvest at the middle of July. Ploughing to 22 cm depth was performed as primary tillage in CN, while the soil in CS was prepared with a disc harrow to 10 cm depth. A fine tine harrow cultivator prepared the seedbed in both CN and CS. Additional no till (NT) fallow treatment was included in the experiment where the stubble was left undisturbed.

The selection of CC for the trial was based on our prior research, which examined the performance of individual CC species and their ability to suppress weeds under the humid continental climatic conditions of central Europe (Leskovšek and Simončič, 2013; Adamič and Leskovšek, 2023). Based on those studies oilseed radish (*Raphanus sativus* L. cv., Apoll; 30 kg ha^{-1}) and berseem clover (*Trifolium alexandrinum* L. cv., Alex; 30 kg ha^{-1}) were selected for individual CC species testing. Besides the two single CC species, three CC species widely used by the local farming community were included in the simple five-species CC mixture, namely oilseed radish (*Raphanus sativus* L. cv., Apoll; 3 kg ha^{-1}), niger seed (*Guizotia abyssinica* - (L.f.) Cass., cv., Niger 4 kg ha^{-1}), berseem clover (*Trifolium alexandrinum* L. cv., Alex; 3 kg ha^{-1}), white mustard, (*Sinapis alba* L., cv., Bea; 2.5 kg ha^{-1}), and tillage radish (*Raphanus sativus* var. *longipinnatus* L., cv., Strukturator 0.4 kg ha^{-1}). The diverse seven-species CC mixture used in the study struck a balance between multiple highly productive CC species as well as grass and forb plant functional types and was comprised of common vetch (*Vicia sativa* L., cv., Ebena 10 kg ha $^{-1}$), sorghum-Sudan grass hybrid (*Sorghum bicolor* L. Moench; cv., Nutri honey (5.5 kg ha $^{-1}$), berseem clover (*Trifolium alexandrinum* L. cv., Alex; 5 kg ha^{-1}), linseed (*Linum usitatissimum* L., cv., Comtess, 4 kg ha $^{-1}$), niger seed (*Guizotia abyssinica* - (L.f.) Cass., cv., Niger 2 kg ha^{-1}), tillage radish (*Raphanus sativus* var. *longipinnatus* L., cv., Structurator 2 kg ha $^{-1}$), and safflower (*Carthamus tinctorius* L. var., Lizzy 1.5 kg ha $^{-1}$). CC were sown on 11th August 2020 and 3rd August 2021, using a precision plot drill (Wintersteiger), and to obtain uniform germination conditions the entire experimental field was compacted with a Cambridge roller.

Shortly before the frost, CCs on the CN plots were terminated with ploughing, while a disc harrow was applied to non-inversion CS plots. The following year, the seedbed for the main maize crop was prepared with a power harrow for the CN treatment and a fine tine cultivator for the CS treatment. Intermediate maturation period maize crop (variety DKC 4569) was planted with row spacing of 70 cm and a planting depth set at 4–5 cm. In-row spacing was 16.5 cm, amounting to a seed density of 86,600 seeds per hectare. Before planting, all plots received a base fertilization using 250 kg/ha of an NPK 6:18:34 blend. Maize was sown on April 21, 2021, and April 18, 2022, using a pneumatic planter. Post-emergence weed control was applied uniformly across CN, CS, and NT plots and conducted at the two-leaf growth stage of maize using a commercial herbicide mixture of isoxaflutole (225 g ha $^{-1}$) and thiencarbazone-methyl (90 g ha $^{-1}$). A commercial sprayer (Agromehanika AGS 3000 EN HP, Slovenia) was used equipped with anti-drift nozzles (IDK 120–03; Lechler GmbH, Stuttgart, Germany) and 250 L ha $^{-1}$ of spray volume. Nitrogen was applied in smaller doses tailored to the crop's developmental stages, with three applications between the third (V3) and fifteenth (V15) fully developed maize leaves, totaling 160 kg/ha. The nitrogen was supplied as a blend of ammonium (10.5 %) and urea (23 %) fertilizers. Maize was harvested on October 1, 2021, and October 4, 2022, using a small plot harvester (Quantum, Wintersteiger AG, Ried, Austria). The middle two rows of maize were harvested, and seed moisture content was adjusted to 14 %.

2.3. Weed and cover crop assessments

Within each sub-plot, random sampling areas of 0.5 m^2 were designated to perform four destructive harvests (H1-H4) throughout the season to assess weed and CC aboveground biomass (or dry matter, see Table 1). No observations were recorded from within 1 m wide buffer zones along the outer edges of each sub-plot. Sampled plant material with aboveground weed and cover crop parts were then divided and dried at 60 °C for three days, before weighing the biomass.

Air temperatures measured at a weather station near the experimental field were logged hourly throughout the growing season, and converted to cumulative growing degree days (GDD) for the corresponding harvest times (HT) by using the following equation (Gilmore and Rogers, 1958):

$$GDD = \sum [(T_{\max} + T_{\min})/2 - T_{\text{base}}] \quad (1)$$

where $T_{\max}$ and $T_{\min}$ are daily maximum and minimum air temperatures (°C), respectively. While optimum germination and temperature preferences may differ among individual CC species, a standard T_{base} temperature of 5 °C was utilized to comply with temperature requirements of majority of CC species included in the study (Licata et al., 2023; Starr et al., 2020; Tribouillois et al., 2015).

Absolute growth rate (AGR) was estimated according to Causton and Venus (1981) using the following equation:

$$AGR = (W_2 - W_1) / (t_2 - t_1) \quad (2)$$

where W_1 is the treatment mean of the total shoot dry matter at time t_1 and W_2 the individual total shoot dry matter sample of the subsequent harvest at time t_2.

CC:weed biomass ratio and relative weed suppression efficacy (RWSE) were determined to quantify the level of CC-weed competition.

Table 1
Harvest times (HT) with corresponding days after sowing and growing degree days (GDD) for 2020 and 2021.

Year	HT	Days after sowing	GDD (° C)
2020	1	23	348
	2	35	505
	3	52	668
	4	87	865
2021	1	27	426
	2	43	625
	3	59	789
	4	78	886

CC:weed biomass ratio compares the biomass of CC to the biomass of weeds and provides a good indication of the relative dominance or suppression of weeds by the CC. A higher ratio suggests stronger competition from the CC.

RWSE is a measure of competition intensity provided by CC. RWSE compares the weed performance in the absence of plant competition (tilled weedy control without CC) and the weed performance in a mixture with CC and was calculated using the following equation (Grace, 1995):

$$RWSE = [(WB_{noCC} - WB_{cc})/WB_{noCC}] \times 100 \quad (3)$$

where WB_{noCC} is the weed biomass in tilled fallow plots without CC and WB_{cc} is weed biomass in plots with CC.

2.4. Statistical analysis

Statistical analysis of the data and graphing were performed using (R Core Team, 2022). Due to significant effect of the experimental season, data of each year were analyzed separately and examined for homogeneity of variance using Levene's tests prior to the statistical analysis. The effects of CC type, tillage practice and harvest time, and their interactions on each of the response variable (CC and weed dry biomass production, absolute growth rate, CC:weed biomass ratio, RWSE and dry maize grain yield) were evaluated using linear mixed models (*lme4* R package). Blocks were used as a random effect factor in these models.

Tukey's post-hoc test was performed to assess the effects of CC type, tillage practice and their interaction at each harvest term separately, at 0.05 significance level.

3. Results

3.1. Environmental conditions during cover crop growth

The weather conditions during the 2020 and 2021 experimental seasons were favorable, with seasonal air temperatures and precipitation levels remaining similar to long-term averages (2020: 11.9 °C and 1315 mm; 2021: 11.5 °C and 1240 mm; see Fig. 1). Additionally, post-sowing soil moisture was ample, with 120 mm of precipitation recorded in August for both seasons, providing optimal conditions for CC germination and early establishment. While some periods, such as September 2021, experienced drier conditions, water availability generally did not constrain cover crop development during later growth stages. The 2022 season was significantly warmer (13.2 °C), with only 360 mm of precipitation during the critical maize growth period (May-September), compared to markedly higher precipitation observed in the previous season (650 mm). GDD (Growing Degree Days) accumulation from sowing to each sampling period in 2021 was 70–121 °C higher than in the previous season. However, by the final assessment, the difference in heat units was minimal, with only 21 °C more observed in 2021 (Table 1).

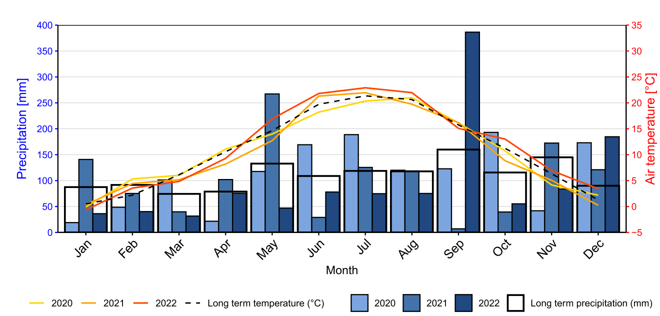

Fig. 1. Mean air temperatures and precipitation at the experimental location between years 2020–2023 compared to the long-term average for the period 1951–1994.

3.2. Seasonal dynamics of cover crop dry matter accumulation

All CCs exhibited significantly greater dry matter production in 2021 compared to 2020, and this was observed within each assessment period ($p < 0.001$). Dry matter accumulated steadily in all CC throughout the growing period, except for oilseed radish, which saw a drop in dry biomass at the final sampling in 2020 (Fig. 2).

However, CC displayed different responses when comparing individual CC performance within each HT (Table 2). Across the tillage practices and years, oilseed radish had the greatest dry matter production in the period following seed germination (H1) accompanied by notable higher absolute growth rates (Fig. 3).

In 2020, oilseed radish overperformance in comparison with berseem clover and both CC mixtures extended up to the H2 and H3 periods, respectively. Berseem clover and the simple CC mixture reached their peak absolute growth rate values later (in the H3 and H4 period), resulting in the highest final dry matter accumulation of 270 ± 33 g DM m^{-2} and 970 ± 215 g DM m^{-2}, in 2020 and 2021, respectively. The simple CC mixture tended to yield more compared to the diverse CC mixture, but significant increases were limited to the early period (H2) in 2020 and the final assessment (H4) in 2021.

The performance of individual CC for each tillage practice differed between years and within specific sampling periods (Table 2). CN generally promoted CC growth, though substantial dry matter increases were inconsistent across CC species and assessment periods (Fig. S1).

Absolute growth rate of oilseed radish and the simple CC mixture were also facilitated by CN, although this effect was limited to the H2 and H3 periods of 2021, respectively. In contrast, CS encouraged absolute growth rate for berseem clover only at H3 of the 2020 season (Fig. S2).

3.3. Cover crop and weed competition

Initial weed densities varied significantly between the seasons, with 88 and 164 weeds per square meter recorded in 2020 and 2021, respectively ($p \leq 0.001$). Despite these differences, the weed species composition remained relatively stable, being dominated by annual broadleaf species. In 2020, more than 80 % of the weed population consisted of *Chenopodium polyspermum* L., *Stellaria media* (L.) Vill., *Lamium purpureum* L., and *Capsella bursa-pastoris* (L.) Medik. In 2021, *Galinsoga parviflora* (Cav.) and *Amaranthus retroflexus* L. were additionally prevalent. Perennial and monocotyledonous weed species were observed infrequently and at very low densities (≤ 0.5 m^2).

In both years, the dynamics of weed development on plots without CC presence (including different soil preparation procedures) was inversely proportional to the intensity of the tillage operations (Fig. S3). Weed dry matter accumulation on NT peaked earlier (at H2), and then decreased towards the end of the growing season. In contrast, weed biomass on CS and CN generally increased throughout the growing period and exhibited maximum levels of biomass accumulation at H3 and H4 respectively. Tillage practice had only moderate effect on weed development on CC plots, weed enhancement with CS and CN sustained for a longer period and exhibited maximum biomass production later at the H3 and H4, respectively (Fig. S4).

The competition intensity imposed by CC on weeds was significantly stronger in 2021 ($p < 0.001$) with contrasting CC response between the seasons (Table 2). In the 2020 season, all CCs displayed an increasing trend of the CC:weed biomass ratio reaching peak values in the H3-H4 period (Fig. 4). In 2021, however, only oilseed radish demonstrated a consistently high CC:weed biomass ratio throughout the entire observation period. Diverse CC mixture had consistently lower CC:weed biomass ratio during the early period (H1), with no differences between CCs at the final observation.

Relative weed suppression efficacy (RWSE) also exhibited an increasing trend with peak values in the H3-H4 period (Fig. 5). The diverse CC mixture had lower RWSE levels during the early (H1-H2) period, with no differences with other CCs found in the subsequent assessment. Moreover, oilseed radish and simple CC mixture were better able to maintain high weed suppression levels across the period ($p \leq 0.01$). The most effective weed biomass reductions across years and tillage practices were 85 % for the simple CC mixture and 81 % for oilseed radish. These were followed by reductions of 75 % and 73 % for berseem clover and the diverse CC mixture, respectively.

While CC:weed ratio was unaffected by the presowing tillage operations (Fig S5), CS resulted in different inter-seasonal CC response to weed suppression (Fig. S6). In 2020, tillage practice had no effect on RWSE, while an increase at H1 was seen with both CC mixtures lasting up to H3 with berseem clover in the subsequent season.

Overall, no clear correlation between CC biomass and its ability to suppress weeds (expressed as RWSE) was found (Fig. 6). There was a

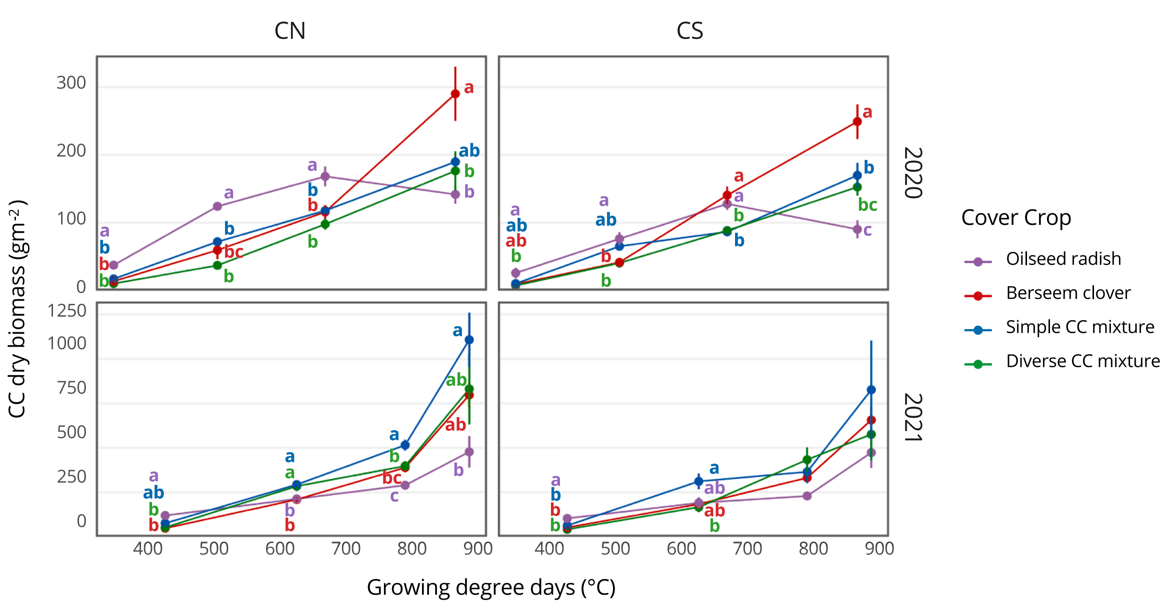

Fig. 2. Aboveground dry biomass over thermal time for the different CC treatments (oilseed radish, berseem clover, simple CC mixture and diverse CC mixture) under conventional (CN) and conservation (CS) tillage practice for years 2020 and 2021. Points represent average values, while the bar errors correspond to ± 1 standard error. Different letters represent significant differences ($p < 0.05$) among cover crop treatments within each sampling date.

Table 2
ANOVA results from the linear mixed model applied on the following parameters: CC and weed dry matter, absolute growth rate, CC: Weed biomass ratio and relative weed suppression efficacy (RWSE).

Year 2020								
Parameter	Source of variation	TP	CC type	HT	TP x CC	CC x HT	TP x HT	TP x CC x HT
CC dry matter	DF	1	3	3	3	9	3	9
	F-value	8.58	13.24	260.28	2.40	19.24	1.73	0.87
	P-value	**< .0001**	**< .0001**	**< .0001**	**< .0001**	**< .0001**	**< .0001**	**< .0001**
Weed dry matter	DF	1	4	3	4	12	3	12
	F-value	17.12	239.33	34.40	14.08	38.14	10.75	13.02
	P-value	**< .0001**	**< .0001**	**< .0001**	**< .0001**	**< .0001**	**< .0001**	**< .0001**
Absolute growth rate	DF	1	3	1	3	3	1	3
	F-value	2.60	1.13	0.08	1.45	8.57	1.71	2.27
	P-value	0.24	0.14	0.09	0.88	**< .0001**	0.93	0.89
CC:weed biomass ratio	DF	1	3	1	3	3	1	3
	F-value	0.82	1.87	5.99	1.24	3.06	1.75	1.09
	P-value	0.37	0.14	**0.02**	0.30	**0.03**	0.19	0.36
RWSE	DF	1	3	1	3	3	1	3
	F-value	2.42	1.07	121.57	1.43	6.07	0.01	0.14
	P-value	0.12	0.37	**< .0001**	0.24	**< .001**	0.94	0.94
Year 2021								
Parameter	Source of variation	TP	CC type	HT	TP x CC	CC x HT	TP x HT	TP x CC x HT
CC dry matter	DF	1	3	1	3	3	1	3
	F-value	4.11	8.94	274.05	0.20	8.17	3.28	0.43
	P-value	**0.05**	**< .0001**	**< .0001**	0.90	**< .0001**	0.07	0.73
Weed dry matter	DF	1	3	1	3	3	1	3
	F-value	10.64	19.06	15.98	3.59	0.91	0.48	2.09
	P-value	**< .01**	**< .0001**	**< .0001**	**0.02**	0.44	0.49	0.11
Absolute growth rate	DF	1	3	1	3	3	1	3
	F-value	3.08	4.33	42.08	0.41	2.21	3.27	1.18
	P-value	0.08	**0.01**	**< .0001**	0.75	0.09	0.07	0.32
CC:weed biomass ratio	DF	1	3	1	3	3	1	3
	F-value	1.59	7.06	31.63	0.62	1.70	0.56	0.54
	P-value	0.21	**< .001**	**< .0001**	0.60	0.17	0.46	0.66
RWSE	DF	1	3	1	3	3	1	3
	F-value	17.74	14.10	28.46	1.73	1.32	4.53	0.86
	P-value	**< .0001**	**< .0001**	**< .0001**	0.16	0.27	**0.04**	0.46

Note: CC = Cover Crop, TP = Tillage Practice, HT = Harvest Time, RWSE = Relative Weed Suppression Efficacy

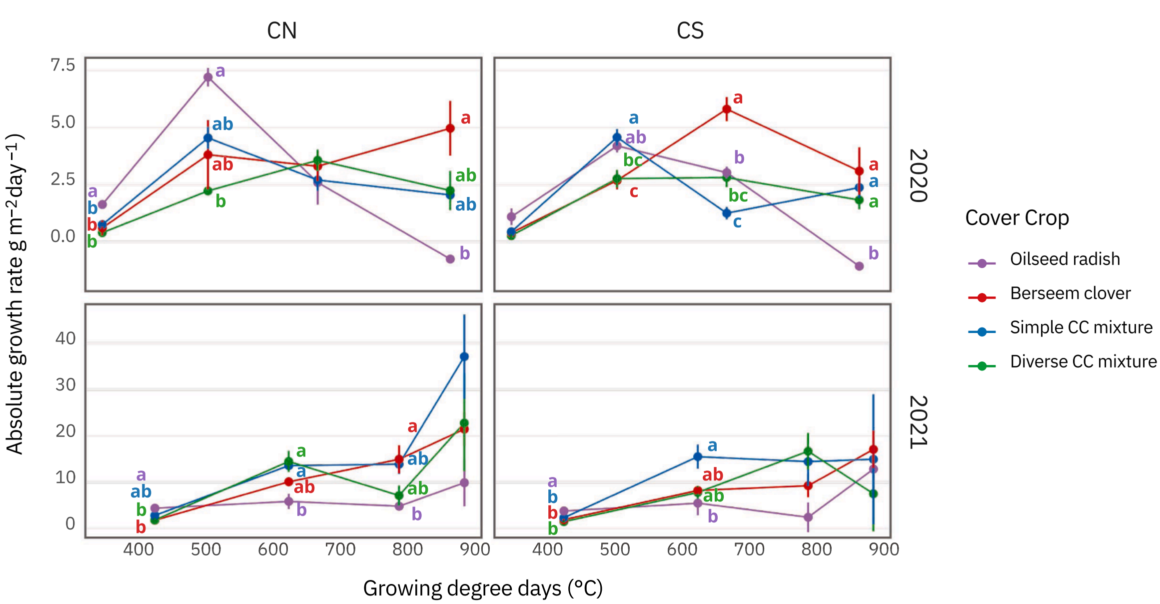

Fig. 3. Absolute growth rate over thermal time for the different CC treatments (oilseed radish, berseem clover, simple CC mixture and diverse CC mixture) under conventional (CN) and conservation (CS) tillage practice for years 2020 and 2021. Points represent average values, while the error bars correspond to ± 1 standard error. Different letters represent significant differences ($p < 0.05$) among cover crop treatments within each sampling date.

large variability in weed biomass and RWSE at similar CC biomass values, particularly in 2021 when both weed and CC biomass values were higher. *Vice versa*, weed biomass was rather low in some cases despite relatively low CC biomass production. The expected negative relationships between CC biomass and RWSE or weed biomass were not observed, neither in the last harvesting term when CC biomass was the largest nor in earlier harvesting terms.

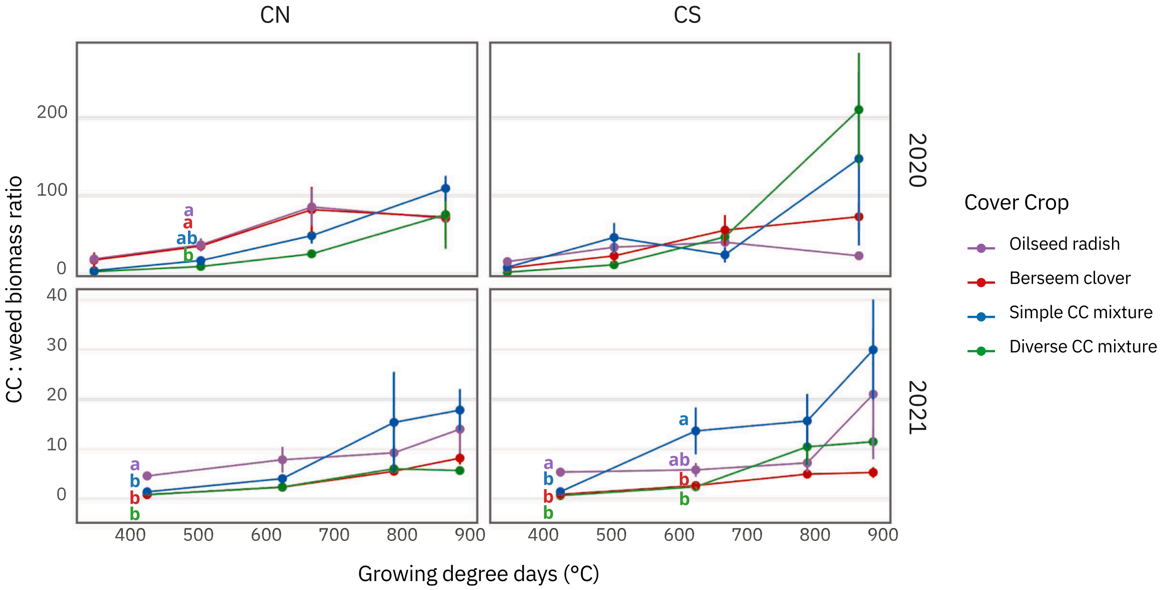

Fig. 4. CC: Weed biomass ratio over thermal time for the different CC treatments (oilseed radish, berseem clover, simple CC mixture and diverse CC mixture) under conventional (CN) and conservation (CS) tillage practice for years 2020 and 2021. Points represent average values, while the bar errors correspond to ± 1 standard error.

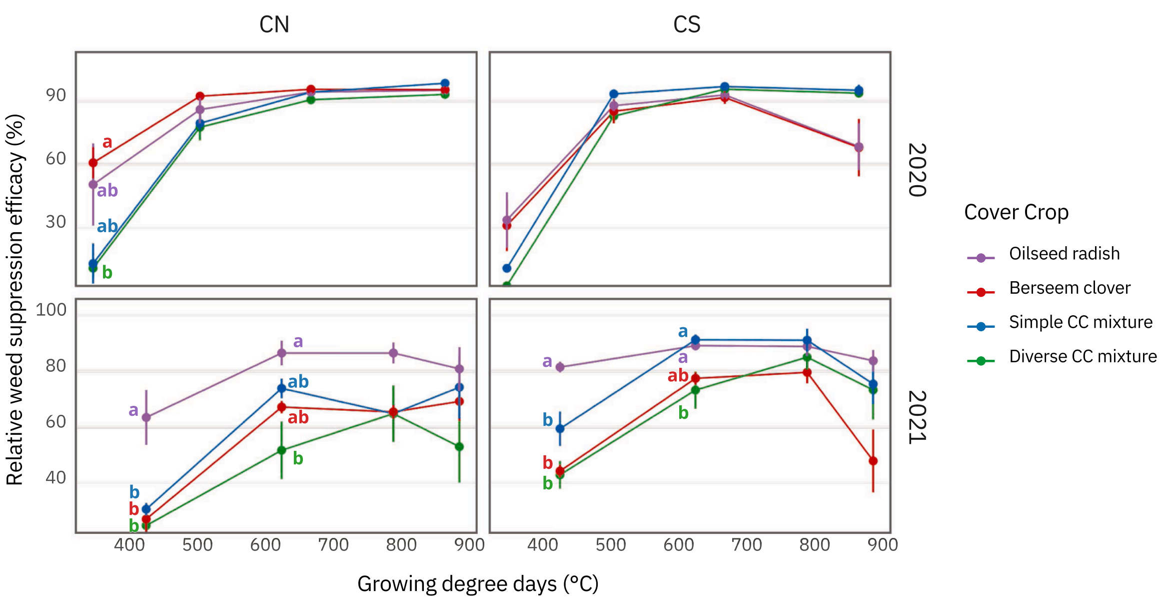

Fig. 5. Relative weed suppression efficacy (RWSE) over thermal time for the different CC treatments (oilseed radish, berseem clover, simple CC mixture and diverse CC mixture) under conventional (CN) and conservation (CS) tillage practice for years 2020 and 2021. Points represent average values, while the bar errors correspond to ± 1 standard deviation. Different letters represent significant differences ($p < 0.05$) among cover crop treatments within each sampling date.

3.4. Yield of the subsequent maize crop

When averaged across cover crop (CC) treatments, maize dry grain yield in 2022 (8.6 ± 0.3 t ha^{-1}) was significantly higher than in the previous season (5.9 ± 0.2 t ha^{-1}) (Table 3). Neither tillage practice nor CC treatment had a significant effect on maize yield. Although not statistically significant, both berseem clover and the simple CC mixture generally resulted in higher yields compared to the fallow plots, with an average increase of 0.7 t ha^{-1} across seasons and tillage practices.

4. Discussion

The present study shows the results from comprehensive evaluation of CC-weed relationship and their carry-over effect on the subsequent maize crop across the two consequent seasons

in temperate regions of central Europe. Given the increasing adoption of less intensive soil management, the response of CC and weeds was examined under two distinct levels of soil disturbance. To further explore advantages of CC mixtures, two functional diverse CC mixtures were compared over typical representatives of single CC species, a crucifer oilseed radish and legume berseem clover. This allowed us to evaluate CC response over time and deliver quantitative evaluation of weed suppression pathways and assess its effect on the subsequent maize crop.

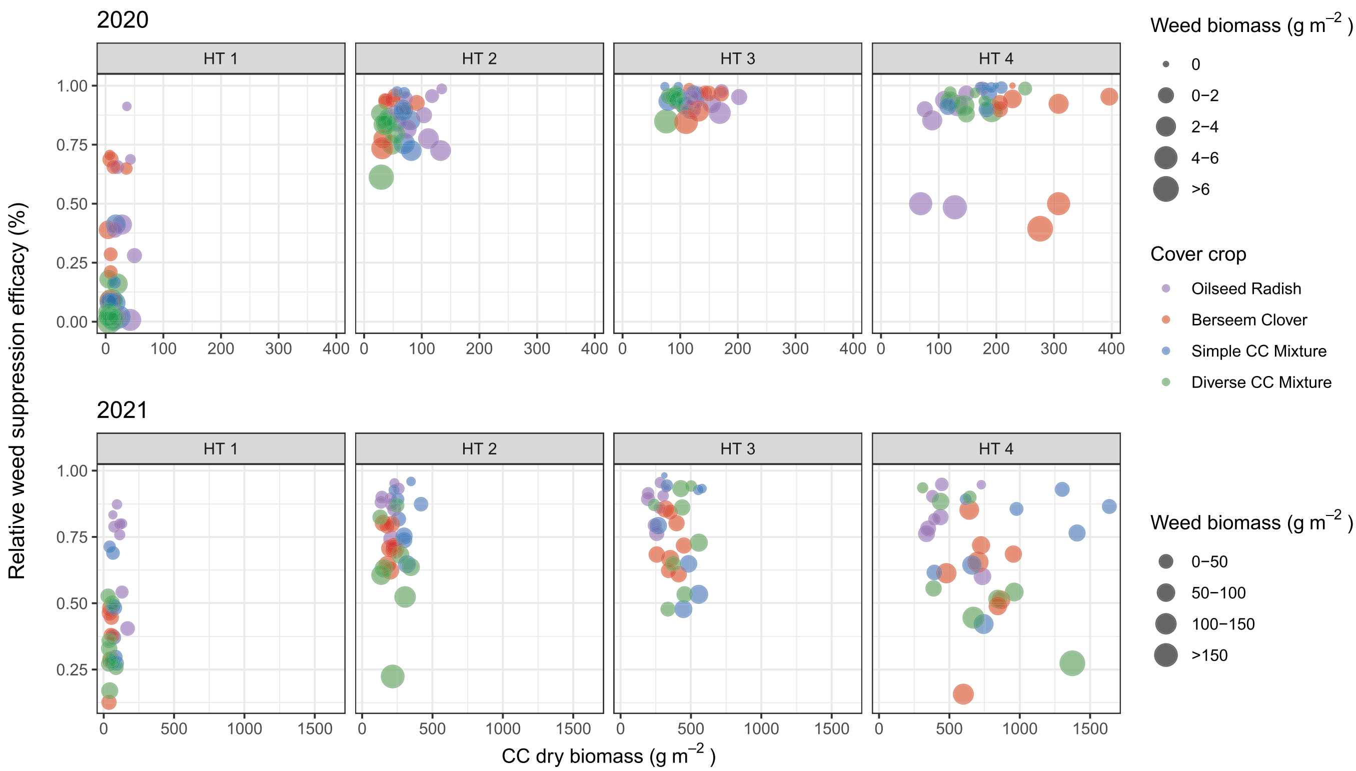

Fig. 6. Relationship between CC biomass and relative weed suppression efficacy (RWSE) for individual CC treatments (oilseed radish, berseem clover, simple CC mixture and diverse CC mixture) at designated harvest times (HT1-HT4). Circle sizes represent distinct weed biomass classes, illustrating the level of weed suppression across the range of CC productivity.

4.1. Advantages of CC mixtures over single species are more evident in extended growing period and increased nutrient availability

Field studies investigating CC performance across diverse agronomic and environmental conditions often deliver heterogeneous results (Brainard et al., 2011; Brust et al., 2014; Osipitan et al., 2019). As observed in the 2020 experiment, berseem clover showed similar growth responses while oilseed radish exhibited markedly greater absolute growth rate (2.9 vs 1.3 kg dry biomass ha^{-1} GDD^{-1}) in comparison with a 70-day study of Tribouillois et al. (2015) conducted under non-limiting resource supply and a higher total heat unit accumulation (1139 °C GDD vs 885 °C GDD). During the 2021 season, a significant increase in CC performance was observed, largely attributed to eight days earlier sowing and increased residual levels of phosphorus and potassium in the soil.

The early dominance of oilseed radish over berseem clover and the two CC mixtures in terms of growth rates within the initial three to four weeks post-sowing is unsurprising. Brust et al. (2014) similarly noted its robust growth in a multiyear/multisite study, driven by its quick germination and fast biomass production. Moreover, high growth rates in the first weeks after sowing found in both years and across tillage practices, suggest a strong initial competitive advantage of oilseed radish for a wide range of environmental conditions and management practices. The initial advantage of oilseed radish ended by H2 or H3 the latest underscoring importance of early establishment in short-duration growing periods (Nilsson et al., 2024). It should be emphasized that in 2020 oilseed radish was heavily affected by the cabbage stem flea beetle (*Psylliodes chrysocephala*) causing severe defoliation and biomass decline. From the period five weeks after sowing onwards, only the growth of the simple CC mixture was consistently higher than oilseed radish. This resulted in impressive final production between 8 and 11 t ha^{-1} of dry biomass during the 2021 season. This growth advantage was most likely driven by the oilseed radish, white mustard and niger seed which reliably produce substantial biomass across varying environmental conditions (Brust et al., 2014; Büchi et al., 2020).The sustained growth from October until frost termination is likely attributable to berseem clover, which exhibited high growth rates and productivity during the autumn in a single stand.

Diverse CC mixture had slower development during the initial period after sowing, with 20–25 % less dry biomass accumulated than simple CC mixture which can partly be attributed to the slow development of safflower and common vetch. Furthermore, the calculation method of absolute growth rate in the initial period after sowing affected these results as for H1, CC seed biomass was considered as the initial biomass value. Common vetch and the sorghum-Sudan grass hybrid in the diverse CC mixture have relatively high 1000-seed weights (70.9 and 21.2 g) in comparison to the prevailing species in the simple CC mixture, oilseed radish and berseem clover (12.0 and 3.1 g, respectively), thus leading to a higher starting weight for the diverse CC mixture.

Attempts to enhance drought resilience by increasing the functional diversity of more complex CC mixtures, such as those including safflower and sorghum–Sudan grass, did not yield biomass benefits, likely because water availability was not a limiting factor in either season (Pabuayon et al., 2019; Schittenhelm and Schroetter, 2014). Moreover, slower-growing legumes like common vetch generally resulted in lower productivity (Büchi et al., 2020). The advantage of simple CC mixture over more diverse one might have been smaller if below-ground biomass had been considered, given that species like safflower and tillage radish are known for significant root biomass allocation (Hudek et al., 2022).

While temporal niche differentiation is crucial (Smith et al., 2020), spatial niche differentiation appears less important (Elhakeem et al., 2019). Florence et al. (2019) also argued that increased biomass in diverse CC mixtures is driven by the inclusion of low-yielding species like safflower and common vetch, rather than by niche complementarity or enhanced resource use efficiency.

Table 3
Dry grain yield of maize (14 % moisture content) as influenced by tillage practices (TP: conventional – CN, conservation – CS) and cover crop (CC) species selection across two growing seasons (2021–2022). ANOVA results were obtained using a linear mixed model, with significance levels indicated.

Year (Y)	Tillage practice (TP)	CC species (CC)	Grain yield (t ha^{-1})
2021	CN	Fallow	7.6 ± 0.8
		Oilseed radish	8.4 ± 0.8
		Berseem clover	9.0 ± 0.0
		Simple CC mixture	9.1 ± 1.5
		Diverse CC mixture	9.1 ± 0.5
	CS	Fallow	8.5 ± 0.8
		Oilseed radish	7.8 ± 0.7
		Berseem clover	9.4 ± 0.1
		Simple CC mixture	8.9 ± 0.4
		Diverse CC mixture	7.8 ± 0.4
2022	CN	Fallow	6.1 ± 0.5
		Oilseed radish	5.9 ± 0.6
		Berseem clover	5.6 ± 0.5
		Simple CC mixture	6.0 ± 0.4
		Diverse CC mixture	5.6 ± 0.4
	CS	Fallow	5.3 ± 0.2
		Oilseed radish	5.8 ± 1.0
		Berseem clover	6.4 ± 0.2
		Simple CC mixture	6.3 ± 0.3
		Diverse CC mixture	5.7 ± 0.3
Source of variation	**DF**	**F**	**p**
Year (Y)	1	107.66	**< 0.001**
Tillage practice (TP)	1	0.03	0.8662
CC species (CC)	4	1.50	0.2212
Y x TP	1	0.21	0.6499
Y x CC	4	0.46	0.7657
TP x CC	4	0.48	0.7524
Y x TP x CC	4	0.95	0.4443

4.2. More intensive tillage facilitates CC growth but also promotes weed development

More intensive pre-sowing tillage only had a minor influence on the CC-weed competitive relationship, as both CC and weed growth were facilitated by CN tillage. In contrast, CC and weed biomass production tended to be moderately hindered by CS. This may be related to shallow tillage leading to lower CC establishment, as shown in a study with similar soil type and environmental conditions (Hösl and Strauss, 2016). As a result, CC:weed biomass ratio was largely unaffected by the soil management procedures, exhibiting only seasonal differences.

Similar to CC productivity, a large amount of weed biomass was observed in 2021, likely related to the combined effect of increased nutrient availability and earlier sowing. Previous studies have shown that increased levels of nitrogen and phosphorus were found to stimulate the growth and emergence of weeds (Blackshaw et al., 2003; Blackshaw et al., 2004; Brainard et al., 2011). As observed in 2021 season, weed growth can be particularly vigorous when early CC sowing coincides with a peak in germination of summer annual weeds (Myers, et al., 2004). To reduce weediness, CC sowing can be delayed until mid-August or early September (Baraibar et al., 2018) as relative timing of weed emergence with respect to the crop is more important than weed density (Knezevic et al., 1994; Bosnic and Swanton, 1997).

In the absence of CC, soil preparation with the less intensive CS tillage decreased the autumn weed growth in comparison to CN. With the latter, weed development was interrupted only by winter frost. Conversely, omitting tillage (as seen in the NT control) led to accelerated weed biomass accumulation and peak weed occurred at least two months earlier compared to the tilled plots. Teasdale et al. (1991) also reported that weed seeds remaining on the soil surface led to a large increase in the weed density in the first year following NT practice, whereas Fisk et al. (2001) noted that tillage before CC sowing did not significantly affect weed development in the autumn. While NT treatment with CC was not examined, recent studies suggest that effective weed control is likely related to early CC establishment (Merkle et al., 2024). However, CC may not be crucial for weed management in no-till and herbicide-free systems, especially at low levels of biomass production (Rouge et al., 2023).

4.3. Early seasonal competition is important but not decisive for final weed suppression

In our study, the intensity of competition imposed on weeds was much stronger in the 2020 season. This might be due to the plasticity of weeds, enabling them to benefit more from the increased resource availability in that year, thereby modifying the CC-weed interaction dynamics (Kaur et al., 2018). Oilseed radish tended to be the strongest competitor during the initial five to six week period after sowing, while the diverse CC mixture was initially inferior in terms of its competitive ability. Shallow-angled leaves that favour rapid canopy development also increase the competitive ability of oilseed radish and white mustard (Brennan and Smith, 2005, Marshall and Pomeranz, 1982, Walker et al., 1988). Sturm et al. (2017) reported that the enhanced competitive ability of oilseed radish is related to earlier light interception as a result of faster soil coverage and weed shading.

The intra-seasonal dynamics of the relative weed suppression efficacy (RWSE) followed those of CC biomass production and the increasing trend of absolute growth rate over the assessment period. All CC included in the experiment significantly reduced weed biomass compared to the unsown fallow plot, with relative weed biomass reductions of up to 97 % in the 2020 season. Weed suppression levels of up to 96 % were also reported by Brust et al. (2014) under similar conditions with CC and weed biomass measuring 220 g m^{-2} and 150 g m^{-2}, respectively

Oilseed radish had greater initial levels of RWSE in the period 3–4 weeks after sowing, with a 30 % increase compared to the second most competitive simple CC mixture. Moreover, oilseed radish was the only CC species that maintained a high level of weed suppression throughout the growing period, delivering notable levels of final RWSE (81–82 %) across both seasons. This effect is likely driven by the high dry matter production of oilseed radish (Baraibar et al., 2018) and the well-documented allelopathic potential of Brassicaceae species (Haramoto et al., 2005), which can contribute up to 28 % of overall weed suppression (Sturm et al., 2018).

The simple CC mixture was the most effective in suppressing weeds when considering the whole season. Although not observed in our study, there are studies that found greater weed suppression in more diverse CC mixtures, for example through a more efficient use of light (Tribouillois et al., 2015; Suter et al., 2017). The lack of effect in our diverse CC mixture could be related to less-aggressive species like safflower, linseed and legumes where weed suppression may be lower and more dependent on climatic conditions that influence CC establishment and growth (Brainard et al., 2011). With only a limited number of single species we can only partly claim that CC mixtures are more weed suppressive than the most productive monocultures (Florence and McGuire, 2020; Smith et al., 2020).

The outcome of weed suppression was not determined by the initial level of RWSE. Despite significantly lower initial RWSE than oilseed radish (30 % vs. 60 %), the simple CC mixture was shown to increase RWSE in the subsequent period and delivered impressive 74 % and 97 % reductions of weed biomass at the end of the 2020 and 2021 season, respectively. This gain in both experiments in the late autumn growing period likely resulted from inclusion of white mustard and niger seed, both very competitive species recognized for stable production of large amounts of biomass and good weed control (Büchi et al., 2020; Brennan and Smith, 2005). These findings also suggest the significance of initiating CC mixtures early in the season, as an extended growing period allows for a greater number of species to achieve their full biomass potential.

4.4. High CC productivity does not ensure effective weed suppression or consistently improve maize yields

Greater CC biomass production did not regularly improve the level of weed suppression, indicating that biomass alone is not sufficient to limit weed growth. This was especially noticeable in 2021, with enormous CC biomass accumulation in comparison to the previous season, but lower levels of weed suppression across all CC. A possible explanation for the observed pattern might be sufficient ecological niche differentiation between CC species used in this study and the weed species present of our experimental plots. Specific CC-weed responses have been reported in many studies, yet the mechanisms still need to be identified (Moonen and Barberi, 2004; Alonso-Ayuso et al., 2018; Rouge et al., 2022). Inconsistency was observed in our study also when comparing the performance of individual CCs and CC mixtures. For example, despite oilseed radish's lower biomass production during the second growth period, it demonstrated remarkable weed suppression capabilities. Likewise, diverse CC mixture exhibited similar biomass production compared to the simple CC mixture in the early period (up to H2) of 2021 experiment, yet the latter exhibited higher levels of weed suppression. This observation suggests a potential role of allelopathic compounds released by oilseed radish and white mustard, although disentangling this effect from passive, indirect competition poses a challenge for future field studies (Mahé et al., 2022). Furthermore, Adeux et al. (2021) proposed that crucifer species, such as oilseed radish, may exhibit enhanced weed suppression compared to legumes at lower cover crop (CC) productivity levels (e.g., 200 g dry biomass m^{-2}), attributed to a combination of allelopathy, nitrophilous characteristics, and rapid soil coverage. Consistent with these findings, our study also found that despite berseem clover's substantial final biomass, it did not achieve the level of weed smothering comparable to oilseed radish's weed suppression effect.

Maize yield observed in the 2021 experiment were in the range of regional yields of rain feed maize (Schils et al., 2018), while they were markedly lower in the 2022 season as a result of long-term water shortage. Tillage practices used for CC establishment and maize presowing soil management had no influence on maize yields. This could be because of small differences in the soil disturbance level between the CN and CS, but more likely because the tillage treatments were imposed only for a short study period. As noted by Cooray et al. (2023), the yield benefits from tillage practices vary across different crops. For main crop maize, grain yield increased proportionally with reductions in tillage intensity. DeFelice et al. (2006) further highlighted the limitations of short-term experiments in providing fair comparison as yield benefits from conservation tillage practices may only become evident over time due to the gradual accumulation of soil organic carbon, microbial biomass, and structural integrity (Sindelar et al., 2015).

There is an ongoing debate about the impact of cover crops on main crop yields, with studies reporting positive influence (Adeux et al., 2021; Vendig et al., 2023). Yet, other investigation showed no effect (Cottney et al., 2022) or even negative yield response (Deines et al., 2023).

Use of CC had limited influence on main crop productivity as only berseem clover and simple CC mixture insignificantly improved maize dry grain yield with an average of 0.7 t ha^{-1} in comparison to tilled fallow without CC. These findings align to some extent with previous studies advocating for diverse cover crop mixtures and legume integration with maize as yield-enhancing strategies (Wojciechowski et al., 2023; Peng et al., 2024). The effect of soil-mediated factors like nitrogen supply on crop productivity was observed in numerous studies emphasizing the complexity of interactions between cover crop management and environmental variables in determining the carry-over effects on succeeding crops (Adeux et al., 2021; Rouge et al., 2023). A greater yield improvement expected with berseem clover may thus be attributed to the humid conditions at the study site (annual precipitation exceeding 1000 mm), which typically reduces the nitrogen-fixation capacity of these crops and may negate the yield benefits (Rusinamhodzi et al., 2011; Daryanto et al., 2018). The simple CC mixture produced an excessive amount of biomass in 2022, yet no reduction in maize yield was observed in the subsequent year due to inorganic nitrogen immobilization (Finney et al., 2016; Adeux et al., 2021). However, in the diverse CC mixture, where sorghum-Sudan grass hybrid dominated, this effect might have been more significant. Oilseed radish produced yields similar to those in fallow plots. The lack of maize yield response to oilseed radish suggests that its role in yield improvement is often limited, even in mixtures with grasses and legumes (Engedal et al., 2023; Wojciechowski et al., 2023). This may be due to the excessive depletion of soil mineral nitrogen (Adeux et al., 2021), the high organic matter levels at the study site (Bourgeois et al., 2022), and the intensive maize fertilization, which could have reduced its benefits on crop yield (Fan et al., 2021).

5. Conclusions

High levels of weed suppression using CCs confirm their importance as a key diversification and weed management strategy. The results from this study indicate negligible effect of tillage establishment practice on CC productivity, while competitive species like oilseed radish can deliver a high degree of season-long weed suppression comparable to CC mixtures. Maize yields, and the level of weed control were poorly related with CC biomass production, with rapid early growth potentially being a more important functional trait explaining CC weed suppression ability. Moreover, to improve CC-weed competitive outcomes, the functional traits most relevant for weed suppression should be identified for a specific level of resource availability, management practice or desired ecosystem service. This would allow optimization of CC management towards more agro-ecological CC strategies which will contribute to the overall stability and resilience of crop production and food security.

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CRediT authorship contribution statement

Adamič Zamljen Sergeja: Writing – review & editing, Investigation, Formal analysis, Data curation. **Leskovsek Robert:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Eler Klemen:** Writing – review & editing, Visualization, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.109530.

Data availability

Data will be made available on request.

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