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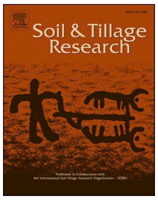

Weed seed bank response during the early conversion period to less intensive tillage systems

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ABSTRACT

In recent decades, adoption of less intensive soil tillage practices has increased due to numerous environmental and economic benefits. Our investigation was performed during 2020–2022 and examined germinable and persistent weed seed bank response in the early conversion period from conventional (CN) to conservation (CS) and no-tillage (NT) system under the humid, temperate climate of central Europe. The germinable seed bank determined in the spring cropping period was up to four times greater compared to the autumn cropping period. By using NT, consistently lower germinable weed seed banks were seen compared to CN or CS with observed reductions of weed seed density 45–75%. Final abundance of persistent weed seed bank (117,000 seeds m^{-2}) was considerably greater than average size of germinable weed seed bank across years (27,000 seeds m^{-2}). The vertical distribution of germinable weed seed bank in the soil did not differ between the tillage treatments, while using CS and CN more persistent weed seeds were accumulated in the intermediate (5–10 cm) or the bottom (10–20 cm) soil layer. CS and NT facilitated greater weed species diversity and evenness in both germinable and the persistent weed seed bank. Our results showed that introduction of different tillage systems had an almost immediate response in the size of germinable weed seed bank in the soil. Thus, when converting new fields to alternative tillage systems with high weed pressure, weed seed bank studies are advocated to select systems that reduce germinable seed in the seedbank. This method will enable coherent integration of non-chemical weed management strategies and support existing IWM methods in transition to sustainable agroecological based weed management strategies.

1. Introduction

Weeds remain the most important global biotic factor in crop production because of their potential to induce substantial yield losses in various types of crops (Oerke, 2006). The significance of weed management for achieving ecological intensification in agriculture is widely recognized, as current weed control measures rely heavily on chemical approaches (Chikowo et al., 2009; Petit et al., 2015). In response to growing societal pressure to reduce the ubiquity of synthetic herbicides, farming practices are adapting, revitalizing interest in soil seedbank studies both from agronomic and ecological perspectives (Mahé et al., 2020).

Soil weed seed banks represent a large reservoir of viable seeds and propagules and therefore the main source of future weed infestation (Chauhan and Johnson, 2010; Shrestha et al., 2002). The weed seed bank size is a reflection of past and current soil and weed management practices regulating the addition of new weed seeds into the weed seed bank (Buhler et al., 1997; Baraibar et al., 2009). Moreover, the changes in the weed seed bank are also impacted by processes of seed predation and degradation, thereby influencing weed emergence events (Auffret and Cousins, 2011; Leon and Owen, 2004).

Among the agronomic factors interacting with weed management, tillage and crop rotation have been identified as exerting a dominant influence on weed community size and composition (Buhler et al., 2001; Hosseini et al., 2014; Otto et al., 2023). Tillage is a fundamental agricultural practice that involves mechanical manipulation of soil to prepare the seedbed, control weeds, and manage crop residues (Hobbs et al., 2008). Less intensive tillage practices are being adopted globally in response to major soil degradation (erosion, run-off, nutrient leaching, and soil fertility loss) caused by conventional practices (Holland, 2004; Soane et al., 2012; Tilman et al., 2002).

Long-term studies investigating the effects of management practices on weed community dynamics report considerable variations in weed density, emphasizing complex interactions between crop rotation,

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tillage, and weed management (Otto et al., 2023; Ruisi et al., 2015; Sosnoskie et al., 2006). Because weed composition is subject to considerable natural fluctuations in both time and space, the initial state and distribution of the weed seed bank have been identified as important factors influencing the weed response to tillage (Barberi et al., 1997; Légère and Samson, 1999; Mohler, 1993). Previous studies showed that tillage systems affect the composition and density of the weed communities, primarily by altering the vertical distribution of seeds in the soil (Buhler et al., 1994; Scherner et al., 2016). Thus, certain weed seedlings may fail to emerge when buried deeply within the soil, while low–soil-disturbance tillage systems lead to the accumulation of seeds near the surface, thereby favouring germination of species with greater light requirements and facilitating weed seed predation (Baraibar et al., 2009; Chauhan and Johnson, 2010; El Titi, 2002). Conservation agriculture and reduced tillage may also increase total weed abundance and lead to weed community shifts from annual dicots to grassy annuals and perennials (Derrouch et al., 2021; Scherner et al., 2016).

The adoption success of alternative tillage systems is closely related to the soil and environmental constraint, as humid climate and undrained soils have been found to limit the effectiveness of reduced tillage (Engell et al., 2022; Soane et al., 2012). Moreover, short-term changes in weed seed bank dynamics related to conversion to alternative soil management practices in less favourable soil types and humid environmental conditions across Europe have been examined to a lesser extent. There is indication that conversion to no-till can lead to considerable weed seed build up in the early phase (Moonen and Barberi, 2004). Conversely, a tillage study performed on heavier soil type and humid conditions in Switzerland showed only moderate decrease of the weed seed bank to shallow tillage (Krauss et al., 2020).

Weed community characteristics and the level of weed infestation are predominantly influenced by the density and composition of the weed seed bank. Yet, even in mature experiments weed seed banks have been poor predictors of aboveground weed communities which has been attributed to management strategies and environmental variability (Feledyn-Szewczyk et al., 2020; Davis et al., 2005). Given the complex effects of tillage on weed seed germination and emergence, numerous studies imply a significant role of physical and environmental changes affecting seasonal dynamics of weed seed dormancy (Nichols et al., 2015; Samarajeewa et al., 2005; Sharma et al., 2020). Hence, with variable dormancy, weed seed banks can either be relatively transient or persistent, where significant numbers of weed seed accumulate in the soil (Fenner, 1985; Karssen, 1982). As such, conservation tillage techniques have been found to favour persistent weed seed bank (Benech-Arnold et al., 2000; Santín-Montanyá et al., 2013)

Thus, understanding the short-term, site-specific effects of tillage practices on soil weed seed banks and the mechanisms underlying transitions within weed communities is crucial for reducing dependence on chemical herbicides and developing more sustainable weed control methods, with minimal impact on the agroecosystem. In this context, this study aimed to quantify the response of the germinable and persistent weed seed bank and determine the changes in weed species composition and diversity in response to variation in the soil and environmental conditions.

2. Material and methods

2.1. Study site and weather conditions

The study was conducted between 2020 and 2022 using an experimental field and net covered greenhouse (hereafter referred as greenhouse) of the Agricultural Institute of Slovenia (AIS) (Infrastructure Centre Jablje pri Mengšu, 46°08’33.9’’ N, 14°34’21.5’’ E, 309 m a.s.l.). The field is dominated by shallow to medium/deep alluvial eutric brown soil on calcareous pebble and sand. The soil was well drained with a silty-loam soil texture comprising 25% clay, 37% silt, and 38% sand. Due to their high pebble content, these soils have limited water storage capacity and are therefore prone to summer droughts. Soil samples were taken from depths ranging from 0 to 20 cm in March 2020, i.e., before the beginning of this study. Soil analysis was performed at the Agricultural Institute of Slovenia in Ljubljana for the following parameters: pH (in KCl) and available phosphorus and potassium (P_2O_5, K_2O, respectively) employing the calcium-acetate-lactate method from Santner et al. (2015). Soil organic carbon was high (2,5%) and determined with Walkley–Black method (FAO, 2019). The results showed neutral pH (7.6) and abundant supply with available phosphorus (30 mg kg^{-1} P_2O_5) and potassium (35 mg kg^{-1} K_2O). Precipitation and temperatures were measured using weather stations located near the experimental field (Adcon, A753GSM) and inside the greenhouse (A753). Temperatures and precipitation data from the experimental site were compared to long-term averages for period 1951–1994 (Fig. 1). Mean temperatures in years 2020, 2021 and 2022 were 10.8°C, 10.3°C and 11.6°C while the long-term average is 10.9°C. Total annual precipitation in years 2020, 2021 and 2022 was 1316 mm, 1238 mm, and 1048 mm, while the long-term average is 1294 mm. Compared to the ambient 30-year temperature average for Ljubljana (12.1°C), mean temperatures in the greenhouse at the time of germination period (January to December) were similar in all years (12.0°C in 2020, 11.5°C in 2021, and 12.8°C in 2022).

2.2. Crop and weed management history at the study site

For the two decades preceding the initiation of the long-term experiment, the study site underwent diverse rotations. Key crops incorporated in the rotation encompassed winter wheat (*Triticum aestivum* L.), winter barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), crimson clover (*Trifolium incarnatum* L.), and grass-clover mixture. Periodically, forage rape (*Brassica napus* L. var. napus f. biennis) was also cultivated. Plant residues were predominantly left on-site, with occasional removal of winter wheat straw. Initially, bare winter furrowing was practiced until 2005 when cover crops were integrated into the rotation. Winter-hardy species such as forage rape and crimson clover were sown alone or in combination on the stubbles of winter cereals in September. Mechanical termination of cover crops was conducted through ploughing as part of primary tillage in spring. Soil management followed conventional practices involving ploughing to a depth of 25 cm, with seedbed preparation using a fine-tine spring cultivator to a depth of 10 cm. Occasionally, a power harrow was employed, particularly when soil conditions were unsuitable for small-seeded species like crimson clover or grass-clover mixture. Since the introduction of cover crops, shallow tillage up to 15 cm has been employed using a stubble cultivator with wing shares. Fertilization practices were informed by soil analyses conducted every two to three years, with nutrients primarily supplied through mineral fertilizers. Solid manure containing wheat straw bedding was applied three times over a 20-year period at a rate of 20 t ha^{-1} on winter wheat stubbles. Phosphorus and potassium inputs ranged from 50 to 80 kg and 130–180 kg ha^{-1}, respectively. Nitrogen application predominantly utilized calcium ammonium nitrate and amid (e.g. urea). Starting from 2015, slow-release nitrogen fertilizers containing the nitrification inhibitor dicyandiamide have been employed. Seasonal nitrogen inputs ranged from 60 kg ha^{-1} in crimson clover to 250 kg ha^{-1} in maize, with winter cereals and forage rape typically receiving 160 kg ha^{-1}. Weed management primarily relied on pre-emergence herbicide application, with additional post-emergence treatments in maize and soybean to control perennial weeds or grasses. No herbicides were applied in the grass-clover mixture and forage rape. Key active substances utilized in maize included pendimethaline, S-metolachlor, mesotrione, terbuthylazine, and nicosulfuron. Weed control in winter cereals involved herbicides containing diflufenican, iodosulfuron and pinoxaden, while bentazone and cycloxydim were used in crimson clover. Fungicide treatments were limited to two applications in winter cereals, and pest control with insecticides was similarly restricted to two applications in winter cereals, oilseed, and

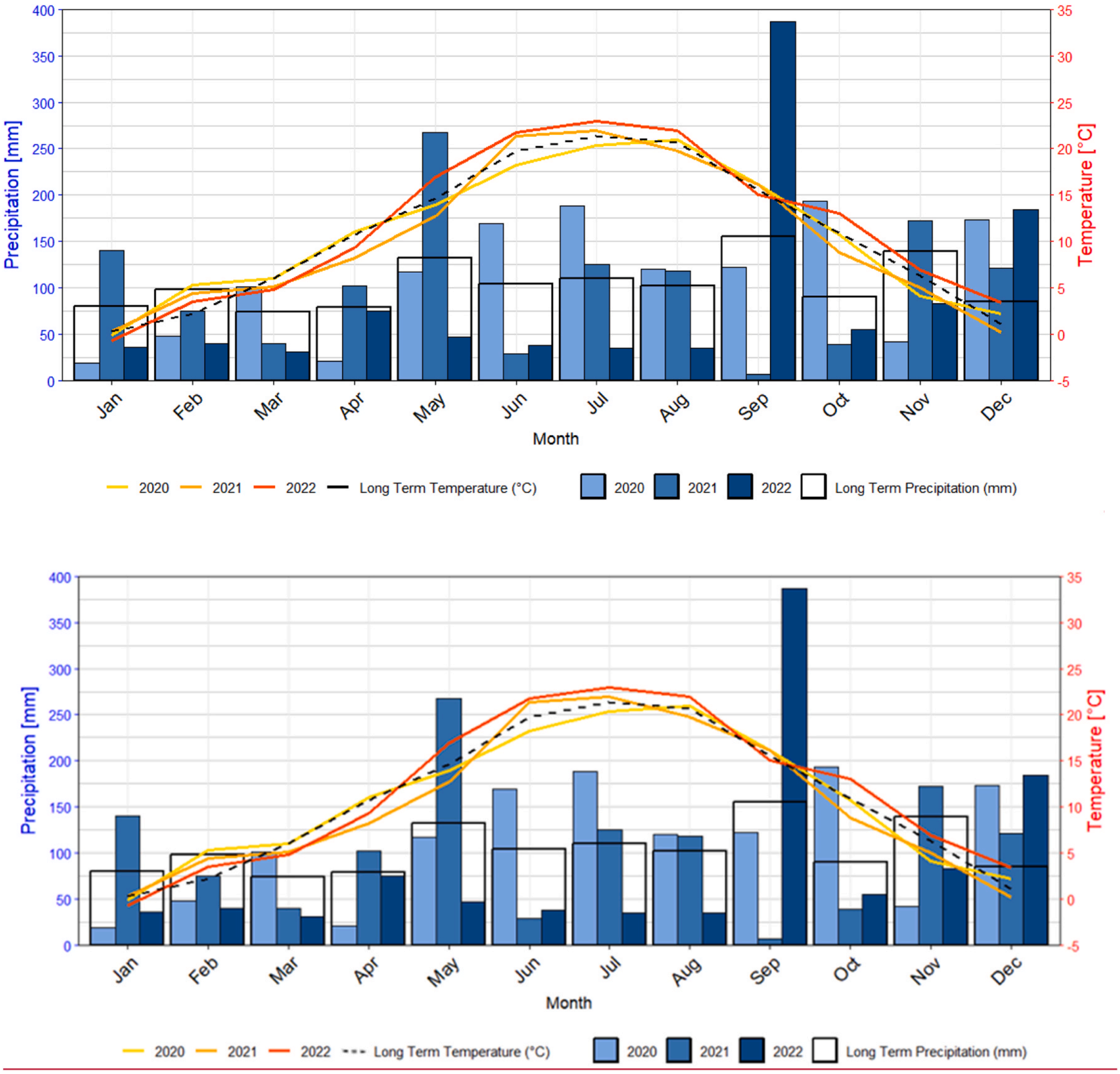

Fig. 1. Mean temperatures and precipitation at the experimental field in Jablje (Slovenia) between 2020 and 2022 compared to long-term average temperatures and precipitation in the period 1951–1994.

forage rape.

2.3. *Experimental setup and soil seed bank sampling*

Three different tillage systems were used as part of a newly established long-term tillage experiment: conventional (CN), conservation (CS), and no tillage (NT). Plots, representing the three tillage systems were 50 × 25 m in size and the trial was arranged in a randomized block design, with four replications. The tillage methods were established in the autumn of 2018. In all years from autumn 2018 onwards the soil in CN tillage was prepared conventionally with ploughing (depth, 22 cm), furrows were closed with a drag leveling bar and the pre-sowing soil preparation was carried out with tine cultivator or a rotary harrow. In CS tillage the soil was tilled with a disc harrow (depth, 8 cm), while seedbed was prepared with tine cultivator. In the NT system, the only ground penetration action undertaken was the formation of seeding furrows, preserving the soil surface largely undisturbed. Weed management between CN and CS did not differ, while additional pre-sowing or post-harvest treatment was implemented in NT (glyphosate 1800 g a.i. ha^{-1}). Detailed description of the tillage treatments and weed management can be found in Table 1.

Soil sampling for weed seed bank analysis was carried out before commencing tillage operations in the spring and autumn cropping period. Soil seedbank assessments for autumn cropping period were performed at the end of October or beginning of November, while soil seed bank for spring cropping period was determined in between March and the beginning of May. The long-term trial was established in autumn 2018 with winter wheat (*Triticum aestivum* L.). After the winter wheat harvest, plant residues were left on the field and mulched. On 10 August 2019 a cover crop of berseem clover (*Trifolium alexandrinum* L.) was sown. Berseem clover was terminated by frost in November 2019 and the soil was left covered with a thick layer of dead plant residues. In March 2020 first soil sampling for weed germination assessment was performed in the spring cropping period before implementing tillage operations. After the sampling, CN, CS and NT soil management operations were executed and early variety of soybean was sown at the last decade of May 2020. After the soybean harvest, first soil samples were taken for the autumn cropping period, followed by establishment of winter barley (*Hordeum vulgare* L.) in October 2020. After the harvest plant residues were left in the field and non-winter hardy cover crop mixture was sown in July 2020. Second soil sampling in the spring cropping period was performed in dead cover crop mulch. Again, the three soil tillage managements were used to establish the maize crop (*Zea mays* L.) in April 2021. Maize was harvested in October 2021,

Table 1
Description of the three different tillage treatments, equipment used, the level of soil disturbance, and weed management.

Tillage treatment	Equipment used	Cultivation depth and the level of soil disturbance	Weed management
Conventional tillage (CN)	Moldboard plough, fine spring tine cultivator or power harrow and seed-drill with disc openers	Soil inverted and loosened down to depth of 18–20 cm during primary tillage. Soil mixing to 5–8 cm depth during seedbed preparation.	Conventional with one to two pre- and post-herbicide treatments per season with total TFI[a] of 1.5. Crop sequence for the period 2019–2022 included winter wheat + cover crop, soybean, winter barley + cover crop and maize.
Conservation tillage (CS)	Disc harrow or field cultivator, fine spring tine cultivator and seed-drill with disc openers	Soil loosened down to depth of 10–12 cm during primary tillage. Soil mixing to 5–8 cm depth during seedbed preparation. Planting furrows 3–5 cm deep with surface disturbance in the furrow line.	
No tillage (NT)	No-till seed-drill with disc coulters	Planting furrows 3–5 cm deep with surface disturbance in the furrow line	Conventional with two to three pre- and post-herbicide treatments per season with TFI of 2.5. Crop sequence for the period 2019–2022 included winter wheat + cover crop, soybean, winter barley + cover crop and maize.

with n: number of years in the crop sequence, T: total number of pesticide treatments, D: applied rate in commercial product, DAp: approved/registered rate for the commercial product (Kudsk, 2018).

[a] Values indicate Treatment frequency index, number of full rate treatment: $TFI = 1/\mathrm{n}\sum_{t=1}^{t=T} D_{t/}/DApt$

followed by second soil sampling in the autumn cropping period. Winter wheat was established then and in the following spring cropping period the third and concluding sampling session was executed. Soil samples were collected from randomly assigned subplots measuring 25 m^2 (5 m x 5 m) and from three different soil depth intervals (0–5 cm, 5–10 cm, and 10–20 cm), using four repetitions. Sampling was carried out with a 52 mm diameter soil probe, mixing composite samples. A total of 36 samples were collected (3 tillage systems, 3 depths, and 4 repetitions) and stored at 4°C for one week in the dark until commencing the germination experiment to break the dormancy of weeds. Soil samples were then weighed, and the seed bank contents adjusted to express the weed seed content of 1 m^2 of each soil layer using soil bulk densities (described below). Only in spring of 2022, sub-samples of soil seed bank were taken to assess total weed seed bank abundance and species composition with flotation method. Soil samples were added to 1 L of tap water and dispensed into a 2 mm mesh-size sieve, rinsing them with more water to break down larger soil particles. The remains were then carefully collected with a spoon and placed into plastic containers that were sealed with a lid. Then, the process was repeated using sieve with a 0.355 mm mesh size. Weed seeds in the rinsed samples were counted and examined under a microscope for taxonomic identification at the seed laboratory of the Agricultural Institute of Slovenia.

2.4. Determination of soil bulk density

Soil bulk density was assessed using Kopecky cylinders following the ISO standard (ISO 11272, 1993). Intact soil samples enclosed in Kopecky cylinders were collectively weighed and subsequently dried at 105 °C for 48 hours until reaching constant weight. The dried samples, along with the cylinders, were then weighed again and passed through a 2 mm sieve to separate out particles larger than 2 mm. The volume and weight of the rocks were determined by measuring the volume of water displaced in a graduated cylinder upon immersion of the rocks. Empty Kopecky cylinders were weighed, and the difference between the weights of empty and full cylinders was used to ascertain the mass of the solid phase of the soil sample. Bulk density was calculated based on the ratio of the mass of the solid phase to the volume of the cylinder (100 cm^3), while also considering the mass and volume of the rocks.

2.5. Germination experiment and assessment

Before the onset of germination experiments, all samples were passed through sieves with a mesh size of 1 cm to break up soil clods and remove large debris. Then, samples were placed into 39 cm × 29 cm × 6.5 cm flat plastic trays filled with glass wool for greater water holding capacity before adding 1000 mL of sieved sample and mixture of sterilized peat (70%), vermiculite (20%) and fine sand (10%). Samples were kept in a greenhouse and irrigated using a fogging irrigation system, with irrigation intensity and frequency adjusted based on outside weather conditions. Samples were kept moist to avoid the formation of crusts which would have impeded seedling emergence. The plastic trays were placed in the greenhouse using natural lighting only. Seedlings were allowed to develop until their species could be identified and were then removed. This process was repeated until no more seedlings emerged. Total number of germinating weed seeds from the surface of each soil sample (ten 52 mm diameter cores equal to 212.4 cm^2 field area) were scaled up to the number of seedlings per m^2. Although this typically underestimates the total weed seedbank density, it still provides accurate estimates of relative differences between cropping systems (Ball and Miller, 1989).

2.6. Weed species abundance and diversity indices

Using the germinable weed seed density data and the total weed seed number data diversity indices were calculated for germinable and persistent weed seedbank. Species richness of the weed community was determined by calculating the Margaleff's diversity index (D):

$$\mathrm{D} = \frac{S-1}{\ln(N)}$$

where S is the number of species present in the population and N is the average total weed density (plants m^{-2}) within the individual sample. The weed species diversity (Shannon-Weiner diversity index - H′) was calculated by the following equation:

$$\mathrm{H}' = -\sum_{i=1}^{s} p_i(\ln pi)$$

where pi is the proportion of individuals belonging to the ith species and S is the total number of species distributed among different species in a weed community. Species evenness (Pielou's index - E) is a measure of weed species uniformity in a weed community and was determined using following equation:

$$\mathrm{E} = \frac{H'}{\ln(k)}$$

where k is the number of species in our samples. A high degree of species evenness indicates a relatively equal distribution of individuals among the weed species germinated from the soil samples.

2.7. Statistical analysis

Statistical analysis and model fitting were performed using R 4.1.3 software, with data visualization using the ’ggplot2’, `gridExtra`, `ggpubr` and `patchwork` packages (R Core Team, 2022). Assumptions for normal distribution and homogeneity of variances were verified using the Shapiro–Wilk and the Levene test. Soil germinable weed seed bank and the diversity indices data were analysed using Linear Mixed Models (LMM), where tillage system, cropping period, soil depth and experimental year were the fixed effects, and the replications were the random effects. When ANOVA indicated statistical differences, Tukey’s post-hoc test for multiple comparisons was used ($p \leq 0.05$). An autoregressive correlation structure and variance was assumed between years; thus, autocorrelation function was used and patterns of serial correlation checked with post-hoc analysis of the residuals. Persistent weed seed bank and diversity indices data obtained from the flotation experiment were subjected to factorial ANOVA considering tillage system, cropping period, soil depth as main factors. Means obtained by ANOVA were compared using Tukey’s post-hoc test for multiple comparisons ($p \leq 0.05$).

3. Results

3.1. Seasonal abundance and vertical distribution of germinable weed seed bank

Inter-seasonal comparison showed significant fluctuations of germinable weed seed bank densities throughout the experimental period (Fig. 2). The weed seed bank determined in the spring cropping period for year 2020, significantly increased by half in the next season, while the final number of germinated weed seeds was 55% lower compared to the initial seed bank assessment. Similar but non-significant difference between the experimental seasons were seen also in the autumn cropping period, where the final size of germinable weed seed bank was reduced by 70% compared to the weed seed density found in the previous year.

Across the tillage systems and years, autumn cropping period weed seed bank was markedly smaller, yielding only 23% of the weed seed bank determined in the spring cropping period (Table 2; Fig. 2). Compared to CN and CS the low disturbance NT consistently reduced the weed seed banks as observed in all seasons and both cropping periods. Using NT in the spring cropping period resulted in a notable 72–75% weed seed decrease. Weed seed decline with NT in the autumn cropping period was pronounced with 56% and 45% less germinated weed seeds compared to the CN and CS plot, respectively.

Table 2
Statistical results from the linear mixed model applied on the germinable weed seed density and seed distribution including soil depth (SD) at the significance levels *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

		Total weed seed density		Weed seed distribution including soil depths (SD)	
Factor	DF	F	p	F	p
Tillage system (TS)	2	8.32	<0.001	15.04	<0.001
Cropping period (CP)	1	57.15	<0.001	103.3	<0.001
Soil depth (SD)	2	/	/	0.34	0.71
Year (Y)	1	1.18	0.28	2.14	0.14
TS x CP	2	2.53	0.09	4.57	<0.05
CP x Y	2	0.02	0.89	0.03	0.85
TS x Y	2	0.08	0.92	0.16	0.86
TS x SD	4	/	/	3.13	<0.05
CP x SD	2	/	/	0.45	0.64
SD x Y	2	/	/	0.35	0.70
TS x CP x Y	2	0.44	0.65	0.82	0.44
TS x CP x SD	4	/	/	3.35	<0.001
TS x SD x Y	4	/	/	0.45	0.77
CP x SD x Y	2	/	/	0.06	0.94
TS x CP x SD x Y	4	/	/	0.53	0.72

The various tillage treatments resulted in a relatively homogenous distribution of the germinable weed seed bank down the soil profile, but the weed seed density within the individual soil depth was strongly influenced by the tillage system (Fig. 3). This effect was however not consistent across the cropping period and soil depth, as indicated by the significant three-way interaction among the factors (Table 2). For example, intensity of the tillage operations had no effect on the weed seed abundance in the top (0–5 cm) soil layer in both cropping periods. However, when comparing the three tillage systems in the intermediate (5–10 cm) soil layer NT resulted in significant reduction of germinated weed seeds compared to the CS treatment as 87% and 56% less weed seeds were observed in the autumn and spring cropping period, respectively. Similar levels of weed seed bank reductions were seen also in the bottom soil layer (10–20 cm), where NT was found to have substantially lower weed seed number in comparison to the CN. Tillage system had no effect on the weed seed distribution between equivalent soil depths (0–10 cm vs 10–20 cm) in both cropping periods with 55–65% germinable weed seeds located in the 10–20 cm soil depth. Only NT in the autumn cropping period resulted in the non-significant share of 56% weed seeds placed in the 0–10 cm soil depth.

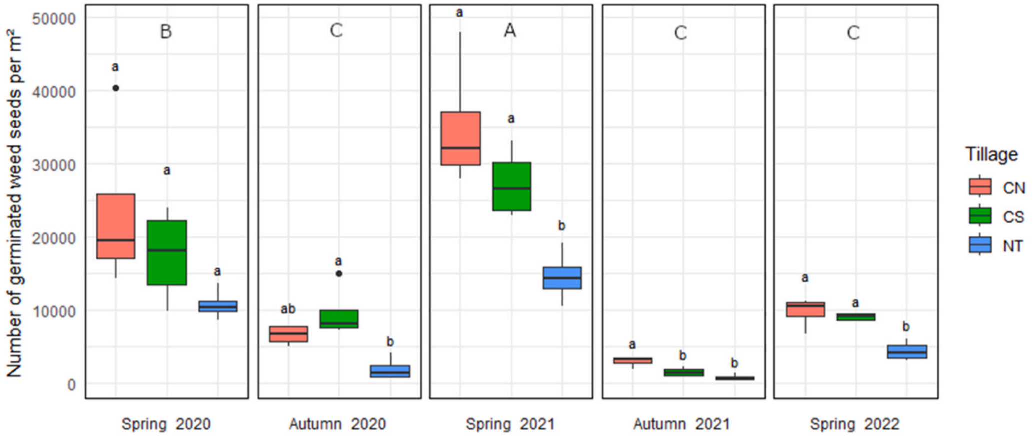

Fig. 2. Total density of germinable weed seeds during the spring and autumn cropping period for years 2020–2022 under conventional tillage (CN), conservation tillage (CS), and no tillage (NT). Boxplots with different uppercase letters indicate significant inter-seasonal differences ($p < 0.05$) in weed densities, while lowercase letters indicate significant differences in weed densities between the different tillage treatments within each year and cropping period.

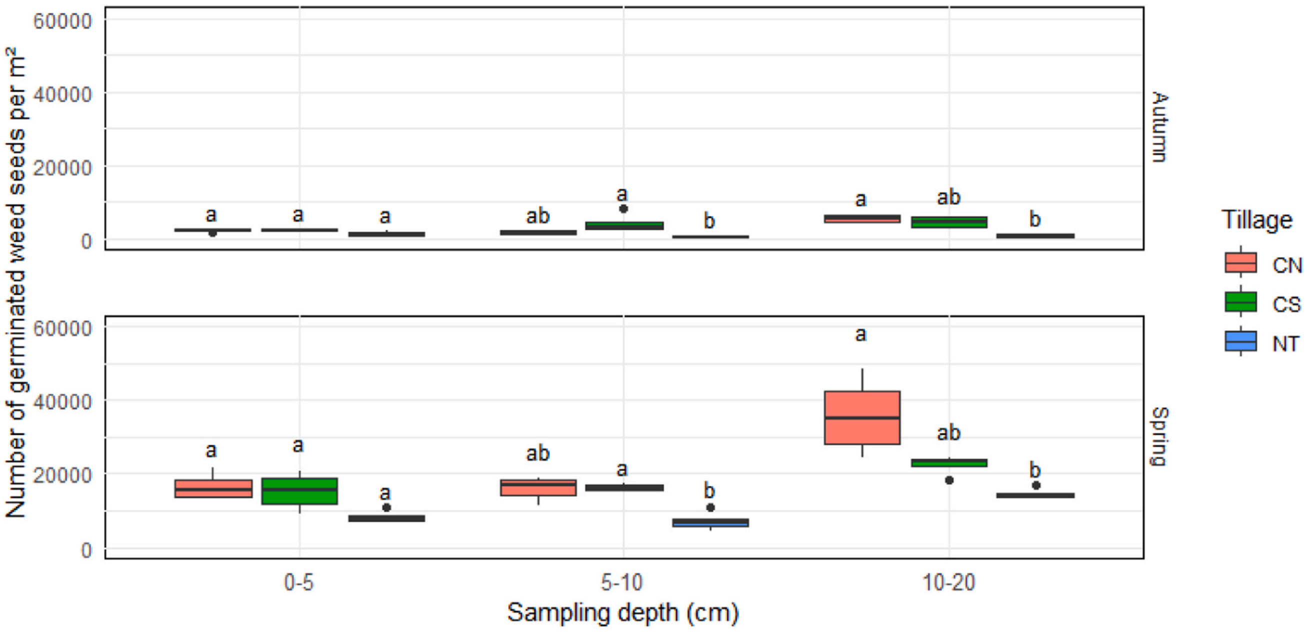

Fig. 3. Vertical distribution of germinable weed seeds during the spring and autumn cropping period under conventional tillage (CN), conservation tillage (CS), and no tillage (NT) for years 2020–2022. Boxplots with different lowercase letters indicate significant differences ($p < 0.05$) in weed densities between the different tillage treatments within each cropping period and soil depth.

3.2. *Weed diversity indices of the germinable weed seed bank*

Throughout the experimental period 15–21 weed species were determined in the spring cropping period with prevailing weed species *Chenopodium polyspermum* L. *and Galinsoga parviflora* (Cav), ranging from 53% in NT to 69% in CN tillage system. In the autumn cropping period weed population was comprised of 5–9 weed species and weed community was dominated by *Lamium amplexicaule* L. *and Viola arvensis* Murray. In CS they represent 59% of all weeds, while in CN and NT tillage system they account for 66% of all weed species determined in the study.

Significant inter-seasonal variations were observed in the spring and

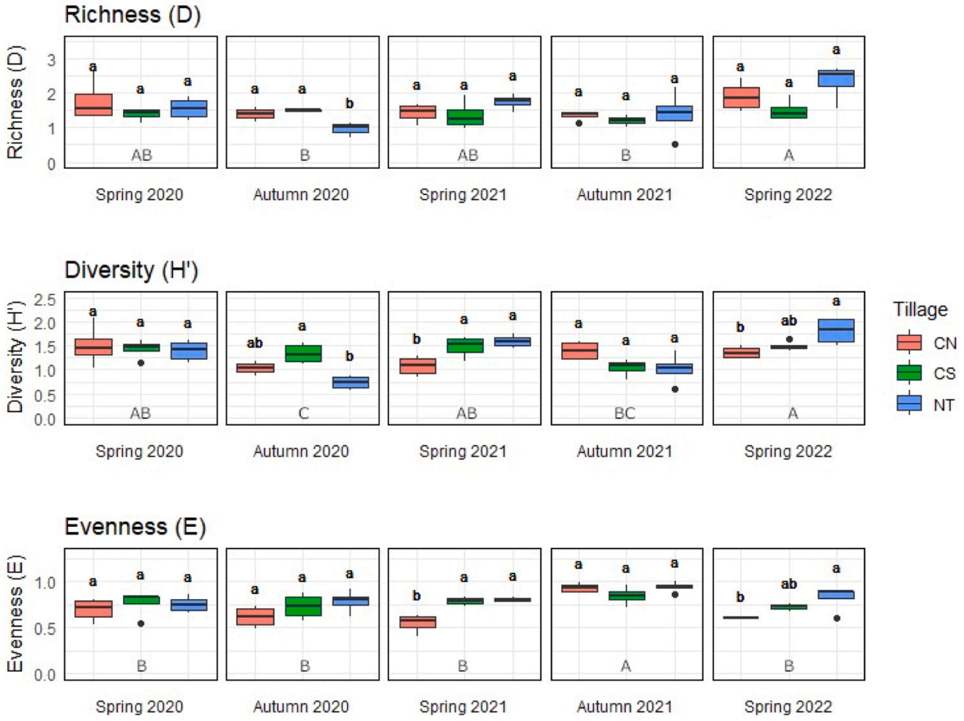

Fig. 4. Weed seed species richness (D) diversity (H'), and evenness (E) during the spring and autumn cropping period for years 2020–2022 under conventional tillage (CN), conservation tillage (CS), and no tillage (NT). Boxplots with different uppercase letters indicate significant inter-seasonal differences ($p < 0.05$) in weed diversity indices, while lowercase letters indicate significant differences in weed diversity indices between the different tillage treatments within each year and the cropping period.

autumn cropping season; however the final evaluations of weed species indices were in line with the values recorded at the initiation of the study (Fig. 4). When averaging across the years and tillage systems, weed community observed in the spring cropping period was richer compared to the weed population determined in the autumn cropping period (Table 3). Moreover, weed species richness was promoted also by more intensive tillage operations within CN and CS as seen in the autumn cropping period of year 2020. Contrasting temporal response of weed species diversity and evenness was seen for each cropping period (Table 3). In the autumn cropping period, CS was found to promote greater diversity in comparison to NT, but this effect was limited to the year 2020. In the spring cropping period, both weed species diversity and evenness were facilitated by CS and NT, but this trend was noticeable in the later experimental period.

3.3. *Abundance and vertical distribution of the persistent weed seed bank*

The weed seed density and weed seed distribution along the soil depth of persistent weed seed bank were not affected by the tillage system (data not shown). Majority of weed seeds with NT (49%) was found in the topsoil layer (0–5 cm) and with CS (56%) in the intermediate soil layer (5–10 cm) while using CN similar share of seeds was observed in the bottom soil layer (41%) (Fig. 5). The tillage systems however changed persistent weed seed distribution pattern along the soil depth as indicated by significant two-way interaction ($p \leq 0.001$). Surface weed seed density increased with decreasing tillage intensity, so most weeds were found in the NT (+110%). Contrasting pattern was seen in the intermediate soil layer, where NT resulted in 25% and 44% lower number of germinated weed seeds compared to the CN and CS plot, respectively. In the bottom soil layer, a significantly greater weed seed build up was seen with use of CN, while weed seed accumulation with CS and NT was similar (Fig. 5). When comparing vertical weed seed distribution among the tillage treatments and considering the combined content of the top two soil layers (0–5 cm and 5–10 cm), a significantly higher proportion of persistent weed seeds was detected in the top 0–10 cm soil layer with CS (82%) and NT (62%) ($p < 0.001$). However, this effect was not evident with CN, as a relatively uniform distribution was observed between the upper (47%) and lower (53%) soil depth.

3.4. *Weed indices of the persistent weed seed bank*

The total weed species determined in the persistent weed seed bank was 8, 4 and 14 for the CN, CS and NT, respectively. *C. polyspermum* and *L. amplexicaule* were the most common species determined in the study, accounting for 93%, 52% and 64% in CN, CS and NT, respectively. *Poa annua* L. was found only in the NT plot. Due to low number of weed species observed within the individual soil layer, weed indices data were calculated for the entire soil depth. Weed species richness, diversity and evenness of persistent weed seed bank increased with decreasing intensity of the tillage operations (Fig. 6). Using NT resulted in significantly greater weed species diversity and evenness, while no differences were found between the CN and CS ($p \leq 0.05$). Weed species richness was only marginally affected by the tillage system ($p = 0.09$), however NT had greater species richness (92%) in comparison to the most intensive CN tillage system (Fig. 6).

Table 3
Statistical results from the linear mixed model applied on the weed species richness (D), weed species diversity (H′) and weed species evenness (E) data in germinable weed seed bank at the significance levels *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

		Weed richness		Weed diversity		Weed evenness	
Factor	DF	F	p	F	p	F	p
Tillage system (TS)	2	1.90	0.16	0.65	0.52	11.24	<0.001
Cropping period (CP)	1	8.39	<0.01	25.58	<0.001	14.25	<0.001
Year (Y)	1	10.29	<0.01	11.76	<0.01	0.29	0.60
TS x CP	2	1.48	0.24	5.49	<0.01	1.98	0.15
CP x Y	1	0.73	0.40	0.35	0.56	20.59	<0.001
TS x Y	2	5.86	<0.01	7.05	<0.01	0.81	0.45
TS x CP x Y	2	0.45	0.64	4.03	<0.05	3.04	0.06

4. Discussion

4.1. *Seasonal variation and abundance of germinable and persistent weed seed bank*

Our investigation examining a weed seed bank response to a change in the intensity of the tillage practice showed both inter-seasonal but also intra-seasonal variability of germinable seedbank abundance over the course of the 2-year study period. When comparing seed bank sizes between studies, germinable seed bank size determined in our study (up to 35,000 seeds m^{-2}) is relatively low, while instances of highly abundant seed banks, reaching up to 222,000 seeds m^{-2}, have also been documented (Romaneckas et al., 2021). To what extent weed seed banks were affected by weather conditions remains unclear as no extreme drought or flooding events were observed during the course of experiment that might affect weed seed production (Singh et al., 2022), increase dormancy level (Battla and Benech-Arnold, 2007) or cause seed death (Dahlquist et al., 2007). Still, the lowest germinable weed seed bank in the spring was determined in 2021 with relatively dry three-month period that could induce dormancy.

In our study, seasonal periodicity of crop growing cycle and the intensity of the tillage operations were important drivers in determining the weed seed bank sizes. Earlier studies have also demonstrated that weed composition is subject to considerable temporal and spatial variability, which also depend on weed species and cropping systems (Barberi et al., 1997). It has been repeatedly shown that arable weed seed banks in the intensive cropping systems are in decline due to extensive herbicide inputs. Nevertheless, our results indicate that also continuous use of moderate herbicide inputs (treatment frequency index (TFI) up to 1) may allow long-term impoverishment of weed seed banks (Montull et al., 2014; Fonderflick et al., 2020; Köllmann and Waldhardt, 2022). The notable increase in weed seed bank density during the spring cropping period could be linked to the residual effect of weed management strategies employed in previous winter cereal crops. Specifically, the timeframe between herbicide application in spring crops and subsequent autumn sampling endured for only four months (from June to September). Conversely, this duration extended to nearly a year (from April to the subsequent March season) during the spring cropping phase. Felix and Owen (2001) also speculated that substantial increase within a single growing season is likely because of the effects of weed management, while moderate seasonal differences in the weed seedbank could also derive from weed seed predation and fungal attack.

We found that the size of the germinable seed bank determined in the germination experiment was up to four times lower compared to the persistent weed seed bank recovered in the flotation experiment. This seed bank disparity indicates a significant role of weed seed dormancy mechanisms regulated by complex interaction between hydric, thermal and gaseous environment in the soil (Benech-Arnold et al., 2000; Ghersa et al., 1992; Travlos et al., 2020). Moreover, weed seeds tend to accumulate more readily in clay soils often entering dormancy that create hypoxic burial conditions and extended seed longevity (Benvenuti and Mazzoncini, 2019). These specific conditions required to induce weed seed dormancy were possibly present in the field conditions but likely missing in the greenhouse germination experiment (Ghersa et al., 1992; Travlos et al., 2020). Considering the share of fully dormant seeds, the re-activation extent of breaking the dormancy in the persistent weed seed bank in the following seasons remains an important future objective of this long-term experiment.

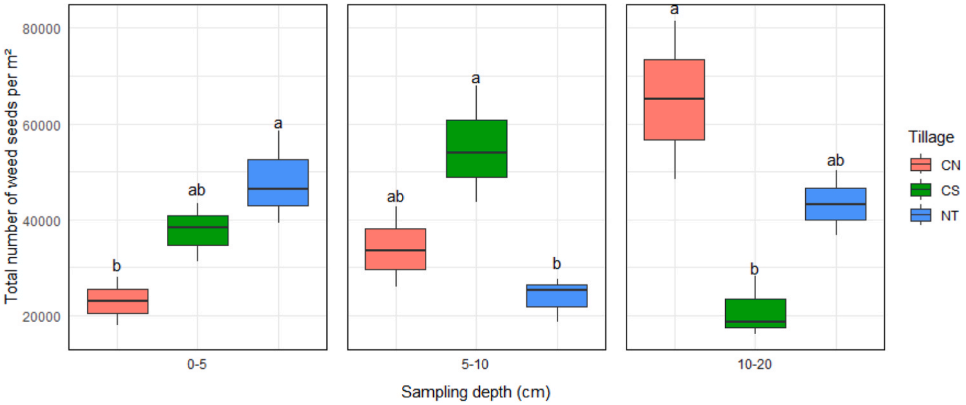

Fig. 5. Vertical distribution of persistent weed seed bank under conventional tillage (CN), conservation tillage (CS), and no tillage (NT) in 2022. Boxplots with different lowercase letters indicate significant intra-seasonal differences ($p < 0.05$) in weed densities between the different tillage treatments.

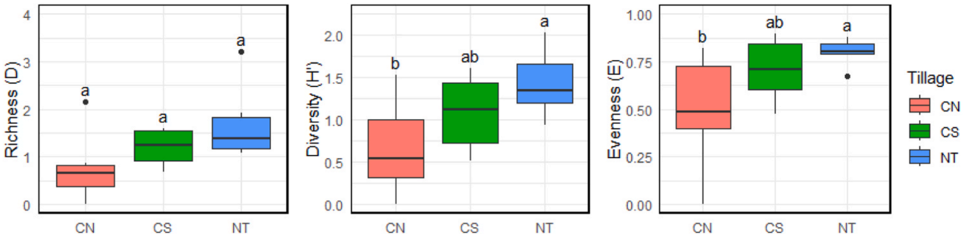

Fig. 6. Weed seed species richness (D), diversity (H') and evenness (E) of persistent weed seed bank in 2022 spring cropping season. Tillage treatments included conventional tillage (CN), conservation tillage (CS), and no tillage (NT). Boxplots with different lowercase letters indicate significant differences ($p < 0.05$) in weed density between the tillage treatments.

4.2. Weed seed bank response to tillage intensity

Our study demonstrated that regardless of the cropping period, using NT had an immediate response and consistently reduced the total amount of germinated seeds in a range from 56% to 72%, compared to the CN tillage practice. A weed seed augmentation study from Smith et al. (2009) found only minor differences in weed densities between the beginning and end of a three-year transition period, from the full-tillage system to a reduced tillage system. Similar results were also obtained by Maxwell et al. (2007) who observed that an increase in wild oat (*Avena fatua* L.) seeds led to an increase in wild oat soil seed bank densities in the following two growing seasons, although no residual effects remained in subsequent seasons.

The decline in total number of germinated weed seeds observed in NT is most likely due to increased predation facilitated by the absence of soil disturbance and the accumulation of weed seeds near the soil surface (Trichard et al., 2013). Annual fresh seed predation rates can reach as high as 90% which not only affect weed seed germination in the current growing season but also reducing the potential for weed infestation in the subsequent growing season (Davis et al., 2013; Westerman et al., 2003). Landscape complexity could also play an important role in weed seed removal since our experiment was conducted using small crop fields with permanent grassland in the surrounding plots, increased seed predation observed in the upper soil layer could also derive from a greater richness and abundance of granivorous carabids (Menalled et al., 2007). CS also resulted in generally lower weed seed numbers, but due to greater variability the significant effect of CS was evident only in one season. Some previous studies showed that experimentally manipulated tillage practices revealed contrasting results on the weed seed bank size. Our study is in line with investigations showing that tillage and soil disturbance increase light exposure and nitrogen mineralization and thus stimulate weed germination (Hossain and Begum, 2015; Travlos et al., 2020). Conversely, Ruisi et al. (2015) found that the size of the weed seed bank was not influenced by the tillage system, despite significant alterations in weed composition due to tillage practices lead to vertical mixing.

Germinable weed seed bank distribution over the studied soil depths was not affected by tillage, but more weed seeds with NT were found in the topsoil layer, while using CS and CN more persistent seeds were observed in the intermediate or the bottom soil layer. As only limited amount of fresh weed seeds can enrich the weed seed bank in the intermediate soil layer of NT, higher microbial activity and degradation rates are suggested to induce more unfavourable conditions which decrease seed viability (Nikolić et al., 2020).It has been regularly shown that the proportion of weed seeds near the surface is a function of level is a function of level of soil disturbance (Feledyn-Szewczyk et al., 2020), but studies where less intensive tillage have not altered vertical weed seed distribution can be found too (Santín-Montanyá et al., 2013). In tillage systems characterized by high soil disturbance, a majority of weed seeds become buried, while those with low disturbance levels leave the weed seeds on the soil surface (Choudhary, 2023). Contrasting results of vertical weed seed distribution in our study could be linked to the irregular vertical and lateral movement of weed seeds, primarily influenced by soil structure and compaction, which in turn affects the arrangement of tillage machinery (Colbach et al., 2000). In addition, tillage operations themselves can lead to changes in dormancy levels within soil profiles (Ghersa et al., 1992). A lasting impact on the evenness of weed seed distribution could also derive from periodic shallow tillage of stubbles performed in the past to establish cover crops.

4.3. Weed diversity in the germinable and persistent weed seed bank

The number of weed species identified in our study was rather limited, but even in weed communities with relatively high species diversity, few dominant species typically represented most of the weed population (Otto et al., 2012). Weed species composition and seed density generally differ for different tillage systems. Shallow tillage, which retains seeds at the soil surface favours weeds with larger seeds while the NT system typically yields a greater population of small-seeded annual weeds (Colbach et al., 2014; Hernández Plaza et al., 2015; Yenish et al., 1992). The abundance of perennial and grass species commonly increases with the reduction in the intensity of soil tillage (Buhler et al., 1994; Derrouch et al., 2021). However, in both the CS and NT treatments, no seeds of perennial weed species were found in the seed banks. Nevertheless, moderate population of field bindweed (*Convolvulus arvensis* L.) persisted across all experimental treatments throughout the study period. Given the challenge of managing this perennial weed species with shallow or no-till methods, our observations suggest potential limitations of seed bank analysis in detecting species employing successful vegetative propagation strategies (Willeke et al., 2015).

In our study, weed composition of the germinable seed bank varied between the years but demonstrated significant change in response to the implemented tillage practice. Although this effect was largely limited to spring cropping period, both CS and NT were found to facilitate more even and more diverse weed community as early as in the third year of conversion to less intensive tillage system. A rapid shift of weed composition in response to tillage management has been demonstrated also in previous research emphasizing the role of herbicides in the weed community assembly (Ryan et al., 2010). Santín-Montanyá et al. (2013) also reported that weed diversity of the transient seed bank did not appear to be affected by tillage and/or depth, while in the current study CS and NT were found to increase weed species diversity in the persistent weed seed bank. Differential response of weed seed abundance and diversity in the persistent weed seed bank was likely influenced by the effects of tillage system interacting with the soil matrix and weed seed bank factors. Varied seasonal outcomes regarding weed diversity in response to tillage intensity have been documented in other studies as well, highlighting the importance of an extended observation period (Feledyn-Szewczyk et al., 2020). The diversity levels observed in our study is similar to other reports observing significant impact of increasing farming intensity which not only decreased weed seed bank but also reduced weed species richness and diversity (Fonderflick et al., 2020; Fracchiolla et al., 2018).

4.4. Prospects and implications for weed seed bank management

In the present era of agricultural intensification and simplification, novel soil and crop management measures continuously induce changes in weed flora. Understanding how less intensive conservation tillage (CS) and no-tillage (NT) soil management practices shape seed bank composition may thus allow predictions of future challenges in support of transition to weed management less reliant on herbicides.

A recent integrated weed management (IWM) framework proposed a proactive approach employing multiple tactics of weed control, including seed bank exhaustion as a key element for control of annual and perennial weed species that spread through seeds (Blumenthal and Jordan, 2001; Riemens et al., 2022). Although weeds can be targeted at various stages of their life cycle, the most effective strategy to mitigate future weed management challenges is to minimize current contributions to the weed seed bank. Managing weed communities requires long-term strategies, in contrast to the more immediate approaches often sufficient for most insects, pests and diseases. It has been shown that comprehensive weed eradication program must be maintained more than 5 years to keep the soil seed bank at a minimum level. However, due to seed longevity or failures in the weed control program, surviving weed populations can rapidly replenish the soil's seed bank (Burnside et al., 1986). Combining multiple tools and techniques is a valid IWM approach that may allow low levels of germinable weed seed bank but weed control in NT without herbicides remains a significant challenge (Anderson et al., 2015; Moonen and Barberi, 2004). It has been suggested that cover crops present the highest potential to diminish the seedbank by decreasing seed viability, preserving weed seed dormancy, and minimizing germination triggers (Sias et al., 2021). However, recent studies showed that the role of cover crops in weed control within no-till and herbicide-free systems may be less significant, particularly with low levels of cover crop biomass production (Rouge et al., 2023).

To our knowledge, this is the first study to investigate the early response of both germinable and persistent weed seed banks under specific soil and climatic conditions. Weed seed bank results that emerged in our study could prove valuable for transitioning to alternative tillage systems. Moreover, they facilitate long-term planning and enable better integration of non-chemical weed management strategies at the cropping system scale (Adeux et al., 2019a). Specifically, the relatively low level of germinable weed seeds of less competitive weed species observed in the autumn cropping period indicates the possibility of substituting herbicides with mechanical weed control methods in winter cereal cultivation. Additionally, introducing early–sowing crops like field pea into the rotation sequence could diversify weed selection pressure and disrupt various phases of the weed species' life cycle in the spring crops (Derksen et al., 2002).

A comprehensive French study demonstrated that a well-balanced, diversified grain-based cropping system, which primarily relies on preventive measures and mechanical weeding, can reach high productivity while enabling up to 65% herbicide reduction and maintain low weed densities over time (Adeux et al., 2019a). The size of the persistent weed seed bank underscores the critical role of dormancy in weed population dynamics, highlighting the need for comprehensive understanding of the mechanisms controlling dormancy to improve weed management practices (Benech-Arnold et al., 2000; Foley, 2002). With limiting number of diverse spatial-temporal weed management tools available in conservation agriculture, strategic tillage was proposed to interrupt the dormancy of weed seed species, diversify selection pressures, and address specific challenges inherent in NT practices (Crawford et al., 2015; Santín-Montanyá et al., 2013). However, long-term benefits associated with conservation agriculture may be at risk and future studies are needed to assess how occasional tillage interventions impact the overall performance of conservation agriculture systems over time (Cordeau et al., 2020).

Our findings demonstrate that transitioning to CS and NT tillage practices does not adversely impact on the level and composition of the germinable weed seedbank. Moreover, NT typically resulted not only in the lowest levels of weed seed abundance across all studied soil depths but also in greater evenness and diversity of weed community at relatively early transition phase. In addition to tillage, diversified crop rotation was also found to foster weed communities characterized by species richness, while the role of cover crop mixtures on promoting weed diversity might be less pronounced (MacLaren et al., 2019; Sosnoskie et al., 2006). Our research did not specifically address individual weed control measures, yet introduction of occasional ploughing, repeated false seedbed preparations or mechanical weeding could enable a high level of herbicide reduction over time (Adeux et al., 2019b). Moreover, a recent study proposed a more ecological approach through regulating weed populations and conserving diversity to minimize the impacts, rather than eliminating weeds (MacLaren et al., 2020). A significant shift in the perception of weed control is necessary, as IWM systems with reduced herbicide inputs, except those incorporating forage crops, will need to adapt to rising weed populations over time (Summers et al., 2021).

5. Conclusions

In summary, the different tillage systems implemented in the given environmental conditions had a clear impact on both size and to a lesser extent weed diversity in the germinable weed seedbank. The present study demonstrated that the number of germinated weed seeds at all soil depths was considerably reduced already eighteen months after implementation of NT. Furthermore, the germinable weed seed bank with NT exhibited a consistent decrease over the subsequent two seasons of seed bank assessments. Nevertheless, the overall response of the persistent weed seed bank to the implemented soil management practices and the influence of seed dormancy needs further investigation.

In a time of decreasing herbicide availability and limited non-chemical weed control measures we advocate weed seed bank assessment studies as a part of IWM strategies. Determination of size and composition of weed seed bank can support the existing IWM methods enabling coherent integration of non-chemical weed management strategies and effectively mitigate weed proliferation and seed bank buildup during the transition process. This can promote herbicide-independent arable production and facilitate development of new agroecological based strategies favouring diverse weed species communities within CS and NT systems, contributing to overall ecosystem resilience and stability.

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CRediT authorship contribution statement

Robert Leskovšek: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Sergeja Adamič Zamljen:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Anže Rovanšek:** Writing – review & editing, Investigation, 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.

Data Availability

Data will be made available on request.

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