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Vertical distribution and composition of weed seeds within the plough layer after eleven years of contrasting crop rotation and tillage schemes

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ABSTRACT

Tillage methods and crop rotation are probably the two most important cropping factors affecting weed communities, particularly when herbicide use is restricted. This study examined weed dynamics following eleven years of different tillage and crop rotation treatments. The aboveground grass weed flora was recorded each year and the content and vertical location of individual weed seeds within the plough layer (0–20 cm) were determined after 11 years of continuous mouldboard ploughing (P), pre-sowing tine cultivation to 8–10 cm soil depth ($H_{8\text{-}10}$) and direct drilling (D). The content of weed seeds, especially grass weeds, was determined for three distinct soil layers (0–5, 5–10 and 10–20 cm), reflecting the cultivation depths of the tillage treatments. The annual grass weeds, *Apera spica-venti* and *Vulpia myuros*, were promoted by non-inversion tillage and in the case of *V. myuros* also by frequent cropping of winter cereals. The two non-inversion tillage treatments caused a strong stratification of weed seeds within the plough layer, with the majority of the seeds being accumulated in the upper soil layers, at 0–5 and 5–10 cm, and markedly less so in the 10–20 cm layer. Ploughing resulted in a more even distribution between the three layers. It is suggested that in cases where severe grass weed problems have built up in a non-inversion tillage system and where changes in crop rotation are ineffective or undesirable, inversion of the upper soil layer with the lower one could be considered a management option.

1. Introduction

The soil seed bank represents the principal source of future weed infestations in arable cropping systems and, as such, is a potential target for management (Norris, 2007). Weed seed density and diversity in the seed bank and the aboveground weed community reflect cropping history (Cardina et al., 2002; Davis et al., 2006), crop and weed management (Barberi and Cascio Lo, 2001; Menalled et al., 2001), tillage (Blackshaw et al., 2001) and local environmental conditions. Seed bank management is becoming increasingly important in future cropping systems because restrictions on herbicide use (Hillocks, 2012) and problems with herbicide resistance (LeBaron, 1989; Harker and O'Donovan, 2013) make chemical solutions less reliable. Preventive, cultural and even physical methods will play a greater role in future weed control programmes. However, non-chemical methods are usually weaker than herbicides (Lutman et al., 2013), which reinforces the need for a better understanding of the weed seed bank dynamics in relation to crop rotation and tillage to improve the outcome of control measures. In particular, non-inversion tillage systems, where weed seeds accumulate in the upper soil layers, are known to depend on herbicide usage (Melander et al., 2013).

Crop rotation, regardless of herbicides, is an important measure for diversifying weed communities (Radosevich et al., 1997). There are several mechanisms responsible for this effect, including allelophathy, changes in fauna and disturbance patterns, which could diversify selection pressures by influencing seed bank dynamics (Sosnoskie et al., 2009). Rotation also affects species communities by determining the tillage frequency and effects attributed to cropping practices, such as herbicide programmes, crop seed rate and sowing time (Smith and Gross, 2007). Furthermore, where crops are rotated, more diversity is expected than in monocultures (Dorado et al., 1999) where monotonous cropping exerts the same selection pressure on the weed community, favouring species with phenotypes and phenology similar to the crop, such as grass weeds in winter cereals (Koocheki et al., 2009).

Tillage systems also affect the composition and density of the weed communities, mainly by modifying the vertical distribution

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of seeds in the soil (Buhler et al., 1994), affecting weed emergence, management and seed production. Conservation tillage systems involving minimum soil disturbance are expanding in several parts of the world. The availability of efficient non-selective herbicides, such as glyphosate, is a major driver in this development because these chemicals render tillage unnecessary for weed management purposes (Givens et al., 2009).

There are many advantages associated with reducing tillage intensity. In particular it saves time, prevents soil erosion (Melander et al., 2013), saves on expenditure for machinery and fuel (Davies and Finney, 2002; Morris et al., 2010), enhances soil quality and reduces nutrient and pesticide losses through leaching (Holland, 2004; Morris et al., 2010). However, reduced tillage systems often have a greater reliance on herbicides, which can increase the prevalence of some weed species, such as herbaceous perennials, woody shrub (Zanin et al., 1997), annual grasses and wind-disseminated species (Streit et al., 2003).

Reductions in tillage frequency and depth generally place the majority of weed seeds close to the soil surface, which can reduce seed longevity compared to mouldboard ploughing that locates the seeds more deeply (Cardina et al., 2002; Sosnoskie et al., 2009). Information about the precise location of weed seeds following current non-inversion tillage techniques, such as pre-sowing tine tillage and direct drilling predominantly working in the 0–10 cm and 0–5 cm soil layers respectively, is still limited. There could be location specific responses from seeds that can influence seed germination and predation. Studies with seeds of different grass weed species that simulate distinct locations of grass seeds in the upper soil layers have clearly demonstrated a dynamic response to burial depth (Jensen, 2010). The greatest seed losses involved seeds on the soil surface or in close proximity to the surface, but the magnitudes were very species dependent. In contrast, the content of viable weed seeds below the cultivation depth of non-inversion tillage can decline substantially over time due to the small input of new seeds. This suggests that an inversion of soil layers could be a management option against a severe weed problem.

The combined effects of crop rotation and tillage on weed flora deserve special attention despite contrasting results (Omani et al., 1999). Some authors report that tillage influences seed bank size and composition more than crop rotation (Barberi and Cascio Lo, 2001), while others find crop sequence to be more important due to alternations in herbicide use (e.g. Ball, 1992; Cardina et al., 2002). Furthermore, the effects of herbicide practices on the weed seed bank mainly depend on the local weed species composition (Fleix and Owen, 1999).

In this study, the content and vertical location of individual weed species were investigated within the plough layer (0–20 cm), following eleven years' cropping with three crop rotations subjected to three tillage regimes. The aim of this study was to examine the content of weed seeds, especially grass weeds, in three distinct soil layers reflecting the cultivation depths of (i) mouldboard ploughing, (ii) pre-sowing tine tillage and (iii) tine-based direct drilling, and to compare the aboveground grass weed flora with the grass seeds retrieved from the different soil layers. It was hypothesised that frequent cropping of winter cereals in non-inversion tillage systems would result in a high abundance of winter annual grass weeds, with seeds distinctly located within the cultivation depth of the tillage methods applied.

2. Materials and methods

2.1. Crop rotation and tillage experiment

The experiment was established on a sandy loam at the Flakkebjerg Research Centre (55°19′ N, 11°23′ E), Denmark, in the autumn of 2002. The experimental field had been cropped and cultivated according to normal agricultural practices prior to the start of the experiment, with oat (*Avena sativa* L.) being the last preceding crop. The soil is based on ground morainic deposits from the last glaciations and is classified as a Glossic Phaeozem according to the WRB (FAO) system (Krogh and Greve, 1999). The clay (<2 μm), silt (2–20 μm), fine sand (20–200 μm), coarse sand (200–2000 μm) and organic carbon contents of the soil (0–25 cm) were 147, 137, 426, 270 and 12 g kg^{-1} respectively. The mean annual temperature (1961–90) at Flakkebjerg is 7.7 °C and annual precipitation is 558 mm (Olesen, 1993).

The experimental design was a split plot with four replications. The main plot factor was crop rotation, while subplots evaluated soil tillage. Each tillage plot consisted of two 2.5 m-wide and 40 m-long tillage strips, allowing special treatments and recordings to take place in six sub-subplots, each measuring 12.5 × 2.5 m gross area and 10.0 × 1.5 m net area. In this study, all measurements were taken in one and the same sub-subplot within each tillage treatment. The experiment had four crop rotations: R1, R2, R3 and R4. However, only three crop rotations (R1, R2 and R4) were included in the study with the specific crop sequences shown in Table 1. Crop rotation R3 was similar to R4 but the straw was removed, as opposed to the other three rotations where the straw was chopped and retained after harvest. Rotation R1 had a winter wheat monoculture, except for a one-year break in 2011, resulting in approximately 91% autumn-sown crops. R2 also had 91% autumn-sown crops in the sequence, but three different winter crops were included. R4 had only 55 % autumn-sown crops and was generally the most diversified crop sequence.

In the period 2002–2006, the tillage systems considered in this study were direct drilling (D), harrowing to 8–10 cm (H_{8-10}) and mouldboard ploughing (P) to 20 cm soil depth. The H_{8-10} treatment was only stubble cultivated using a rotary harrow (Bomford Dyna Drive), applying one pass just after harvest to 3–4 cm soil depth and then again just before crop sowing to 8–10 cm depth. The crops were sown with a single-disc drill (Gaspardo Scan-Seeder DP300) in D and H_{8-10} and with a traditional seed drill (Nordsten Lift-o-matic CLH300) in P after seedbed harrowing. In all treatments, crops were sown at the same row distance of 17.5 cm. Since there was poor crop establishment and growth in some years in treatment D (Hansen et al., 2010), the single-disc drill was replaced by a chisel coulter (Horsch Airseeder CO 3) from 2006. Moreover, the Dyna Drive harrow for H_{8-10} was also replaced by a Horsch Terrano 3 FX stubble tine cultivator from 2006 onwards. Ploughing took place just before the autumn-sown crops were sown, and in the case of the spring-sown ones in late autumn (with

Table 1
Crop sequences R1, R2 and R4 and straw management at Flakkebjerg.

Year	R1	R2	R4
2003	Winter wheat	Winter barley	Winter wheat
2004	Winter wheat	Winter oilseed rape	Spring barley
2005	Winter wheat	Winter wheat	Peas
2006	Winter wheat	Winter wheat	Winter wheat
2007	Winter wheat	Winter barley	Winter wheat
2008	Winter wheat	Spring oat[b]	Winter barley
2009	Winter wheat	Winter wheat	Spring oat
2010	Winter wheat	Winter wheat	Winter wheat
2011	Spring oat	Winter barley[a]	Spring barley
2012	Winter wheat	Winter oilseed rape	Spring oat
2013	Winter wheat	Winter wheat	Winter wheat
2014	Winter rye	Winter wheat	Winter wheat
Straw	*Retained*	*Retained*	*Retained*

[a] Spring barley in plots with direct drilling (D) at Flakkebjerg where winter barley was damaged by frost.
[b] Re-sown with oat in the spring after winter oilseed rape was severely damaged by slugs.

seedbed harrowing in spring). Glyphosate, (Roundup Bio, 360 g a.i. l^{-1}, Monsanto) at a dose of 540 g a.i. ha^{-1} was used in all the non-inverted tillage treatments to kill weeds and volunteer crop plants prior to drilling.

In contrast to the disc drill, the Horsch Airseeder does not establish the crop in distinct rows but spreads the seeds in bands. In order to obtain the same plant distribution in all plots irrespective of tillage treatment, all treatments sown in autumn 2006 and spring 2007, including the ploughed treatments, were sown using the chisel coulter. However, sowing with chisel coulters in the ploughed treatments did not prove satisfactory. To avoid the problem in the following years, it was decided that seeding in the ploughed treatments should be undertaken using a traditional seed drill (Nordsten Lift-o-matic CLH300) with a row distance of 12.5 cm.

All crops except peas were fertilised with 100 kg ha^{-1} NH_4-N in pig slurry and the remainder of the fertiliser recommendation was provided by a mineral fertiliser. For winter rape, 30 kg ha^{-1} N as a mineral fertiliser was applied in autumn. All other crops received the total amount of manure/fertiliser in spring. The target fertiliser applications (NH_4-N in slurry plus N in mineral fertiliser) were winter wheat 165, winter rape 171, winter barley 139, spring barley 117 and oat 92 kg N ha^{-1}. Analyses of slurry were performed prior to application and the N content was used to target an application rate of 100 kg ha^{-1} NH_4-N. At spreading, slurry samples were collected to determine the actual N content. The slurry was applied with trailing hoses to winter crops and injected after ploughing, but before sowing, to spring crops. The slurry was injected shortly before sowing in plots with spring crops and non-inversion tillage.

Apera spica-venti (L.) P. Beauv. was artificially introduced in the experimental area in 2002, as explained in Melander et al. (2008). The purpose was to create a rapid and dynamic response to crop rotation and tillage effects. Weeds were controlled as outlined in Melander et al. (2008) for the 2003–2006 period. The sub-subplots used for the seed bank estimations (see Section 2.3 below) and annual counts of grass weed panicles (see Section 2.2 below) originated from the sub-subplots with a 70% target control level against *A. spica-venti* and a 90% target control level against broadleaved weed species. However, the herbicide strategy was changed in the 2007–2013 period from spring application to autumn application in winter cereals, using a mixture of pendimethalin (Stomp®, 400 g a.i/L, Basf A/S) and prosulfocarb (Boxer®, 800 g a.i/L, Syngenta Crop Protection A/S). Autumn application was not undertaken in 2009 because of heavy rainfall events that made access to the field plots with machinery impossible. Instead spraying was postponed until the spring, using iodosulfuron + mefenpyr-diethyl (Hussar OD®, 100 + 300 g a.i/L, Bayer CropScience) against grass weeds plus other relevant herbicides against broadleaved weeds. The 70% target control level against *A. spica-venti* was abandoned from autumn 2010, and hereafter all doses were raised to target 90% control of grass weeds due to increasing grass weed problems in the non-inverted tillage treatments. Herbicide choices and doses against broadleaved weeds and doses of the graminicides were based on the recommendations of Crop Protection Online, a Danish decision support system for chemical weed management (Rydahl, 2004).

2.2. Annual counts of grass weed panicles

The production of grass weed panicles in July reflects the potential input of grass seeds to the seed bank. Panicles of *A. spica-venti* were counted in mid-July in all years in the period 2003–2013. The annual grass weed *Vulpia myuros* (L.) K.C. Gmel was first observed in 2007, after which time it rapidly invaded the experiment, showing a pronounced response to crop rotation and tillage treatments. Hence panicles of *V. myuros* were included in the summer counts of *A. spica-venti* from 2007. All counts were carried out in four randomly-placed 0.25 m^{-2} quadrats per sub-subplot. However, it was not possible to count panicles and stems

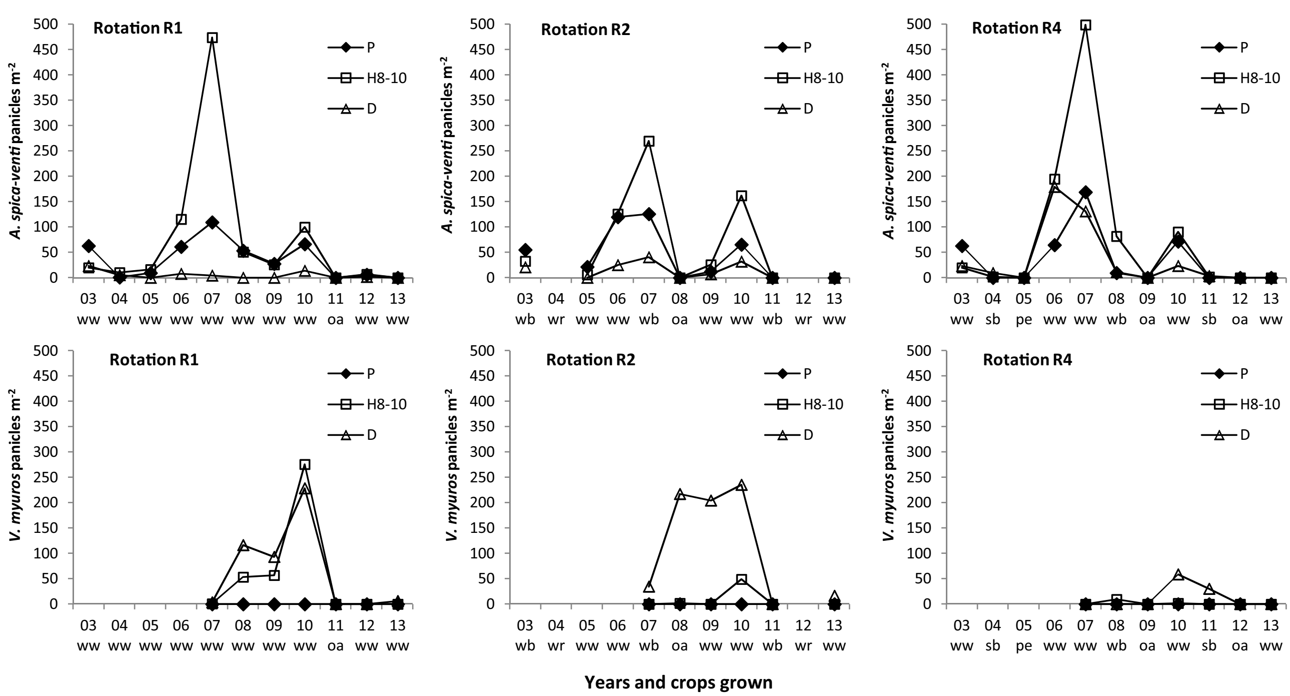

Fig. 1. Mean numbers of panicles of *A. spica-venti* and *V. myuros* counted in all combinations of crop rotation (R1, R2 and R4) and tillage treatments (P, H8-10 and D) in each year in the period 2003–2013. No counts were made in winter oilseed rape in R2 in 2004 and 2012. *V. myuros* was not present until 2007, after which time it rapidly spread in the experiment. PE: pea, SB: spring barley, WB: winter barley, WW: winter wheat, WR: winter oilseed rape, OA: oat.

in winter oilseed rape in 2004 and 2012 due to a dense and impassable crop stand, especially in P and $H_{8\text{-}10}$.

2.3. Soil sampling

Soil samples were collected by the end of March 2014 from three rotations (R1, R2 and R4), three tillage systems (D, $H_{8\text{-}10}$ and P) and three soil layers (0–5, 5–10 and 10–20 cm). Sampling was performed using an auger with a 5 cm diameter. Ten samples were taken for each soil layer and were then mixed, placed in plastic bags and stored at 4 °C in darkness until set for germination. In total, 108 soil samples were germinated (3 rotations × 3 tillage methods × 3 layers × 4 blocks = 108).

2.4. Germination in the glasshouse

The soil samples were germinated in a glasshouse in the Agroecology department of Aarhus University. Polystyrene boxes (248 × 182 × 64 mm length, width and height, respectively) were prepared prior to receiving the soil samples. Four cracks were made in the bottom of each box where fibre fabric wicks (20 × 210 mm wide and long, respectively) were positioned to allow water uptake from a watered Table on which all the boxes were placed. A layer of fibre textile was put on the wicks on the bottom of the boxes, and on top of that a 30 mm layer of mixed sterilised potting compost (800 g per box) and sand (0.1–3 mm particle size, 1550 g per box). The compost-sand mixture was saturated with water and a fibre textile placed on the surface on which the soil samples were then stored.

Sub-samples (1 L of soil) of the primary soil samples were used for the assay. A sample of the remaining soil was taken to determine the water content by weighing the samples before and after drying in the oven at 85 °C for 24 h. The sub-samples were weighed and the seed bank contents adjusted to express the weed seed content of 1 m^2 of each soil layer using previously obtained soil bulk densities.

Germination took place at a minimum of 10 °C with light conditions following the outdoor 24-h rhythm. The number of emerged seedlings was counted at species level and removed whenever species could be identified. All samples were dried for three weeks by shutting off the water supply when emergence of new seedlings appeared to have ceased. Thereafter, samples were turned over, watered again and set for germination to continue counting emerged plants. The drying and turning procedure was performed twice during spring of 2014, once in the following autumn of 2014 and finally once in the winter of 2015. At the last drying, 200 mL of a 2% potassium nitrate (KNO_3) solution was added to the soil samples to stimulate germination of remaining

Table 2
Least squares means (LSM, log-transformed and back-transformed values) of the number of panicles of *A. spica-venti* and *V. myuros* and the sum of the two species produced over eleven years (2003–2013) shown for each level of tillage within the crop rotation. Maximum standard errors of differences (SED) between LSMs are given in italics.

Grass weed	Crop rotation	Tillage	LSM (panicles m^{-2}) Log-trf.	Back-trf.	Significance between levels (Tukey test)
Total (2003–2013)	R1	1. Ploughing	2.331	10.29	1 *vs.* 2, (*P* = 0.9346)
		2. $H_{8\text{-}10}$	2.788	16.25	1 *vs.* 3, (*P* = 0.9881)
		3. Direct	1.986	7.29	2 *vs.* 3, (*P* = 0.3793)
		SED	*0.3582*		
	R2	1. Ploughing	1.724	5.61	1 *vs.* 2, (*P* = 0.9989)
		2. $H_{8\text{-}10}$	1.977	7.22	1 *vs.* 3, (*P* = 0.6699)
		3. Direct	2.413	11.17	2 *vs.* 3, (*P* = 0.9642)
		SED	*0.3792*		
	R4	1. Ploughing	1.149	3.16	1 *vs.* 2, (*P* = 0.7291)
		2. $H_{8\text{-}10}$	1.806	6.09	1 *vs.* 3, (*P* = 0.8960)
		3. Direct	1.680	5.37	2 *vs.* 3, (*P* = 1.0000)
		SED	*0.3808*		
A. spica-venti (2003–2013)	R1	1. Ploughing	2.301	9.98	1 *vs.* 2, (*P* = 0.9999)
		2. $H_{8\text{-}10}$	2.444	11.52	1 *vs.* 3, (*P* < 0.0001)
		3. Direct	0.567	1.76	2 *vs.* 3, (*P* < 0.0001)
		SED	*0.2903*		
	R2	1. Ploughing	1.730	5.64	1 *vs.* 2, (*P* = 0.9998)
		2. $H_{8\text{-}10}$	1.894	6.65	1 *vs.* 3, (*P* = 0.1251)
		3. Direct	0.858	2.36	2 *vs.* 3, (*P* = 0.0322)
		SED	*0.3083*		
	R4	1. Ploughing	1.188	3.28	1 *vs.* 2, (*P* = 0.4861)
		2. $H_{8\text{-}10}$	1.833	6.25	1 *vs.* 3, (*P* = 0.9989)
		3. Direct	1.397	4.04	2 *vs.* 3, (*P* = 0.8887)
		SED	*0.3082*		
V. myuros (2007–2013)	R1	1. Ploughing		0.00	
		2. $H_{8\text{-}10}$	1.869	6.48	2 *vs.* 3, (*P* = 0.9304)
		3. Direct	2.279	9.77	
		SED	*0.4325*		
	R2	1. Ploughing		0.00	
		2. $H_{8\text{-}10}$	0.561	1.75	2 *vs.* 3, (*P* = 0.0002)
		3. Direct	2.887	17.94	
		SED	*0.4446*		
	R4	1. Ploughing		0.00	
		2. $H_{8\text{-}10}$	0.428	1.53	2 *vs.* 3, (*P* = 0.8451)
		3. Direct	0.937	2.55	
		SED	*0.4325*		

viable seeds. The experiment was completed at the end of March 2015, providing a germination period of one year.

2.5. Data analysis

Crop rotation and tillage effects on panicle density counted annually plus germinable weed seeds in the soil samples were analysed using a general linear mixed model. For grass weed panicles, the dependent variable was the number of panicles m^{-2} produced annually in all eleven years (2003–2013) for *A. spica-venti* and in all seven years (2007–2013) for *V. myuros*. However, the data only accounted for nine and six years, respectively, for rotation R2, as the panicles were not counted in oilseed rape in 2004 and 2012. It was assumed that the contribution from those two years was negligible considering the competitiveness of the crop stand and the generally low occurrence of grass weeds in the other crop rotations in 2004 and 2012 (Fig. 1). For panicle density, the full model included fixed effects of crop rotation and tillage; random effects included year, block and their interactions with rotation. The repeated nature of the panicle data with recordings made over time in the same plots was accounted for by including year as a repeated effect with plot as the subject. An autoregressive correlation structure and variance was assumed between years. For the content of germinable seeds in soil samples, the fixed effects also included soil layer in addition to rotation and tillage; random effects were the block and its interactions with rotation and tillage.

Parameters were estimated using residual likelihood estimations. Calculations were made using the mixed procedure of SAS (SAS Institute Inc., 2010 release 9.2), and means were calculated as least square means (LSM). Models were reduced by excluding non-significant effects based on likelihood ratio tests and Akaike's information criterion (Akaike, 1974). The denominator degrees of freedom (DDF) in *F*-tests and *t*-tests for mean separations were calculated according to Kenward and Roger (1997). Probability values for multiple mean separations were adjusted according to the Tukey-Kramer method. Data were transformed whenever necessary to obtain homogeneity of variance. The specific transformations are given in Tables 2 and 3.

3. Results and discussion

3.1. Grass weed panicles

The occurrence of *A. spica-venti* and *V. myuros* in different combinations of crop rotation and tillage treatments over an eleven-year period (2003–2013) is given in Fig. 1. *A. spica-venti* incidence was most severe in $H_{8\text{-}10}$ tillage treatment, with notable outbreaks in 2006, 2007 and 2010. The 70% target control level failed in 2006 (Melander et al., 2008) and 2007. The grass weed infestation in 2010 was a result of the herbicide application being postponed from autumn 2009 to spring 2010 in which period the grass plants (both *A. spica-venti* and *V. myuros*) had grown too large for effective control.

The mixed analyses on *A. spica-venti* panicle density showed that the high infestation level in $H_{8\text{-}10}$ did not differ between the three crop rotations (R1, R2 and R4). In general, crop rotation had only a minor influence on *A. spica-venti* panicle production (main effect $P = 0.4188$), while tillage effects were significant within crop rotations R1 and R2, as shown in Table 2.

While *A. spica-venti* generally had the lowest presence under D in R1 and R2, *V. myuros* was most prevalent under D in R2. In R1and R4, the occurrence of *V. myuros* was more similar between $H_{8\text{-}10}$ and D (Fig. 1). Crop rotation interacted with tillage ($P = 0.0064$), showing that R1 and R2 with frequent cropping of winter crops had the highest incidence of *V. myuros* (Table 2). The more mixed crop rotation, with the inclusion of spring-sown crops (R4), clearly hampered *V. myuros* panicle production. Similar results have also been observed by Ball et al. (2008), where the inclusion of spring cereals in crop rotation disrupts the life cycle of *V. myuros*.

Table 3
Interactions between tillage systems (P, $H_{8\text{-}10}$ and D) and soil layers (0–5, 5–10 and 10–20 cm) shown as treatment means (log-transformed LSMs with back-transformed values in parentheses corresponding to seeds m^{-2}) for the total content of monocotyledonous and dicotyledonous germinable seeds respectively, and the most frequent individual weed species in the soil seed bank.

Species	Ploughing			$H_{8\text{-}10}$			Direct drilling			SED[a]
				Soil layer(cm)						
	0–5	5–10	10–20	0–5	5–10	10–20	0–5	5–10	10–20	
C. album	5.33a (205)	4.97a (143)	4.95a (140)	5.02a (150)	4.50a (90)	4.36a (77)	5.77a (320)	4.95a (140)	4.01a (54)	*0.848*
C. bursa-pastoris	5.13a (168)	4.77a (117)	5.54a (254)	5.51a (246)	4.12a (61)	4.66a (105)	5.39a (218)	4.60a (98)	4.94a (140)	*0.727*
T. maritimun	4.85a (127)	4.83a (124)	5.77a (320)	5.78a (323)	4.66a (105)	4.07a (58)	4.41a (81)	4.07a (58)	3.91a (49)	*0.668*
P. rhoeas	3.99a (53)	3.10a (21)	4.42a (82)	5.59a (267)	4.36a (77)	3.91a (49)	4.62a (100)	3.24a (26)	2.91a (17)	*0.782*
V. arvensis	4.81a (122)	4.75a (115)	5.46a (234)	5.58a (264)	4.75a (115)	3.61a (34)	5.53a (251)	3.93a (50)	3.79a (44)	*0.846*
Veronica spp.	3.71ab (40)	3.80ab (80)	4.74ab (113)	5.05a (155)	3.55ab (54)	2.82b (16)	5.66a (286)	3.76ab (42)	2.89b (17)	*0.782*
P. aviculare	3.88ab (47)	3.70ab (40)	5.51a (246)	3.59ab (36)	1.95bc (7)	0.72c (2)	3.73ab (41)	2.85ab (16)	0.69c (2)	*0.735*
Total dicots	7.04b (1140)	6.90b (992)	7.59a (1977)	7.47a (1754)	6.80b (897)	6.37c (583)	7.54a (1881)	6.72b (828)	6.32c (555)	*0.203*
A. spica-venti	4.32a (74)	4.29a (72)	4.96a (142)	4.88a (131)	3.28ab (26)	0.77b (2)	4.35a (77)	2.94ab (18)	1.26b (3)	*0.677*
P. annua	6.22a (502)	6.29a (538)	6.85a (943)	6.77a (871)	5.30a (200)	3.77a (43)	6.23a (507)	4.77a (117)	3.18a (24)	*0.649*
Total monocots	6.46a (638)	6.51a (665)	7.10a (1211)	7.02a (1118)	5.67a (289)	3.94b (50)	6.40a (601)	4.97ab (143)	3.49b (32)	*0.538*

Different letters alongside means in rows indicate significant differences at $P < 0.05$.
[a] SED is the maximum standard error of differences between means.

Mouldboard ploughing (P) caused the strongest prevention of *V. myuros* growth of all treatments, with no records of the grass weed in any rotations. Lawrence and Burke (2014) also observe that *V. myuros* seeds are unable to germinate under intensive tillage since germination and seedling establishment are more easily achieved with little soil disturbance. The seeds are also sensitive to deep

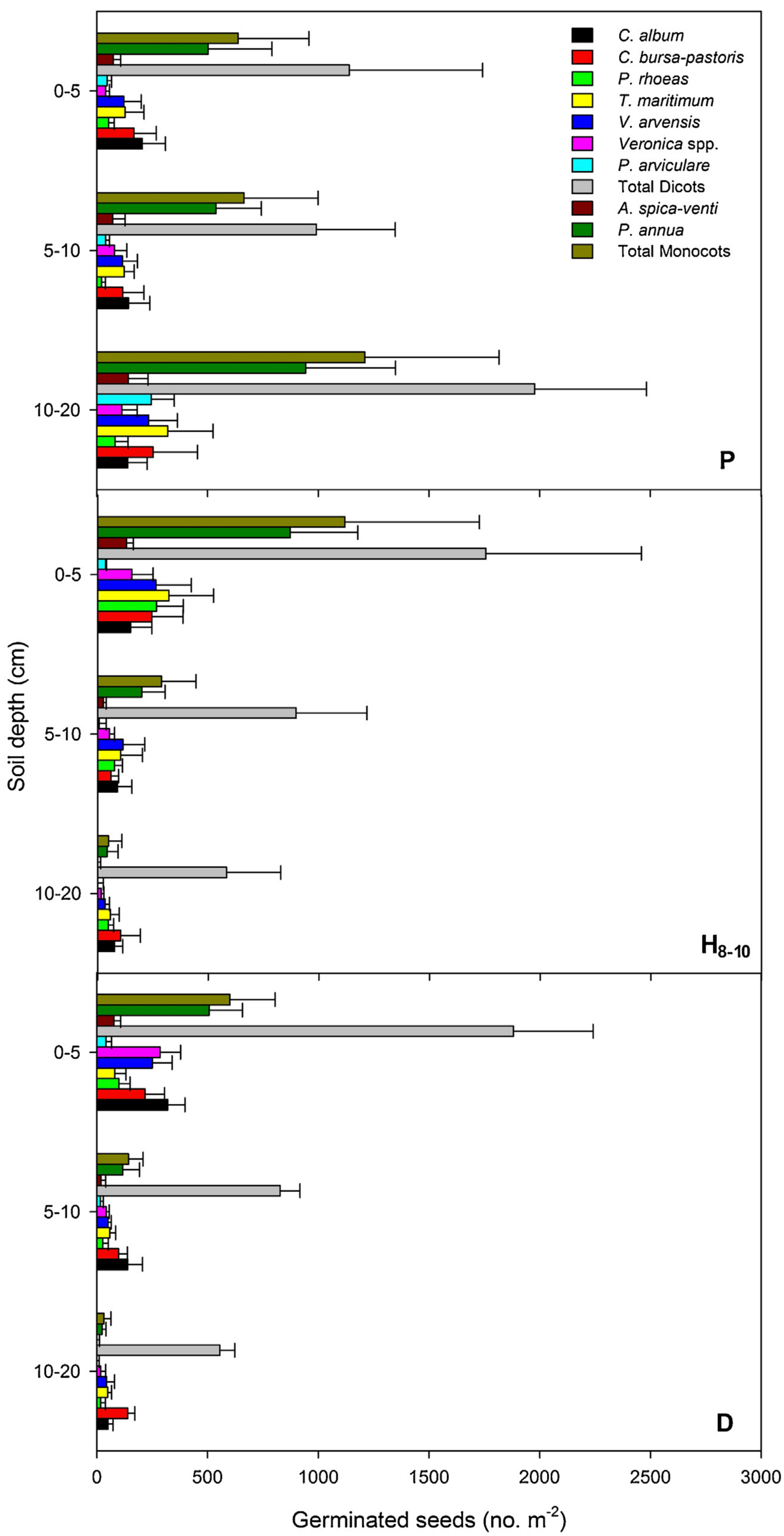

Fig. 2. Mean values of germinable seeds content (m^{-2}) of the most frequent weed species found in the three soil layers, 0–5, 5–10 and 10–20 cm, removed from the three tillage treatments, P, $H_{8\text{-}10}$ and D. Data were pooled across the three crop rotations R1, R2 and R4. Bars on each column are standard errors of the means.

burial, which usually prevents them from emerging (Dillon and Forcella, 1984). Increasing problems with *V. myuros* have also been reported elsewhere, with its spread appearing to follow the adoption of minimum tillage systems (Dowling, 1996; Ball et al., 2008). *V. myuros* infestations tend to increase rapidly once seeds are placed on or close to the soil surface, where they can easily germinate due to their requirement for light (Ball et al., 2008). Another important aspect is the low efficacy of many post-emergence herbicides against *V. myuros*, which of course contributes strongly to its proliferation (Dillon and Forcella, 1984).

Furthermore, other authors have been reporting that the annual or occasional use of P, once every four years, in a no-tillage cropping system shows benefits for weed management, offering an efficient strategy to reduce resistance and the overall weed populations (Renton and Flower, 2015). Therefore, these results are in accordance with the findings of the present study, where P showed to be able to reduce severe grass weeds problems, such as *V. myuros*, associated with D.

The total production of grass weed panicles was greatest with $H_{8\text{-}10}$ in R1 (Table 2), although this tillage regime did not differ significantly from $H_{8\text{-}10}$ in the other two rotations. Main effect of crop rotation was significant ($P = 0.0211$) with R4 having the lowest density of grass weeds and R1 the greatest. In general, tillage effects did not differ within rotations presumably because the higher occurrence of *V. myuros* in D counterbalanced the low presence of *A. spica-venti* in the same plots.

3.2. Vertical distribution and composition of weed seeds

Total emergence of the most predominant weed species in the soil samples for seed bank estimation is shown in Fig. 2 for the three soil layers and the three tillage regimes. The figures are averages for the three crop rotations since this factor was non-significant. Statistical differences between soil layers and tillage treatments for each species and weed group (dicotyledonous (dicots) and monocotyledonous (monocots)) are shown in Table 3. The majority of seeds germinated within the first month of the one year germination period being settled in all treatments combinations. Another great flux of seedling emergence was observed after the first occasion when the soil was dried and turned over. However *A. spica-venti* and *V. myuros* emergence was only observed in the autumn, four to five months after the trial began.

Grass seed production of both *A. spica-venti* and *V. myuros* declined markedly in all crop rotations from 2011 to 2013 (Fig. 1) due to the more intensive herbicide management of the two species. Since the seeds of both grasses are short lived in soil, the effects of the previous year's crop rotation had probably been eliminated by the time of soil sampling. Furthermore the dicot species did not show crop rotation differences, but this weed group did not demonstrate the same dynamic behaviour in response to crop rotation as the grasses during the eleven years of the experiment (data not shown).

The tillage methods $H_{8\text{-}10}$ and D stratified both dicot and monocot species in total in a similar pattern, with most seeds being located in the upper soil layer (0–5 cm). In contrast, seed contents in the 10–20 cm layer were substantially lower, especially grass seeds, despite having been retrieved from a larger soil volume. For example, when comparing a soil volume of similar size in D, the 10–20 cm layer had 85% and 97% less germinable seeds of total dicot and total monocot species respectively than the 0–5 cm layer. Tillage treatment $H_{8\text{-}10}$ placed slightly more seeds in the 5–10 cm layer than D, although the distribution patterns were very similar. The 5 cm deeper cultivation with $H_{8\text{-}10}$ only caused minor downward seed movement similar to other studies. P placed seeds more evenly among the three soil layers, although with a tendency for more seeds to be located in the 10–20 cm layer, partly due to the larger soil volume in this layer compared to the other two.

In general, more germinable seeds of dicot species were retrieved than of monocot species across all tillage treatments and soil layers. The seed bank of dicot species usually contains more species with greater longevity when incorporated in soil than seeds of grass species (Melander, 1994). P accumulated more seeds than the other tillage methods, probably because deep burial protects seed from predation and induces secondary dormancy that prevents weed seeds from unsuccessful germination (Ball, 1992; Cardina and Sparrow, 1996).

Examining the responses of individual species further, *P. annua* was the monocot with the greatest abundance in all tillage regimes and soil depths, but with a layer stratification similar to the other grasses (Table 3). The main reason for the greater seed bank of this grass than *A. spica-venti* and *V. myuros* was that *P. annua* seeds are able to survive longer in the soil when incorporated. This became particularly pronounced in this study where the latest input of new seeds took place at the end of the previous growing season. Thus, the seed bank of these autumn germinator weeds could have been underestimated since the seeds were already a year old by the time they became non-dormant and willing to germinate. Also the fact that *P. annua* can germinate in spring may have resulted in more viable seeds being retrieved since the lag period of four to five months may have caused further mortality of *A. spica-venti* and *V. myuros*. Moreover, the reduced panicle production of *A. spica-venti* and *V. myuros* observed in the three years prior to soil sampling (Fig. 1) probably resulted in a smaller seed density of these species, since they are extremely dependent on annual seed shed to maintain the seed bank (Jensen, 2009).

Chenopodium album L., *Capsella bursa-pastoris* (L.) Medikus, *Tripleurospermum maritimum* (L.) W. D. J. Koch, *Papaver rhoeas* L. and *Viola arvensis* L. were among the most prevalent dicot species to emerge from the soil samples. The tillage treatments did not stratify these species in the same way as the grasses (Table 3), but the less prevalent dicots, *Veronica* spp. and *Polygonum aviculare* L., were distributed in a very similar manner to the grasses following the tillage treatments.

The hypothesis that frequent cropping of winter cereals in non-inversion tillage systems would result in a high abundance of annual grass weeds, with seeds distinctly located within the cultivation depth of the tillage methods applied, was only partly supported. Higher incidences of *V. myuros* with frequent cropping of winter cereals were seen between 2007 and 2011, however this was not reflected in the seed bank. Nevertheless, the distinct stratification of grass seeds according to cultivation depth did hold true.

It should be noted that germinable grass seeds were still present in the 10–20 cm soil layer after 11 years of continuous application of D. Weed seeds are not only moved by cultivation, but may be carried by water and invertebrates or subject to the passive action of gravity, freezing-thawing cycles, and falling into cracks and fissures created when soil dries out in summer or in burrows created by earthworms (Smith et al., 2005; Eriksen-Hamel et al., 2009).

4. Conclusions

An eleven-year period with different crop sequences and continuous tillage regimes demonstrated a dynamic occurrence of grass weeds in particular. Non-inversion tillage systems caused severe outbreaks of the annual grass weeds *A. spica-venti* and *V. myuros* in several years, while mouldboard ploughing significantly prevented the establishment of *V. myuros* in particular. Differences in the aboveground grass weed flora caused by crop rotation were not reflected in the weed seed bank determined in the eleventh year. However, the tillage method greatly determined the vertical distribution of weed seeds within the plough layer, notably by accumulating seeds in the upper 0–5 cm layer following non-inversion tillage.

For the management of severe weed problems in non-inversion tillage systems, an inversion of the upper soil layer with soil from the 10-20 layer could almost remove a weed problem, at least for a period. This might be relevant where weed problems have built up over the years due to herbicide resistance and reluctance among farmers to change their cropping practices, such as crop rotation. From a weed control point of view, one inversion could significantly alter the weed seed bank, but it may conflict with other considerations, such as the beneficial soil properties that can be created by the ongoing application of non-inversion tillage.

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