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Incompatibility between fertility building measures and the management of perennial weeds in organic cropping systems

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ABSTRACT

Fertility building measures are important components in improving the productivity of organic cropping systems without livestock. However, some measures seem to be incompatible with the control of perennial weeds that can have adverse effects such as significant weed competition. The influence of fertility building measures (N_2-fixing crops in the crop sequence, cover crops and manuring) and the abundance of perennial weeds were studied in a long-term crop rotation experiment at two locations in Denmark. The aim was to gain insight into the factors that influence the growth of perennial weed species occurring in mixed stands. Data were obtained from three cycles of four-year arable crop rotations comprising various cash crops in rotations with and without annual whole-year grass-clover as green manure and subjected to four treatment combinations: with and without animal manure and with and without cover crops. Severe outbreaks of perennial weed problems did not occur at the location that had the highest soil fertility, whereas the other site demonstrated dynamic growth of *Cirsium arvense* and *Elytrigia repens*. Grain legumes tended to promote the growth of *C. arvense*, while manuring was neutral to *C. arvense* but beneficial to *E. repens*. Cover crops assisted the growth of *E. repens* since prolonged mechanical interventions were not possible. Compatibility was only achieved with grass-clover and *C. arvense*, meaning that green manure crops suitable for cutting and mulching could offer an important management option against *C. arvense* but not against *E. repens*.

1. Introduction

Crop productivity in organic cropping systems is particularly constrained by nutrient deficiency and weeds, especially arising from infestations with perennial weeds (Turner et al., 2007; Alrøe and Halberg, 2008; Salonen et al., 2011). Normally, expedient sequencing of crops in crop rotations with the inclusion of perennial forage crops that are suitable for mowing facilitate the management of weed populations (Ominski et al., 1999; Schwarz and Moll, 2010). However, for arable growers without livestock, forage crops have little value apart from acting as a green manure crop that primarily adds nitrogen (N) to the cropping system (Olesen et al., 2002 Alrøe and Halberg, 2008). Crop production without livestock presents a particular challenge in that the supply of nutrients, particularly of N, might become inadequate (Berry et al., 2002; Olesen et al., 2007, 2009). Crops grow less vigorously and may suppress weed growth inadequately in comparison to crops that are amply supplied with nutrients. The problem is particularly significant in areas where dairy producers and other types of animal husbandry are scarce and the surplus of nutrients for export to arable growers is limited (Alrøe and Halberg, 2008).

Supplementary measures are therefore needed to raise the fertility of the soils to ensure the crops have a sufficient nutrient supply. The inclusion of symbiotic N_2 fixation in leguminous green manures and grain legumes are important fertility building methods in Northern European cropping systems for organic arable crops (Olesen et al., 2007). In practise, growers predominantly choose N_2-fixing cash crops to ensure sufficient revenues from the cropping system because the benefits of green manures are more long-termed and thus harder to justify economically (Noe et al., 2015). Grain legumes and mixtures of these with cereals can be important sources of N, but they can also promote the propagation of perennial weeds (Permin, 1982). For example, Rasmussen et al. (2014) estimate that the increase of *Elytrigia repens* (L.) Desv. ex Nevski is four times greater in a mixture of grain legumes and spring barley (*Hordeum vulgare* L.) than in winter wheat (*Triticum aestivum* L.) preceded by a spring cereal crop.

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The inclusion of cover crops in crop rotations is another important fertility building measure that is increasingly employed in organic cropping systems (Doltra and Olesen, 2013). The primary purpose of growing cover crops in Northern Europe has traditionally been to minimise nutrient losses through leaching (Thorup-Kristensen et al., 2003; Askegaard et al., 2011). However, cover crops also have other fertility building purposes, such as reducing soil erosion (Langdale et al., 1991) and improving chemical, biological and physical soil properties (Teasdale and Mohler, 1993; Abdollahi and Munkholm, 2014). The beneficial effects of cover crops depend on the choice of cover crop species, the success of establishment and the time they take to cover the soil (Doltra and Olesen, 2013). In particular, prolonged growth after crop harvest is in conflict with the management of perennial weeds (Askegaard et al., 2011; Melander et al., 2013). Problematic infestations with perennial weeds usually require periods in which a mechanical weed control campaign can be implemented to reduce population size and the regenerative capacity of surviving belowground propagules. Mechanical interventions are preferably applied post-harvest because earlier employment in the growing season, such as summer-fallowing (Rasmussen et al., 2014), would obstruct the growing of a valuable cash crop and ultimately have a severe impact on revenues.

Despite that fertility building measures have some inherent disadvantages for the management of perennial weeds, little is known about the actual responses in the context of a long-term organic cropping system in which several cropping factors interact simultaneously. Particularly, when two or more perennial species occur simultaneously in mixed stands in which individual species may respond differently to fertility building measures. The present study treats the dynamics of perennial weeds at two locations having different soil types, climates and soil fertilities. The purpose was to examine the biomass production and reproduction of mixed stands of perennial weeds in organic cropping systems that make use of the fertility building methods of N_2-fixing crops in the crop sequence, cover crops and manuring. The study also included animal manure application because this nutrient source still plays an important role for the N supply despite that N rates might be restricted by the availability of manure (Alrøe and Halberg, 2008) and because manuring will affect crop/weed interactions (Gallandt et al., 1999). The hypotheses were:

(a) Grass-clover as a source of N in the crop rotation causes less growth of perennial weeds than grain legumes or mixtures of grain legumes and cereals.
(b) Cover crops and manuring promote the growth of perennials.

2. Materials and methods

2.1. Crop rotation experiment

The crop rotation experiment was established in the autumn of 1996 at three locations in Denmark with the main objective of exploring the possibilities for both short-term and long-term increases in organic cereal production by manipulating the crop-rotation design on different soil types. The performance of the crop rotations was evaluated in terms of crop production, nutrient leaching, soil fertility and the occurrence of weeds, pests and diseases. Full details of the design of the experiment can be found in Olesen et al. (2000). This study includes data from two locations where perennial weed species occurred in mixed stands: Flakkebjerg (55°20′N, 11°23′E) on Western Zealand and Foulum (56°30′N, 09°35′E) in Central Jutland. The soils are loamy sand (Mollic luvisol according to WRB (FAO)) with a 8.8% clay content in the 0–25 cm topsoil at Foulum and sandy loam (Glossic Phaeozem) with a 15.5% clay content at Flakkebjerg. A detailed characterisation of the locations is presented in Djurhuus and Olesen (2000). The mean temperatures and rainfall during the main growing season (April to July) are shown for each year and for each site in Table 1.

From the start of the experiment in 1997, three experimental factors were included in a full factorial design: (i) crop rotation (O_2 and O_4), (ii) cover crop (with (+CC) and without (−CC)) and (iii) manure (with (+M) and without (−M)). Both crop rotations O_2 and O_4 were grown during three cropping cycles: first cycle 1997–2000, second cycle 2001–2004 and third cycle 2005–2008. All combinations of factors and all crops in the four-cycle rotations were present in two replicates every year in the first two cycles (1997–2004), resulting in a total of 64 plots. The use of only two replicates was mainly decided to accommodate all the treatments in relation to land limitations and running costs as explained in Chirinda et al. (2010). Due to declining soil fertility, the combination -M-CC was converted to a conventional treatment from 2005 and thus not included in the analyses here. Three combinations of factors (+M+CC, +M−CC and −M+CC) and all crops were therefore present in two replicates in each rotation every year in the third cycle (2005–2008), resulting in a total of 48 plots. The plot sizes were 12 × 18 m at Foulum and 13 × 13 m at Flakkebjerg. Each replicate was laid out as a complete block, which was further subdivided into two sub-blocks. The sub-blocks were introduced to better cover soil variation and reduce random error. They were constructed by confounding the threeway interactions between crop rotation, cover crop and manure treatments with the sub-blocks - meaning that the main effects and the two-way interactions could be estimated with good precision. Plots were randomised within sub-blocks.

Since each crop within a crop rotation was grown every year, each crop rotation had four crop sequence entries as shown in Table 2 for O_2 and Table 3 for O_4. The crops were spring barley with undersown grass-clover in O_2, winter wheat, winter triticale (*Triticosecale*), lupin (*Lupinus angustifolius* L.), a mixture of peas (*Pisum sativum* L.) and spring barley, termed 'pea:barley', spring oat (*Avena sativa* L.), faba bean (*Vicia faba* L.), potato (*Solanum tuberosum* L.) and a mixture of lupin and spring barley, termed 'lupin:barley'. Potato was introduced in both rotations after eight years to enhance the possibilities of effective control of perennial weeds. The grass-clover mixture for green manure in O_2 was

Table 1
Temperature and rainfall from April to July at Flakkebjerg and Foulum during 12 experimental years, including the average (normal) climate for 1961–1990.

Location	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	Normal
Mean temperature (°C)													
Flakkebjerg	11.9	12.1	12.5	12.7	12.4	13.6	13.1	11.7	12.3	13.2	13.4	13.2	11.8
Foulum	11.4	11.3	11.8	11.9	11.2	12.6	12.5	11.4	11.9	12.6	12.6	12.6	11.4
Rainfall (mm)													
Flakkebjerg	164	232	254	139	163	229	248	250	156	185	173	132	203
Foulum	284	228	261	188	189	241	264	236	212	198	225	149	219

Table 2
The crops grown in the four entries of crop rotation O_2 at Flakkebjerg and Foulum during the three cycles: (1) 1997–2000, (2) 2001–2004, (3) 2005–2008. Manure N applications for each crop are shown as '+M'-treatments. Crops succeeded by a cover crop CC are shown with a '+'.

Cycle	Year	Entry 1			Entry 2			Entry 3			Entry 4		
		Crops	+M[a]	CC	Crops	+M[a]	CC	Crops	+M[a]	CC	Crops	+M[a]	CC
1	1997	S. barley:ley	50		Grass-clover	0		W. wheat	50	+[b]	Pea:barley	0	+[c]
	1998	Grass-clover	0		W. wheat	50	+[b]	Pea:barley	0	+[c]	S. barley:ley	50	
	1999	W. wheat	50	+[b]	Pea:barley	0	+[c]	S. barley:ley	50		Grass-clover	0	
	2000	Pea:barley	0	+[c]	S. barley:ley	50		Grass-clover	0		W. wheat	50	+[b]
2	2001	S. barley:ley	50		Grass-clover	0		W. wheat	50	+[b]	Lupin	0	+[c]
	2002	Grass-clover	0		W. wheat	50	+[b]	Lupin:barley	0	+[c]	S. barley:ley	50	
	2003	W. wheat	50	+[b]	Lupin:barley	0	+[c]	S. barley:ley	50		Grass-clover	0	
	2004	Lupin:barley	0	+[c]	S. barley:ley	50		Grass-clover	0		W. wheat	50	+[b]
3	2005	S. barley:ley	60		Grass-clover	0		Potato	110		Oat	60	+[c]
	2006	Grass-clover	0		Potato	110		W. wheat	110	+[c]	S. barley:ley	60	
	2007	Potato	110		W. wheat	110	+[c]	S. barley:ley	60		Grass-clover	0	
	2008	W. wheat	110	+[c]	S. barley:ley	60		Grass-clover	0		Potato	110	

[a] +M: manure application target rates. Unit: NH_4-N ha^{-1} in 1st and 2nd cycle and kg total-N ha^{-1} in 3rd cycle.
[b] Monocultures or mixtures of non-N_2-fixing cover crops.
[c] Mixtures of N_2-fixing and non-N_2-fixing cover crops.

undersown in the spring barley and ploughed in autumn at Flakkebjerg and in spring at Foulum. The mixture consisted mainly of perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and red clover (*Trifolium pratense* L.). All the crops were grown according to Danish standards for organic crops. The cereal and grain legume crops were grown for grain harvest and the potatoes for tuber harvest. The grass-clover was managed as a green manure crop, with the cuttings left on the soil surface. However, in rotation O_2/+M during 2005–2008, grass-clover cuttings were removed from the plots.

The cover crops were primarily undersown in the cereal or grain legume crops in spring, except for Flakkebjerg during the last cycle (2005–2008) where they were established post-harvest. In cases where it was considered that perennials weeds were becoming a problem, the cover crop was abandoned to allow for post-harvest cultivations and the cover crops reseeded after the cultivations. The cover crops consisted of non-N_2-fixing species in monoculture, non-N_2-fixing species mixed with N_2-fixing species or pure N_2-fixing species (clover) (Tables 2 and 3). Askegaard et al. (2011) provide more details about the specific species used.

In the manure treatments, slurry was applied in spring as pig slurry at Foulum and as anaerobically digested slurry at Flakkebjerg during the first and second cycles, while just pig slurry was used at both locations in the third cycle. No slurry was applied to grass-clover, grain legume crops or legume:cereal mixtures. For spring-sown crops, the manure was worked into the soil or injected prior to sowing. For autumn-sown crops, the slurry was applied in spring using trail hoses on top of the soil, but beneath the crop canopy. The amount of total ammonium nitrate (NH_4-N) applied in each crop and year in the first and second cycles are shown in Tables 2 and 3. The NH_4-N rates corresponded to 40% of the N requirement of the specific rotation, according to a Danish national standard for conventional crops (Anonymous, 1997). The manure application rates in the third cycle were changed to correspond with a revised Danish national standard allowing the import of animal manure of conventional origin to organic

Table 3
Crops grown in the four entries of crop rotation O_4 during the three cycles: (1) 1997–200, (2) 2001–2004, (3) 2005–2008. Differences in crop species between sites is shown with a '/' where the first crop name corresponds to Flakkebjerg and the second to Foulum. Manure N applications for each crop are shown as '+M'-treatments. Crops succeeded by a cover crop CC are shown with a '+'.

Cycle	Year	Entry 1			Entry 2			Entry 3			Entry 4		
		Crops	+M[a]	CC	Crops	+M[a]	CC	Crops	+M[a]	CC	Crops	+M[a]	CC
1	1997	Oat	40	+[d]	W. wheat	70	+[d]	W. wheat	70	+[d]	Pea:barley	0	+[c]
	1998	W. wheat	70	+[d]	W. wheat	70	+[d]	Pea:barley	0	+[c]	Oat	40	+[d]
	1999	W. wheat/triticale	70	+[d]	Pea:barley	0	+[c]	Oat	40	+[d]	W. wheat	70	+[d]
	2000	Pea:barley	0	+[c]	Oat	40	+[d]	W. wheat	70	+[d]	W. wheat/triticale	70	+[d]
2	2001	W. wheat/W. rye	50	+[c]	Oat	50	+[c]	S. barley	50	+[b]	Lupin	0	
	2002	Oat	50	+[c]	S. barley	50	+[b]	Lupin:barley/lupin	0		W. wheat	50	+[c]
	2003	S. barley	50	+[b]	Lupin:barley/lupin	0		W. wheat	50	+[c]	Oat	50	+[c]
	2004	Lupin:barley/lupin	0		W. wheat	50	+[c]	Oat	50	+[c]	S. barley	50	+[b]
3	2005	S. barley	60	+[c]	Potato	110		W. wheat/ oat	110/ 60	+[c]	Faba bean/pea:barley	0	+[c]
	2006	Faba bean	0	+[c]	W. wheat	110	+[c]	S. barley	60	+[c]	Potato	110	
	2007	Potato	110		S. barley	60	+[c]	Faba bean	0	+[c]	W. wheat	110	+[c]
	2008	W. wheat	110	+[c]	Faba bean	0	+[c]	Potato	110		S. barley	60	+[c]

[a] +M: manure application target rates. Unit: NH_4-N ha^{-1} in 1st and 2nd cycle and kg total-N ha^{-1} in 3rd cycle.
[b] Monocultures or mixtures of non-N_2-fixing cover crops.
[c] Mixtures of N_2-fixing and non-N_2-fixing cover crops.
[d] White clover.

farmland, corresponding to 70 kg total-N ha^{-1} $year^{-1}$ (Anonymous, 2005).

2.2. Management of perennial weeds

The grass-clover in rotation O_2 was generally mown three times per growing season in May and July and at the end of August. Potato ridges in O_4 in the third cycle were cultivated once or twice close to crop emergence using a rotary cultivator to control annual weeds and build up the ridges. However, perennial weeds were also hampered by this treatment and the harvest of potato tubers in particular caused considerable uprooting of *E. repens* rhizomes that were subsequently removed from the plots. Post-harvest cultivation was generally conducted in plots after the grain legume crops, cereal crops (other than spring barley with undersown grass-clover), potato crops and when abandoning grass-clover, in plots without cover crops and occasionally also with cover crops, as mentioned earlier. Decisions for employing post-harvest cultivations were based on a visual assessment of the weed infestation of each plot. Post-harvest cultivations were followed by mouldboard ploughing to 20 cm soil depth in late autumn at Flakkebjerg and in spring at Foulum. A stubble cultivator with goosefoot shares mounted on vibrating S-shaped tines slicing the soil across the full working width was generally used. However in a few cases a power rotary cultivator, intensifying root and rhizome fragmentation and uprooting, was used where only one pass was executed. Due to the wide variation in use of implements and the timing of mechanical interventions, the analysis of the impact of these measures on the growth of perennial weeds was simplified to the number of cultivations undertaken post-harvest. Cultivation comprised tine cultivation or rotary cultivation. Table 4 gives an overview of the number of cultivations conducted for each location, cycle, crop rotation and cover crop treatment.

In addition to mowing and post-harvest cultivations, aboveground shoots of mainly *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L. were hand-weeded plot-wise in mid-July each year in particularly weedy plots. However, at the Flakkebjerg site, *C. arvense* was consequently removed by hand-pulling in all plots and years in 1999, 2000 and during the second cycle because infestations were particularly high. Primarily tall and visible shoots strong enough for hand-pulling were removed.

2.3. Recording perennial weeds

The principal perennial weed species were *C. arvense* and *E. repens* at Flakkebjerg and *C. arvense*,*S. arvensis* and *E. repens* at Foulum. Aboveground perennial weed biomass production was recorded at the end of June for all crops and years except in 2005 and for grass-clover in all years. Two 0.5 m^2 quadrates were randomly placed in each plot and all aboveground plant material within the quadrate cut at ground level. Perennial weeds were separated from other plant material and the perennial weed biomass was oven-dried for 24 h at 80 °C to obtain dry matter (DM). All the hand-pulled plant material of *C. arvense* at Flakkebjerg was recorded as fresh weight from the whole plot.

The incidence of *E. repens* at Flakkebjerg was assessed by counting the number of spikes in five 0.1 m^2 quadrates per plot in mid-July in all years and crops except for grass-clover, where mowing made this impossible. The spike number does not reflect the total number of shoots in a quadrate, as many shoots may not have produced flower stands at the time of recording. Nevertheless, dynamics in spike numbers over time can reflect changes in the population size of *E. repens* and thus its reproductive capacity, as argued in Rasmussen et al. (2014).

2.4. Data analyses

General linear mixed models with normally distributed data were used to test the fixed effects of location, crop rotation cycle, entry point, crop rotation, cover crop and manuring on the dry matter of perennial weeds and on the fresh weight of *C. arvense*. The fixed effects of treatments on *E. repens* spike number was analysed using a generalised linear mixed model with a Poisson distribution of errors. In a full model, the random effects were the year within the cycle, the block within the location, the subblock within the location and block, the year x block within the cycle and location, the interaction year x crop rotation x cover crop x manure within the cycle, and plot. However, the occurrence of perennial weeds was insignificant at the Foulum site and thus simpler models were used where the location was excluded. In some instances the cycle was excluded if dynamic weed behaviour only occurred within a specific cycle. The location-wise analyses were also performed with the number of post-harvest cultivations included as a covariate to test whether these affected perennial weed growth. The repeated nature of the weed data with recordings made over time in the same plots was accounted for by including year as a repeated effect, with plot nested under location as the subject. An autoregressive correlation structure and variance was assumed between years.

The parameters of the linear models were estimated using residual likelihood estimations. Models were reduced by excluding non-significant effects based on likelihood ratio tests and Akaike's information criterion (Akaike, 1974). Calculations were made using the mixed procedure of SAS (SAS release 9.2, SAS Institute Inc., Cary, N.C.), and means were calculated as least square means (LSM). In the case of *E. repens* spike number, calculations were

Table 4
The number of post-harvest cultivations conducted for each location, cycle, crop rotation and cover crop to control perennial weeds during each cropping cycle (four years).

Location	Cycle	Crop rotation	Number of post-harvest stubble cultivations	
			Without cover crop	With cover crop
Flakkebjerg	1997–2000	O_2	10	4
		O_4	3	0
	2001–2004	O_2	11	0
		O_4	8	0
	2005–2008	O_2	14	4
		O_4	8	12
Foulum	1997–2000	O_2	2	0
		O_4	0	0
	2001–2004	O_2	4	0
		O_4	4	0
	2005–2008	O_2	1	7
		O_4	7	7

made with the glimmix procedure of SAS that features a Poisson distribution. Pair-wise comparisons between LSMs were based on *t*-tests, with probability values adjusted according to the Tukey method. Contrasts were used to test (*F*-tests) differences between groupings of treatments using the contrast statement of mixed. The 5% level or less indicated a significant difference between means or contrasts. The denominator degrees of freedom (DDF) in *F*-tests and *t*-tests for mean separations were calculated according to Kenward and Roger (1997). All data were log-transformed to obtain homogeneity of variance.

3. Results

3.1. Perennial weed biomass

The growth of perennial weeds was generally more prevalent at Flakkebjerg than at Foulum ($P < 0.001$) (Fig. 1). Perennial weeds were barely present during the first cycle at Foulum and then slightly increased during the second cycle, reaching a level that remained stable throughout the third cycle. Further analysis of the perennial weed biomass data at Foulum with reasonable robustness was not possible due to the low and patchy presence of perennials. No perennial weed biomass was recorded at Flakkebjerg at the start of the experiment in 1997. Thereafter perennials gradually spread in the experiment, resulting in a sufficient occurrence in the second and third cycles to undertake statistical analyses. Manure, cover crop and the co-variate post-harvest cultivation did not explain any of the variations and it was possible to reduce the model to the effects seen in Table 5. The third cycle generally had three times more biomass than the second cycle (main effect of 'cycle', $P = 0.0597$ in Table 5) and crop rotation O_4 was 89% more infested with perennial weed biomass than rotation O_2 when averaging both cycles and all other factors (main effect of 'rotation', $P = 0.0042$ in Table 5). There was a three-way interaction between cropping cycle, crop rotation and entry point ($P = 0.0235$ in Table 5). Entry points differed when making a comparison across cycles and crop rotations, but not when comparing entries within cycle and crop rotation based on Tukey tests (data not shown). This suggests that the differences between crop rotations for each cycle were unaffected by entry point.

The main effects of the different crops on perennial weed biomass within the two crop rotations are shown in Table 6 for the second and third cycles. Lupin was generally more infested with perennial weeds than the cereals, although winter wheat had markedly more weeds when it did not follow grass-clover. Mixtures of lupin and barley had weed infestations in between lupin and cereals, whereas potatoes responded in a similar way to spring barley. However most of the comparisons of crops within cycle and crop rotation were non-significant when *P*-values had been adjusted according to the Tukey test (Table 6).

3.2. Fresh weight of *C. arvense* at Flakkebjerg

The experiment was gradually infested with *C. arvense* during the first cycle, reaching numbers for whole plots appropriate for statistical analyses in 1999 and 2000. The population developed further during the second cycle with biomasses clearly being affected by crop rotation and entry point (Fig. 2). As for perennial weed biomass, manure and cover crop, and the co-variate post-harvest cultivation were non-significant and the model could be reduced to the effects seen in Table 5. The effects of crop rotation and entry point were prevalent in both the first and second cycles, but the effects of entry point could not be estimated during the third cycle due to unbalanced data. However, cereals and grain legumes in the third cycle were generally infested with *C. arvense* more than twice as much as similar crops in the second cycle ($P = 0.0193$). Crop rotation O_4 had 207% more *C. arvense* biomass than O_2 across the two first cycles. The difference ceased during the third cycle when comparing spring barley and winter wheat grown in rotation O_2 *versus* the same two crops in rotation O_4 ($P = 0.1824$ for barley and $P = 0.6251$ for wheat).

Fig. 2 illustrates the three-way interaction between cycle, rotation and entry point for the first two cycles. Entry 3 in crop rotation O_2 had more *C. arvense* biomass in the first cycle compared to the other entries (1, 2 and 4) in O_2 ($P < 0.0001$). This situation lasted for O_2 throughout the second cycle, but the difference between entry 3 and 1 was not significant ($P = 0.1068$). Only minor differences were seen among the four entries in crop rotation O_4 in the first cycle. However entries 1, 3 and 4 in O_4 were markedly more infested than entry 2 in O_4 in the second cycle ($P = 0.0002$), while differences among the three entries 1, 3 and 4 were only negligible.

The dynamic occurrence of *C. arvense* in individual crops within years, entries and rotations is evident in Fig. 2, with lupin in 2001 and the oat and lupine barley mixture in 2004 being particularly infested. Again the infestation in winter wheat was less when it followed grass-clover in O_2 rather than a legume crop in O_4 ($P = 0.0168$).

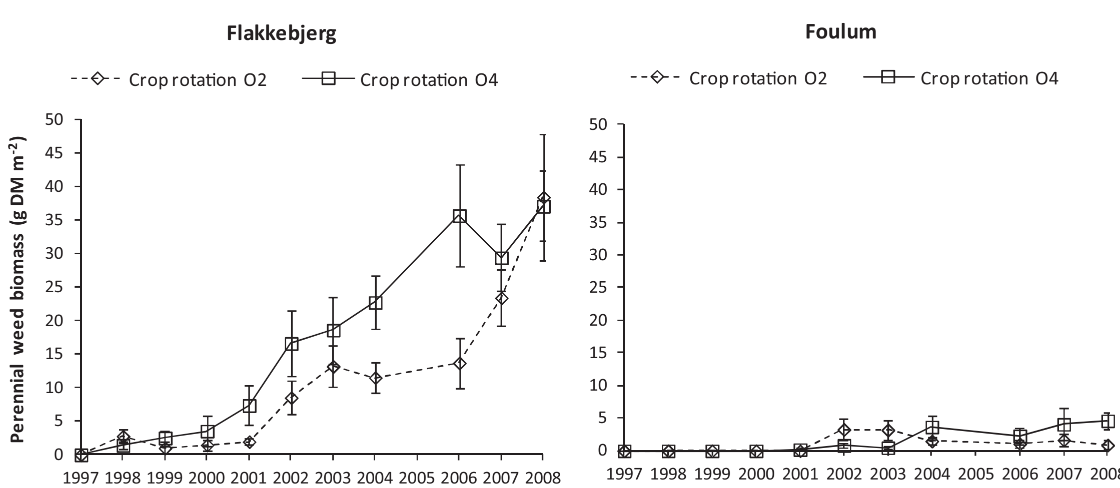

Fig. 1. The development of mean biomass of perennial weeds from 1997 to 2008 at the two locations, Flakkebjerg and Foulum, shown for each crop rotation O_2 and O_4. Observed values are means across all other treatment factors and vertical bars are standard errors of means.

Table 5
Effects of cycle, entry point, crop rotation, cover crop, manuring and their interactions on log-transformed perennial weed biomass, *C. arvense* fresh weight and *E. repens* spike number (Poisson distribution) at Flakkebjerg. All the effects shown are those obtained after model reduction. Perennial weed biomass cover all three cycles, while *C. arvense* only includes cycles 1 and 2 and *E. repens* only cycle 2. DF = degrees of freedom and DDF = denominator degrees of freedom.

Variables	Factors	DF	DDF	*F*-value	Level of significance
Perennial weed biomass ($g\,m^{-2}$)	Cycle	1	5.3	5.71	$P = 0.0597$
	Rotation	1	45.6	9.11	$P = 0.0042$
	Entry	3	46.7	1.14	$P = 0.3420$
	Cycle × rotation	1	104	0.26	$P = 0.6133$
	Cycle × entry	3	109	2.16	$P = 0.0965$
	Rotation × entry	3	46.7	0.42	$P = 0.7429$
	Cycle × rotation × entry	3	109	3.29	$P = 0.0235$
C. arvense fresh weight ($g\,m^{-2}$)	Cycle	1	4.2	4.08	$P = 0.1101$
	Rotation	1	64.4	12.32	$P = 0.0008$
	Entry	3	64.9	20.4	$P < 0.0001$
	Cycle × rotation	1	66.6	1.72	$P = 0.1942$
	Cycle × entry	3	283	7.72	$P < 0.0001$
	Rotation × entry	3	64.9	5.03	$P = 0.0034$
	Cycle × rotation × entry	3	283	4	$P = 0.0082$
E. repens spikes (no m^{-2})	Rotation	1	33.42	3.92	$P = 0.0561$
	Cover crop	1	33.55	21.07	$P < 0.0001$
	Manure	1	32.67	4.4	$P = 0.0438$
	Rotation x cover crop	1	33.96	4.76	$P = 0.0362$

Table 6
Least squares means (LSM_{transf}, log-transformed values with detransformed values in parentheses) of perennial weed biomass shown for each crop within levels of crop rotation and cropping cycle at Flakkebjerg. SED = maximum standard error of differences between LSM_{transf}, DDF = denominator degrees of freedom. BA: spring barley, GC: grass-clover, WW: winter wheat, LU:BA: lupin barley mixture, LU: lupin, PO: potatoes, FB: faba bean, OA: oat.

Crop rotation	Cycle	Crops	LSM_{transf} (biomass $g\,m^{-2}$)	DDF	Relative to BA (%)	Significance between crops (Tukey test)
O_2	2001–2004	1. BA	0.9613 (2.52)	8.00	–	1 *vs.* 3, $P = 0.9977$
		2. GC	–	–	–	1 *vs.* 4, $P = 1.0000$
		3. WW	0.5417 (1.62)	8.02	−36	1 *vs.* 5, $P = 0.2638$
		4. LU:BA	1.2010 (3.22)	9.47	+28	3 *vs.* 4, $P = 0.8776$
		5. LU	2.5909 (13.24)	26.6	+425	3 *vs.* 5, $P = 0.0576$
		SED	0.6400			4 *vs.* 5, $P = 0.7152$
	2005–2008	1. BA	2.1916 (8.85)	9.26	–	1 *vs.* 3, $P = 1.0000$
		2. GC	–	–	–	1 *vs.* 4, $P = 0.9372$
		3. PO	2.1855 (8.80)	9.27	−1	3 *vs.* 4, $P = 0.9346$
		4. WW	2.9427 (18.87)	9.26	+113	
		SED	0.4631			
O_4	2001–2004	1. BA	1.0991 (2.90)	8.00	–	1 *vs.* 2, $P = 0.0048$
		2. LU	3.6043 (36.66)	26.7	+1164	1 *vs.* 3, $P = 0.3545$
		3. LU:BA	2.0291 (7.51)	9.43	+159	1 *vs.* 4, $P = 0.8134$
		4. WW	1.7897 (5.89)	7.98	+103	1 *vs.* 5, $P = 0.9126$
		5. OA	1.6784 (5.26)	7.98	+81	2 *vs.* 3, $P = 0.5102$
		SED	0.6419			2 *vs.* 4, $P = 0.1218$
						2 *vs.* 5, $P = 0.0980$
						3 *vs.* 4, $P = 1.0000$
						3 *vs.* 5, $P = 0.9999$
						4 *vs.* 5, $P = 1.0000$
	2005–2008	1. BA	2.8913 (17.92)	9.20	–	1 *vs.* 2, $P = 1.0000$
		2. FB	3.1378 (22.95)	9.20	+28	1 *vs.* 3, $P = 1.0000$
		3. PO	2.7483 (15.52)	9.20	-13	1 *vs.* 4, $P = 0.9977$
		4. WW	3.4206 (30.49)	9.20	+70	2 *vs.* 3, $P = 0.9999$
		SED	0.4588			2 *vs.* 4, $P = 1.0000$
						3 *vs.* 4, $P = 0.9743$

3.3. Spike number of *E. repens* at Flakkebjerg

The rhizomatous weed *E. repens* was the second most prevalent perennial species throughout all three cycles at Flakkebjerg, although less common than *C. arvense*. *E. repens* spread slowly during the first cycle, peaked during the second cycle and maintained its population size during the third cycle, averaging 4 spikes m^{-2}. Spike numbers were high enough in the second cycle to detect some dynamics for statistical analysis (Table 6). In general, rotation O_2 tended to be more infested than O_4 during cycle 2 (main effect of 'rotation', $P = 0.0561$ in Table 5). The cover crop in particular affected *E. repens* and it interacted with crop rotation. No cover crop in rotation O_4 reduced spike numbers by 87% ($P < 0.0002$) as compared to cover cropping in O_4 whereas cover crop versus no cover crop was non-significant in O_2 ($P = 0.3343$). Manure generally increased the density of *E. repens*, averaging 1.2 spikes m^{-2} for no manuring *versus* 5.1 spikes m^{-2} with manuring across crop rotations and cover crop treatments.

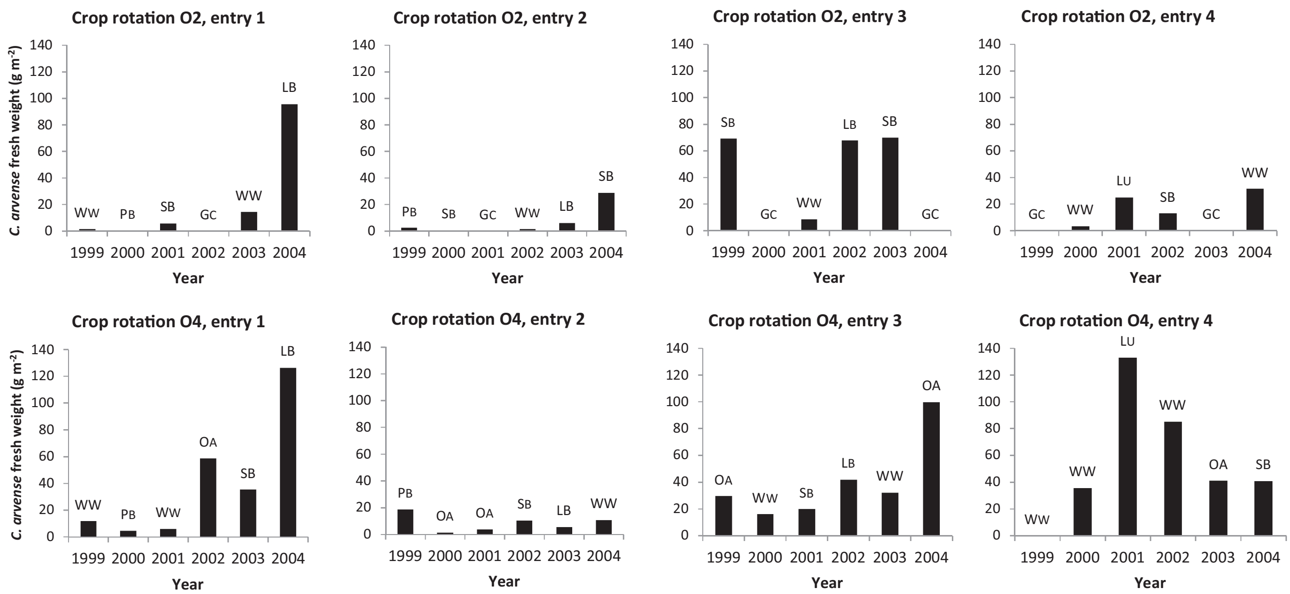

Fig. 2. Mean fresh weight of *C. arvense* across manure and cover crop treatments (with and without for both factors) recorded each year in 1999–2004 in crop rotations O_2 and O_4 and for the crop rotation entries 1–4 at Flakkebjerg (see also Tables 2 and 3). No assessments were made in grass-clover in rotation O_2. Figures are back-transformed means from analyses made on log-transformed data. LB: lupin:barley mixture, PB: pea:barley mixture, SB: spring barley, GC: grass-clover, WW: winter wheat, LU: lupin, OA: oats.

None of the differences seen in cycle 2 were present during the third cycle (data not shown).

There were only minor differences in the *E. repens* infestation level among the crops grown in rotation O_2 when looking at the main effects of individual crops within each rotation (data not shown). In O_4, however, the lupin:barley mixture was 3.5 times more infested than lupin, wheat and oat ($P = 0.0002$) and 1.7 times more than spring barley ($P = 0.0460$).

4. Discussion

C. arvense and *E. repens* propagate vegetatively through an extensive production of roots and rhizomes, respectively (Holm et al., 1977). Detrimental reductions in crop growth are mainly caused by interference from shoot growth of both species arising from vegetative reproduction rather than from seedlings. Significant proliferation of *C. arvense* and *E. repens* was only seen at Flakkebjerg, despite more intensive post-harvest cultivation at this site (Table 4). The crop rotation with grass-clover generally had less perennial weed biomass than the rotation with grain legumes or mixtures containing grain legumes as the only N_2-fixing main crops. This effect was mainly driven by the dominant occurrence of *C. arvense*, which responded strongly to grass-clover in the crop rotation. Therefore hypothesis (a), stating that grass-clover in the crop rotation causes less presence of perennial weeds, holds true for perennial weeds in general and *C. arvense* in particular. Neither perennial weed biomass nor *C. arvense* responded to manure or cover crops, whereas *E. repens* was promoted by both factors. Hence hypothesis (b), suggesting that cover crops and manuring promote the growth of perennials, is only valid for *E. repens*.

The fact that the grass-clover was repeatedly mown greatly reduces *C. arvense* stands, with a detectable effect in subsequent crops (Häusler et al., 2004; Graglia et al., 2006). Lukashyk et al. (2008) suggest that the frequency of defoliation caused by mowing corresponds to the reduction of root assimilates, eventually depleting the food reserves in roots and consequently weakening the regenerative capacity. The ability of grass-clover to add more N to the cropping system than grain legumes is another aspect likely to have determined the crop rotation effects seen on the growth of perennial weeds. Crop rotation O_2 also had more N_2-fixing crops during the first and second cycles than O_4. Previous analyses of crop yields also showed that yields were generally higher in rotation O_2 with grass-clover compared to O_4 (Askegaard and Olesen, 2011). Crop fitness and the crops' suppressive ability against weeds are likely to have benefitted from higher N availability. However, *E. repens* responded to grass-clover in the rotation in a contrasting way to *C. arvense*, with higher infestation levels in O_2, rejecting hypothesis (a) for *E. repens*. Other studies confirm that mowing grass-clover between one and three times during the growing season does not offset rhizome propagation and may even lead to more *E. repens* in subsequent crops (Albrecht, 2005; Graglia et al., 2006; Rasmussen et al., 2014). Defoliation needs to be more frequent to suppress rhizome growth significantly (Turner, 1968). Crop rotation differences disappeared in the third cycle because the measures to control *E. repens* in potatoes were taken in both rotations. Both *C. arvense* and *E. repens* tended to grow more vigorously where grain legumes were grown, either solely or in mixtures, although not as markedly as is observed for *E. repens* in Rasmussen et al. (2014). The N fixated by legumes presumably acts as an important N source for the augmentation of belowground propagules in the post-harvest period and probably even in the subsequent year (Nadeau and Born Vandern, 1990; Ringselle et al., 2015).

The sequence of crops reflected by the different entry points in each crop rotation surely had an influence on *C. arvense* growth in the period 1999–2004, while the effects on perennials in general were less clear. For crop rotation O_2, the years in which grass-clover was grown appeared important for the suppression of *C. arvense*. Grass-clover came later in entry 3 (having most *C. arvense* growth) than in the other entries (see Table 2), suggesting that grass-clover should be introduced early in the sequence to impede the proliferation of *C. arvense*. The picture for entry effects in rotation O_4 is less clear and more difficult to explain. The infestation level of *C. arvense* in entry 2 remained low in O_4 throughout the period, irrespective of the crops grown.

Treatments with no cover crop made room for more post-harvest cultivations in contrast to cover cropping, especially during the first and second cycles, but only *E. repens* was reduced by the cultivations. *C. arvense* and perennials in general were both neutral to cover crop and manuring, while *E. repens* responded to manure application. Insignificant or varying responses of *C. arvense* to shallow post-harvest cultivations or cover-crop growing have also been observed in other studies (e.g., Brandsæter et al., 2011; Melander et al., 2012). Brandsæter et al. (2010) and Tørresen et al. (2010) infer that post-harvest measures against *C. arvense* populations showing little or varying readiness of adventive buds on roots to sprout in late summer and autumn would have a limited effect since innate bud dormancy makes it difficult to exhaust the carbohydrate reserves in the roots. The rather intensive hand-pulling of *C. arvense* in this study is another aspect likely to have masked any effects of the post-harvest treatments. Depleting the carbohydrates in the rhizomes of *E. repens* by post-harvest tillage is more easily achieved due to the readiness of rhizome buds to sprout after disturbance. This results in the weakened re-growth capacity of rhizome fragments that will eventually reduce population size, as achieved for the treatments without cover crops. However it was not possible to quantify the effect of each post-harvest cultivation because the treatments were not tailored to a specific strategy against *E. repens*.

C. arvense and perennial weeds in general did not respond to manuring while the growth of *E. repens* was promoted by manure application in contrast to results from coarse sand (Rasmussen et al., 2014) where manuring suppressed *E. repens*. However, the crops in this study benefitted relative to *E. repens* from the supply of manure because crop biomasses and yields are increased (Olesen et al., 2007, 2009). Contrasting results have also been reported in other studies, showing that organic amendments (legume residues, manure and compost) can lower weed biomasses and improve crop yields (Gallandt et al., 1998), while in other cases amendments can even improve weed fecundity and weed competitiveness against the crop (Liebman et al., 2004; Menalled et al., 2004). Therefore organic amendments to soil may not always contribute to effective weed management or obviate the need for effective control practices.

Outbreaks of any noticeable infestations with perennial weeds were absent at the Foulum location during all three cycles. The slightly warmer and drier climate at Flakkebjerg would not be a plausible explanation for the greater prevalence of *C. arvense* and *E. repens* at this site compared to Foulum. Both perennials prefer a cool and moist climate, which is more typical of the Foulum site (Holm et al., 1977; Donald, 1994). Also the soil type at Flakkebjerg is not known to favour the two perennials more than the soil type at Foulum. In previous publications, crop yields have mostly been reported to be higher at Foulum as compared to Flakkebjerg, apart from winter cereals, during the twelve years of experimentation (Olesen et al., 2007, 2009; Askegaard and Olesen, 2011). The initial soil fertility was greater at Foulum due to the cropping history prior to the start of the experiment (Olesen et al., 2007). This suggests better conditions for crop growth at Foulum and may have favoured crops more than weeds in contrast to Flakkebjerg. Furthermore, the N-use efficiency of manure applied to cereals was found to be higher at Foulum than Flakkebjerg (Olesen et al., 2007, 2009).

5. Conclusions

Incompatibility between fertility building measures and the management of perennial weeds was not seen at the site (Foulum) that was assumed to have highest soil fertility, but it was present in Flakkebjerg. Grain legumes can promote infestations with perennials unless careful precautions are taken. Grain legumes should preferably be preceded by crops that make room for the employment of effective control interventions to minimise the amount of belowground propagules left for growth in the legume crop. However, cereals such as barley, oat and wheat, which are known to be more competitive than legumes against perennials, are no safeguard against perennial weed problems.

Compatibility between fertility building measures and the management of perennial weeds was only achieved with grass-clover and *C. arvense*, meaning that green manure crops suitable for mowing can be an important management option against *C. arvense* but not against *E. repens*. No justification was found for abandoning a cover crop to allow for post-harvest tillage against *C. arvense* where that species is the main perennial weed problem.

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