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Weed density and diversity in a long-term cover crop experiment background

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ABSTRACT

Cover crops (CC) are biological tools with a great potential for weed control, but the suppression level depends on the CC species and management. A 2-year study was performed in the eighth year of a long-term experiment located in Central Spain to study the effect of replacing winter fallow by barley (*Hordeum vulgare* L.) or vetch (*Vicia sativa* L.), on the weed control. Moreover, two CC termination dates were evaluated. Weed biomass, density, diversity, population composition and the seed bank were assessed. Ground cover and CC biomass, soil inorganic N and topsoil water content were determined throughout the season. Barley achieved a greater weed control compared to vetch in winter and early spring. Later in May, both CC residues decreased weed density compared to fallow (63% in 2015, 55% in 2016), and reduced the density of some broadleaf species (i.e. *Xanthium spinosum* L. reduced > 50%). The weed seedbank density was not affected by CC but the effect on specific species confirmed the control over *Xanthium* spp. (78% reduction), and also warned of the incomplete weed control by CC. The year in which the biomass and ground cover increased between termination dates, delaying the CC termination reduced weed density > 75%. Therefore, delaying the termination date was a mean to increase weed control but should be performed with caution to avoid pre-emptive competition with the cash crop. Results underline the relevance of CC species and the termination date as management tools for weed control, and must be considered to plan specific management strategies in different scenarios.

1. Introduction

The interest of replacing winter fallow by cover crops (CC) has increased in last decades mainly due to the beneficial effects on soil and water quality (Thorup-Kristensen et al., 2003). Given the economic relevance of crop losses by weeds and the negative effects of herbicides, as the environmental persistence or herbicide-resistant weeds increase (Oerke, 2006; Zimdahl, 2013), weed suppression benefits from CC have become highly relevant as well (Teasdale et al., 2007). However, there is still a need to clarify what are the best management choices to enhance weed control capabilities of CC (Schipanski et al., 2014). In addition, the result of introducing CC for weed communities may take several years to manifest (Moonen and Barberi, 2004) and information from long-term experiments may help to design strategies to optimize weed control.

As living crops or as mulch, CC compete for resources as light, water and nutrients (Teasdale and Mohler, 1993). Also, chemical control has been reported in CC that release allelopathic substances that reduce weed density (Sturm et al., 2016). However, CC impact on weed density and diversity is still not clear as it depends on CC type and management, among other factors (Campiglia et al., 2012; Radicetti et al., 2013).

Cover crop biomass is an important factor in weed control (Teasdale and Mohler, 2000) and can explain differences between CC species performance (Campiglia et al., 2012; Buchanan et al., 2016). Further, the impact of the CC on the soil N and water content must be considered. The soil N depletion by the growing CC might contribute to weed control, but the N release from residue after CC termination might enhance weed emergence (Blackshaw et al., 2003). As legumes incorporate atmospheric N to the soil, they should be considered as another variable leading to differences between CC (Blum et al., 1997). Likewise, under no-tillage practices, CC mulch preserves soil moisture that may promote weed density (Teasdale and Mohler, 1993). However, few field experiments connected soil N availability and moisture with the weed control by CC residues.

The CC termination date is crucial for weed control (Mirsky et al.,

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2011). In some experiments the termination date delay increased CC biomass and led to better weed control and changes in the composition of the weed population (Mirsky et al., 2011). However, other authors did not observe an impact of termination date (Duiker and Curran, 2005). The termination date effect on water and N availability has been reported (Alonso-Ayuso et al., 2014) but few studies related the termination date effect on weed control with soil water and N. Only the study by Wayman et al. (2015) focus in the correlation between CC termination date, soil N availability and weed density in a field experiment, but results were not conclusive. In addition, none of previous studies integrating CC termination date and weed control were performed in semiarid regions in which the termination date delay could entail a pre-emptive competition risk (Nielsen and Vigil, 2005). Therefore, more comprehensive research is needed to understand the impact of CC and management practices on environmental variables that affect weed control.

The objectives were to study the effect on weed density and diversity of i) replacing fallow by a grass or a legume winter CC in a long-term field experiment, and ii) terminating the CC in two dates in spring. Topsoil water and N availability were periodically measured to explain weed control. The study was conducted in an irrigated area with intensive crop management. Weed competition is a major concern in the area, but relying only on chemical control could lead to environmental pollution of a shallow aquifer (≈5 m bellow surface). The use of CC is not widespread in the region and its use could contribute to control weeds and diminish herbicide application.

2. Materials and methods

2.1. Field experiment

The study was performed in a field experiment located in Aranjuez (Central Spain) that was established in 2006. Year after year a winter CC-irrigated cash crop rotation was carried out in the same plots. Two CC species, barley (*Hordeum vulgare* L.) and common vetch (*Vicia sativa* L.), were compared with a fallow treatment. Then, the cash crop – maize (*Zea mays* L.) or sunflower (*Helianthus annuus* L.) were sowed through CC residues. The design corresponded to 4 replications randomly distributed in 12 plots (12 × 12 m^2). Detailed information can be found elsewhere (Garcia-Gonzalez et al., 2016). The soil is classified as a *Typic Calcixerept* (Soil Survey Staff, 2014) and had a silty clay loam topsoil (pH ~8.1; soil organic matter ~1.9%). The climate of the area is Mediterranean semiarid (Papadakis, 1966). Weather measurements during the experiment were recorded by aCR23X micrologger in a Campbell Scientific station located less than 100 m from the field experiment.

The current study was performed eight years after the establishment of that long-term experiment to show the legacy of CC treatments on the weed assembly over two seasons (2014–2015, 2015–2016). Cover crop species (barley, 180 kg ha^{-1}; vetch, 150 kg ha^{-1}) were sown in early October each year and buried with a shallow cultivator (~5 cm). Fallow plots received as well a shallow cultivator pass. None of the plots received fertilization, irrigation, or any type of weed control during the autumn-winter period. Cover crops were terminated in spring with glyphosate (1.07 kg a. e. ha^{-1}) and chopped when dry. The termination date, was considered a second factor to study. Both years, a 4 m^2 microplot was established and corresponded to the first termination date (FT, March 13th, 2015; March 17th, 2016). At this time barley was at the end of the booting, and vetch at the stem elongation stage, and the herbicide was applied by a backpack sprayer in the microplot. The rest of the main plots corresponded to the second termination date (ST, April 20th, 2015; April 14th, 2016), when barley was at the middle of heading and vetch at the stem elongation stage. The herbicide was applied with a tractor sprayer, after covering microplots. Both termination dates, glyphosate was sprayed as well over fallow plots.

Cash crops, sunflower in 2015, and maize in 2016, were sown (April 29th, 2015; April 18th, 2016) by direct drilling through the CC mulch (80,000 plants ha^{-1}). On May 29th, 2015, tribenuron-methyl (18.5 g a. e. ha^{-1}) was applied over sunflower (5 extended leaves). On May 18th, 2016, a mix of mesotrione (12 g a. i. ha^{-1}) and S-metolachlor (120 g a. i. ha^{-1}), was applied over maize (3 unfolded leaves).

Irrigation was applied from May to July for sunflower, and from May to September for maize. A sprinkler delivery system was used and irrigation water applied was ≈80% of cash crop evapotranspiration calculated by the FAO method (Allen et al., 1998) due to water scarcity.

Sunflower did not receive fertilizer application, while maize received two split application of N-fertilizer (at the end of May and June). Each CC treatment received a different rate according to the N available in the soil, the expected maize N uptake, and the estimated N mineralized from barley and vetch residues. Therefore, 196, 110 and 150 kg N ha^{-1} were applied on barley, vetch and fallow plots respectively.

In 2015, sunflower heads were damaged by birds. Thus, at early September 3 rows $plot^{-1}$ were hand harvested and the seed yield was estimated through the head diameter, as the head diameter and the seed weight were highly correlated in a calibration obtained from seed-full sunflower heads (R^2 = 0.99). No differences between CC treatments were found in sunflower commercial grain yield (1528 ± 161 kg ha^{-1}). For maize, at early October 2016, two 8-m stripes of the central rows in each plot were harvested with an experimental combiner and maize yield was recorded. No differences were found between CC treatments in commercial grain yield (8.0 ± 0.8 Mg ha^{-1}). A timeline with the main field labors and sampling performed from 2014 to 2016 is presented in Fig. 1.

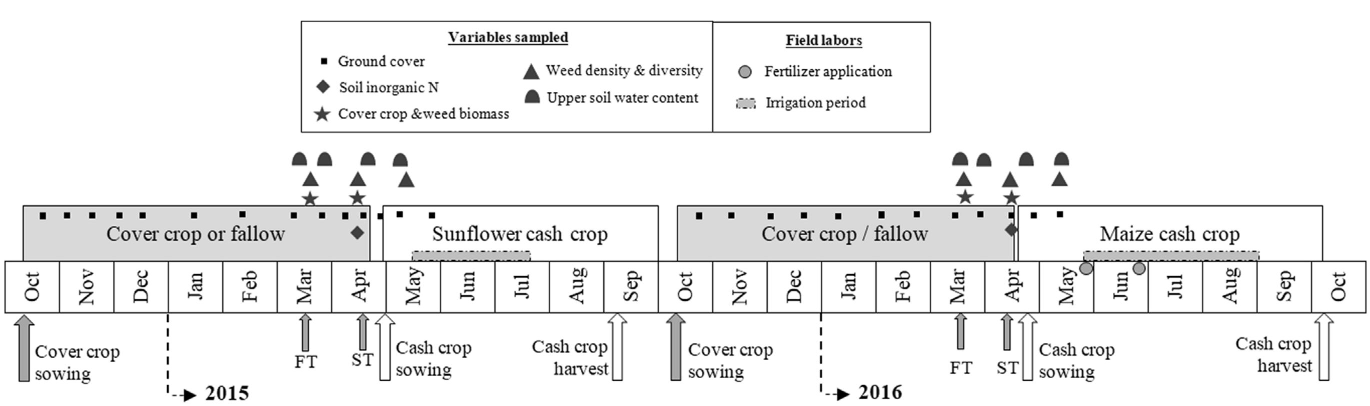

Fig. 1. Schematic timeline showing the field works and sampling performed on the experimental site from 2014 to 2016.

2.2. Cover crops: biomass and ground cover

Prior to each termination date, the aboveground biomass of four 0.25 m^2-size quadrats per plot was hand harvested. For FT, squares were taken outside the microplots but close to them. The CC and weed biomass were separated, oven-dried and weighed.

Ground cover (GC) evolution was determined by taking digital images from a nadir perspective at a 1 m height of four quadrats (0.5 × 0.5 m^2) in each plot every 2–3 weeks. Until CC termination, GC was determined by analyzing images with Sigma-Scan Pro®. For each image, a layer with pixels contained in the range of hue and saturation corresponding to the CC green color was created, and then divided by the total number of pixels in the image to obtain the GC value (Ramirez-Garcia et al., 2015). After CC termination, GC was determined by analyzing images with SamplePoint (Booth et al., 2006). A grid with 100 crosshairs was overlaid on each image. The number of crosshairs intersecting CC residue or soil were counted, and GC was calculated as the proportion of residue points in each image (Quemada and Daughtry, 2016).

2.3. Topsoil N availability and water content

Soil inorganic N (Nmin) was determined prior to cash crop sowing. For CC treatments, samples were taken from FT and ST plots. In fallow, soil samples were only taken from large plots. Two soil cores were taken from each plot to 20 cm depth, and combined. A subsample was extracted with 1M KCl (~30 g soil: 150 ml KCl), then centrifuged (10 min, 1200 rpm) and decanted. The Griess-Ilosvay method was used to determine nitrate concentration (Keeney and Nelson, 1982), while NH_4^+ was determined by the salicylate-hypochlorite method (Crooke and Simpson, 1971).

The topsoil water content (WC) was determined using a soil-moisture sensor (Decagon 5TM, Decagon Devices, Pullman, WA, USA) previously calibrated in the laboratory with undisturbed soil. Different measurements were performed from mid-March to mid-May. Each date, seven measurements were taken inside each microplot and plot and a representative value was obtained as the average of the readings.

2.4. Weed control

Weed biomass was determined at CC termination using the same procedure as described above. In spring, weed density and diversity were determined at three sampling times: 1) at mid-March, before FT, 2) at mid-April, at ST, and 3) at mid-May. At each sampling, weeds were counted and identified in four 0.25 m^2-size quadrats randomly marked in each plot and microplot. Shannon index (H) was used to evaluate diversity (Marrugan, 1988). In the identification, in some cases it was only possible to reach the genus level. The density was studied only in species with a relative abundance > 15% (Derksen et al., 1993). At mid-May, weeds were classified as: annual broadleaf, annual grasses and perennials species. Each group was expressed as a percentage by adding the species and then dividing them by the total weed density.

For each group, the relative neighbor effect index (RNE) was calculated for barley and vetch vs. fallow (Markham and Chanway, 1996). This index compares the performance of weed flora growing with a CC and those growing with no-CC. The index was calculated as: $RNE = \frac{Nfallow - Ncc}{x}$, where Nfallow is the weed density in fallow treatment and Ncc the weed density in the presence of a CC (either vetch or barley). The term "**x**" depends on which weed density is greater. If Nfallow > Ncc, then x = Nfallow, and vice versa. A positive RNE value from 0 to 1 indicated suppression of the weed by the CC; a RNE of 0 indicated no effect of the CC on the weed; and negative values (0 to −1) indicated facilitation of the weed by the CC (Smith et al., 2015).

In February 2016, six soil samples (4.8 cm diameter x 5 cm depth) were collected from each plot and air dried in the laboratory to assess the weed seedbank. Seeds were extracted following Malone methodology (Malone, 1967), with modifications in the dispersant solution preparation. After extraction, seeds were separated, identified and counted. Apparent seed viability was assessed by applying pressure (Ball and Miller, 1989).

2.5. Statistic analysis

Data were analyzed separately by year because significant interaction between year*treatment were observed for most of the variables studied.

Variables that were determined before April (weed density, weed diversity and topsoil WC in March) and weed seebank variables were analyzed with a one-way ANOVA, because only differences between barley, vetch and fallow treatments were assessed. Means were separated by Tukey test ($P < 0.05$). Normality and variance homogeneity assumptions were checked.

To assess the effect of the CC and the termination date factors in the variables CC biomass, weed biomass, soil water and soil inorganic N, weed density and diversity determined from April, a generalized mixed linear model (GLMM) was used. The CC treatment factor, the termination date factor and the interaction between them were included as fixed effects, whereas the plot was included as random effect.

Analyses were performed with the statistical software package R ver. 3.2.3. (R Core Team, 2015).

3. Results

3.1. Weather

The cumulative rainfall was ~300 mm, similar to the 30-year average, in both experimental periods but the seasonal distribution differed (Fig. 2). The first season, more precipitation occurred during autumn (165 mm) than spring (73 mm); while the second was characterized by a dry autumn (42 mm) and a rainy spring (187 mm). The

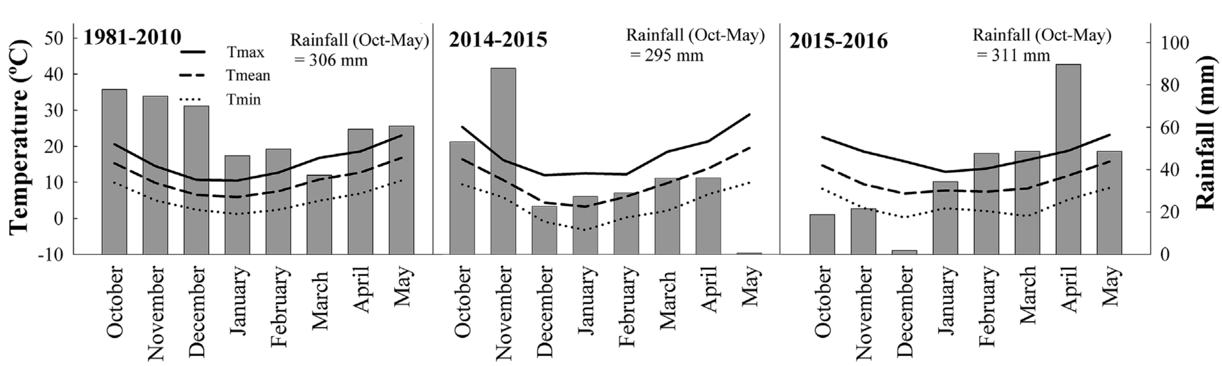

Fig. 2. Monthly rainfall (bars) and average maximum, mean and minimum temperature from 1981 to 2010 and during the study in Aranjuez (Madrid, Spain).

Table 1
Effect of the cover crop treatment (barley, vetch and fallow) and the termination date (FT, first termination; ST, second termination) on the cover crop biomass (kg DM ha^{-1}) and on the topsoil inorganic N (kg N ha^{-1}) measured in April, for each experimental year (means ± standard error).

	Cover crop biomass (kg DM ha^{-1})			Topsoil inorganic N, April (kg N ha^{-1})		
2014–2015						
barley	2474.2 ± 545.9 a			8.7 ± 1.1 a		
vetch	2888.7 ± 522.4 a			14.7 ± 2.0 b		
fallow	–			14.0 ± 0.8 ab		
FT	1466.7 ± 182.5 a			14.2 ± 1.3 b		
ST	3896.3 ± 264.1 b			10.8 ± 1.2 a		
	df	F	P	df	F	P
CC	1	1.2	0.322	2	5.5	0.028
TD	1	171.4	< .0001	1	14.0	0.005
CC x TD	1	0.0	0.967	2	3.5	0.075
2015–2016						
barley	1681.7 ± 144.4 b			7.2 ± 0.9 a		
vetch	1040.7 ± 35.9 a			26.5 ± 2.9 b		
fallow	–			17.6 ± 2.1 b		
FT	1092.6 ± 131.7 a			18.1 ± 3.0 a		
ST	1629.9 ± 159.2 b			16.1 ± 2.8 a		
	df	F	P	df	F	P
CC	1	28.7	0.002	2	15.5	0.001
TD	1	67.4	0.000	1	1.8	0.215
CC x TD	1	0.2	0.707	2	0.5	0.644

Within variable and year, degrees of freedom (df), F and *P* values obtained for the cover crop (CC) and the termination date factors (TD) and the interaction between them are presented. Letters indicate significant differences between treatments ($P < 0.05$, Tukey test.).

first season, the temperature was similar to the 30-year average, whereas in the second winter was warmer and spring cooler.

3.2. Cover crops: biomass and ground cover

In 2015, no differences between CC species in biomass were observed (Table 1). In 2016, values were lower than in 2015 and barley had higher biomass than vetch. Between termination dates, CC biomass increased 3-fold in 2015, whereas the increase was lower in 2016.

Regarding GC, in mid-March 2015 no differences were observed (51 ± 3%, df = 1, F = 2.7, $P = 0.1$) while in 2016 barley attained a higher GC (50%) than vetch (36%) (F = 28.9, $P < 0.0001$) (Fig. 3). After CC termination, residues attained a GC > 75%. At mid-May 2015, no differences were found in GC between CC species (74 ± 3%; df = 1, F = 1.4, $P = 0.3$) but in 2016, barley residue GC > vetch (43 vs. 20%; F = 26.8, $P < 0.005$). As observed for CC biomass, GC values were lower in 2016 than in 2015, and by mid-May 2016, both CC residues had a GC < 50%. Differences in GC increase between termination dates were found only in 2015 (vetch: F = 23.4, $P < 0.005$; barley: F = 8.8, $P < 0.05$). Nevertheless, both years ST residues maintained higher soil GC until mid-May (2015: $P < 0.005$, 2016: $P < 0.005$). Regarding fallow treatment, at mid-March weed coverage was < 6% (2015: 5 ± 1%; 2016: 6 ± 1%), and at mid-April < 20% (2015: 20 ± 2%; 2016: 12 ± 2%). By mid-May, the 38 ± 7% of fallow plots were covered by weeds in 2015, and 26 ± 5% in 2016.

3.3. Topsoil N availability and water content

At mid-April 2015, barley treatment showed a Nmin lower than vetch, and in 2016, a Nmin lower than vetch and fallow. Both CC had a lower Nmin (~40%) when terminated later (ST) but differences were only significant in 2015 (Table 1).

Topsoil WC results are presented in Fig. 4. In March, fallow had a higher topsoil WC than CC (df = 2; 27/03/2015: F = 15.0, $P < 0.005$;

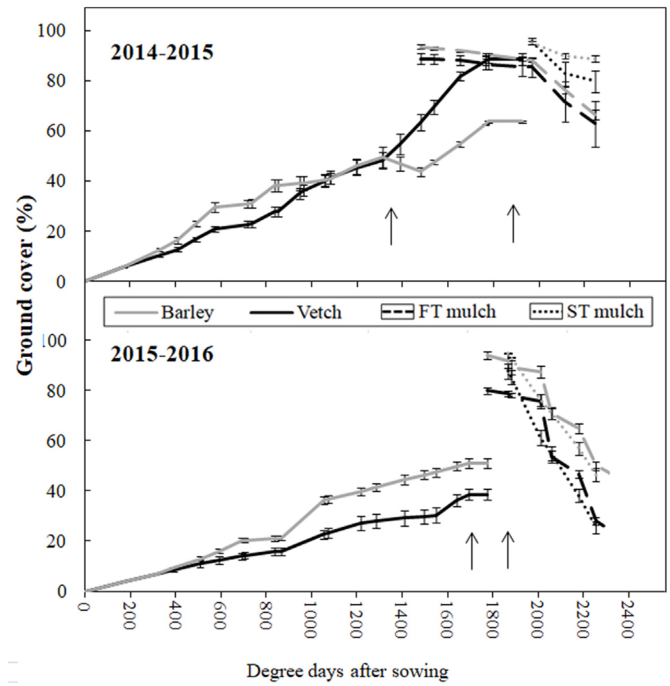

Fig. 3. Barley and vetch ground cover evolution, from early October to mid-May, in both experimental periods. Solid lines represent the living crop coverage and dashed lines represent residue cover. Arrows represent the first and second termination dates. Small bars represent the standard error.

17/03/2016: F = 9.6, $P < 0.01$). At late-April 2016, CC showed greater values than fallow (df = 2, F = 10.3, $P < 0.005$). Concerning the termination date, at the end of March, FT presented a higher topsoil WC than ST (df = 1; 27/03/2015: F = 24.7, $P < 0.001$; 31/03/2016: F = 29.8, $P < 0.0005$), and an interaction with the CC factor was observed: in barley and vetch, differences between termination dates were found, while not in fallow (df = 2; 2015: F = 10.3, $P < 0.005$; 2016: F = 27.4, $P < 0.0001$). An early termination showed as well a higher WC in May 2015 (F = 13.8, $P < 0.005$). The opposite effect, ST higher than FT was observed at the late-April sampling in 2016 (F = 7.2, $P < 0.05$).

3.4. Weed control

Weed biomass, weed density and diversity results are presented in Table 2.

Both years at CC termination, CC treatments presented a lower weed biomass than fallow (df = 2; 2015: F = 5.6, $P < 0.05$; 2016: F = 16.3, $P = 0.001$), and from an early to a late termination date, weed biomass increased (df = 1; 2015: F = 4.9, $P = 0.05$; 2016: df = 1, F = 34.1, $P < 0.0005$). An interaction between CC treatment and the termination date occurred in 2016 (F = 19.3, P < 0.001), explained by a higher weed biomass increase in fallow than in CC plots.

At mid-March 2015, weed density was larger in vetch and fallow compared to barley (df = 2; F = 12.4, $P < 0.005$), and in 2016, larger in vetch than in the others (F = 23.8, $P < 0.0005$). At mid-April, weed density was larger in fallow than in CC in 2015 (F = 11.8, $P < 0.005$), and greater than barley in 2016 (F = 4.0, $P = 0.05$). At mid-May, CC showed a lower weed density than fallow (2015: F = 6.0, $P < 0.05$; 2016: F = 7.3, $P = 0.01$). At mid-May 2015, differences between termination date treatments were found: FT weed density was 5-times larger than in ST (df = 1, F = 19.2, $P < 0.005$).

The Shannon diversity index was higher in fallow compared to barley, except in April and May 2016, in which no differences were observed.

Throughout the experiment, around 30 weed species were identified

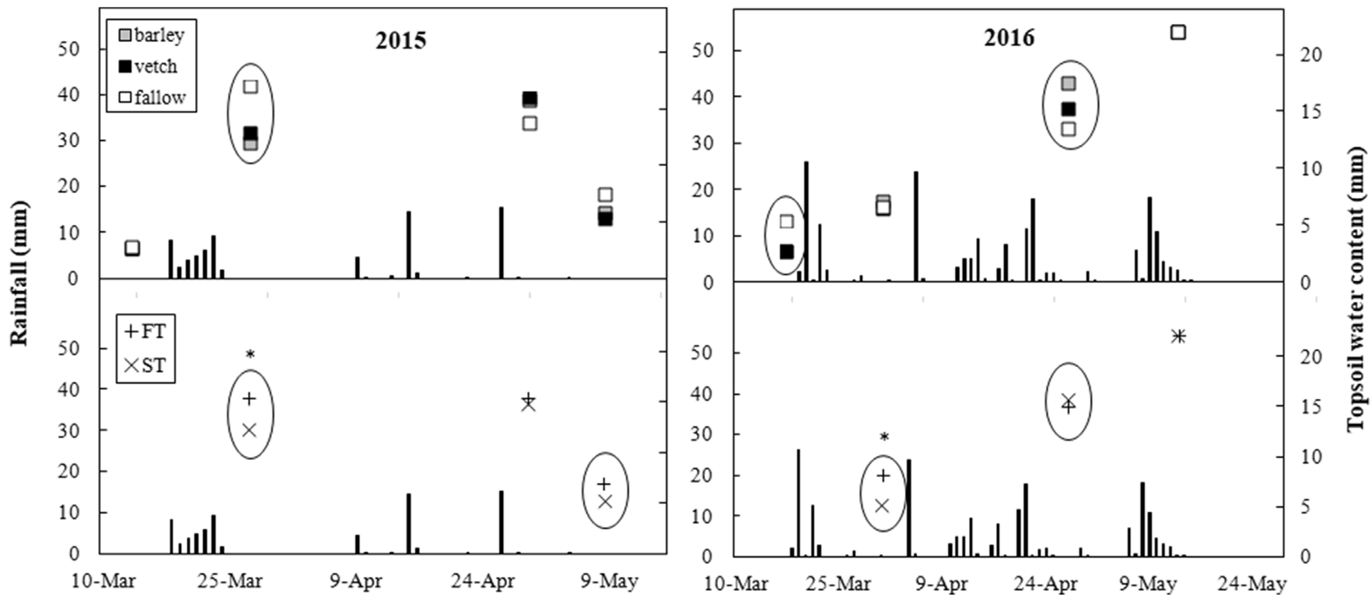

Fig. 4. Topsoil water content (mm), represented by small squares, measured at different times in spring for cover crop treatments (barley, vetch, fallow); and termination date treatments (FT, first termination date; and ST, second termination date) in the two experimental periods. Squares enclosed indicate differences between treatments, at P < 0.05, Tukey test. An asterisk indicate significant interaction between factors for that sampling date. Black bars indicate daily rainfall (mm).

(Table 3). There was a greater presence of dicotyledonous (73%), and annual species (87%). Spring-summer species represented 57%.

Through the two experimental periods, weed density changes were observed at a species level. At mid-March, vetch treatment had the largest *Lamium amplexicaule* L. density (df = 2; 2015: 5 vs. 0 seedling m^{-2}, F = 27.8, $P < 0.0005$; 2016: 15 vs. 1 seedl. m^{-2}, F = 31.8, $P < 0.0001$). At this time, fallow showed a higher density than barley of *Sonchus oleraceus* L. in 2015 (1.5 vs. 0 seedl. m^{-2}, F = 5.7, $P < 0.05$), and of *Senecio vulgaris* L. in 2016 (3.5 vs. 0 seedl. m^{-2}, F = 14.8, $P = 0.001$).

Later, by mid-April, *Xanthium spinosum* L. density was larger in fallow than in CC treatments (2015: 6 vs. 0 seedl. m^{-2}, F = 69.7, $P < 0.0001$; 2016: 8 vs. 1 seedl. m^{-2}, F = 12.1, $P < 0.005$). At this time some winter species, as *Convolvulus arvensis* L. both years, and *S. oleraceus* in 2015, showed a higher abundance in the treatment terminated later (ST) than in the terminated earlier (FT). In 2016, *L. amplexicaule* density was again larger in vetch than in other treatments (2 vs. 0 seedl. m^{-2}, F = 7.5, $P = 0.01$).

At mid-May, *X. spinosum* density was greater in fallow than in CC plots (6 vs. 2 seedl. m^{-2}, F = 19.2, $P < 0.001$), and greater than barley in 2016 (20 vs. 1 seedl. m^{-2}, F = 4.6, $P < 0.05$). In 2015, *Chenopodium album* L. density was larger in fallow than in barley (F = 4.4, $P < 0.05$). Concerning the effect of the termination date at mid-May, in 2015 *Setaria* spp., *X. spinosum* and *Ch. album* showed a higher weed density in FT plots than in ST plots (4 vs. 2 for *X. spinosum*; 56 vs. 21 for *Setaria* spp.; 4 vs. 1 seedl m^{-2} for *Ch. album*). In 2016, *Setaria* spp. showed the opposite effect (ST > FT, 38 > 18 seedl. m^{-2}, F = 7.3, $P = 0.02$).

At mid-May 2015, fallow and vetch had a greater proportion of annual broadleaf species compared to barley while in 2016, only fallow had a larger proportion of broadleaf species than barley (Table 4; Fig. 5). In 2015, barley showed a greater proportion of annual grasses than vetch and fallow, but only a trend was observed in 2016 ($P < 0.1$). No differences between treatments were found for perennial species. Concerning the termination date, no differences were observed in 2015. In 2016 the proportion of annual broadleaf species was higher in FT treatments than in ST, while the opposite was observed for the annual grasses group.

A total of 17 weed species were identified in the seedbank (Table 3). All of them were annual species, and 90% of summer weeds. A 2% of

Table 2
Effect of the cover crop treatment (barley, vetch and fallow) and the termination date (FT, first termination; ST, second termination) on the weed biomass measured at cover crop termination (kg DM ha^{-1}) and the weed density (seedling m^{-2}), and weed diversity determined at different times in spring (means ± standard error).

	Weed biomass (kg ha^{-1})	Weed density (seedling m^{-2})			Weed diversity (Shannon index)		
		mid-March	mid-April	mid-May	mid-March	mid-April	mid-May
2014–2015							
barley	10.5 ± 6.5 a	0.8 ± 0.6 a	10.5 ± 3.6 a	57.6 ± 13.6 a	0.0 ± 0.0 a	0.04 ± 0.04 a	0.42 ± 0.13 a
vetch	58.1 ± 21.2 a	8.0 ± 2.8 b	11.6 ± 3.4 a	59.6 ± 19.6 a	0.61 ± 0.45 ab	0.80 ± 0.26 ab	0.89 ± 0.12 ab
fallow	464.2 ± 230.5 b	7.3 ± 1.4 b	60.0 ± 3.8 b	159.6 ± 52.6 b	1.25 ± 0.39 b	1.36 ± 0.22 b	1.04 ± 0.15 b
FT	47.2 ± 19.1 a	–	28.9 ± 10.8 a	147.3 ± 33.1 b	–	0.54 ± 0.17 a	0.64 ± 0.12 a
ST	308.0 ± 161.9 a	–	25.8 ± 6.8 a	37.3 ± 10.1 a	–	0.92 ± 0.26 a	0.93 ± 0.13 a
2015–2016							
barley	1.1 ± 0.5 a	1.0 ± 0.8 a	4.3 ± 1.2 a	43.6 ± 5.8 a	0.16 ± 0.18 a	0.32 ± 0.14 a	1.06 ± 0.12 a
vetch	68.1 ± 26.8 a	19.3 ± 2.7 b	9.9 ± 3.6 ab	48.9 ± 7.4 a	0.54 ± 0.31 ab	0.84 ± 0.17 a	1.32 ± 0.18 a
fallow	237.2 ± 78.8 b	7.5 ± 2.0 a	13.4 ± 1.6 b	102.9 ± 18.9 b	1.19 ± 0.27 b	0.76 ± 0.23 a	1.43 ± 0.08 a
FT	33.1 ± 12.4 a	–	8.4 ± 1.7 a	56.4 ± 7.5 a	–	0.50 ± 0.15 a	1.35 ± 0.07 a
ST	171.1 ± 60.5 b	–	9.9 ± 2.5 a	73.8 ± 15.9 a	–	0.78 ± 0.16 a	1.19 ± 0.14 a

Within year and variable, letters indicate significant differences between treatment, at P < 0.05, Tukey test.

Table 3
Weed species observed through the study. Weeds are characterized as dicotyledonous (Dicot.) or monocotyledonous (Monoc.) and according to the life cycle.

Botanic name	Plant taxonomy	Life Cycle
**Abutilon theophrasti* Medicus	Dicot.	Annual
**Amaranthus* spp.	Dicot.	Annual
Anacyclus clavatus (Desf.) Pers.	Dicot.	Annual
Bromus diandrus Roth.	Monoc.	Annual
Capsella bursa-pastoris (L.) Medicus	Dicot.	Annual
**Chenopodium album* L.	Dicot.	Annual
Convolvulus arvensis L.	Dicot.	Perennial
Conyza bonariensis (L.) Cronquist	Dicot.	Annual / Biennial
Cyperus rotundus L.	Monoc.	Perennial
**Datura stramonium* L.	Dicot.	Annual
**Echinochloa crus-galli* (L.) Beauv.	Monoc.	Annual
**Fumaria officinalis* L.	Dicot.	Annual
Fumaria parviflora L.	Dicot.	Annual
**Lamium amplexicaule* L.	Dicot.	Annual
Malva sylvestris L.	Dicot.	Biennial / Perennial
Papaver rhoeas L.	Dicot.	Annual
Plantago lanceolata L.	Dicot.	Perennial
Polygonum convolvulus L.	Dicot.	Annual
**Polygonum aviculare* L.	Dicot.	Annual
**Portulaca oleracea* L.	Dicot.	Annual
Salsola kali L.	Dicot.	Annual
**Setaria* spp.	Monoc.	Annual
Senecio vulgaris L.	Monoc.	Annual
Sonchus oleraceus L.	Dicot.	Annual
Sorghum halepense (L.)	Monoc.	Perennial
Taraxacum gr. *officinale* Weber	Dicot.	Perennial
Veronica hederifolia L.	Dicot.	Annual
**Xanthium spinosum* L.	Dicot.	Annual
**Xanthium strumarium* L.	Dicot.	Annual

(*) indicate species present in the seedbank assessed.

Table 4
Effect of the cover crop treatment (barley, vetch and fallow) and the termination date (FT, first termination; ST, second termination) on the proportion (%) of annual broadleaf, annual grasses and perennial species groups on the weed density measured at mid-May (means ± standard error).

%	Annual broadleaf			Annual grasses		Perennial species	
2014–2015							
barley	2 ± 1 a			79 ± 8 b		19 ± 8 a	
vetch	18 ± 6 b			44 ± 11 a		38 ± 12 a	
fallow	25 ± 9 b			33 ± 9 a		42 ± 13 a	
FT	12 ± 6 a			53 ± 11 a		35 ± 11 a	
ST	18 ± 6 a			50 ± 8 a		32 ± 7 a	
	df	F	*P*	F	*P*	F	*P*
CC	2	13.4	0.002	8.6	0.008	1.4	0.291
TD	1	0.9	0.370	0.1	0.759	0.1	0.811
CC x TD	2	0.1	0.880	2.8	0.111	1.5	0.283
2015–2016							
barley	30 ± 6 a			60 ± 8 a		9 ± 5 a	
vetch	43 ± 8 ab			43 ± 9 a		13 ± 7 a	
fallow	57 ± 8 b			35 ± 6 a		9 ± 5 a	
FT	56 ± 5 b			34 ± 5 a		9 ± 5 a	
ST	31 ± 6 a			57 ± 7 b		12 ± 4 a	
	df	F	*P*	F	*P*	F	*P*
CC	2	5.9	0.023	3.4	0.079	0.2	0.855
TD	1	16.4	0.003	12.0	0.007	0.4	0.540
CC x TD	2	0.0	0.954	0.3	0.728	1.2	0.338

Within group and year, degrees of freedom (df), F and *P* values obtained for the cover crop (CC) and the termination date factors (TD) and the interaction between them are presented. Letters indicate significant differences between treatments ($P < 0.05$, Tukey test.).

seeds could not be identified. The seedbank density and diversity did not differ between treatments (Density: 4457 ± 378 seeds m^{-2}, F = 1.6, $P = 0.3$; Shannon index: 1.45 ± 0.06, F = 1.3, $P = 0.3$) but differences were found for some species (Table 5): *Xanthium* spp. density was lower in CC compared to fallow. *Setaria* spp. density was greater in barley. Vetch had the highest *Lamium amplexicaule* densities, and higher *Amaranthus* spp. *Portulaca oleracea* L. densities than fallow.

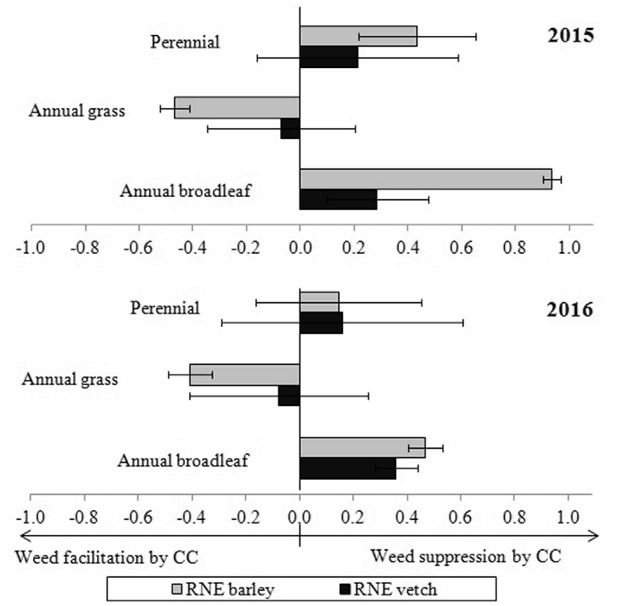

Fig. 5. Relative neighbor effect (RNE) of barley and vetch vs. fallow treatments for the three weed groups, calculated at mid-May each experimental year. Bars represent the standard error.

Table 5
Total seedbank density (seeds m^{-2}), diversity (Shannon index), and seedbank density for relevant weed species, for treatments: barley, vetch and fallow (mean ± standard error).

	Weed seedbank density (seeds m^{-2})		
	barley	vetch	fallow
Total density	5229 ± 925 a	4381 ± 739 a	3761 ± 183 a
Diversity (Shannon I.)	1.34 ± 0.12 a	1.45 ± 0.10 a	1.54 ± 0.11 a
Datura stramonium L.	92 ± 0 a	69 ± 26 a	46 ± 31 a
Polygonum aviculare L.	46 ± 31 a	23 ± 26 a	0 ± 0 a
Amaranthus spp.	1239 ± 218 ab	1583 ± 205 b	894 ± 67 a
Chenopodium album L.	849 ± 594 a	665 ± 576 a	872 ± 254 a
Setaria spp.	2408 ± 438 b	1193 ± 216 a	1124 ± 170 a
Portulaca oleracea L.	69 ± 51 ab	229 ± 68 b	46 ± 31 a
Echinochloa crus-galli L.	229 ± 140 a	92 ± 106 a	161 ± 67 a
Xanthium spp.	115 ± 51 a	69 ± 26 a	367 ± 97 b
Fumaria officinalis L.	23 ± 26 a	46 ± 31 a	69 ± 51 a
Lamium amplexicaule L.	0.0 ± 0 a	344 ± 100 b	0.0 ± 0 a

Species with rel. abundance > 10% are shown, and summed frequency of the species > 97%. Letters indicate significant differences between cover crop treatments, at $P < 0.05$, Tukey test.

4. Discussion

Our results showed that replacing a winter fallow by a cover crop, either common vetch or barley, in a long-term experiment located in a semiarid region had an effect on the weed density and diversity, and on the seedbank of some specific weed species. The termination date delay contributed to a better weed control in 2015, whereas in 2016 no differences between termination dates were found. The work sought to integrate different CC variables and changes in the topsoil WC and N availability that explained results partly.

During the winter period, barley CC achieved a great control as at mid-March weed biomass and density were almost negligible. Vetch reduced weed biomass but the weed density was similar to fallow. During this period the main driver of differences between CC species in weed control was soil N availability because both years Nmin was three times higher in vetch than in barley, but both CC had a similar CC biomass, GC and topsoil WC at this time in 2015. Besides, at this time weed coverage in fallow was < 3%. In agreement, various authors showed the winter weeds density increase to soil N levels. Increasing N application enhanced root and shoot weed biomass in a pot experiment (Blackshaw et al., 2003) and winter weed biomass in a field trial (Nelson, 2015).

In mid-April 2015, both CC reduced the weed density by 83% compared to fallow, but in 2016 barley showed a better weed control ability as barley reduced weed density by 69% compared to fallow, while vetch was similar to fallow. Climate conditions explained differences between species observed in weed density: in 2015, autumn precipitation favored a proper establishment and CC led to GC > 60% and a biomass > 3.5 Mg ha^{-1}. Under these conditions, both CC showed a similar weed control compared to fallow. The second year, low autumn precipitation hindered CC establishment, but barley recovered better than vetch, and maintained a low weed density by mid-April. The better aptitude of barley under dry conditions was already observed in past works (Gabriel and Quemada, 2011). In a 3-year study, Barberi and Mazzoncini (2001) found that weed biomass prior to CC termination was always lower in a rye treatment, while in legume CC only one of the years. In other study, Hayden et al. (2012) observed that grass CC achieved better winter weed control and less variable than a legume CC.

Other authors attributed a weaker or variable weed suppressive effect of legumes to the N added by N fixation (Blum et al., 1997). However, in our study vetch led to higher Nmin both years, even the first year in which no differences in weed control were found between species.

By mid-April, CC biomass was the main driver in the weed control. Results agreed with authors who indicated CC biomass as the main variable influencing weed control (Campiglia et al., 2010). Even more, in our study CC biomass was lower than the amount reported for most authors but it was still the main driver for weed control. Teasdale et al. (2007) reported in a review that more than 5 Mg ha^{-1} of CC residue was needed to achieve a complete weed control, and with 3–5 Mg ha^{-1} could not have an acceptable result. In our study, an acceptable weed control was achieved from ~2 Mg ha^{-1}, as was reported by Buchanan et al. (2016) in a vegetable production system. Nmin and weed density observed were not in line: vetch had higher Nmin but weed density was now higher in fallow. Results agreed with Wayman et al. (2015) that did not find correlation between Nmin increase and weed cover in spring. Besides, no differences were observed in topsoil WC.

By mid-May, both CC residues reduced weed density by 63% in 2015, and 55% in 2016, when compared to fallow, mainly over annual broadleaf species. It was expected a greater weed density reduction with barley residues that stayed longer in the surface, but differences between CC species were not observed.

Regardless of the accumulated biomass, after CC termination CC residues covered soil surface > 75%, and despite the faster legume decomposition (Fig. 3), both CC covered the soil sufficiently during the cash crop early stages to contribute to a weed density reduction compared to fallow.

At late-April 2016, CC mulch had a higher topsoil WC than fallow. Some authors attributed a variable weed control to the higher moisture under mulch that could enhance weed emergence (Teasdale and Daughtry, 1993). Nevertheless, we observed a larger weed density in fallow. Therefore, the final result was that in spring the mulch enhanced weed control and topsoil water content. It is worth noting that increasing soil WC storage by the mulch is beneficial for the cash crop that could benefit during the emergence. Thus, from our results we conclude that weed control until mid-April depended on CC species, but afterwards both CC residues achieved a similar control compared to fallow. Our results differed from Barberi and Mazzoncini (2001) who found that CC mulch did not reduce weed density compared to the control in spring. However, other findings in Mediterranean climates were in line with our results and both legume and grass CC mulches reduced weed density compared to fallow (Campiglia et al., 2012; Radicetti et al., 2013).

Through the two experimental seasons, barley showed a low weed diversity, mainly in 2015, when Shannon index values were significantly lower than vetch and fallow at different times in spring. Our results agreed with Campiglia et al. (2012) that observed lower diversity for oat compared to other CC treatments; but were opposed to other authors that did not observe effects in total weed diversity (Smith et al., 2015; Buchanan et al., 2016). Diversity differences between treatments indicated that weed species were not suppressed equally by both CC and this was confirmed in the effect observed in some specific species density. During the winter period the *L. amplexicaule* density was promoted by vetch, mainly attributed to N fixation. Nelson (2015) reported a great response of this species to N application. In spring 2015, barley reduced in a 90% the annual broadleaf group density compared to fallow and vetch, while in 2016 both CC species reduced the weed density but the decrease was lower than in 2015 (55%). Among this group, it was remarkable CC control over *X. spinosum*, considered a harmful weed in irrigated crops. Some studies were found about *X. spinosum* control with chemicals or bioherbicides (Arregui et al., 2001; Chittick and Auld, 2001), but no one focused on CC control. In spring 2015, *C. album*, a relevant harmful weed in summer cash crops, had a lower presence in barley, but we could not confirm it in 2016. Our first year results agreed with Sturm et al. (2016) who found in a greenhouse experiment that a winter grass CC reduced *C. album* density. At mid-May 2015, annual grasses proportion was larger in barley than in fallow and vetch, and both years perennial weeds were unaffected. Mirsky et al. (2011) did not found differences between CC species in annual grasses nor perennial weeds.

Long-term field experiments are very valuable to assess the effect of agricultural practices in the weed seedbank (Cardina et al., 2002). In previous mid-term studies, CC did not have a clear effect on the seedbank (Moonen and Barberi, 2004; Buchanan et al., 2016), so the question of the long-term effect remained open. In our study, even after 10 years of winter legume or grass CC we could not find an effect on the total seedbank density or diversity when comparing with the fallow, confirming the low effect of winter soil coverage on the seedbank. In contrast, other agricultural practices such inverted tillage are known to affect the seedbank (Moonen and Barberi, 2004).

In our experiment, even if there were not differences in total diversity, we observed that CC affected the presence of specific species in the seedbank. This was the case in *L. amplexicaule* promoted by vetch, and *Xanthium* spp. more present in fallow treatment. Moreover, these species-specific effects in the seedbank were on line with weed densities observed aboveground. Nevertheless, some differences between seedbank and aboveground weed densities were observed. The emergence of *Setaria* spp. and *P. oleracea* was delayed in CC plots one of the years, but in the seedbank were more abundant in CC treatments. This indicates that winter CC had a limited effect on controlling summer weed species: the residue mulch may delay weed density in the first phases of the cash crop but it may favor weed appearance later in the season. Also Moonen and Barberi (2004) observed that winter CC affected not only winter weed species but also species related to the summer cash crop. Overall, these results endorsed the relevant role of winter CC in an integrated weed management, even if CC are not expected to provide a complete weed control throughout the whole cash crop season (Teasdale et al., 2007). The termination date had an impact on weed variables but results were inconsistent through our study as they differed from year to year. Differences between FT and ST in weed biomass were found only in 2016 for barley and fallow. The general lack of differences agreed with other authors who also did not observe differences in weed biomass between CC termination dates (Duiker and Curran, 2005; Wayman et al., 2015). In terms of weed density, the effect was observed by mid-May 2015: CC continued growing intensively, and great differences in biomass and GC occurred between dates. Differences between years were attributed to climatic conditions: ~450ºCdays were cumulated between termination dates in 2015, whereas half in 2016 (Fig. 3). In line with our results, Mirsky et al. (2011) observed that the termination date delay led to cumulated biomass increase that resulted in a lower weed density.

Similarly for Nmin, differences between FT and ST were found only in 2015, in which the biomass increase between termination dates led to a higher soil Nmin depletion. Therefore, Nmin was considered a driver leading to changes in weed control, together with GC and biomass. In terms of topsoil WC, the termination date delay led to a higher soil water extraction, but later the mulch coming from the second termination date was not able to preserve more water and no differences were observed between termination date treatments. Differences in topsoil WC observed in both years were expected to explain weed density results, but weed differences appear only in one of the years. Therefore, in this experiment it could not be concluded that topsoil WC was a main driver in weed control. In regions with scarce water in spring, the termination date may have an effect not only on weed control but also on water availability for the subsequent cash crop, so it should be decided with caution (Alonso-Ayuso et al., 2014).

Specific-species changes were observed as response to the termination date although results were not maintained from year to year. The ST treatment reduced the density of certain broadleaf species, i.e. *X. spinosum* and *C. album*, and maintained a higher density of winter weed species. Mirsky et al. (2011) found as well an impact on certain broadleaf species (*C. album* or *Ambrosia artemisiifolia* L.) and in certain grasses (*Setaria* spp.), but not in perennial weeds.

This work contributes to increase the knowledge of CC management in Mediterranean semiarid regions, confirming the relevance of the termination date as it led to changes in the weed community among other factors. However, other management issues must be taken into account as the termination itself to compare chemical termination, currently in the spotlight, with other methods, in terms of cost effectiveness, environmental impact and economic sustainability.

5. Conclusions

Replacing winter fallow by cover crops in annual rotations had an impact on the weed density and should be considered an interesting tool for weed control that could reduce herbicide application. Barley suppressed winter weeds better than vetch but the mulch from both cover crop species was able to reduce weed density later in spring compared to fallow. Shannon diversity index was lower after barley CC throughout the experiment.

Nitrogen availability was the main driver leading differences between barley and vetch in winter weed control, while CC biomass and ground cover were drivers after CC termination. Cover crop biomass from 2 to 4 Mg ha^{-1} was enough to ensure a low weed density that would contribute to reduce chemical or physical weed treatments during the cash crop period compared to fallow. In spring, moisture preserved by the mulch that favored cash crop sowing was not a drawback for weed control.

After a 10-year crop rotation, with or without winter CC between consecutive irrigated summer cash crops, the weed seedbank density and Shannon diversity index were not affected by CC. However, seedbank results endorsed CC control of *X. spinosum* observed in the aboveground. The CC control was partial as *L. amplexicaule* seed density was larger in vetch, and *Setaria* spp. were more abundant in barley treatment seedbank.

The CC termination date had an impact on weed control one of the two years, in which biomass and ground cover increased substantially between dates. In that case, a late termination date delayed the weed emergence and increased diversity in the field, but entailed a topsoil water depletion that should be considered in water limited scenarios.

Climatic conditions played a relevant role as they were behind differences found each year between CC species and between termination dates. Therefore, more research is needed to understand the drivers of the CC: weed interaction and to design management practices leading to an optimal weed control in specific sites and scenarios.

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