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Mulch-Based No-Tillage Effects on Weed Community and Management in an Organic Vegetable System

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Abstract: Weeds can cooperate with the agroecosystem's functioning by providing ecosystem services. Effective weed management should mitigate negative weed–crop interference, while maintaining a functional and balanced weed community. In a two-year trial, the in-line/roller crimper (RC) was used to terminate an agroecological service crop (ASC; here barley, *Hordeum vulgare* L.) before organic zucchini (*Cucurbita pepo*, L.) and compared with green manure (GM) ASC and tilled no-ASC with Mater-Bi mulch on the rows (No_ASC). Zucchini yield, soil N availability, weed density/cover, biomass, and community composition were assessed. Analysis of variance, exploratory statistical analysis, and non-parametric inferential approaches were run, respectively, on agronomic data, species-specific weed frequencies, and Shannon diversity. Zucchini yield was the highest in No_ASC, due to soil N immobilization under high C:N barley residues in GM and RC. Multivariate analysis discriminated RC from tilled systems, outlining a specific *ensemble* of weed species correlated to Shannon diversity. From zucchini fruit set, RC selectively favored *Polygonum aviculare* L. and *Helminthotheca echioides* (L.), reasonably because of their oligotrophy and creeping habit. Their dominance finally caused low RC weed control. Results highlight strong weed selective pressure by the mulch-based no-tillage. Understanding the mechanisms underpinning the impact of soil management practices on weed community can drive towards a tailor-made and more effective weed management.

Keywords: agroecology; cover crops; ecological weed management; weed biodiversity; *Amaranthus retroflexus*; *Portulaca oleracea*

1. Introduction

Organic agroecosystems, from an agroecological perspective, should be managed to maintain and/or enhance ecological services provided by their functional elements (agrobiodiversity). For this reason, these systems are characterized by high complexity in terms of plant species biodiversity, both cultivated and weeds [1,2]. Hence, the maintenance of a balanced and diversified weed community, minimizing the predominance of any one species, plays a role in supporting biological diversity [3] and, consequently, regulating processes such as nutrient cycling and pest control, which determine functioning and resilience of agroecosystems at large [4]. Agronomic practices to manage soil and biodiversity shape the interaction between weed species, acting as a selective pressure in both the short- and long-term. In organic vegetable systems managed under rotation schemes, the use of agroecological service crops (ASCs; i.e., cover crops, catch crops, break crops, living mulch) is one of the main agroecological practices used to manage soil fertility and improve biodiversity in time and space, providing ecological services to the agroecosystem [5–7]. The ASCs can alter the weed community through direct species-specific interference (competition and allelopathy), as well as affecting resources availability (e.g., nutrients, light, water indirectly). Actually, ASCs may affect soil nitrogen (N) dynamics [8] through phenomena such as pre-emptive competition [9], N fixation in case of legumes [10], and N immobilization in case of high C:N ratio of the residues, e.g., in grass species [11]. These processes, besides the direct effects on vegetable crop nutrition and yields, could alter the soil seed-bank composition in the long run, driving the weed community toward a positive response to N (i.e., prevalence of nitrophilous weed species) or to a low-N suite of traits [12].

After ASC killing, the weed community can be driven towards different responses, depending on the chosen termination strategy. The way to terminate ASCs determines indeed the soil tillage management, acknowledged as one of the main drivers influencing the composition of the weed flora in terms of density and diversity, even more so than crop rotations [13]. Soil tillage incorporating the ASC biomass into the soil as green manure (GM) is certainly the most common strategy in organic farming. After termination, the ecological niche previously occupied by the ASC becomes available for weed development. However, green manure decaying residues may affect weeds through physical, biotic, and allelopathic interactions [14]. According to soil conditions (e.g., temperature, moisture, and microbial activity), allelopathic compounds in crop residues could be released into the soil and selectively affect weed emergence [15]. Moreover, tillage practice changes weed seed depth in the soil, which plays a role in weed species shifts [16]. As an alternative to termination of ASCs by green manure, the conservation tillage strategy named in-line/roller crimper (RC) has been identified as a feasible option to terminate ASCs and simultaneously prepare the transplanting bed for the following vegetable cultivation [6,17,18]. The RC allows to flatten the ASC, leaving the soil undisturbed and obtaining a natural mulch generally able to contrast seedling emergence and delay the development of spontaneous plants. Despite several studies evaluating the RC implications on cash crop yield and weed control, little is reported about its effects on weed community composition shift, both in the short- and long-term. In the literature, information on the effects of the RC on vegetable yield are contrasting, depending on several factors such as the pedoclimatic conditions, the ASC, and cash crop species and cultivar. Particularly, the choice of cash crop cultivar suitable for no-till soil management is fundamental to ensure yield maintenance [19]. For what concerns weed control, the literature reports strong evidence of the suppressive ability of RC against weeds, particularly when high ASC biomass is produced [6,17,20,21]. Besides these effects, several features of the no-till RC technique can also potentially have an impact on weed community composition. In comparison with tilled systems, one of the critical issues of the reduced/no-tillage practice is related to the potential colonization of perennial weed species [22,23], commonly perceived as difficult to manage, because of the seed accumulation at the soil surface and reduced disturbance of vegetative propagules [24–26]. Similarly, wind-dispersed seed species and plants with low seed longevity, unable to emerge from deep soil layers, take advantage from no-till management [27]. Prostrate and creeping weeds can generally escape mowing due to their habits [28], being therefore also reasonably more adaptable to survive the flattening than erect species and grow through the textures of the ASC mulch.

In this study, a *Poacea*, barley (*Hordeum vulgare* L.) was introduced as the ASC before zucchini cash crop (*Cucurbita pepo* L.) in an organically-managed cropping system. Two termination strategies, till green manure and the no-till RC, were tested and compared with a control (tilled, no_ASC treatment, with Mater-Bi mulch on the zucchini rows) in a two-year experiment. We assumed that soil management (ASC presence and soil tillage) would influence the system performance in terms of crop yield, soil N availability, weed development, and community composition, in view of the mechanisms above suggested. We specifically hypothesize that: (i) Use of the barley as the ASC would reduce the zucchini yield due to pre-emptive competition and N immobilization, in comparison to the no_ASC system; (ii) no-till RC would mitigate zucchini yield reduction in respect to GM; (iii) RC would reduce the weed biomass and density in respect to the no_ASC and the GM treatments; and, finally, (iv) RC would differentiate weed community in respect to tilled GM and no_ASC, shifting towards weeds with ecological profiles more favored to mulch presence and no-till conditions.

2. Materials and Methods

2.1. Site, Climate, and Soil

A two-year field experiment was carried out in 2014–2015 at the MOVE-LTE (MOnsampolo VEgetables Long-Term organic Experiment) in central Italy (42°53′ N, 13°48′ E). The site is characterized by a "thermo-Mediterranean" climate [29], with an average total annual precipitation of 564 mm and temperatures averaging 9 °C and 20 °C in the October–March and April–September growing periods, respectively. Mean monthly temperatures and rainfall during the studied period (October 2013–August 2015), compared with the long-term average values (30 years), are shown in Figure 1.

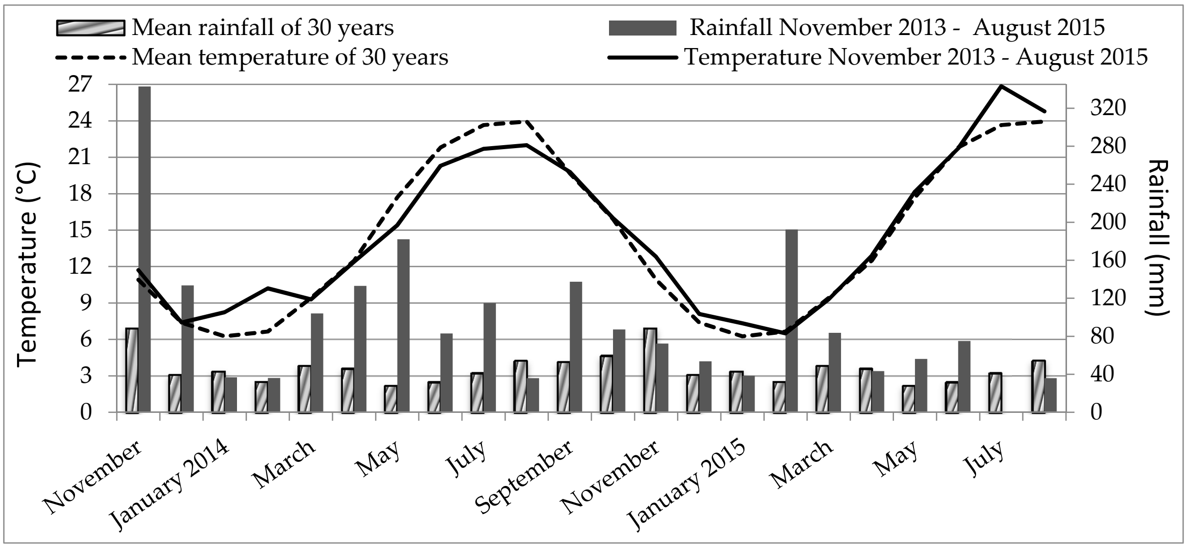


Figure 1. Mean monthly temperature and rainfall of the experimental trial during the barley and zucchini cycles compared with the mean long-term values (30 years).

2.2. Experiment Setup and Treatments

Barley (cv. Trasimeno) ASC was cultivated before zucchini (cv. Zuboda) in a randomized block design (RBD) with three blocks. The vetch (*Vicia villosa* Roth) green manure followed by tomato (*Solanum lycopersicum* L.) preceded barley, while zucchini replaced melon (*Cucumis melo* L.) in the rotation of the MOVE-LTE [30]. The main factor was the soil management (Man): (i) Without ASC or control (No_ASC), tilled at ASC termination and mulched with Mater-Bi, a commercial thermoplastic matrix based on starch, on the rows; (ii) ASC chopped and incorporated with a rotary disk as green manure (GM); and (iii) ASC terminated with the no-till strategy in-line/roller crimper (RC). Barley was sown on 31 and 21 October in 2013 and 2014, respectively, at a rate of 200 kg ha^{-1}, and it was terminated on 30 and 20 April in 2014 and 2015, respectively, at full flowering stage. Detailed information about the machinery utilized for the RC is reported in Canali et al. [17]. The No_ASC and GM plots (7 × 8 m) were tilled with a rotary tiller (DL 2500; Maschio SPA, Padua, Italy) to a depth of 15 cm and disked (15-cm-deep) according to standard agronomic practices used by organic farmers in the area, thus ensuring full ASC incorporation into the soil in the GM treatment and a properly prepared field in the No_ASC one.

Zucchini seedlings were 3 weeks old and were hand-transplanted at an inter-row × row distance spacing of 1.5 × 0.8 m (0.83 plants m^{-2}) on 6 and 8 May in 2014 and 2015, respectively. The zucchini harvest started on 6 and 5 June and was completed on 13 and 3 August in 2014 and 2015, respectively, with a cropping cycle of 98 days in 2014 and 89 days in 2015. Weed management was carried out in accordance with the common/standard practice of the area, with mowing in the inter-rows on 10 June and 3 July in 2014 and on 28 May and 30 July in 2015. In each experimental year, hand weeding on the rows was also carried out during the first mowing operations. In 2014 and 2015, 1770 and 2150 m^3 ha^{-1} were distributed by micropipe irrigation, respectively. At the time of zucchini transplanting, the trial was fertilized using off-farm animal manure-based organic fertilizers (Superstallatico—Nuova Concimer, S. Severino Marche, MC, Italy) permitted for use in organic farming according to the European regulation in force, corresponding to 40 kg ha^{-1} of N distributed just to the crop plants. An additional 10.5 and 8.5 kg ha^{-1} of N and K_2O, respectively, were distributed by drip fertigation along the cropping cycle (Goldust—Ico-hydro srl, Mutignano, BA; Prodigy 4 and Prokton—Intrachem Bio Italia, Grassobbio, BG).

2.3. Measurements

2.3.1. ASC and Cash Crop Yields

At ASC termination, aboveground biomass of barley was measured by mowing all the plant biomass at ground level within a half-meter-square area. Throughout the season, all mature zucchini fruits were collected, sampling the three central zucchini plants from each plot, and selected according to local market standards to obtain fresh marketable yield. At each harvest time, an aliquot of the fruit samples was subsampled at plot level and frozen to constitute the final samples. The ASC fresh biomasses and the final thawed fruit samples were dried at 105 °C for 24 h to obtain dry weight.

2.3.2. Soil Mineral Nitrogen

Soil mineral N (SMN; NO_3^-–N + NH_4^+–N, recorded at 0–30 cm soil depth) was also determined at 13, 23, 49, and 90 days after zucchini transplanting (DAT) in 2014 and at 11, 13, 44, and 88 DAT in 2015. These DAT corresponded to barley termination, zucchini start of flowering, zucchini fruit set, and harvest. The samplings were done in the inter-rows, near the zucchini plants. In the second year of the experiment, an in-depth study was conducted and SMN was also measured under the Mater-Bi mulch of the control (on the rows). The SMN was extracted by 2 M KCl (1:10, w/v) and measured by continual flow colorimetry according to Krom [31] and Henriksen and Selmer-Olsen [32] for NH_4^+–N and NO_3^-–N, respectively. All of the soil laboratory tests were carried out in triplicate to control intra-laboratory variability.

2.3.3. Weed Biomass and Density

Weed total density was recorded at 35 and 20 days after soil management operations (barley termination/green manure/tillage of the control) in 2014 and 2015, respectively, corresponding to zucchini start of flowering. In each plot, the weed density was recorded by using a 0.25 × 0.25 m frame with three replications per plot. Moreover, at zucchini fruit set, at 62 and 55 days after soil operations in 2014 and 2015, respectively, weed aboveground biomass was collected within a 1.0 × 0.5 m frame, before the second weed control. Each weed sampling was performed in the inter-rows, close to the zucchini plants, before the weeding procedures.

An aliquot of all the sampled biomasses were dried at 105 °C for 24 h to obtain dry weight.

2.3.4. Ecological Characterization of Weed Community

To analyze weed community, species occurrence (frequency) was estimated in each plot with species-specific density samplings performed when weeds had similar weed density in the two years, namely at 62 and 24 days after termination in 2014 and 2015, respectively. Moreover, at zucchini fruit set and final harvest, species-specific weed cover abundance was assessed by visual estimation according to the Braun–Blanquet scale [33], as modified by Pignatti [34]. Each Braun–Blanquet class was converted to its midpoint cover value and graphed as cover according to Wikum and Shanholtzer [35].

The weed species-specific density was recorded by using a 0.25 × 0.25 m frame with three replications in 2014 and by a 0.25 × 0.25 m frame with six replications in 2015, while weed species-specific cover was assessed with 1 × 1 m frame with three replications in both the years. The weed samplings were performed in the inter-rows.

The weed community diversity was assessed by calculating Shannon Weaver index [36]:

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

where: "pi" = proportion of a given species relative to the total from all species found in the i-th sample; "ln pi" = natural logarithm of pi; "s" = number of species found in the i-th sample.

Moreover, with the aim to assess the potential impact of the different soil management strategies on weed community structure, weeds were categorized according to life-forms derived from Raunkier biological types (therophytes, geophytes, and hemicryptophytes) [37]. The mean cover-weighted Ellenberg Nitrogen (N), as revised by Pignatti [38] and extensively used as indirect indicators of abiotic conditions [39], here the soil nitrogen availability, was associated with the recorded species. Furthermore, weeds were categorized on the basis of their growth habit [40].

2.4. Statistical Analysis

Results related to SMN, barley biomass, total zucchini yield, and total weed density and biomass were analyzed using univariate analysis of variance (ANOVA) considering year (Y) as the random factor and soil management (Man) as the main factor. Mean comparison was carried out according to the least square difference (LSD) statistic and the Duncan multiple range test (DMRT), respectively, for two and more than two comparisons, testing for significance at $p \leq 0.05$. A principal component analysis (PCA) was run on a total of 17 variables (16 weed species *plus* the related Shannon H' diversity index) calculated for 36 treatments (RC, GM, and fallow (FA)) with the aim to characterize weed ecological niches (using weed species–species proportion in total sample). A biplot (weed species vs. treatments) considering the two most important components was generated with the aim to identify the latent relationship between cases (treatments) and variables (weed species). The direction and length of the arrows in the biplot indicate the direction and magnitude in which each variable contributes to the location of each case in the plot. The angle between each arrow and the axes is inversely proportional to the correlation between each variable and the axes constructed in the biplot. Non-metric multidimensional scaling (NMDS) and hierarchical clustering (using Euclidean distances' metric and Ward's agglomeration rule) were also run with the aim to assess similarity among the three soil management systems based on the weed. Both techniques were run on the original data matrix (17 variables × 36 cases, see PCA) after variable standardization. More specifically, hierarchical clustering using two separate dendrograms allowed discrimination of (i) RC treatments (I and II years) from tilled (GM and FA) treatments, and (ii) identified similarities among weed species. The NMDS discriminated the first year from the second year RC treatments, in turn illustrating the multivariate relationship between treatment type and the level of biodiversity (H' Shannon diversity index).

Two pair-wise correlation approaches (using both Pearson moment-product coefficients and Spearman rank coefficients) were used to assess the contribution of each species to community diversity (Shannon H' diversity index), testing for significance at $p < 0.05$ after Bonferroni's correction for multiple comparisons. Analysis of correlation coefficients outline the pair-wise relationship between Shannon H' diversity and the binary pattern of presence/absence of each species recorded in the samples. Pearson and Spearman coefficients, respectively, revealed linear and non-linear correlations in the sample. Results indicate species characterizing diversified populations and other species reflecting less diversified populations (i.e., species with exclusive or dominant niche). The presence of a significant, non-linear association among biodiversity and species reflects complex and unpredictable ecological patterns that are more difficult to practically manage. Pearson and Spearman coefficients assume a similar test statistic and significance level for linear relationships; a marked difference between these two coefficients indicates a more complex form of association between the corresponding variables. Statistical analysis was carried out using Statistical Package for Social Science (SPSS) 16.0 and PAST (3×. Oslo, Norway) packages.

3. Results

Climatic variability was rather high over the two study years (Figure 1).

The rainfall during the first experimental year was 786 mm during ASC cycle (from November 2013 to April 2014) and 416 mm during zucchini cycle (from May to July 2014), which are considered high according to historically recorded data for the same periods (297 and 155 mm, respectively). The mean air temperatures were 9.9 °C during the ASC cycle and 19.9 °C during zucchini cycle, rather close to the historically recorded mean values (8.9 and 21.8 °C, respectively). However, in February, a value up to 3.6 °C higher than the long-term trend was observed, while in July, mean air temperatures were 1.9 °C lower than the long-term mean. In the second experimental year (2014–2015), rainfall was 484 mm during the ASC cycle and 168 during zucchini cycle. Wheatear variability was high; in February and March in particular, rainfall reached 276 mm with respect to the mean historically recorded value of 81, and in May and June it was about twice (131 mm) the mean trend (60 mm), followed by a month (July) with no rainfall. During the whole second year of the trial, air temperatures were close to the long-term mean (9.5 and 22.9 °C, the mean values during the ASC and zucchini cycles, respectively) except for July, with values up to 3.2 °C higher.

3.1. ASC and Cash Crop Yields

The ANOVA results for the barley biomass, zucchini fruit marketable yields, weed biomass and density are reported in Table 1.

Table 1. Barley biomass, zucchini marketable yield, and weed density and biomass, 2014–2015.

	Levels	Barley Biomass		Zucchini Yield		Weed Biomass		Weed Density	
				Mg ha^{-1} (dry matter)				N m^{-2}	
Y [1]	2014	16.86	a	17.13	b	0.93		33.91	
	2015	10.52	b	22.11	a	1.23		79.26	
	Sig.	*		**		n.s.		n.s.	
Man [2]	No_ASC [3]			33.25	a	0.45	b	88.33	
	GM [4]			17.00	b	0.97	b	70.22	
	RC [5]			8.60	c	1.81	a	84.67	
	Sig.			***		**		n.s.	
Y × Man	No_ASC I			31.28	a			113.33	ab
	GM I			11.53	c			96.00	ab
	RC I			8.57	c			41.33	b
	No_ASC II			35.22	a			63.33	ab
	GM II			22.47	b			44.44	b
	RC II			8.64	c			128.00	a
	Sig.			*		n.s.		*	

Note: The mean values in each column followed by different Roman letters are significantly different according to LSD and DMRT (for two and more than two comparisons, respectively), at the reported probability level. n.s., not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. [1] Year; [2] Soil management; [3] Fallow; [4] Green manure; [5] Roller crimper.

The Y × Man interaction was significant for zucchini yield and weed density.

At termination, barley above ground biomass was significantly higher in 2014 than 2015 (+37.6%). Zucchini yield was significantly higher in No_ASC than other treatments. In particular, GM in the first experimental year (GM I) and RC in both the years (RC I, RC II) had the lowest zucchini yields, while GM II experienced intermediate values. Weed above ground biomass was generally lower in 2014 than 2015 and followed the trend RC > GM > No_ASC, with a significant difference between RC and No_ASC. For what concerns weed density, RC II had the highest value, with significant differences with respect to RC I and GM II. In both years, GM showed a decreasing trend in weed emergence compared to No_ASC (not significant differences).

3.2. Soil Mineral Nitrogen

The SMN did not show any significant Y × Man interaction at all the considered phenological stages (Table 2).

Table 2. Soil mineral N (SMN) during zucchini cycle (2014–2015).

Factor	Level	SMN (mg kg^{-1})					
		Barley Termination	Zucchini Flowering		Zucchini Fruit Set	Zucchini Harvest	
	DAT [3] 2014/2015	−13/−11	23/13		49/44	90/88	
Y [1]	2014	26.76	14.24	b	26.34	16.75	b
	2015	24.12	61.33	a	28.72	40.53	a
	Sig.	n.s.	***		n.s.	***	
Man [2]	No_ASC [4]	28.80	47.27	a	24.47	26.82	
	GM [5]	24.15	29.22	b	32.96	28.85	
	RC [6]	23.37	36.86	b	25.16	30.26	
	Sig.	n.s.	**		n.s.	n.s.	
Y × Man	Sig.	n.s.	n.s.		n.s.	n.s.	

Note: The mean values in each column followed by different Roman letters are significantly different according to LSD and DMRT (for two and more than two comparisons, respectively), at the reported probability level. n.s., not significant; ** $p \leq 0.01$; *** $p \leq 0.001$. [1] Year; [2] Soil management; [3] Days after transplanting; [4] Fallow; [5] Green manure; [6] Roller crimper.

At barley termination, soil N availability was the same in both the years of the trial. Conversely, Y affected SMN content during the zucchini cycle, with mean values significantly higher in 2015 than 2014 at zucchini fruit set and harvest (+331% and +142%, respectively). As far as Man factor is concerned, at barley termination, a trend of lower SMN values in barley treatments with respect to No_ASC was observed. The Man factor significantly affected SMN at zucchini start of flowering, where barley presence reduced SMN with respect to No_ASC, regardless of the termination method. At zucchini fruit set, SMN values decreased in No_ASC and RC treatments with respect to the previous stage, while remaining constant in GM, the differences among treatments being not statistically significant. At zucchini harvest, similar SMN values were recorded among the three treatments. In the second year of the experiment, the SMN measured under the plastic mulch of the control was not significantly different from No_ASC values in each phenological stage (data not shown).

3.3. Ecological Characterization of Weed Community

Weed presence under the three tillage systems was reported and classified into biological groups (BG), habit, and Ellenberg N-score, for the two-year experiment (Table 3).

3.3.1. Principal Component Analysis

The PCA extracted two components accounting for 32.9% of the total variability in the sample dataset (the first axis explained 18.0% of the total matrix variance). Ordination of the studied weed species along Components 1 and 2 is shown in Figure 2.

Table 3. Ecological characterization of weed communities (2014–2015).

Species	Common English Name	EPPO [1] Code	Raunkiaer BG [2]	Habit	N [12]	I Year	II Year
Amaranthus retroflexus L.	Redroot pigweed	AMARE	T [3] scap [7]	Erect	9	*	*
Anagallis arvensis L.	Scarlet pimpernel	ANGAR	T rept [8]	Prostrate	6	*	*
Beta vulgaris L.	Beet	BEAVX	H [4] scap	Erect	5		*
Cichorium intybus L.	Common chicory	CICIN	H scap	Erect	5	*	
Convolvulus arvensis L.	Field bindweed	CONAR	G [5] rhiz [9]	Prostrate/Creeping	5		*
Echinocloa crus-galli (L.) P. Beauv.	Cockspur	ECHCG	T scap	Erect	8	*	
Hedera helix L.	Common ivy	HEEHE	P [6] lian [10]	Creeping	x		*
Helminthotheca echioides (L.) Holub	Bristly oxtongue	PICEC	T scap	Erect	2		*
Lolium spp	Tufted grass	LOLSS	T scap	Erect	6		*
Matricaria chamomilla L.	Chamomile	MATCH	T scap	Erect	5		*
Plantago lanceolata L.	Ribwort plantain	PLALA	H ros [11]	Erect	x		*
Polygonum aviculare L.	Common knotgrass	POLAV	T rept	Prostrate/Creeping	1	*	*
Portulaca oleracea L	Common purslane	POROL	T scap	Prostrate/Creeping	7	*	*
Rumex crispus L.	Curly dock	RUMCR	H scap	Erect	5	*	*
Setaria viridis L.	Green foxtail	SETVI	T scap	Erect	7		*
Sonchus oleraceus L.	Common sowthistle	SONOL	T scap	Erect	6	*	*

[1] European and Mediterranean Plant Protection Organization; [2] Raunkiaer biological group; [3] Therophytes; [4] Hemicryptophytes; [5] Geophytes; [6] Phanerophytes; [7] Scapose; [8] Reptant; [9] Rhizomatous; [10] Liane; [11] Rosulate; [12] Ellenberg N-score. Asterisks indicate the presence of the species in the associated experimental year.

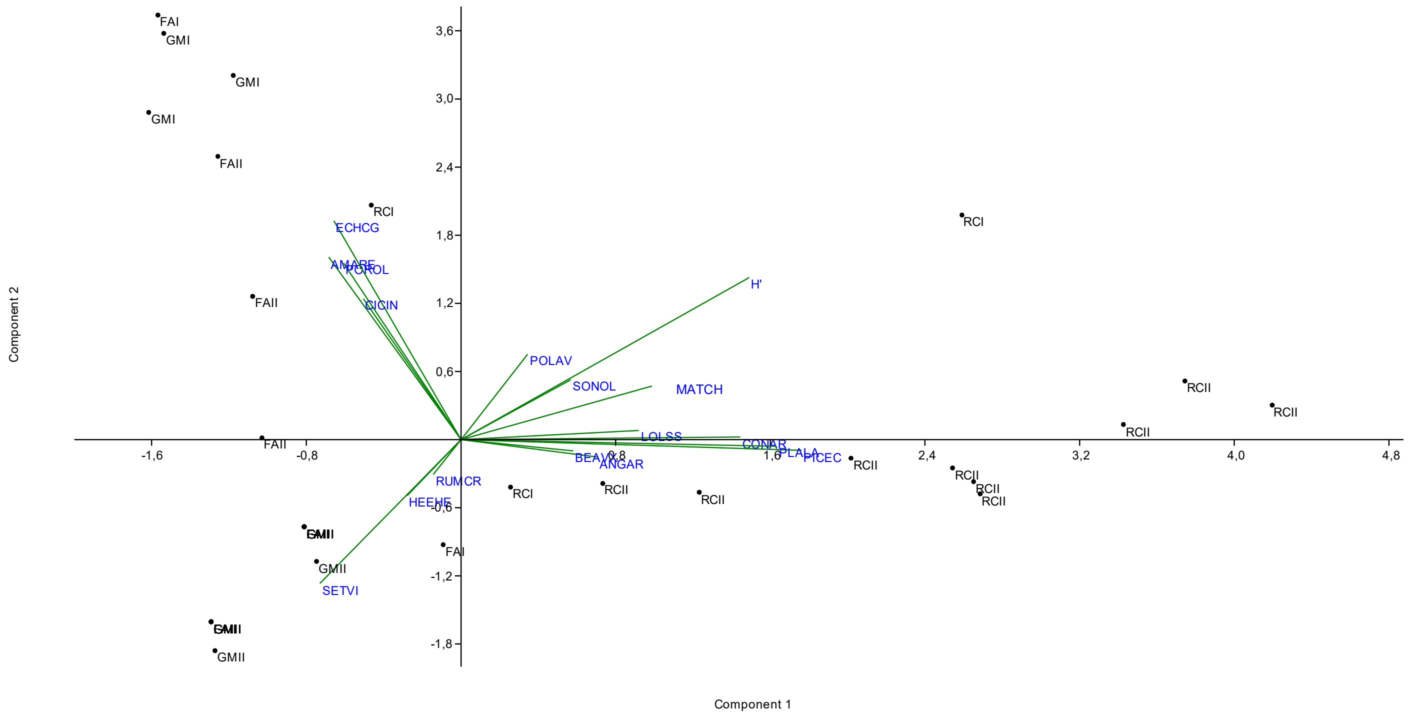
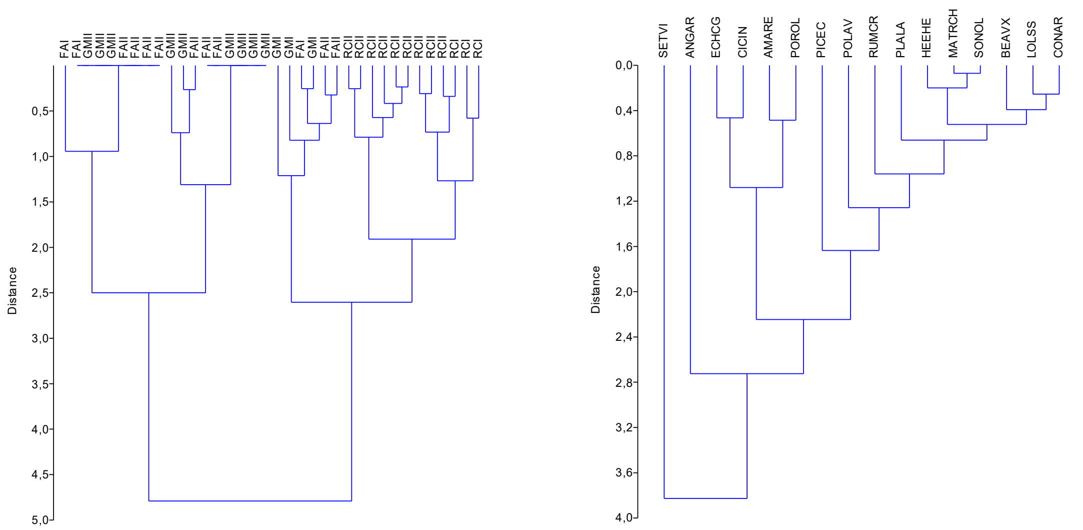


Figure 2. PCA biplot ordering the experimental trials relative to weed frequency and Shannon diversity index (H′). FA, fallow; GM, green manure; RC, in-line/roller crimper.

Soil tillage systems were discriminated based on weed frequency. The first component separated the RC system from GM and No_ASC, and was associated with the following species: *Helminthotheca echioides* (L.) Hollub (PICEC), *Convolvulus arvensis* L. (CONAR), *Plantago lanceolate* L. (PLALA), *Anagallis arvensis* L. (ANGAR), *Beta vulgaris* L. (BEAVX), *Matricaria chamomilla* L. (MATCH), *Sonchus oleraceus* L. (SONOL), and *Polygonum aviculare* L. (POLAV). These species positively contributed to the global diversity of the sample (Shannon H′ diversity). The species *Cichorium intibus* L. (CICIN), *Portulaca oleracea* L. (POROL), *Amaranthus retroflexus* L. (AMARE), and *Echinocloa cruss-galli* L. (ECHCG) were correlated with No_ASC and GM systems and uncorrelated with Shannon H′ diversity. *Setaria viridis* L. (SETVI) was not correlated with either species groups.

3.3.2. Hierarchical Clustering

Results of hierarchical clustering (Figure 3a) contributed to discriminate RC treatments from tilled ones. RC samples were grouped together, and were rather clearly segregated from the major cluster mixing No_ASC and GM. A second clustering run on species frequency (Figure 3b) confirmed basic PCA results, grouping the species PICEC, POLAV, *Rumex crispus* L. (RUMCR), PLALA, *Hedera elix* L. (HEEHE), and MATCH. At the same time, this dendogram confirmed the distinctive spatial distribution of SETVI with respect to the other species. Moreover, a similar spatial distribution was observed for AMARE, POROL, CHICIN, and ECHCG.



(a) (b)

Figure 3. Hierarchical clustering run on (**a**) different treatment types (tillage systems) and (**b**) weed species. FA, fallow; GM, green manure; RC, in-line/roller crimper.

3.3.3. Non-Metric Multi-Dimensional Scaling

The NMDS analysis discriminated RC treatments in respect with the other variables (Figure 4). Line vectors running from the centroid of the NMDS ordination plot to each individual treatment illustrate pair-wise correlations between Shannon H′ diversity and each of the plant samples in the treatments. The line vector highlights a latent shift of H′ toward RC, indicating that species diversity increases in samples with the greatest association with RC, particularly RC I. The No_ASC and GM treatments were less clearly separated from the rest of the sample.

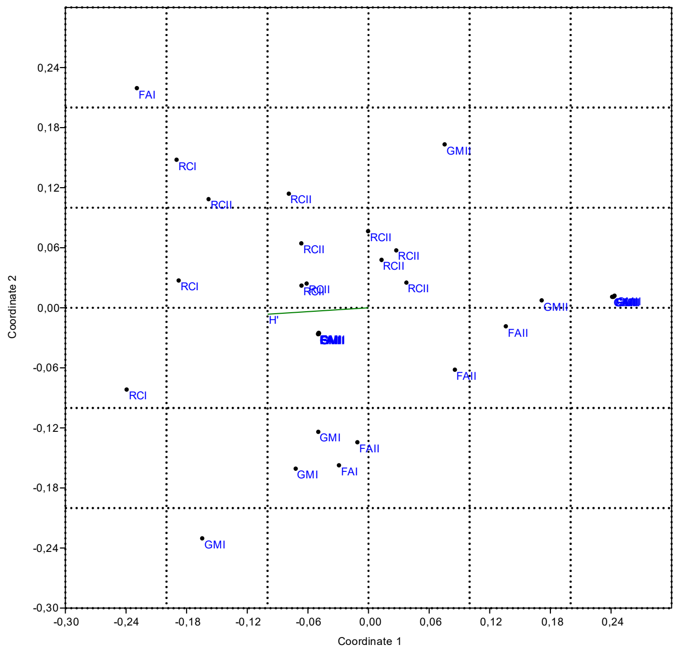


Figure 4. Two-dimensional non-metric multi-dimensional scaling ordination of weed frequencies/biodiversity (Shannon H′ diversity index) and tillage systems. FA, fallow; GM, green manure; RC, in-line/roller crimper.

3.3.4. Correlation Analysis

Pearson and Spearman correlation coefficients are reported in Table 4. This analysis outlines positive and significant pair-wise correlations between H′ and AMARE, ANGAR, CONAR, MATCH, PICEC, PLALA, and POROL. Such results suggest that these species contribute the most to the sample's biodiversity; in other words, these species were systematically found in samples with high biodiversity. The presence of species associated to biodiversity in the studied systems was quite different if considering linear or non-linear relationships; more specifically, ANGAR, AMARE and MATCH showed a non-linear correlation with the sample's biodiversity.

Table 4. Pearson and Spearman correlation coefficients between weed frequency and Shannon H′ diversity index (bold indicates significant coefficients at $p < 0.05$ after Bonferroni's correction for multiple comparisons).

Species	EPPO [1] Code	Pearson	Spearman
Amaranthus retroflexus L.	AMARE	0.30	**0.33**
Anagallis arvensis L.	ANGAR	0.14	**0.39**
Beta vulgaris L.	BEAVX	0.20	0.21
Cichorium intybus L.	CICIN	0.10	0.19
Convolvulus arvensis L.	CONAR	**0.43**	**0.53**
Echinocloa crus-galli (L.) P. Beauv.	ECHCG	0.21	0.21
Hedera helix L.	HEEHE	−0.02	−0.04
Helminthotheca echioides (L.) Holub	LOLSS	0.29	0.29
Lolium spp	MATCH	0.30	**0.33**
Matricaria chamomilla L.	PICEC	**0.60**	**0.65**
Plantago lanceolata L.	PLALA	**0.49**	**0.61**
Polygonum aviculare L.	POLAV	0.15	0.25
Portulaca oleracea L	POROL	**0.32**	**0.31**
Rumex crispus L.	RUMCR	0.02	0.02
Setaria viridis L.	SETVI	−0.32	−0.15
Sonchus oleraceus L.	SONOL	0.20	0.21

[1] European and Mediterranean Plant Protection Organization.

4. Discussion

4.1. Zucchini and Barley Yields, Soil Mineral N

Barley development was significantly affected by Y, reasonably due to the variation of the weather conditions. In January–February of the first year, the higher recorded temperatures than the mean trend, in conjunction with the high rainfall in the spring months, could have favored the growth of the cereal. However, at barley termination, SMN was similar in the two years and under barley/no barley treatments, therefore no significant pre-emptive competition effect was observed. Conversely, at zucchini flowering, barley seemed to shorten SMN, as it was significantly lower in 2014 with respect to 2015 and significantly higher in the control with respect to GM and RC in both experimental years. This was probably due to immobilization events that occurred under the high C:N input by barley biomass (C:N = 47, as mean value of the two years, data not shown) [40]. Additionally, in the RC treatments, the presence of the barley mulch may have decreased the temperature of the soil surface [41], slowing down the mineralization of the organic residues. Moreover, the exceptional rainfall that occurred from May to July 2014 may have reasonably caused leaching of SMN, decreasing its content in 2014 at zucchini flowering and harvest with respect to 2015. The above SMN dynamics influenced the zucchini performance. Indeed, zucchini yield reflected the nitrogen availability in the soil, as it was higher in the control, with respect to GM and RC, in both the years. This result is partially in accordance with our first hypothesis. Indeed, we observed a significant reduction in zucchini yield in barley treatments, which was likely due to N immobilization and/or leaching and not to pre-emptive competition. However, the effects of weed development can also contribute to N sequestration; therefore, our first hypothesis could not be clearly confirmed or denied, since weed competition effects could hardly be separated from the above mechanisms in our experimental design. Actually, the Mater-Bi mulch on No_ASC rows could also have positively affected zucchini yield by eliminating any negative interference with weeds. The zucchini yield reduction with respect to the control was particularly severe in RC, in contrast to our second hypothesis. Contrary to our findings, previous studies have demonstrated the feasibility of zucchini cultivation under RC management. Canali et al. [17], in a two-year field experiment carried out in the MOVE-LTE, found that zucchini cultivated under RC barley yielded 69% more than the zucchini preceded by GM barley, and similarly to a no-barley control; likewise, Ciaccia et al. [18] found that zucchini fruit yield under barley RC was higher than GM and not different from no-barley control in two consecutive years. In our study, we used an early cultivar of zucchini (Zuboda). Generally, early cultivars of vegetables are not inclined to vegetative development; therefore, we can hypothesize a poor competitive attitude and scarce adaptability of Zudoba for cultivation under no-till conditions. Our results confirm the importance of the cash crop cultivar choice for the proper design of the vegetable system when RC is used for soil management.

4.2. Weed Community and Management

The barley biomass at termination (i.e., the mulch thickness in RC) was an important factor determining weed emergence. In 2014, despite the abundant rainfall, RC had the lowest weed density value at zucchini flowering, while the reverse trend was observed in 2015, when the barley dead mulch was not dense enough to act as barrier for weed emergence. By this, the RC experienced the highest weed density, even if not significantly different from the control. This finding is in accordance with other empirical works, suggesting a threshold below which the ASC biomass cannot produce a weed suppressive mulch [42]. At zucchini fruit set, in neither of the two years did the RC produce an effect against weeds in terms of biomass (Table 1). This result did not allow us to confirm our third hypothesis. In 2015, the RC contributed to weed development more than the control did, despite the lower N availability in the soil. These findings contrast with other studies that highlight the advantage of using RC in terms of weed management in Mediterranean vegetable systems [6,17,18]. Here, to understand the mechanism underpinning the RC performance on weeds, its effects on weed community composition should be considered. The exploratory analysis of weed community separated RC, particularly RC II, from GM and No_ASC. The RC resulted indeed associated with an *ensamble* of species cooperating in the establishment of a community with a higher level of biodiversity than the other treatments, as highlighted by the correlation pattern of Shannon H′ diversity. This pattern (ANGAR, BEAVX, CONAR, LOLSS, MATCH, PICEC, PLALA, POLAV, and SONOL) was therefore favored through different mechanisms during the first half of the zucchini cycle. The CONAR and POLAV are classified as prostrate/creeping (Table 2), which are reasonably able to survive the rolling and are well suited to develop between the web of the RC mulch. A similar mechanism may have favored the prostrate ANGAR in finding its ecological niche by easily developing through the barley mulch texture. The PLALA and BEAVX (perennial species) could have been encouraged by the no-till conditions. Indeed reduced/no-till practices commonly increase the abundance of perennials [24,43,44], since their propagules are not buried to depths unfavorable to emergence and not uprooted and killed as under tillage [45]. POLAV and PICEC, the latter being strictly correlated with RC II, had the lowest Ellenberg N score (2 and 1, respectively), associated with an oligotrophic attitude, suggesting that they can be easily found in environments with low levels of nitrogen in the soil [46]. In our study, the oligotrophy of POLAV and PICEC could have driven them to occupy the ecological niche leaved by nitrophilic species, being more disadvantaged in an environment where nitrogen immobilization or other mechanisms causing nitrogen deficiency take place [47–49]. After Green Revolution, in response to the intensification of agriculture with high nutritional inputs, oligotrophic species and associated biodiversity have declined in agroecosystems, while nitrophilic species, more competitive in N-rich soils, have been maintained [12,46], becoming the most problematic species in intensive cropping systems. In this context, our result, from an ecological perspective, highlights the potential contribution of low-input agriculture in the conservation of spontaneous flora. From zucchini fruit set, a condition of dominance of few of the selected species, which developed a considerable amount of biomass, began. This was reasonably the cause of the rejection of our third research hypothesis. Actually, weed soil cover samplings (by visual estimation) in RC plots showed a mean cover of 50% by POLAV and 63% by PICEC, in 2014 and 2015, respectively (data not shown). At zucchini harvest, soil cover by POLAV and PICEC was 79% and 88%, respectively (data not shown), confirming their dominant behavior until the final phases of the cash crop cycle. The creeping habit of POLAV and the rosette initial stage of PICEC have reasonably given them an additional advantage with respect to the other selected species, since these traits can also easily help the plant in escaping the mowing [50].

The tilled systems (GM/No_ASC), on the other hand, resulted more associated with CICIN, POROL, AMARE, and ECHCG, as showed by the PCA. The cluster analysis highlighted a similar spatial distribution of these species, suggesting a (partly) overlapping ecological niche. Moreover, they were uncorrelated with Shannon H′ diversity, indicating the association of these species with systems regardless of the biodiversity level. It is interesting to observe that these species were completely controlled by the mulch-based no-till strategy (around zero mean soil cover in RC treatments, both at zucchini mid-season and harvest, data not shown). The species POROL and AMARE are small-weight seed species. Compared to species with larger seeds, germination and growth of small-seeded annuals could suffer from restricted light availability, physical growth barriers, and the allelopathy of surface residues [51]. Thus, we can hypothesize that they may have been least favored in RC due to no-till compact soil, less aerated than tilled one, and to the further impediment of the mulch, which prevents radiation and decrease soil surface temperature. This finding is in accordance with Teasdale and Mohler [42], who found high sensitivity of AMARE to mulches, regardless of mulch material, indicating the limiting light conditions as the main cause restricting the seedlings growing around obstructing mulch. From a weed management perspective, this last result is quite significant, since RC has proven the ability to control such common and, particularly in case of AMARE, highly competitive and nitrophilic weed species (C-R species) [52].

The results from the analysis of the weed community, structured under the compared treatments, confirm our fourth research hypothesis, as a relevant differentiation among tilled and no-till RC systems was observed. The mulch-based no-till practice showed a strong selective pressure on weeds in the short-term, shifting the community towards a suite of traits able to survive the flattening, which overcame the mulch barrier and favored soils with reduced N availability.

These results suggest the need to properly manage the agroecosystem by designing a suite of complementary soil management practices to minimize the selective pressure on weeds.

Similar studies should be carried out comparing the effect of repeated mulch-based RC over time with tillage and no/reduce tillage alternation on weed community structure, dynamics, and distribution of traits.

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