



Targeted timing of hairy vetch cover crop termination with roller crimper can eliminate glyphosate requirements in no-till sunflower

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Abstract

No-till cropping systems with cover crops can improve soil health, but often rely on glyphosate, which is a contentious herbicide. In this study, we investigated whether a system based on the direct sowing of sunflower (*Helianthus annuus*) in the dead mulch of a roller-crimped hairy vetch (*Vicia villosa*) could be competitive with a system where glyphosate is also sprayed to terminate the cover crop and to control weeds. We hypothesized that optimum timing of roller-crimping would be key to eliminate glyphosate requirements while maintaining sunflower performance. In a 3-year on-farm experiment, we compared three vetch termination stages (early: pre-flowering; Intermediate: beginning of flowering; late: 70% flowering) and three glyphosate rates (Nil, half and full, i.e. 1440 g of active ingredient per hectare). Vetch biomass increased progressively from early to late termination stages, and ranged between 414 and 658 g m⁻². Higher vetch biomass was correlated with lower weed biomass. Treatments had inconsistent effects on weed diversity and composition, largely determined by the interactions between treatments and seasonal (different years) or local factors (different fields). Glyphosate-based treatments seemed to select for aggressive weed species, but no clear species filtering effect based on ecological or functional traits was detected. Shannon H' was positively correlated with sunflower grain yield below a weed dry biomass threshold of 150 g m⁻². Crop yield with early termination stage was a failure without glyphosate application. However, crop yield with late vetch termination was acceptable, being at par or 15% higher (mean of first and second years) in no-glyphosate compared with glyphosate-based treatments. Crop gross margins showed the same trend (+33% for no-glyphosate compared with glyphosate-based treatments). This study, for the first time, shows that targeted timing of roller-crimped hairy vetch in no-till sunflower can result in equal agronomic and economic performances as addition of glyphosate.

Keywords Agroecosystem service · Conservation agriculture · Cropping system diversification · Dead mulch · Ecological weed management · Functional trait · Gross margin · Integrated weed management · Legume · Weed diversity

1 Introduction

Mouldboard ploughing is considered as one of the agricultural practices with the highest potential contribute to soil health deterioration and a major contributor to greenhouse gases emissions in certain pedoclimatic conditions (Lal 2009). Conservation tillage, i.e. reduced (non-inversion) tillage and

no-till, is well known for its positive impact on soil structure and on chemical and biological fertility, with clear effects on soil erosion reduction (Cooper et al. 2020; Ryken et al. 2018). However, conservation tillage alone is not sufficient to reap all the benefits of conservation agriculture, which stem from context-based application of all its three components, i.e. reduced or no-till, crop rotation and permanent soil cover (Kassam et al. 2009). This highlights the key concept that conservation tillage can improve sustainability only when framed into diversified cropping/farming systems (Francaviglia et al. 2019). In fact, continuous use of no-till in overly simplified cropping systems can increase soil compaction and facilitate selection of harmful organisms (e.g. pests, pathogens and weeds) that are well adapted to a disturbance regime given by repeated application of the same agricultural practices across time and space (Flower et al. 2019).

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In rotational (e.g. arable) systems, cover crops constitute an important element of diversification. An increasing body of literature supports the numerous benefits that insertion of cover crops — especially legumes — in arable cropping systems can give to crop production and to the provision of several agroecosystem services (above all, weed suppression, nutrient cycling and supply, soil water conservation), when they are shallowly incorporated into soil or used as a dead mulch in no-till systems (Teasdale et al. 2008; Palm et al. 2014; Vincent-Caboud et al. 2019). In Mediterranean climates, successful application of conservation agriculture systems normally implies the use of winter cover crops grown before a spring sown cash crop such as sunflower (*Helianthus annuus* L.), maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.) or sorghum (*Sorghum bicolor* (L.) Moench). When the cash crop is a non-legume species, one key ecosystem service provided by cover crop use is nitrogen supply. As such, a legume cover crop (e.g. hairy vetch, *Vicia villosa* Roth) being able to supply an adequate amount of N through symbiotic N₂-fixation, is the optimal choice (Costantini et al. 2020). On the other hand, non-legume cover crops can increase N availability to the cash crop in situations of N excess in the soil, thanks to their capacity to act as catch crops and to reduce nitrogen losses.

The development of no-till farming — with or without cover crops — has brought about a substantial increase in the use of burndown herbicides, of which glyphosate is by far the most widespread. It has been estimated that glyphosate use in the USA has increased ca. 200-fold from 1974 to 2014, and that — globally — glyphosate use has risen 15-fold since the introduction of glyphosate-resistant crops in 1996 (Benbrook 2016).

Despite the initial claims of harmlessness due to the apparent limited persistence of glyphosate in the environment, an increasing body of scientific literature is highlighting ecotoxicological and toxicological problems associated to the active ingredient, its metabolites (mainly aminomethyl phosphonic acid, AMPA) and adjuvants (mainly polyoxyethylene amine, POEA), mainly linked to chronic exposure (e.g. Kwiatkowska et al. 2017; Mesnage et al. 2016; Perego et al. 2017; Van Bruggen et al. 2018). In addition, glyphosate and its associated chemicals are consistently found in soil, surface- and groundwater, especially in regions where glyphosate-resistant crops are commonly used (Battaglin et al. 2014), and can persist in sediments, where their microbial degradation is slowed down (Wang et al. 2016). Residues of glyphosate and AMPA are also found in plant products and scale up the trophic chain, explaining the high occurrence (from 30 to 80%) of farm animals and humans showing glyphosate residues in their urine in both USA and Europe (Niemann et al. 2015). Sublethal glyphosate concentrations may reduce plant resistance to pathogens (e.g. *Fusarium* spp.), possibly due to the detrimental herbicide effect on beneficial endophytic and rhizosphere microorganisms (Finckh et al. 2015).

For all these reasons, glyphosate use is under scrutiny, and there are discussions on its possible phasing out, e.g. in the European Union (Kudsk and Mathiasen 2020). In this context, innovative no-till based cropping systems that can reduce or eliminate glyphosate use without jeopardising crop yield and profitability are urgently needed.

In no-till cropping systems, the major alternatives to the use of glyphosate for cover crop termination are mechanical or physical methods, such as flail choppers, mowers, flame weeders and roller crimpers (Vincent-Caboud et al. 2019). The choice and effectiveness of each method depend on cover crop characteristics (e.g. ability to regrow, stem height and stiffness, phenological stage at termination, biomass amount) and operational issues (e.g. working speed and time, fuel consumption, costs). Furthermore, management objectives in terms of ecosystem service provision play an important role on choice of cover crop termination. When prompt nutrient release from cover crop biomass is sought, termination should produce small plant fragments, as ensured by choppers (Creamer and Dabney 2002). In contrast, when weed emergence reduction is prioritized, flail mowers can create a better dead mulch due to stems with long fibre, but they separate stems from roots and spread stems unevenly on soil surface (Vincent-Caboud et al. 2019). Roller-crimpers are gaining attention as mechanical termination tools because they can achieve multiple goals: (i) quick and effective cover crop termination and reduced regrowth; (ii) creation of a long-lasting, homogeneous and weed suppressive dead mulch; (iii) partial and progressive decomposition of the dead mulch due to the crimped parts of the stems, with consequent nutrient release from cover crop biomass mineralization (Vincent-Caboud et al. 2019). Nevertheless, difficulties in achieving a high termination rate with creeping or highly productive cover crops (e.g. hairy vetch) have been reported, especially with early termination (Vincent-Caboud et al. 2019). Roller-crimpers are considered very effective for cover crop termination from the 70% flowering stage in forbs or anthesis in grasses (Miville and Leroux 2018). In Mediterranean climates, early termination of cover crops (e.g. by the end of March) can be a crucial issue to enable timely sowing of spring arable cash crops and thus to avoid excessive drought stress in summertime (Teasdale et al. 2012). To increase the effectiveness of roller-crimpers, several adaptations of the classical chevron design (Rodale Institute 2018) have been tested, ranging from different designs (Kornecki et al. 2009), increased weight and combination with flaming (Frasconi et al. 2019). In addition, changes in operational characteristics (e.g. direction of roller-crimping or number of passes) have been tested, with variable results (Vincent-Caboud et al. 2019).

To increase the effectiveness of the chevron-design roller-crimper type for early cover crop termination, a promising solution is to combine roller-crimping with direct drilling of the subsequent cash crop in one pass, with a front-mounted

roller-crimper and a rear-mounted no-till driller (Figure 1). In this way, double disturbance of the cover crop is achieved, with the roller-crimper crimping the cover crop stems orthogonally and the metal discs of the drilling machines cutting them and opening the sowing furrow along the crop row direction.

The objective of this study was to investigate whether an innovative no-till system based on roller-crimped hairy vetch cover crop followed by sunflower directly sown in the legume dead mulch could be agronomically and economically competitive with the reference system, managed in the same way, but glyphosate was also sprayed to increase cover crop termination and weed control. In particular, we aimed to optimise the timing of cover crop termination by roller-crimper to reduce or eliminate glyphosate use while maintaining sunflower performance; to assess so, we built up a 3-year factorial study in which three termination time and three glyphosate rate were the factors. We hypothesised that (i) optimal roller-crimping timing maximises cover crop biomass and minimises regrowth as to enhance weed suppression and supply adequate nitrogen to the subsequent cash crop, thus maintaining crop yield and economic return even with limited or no glyphosate application; (ii) weed community composition and diversity change upon roller-crimping timing, with possible carry-over effects on sunflower performance. To the best of our knowledge, no previous papers have documented the effects of the combination between roller-crimping and glyphosate application rate on sunflower agronomic and economic performances and on weed shifts in Mediterranean conditions.



Fig. 1. Roller crimper (chevron design Rodale-type built by the farmer) mounted on the front of the tractor and coupled with a rear-mounted direct drill machine (Semeato SPE 06), carrying out hairy vetch (cv. Haymaker Plus) termination and sunflower (hybrid LG 55-57 HO) no-till sowing in one pass. Picture taken by Daniele Antichi at the farm hosting the trial (Tuscany, Italy) the 19th March 2014.

2 Material and methods

2.1 Experimental set-up and field management

A 3-year field experiment was carried out from 2012 to 2015 at a commercial arable farm in Lorenzana, Pisa, Central Italy (43.58 N, 10.53 E). The trial was run each year on a different field of ca. 2 ha size, each following durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.) and located in the same area. The mean soil characteristics of the three fields (0–30 cm) were determined at the beginning of each experimental year by collecting 9 soil cores per field and resulted in: clay content 8.38 g 100 g⁻¹, sand content 65.49 g 100 g⁻¹, soil organic matter concentration (Walkley-Black method) 1.48 g 100 g⁻¹, pH 7.84, total N (Kjeldahl method) 0.91 g kg⁻¹, available P (Olsen method) 20.07 mg P₂O₅ kg⁻¹. The soil was a Typic Xeropsamment, with sandy loam texture (USDA 1999). The trial had a two-way factorial design with three cover crop termination dates (early, i.e. before hairy vetch flowering; intermediate, i.e. at the beginning of vetch flowering; late, i.e. at 70% of hairy vetch flowering) and three glyphosate rates (nil, half and full rate, where the latter was 1440 g a.i. ha⁻¹, applied as 200 L ha⁻¹ water solution of Roundup 360 Power, Monsanto Agricoltura Italia). Elementary plots were 6 m wide and 60 m long.

Main tillage (chisel ploughing at 40 cm depth) was done in August 2012, 2013 and 2014, before the seedbed preparation for the cover crop, i.e. one pass of rotary harrow that was coupled with broadcast application of 300 kg ha⁻¹ of 6–23–0 fertiliser as a starter. Hairy vetch (cv. Haymaker Plus) was broadcast sown at 35 kg seeds ha⁻¹ on August 28th, 2012; October 2nd, 2013 and September 17th, 2014 (with variations due to weather conditions), and did not receive any other input. Cover crop was terminated on April 22nd, 2013; March 19th, 2014 and March 24th, 2015 (early stage); May 13th, 2013; May 12th, 2014 and April 16th, 2015 (intermediate stage); June 7th, 2013; June 3rd, 2014 and May 13th, 2015 (late stage). Where applied, glyphosate was sprayed the day before the pass of the roller crimper.

The roller crimper was a chevron design Rodale-type built by the farmer with the scientific support of the University of Pisa. The main characteristics of the roller are reported in Frasconi et al. (2019), with a slight modification (narrower blade insertion angle on the roll body, made with heat-treated steel) done to make it more aggressive. The roller crimper was mounted on the front of the tractor and was coupled with a rear-mounted direct drill machine (Semeato SPE 06), to carry out cover crop termination and sunflower no-till sowing in one pass.

Sunflower (hybrid LG 55-57 HO) was sown at a rate of 6.67 seeds m⁻² with a 45-cm inter-row distance. At sowing, lambda-cyhalothrin and metaldehyde were applied as granules in the seed furrows to prevent from seed predation,

respectively, from insects and slugs. The cash crop was grown in rainfed conditions and did not receive any fertilization nor herbicide treatment until harvest, which occurred on September 24th, 2013; September 4th, 2014 and August 26th, 2015 (early and intermediate termination stages) or on October 18th, 2013 and September 25th, 2014 (late termination stage). In 2015, harvest data at the late termination stage could not be collected because the crop was erroneously harvested by the farmer beforehand.

2.2 Weed assessments

Weeds were assessed both at cover crop termination and in the following sunflower crop. At cover crop termination, hairy vetch and total aboveground weed biomass were sampled in three areas of $0.5 \text{ m}^2 \text{ plot}^{-1}$. In the same areas, per cent cover of hairy vetch and of each weed species was visually estimated. At sunflower harvest, BBCH 89 (Hess et al. 1997), total aboveground weed biomass and weed cover by species were assessed on six samples plot^{-1} (replicates) of 1 m^2 , nested in the sampling areas selected for crop biomass and yield assessment. In 2014 and 2015, crop and weed cover were visually assessed also at sunflower 5-leaf stage, BBCH 15, using the same sample number and size. Biomass samples were oven-dried at 60°C until constant weight.

2.3 Crop yield and gross margin

Sunflower yield was assessed on six samples plot^{-1} (replicates) of $2 \text{ m length} \times 1 \text{ m width}$ (two crop rows), after separating biomass into grain, heads and stubble. Each component was oven-dried at 60°C until constant weight.

Crop gross margin was calculated as the difference between revenue (grain yield \times unit market price) and production costs on a hectare basis. We used the yearly sunflower grain market price as reported by the farmer, based on the contract price agreed with buyers, including taxes and value-added tax. Production costs included the costs of field operations (fuel, tractor driver's labour, usual maintenance) as accounted by the farmer and averaged over several years, and the actual costs of supplies (cover crop and cash crop seeds, glyphosate) purchased yearly by the farmer, including value-added tax.

2.4 Statistical analysis

2.4.1 Weed biomass and cover

Total weed biomass and weed diversity indices (species richness, Shannon diversity index, inverse Simpson index, Pielou equitability) based on species cover, at harvest in 2013 or at sunflower 5th leaf stage (BBCH 15) in 2014 and 2015, were analysed by linear mixed effect models assuming Gaussian

distribution and an identity link function, with termination stage, glyphosate rate and year as fixed factor (three levels each). In all cases, a random intercept model was adopted: years and replicates within years were added as random effects.

To study the effect of cover crop biomass on weed biomass at cover crop termination and at sunflower harvest, the interaction effect between all the fixed factor studied and their interactions (cover crop termination timing, glyphosate application, year) and the cover crop biomass was added to the mixed model. A random intercept model was adopted: year and replicates within years were added as random effects to the models.

2.4.2 Weed community composition and functional analysis

The effect of cover crop termination stage, glyphosate rate and their interaction on weed community composition was tested by means of a permutational analysis of variance (PERMANOVA) (Anderson 2001) using the Bray–Curtis dissimilarity index. The significance of the factors was tested by means of F tests based on sequential sums of squares from permutations of the raw data (9999 tries). The diversity matrix was also used to perform a multivariate ordination through non-metric multi-dimensional scaling (NMDS) (1000 traces, 20 tries). Multivariate analyses were run with *vegan* package for R (Oksanen et al. 2013).

Weed community composition was also analysed through a functional approach (Bàrberi et al. 2018), focusing on species biological and ecological response traits that could potentially be affected by the treatments. For each weed species, values of Raunkiaer life form, growth form, life span \times regeneration form, Grime's life strategy, specific leaf area, plant height, seed weight, seasonality of germination and affinity to soil nutrient conditions were assigned upon information from a dedicated weed trait database (Armengot et al. 2016; Bàrberi et al. 2018). A principal component analysis (PCA) on the species trait matrix was applied to detect redundant information and select the most informative traits. Three RLQ analyses (one per year) were run to highlight the combinations of traits with the highest covariance with combinations of agronomic factors. These analyses were based on three tables: the R-table (cover crop termination stage and glyphosate rate), the Q-table (with 6 selected traits resulting from the PCA: Grime's life strategy, specific leaf area, plant height, seed weight, seasonality of germination, affinity to soil nutrient conditions), and the L-table (weed species cover at harvest in 2013; and at sunflower 5th leaf stage in 2014 and 2015). By using the fourth corner analysis (Dray and Legendre 2008), we tested whether weed species are distributed independently of the agronomic treatments and of their traits. In this respect, permutation models (with 999 permutations)

were applied. RLQ and fourth corner analyses were performed with the library `ade4` of R (Chessel et al. 2004).

2.4.3 Vetch biomass at vetch termination stage

The effect of cover crop termination stage, year and their interaction on vetch biomass was analysed by linear mixed effect models assuming Gaussian distribution and an identity link function, with termination stage and years as fixed factor (three levels each). In all cases, a random intercept model was adopted: years and replicates within years were added as random effects.

2.4.4 Crop yield and gross margin, and relationship between yield and weeds

The effect of cover crop termination stage, glyphosate rate, year, and their interaction on crop yield and gross margin data was studied following the same method as described in the Section 2.4.1.

To study the effect of weed biomass and diversity on sunflower agronomic performance, crop yield data were studied by testing the additive and the interaction effect of weed biomass at harvest and weed diversity indices (Shannon) over the effect of the three fixed factors tested (cover crop termination stage, glyphosate rate and year). Model selection was based on the Akaike selection criteria (Zuur et al. 2009); only the most conservative model respecting the residual diagnostics was selected. The analysis was run with the `lme4` package of R (Bates et al. 2014), normality and homoscedasticity of residual were assessed with the `DHARMA` package of R (Hartig 2020).

Post hoc analyses were performed with Tukey's HSD test using least square means in the `emmeans` package of R (Lenth 2019). All statistical analyses were performed using the software R, version 3.6.2 (R Core Team 2019).

3 Results and discussion

3.1 Cover crop and weed biomass

Final cover crop biomass was significantly affected by year, termination stage and their interaction. Vetch dry biomass in the first year (Figure 2) was more than double that in the third year (548 vs. 256 g m⁻², $P < 0.05$), while in the second year, it was intermediate (368 g m⁻²). The low rainfall that occurred in fall 2014 and spring 2015, especially in May (Figure 3) was likely the reason of the low biomass production in the third year. Overall, the amount of vetch biomass produced in this trial is in line with what previously observed under similar conditions by Farneselli et al. (2020) that found a dry mass production of vetch in two year of respectively 377 and 594 g m⁻².

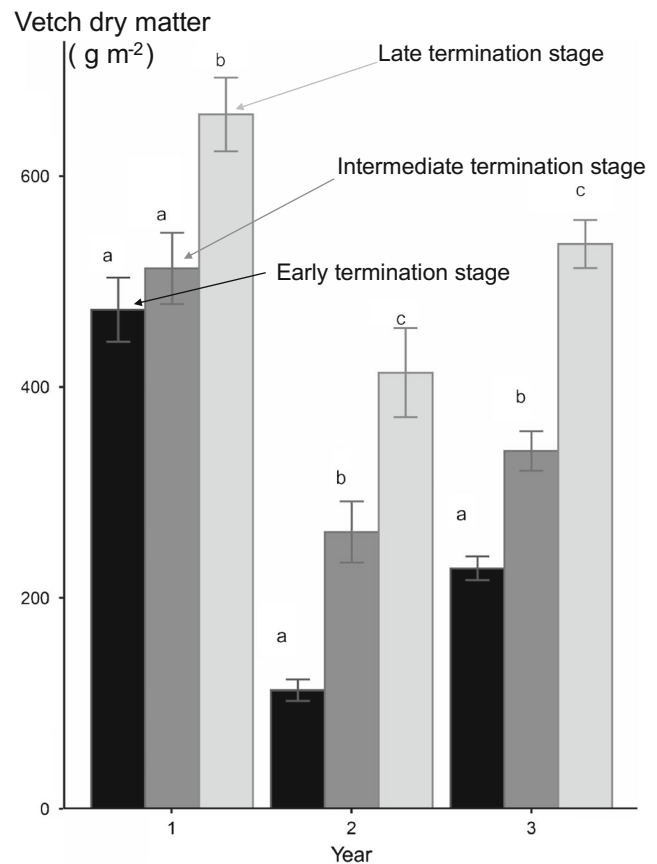


Fig. 2. Hairy vetch aboveground dry biomass in the three experimental years (2013 = year 1; 2014 = year 2; 2015 = year 3) as affected by termination stage, across all glyphosate rates. Within each year, treatments with the same letter are not significantly different at $P \leq 0.05$ (Tukey's HSD test). Confidence intervals at 95% of the actual data (1.96 times the standard error), are shown on top of each bar.

Hairy vetch biomass increased progressively from the early to the late termination stage, although in the first year biomass at the early and intermediate stages were not significantly different (Figure 2). Although they did not focus on subsequent cash crop, Price et al. (2019) confirmed the importance of hairy vetch late termination period to increase cover crop termination rate, making glyphosate use unnecessary; this effect was explained with a 2–3 times higher vetch biomass in the 2nd versus the 1st termination period (4 weeks apart).

Overall, the weed dry biomass at cover crop termination across the 3 years was low (maximum 100 g m⁻² in the second year). Good weed suppression by hairy vetch was observed in previous studies across a number of different conditions (e.g. see Dorn et al. 2015). Total weed biomass at cover crop termination diminished when vetch biomass was higher, but only in 2 years out of three (Figure 4). In year 3, lack of such correlation could be due to the low amount of both weed and hairy vetch biomass observed.

The weed suppressive capacity of hairy vetch (first 2 years) was likely due to competition for light and water, especially

Fig. 3. Monthly values of total rainfall and mean minimum and maximum air temperatures registered at the experimental site from September 2012 to October 2015 (Source: Hort@ weather station, Piacenza, Italy).

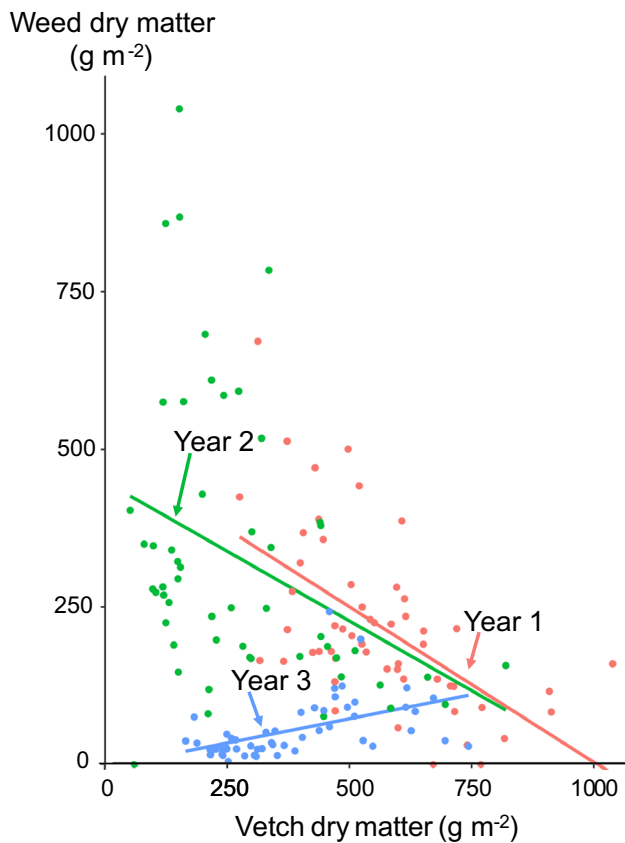
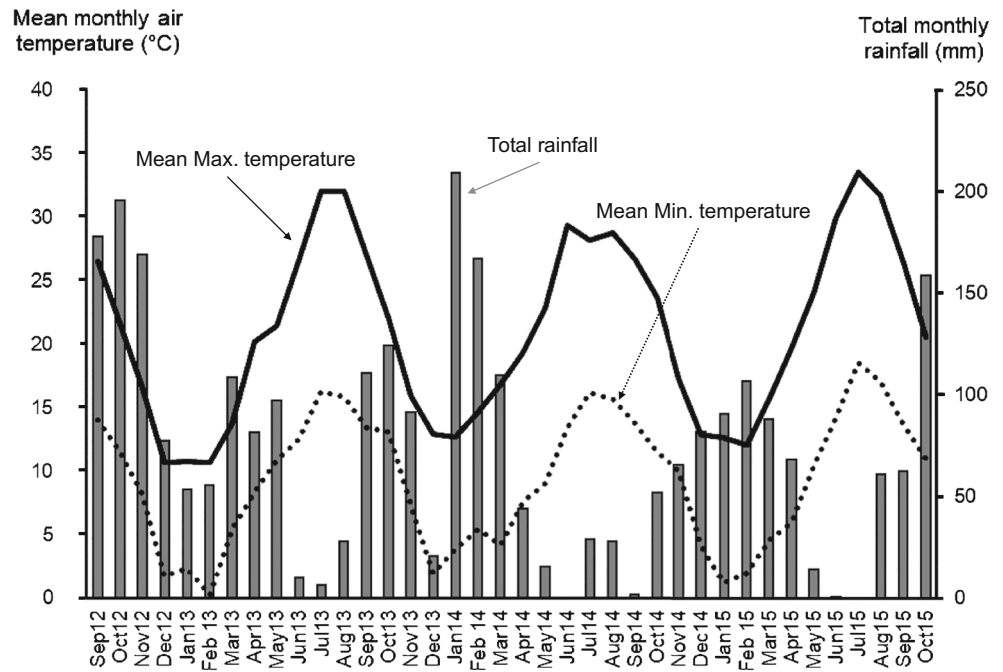


Fig. 4. Relationship between hairy vetch aboveground dry biomass and total weed biomass at cover crop termination in the three years of trial: 2013 (year 1), 2014 (year 2) and 2015 (year 3). Data were pooled across vetch termination stages. The slope of the regression line was significantly different from zero in year 1: $y = 490 - 0.59x$ (t ratio -4.076 ; $P < 0.001$) and year 2: $y = 440 - 0.44x$ (t ratio -4.044 ; $P < 0.001$) but not in year 3 (t ratio 1.186 ; $P = 0.237$).

from early spring. In Mediterranean conditions, hairy vetch biomass can reach up to 8 t d.m. ha^{-1} (Tosti et al. 2012). Instead, we do not expect significant competition for nutrients, due to the high N-fixing capacity of vetch, which can supply up to $275 \text{ kg fixed N ha}^{-1}$ (Tosti et al. 2014), and to its symbiosis with arbuscular mycorrhizal fungi (AMF) and associated P solubilizing bacteria (Njeru et al. 2014). Normally, legume cover crops can stimulate weed growth, especially of high N-demanding species (Campiglia et al. 2015). Nevertheless, hairy vetch is considered a mild allelopathic cover crop (Hill et al. 2007), a reason which might explain, in addition to high biomass production and soil cover, the low average value of total weed biomass across termination stages.

Total weed biomass at sunflower harvest, averaged across treatments, was 100.6 , 88.2 and 170.5 g m^{-2} in years 1, 2 and 3 (calculated only on early and intermediate termination stage) respectively. In general, these values reveal a good weed suppression ability of the combination of vetch cover crop and no-till sowing in sunflower. It is worth mentioning that sunflower is well known to be a competitive crop against weeds, provided early weed control and good crop establishment is achieved (Johnson 1971) and sunflower is also an allelopathic crop (Rawat et al. 2017). In a study conducted in similar conditions, we reported no effect of the combination between hairy vetch cover crop and reduced tillage on weed biomass at sunflower harvest (Adeux et al. 2021). In the present study, weed biomass at sunflower harvest was influenced by cover crop termination stage and glyphosate rate differently upon year (Table 1). In 2013, no differences among treatments were observed, possibly due also to quite large variation in weed biomass across replicates. In 2014, total weed biomass was

Table 1 Total weed aboveground dry biomass (g m^{-2}) measured at sunflower harvest across 3 years in the nine combinations between three hairy vetch termination stages and three glyphosate rates. NA, not assessed. Within a year, treatment means followed by the same letter are not significantly different at $P \leq 0.05$ (Tukey's HSD test). Values in parentheses are standard deviations.

Year	Vetch termination stage	Glyphosate rate	Weed dry biomass (g m^{-2})
1	Early	Nil	63.6 (39.9) a
	Early	Half	20.7 (18.3) a
	Early	Full	30.6 (28.9) a
	Intermediate	Nil	132.6 (232.6) a
	Intermediate	Half	165.5 (87.0) a
	Intermediate	Full	93.9 (103.2) a
	Late	Nil	104.0 (129.5) a
	Late	Half	142.8 (84.1) a
	Late	Full	151.8 (74.3) a
2	Early	Nil	174.7 (57.4) cd
	Early	Half	130.6 (52.5) bcd
	Early	Full	222.8 (78.0) d
	Intermediate	Nil	80.6 (70.6) ab
	Intermediate	Half	31.1 (19.6) a
	Intermediate	Full	76.8 (54.0) abc
	Late	Nil	19.9 (10.6) ab
	Late	Half	11.1 (10.8) a
	Late	Full	45.9 (40.0) abc
3	Early	Nil	314.0 (111.4) d
	Early	Half	228.4 (168.1) cd
	Early	Full	136.3 (99.2) bcd
	Intermediate	Nil	145.9 (126.1) abc
	Intermediate	Half	97.6 (49.5) ab
	Intermediate	Full	100.6 (55.9) a
	Late	Nil	NA
	Late	Half	NA
	Late	Full	NA

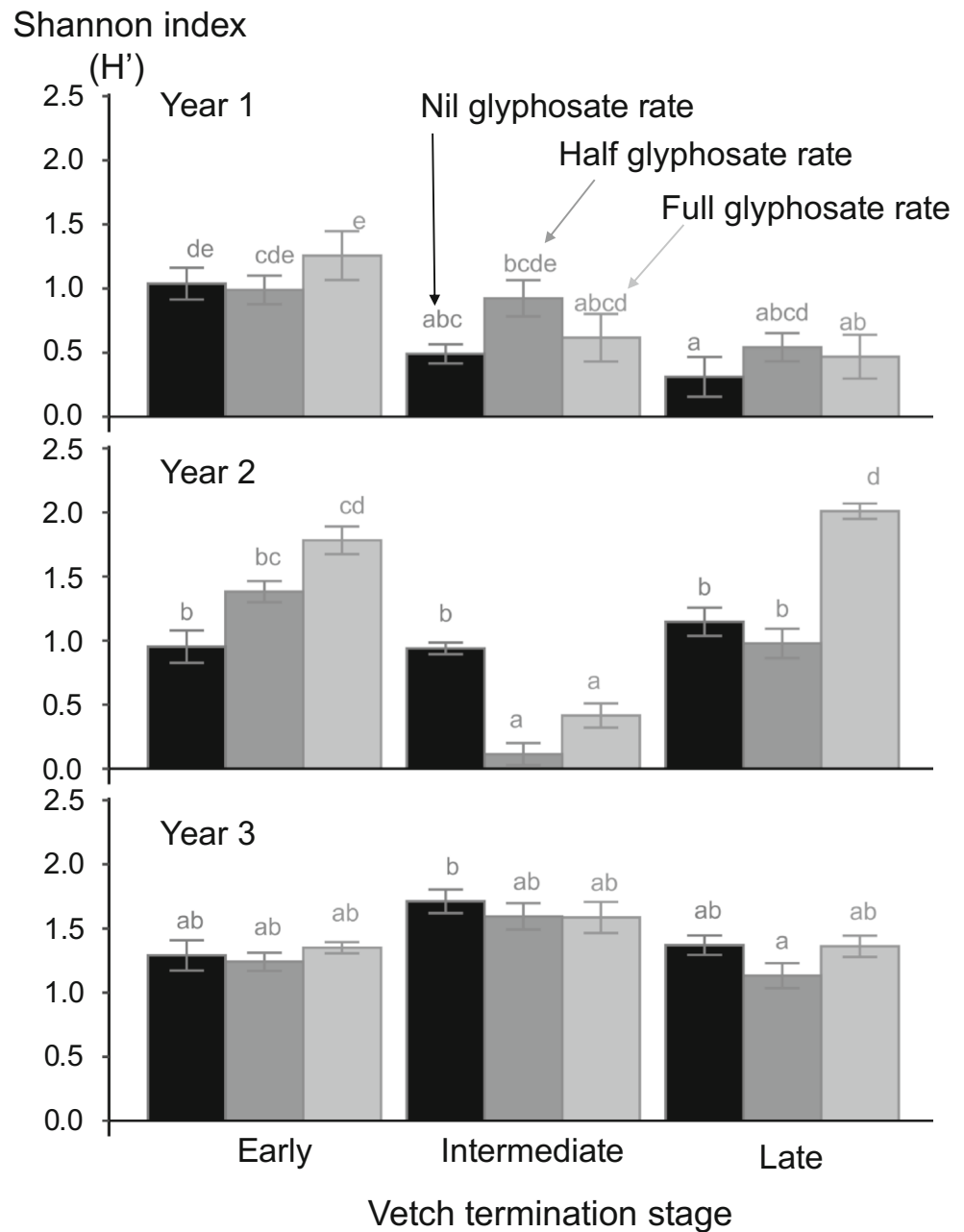
lower in sunflower following intermediate and late vetch termination stages (on average 64% and 85% reduction respectively, compared to early termination), with no significant differences among glyphosate rates within each termination stage. Besides the high competition against weeds due to higher vetch biomass at the intermediate and late termination stages (Figure 2), we argue that the low rainfall that occurred from April to end of September 2014 (Figure 3) might have further reduced weed biomass in sunflower. In 2015, there were no differences in weed biomass at sunflower harvest between the full and half glyphosate rates at each single cover crop termination stage (early or intermediate; data were not collected in the late stage treatment).

3.2 Weed diversity

Diversity indices calculated on weed species cover at sunflower harvest in 2013 and at sunflower 5th leaf stage in 2014 and 2015 showed similar trends: all indices were significantly affected by cover crop termination stage and

glyphosate rate, in a way different upon year (triple interaction significant at $P < 0.001$). Shannon H' was significantly ($P < 0.001$) correlated with the inverse Simpson index ($r = 0.98$), with species richness ($r = 0.85$) and with Pielou equitability ($r = 0.70$); as such, only H' results are presented (Figure 5) since H' already give information on species richness and equitability. Glyphosate rate did not affect H' within each termination stage in years 1 and 3 ($P > 0.05$), while inconsistent results were found in year 2: higher diversity was observed with higher glyphosate rate at early and late stages (+0.829 and +0.862 H' with full dose compared with no glyphosate), whilst a weed diversity reduction was found at the intermediate termination stage (no glyphosate had +0.524 H' than full rate). Similarly, vetch termination stage showed different effects upon year. In year 1, a delay in roller crimping caused a weed diversity decrease (H' decreased by 0.654 from early to late stage). Intermediate vetch termination stage resulted in the lowest Shannon diversity in year 2 ($H' = 0.490$) and the highest ($H' = 1.630$) in year 3.

Fig. 5. Shannon diversity index (H') calculated at sunflower harvest in 2013 (year 1) and at sunflower 5th leaf stage in 2014 (year 2) and 2015 (year 3) in the nine treatments (three vetch termination stages \times three glyphosate rates). Within each sampling period, treatments with the same letter are not significantly different at $P \leq 0.05$ (Tukey's HSD test). Confidence intervals at 95% of the actual data (1.96 times the standard error) are shown on top of the bars.



Despite a general perception that glyphosate should reduce weed diversity, different long-term studies focused on glyphosate-based cropping systems (glyphosate tolerant GM crops) have found limited connection between glyphosate use and weed diversity reduction (Schwartz et al. 2015; Young et al. 2013). In our work, we tried to disentangle the effect of glyphosate application from that of the cropping system, by comparing the effect of full and half glyphosate rates with the nil glyphosate rate. The general trend of our data depicts an unclear pattern linked to glyphosate application. Our results are partially in accordance with Koning et al. (2019), one of the few studies on glyphosate effects in non-GM crops at application rates similar to ours (i.e. 1080 g a.i. ha⁻¹ as full

rate): they also found an unclear effect of glyphosate application on weed diversity, but they detected a clearly negative effect on species richness, with a threefold number of applications compared to our study, and a clearer effect on weed community composition, similar to our case (see the Section 3.3).

Cover crop termination stage is considered a key element to estimate cover crop effect on weed management (Mirsky et al. 2011), but few studies have focused on termination stage effect on weed diversity. An exception is Alonso-Ayuso et al. (2018), who — like us — reported the lack of a general trend on weed richness and diversity as affected by cover crop termination stage.

Table 2 Results of permutational multivariate analysis of variance (PERMANOVA) of weed community composition as influenced by glyphosate rate (GR), vetch termination stage (VTS) and their interaction, based on 9999 permutations, stratified by replication (6) in each year. Significant effects ($P \leq 0.05$) are highlighted in bold; df = degrees of freedom.

Year	Effect	df	R ²	F value	P value
Year 1	Glyphosate rate	2	0.033	1.011	0.499
	Vetch termination stage	2	0.105	3.242	0.002
	GD × VTS	4	0.135	2.087	0.001
	Residuals	45	0.728		
Year 2	Glyphosate rate	2	0.12276	10.349	0.001
	Vetch termination stage	2	0.43356	36.550	0.001
	GD × VTS	4	0.17679	7.452	0.001
	Residuals	45			
Year 3	Glyphosate rate	2	0.12836	7.3619	0.001
	Vetch termination stage	2	0.28766	16.4981	0.001
	GD × VTS	4	0.19166	5.4962	0.001
	Residuals	45	0.39231		

3.3 Weed community composition

Results of PERMANOVA showed that weed community composition was significantly affected by the glyphosate rate × vetch termination stage in all 3 years (Table 2). Bi-dimensional NMDS analysis (Figure 6) showed that weed communities (Table 3) tended to cluster mainly by vetch termination stage, but glyphosate rate also determined a stratification of samples, in particular in years 2 and 3. In year 1, the full glyphosate rate applied at late termination stage resulted in the most different weed composition, compared to the other combination of treatments at sunflower harvest, characterized by *Poa annua* and *Echinochloa crus-galli*. Weed communities sampled at sunflower 5th leaf stage (years 2 and 3) showed a greater differentiation among treatments than those

sampled at sunflower harvest (year 1). In year 2, the effect of glyphosate rate was highest when applied at the late termination stage, as indicated by the larger distance from the axes origin of nil glyphosate application respect to full and half rate. In year 3, the effect of vetch termination period on weed community composition was stronger (more visible along axis 1) than that of glyphosate rate (more visible along axis 2). *Beta vulgaris*, *Ammi majus*, *Phalaris* sp. and *Matricaria* sp. were favoured by lack of glyphosate application, while *Amaranthus retroflexus* and *Chenopodium album* were characteristic of plots where glyphosate was applied, regardless of rate. (Table 3)

As expected, macrothermal species such as *E. crus-galli* and *A. retroflexus* were associated to cover crop late termination stage, where they largely contributed to total weed biomass observed at sunflower harvest in 2013 (pers. obs.). This was the wettest of the 3 years from April to October (the standard sunflower growing period in our area), an issue that likely favoured the growth of these highly competitive species (Mirshekari et al. 2010; Vázquez-García et al. 2021).

3.4 Weed community functional analysis

In all 3 years, RLQ and fourth corner analysis did not highlight any significant association between treatments and the selected ecological or functional traits. In accordance with other authors (Alonso-Ayuso et al. 2018; Mirsky et al. 2011) cover crop termination stage emerged as an important driver of weed community composition. However, no clear general trend was observed, likely due to the prevailing effect of seasonal weather conditions and specific weed seed bank in each field over treatment effect, which likely also influenced the differences observed in weed biomass in sunflower.

Although we detected a clear effect of vetch termination stage and glyphosate rate on weed species assemblages, their effect on weed ecological or functional traits was uncertain. Koning et al. (2019) showed that glyphosate selected for

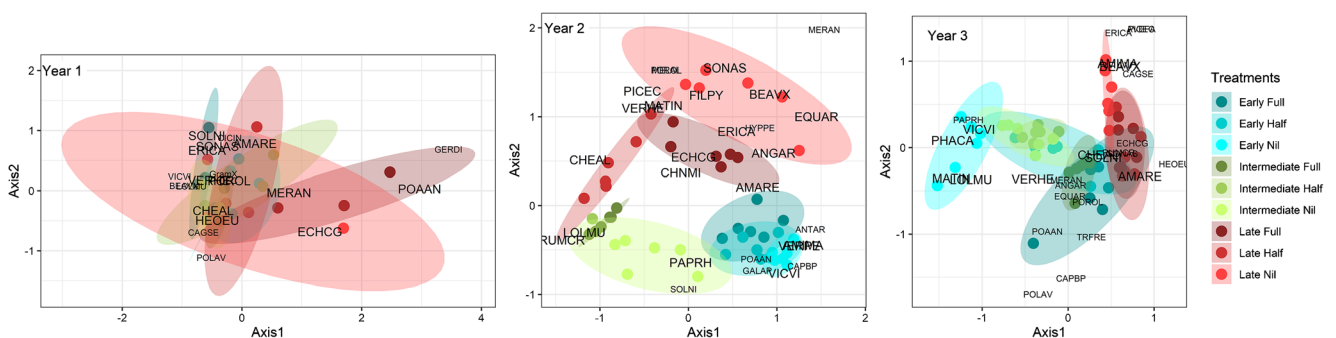


Fig. 6. Non-metric multidimensional scaling (NMDS) ordination of weed communities in the three years (2013, year 1: sunflower harvest; 2014, year 2 and 2015, year 3: sunflower 5th leaf stage) for the three vetch termination stages (early, intermediate or late) × three glyphosate rates (full, half or nil) combinations. Markers represent sampling areas.

Species are indicated with their EPPO-Bayer codes reported in Table 3; the 10 most abundant species are highlighted with a larger font size. Ellipsoid size represents the extent of weed species compositional variation within each treatment. Stress: year 1 = 0.170, year 2 = 0.171, year 3 = 0.186; $n = 54$.

Table 3 Weed species found during the 3-year trials. The EPPO code, the main functional groups (monocot/dicot or neither; Raunkiaer life form; Grime life strategy (R = ruderal; C = competitive; S = stress tolerant) and presence (1) or absence (0) in each year of the trial. *Gram X was a monocot not recognized at species level that was later attributed to *Bromus sterilis*.

Species	EPPOcode	Monocot/Dicot	Raunkiaer life form	Grimes life strategy	Year 1	Year 2	Year 3
<i>Amaranthus retroflexus</i>	AMARE	Dicot	Therophyte	CR	1	1	1
<i>Ammi majus</i>	AMIMA	Dicot	Therophyte	CR	0	1	1
<i>Anagallis arvensis</i>	ANGAR	Dicot	Therophyte	R	0	1	1
<i>Anthemis arvensis</i>	ANTAR	Dicot	Therophyte	CR	0	1	0
<i>Avena fatua</i>	AVEFA	Monocot	Therophyte	CR	0	0	1
<i>Beta vulgaris</i>	BEAVX	Dicot	Hemicriptophyte	CS	1	1	1
<i>Chaenorhinum minus</i>	CHNMI	Dicot	Therophyte	R	0	1	1
<i>Calystegia sepium</i>	CAGSE	Dicot	Geophyte	C	0	1	0
<i>Capsella bursa pastoris</i>	CAPBP	Dicot	Therophyte	R	1	0	1
<i>Chenopodium album</i>	CHEAL	Dicot	Therophyte	CR	1	1	1
<i>Cichorium intybus</i>	CICIN	Dicot	Hemicriptophyte	C	1	0	0
<i>Conyza canadensis</i>	ERICA	Dicot	Therophyte	CR	1	1	1
<i>Echinochloa crus-galli</i>	ECHCG	Monocot	Therophyte	CR	1	1	1
<i>Equisetum arvense</i>	EQUAR	Neither	Geophyte	CR	0	1	1
<i>Filago pyramidata</i>	FILPY	Dicot	Therophyte	SR	0	1	0
<i>Galium aparine</i>	GALAP	Dicot	Therophyte	CR	0	1	0
<i>Geranium dissectum</i>	GERDI	Dicot	Therophyte	CR	1	0	0
<i>Bromus sterilis</i>	GramX*	Monocot	Therophyte	CR	1	0	0
<i>Heliotropium europaeum</i>	HEOEU	Dicot	Therophyte	R	1	0	1
<i>Hypericum perforatum</i>	HYPPE	Dicot	Hemicriptophyte	CSR	0	1	0
<i>Lolium multiflorum</i>	LOLMU	Monocot	Therophyte	C	1	1	1
<i>Matricaria perforata</i>	MATIN	Dicot	Therophyte	CR	0	1	1
<i>Mercurialis annua</i>	MERAN	Dicot	Therophyte	R	1	1	1
<i>Papaver rhoeas</i>	PAPRH	Dicot	Therophyte	CR	0	1	1
<i>Phalaris canariensis</i>	PHACA	Monocot	Therophyte	R	0	0	1
<i>Picris echioides</i>	PICEC	Dicot	Therophyte	CSR	1	1	1
<i>Poa annua</i>	POAAN	Monocot	Therophyte	R	1	1	1
<i>Polygonum aviculare</i>	POLAV	Dicot	Therophyte	R	1	0	1
<i>Portulaca oleracea</i>	POROL	Dicot	Therophyte	R	1	1	1
<i>Rumex crispus</i>	RUMCR	Dicot	Hemicriptophyte	C	0	1	1
<i>Silene alba</i>	MELAL	Dicot	Hemicriptophyte	CSR	0	1	0
<i>Solanum nigrum</i>	SOLNI	Dicot	Therophyte	R	1	1	1
<i>Sonchus asper</i>	SONAS	Dicot	Therophyte	CR	1	1	1
<i>Trifolium repens</i>	TRFRE	Dicot	Hemicriptophyte	CSR	0	0	1
<i>Veronica hederifolia</i>	VERHE	Dicot	Therophyte	R	1	1	1
<i>Veronica persica</i>	VERPE	Dicot	Therophyte	R	0	1	0
<i>Vicia villosa</i>	VICVI	Dicot	Therophyte	CR	1	1	1

groups/traits like legumes or hemicryptophytes, and against traits like propagation by rhizome or non-seasonal annual weeds. No such selection effect was evident in our study, suggesting that the combined effect of roller-crimper and vetch dead mulch, largely influenced by site conditions, was more important than that of glyphosate in filtering weed species. We note that the weed community of each field was only

studied for a single year on different fields, potentially masking weed selection effects that would appear in the long term, e.g. by studying the effect on abundance and composition of the weed seed bank. In this perspective, it would be important to check whether the apparent selection of competitive species like *Amaranthus retroflexus* and *Echinochloa crus-galli* in glyphosate-treated plots is confirmed.

3.5 Weeds affecting crop yield

The most conservative model (lower Akaike information criterion and Bayesian information criterion) to study the effect of weeds on crop yield considered both the log of weed biomass and weed diversity (Shannon H' index) with 24 different intercepts given by the interaction between year, glyphosate rate and vetch termination stage. Weed biomass and diversity interacted significantly, while glyphosate rate and year, and the triple interaction (vetch termination stage \times glyphosate rate \times year) affected significantly crop yield but had no significant interaction with either weed biomass or weed diversity (Table 4).

As shown in Figure 7, higher weed biomass determined lower crop yield, but this effect was influenced by weed diversity until 150 $g\ m^{-2}$ of weed biomass (i.e. 74.3% of samples). At biomass values lower than this threshold, higher weed diversity resulted in higher sunflower yield irrespective of weed biomass level, whilst above the threshold such effect vanished. This finding supports recent scientific evidence indicating a positive effect of weed diversity on reduction of crop yield loss (Adeux et al. 2019), suggesting that targeted management of cropping system diversification — of which dead mulch systems are an important component — allows to maintain crop yield by keeping weed community diversity, as long as the total weed biomass is kept suppressed. This evidence could be explained by the reduced presence of competitive weed species, the most detrimental for crop yield, in a diversified plant community (Adeux et al. 2019).

3.6 Crop yield and gross margin

In all years, grain yield of sunflower following termination of hairy vetch with roller crimper was largely determined by termination stage. In particular, in the first 2 years late termination resulted in comparable yield, which was at par in no-glyphosate than glyphosate-based treatments (Figure 8).

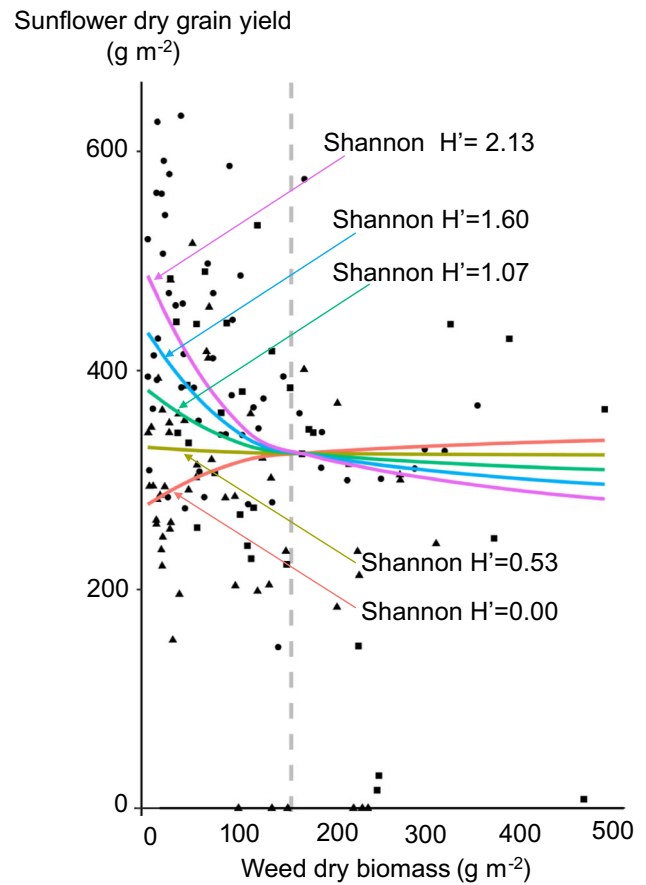


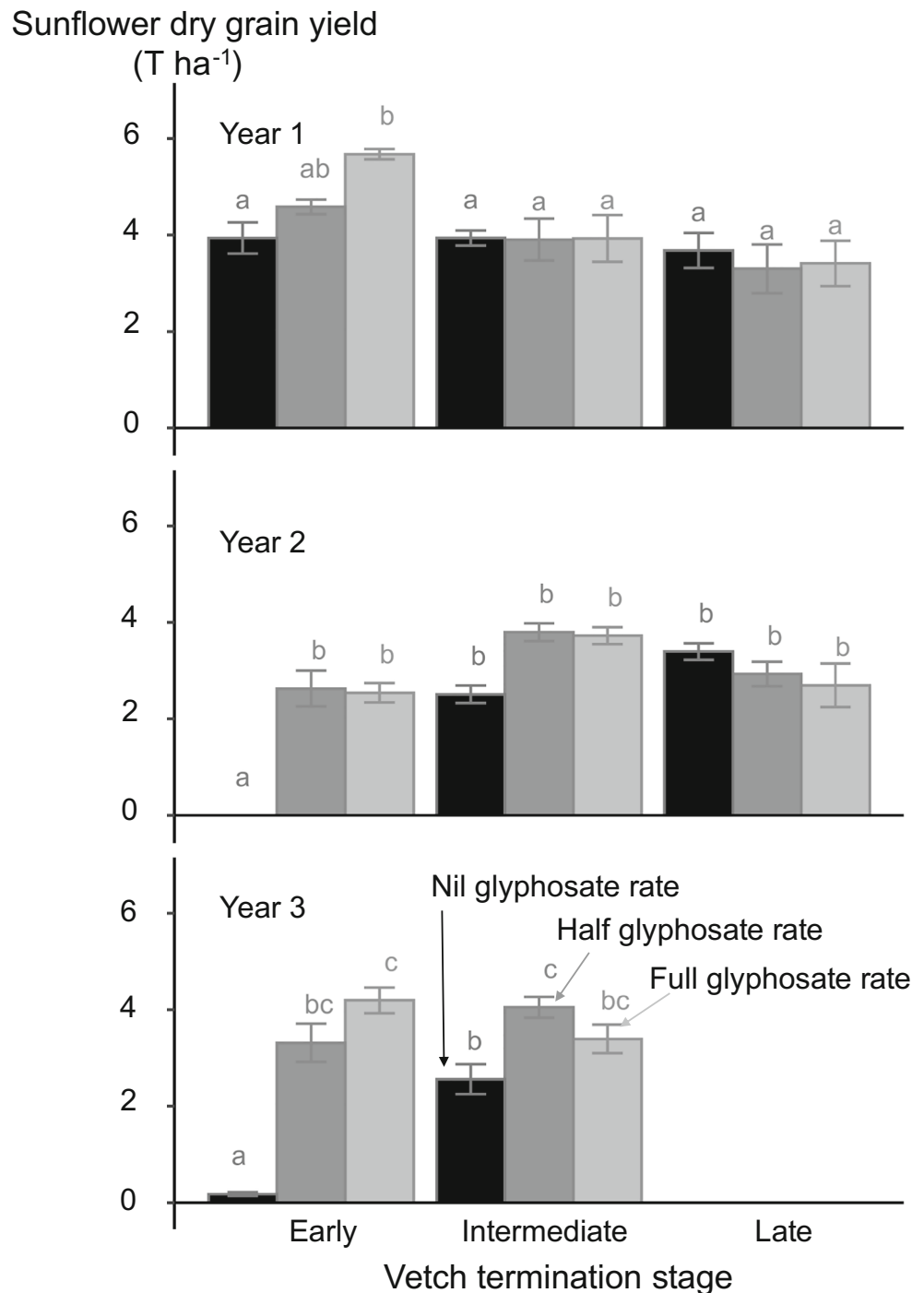
Fig. 7. The effect of weed biomass on crop yield as influenced by weed diversity, upon the most conservative model. Circles, triangles and squares represent data collected respectively in years 1, 2 and 3. The interaction between weed biomass and diversity is represented by the different curves, calculated on five levels of weed diversity as reported in the legend. The dashed vertical line represents the weed biomass threshold below which a significant positive effect of weed diversity on sunflower yield can be detected.

Without glyphosate application, early cover crop termination resulted in sunflower crop failure in 2 years out of three, with the only exception of 2013, when grain yield in the no-glyphosate

Table 4 Type III ANOVA table for the most conservative model expressing the effect of weed biomass and diversity on crop yield; df = degrees of freedom; NS = not significant.

Model parameter/effect	Chi-square	df	P value (>chi-square)
Intercept	24.22	1	<0.001
Log (weed biomass)	0.95	1	NS
Weed diversity (Shannon H')	5.63	1	<0.05
Vetch termination stage	4.03	2	NS
Glyphosate rate	12.89	2	<0.01
Year	5.15	2	<0.10
Log (weed biomass) \times weed diversity (Shannon H')	4.68	1	<0.05
Vetch termination stage \times glyphosate rate	10.72	4	<0.05
Vetch termination stage \times year	5.17	3	NS
Glyphosate rate \times year	27.46	4	<0.001
Vetch termination stage \times glyphosate rate \times year	15.01	6	<0.05

Fig. 8. Sunflower dry grain yield biomass as affected by hairy vetch termination stage and glyphosate rate in the three experimental years. Within each year, treatments with the same letter are not significantly different at $P \leq 0.05$ (Tukey's HSD test). Lines on top of each bar represent standard deviations.



treatment (3.9 t ha⁻¹) was comparable to that in the half dose treatment and 31% lower than in the full dose treatment (5.7 t ha⁻¹). This result was possibly due to the high biomass produced by vetch even at early termination date in 2013, resulting in higher weed suppression and possibly also higher N availability for sunflower. In 2014 and 2015, sunflower did not produce any yield due to difficulties in crop establishment, as the vetch was not completely killed by the roller and its thickness hampered crop emergence. No yield difference was ever observed between the half and full glyphosate doses. Roller-crimper passed at the

intermediate termination stage did not determine any significant differences in sunflower yield among treatments, with the only exception of 2015, when yield in the half dose glyphosate treatment was 58% higher than in the no-glyphosate treatment (4.1 vs. 2.6 t ha⁻¹).

Obviously, sunflower gross margin was largely determined by crop yield potential, also due to the stability of grain market price across the 3 years (i.e. 380, 360 and 350 € t⁻¹ grain, respectively for 2013, 2014 and 2015) and the actualization of production costs for the three systems (i.e. 653, 703 and 711

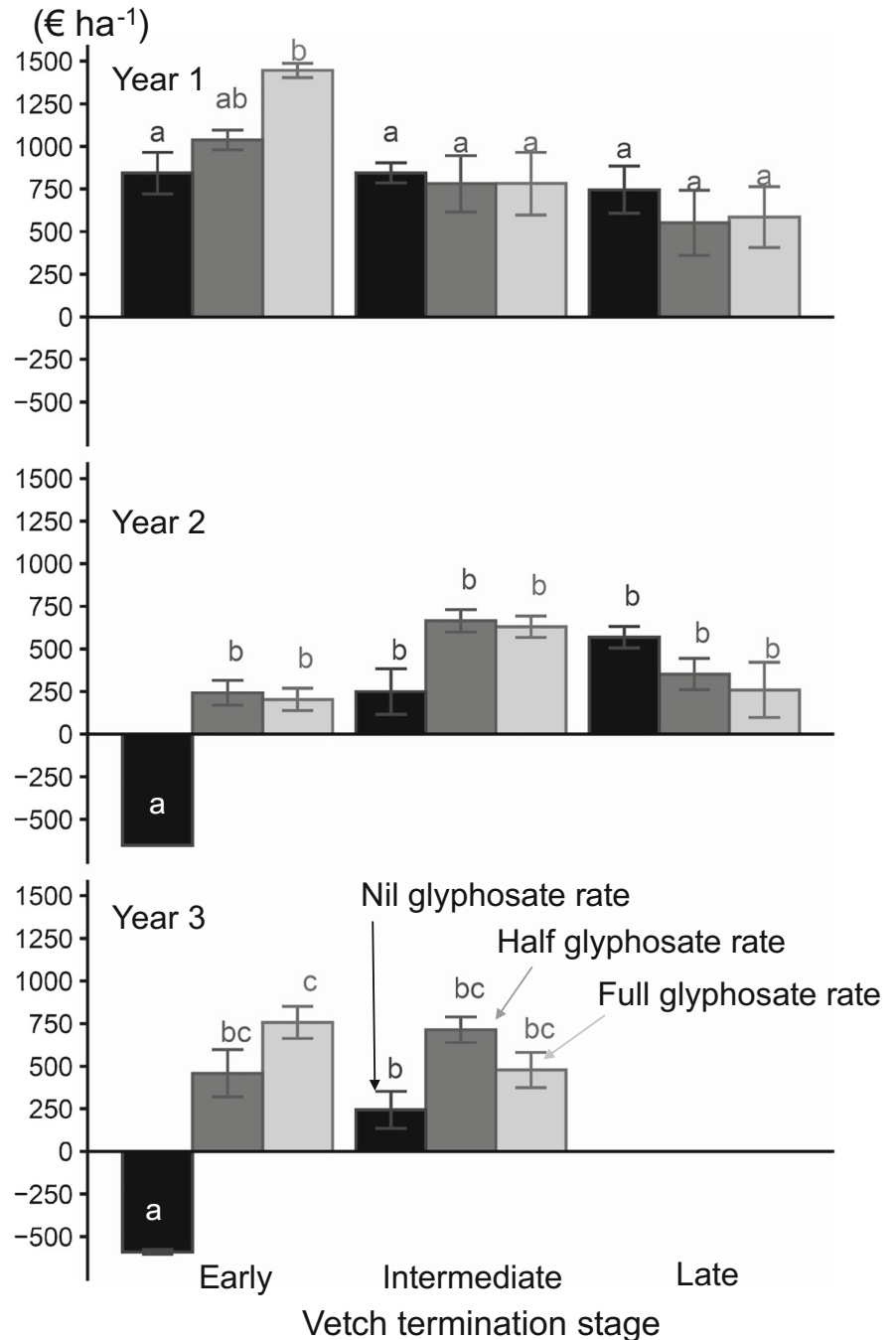
€ ha⁻¹ for nil, half and full glyphosate rate, respectively) that differentiated only due to the buying cost of glyphosate and its cost of spraying. Gross margin (Figure 9) was always positive, with the exception of the no-glyphosate treatment in the early termination period in 2014 (-653 € ha⁻¹) and 2015 (-590 € ha⁻¹). In 2013, the highest gross margin was observed in the full dose glyphosate treatment in the early termination period (1447 € ha⁻¹). In 2014, all treatments but the no-glyphosate treatment in the early termination period resulted in similar

gross margin (on average 397 € ha⁻¹). In 2015, use of the roller-crimper with no glyphosate application at the late cover crop termination period determined the highest absolute sunflower gross margin (1209 € ha⁻¹), which was significantly higher than in three out of six glyphosate-based treatments (nearly 2.5-fold, corresponding to a net gain of 721 € ha⁻¹).

The scientific literature on crop yield and gross margin following a roller-crimped cover crop in trials that include a glyphosate-treated comparison is relatively limited, and

Fig. 9. Sunflower gross margin as affected by hairy vetch termination stage and glyphosate rate in the three experimental years (2013 = year 1; 2014 = year 2; 2015 = year 3). Within each year, treatments with the same letter are not significantly different at $P \leq 0.05$ (Tukey's HSD test). Lines on top of each bar represent standard deviations.

Sunflower gross margin



mainly relates to the classical application on rye (*Secale cereale* L.) cover crop in the USA; instead, studies including a legume cover crop are very scarce. Kornecki (2020) showed no difference in seed cotton (*Gossypium* spp.) lint yield between roller-crimping and glyphosate ($1.06 \text{ kg a.i. ha}^{-1}$), regardless of roller-crimper type and number of passes. Similarly, Price et al. (2009) observed no significant differences in cotton yield across different locations and years between roller-crimped and glyphosate-terminated rye, black oat (*Avena strigosa* L.) or winter wheat (*Triticum aestivum* L.) cover crops, with one only exception where full or half glyphosate rate (0.84 and $0.42 \text{ kg a.i. ha}^{-1}$ respectively) had higher yield than 25% rate or no glyphosate. Still on cotton, no yield differences were observed between roller-crimped or glyphosate-treated ($1.06 \text{ kg a.i. ha}^{-1}$) rye or crimson clover (*Trifolium incarnatum* L.) cover crops across 3 years (Kornecki et al. 2015). In Argentina, no significant soybean yield differences between roller-crimped and glyphosate-treated ($1.5 \text{ kg a.i. ha}^{-1}$) rye, hairy vetch or triticale (x *Triticosecale*) were detected (Baigorria et al. 2019). In Spain, Alonso-Ayuso et al. (2020) observed basically no yield differences in 2 years out of three between roller-crimping and glyphosate ($1.07 \text{ kg a.i. ha}^{-1}$) in irrigated maize following a barley (*Hordeum vulgare* L.) vetch cover crop mixture, across several post-emergence weed control treatments including no weed control.

Overall, currently available scientific evidence indicates that no or limited differences in subsequent crop yield exist between roller-crimped and herbicide-treated cover crops, across a range of cover crops, subsequent crops and environments. In addition, it should be stressed that roller-crimping has much lower environmental impact (e.g. energy consumption) and higher energy productivity than herbicide-based treatments (Baigorria et al. 2019; Alonso-Ayuso et al. 2020).

4 Conclusion

Despite some year-to-year variations due to seasonal and field conditions, results of this study confirmed our hypothesis that appropriate timing of hairy vetch cover crop termination maximises cover crop biomass and improves enhances provision of important agroecosystem services like the effect of the cover crop on weed suppression, thereby reducing or even eliminating the need to use a contentious herbicide like glyphosate in a no-till system perspective. These outcomes highlight how cover-crop based no-till system could be implemented also in farming systems without the application of herbicides, e.g. in organic farming. Although we did not measure nitrogen supply to the subsequent cash crop, we can hypothesise that, besides the effects on weed reduction, the good level of sunflower grain yield that we observed at intermediate and late termination date of the cover crop might also be due to the considerable amount

of N left by hairy vetch, especially where cover crop biomass was higher, i.e. at the late termination stage. As expected, the positive effect on crop yield consequent to optimal timing of roller-crimping had also positive effects on sunflower gross margin, highlighting that through targeted management of a legume dead mulch system it is possible to maintain cash crop yield and economic return by reducing or even eliminating glyphosate use under no-till conditions. Further research is needed to test whether the positive effect of the timing of roller-crimping on cash crop yield is confirmed or not in different pedoclimatic conditions, and particularly in drier areas where the risk of drought could be higher, leading to crop yield depletion. In such challenging conditions, it would be important to identify strategies to improve roller crimper effectiveness at earlier cover crop termination stages. Also in our study, without glyphosate application, early cover crop termination resulted in sunflower crop failure in 2 years out of three.

We did not observe consistent effects of dead mulch treatments on weed community composition and diversity; therefore, we cannot speculate on possible carry-over effects of weed shifts on sunflower performance, as we hypothesised. Nevertheless, glyphosate-based treatments seemed to be characterised more often by higher relative abundance of competitive weed species; a trend that was shown by previous works and that should be confirmed in longer-term studies and in a range of different pedoclimatic conditions and cropping systems. A very interesting result that came out of our study is that, at low weed biomass levels, keeping the diversity in the weed community is beneficial for subsequent cash crop yield: this finding is supported by recent scientific evidence and is likely connected to lower presence of competitive species in diversified weed communities.

This study, for the first time, shows that targeted timing of a roller crimped hairy vetch cover crop in no-till sunflower can result in equal agronomic and economic performances than cover crop termination using glyphosate. As such, our results can contribute to the increasing body of literature highlighting the provision of multiple agroecosystem services through cropping system diversification aimed at reducing the use of contentious inputs.

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Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Code availability Code for data analysis is available at https://www.dropbox.com/sh/fdxhm7lpbyvr8r8/AAcCaOX51_KrUyEa2tyv7kTPxa?dl=0 for review process.

Declarations

Ethics approval Not applicable.

Consent to participate Verbal informed consent was obtained prior to start the trial in Tuscany Farm.

Consent for publication The authors affirm that all farmer participants provided informed consent for publication of data and pictures (Figure 1).

Conflict of interest The authors declare no competing interests.

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