Agron. Sustain. Dev. (2022) 42: 75
https://doi.org/10.1007/s13593-022-00787-3

RESEARCH ARTICLE

Relay intercropping can efficiently support weed management in cereal-based cropping systems when appropriate legume species are chosen

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Accepted: 25 May 2022 / Published online: 4 August 2022

Abstract

Relay intercropping of subsidiary legumes with durum wheat (living mulch) can be a viable option to support ecological weed control and optimize nutrient cycling in cereal-based cropping systems. However, the lack of knowledge on suitable legume species is often identified as the main bottleneck for the successful application of legume living mulches. This study aimed to evaluate the suitability of 12 different legumes for relay intercropping with wheat in two contrasting Mediterranean cereal-based cropping systems respectively characterized by low-input and integrated management. Each legume was monitored from the undersowing in wheat until the following spring and we compared direct drilling to broadcast sowing of legumes. None of the undersown legumes showed a negative effect on the wheat grain yield. Relay intercropping of legumes proved to be an effective solution to control weeds before and after the wheat harvest, provided suitable legumes species are chosen. Suitable legumes reduced the weed biomass up to the 90% during the intercropping and up to 94% in the following spring. On the contrary, legumes such as *Trifolium resupinatum*, *Vicia villosa*, *Medicago truncatula*, and *Medicago scutellata* boosted weed growth in the following spring in comparison with the control. According to the performance of legumes, *Medicago sativa*, *Trifolium repens* and *Medicago lupulina* had the most suitable characteristics for relay intercropping with durum wheat at the Ravenna site, in a highly productive region whereas *Medicago sativa*, *Hedysarum coronarium* and *Trifolium subterraneum* performed better in the low-input system near Pisa, where yields are generally lower. This is the first time that such a diversity in legumes species is tested in the same experiment for relay intercropping under diversified environmental and management conditions. The results of this study can support farmers in selecting the most appropriated legume species for their specific cropping systems and local conditions.

Keywords Living mulch · Crop diversification · Integrated Weed Management · Subsidiary crops

1 Introduction

The increased awareness about the importance of sustainable farming systems has resulted in the development of innovative cropping practices aimed at optimization of resource use and at reduction of the reliance on external inputs while maintaining adequate yield levels (Tilman et al. 2007).

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Agro-ecosystem diversification is a fundamental aspect of sustainable cropping system design and it can be obtained by increasing the number of grown species at crop rotation level (MacLaren et al. 2020; Neve et al. 2018). In this context, intercropping practices and cover crop use offer a wide variety of opportunities for sustainable intensification of conventional agricultural systems through spatial and temporal cropping system diversification (Bedoussac and Justes 2011; Wittwer et al. 2017). In particular, intercropping of a legume subsidiary crop with an annual cash crop (commonly referred to as living mulch) has been proposed as an important agro-ecological management technique that is able to provide agro-ecosystem services such as ecological weed and pest control, reduction of soil erosion, optimization of nutrient cycling and soil resource conservation through the increase of functional biodiversity in the cropping system (Li et al. 2020; Hartwig and Ammon 2002).

The question about how to increase the efficiency of living mulches to control weeds in cereal-based cropping systems has been given increasing attention. In arable crops, weeds are one of the most limiting factors for crop yield (Bàrberi 2002; Oerke 2006; Kruidhof et al. 2008) and to keep the systems economically viable weed control often relies heavily on herbicide applications. None of the alternative tactics proposed in Integrated Weed Management (IWM) used as sole method for weed control are as effective and easy to use as a well-selected herbicide (MacLaren et al. 2020). Therefore, IWM strategies need to be designed based on synergy of different tactics (Kudsk et al. 2020), affecting the various phases of the weed's life cycle (seedbank, plantlet and mature weed) as well as aspects of the entire cropping system (diversification, crop selections and management, field management and combinations of direct control measures).

Legume living mulches are an interesting tactic in more complex weed management strategies. They can reduce weed emergence and growth through competition for limited resources and/or the production of root exudates rich in allelochemical compounds (Hartwig and Ammon 2002; Teasdale et al. 2007).

However, ecosystems services provided by legume are valuable only if productivity of cash crop is preserved (Den Hollander et al. 2007a) and for this reason, a successful intercropping system can only be achieved if legume competition with the main crop is minimized (Hiltbrunner et al. 2007). For this purpose, intercropped species should have specific morphological and phenological characteristics allowing them to grow in a complementary spatial layer in relation to the main crop (Bedoussac and Justes 2010; Den Hollander et al. 2007b).

Several studies reported excellent weed control by intercropped legumes without a negative impact on cereal production (Campiglia et al. 2014) while others reported yield reduction, due to the direct competition (Verret et al. 2017). Moreover, specific weather conditions can alter the performance of the intercropped species. For instance, relatively high temperatures during winter may boost legume biomass production and increase early competition with the main crop. In cereal-based living mulch systems the dominance of the cereal under various weather conditions is fundamental to obtain a yield stability in time (Carof et al. 2007).

One of the solutions to provide a competitive advantage to the cereal is to delay the sowing of the legume (Amossé et al. 2013a). The delayed sowing into an already established crop is defined as relay intercropping (Vandermeer 1989). In such a system, legume growth is restricted by the already established cereal but it is expected to be enough to affect weed presence.

In Mediterranean cropping systems, cereal-legume relay intercropping involves broadcast sowing or direct drilling of the legume in late winter, before wheat steam elongation phase (BBCH GS 30), in order to avoid damage on the main crop during the seeding operation. In such a system, legumes are supposed to persist in the field after the wheat harvest, maintaining the soil covered until the sowing of the subsequent cash crop (Vrignon-Brenas et al. 2016a). In this way, relay intercropping of legumes represents a suitable solution to avoid the bare soil between two subsequent cash crop or between cereal harvest and cover crop establishment (Amossé et al. 2013a).

In Mediterranean cropping systems up to nine months may separate the wheat harvest and the sowing of the subsequent cash crop. Farmers are used to leave the soil uncultivated during this period. Weeds are generally managed before the sowing of the subsequent crop through herbicide application (Bedoussac and Justes 2010). The presence of legumes after the wheat harvest instead, is expected to improve weed control, limit soil erosion and improve soil fertility in the fallow period (Vrignon-Brenas et al. 2016a). This strategy can ultimately result in reduced herbicide and fertilizer applications. Moreover, perennial legumes such as *Medicago sativa* or *Trifolium repens* can be managed, according to farmers' needs, both as cover crop and for forage production. When perennial legumes are used as forage crop, farmers can take advantage from the establishment of the forage crop nine months in advance in comparison with the common timing of the crop rotation, increasing the land use efficiency and reducing the soil tillage.

Annual and annual self-seeding legumes can also be used in relay intercropping systems (Ilnicki and Enache 1992). These legumes are expected to contrast weed growth during the intercropping period by the establishment of a living mulch. Annual and annual self-seeding legumes conclude their growth cycle in late spring, concurrently with wheat. After wheat harvest, the accumulated biomass of annual legumes is supposed to be enough to form a dense dead mulch covering the soil until the subsequent spring. Self-seeding legumes are expected to persist in the field as dead mulch during the dry summer season and re-grow spontaneously from their seeds in autumn, to establish a cover crop until the subsequent summer crop that will be established in spring (Ilnicki and Enache 1992).

The successful application of relay intercropping is further determined by other relevant factors such as:

i) the choice of suitable cereal-legumes combinations (at species and cultivars level) for each given pedo-climatic condition (Amossé et al. 2013b);
ii) the differences between low-input vs high-input cropping system management (Radicetti et al. 2018);
iii) the composition of the weed population in the field, as perennial vs annual or monocotyledonous vs dicotyledonous weeds can react differently in the presence of legumes (Teasdale 1996).

Growth characteristics and weed suppression ability have been largely investigated for the most common legumes sole crop, however their performance as living mulch is still not well studied. Moreover, different legumes species do not have the same efficiency at controlling weeds (Uchino et al. 2011) and their performance may change considerably according to the climate, the cropping system management and the weed population characteristics (Radicetti et al. 2018).

The objective of this work was to identify suitable legumes for relay intercropping with durum wheat as integrated weed management and diversification tool in two contrasting Mediterranean cereal-based cropping systems.

We hypothesized a different behavior and role in the target cropping system for perennial, annual and annual self-seeding legumes, leading to the inclusion of a set of 12 different species from the three groups in the trial. We hypothesized an effect of legume sowing method on legume establishment. We compared direct drilling to broadcast sowing for a subset of legumes in order to evaluate the possible effect of sowing technique on living mulches establishment.

In this study, the target legumes were tested for two consecutive years in two locations under different input levels (a low-input system and an integrated system). Few studies tested such a diversity of legume species, including a focus for low-input vs high-input management system and an in-depth study of the influence of the sowing technique. The evaluation and ranking of the undersown legumes in this study was based on the following: legume emergence and growth in already established wheat, intercropping effects on wheat yield performance and weed control before and after wheat harvest.

2 Material and methods

2.1 Experimental site

This experiment was carried out in two locations in Italy (Pisa and Ravenna) over two consecutive crop seasons (2017/18, 2018/19). The field experiments were set up at the Centre for Agri-Environmental Research “Enrico Avanzi” of the University of Pisa (CiRAA, San Piero a Grado, Pisa, Italy, 43°41′02.08″N, 10°20′35.0″E) and at Horta (Horta, permanent platform for enhancing results from research in the agro-alimentary sector, Cà Bosco farm, Ravenna, Italy, 44°28′56.4″N, 12°10′43.0″E).

The field in Pisa was characterized by a silty-loam and a silt-clay soil (Jahn et al. 2006) respectively in 2017/18 and 2018/19 whereas fields in Ravenna were characterized by silty-clay-loam and clay-loam soil (Jahn et al. 2006) respectively in 2017/18 and 2018/19.

The reference cropping systems in Pisa and Ravenna are different. Ravenna is located in a very productive area of northern Italy where the arable cropping systems are characterized by a winter wheat, forage crop and irrigated summer crop rotation. In Pisa, the reference cropping system is characterized by winter wheat, forage crop and non-irrigated summer crop rotation. The lack of systematical irrigation in the Pisa plain determines the development of reduced-input systems. With respect to the input levels used by the farmers in these two reference cropping systems in the experimental fields the input levels were reduced in order to highlight the benefits provide by the legumes and differences among them (see Fig. 1). According to the different input levels of the cropping systems in each location, the two field experiments were managed as follows: (i) the Pisa site was managed with a low-input system in which no herbicides and fungicides were used, whereas (ii) the Ravenna site was managed as integrated system with optimized use of fertilizers, herbicides and pesticides (timing and doses of each application were optimized through the decision support system grano.net®, developed by Horta S.r.l).

In Pisa wheat was sown on 22 November 2017 and 12 December 2018 and respectively harvested on 26 June 2018 and 10 July 2019. Legumes were undersown on 28 February 2018 and 18 February 2019 and terminated on 14 April 2019 and 15 April 2020.

Total rainfall from wheat sowing to harvest was 766 mm in the first year and 568 mm in the second year whereas from the wheat harvest to the legume termination total rainfall was respectively 414 mm and 856 mm (Fig. 2a). Notably, during the first repetition of the experiment in the 30 days following the legume sowing (from 14 April to 30 March) total rainfall was 215 mm whereas during the second trial year only 28.5 mm of rainfall was measured (Fig. 2a). In Ravenna wheat was sown on 2 November 2017 and 15 November 2018 and respectively harvested on 21 June 2018 and 27 June 2019. Legumes were undersown on 28 February 2018 and 18 February 2019 and terminated on 19 April 2019 and 17 April 2020. Total rainfall from wheat sowing to harvest was 715.3 mm in the first year and 490.4 mm

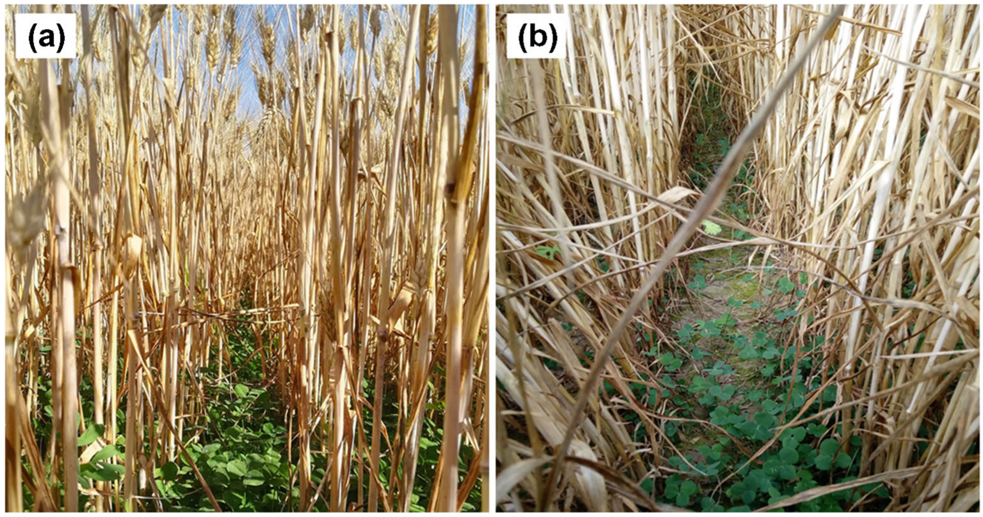

Fig. 1 Relay intercropping of *Medicago lupulina* with wheat. The pictures show that in (a) Pisa, compared with (b) Ravenna, the biomass accumulation of legume (i.e., its height) differs greatly as the result of multiple factors including different input levels. Photographs by Federico Leoni (a) and Matteo Ruggeri (b).

in the second while from the wheat harvest to the legumes termination total rainfall was respectively 599.4 mm and 591.2 mm in the first and the second trial year (Fig. 2b).

2.2 Experimental design and treatments

Durum wheat cv. Minosse was sown at a rate of 350 viable seeds per m^2 in 17 cm (Ravenna) or 18 cm (Pisa) wide inter-rows on fields previously ploughed at 25 cm depth and refined with rotary harrow.

The idea behind this experiment was to test a large diversity in legume species in order to explore the suitability of a wide range of morphological and phenological growth characteristics in the two target systems. Between the legumes available on the market we have chosen twelve different legumes among perennial, annual and annual self-seeding species by contacting experts on pasture legumes and consulting technicians of seed companies. Legumes tested in 2017/18 and their respective seeding rates were (i) four perennial legumes, *Medicago sativa* (cv. Gamma, 40 kg/ha), *Trifolium repens* (cv. Companion, 15 kg/ha), *Hedysarum coronarium* (cv. Carmen, 30 kg/ha), *Medicago lupulina* (cv.-, 40 kg/ha); and (ii) three annual legumes, *Trifolium incarnatum* (cv. Kardinal, 40 kg/ha), *Trifolium resupinatum* (cv. Laser, 10 kg/ha), *Vicia villosa* (cv. Capello, 80 kg/ha); five annual self-seeding legumes, *Trifolium michelianum* (cv. Paradana, 15 kg/ha), *Trifolium subterraneum* (cv. Mintaro, 35 kg/ha), *Medicago polymorpha* (cv. Scimitar, 40 kg/ha), *Medicago truncatula* (cv. Paraggio, 40 kg/ha), *Medicago scutellata* (cv. Sava, 40 kg/ha).

Undersown legumes were reduced in 2018/19 based on poor performance in both sites, excluding one annual legume (*Vicia villosa*) and three annual self-seeding legumes (*Trifolium michelianum*, *Medicago truncatula* and *Medicago scutellata*). Legumes were seeded before wheat stem elongation phase (BBCH 21-29, February-March) in plots (plot area in Pisa: 9 m^2 in 2017/18, 18 m^2 in 2018/19; plot area in Ravenna: 9 m^2 in 2017/18, 9 m^2 in 2018/19). Seeding rate of legumes was adjusted according to a germinability test performed in Petri dishes (100 seeds for each legume species and replicated four times). In each field a control treatment with wheat grown as the sole crop was added to evaluate the incidence of undersown legumes on wheat yield performance. Each treatment was repeated in four randomized blocks per site.

The standard sowing method of the legumes differed between Pisa and Ravenna. Legumes were seeded in late winter through drill sowing in the experiments conducted in Pisa whereas legumes were broadcast in Ravenna. Three legumes, *M. sativa*, *T. repens* and *T. subterraneum*, have been sown both with drill and broadcast method in Pisa and Ravenna in order to detect the influence of sowing technique on legumes establishment.

Durum wheat for grain production was mechanically harvested, and straw was removed from the fields. Undersown legumes were maintained after wheat harvest until spring, in April–May, before the sowing of a summer crop.

2.3 Data collection

Wheat, legume and weed density were evaluated after establishment. At wheat harvest (BBCH 92), wheat, legume and weed aboveground dry matter production (DM, $g \cdot m^{-2}$) were measured. Biomass samples of 0.54 m^{-2} (Pisa, three rows and 3 inter-rows space along 1 m) and 0.51 m^{-2} (Ravenna, three rows and 3 inter-rows space along 1 m) were hand harvested (one point per plot in 2017/18 and two

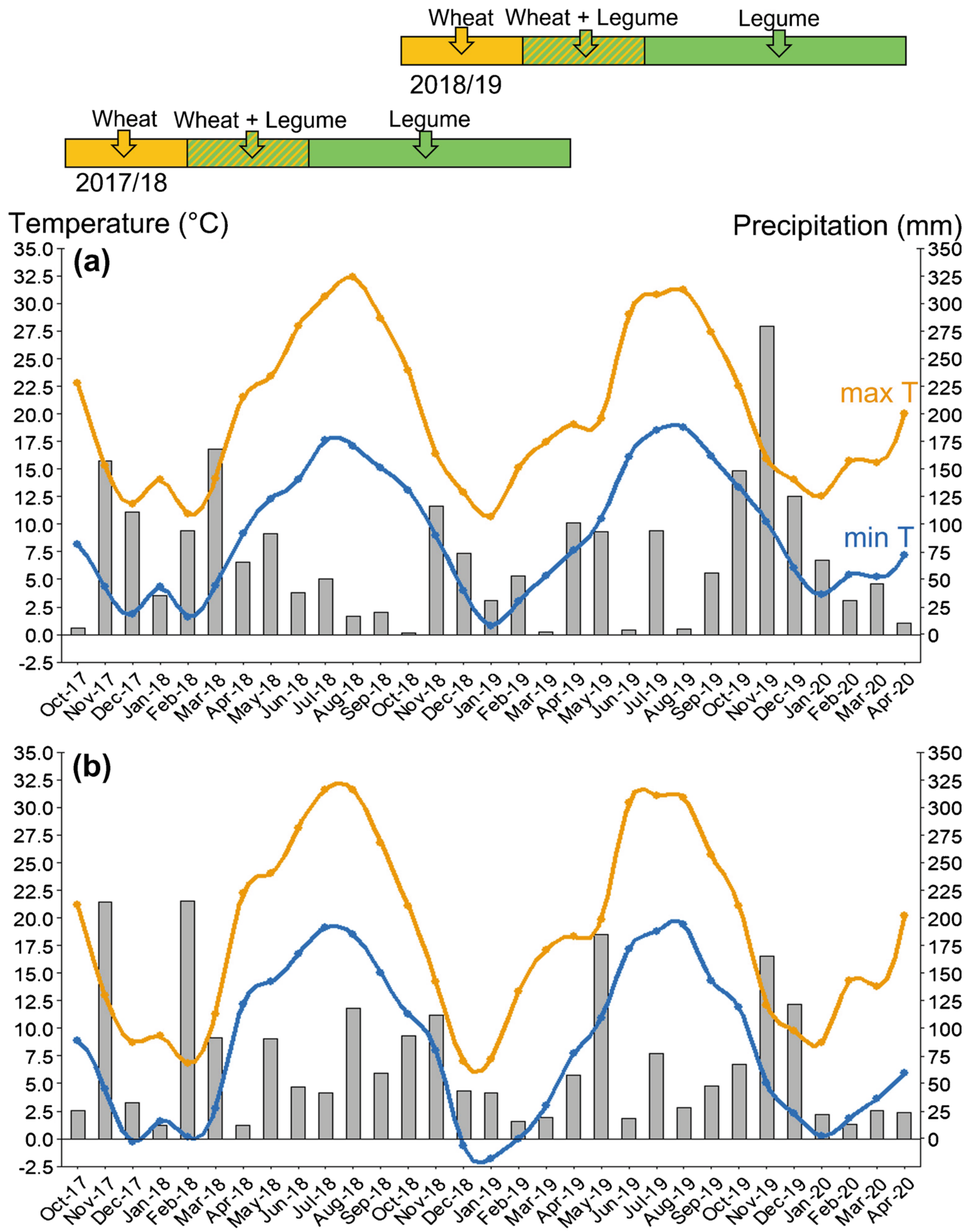

Fig. 2 Maximum (orange dots) and minimum (blue dots) monthly mean temperatures (left y-axis); and monthly mean rainfall (gray bars, right y-axis) from October 2017 to April 2020 in the experimental site in Pisa (a) and Ravenna (b). Site-specific climate conditions are collected by the in situ automatic weather station. Sensors were placed 2 m above ground. Abbreviations: max T, monthly mean maximum temperature; min T, monthly mean minimum temperature.

points per plot in 2018/19). The three components, wheat, legumes and weeds, were separated and weighed after oven-drying (40 °C in air oven). At this sampling time also maximum canopy height (cm) of wheat and legumes was measured randomly in five point per plot (only for 2018/19 growing season).

In the following spring, before sowing of the summer crop, legume and weed aerial dry matter (DM, g·m^{-2}) was determined for all legumes. Biomass samples of 0.27 m^{-2} (Pisa) and 0.51 m^{-2} (Ravenna) were hand harvested (two points per plot in Pisa in 2017/28 and 2018/19 and one point per plot in Ravenna in 2018/19).

Due to logistical and technical issues no biomass sampling was performed in spring 2018 (crop season 2017/18) in Ravenna. As a consequence, data on legume and weed biomass in Ravenna were excluded from the analyses concerning this sampling time.

2.4 Statistical analysis

A Linear Mixed Model was used to evaluate the effects of sowing techniques on legume establishment. In case significant interactions between legume species, year and location were found, differences among treatments were investigated separately for each location and growing season ($P < 0.05$).

The analysis of wheat performance (grain production, straw biomass and Harvest Index) was performed using a Linear Mixed Model. The model was formulated as:

$$\mathrm{Y_{ijkl}} = \mu + \text{Leg-Spec}_i \cdot \text{Year}_j \cdot \text{Location}_k + \text{BLK}_l/\text{Sub} + \epsilon_{ijkl};$$

where Y_{ijkl} is the variable value for each undersown legume i (Leg-Spec$_i$) in the growing season j (2017/18 and 2018/19, Year$_j$), location k (Pisa and Ravenna, Location$_k$) and Block (BLK$_l$). μ represents the grand mean and ϵ_{ijkl} is the residual error. Pseudo-replicates (Sub), were nested in to Block. The model was ran with Leg-Spec$_i$, Year$_j$ and Location$_k$ as fixed effects and BLK$_l$/Sub as random effect. In the case of significant Leg-Spec$_i$ · Year$_j$ · Location$_k$ interactions, differences among treatments were investigated separately for each location and growing season ($P < 0.05$).

To investigate the weed biomass (Y_{ijkl}) at wheat harvest time and in the subsequent spring in response to the different intercropped legumes species, we adopted a model assuming legume species (Leg-Spec$_i$), legume biomass (Leg-Biom$_j$) and year (Year$_k$) and their interactions as fixed terms and blocks (BLK$_l$) as a random term. For each location and sampling time, the model was formulated as:

$$\mathrm{Y_{ijkl}} = \mu + \text{Leg-Spec}_i \cdot \text{Leg-Biom}_j \cdot \text{Year}_k + \text{BLK}_l/\text{Sub} + \epsilon_{ijkl};$$

where μ is the grand mean and ϵ_{ijkl} is the residual error. The model selection was performed by stepwise addition from zero-model of each fixed term by likelihood ratio test. A fixed term was considered significant when its inclusion generated a significant reduction in Akaike information coefficient (AIC). According to this procedure for model selection, the wheat biomass was excluded from the selected the model.

Differences for legume biomass production at wheat harvest time and in the subsequent spring were investigated adopting a model with legume species (Leg-Spec$_i$) and year (Year$_j$) and their interactions as fixed terms and blocks (BLK$_k$ / Sub) as a random term. For each location and sampling time, the model was formulated as:

$$\mathrm{Y_{ijk}} = \mu + \text{Leg-Spec}_i \cdot \text{Year}_j + \text{BLK}_k/\text{Sub} + \epsilon_{ijk};$$

The relationship between weed and legume biomass has been investigated both in Pisa and Ravenna (Pearson correlation, $P < 0.05$) in the subsequent spring. Moreover, a logarithmic regression has been performed to study the relationship between annual, annual self-seeding and perennial legume aerial biomass and weed aerial biomass in spring in Pisa (2017/18 and 2018/19) and Ravenna (2018/19). For each location and year, the logarithmic model formulated as:

$$\mathrm{W}_{ij} = \mu + \log(\text{Leg-Biom}_{ij}) \cdot \text{Cycle}_i + \text{BLK}_j/\text{Sub} + \epsilon_{ij};$$

where W_{ij} is the weed aerial biomass for each legume type i (annual, annual self-seeding and perennial, Cycle$_i$) and Block (BLK$_j$), log(Leg-Biom$_{ij}$) is the logarithm of legume aerial biomass for each legume type i (annual, annual self-seeding and perennial, Cycle$_i$). μ represents the grand mean and ϵ_{ij} is the residual error. Pseudo-replicates (Sub), were nested in to Blocks. The model was run with log(Leg-Biom$_{ij}$) and Cycle$_i$ as fixed effects and BLK$_l$/Sub as a random effect. In the case of significant log(Leg-Biom$_{ij}$) · Cycle$_i$ interactions, differences among treatments were investigated at the average value of legume biomass for each location and year performing a Tukey post hoc test to separate means ($P < 0.10$). Data analysis was performed using R environment for statistical computing (R Core Team 2020). Statistical models were performed using the R/'Lme4' package (Bates et al. 2015). For significant explanatory variables, the Tukey post hoc test was performed to separate means ($P < 0.05$) using the R/'emmeans' package (Lenth et al. 2020). Normality and homogeneity of residuals variance have been graphically studied for the validation of each model using R/'DHARMa' package (Hartig 2020).

3 Results and discussion

3.1 Sowing techniques

In this study, three legumes, *M. sativa* (MEDSA), *T. repens* (TRFRE) and *T. subterraneum* (TRFSU), have been sown both with drill and broadcast method in Pisa and Ravenna (Fig. 3).

Legume drill sowing in an already established wheat stand can be performed using mechanical seed drills. Seeds are placed in the wheat inter-rows space and the distance between wheat and legumes rows is maximized. Broadcast seeding of legumes involves the use of centrifugal seeder combined with a light harrow. When broadcast seeding is performed, light harrowing has a dual functionality; the incorporation of seeds into the soil and mechanical control of smaller weeds. However, with broadcast sowing, seeds are incorporated into the soil only superficially thus decreasing seed contact with the soil, and increasing their susceptibility to unfavorable environmental condition or seed predation (Brennan and Leap 2014). For example, according to the results of this study, in Pisa in 2017/18, 40 days of drought occurred after legume sowing, and broadcast seeded legumes had a significantly lower emergence percentage in comparison with drilled legumes

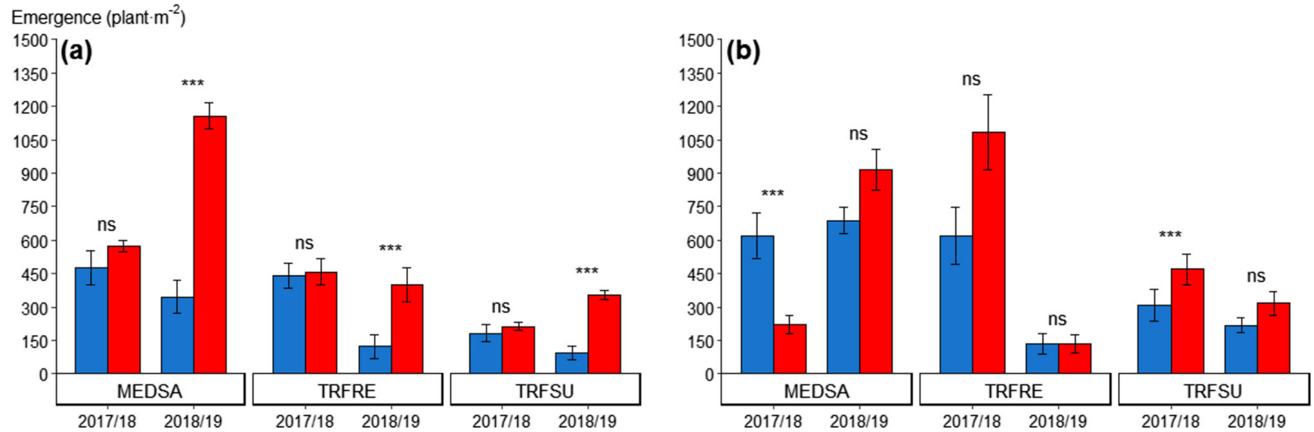

Fig. 3 Seedlings emergence of legumes in Pisa (a) and Ravenna (b) through drill (red) and broadcast sowing (blue) in 2017/18 and 2018/19 growing season. MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; TRFSU: *Trifolium subterraneum*. Significance codes: $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), $P > 0.05$ (ns). Error bars represent standard error (SE).

(MEDSA: 1155 ± 113 vs 345 ± 62 plant·m^{-2}, TRFRE: 402 ± 69 vs 122 ± 35 plant ·m^{-2}; TRFSU: 355 ± 65 vs 95 ± 31 plant·m^{-2}) (Fig. 3).

What has been observed in this study thus confirms that broadcast sowing is a high-risk sowing method for crop establishment and for this reason seeding rate should be increased by 20 to 50% when broadcast sowing is performed (Kearney et al. 2006).

Despite the poor stands resulting from broadcast seeding, it remains difficult to conclude that one method is generally preferable for relay intercropping. Broadcast seeding has clear practical advantages because it is less costly and more rapid and these are important characteristics especially during the winter when time, daylight, favorable weather and soil condition are often a limiting factor for timely sowing (Fisher et al. 2011). Moreover, all farmers have access to simple broadcast seeders. In sub-optimal condition, for example less fertile soils or fields with a higher weed pressure, drill sowing provides some clear advantages related to the higher and more uniform establishment of the undersown legumes, by decreasing the competition between the legume plants and the main wheat crop, thus providing an optimal establishment of legumes after the wheat harvest while guaranteeing sufficient weed control. Therefore, the most suitable sowing technique depends on the local conditions and farmers should evaluate the possible advantages and disadvantages of both sowing techniques in relation to the soil conditions, weed pressure, and the available machinery. The seeding rate should be adjusted accordingly and of course this needs to be included in the analysis of the cost effectiveness of both methods. In this case, both the cost of specific legume species and varieties and their availability on the local seed market are factors that will determine the final decision made by farmers. Positive and negative aspects related with the sowing method adopted have been summarized using a SWOT analysis (see supplementary material SM1).

3.2 Wheat performance

Overall wheat production in Pisa was significantly lower than in Ravenna. In particular, in Pisa in 2017/18 wheat production was very low (1.47 t·ha^{-1}) and it was significantly below to the local production level which was on average 2.56 t·ha^{-1} (ISTAT 2018; Table 1). In 2018/19 instead, wheat production (4.94 t·ha^{-1}) was higher than the local production level that was 2.57 t·ha^{-1} on average (ISTAT 2019; Table 1).

In Ravenna, wheat production was in line with the local production level (ISTAT 2018) and it was on average 6.8 t·ha^{-1} with no significant differences between the two years (Table 1).

The results of this experiment confirmed that delayed sowing of legumes in the already established wheat stand can reduce inter-specific competition between legumes and wheat. Both in Pisa and Ravenna undersown legumes did not have negative effects on the durum wheat yield for any of the species used in this experiment (Table 1). This result is consistent with previous studies on relay intercropping of forage legumes in both conventional (Bergkvist et al. 2011) and organic (Amossé et al. 2013a) cereal-legume associations.

Among the legumes tested in Pisa, *T. resupinatum*, *T. incarnatum* and *V. villosa* overtopped wheat and showed a very vigorous growth during the final part of their life cycle when the competition with wheat for light and space decreased following grain ripening. Results of this experiment showed that late competition with *T. resupinatum* and *V. villosa* did not affect wheat production because the nutrient translocation to the grain had already occurred although a significantly lower HI of *T. resupinatum* (0.30 ± 0.01) and *V. villosa* (0.23 ± 0.01) in comparison with the wheat sole crop (0.35 ± 0.01) was detected ($P < 0.05$). The vigorous legume growth caused problems at harvest though. It is not surprising that problems related

Table 1 Wheat grain production (Yield, t·ha^{-1}), straw biomass (Dry Weight, t·ha^{-1}) and Harvest Index in Pisa (2017/18 and 2018/19) and Ravenna (2017/18 and 2018/19). Leg. Spec.: Legume Species. Different letters (a–b) indicate significant differences at the 0.05 level. Significance codes: $P<0.001$ (***), $P<0.01$ (**), $P<0.05$ (*), $P>0.05$ (ns).

Location	Year	Wheat grain production (Yield, t·ha^{-1})	Straw biomass (DW, t·ha^{-1})	HI
Pisa	2017/18	1.47 ± 0.42 a	4.29 ± 1.02 a	0.31 ± 0.01 a
	2018/19	4.94 ± 0.29 b	12.38 ± 0.73 b	0.39 ± 0.07 b
Ravenna	2017/18	6.94 ± 0.42 a	14.03 ± 1.02 a	0.48 ± 0.01 b
	2018/19	6.77 ± 0.29 a	16.95 ± 0.73 b	0.39 ± 0.01 a
P-value	Legume Species	ns	ns	***
	Location	***	***	***
	Year	***	***	***
	Leg. Spec. x Location	ns	ns	***
	Leg. Spec. x Year	ns	ns	ns
	Location x Year	***	***	***
	Leg. Spec. x Location x Year	ns	ns	ns

with inter-specific competition between legumes and wheat are more relevant in low-input or organic cereal-based system where legumes generally show a higher biomass accumulation than in conventional or integrated systems.

3.3 Wheat and legume canopy height

In order to guarantee the uptake of relay intercropping practices at farm level, wheat mechanical harvest should not be hindered by undersown legumes. In Mediterranean relay intercropping systems, wheat is harvested in early summer whereas undersown legumes are supposed to persist in the field until the subsequent cash crop. In this system, suitable legumes should grow in a complementary space with wheat throughout the entire growing cycle of the main crop and they should not invade the same spatial layer as the harvestable wheat portion. In fact, the presence of legumes at wheat canopy level can hinder the mechanical harvest operations, resulting in grain yield losses and provoking a serious post-harvest problem.

Legume canopy height measured at wheat harvest time can be a good indicator to evaluate the suitability of legumes for relay intercropping systems (Den Hollander et al. 2007a). According to the results of this study, *T. repens*, *M. lupulina*, *H. coronarium*, *T. subterraneum*, *M. polymorpha* and *M. sativa* grew in a complementary layer with respect to the harvestable wheat portion. Canopy height of these legumes was on average 16.5 cm and it was almost six times lower than the maximum canopy height of wheat (94 cm; Fig. 4). Through the use of these legumes,

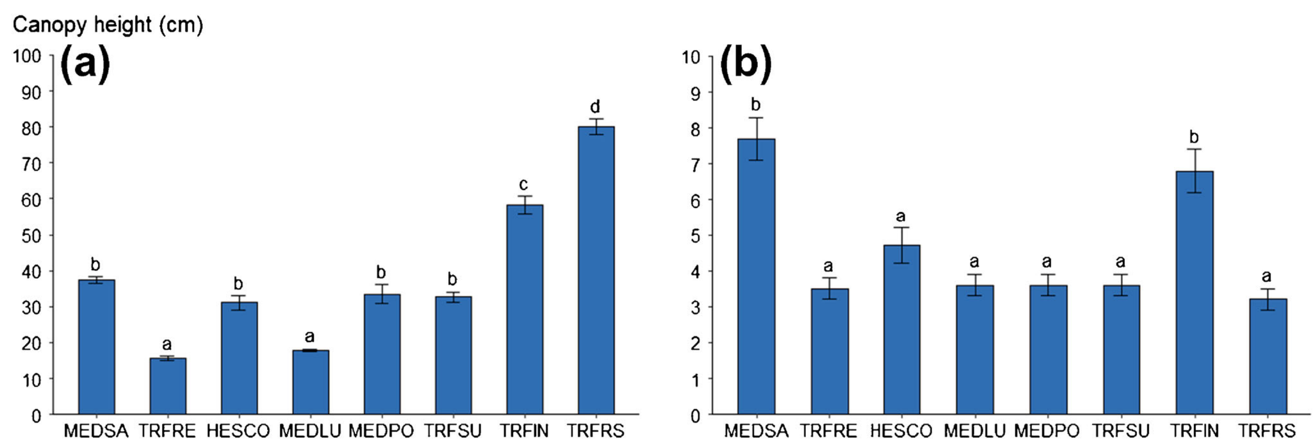

Fig. 4 Canopy height (cm) of the undersown legumes at wheat harvest time in Pisa (a) and Ravenna (b) in 2018/19 growing season. Notice the difference in canopy height scales between Pisa and Ravenna. MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; HESCO: *Hedysarum coronarium*; MEDLU: *Medicago lupulina*; MEDPO: *Medicago polymorpha*; TRFSU: *Trifolium subterraneum*; TRFIN: *Trifolium incarnatum*; TRFRS: *Trifolium resupinatum*; MEDSC: *Medicago scutellata*; MEDTR: *Medicago truncatula*, TRFMI: *Trifolium michelianum*; VICVI: *Vicia villosa*. Different letters (a–d) indicate significant differences at the 0.05 level. Error bars represent standard error (SE).

wheat can be easily harvested by just setting the height of the combine harvester accordingly. On the contrary, the annual legumes *T. incarnatum* and *T. resupinatum* showed a very vigorous growth during the final part of their life cycle and their maximum canopy height was similar with wheat (respectively 58.2 and 80.5 cm) resulting unsuitable for relay intercropping (Fig. 4). Canopy height of all legumes in the Ravenna site ranged from 3.6 to 7.7 cm and therefore harvest was never hindered and no differences in wheat performance were detected.

These results show that the cropping system interferes with the phenotypical expression of the intercropped legumes. More competitive wheat stands are able to suppress potentially high growing annual legumes whereas these legumes come to their full expression in low-input systems where the wheat stand is not able to compete efficiently. Perennial legumes do not normally exhibit such a vigorous growth in their first growing season and may therefore be adapted for both low-input and integrated systems. It is likely that cv Minosse is not competitive in a low-input systems. However, there may be wheat varieties on the market that are competitive also in low-input systems, e.g., varieties originating from organic breeding programs.

3.4 Legumes and weed control during the intercropping period

Cropping system management significantly affected the biomass production and the weed control capacity of living mulches during the intercropping period. Fertilizer application, for instance, supported wheat growth and increased the competitiveness of wheat against the undersown legumes (Vrignon-Brenas et al. 2016a). In Ravenna, where optimal fertilization dosages and timing were provided, total wheat biomass was on average 45% higher than in Pisa and the legume growth in the already well-established wheat was significantly reduced by 90% (Fig. 5). In the low-input system living mulches proved to be a suitable

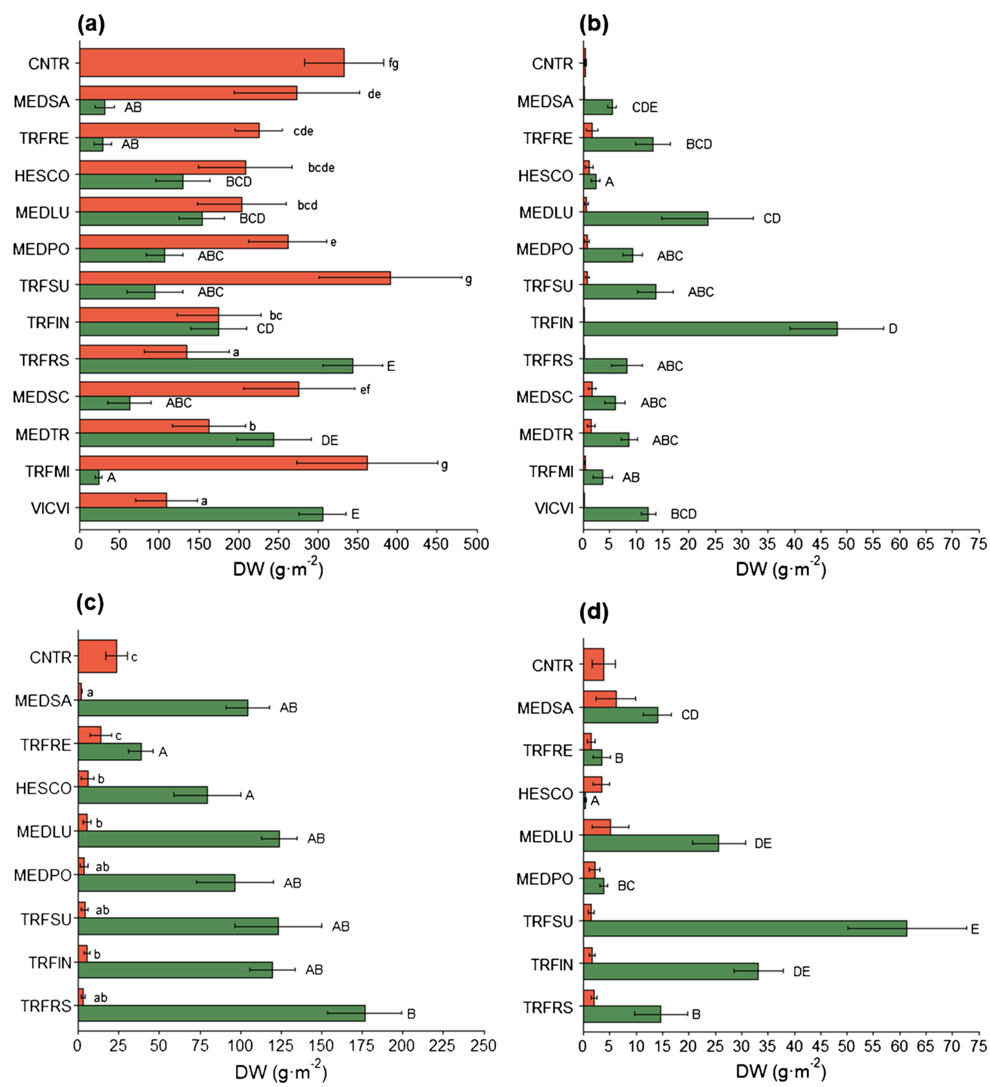

Fig. 5 Legume (green bars) and weed dry aerial biomass (red bars, Dry Weight, g $\cdot$m^{-2}) at wheat harvest time in Pisa in 2017/18 (a), 2018/19 (c) and Ravenna in 2017/18 (b) and 2018/19 (d). CNTR: Control plot (wheat sole stand crop); MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; HESCO: *Hedysarum coronarium*; MEDLU: *Medicago lupulina*; MEDPO: *Medicago polymorpha*; TRFSU: *Trifolium subterraneum*; TRFIN: *Trifolium incarnatum*; TRFRS: *Trifolium resupinatum*; MEDSC: *Medicago scutellata*; MEDTR: *Medicago truncatula*, TRFMI: *Trifolium michelianum*; VICVI: *Vicia villosa*. Different letters (a–e, A–E) indicate significant differences at the 0.05 level. Error bars represent standard error (SE).

Table 2 Factors affecting legume and weed biomass in Pisa and Ravenna at wheat harvest time and in the following spring. Significance codes: $P < 0.001$ (***), $P < 0.01$ (**), $P < 0.05$ (*), $P > 0.05$ (ns).

Factors	Wheat harvest time				Following spring			
	Legume		Weed		Legume		Weed	
	Pisa	Ravenna	Pisa	Ravenna	Pisa	Ravenna	Pisa	Ravenna
Legume Species	***	***	***	ns	***	***	***	***
Legume Biomass	–	–	***	*	–	–	**	***
Year	ns	**	***	*	ns	–	**	–
Leg. Spec. x Leg. Biom.	–	–	***	ns	–	–	ns	–
Leg. Spec. x Year	***	***	***	ns	***	–	***	–
Leg. Biom. x Year	–	–	*	*	–	–	ns	–
Leg. Spec. x Leg. Biom. x Year	–	–	***	ns	–	–	ns	–

strategy to support weed control (Table 2). In Pisa, the above-ground biomass of legumes reached at wheat harvest was sufficient to reduce weeds in comparison with the sole crop (Fig. 5). Our observation of a significant effect of undersown legumes on weed biomass is not in accordance with Amossé et al. (2013a) who reported a reduction in weed density but not in biomass during the intercropping period due to the reduced development of legumes before wheat harvest. In Ravenna instead, presence of undersown legumes was not significantly related with the weed control capacity of legume species used as living mulches (Table 2). In such a system, legume growth was strongly restricted by the wheat competition and weeds were already well controlled by the preventive application of herbicides (Fig. 5).

These results are consistent with previous studies on relay intercropping under different environmental conditions and crop management systems. Such studies highlighted that benefits provided by the use of subsidiary or forage legumes during the intercropping period with cereals are maximised in low-input or organic systems where the absence or the extremely reduced use of external inputs leads to a greater weed population and lower nutrient levels (Nelson et al. 2012). In particular, in Pisa, the most effective legumes in controlling weeds during the intercropping period were, *M. sativa*, *H. coronarium*, *M. lupulina*, *T. incarnatum* and *T. resupinatum* (Fig. 5). Other legumes such as *T. repens* and *T. subterraneum* instead, showed contrasting efficiency in controlling weeds between the two growing seasons (Fig. 5). In particular *T. repens* showed a very slow growth during early establishment and for this reason it had a lower competitiveness against weeds, confirming what has been already reported in a previous study (Brandsaeter and Netland 1999).

In Ravenna, despite the low contribution of undersown legumes in weed control, presence and viability of legumes in already established wheat remains fundamental for their successful establishment and persistence after the wheat harvest, and the advantage this may provide to farmers aiming at the establishment of a perennial alfalfa sward for forage production.

In the experimental fields used in this study, dicotyledonous weeds were the predominant component in the weed community compared to monocotyledonous weeds (see supplementary material SM2). However, many studies reported that efficiency of cover crops in controlling weeds may change according to the composition of the weed population in the field (Teasdale 1996) and that monocotyledonous and dicotyledonous weeds can react differently in the presence of legumes (Hiltbrunner et al. 2007; Leoni et al. 2020). In particular, Brainard et al. (2012) reported that the use of cover crops as sole weed control strategy can be not enough to contrast grass weeds properly and for this reason, the authors recommended the use of supplementary methods for weed control to prevent accumulation of weed seeds in the soil seed bank. After the wheat harvest, the periodic mowing of legumes during the critical period for grass dissemination (August–September for summer weeds and April–May for winter weeds) can reduce the weeds germination and seed multiplication in the long term controlling weeds, without negative effects on the legume sword.

3.5 Legumes and weed control in the following spring

In relay intercropping systems, legumes are expected to persist in the field after wheat harvest (Vrignon-Brenas et al. 2016a). Relay intercropping allows to establish the subsequent forage crop (mostly when perennial species are used) or a cover crop/dead mulch (mostly when self-seeding or annual species are used) immediately after wheat harvest and until sowing of the following cash crop. In Mediterranean cereal-based cropping systems, up to nine months may separate wheat harvest from the following cash crop and farmers are forced to leave the soil uncultivated because soil tillage and crop establishment are almost impossible during the warm and dry summer months. To control weeds in this intercrop period, farmers may use herbicides or cut the vegetation. The results of this experiment confirm what has been reported by Vrignon-Brenas et al. (2016a), i.e., that relay intercropping of legumes can be a suitable solution to maintain a continuous and weed suppressive soil cover during the fallow period and this study identified the most suitable legumes under Mediterranean conditions.

Perennial legumes proved to be the most versatile legumes and they had a high biomass production and good weed control in both Pisa (low-input system) and Ravenna (integrated system; Fig. 6). It has been reported

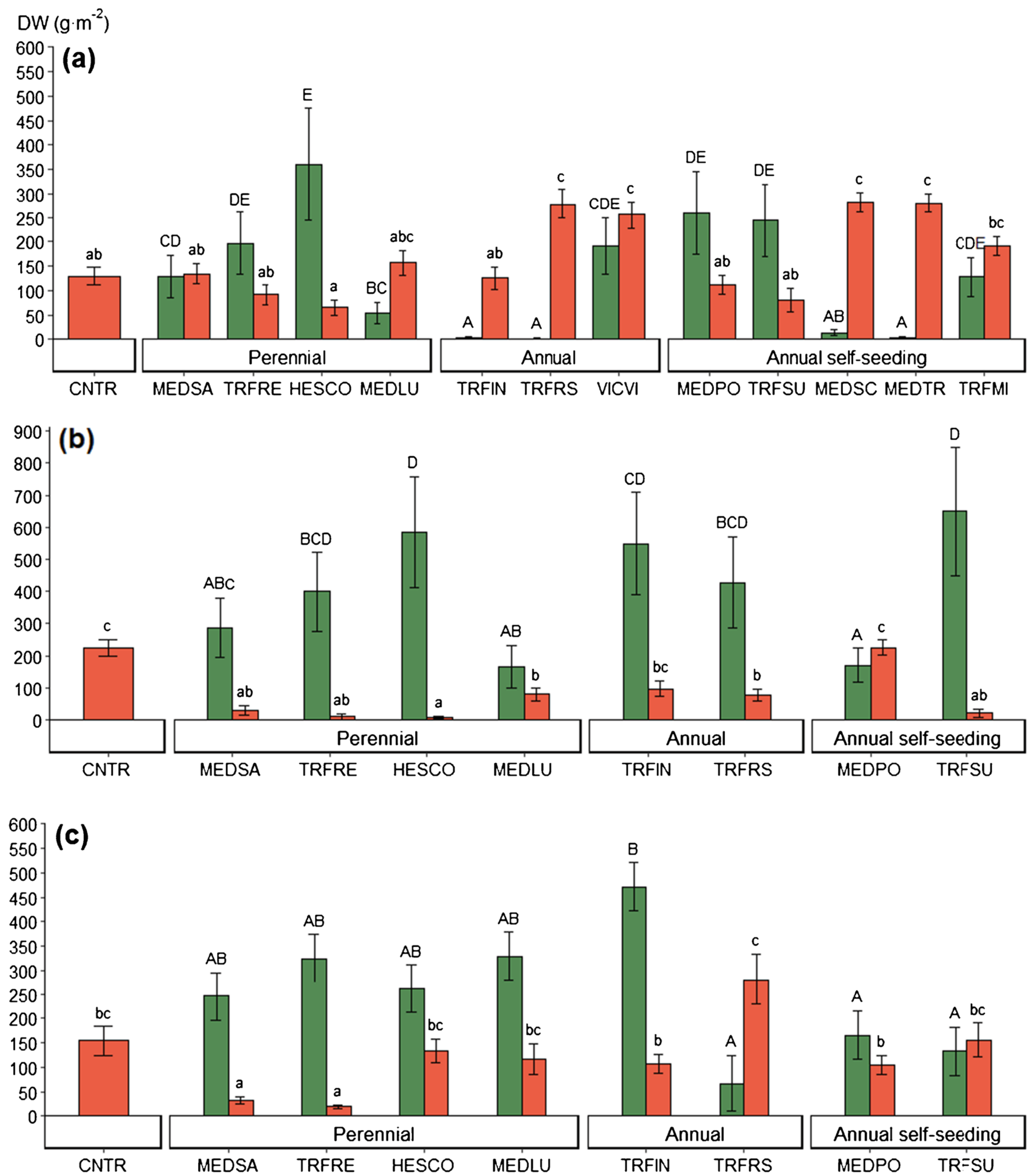

Fig. 6 Legume (green bars) and weed dry aerial biomass (red bars, Dry Weight, g $\cdot m^{-2}$) in spring in Pisa 2017/18 (a) and 2018/19 (b) and Ravenna 2018/19 (c). CNTR: Control plot (wheat sole stand crop); MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; HESCO: *Hedysarum coronarium*; MEDLU: *Medicago lupulina*; MEDPO: *Medicago polymorpha*; TRFSU: *Trifolium subterraneum*; TRFIN: *Trifolium incarnatum*; TRFRS: *Trifolium resupinatum*; MEDSC: *Medicago scutellata*; MEDTR: *Medicago truncatula*, TRFMI: *Trifolium michelianum*; VICVI: *Vicia villosa*. Different letters (a–g) indicate significant differences at the 0.05 level. Error bars represent standard error (SE).

that the persistence of perennial legumes after wheat harvest depends more on the plant density than on their biomass production during the intercropping period (Vrignon-Brenas et al. 2016b). This may be the reason for which they were the most suitable solution in the highly productive integrated systems in Ravenna, where biomass accumulation of legumes during the intercropping period was very low. Among perennial legumes, *M. sativa* and *T. repens* were particularly suitable for both Pisa and Ravenna (Fig. 6). In particular, it has been reported that legumes such as *M. sativa* are physiologically tolerant to shade and, for this reason particularly promising to be used in highly productive cereal system where the competition of the main crop for light is higher (Lorenzo et al. 2019). Among perennial legumes, also *H. coronarium* resulted particularly suitable but only for low-input systems (Fig. 7). On the contrary *M. lupulina* had a higher biomass accumulation in Ravenna than in Pisa where sub-optimal environmental conditions occurred during the summer in terms of temperature and precipitation. In fact *M. lupulina*, as already reported by Whyte et al. (1953), is not drought-resistant and during the summer (Jun–Aug) total precipitation in Ravenna was significantly higher than in Pisa (average of 54 mm vs 34 mm).

Performance of annual self-seeding legumes in the following spring was more dependent on their biomass production during the intercropping period. In fact, the establishment of a dense and suppressive dead mulch during the summer and the proper regrowth in the following autumn require a good biomass and seed production during the intercropping period. For this reason, annual self-seeding legumes resulted more suitable for the low-input system where less competition between wheat and legumes occurred (Radicetti et al. 2018). In particular, among the annual self-seeding legumes, *T. subterraneum* resulted particularly interesting for the low-input system because of the excellent re-growth capacity and good weed control after wheat harvest (Fig. 6).

Annual legumes were less suitable for relay intercropping in both cropping systems. Annual legumes are supposed to conclude their life cycle at the same time as the cereal crop and persist in the field after wheat harvest as a dead mulch until the following crop. Despite the fact that annual legumes have no specific self-seeding ability, following favorable field conditions they can re-grow from the seedbank in autumn as happened in Pisa and Ravenna in the 2018/19 growing season (Fig. 6). However, since performance of annual legumes depended heavily on the environmental conditions, their use in relay intercropping system should be discouraged. The annual legumes *T. resupinatum* and *V. villosa* and the annual self-seeding legumes *M. truncatula* and *M. scutellata* had a very low biomass production and residual effects of these legumes boosted the weed growth and significantly increased the weed pressure in comparison with the control in the subsequent spring (Fig. 6). In agreement with Hiltbrunner et al. (2007) weeds took advantage of the additional N provided by legumes and increase their biomass if not enough dense mulching is established. Overall, biomass production of legumes in spring was significantly correlated with their weed control capacity (Pisa 2017/18: $R^2 = 0.24$, $P < 0.01$; Pisa 2018/19: $R^2 = 0.23$, $P < 0.01$; Ravenna 2018/19: $R^2 = 0.17$, $P < 0.05$). With equal amounts of biomass production, perennial legumes had a higher weed control capacity in comparison with annual and annual self-seeding legumes both in Pisa and Ravenna (Fig. 7). Differences in weed control capacity could be related to different competitiveness between perennial and annual self-seeding legumes during the sum-

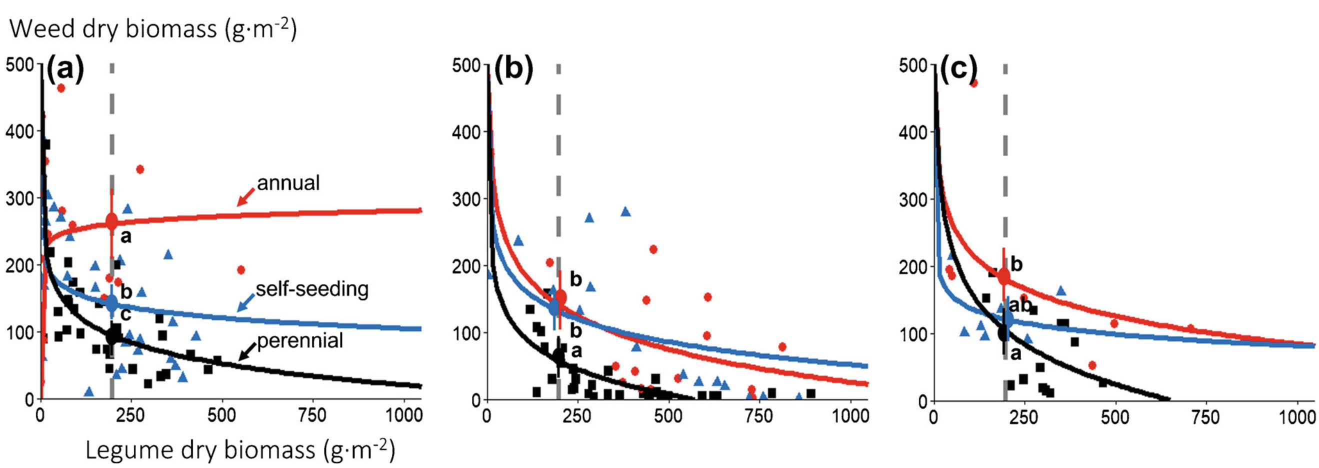

Fig. 7 Relationship between annual, annual self-seeding and perennial legume aerial biomass and weed aerial biomass in spring in Pisa (a: 2017/18, b: 2018/19) and Ravenna (c: 2018/19). The curves represent the logarithm fitted model and dotted line the mean value of legume biomass. Differences between annual, annual self-seeding and perennial legume were evaluated at the point in which grey dotted line intercept the three curves ($P < 0.10$).

mer season (Leoni et al. 2020). As a consequence of their growth cycle, perennial legumes persisted as living mulch during the summer period whereas, residues of annual and annual self-seeding legumes formed a dead mulch. After wheat harvest, annual and annual self-seeding legumes had not enough biomass to establish a suppressive dead mulch and for this reason dead mulches did not suppress weeds as consistently as living mulches during the summer. This may have resulted in an increase in the weed seedbank in annual and annual self-seeding legumes. This ultimately caused a less efficient weed suppression capacity of annual and annual self-seeding in the subsequent spring compared with perennial legumes.

4 Conclusions

Overall, this work highlights that an appropriate choice of legume species is needed to support the successful application of relay intercropping in cereal-based cropping systems and to promote their large-scale use at farm level.

The suitability of legumes for this system changes according to the local environmental conditions and in relation to the management intensity of the cropping system. For this reason, the current experiment was carried out under two different crop management systems. Ravenna is located in a very productive area of northern Italy where water is not a limiting factor during summer and reference cropping systems are characterized by conventional management whereas Pisa is located in central Italy where the reference cropping system is characterized by low-input management and water can be a limiting factor during summer. Under such conditions, *M. sativa*, *T. repens* and *M. lupulina* were the most suitable legumes to be used for relay intercropping systems with durum wheat at the high productivity potential site while *M. sativa*, *H. coronarium* and *T. subterraneum* were the best options for the low-input system. Also the legume relay sowing system affected the success of the legume living mulch establishment and consequently its weed suppression capacity. Broadcast seeding is more prone to adverse agro-pedo-climatic conditions and it is recommended to increase the seeding density to compensate for reduced germination. Drill seeding guarantees a more uniform sward development under variable conditions. With the use of suitable legume species, relay intercropping proved to be a successful agronomical practice to maintain the soil constantly covered, reduce tillage and improve weed control at crop rotation level without negative effects on wheat grain production. Relay intercropping can provide an important contribution to support integrated weed and crop management and significantly reduce the reliance in external inputs such as herbicides and N fertilizers in a wide range of conventional cereal-based cropping systems.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s13593-022-00787-3.

Acknowledgements The authors would like to thank Giacomo Nardi (Sant'Anna School of Advanced Studies) and Christian Benini (Horta) for their excellent technical assistance with the field experiments management.

Authors' contributions Conceptualization, F.L., M.L., S.C., M.R, P.M. and A.C.M.; methodology, F.L., M.L. and S.C.; investigation, F.L., M.L., M.R. and S.C.; data analysis, F.L. and S.C.; writing original draft preparation, F.L.; writing review and editing, F.L., M.L., S.C., M.R, P.M. and A.C.M.; supervision, P.M. and A.C.M.; project administration, P.M. and A.C.M. All authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by Scuola Superiore Sant'Anna within the CRUI-CARE Agreement. The research leading to these results received funding from European Union's Horizon 2020 research and innovation programme under grant agreement No. 727321 (IWMPRAISE). F.L. has a study grant from PhD course in Agrobiodiversity at Sant'Anna School of Advanced Studies, Pisa, Italy.

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

Code availability Code generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Conflict of interest The authors declare no competing interests.

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