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# Legume Ecotypes and Commercial Cultivars Differ in Performance and Potential Suitability for Use as Permanent Living Mulch in Mediterranean Vegetable Systems

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**Abstract:** Weed control in organic conservative vegetable systems is extremely challenging and the use of legume permanent living mulches (pLM) presents an interesting opportunity. The successful use of pLM is largely determined by the choice of appropriate legumes which are able to combine adequate weed control with a marginal competitive effect on the cash crop(s). However, the availability of legumes for such systems is limited and their characterization based on growth traits can support the selection of suitable legumes for conservation organic vegetable systems. The current study investigated weed control capacity and variability in morphological and phenological traits relevant in inter-plant competition among a range of 11 commercial cultivars of legumes and seven ecotypes of *Medicago polymorpha* (bur medic). For commercial cultivars, *Lotus corniculatus* (bird's-foot trefoil) and *Trifolium repens* (white clover) showed the best weed control capacity, while *Trifolium subterraneum* (subterranean clover) and *Medicago polymopha* had more suitable characteristics for a rapid and complete establishment of the pLM. Overall, legume mulches appear more effective in dicotyledonous than in monocotyledonous weed control. *Trifolium subterraneum* cv. Antas and *T. repens* cv. Haifa were identified as the potentially most suitable legumes for use as pLM and their use in mixtures could be a promising solution. In addition, the ecotypes of *Medicago polymorpha* Manciano and Talamone proved to be well adapted for local environmental conditions and they showed a better weed suppression than the commercial cultivars of *Medicago polymorpha*.

**Keywords:** organic farming; weed control; legume screening; dead mulch; clover; bur clover; reduced tillage

## 1. Introduction

The increased need for sustainable agricultural systems has boosted the interest in cropping practices that allow the preservation of crop productivity while reducing the reliance on herbicides and nitrogen fertilisers [1]. The use of subsidiary crops such as permanent living mulches (pLM) in organic Mediterranean vegetable systems may represent a proficient practice to maintain crop productivity, improve soil fertility and support weed control.

Living mulches are subsidiary crops planted either before or together with a main crop and they persist as a living ground cover throughout the growing season. They can die naturally by senescence or they can be killed by mechanical or chemical interventions, and are then left on the soil surface or incorporated into the soil by tillage. In the case pLM, perennial or annual self-seeding species are sown in a cropping system that is designed to maintain them for several growing seasons without the need for reseeding and allowing to minimize soil disturbance [2]. In such systems, cash crops are drilled or transplanted into the established pLM by no- or reduced tillage methods (i.e., superficial strip tillage) [3].

Organic farming systems are often considered unsuitable for conservative tillage. In fact, several practices normally adopted in organic vegetable systems imply frequent soil disturbance. In particular, weed control is usually carried out through mechanical operations, including also ploughing. Moreover, incorporation of organic fertilisers, manures and green manures require soil disturbance. Weed control and nutrient supply in organic conservation agriculture therefore need innovative solutions [4]. The establishment of a pLM can be an agroecological solution to make conservation agriculture applicable in organic vegetable systems [5]. The pLM is expected to establish a dense sward in the inter-row space and to provide benefits to crop production through improved soil fertility, limitation of soil erosion, and improved weed control [6]. In addition, legumes allow farmers to reduce the reliance in nitrogen (N) fertilisation due to N fixation by rhizobia bacteria [7].

Many studies highlighted the weed suppressing ability of LM in different cropping systems [8], and reported weed biomass reduction up to 95% in comparison with the sole crop [9]. Weed suppression mainly takes place through competition for light, space, water and nutrients. Moreover, many species used as a living mulch produce allelopathic compounds able to reduce weed germination and emergence [10,11]. In conventional cropping systems this may lead to reduced herbicide applications and in organic systems this contributes to better weed control. For this reason, organic vegetable farmers, who generally suffer from lack of efficient weed management tools, are extremely interested in this cropping practice [12]. In fact, vegetable crops are generally more susceptible to weed competition than arable crops [13] and strategies for weed control such as crop rotation diversification, mechanical weeding and transplanting methods, often do not result in sufficient weed control. In fact, efficiency of these techniques can be reduced or they cannot be applied due to unfavourable soil conditions caused by adverse weather conditions in the short time window farmers have at disposal [14,15].

Permanent living mulches of legumes have the potential to support weed control and preserve crop productivity in organic vegetable systems, but they are only successful when appropriate legumes are used [16]. Studies carried out on vegetable crops such as cabbage, leek, zucchini, tomato, and potatoes, reported that living mulches reduced weed pressure significantly [17–20]. However, yield loss remains a substantial risk in the presence of living mulches [12,21,22]. Therefore, a successful pLM system should suppress weeds while limiting competition for resources with the crop [23].

Such a balance is largely determined by the use of suitable legume species and cultivar [9,12]. For instance, *Trifolium repens* is a promising legume for this purpose because it is characterised by a low biomass accumulation but it offers an excellent soil cover [21]. *Trifolium incarnatum* instead, is less suitable because it is able to overtop vigorous vegetable crop(s) such as cabbage, causing severe yield losses [13].

Annual self-seeding legumes may also be particularly suitable thanks to the way in which they bury their seeds into the soil [24]. Brandsaeter et al. [25] suggested, for instance, the use of annual self-seeding legumes to support weed control and to limit the competition (both above ground and below ground competition) with the main crop by separating the periods of vigorous growth of living mulch and main crop. Annual self-reseeding legumes (e.g., *Trifolium subterraneum* or *Medicago polymorpha*) form a dense mat, covering the soil during winter. In early spring, the annual self-seeding legumes reach their maximum development while their flowering and senescence take place in late spring. In such a system, weeds are suppressed during the winter and early spring by a living mulch while, in late spring and during the summer, by a dead mulch. In this way, senescence of the living mulch and maximum growth of the main summer crop take place at approximately the same time, minimizing competition for nutrients and water [21].

In general, for a successful use as pLM, a legume should have specific morphological, physiological and phenological characteristics [12]. Breeding and research activities mainly focus on those traits in legumes that optimize fodder production such as high biomass, erect growth habit and high canopy height. These species and cultivar are therefore often not adapted for permanent living mulch systems that require a complete ground cover of dense vegetation, prostrate growth habit and reduced canopy height [9]. For this reason, available legumes need to be characterized by specific growth characteristics and tested for their ability to suppress weed in order to better determine their suitability within a cropping system [26].

Moreover, through screening of genetically diverse resources such as ecotypes, there is a good opportunity to detect legume varieties with the desired growth characteristics. It is of utmost importance that these legumes can be well adapted to the local environmental conditions [27]. In fact, some studies have been carried out in the United States of America [28], France [29], and Italy [30,31] to develop new legume cultivars starting from the screening of local ecotypes aimed at developing cultivars well adapted for specific environmental conditions. These examples show that the screening of ecotypes has been successful for the selections of locally adapted legumes for existing cropping systems. However, this interesting approach has never been applied for the selection of legumes adapted for innovative cropping systems, such as vegetable systems with a permanent living mulch.

The objective of this study is the characterization of a range of promising commercial cultivars and ecotypes of perennial and annual self-seeding legumes for their use as pLM in Mediterranean vegetable systems. If sufficient variation is available, species and cultivars selection is likely to be one of the important means to optimize intercropping systems that contain subsidiary legumes for weed suppression [21]. We hypothesise that legumes characterized by a high biomass production [32] and a fast and complete soil coverage [32–34] can have a good weed control capacity. Moreover, since pLM systems involve the persistence of legumes for more than one growing season, we expected that legumes able to maintain such a characteristics over time can be particularly suitable for this system. Additionally, for annual self-seeding legumes, high self-seeding capacity is an important factor for their persistence in time. According to the literature, we hypothesise also that legume canopy height is an important factor determining above ground competition between legumes and the main crop [21] and for this reason, it should be considered in the screening process. We experimentally tested weed suppression capacity of a range of eleven commercial cultivars of legumes and seven ecotypes of *Medicago polymorpha* and investigated their variability in growth traits which are expected to maximize weed control and limit the inter-specific competition between living mulch and main crop. Legume species used in this experiment are *L. corniculatus*, *T. repens*, *M. polymorpha* and *T. subterraneum*. These species were chosen among the most common ones available on the seed market in Italy considering the general suitability for the soil type at the experimental site, opting for reduced canopy size and prostrate growth habit as preliminary indication of potential use in pLM systems. There is general information available about the growth characteristics of these legumes, but their weed control ability, adaptability for the local environmental condition and performance in time, remain to be explored. Moreover, for each legume species, more than one cultivar has been tested (except for *L. corniculatus*) because, also within each legume species relevant differences in growth characteristics exist [22].

In this study, screening of legumes was extended to ecotypes. We focused on ecotypes of *Medicago polymorpha* as example of an annual self-seeding legume native to Mediterranean basin. In previous studies it has been identified as a potentially interesting species to be used as living mulch [35], also in vegetable cropping systems [17]. Screening is expected to aid in the selection a set of potentially suitable legumes to be used as pLM in organic conservation agricultural systems.

## 2. Materials and Methods

### 2.1. Experiment Site

Two experiments were conducted in a certified organic field at the Centre for Agri-Environmental Research "Enrico Avanzi" of the University of Pisa (San Piero a Grado, Pisa, Italy, 43°41′02.08″ N, 10°20′35.0″ E) across two consecutive growing seasons (from November 2017 to May 2019).

The experimental site was characterized by sandy loam soil [36] with 194 g·kg$^{-1}$ of clay, 292 g·kg$^{-1}$ of silt and, 514 g·kg$^{-1}$ of sand. Additional soil properties were as follows: pH 8.3, organic C 9.3 g·kg$^{-1}$ (Walkeley-Black method [37]), total N 1.1 g·kg$^{-1}$ (Kjendhal method [38]), available P 6.7 mg·kg$^{-1}$ (Olsen method [39]).

The soil was plowed at 25 cm depth and refined by rotary harrowing one week before legume sowing. In November 2017, legumes were seeded in plots of 4.5 m$^2$ (1.5 × 3 m). Seeds were sown in rows (row spacing of 15 cm) with a small-plot precision drill.

Total rainfall and average monthly temperature from legumes seeding (November 2017) to the first spring biomass sampling (May 2018) were 722 mm and 10.6 °C. During the second growing season, from November 2018 to May 2019, total rainfall was only 470 mm and mean temperature 10.8 °C (Figure 1).

Both experiments followed a fully randomized complete block design with four replications and they were conducted in the same experimental field. The first experiment focused on the evaluation of commercial cultivars of perennial and annual self-seeding legumes, and the second experiment on the evaluation of *Medicago polymorpha* ecotypes.

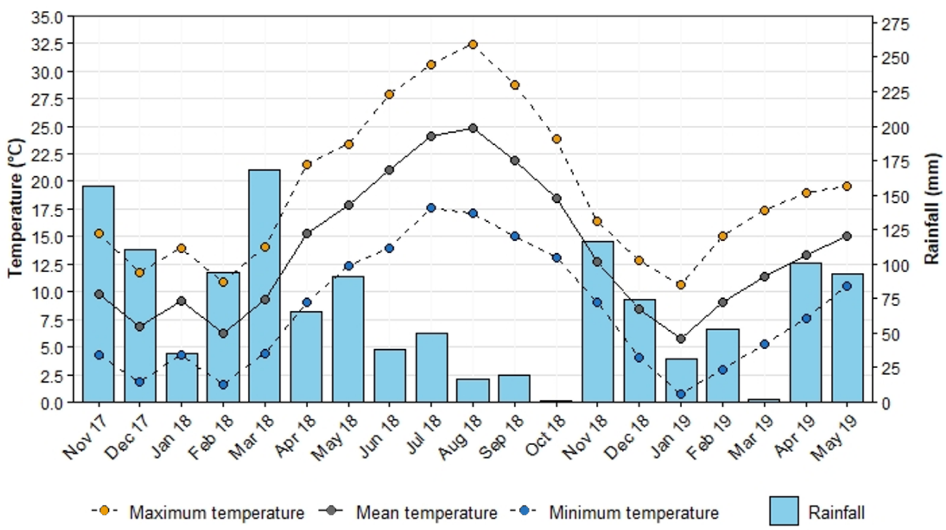



**Figure 1.** Monthly rainfall and temperature (maximum, minimum, mean) from October 2017 to May 2019.

### 2.2. Experiment 1: Evaluation of Commercial Cultivars of Perennial and Annual Self-Seeding Legumes

In this experiment, treatments consisted in 11 commercial cultivars belonging to 4 legume species. More in detail, one commercial cultivar of *Lotus corniculatus* (cv. Leo), 3 of *Trifolium repens* (cvs. Huia, Haifa and RD 84), 3 of *Medicago polymorpha* (cvs. Anglona, Scimitar and Mauguio), 2 of *Trifolium subterraneum* subsp. *brachycalycinum* (cvs. Fontanabona and Antas) and 2 of *Trifolium subterraneum* subsp. *subterraneum* (cvs. Campeda and Dalkeith). Plots where spontaneous vegetation was let grown undisturbed were used as control. Legumes were seeded at recommended seeding rate (Table 1).

**Table 1.** Cultivar names and seeding rates (kg·ha$^{-1}$) of perennial (P) and annual self-seeding (ASS) legumes used in experiment 1.

| Code | Genus and Species | Common Name | Cultivar | Cycle | Seeding Rate |
|---|---|---|---|---|---|
| Lcor | *Lotus corniculatus* L. | Bird's-foot trefoil | Leo | P | 30 |
| Trep1 | *Trifolium repens* L. | White clover | Huia | P | 25 |
| Trep2 | *Trifolium repens* L. | White clover | Haifa | P | 25 |
| Trep3 | *Trifolium repens* L. | White clover | RD 84 | P | 25 |
| Mpol1 | *Medicago polymorpha* L. | Bur clover | Scimitar | ASS | 25 |
| Mpol2 | *Medicago polymorpha* L. | Bur clover | Anglona | ASS | 25 |
| Mpol3 | *Medicago polymorpha* L. | Bur clover | Mauguio | ASS | 25 |
| Tsub(s)1 | *Trifolium subterraneum* L.* | Subterranean clover | Dalkeith | ASS | 35 |
| Tsub(s)2 | *Trifolium subterraneum* L.* | Subterranean clover | Campeda | ASS | 35 |
| Tsub(b)1 | *Trifolium subterraneum* L.** | Subterranean clover | Antas | ASS | 35 |
| Tsub(b)2 | *Trifolium subterraneum* L.** | Subterranean clover | Fontanabona | ASS | 35 |
| CNT | Spontaneous vegetation | - | Control | - | - |

* subsp. *subterraneum*; ** subsp. *brachycalycinum*.

Vegetation sampling was concentrated in spring (M18: 9 May 2018; M19: 7 May 2019), and at the end of summer (S18: 10 September 2018) because, according to the typical crop management of this area, the main vegetable crop(s) can be planted or transplanted into the established LM in these two periods with such timing. Phenological stage of legumes has been determined before each spring samplings (M18 and M19) according to the BBCH scale [40] (*L. corniculatus*, BBCH 60; *T. repens*, BBCH 62; *M. polymorpha*, BBCH 75; *T. subterraneum*, BBCH 63).

The above-ground biomass of the legumes and weeds were hand-harvested in 0.25 m$^2$ quadrates (one point per plot in M18, M19, and two points per plot in S18) at each sampling date. Quadrates were placed randomly avoiding plot edges. Legumes and weeds were separated, oven-dried at 40 °C and the dry biomass (DW) (g·m$^{-2}$) of the two components was recorded.

Legume canopy ground cover was visually estimated by the use of a quadrate (0.25 m$^2$) placed randomly in each plot during 2018 on 26 March, 3 and 30 April, and 7 May, and during 2019, on 20 February, 25 March, 12 April, and 6 May. Weed coverage by species was visually estimated before each spring biomass samplings (M18 and M19). Cumulative soil coverage by legumes in time was described by a logistic function and the ability of a legume to cover the soil rapidly (earliness) was expressed as the earliest point in time at which 50% soil cover was reached by legumes.

Maximum canopy height was measured with the same frequency as legume ground cover in three random sampling points in each plot.

Data on maximum biomass accumulation, canopy ground cover, maximum canopy height and earliness were transformed in an ordinal scale in order to provide a summary of suitability of the tested legumes. For these variables, a relative rank from 1 to 5 was established (1 for very unfavourable values and 5 for very favourable values). The most favourable value were based on the optimal values reported in literature. The other four classes were obtained by dividing the remaining range based our expertise. For instance, legume biomass production >300 DW, g·m$^{-2}$ and soil coverage >90% are expected to provide a good weed control [32,34] whereas, legume canopy height <15 cm is expected to reduce significantly the competition between legumes and the main crop [21,41]. For earliness instead, classes were set according to moment at which legume reach the 50% of the soil coverage (Table 4). More in detail, class limits were set as follows: maximum biomass accumulation (DW, g·m$^{-2}$) **1**: DW < 49, **2**: 50 < DW < 149, **3**: 150 < DW < 199, **4**: 200 < DW < 299, **5**: DW > 300; Canopy ground cover (C, %) **1**: C < 24, **2**: 25 < C < 34, **3**: 35 < C < 59, **4**: 60 < C < 89, **5**: C > 90; Maximum canopy height (h, cm) **1**: h > 31, **2**: 26 < h < 30, **3**: 21 < h < 25, **4**: 16 < h < 20, **5**: h < 15; earliness: **1** = late, **2** = medium-late, **3** = medium, **4** = medium-early, **5** = early.

### 2.3. Experiment 2: Evaluation of *Medicago polymorpha* Ecotypes

In this experiment seven ecotypes and three commercial cultivars of *M. polymorpha* (in common with experiment 1) were tested (Table 2). Experimental design, plot size and repetitions were equivalent to the experiment 1. Legumes were seeded at 25 kg·ha$^{-1}$.

Ecotypes from Central Italy were provided by the Germplasm Bank of the Institute of Genetic Improvement of the University of Perugia and by Pasture Research Centre or the National Research Council (CNR) of Sassari (Figure 2).

**Table 2.** Ecotypes and commercial cultivars of *Medicago polymorpha* (bur clover) used in experiment 2.

| **Code** | **Genus Species** | **Cultivar** * |
|---|---|---|
| Commercial | | |
| Mpol1 | *M. polymorpha* L. | Scimitar |
| Mpol2 | *M. polymorpha* L. | Anglona |
| Mpol3 | *M. polymorpha* L. | Mauguio |
| Ecotypes | | |
| Mpol4 | *M. polymorpha* L. | Pitigliano (Siena, SI) |
| Mpol5 | *M. polymorpha* L. | Manciano (Grosseto, GR) |
| Mpol6 | *M. polymorpha* L. | Talamone (Grosseto, GR) |
| Mpol7 | *M. polymorpha* L. | Principina (Grosseto, GR) |
| Mpol8 | *M. polymorpha* L. | Villa Salto (Sud Sardegna, SU) |
| Mpol9 | *M. polymorpha* L. | San Felice Circeo (Latina, LT) |
| Mpol10 | *M. polymorpha* L. | Tarquinia (Viterbo, VT) |
| CNT | Spontaneous vegetation | Control |

* Ecotype names referred to the place where ecotypes have been collected (Province).

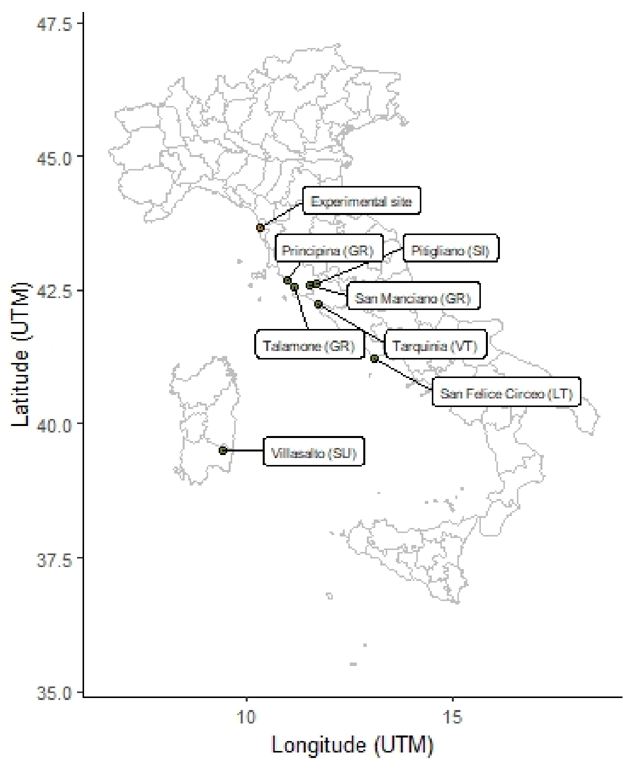



**Figure 2.** Geolocation of *Medicago polymorpha* ecotypes and experimental site.

Legume and weed above ground biomass (DW, g·m$^{-2}$) were collected on 9 May 2018 (M18) and 7 May 2019 (M19) by hand harvesting one biomass sample of 0.225 m$^2$ per plot. Legumes and weeds were separated and weighed after oven-drying (40 °C in an air oven).

The self-seeding capacity (SSC) was also evaluated. For SSC, 25 legumes pods were randomly collected from each plot and opened to count the number of seeds. Subsequently, two iron rings (15 cm diameter) were randomly placed in each plot on September 29th and legume pods within the rings were counted. Pod number within the rings was multiplied by the average number of seeds inside each legume pod, to estimate the number of seeds in each ring. Germinated plants were counted and picked taking care to leave the pods within the ring at 23, 41, and 71 days after placement of the iron rings. Self-seeding capacity of legumes is evaluated as ratio between germinated plants and estimated total seed number inside the rings. The formula used for the SSC evaluation is summarized as follow:

$$SSC(\%) = \frac{G}{N \times \overline{n}} \times 100$$

where SSC is the self-seeding capacity, $G$ (n°/cm$^2$) is the total number of germinated plants in a certain area, $N$ (n°/cm$^2$) is the number of legume pods in a certain area, and $\overline{n}$ (n°/pod) is the average number of seeds in each pod.

### 2.4. Statistical Analysis

Data analysis was performed using R environment for statistical computing [42]. Statistical models were performed using the 'Lme4' package for R [43]. For significant explanatory variables, Tukey post-hoc test was performed to separate means ($p < 0.05$) using the 'emmeans' package for R [44]. For Generalized Linear Mixed Models (GLMM) blocks and pseudo-replicates, when present, were set as random factor while legumes cultivars were used as fixed factor. Type of data and the error distribution of the data determined the distribution with which each variable was analysed. For each model, Kolmogorov-Smirnov test of normality was used to asses the goodness of fit on the scaled residuals of the models performed using the 'DHARMa' package for R [45].

For the evaluation of commercial cultivars of perennial and annual self-seeding legumes, Generalized Linear Mixed Models (GLMM) were used to analyse weed biomass in M18, S18, and M19. For M18 and M19 the Gaussian distribution and log link function was used, while for S18 Gamma distribution and log link function was used. To test the legume (sub)species and cultivar effect on weed suppression, a set of 14 orthogonal linear contrasts was created.

Relationship between weed and legume biomass has been investigated both in M18 and M19 (correlation method: Pearson).

For legume biomass analysis in M18 and M19, a Generalized Linear Mixed Models (GLMM) with Gaussian distribution and identity link function was used. The model was formulated as:

$$Leg_{ijk} = \mu + Trt_i \cdot Year_j + BLK_k + \epsilon_{ijkl}$$

where $Leg_{ijkl}$ is the biomass of each legume i ($Trt_i$) in the growing season j (M18 and M19, $Year_j$) and Block ($BLK_l$); $\mu$ represent the grand mean and $\epsilon_{ijkl}$ is the residual error.

The model was run with $Trt_i$ and $Year_j$ as fixed effects and $BLK_l$ as random effect. Weed biomass has not been included in the model as co-variable because the purpose of the trial was to evaluate the legume biomass production under field conditions. The interaction effect between legume type and weed was not studied because weed biomass was homogeneously distributed among experimental unit. Hence, the legume biomass production is the result of legume identity and the environment, including weeds.

Data on maximum soil coverage reached by legumes in M18 and M19 were analysed with Beta regression model ("logit" as link function) using the 'betareg' package for R [46]. From legumes sowing to the first biomass sampling (May 2018), legume cover was repeatedly monitored, and data were fitted with a logistic curve using 'drc' package for R [47]. From the logistic curve the point in time was calculated at which each legume reached 50% soil cover.

A Generalized Linear Mixed Model (GLMM) with Gaussian distribution and a log link function was used for the analysis of legume canopy height in M18 and M19. Data on biomass accumulation, canopy ground cover, maximum canopy height and earliness were summarised for each legume and represented, after data transformation to an ordinal scale, through radar charts using 'fmsb' package for R [48].

For the evaluation of *M. polymorpha* ecotypes, Generalized Linear Mixed Models (GLMM) were used to analyse weed dry biomass in M18 and M19. For M18 and M19 the Gaussian distribution with log link function and Gaussian distribution with identity link function were used respectively. The legume biomass in M18 and M19 was analysed with Generalized Linear Mixed Models (GLMM) using a Gaussian distribution and log link function. Generalized Linear Models (GLM) with a Gaussian distribution was used to analyse legume self-seeding capacity (SSC).

## 3. Results

### *3.1. Evaluation of Commercial Cultivars of Annual SELF-Seeding and Perennial Legumes*

#### 3.1.1. Weed Biomass

Based on the soil cover sampling just before biomass sampling, the most abundant weed species were identified: *Phalaris paradoxa* L., *Lolium* spp. L., *Poa annua* L. among monocotyledonous weeds and *Picris echioides* L., *Papaver rhoeas* L., *Rumex crispus* L. and, *Sinapis arvensis* L. among dicotyledonous weeds.

In May of both years a negative correlation between total weed biomass and legume biomass was detected (May 2018: R = −0.36, $p$ = 0.031, May 2019: R = −0.43, $p$ = 0.004).

In May 2018, Lcor, Trep, Mpol, Tsub(b), and Tsub(s) reduced significantly total weed biomass (35.66 ± 8.99, 91.28 ± 13.12, 58.99 ± 8.31, 79.05 ± 21.59 and 60.04 ± 15.60 DW g·m$^{-2}$, respectively) in comparison to the control (159.00 ± 21.75 DW g·m$^{-2}$). At this sampling time, presence of legumes significantly affected both monocotyledonous and dicotyledonous weeds. In particular, Lcor and Mpol significantly reduced the monocotyledonous weed biomass, while Mpol and Tsub(b) significantly reduced dicotyledonous weed biomass in comparison with the control (Figure 3).

In May 2019, total weed biomass had tripled in all plots in comparison with the previous year (80.67 ± 14.89 vs 260.22 ± 28.49 DW g·m$^{-2}$, $p$ < 0.05). In May 2019 the weed biomass in all plots contained on average 77% of monocotyledonous weeds and 23% of dicotyledonous weeds. Monocotyledonous weed biomass was not significantly affected by living mulches while legumes significantly reduced the dicotyledonous weed biomass in comparison with the control (Figure 3).

#### 3.1.2. Legume Biomass

Legume above ground dry biomass production was evaluated in May 2018 and in May 2019. Overall, the May 2018 assessment showed perennial legumes produced significantly less biomass in comparison with annual self-seeding legumes (in average 59.98 ± 10.31 vs 257, 29 ± 43, 08 DW g·m$^{-2}$, $p$ < 0.05).

More in detail, *M. polymorpha* cv. Angnona, Scimitar and *T. subterraneum* cv. Campeda, Antas were characterized by significantly higher biomass than *L. corniculatus* cv. Leo, *T. repens* cv. Haifa, Huia and, RD84 and *T. subterraneum* cv. Dalkeit (subsp. *subterraneum*) (Table 3).

In May 2019, biomass of *L. corniculatus* cv. Leo and *T. repens* cvs. Haifa and RD84 were significantly higher in comparison with the previous year (May 2018), while *M. polymorpha* cvs. Anglona and Scimitar and *T. subterraneum* cvs. Campeda, Antas had a significantly lower biomass in May 2019 (Table 3). Among annual self-seeding legumes, only *T. subterraneum* cv. Fontanabona showed a higher biomass in May 2019 in comparison to the previous year (Table 3). No significant variation was observed in dry biomass of *M. polymorpha* cv. Mauguio and *T. subterraneum* cv. Dalkeit among the two years of the experiment.

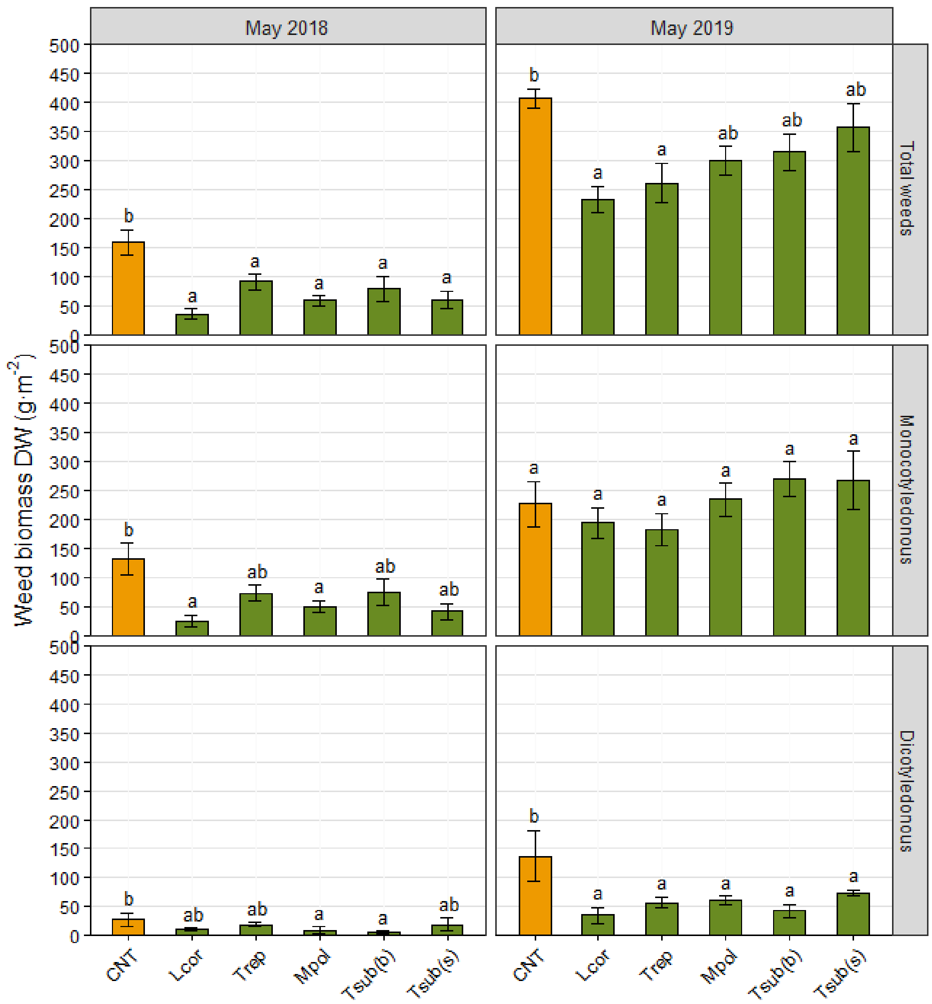



**Figure 3.** Total above ground weed biomass (DW g·m$^{-2}$) and specific biomass of monocotyledonous and dicotyledonous species in May 2018 and May 2019. CNT, control; Lcor, *Lotus cornicutaus*; Trep, *Trifolium repens* all cultivars together; Mpol, *Medicago polymorph* all cultivars together; Tsub(b), *Trifolium subterraneum* all subsp. *brachycalycinum* together; Tsub(s), *Trifolium subterraneum* all subsp. *subterraneum* together. Different letters within each sampling time indicate significant differences at the 0.05 level (Tukey post-hoc test). Error bars represent standard error (S.E).

**Table 3.** Above ground biomass of legumes (DW ± SE, g·m$^{-2}$) in May 2018 and May 2019.

| Legume Species | Cultivar | DW | | | | | |
|---|---|---|---|---|---|---|---|
| | | May 2018 | | May 2019 | | ΔM18–M19 | |
| *L. corniculatus* L. | Leo | 41.61± 8.15 | a | 241.24 ± 39.45 | ab | +199.63±49.4 | *** |
| *T. repens* L. | Haifa | 58.03 ± 13.88 | a | 161.05 ± 26.87 | ab | +103.02±31.1 | *** |
| *T. repens* L. | RD 84 | 82.50 ± 16.11 | a | 174.73 ± 29.66 | ab | +92.23±33.4 | ** |
| *T. repens* L. | Huia | 57.73 ± 11.33 | a | 227.80 ± 39.36 | ab | +220.07±39.4 | *** |
| *M. polymorpha* L. | Mauguio | 180.16 ± 31.20 | ab | 176.52 ± 29.97 | ab | −3.64±42.7 | n.s. |
| *M. polymorpha* L. | Anglona | 455.85 ± 64.59 | d | 137.02 ± 23.32 | ab | −318.83±63.5 | *** |
| *M. polymorpha* L. | Scimitar | 275.76 ± 43.33 | bc | 129.30 ± 21.93 | a | −146.46±46.6 | ** |
| *T. subterraneum* L. | Dalkeith * | 74.67 ± 25.06 | ab | 88.99 ± 15.15 | a | +14.32 ± 31.6 | n.s. |
| *T. subterraneum* L. | Campeda * | 260.24 ± 46.48 | bc | 102.58 ± 17.54 | a | −157.66 ± 44.2 | *** |
| *T. subterraneum* L. | Fontanabona ** | 198.55 ± 30.31 | bc | 288.38 ± 38.59 | b | +89.83 ± 56.1 | *** |
| *T. subterraneum* L. | Antas ** | 335.83 ± 53.36 | cd | 216.42 ± 36.69 | ab | −119.41 ± 59.0 | * |

Different letters (a–d) indicate significant differences at 0.05 level (Tukey post-hoc test). Cultivar: * subsp. *subterraneum*; ** subsp. *brachycalycinum*. DW: *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$), n.s ($p > 0.05$).

### 3.1.3. Canopy Ground Cover

In May 2018, *M. polymorpha* cv. Anglona, Scimitar and by *T. subterraneum* cv. Campeda, Fontanabona and, Anatas reached soil cover of more than 80% (Table 4).

**Table 4.** Point in time at which 50% of soil coverage was reached by legumes in 2018 (C50 ± SE, DAS) and maximum canopy ground cover of legumes in May 2018 and 2019 (Cmax ± SE, %).

| Legume Species | Cultivar | 2018 | | | | 2019 | |
|---|---|---|---|---|---|---|---|
| | | C50 | | Cmax | | Cmax | |
| *L. corniculatus* L. | Leo | | | 18.00 ± 6.02 | a | 81.61 ± 6.07 | bc |
| *T. repens* L. | Haifa | | | 27.32 ± 6.76 | a | 59.38 ± 8.90 | abc |
| *T. repens* L. | RD 84 | | | 39.38 ± 7.67 | ab | 78.99 ± 6.39 | abc |
| *T. repens* L. | Huia | | | 20.84 ± 6.37 | a | 87.29 ± 5.21 | c |
| *M. polymorpha* L. | Mauguio | 134.76 ± 1.06 | b | 64.80 ± 7.53 | bc | 62.49 ± 7.60 | abc |
| *M. polymorpha* L. | Anglona | 129.23 ± 0.55 | ab | 90.29 ± 4.62 | d | 62.67 ± 7.59 | abc |
| *M. polymorpha* L. | Scimitar | 129.64 ± 0.58 | ab | 84.02 ± 5.74 | cd | 57.49 ± 7.76 | ab |
| *T. subterraneum* L. | Dalkeith * | | | 18.36 ± 6.07 | a | 51.25 ± 7.85 | a |
| *T. subterraneum* L. | Campeda * | 127.89 ± 0.82 | a | 95.31 ± 3.25 | d | 63.66 ± 7.55 | abc |
| *T. subterraneum* L. | Fontanabona ** | 127.64 ± 0.87 | a | 94.64 ± 3.48 | d | 83,02 ± 5.88 | bc |
| *T. subterraneum* L. | Antas ** | 127.20 ± 0.72 | a | 95.63 ± 3.13 | d | 84.70 ± 5.21 | c |

Different letters (a–d) within a column indicate significant differences at 0.05 level (Tukey post-hoc test). * subsp. *subterraneum*; ** subsp. *brachycalycinum*.

During the first growing season (from November 2017 to May 2018), *T. subterraneum* cv. Campeda, Fontanabona, Antas reached 50% of soil coverage significantly earlier than *M. polymorpha* cv. Mauguio (Table 4). Earliness in soil cover (C50) is not available for *L. corniculatus* cv. Leo, *T. repens* cv. Haifa, Huia and RD84 and *T. subterraneum* cv. Dalkeit because their maximum canopy coverage was lower than 50%.

### 3.1.4. Canopy Height

In May 2018 *M. polymorpha* cv. Anglona was the tallest legume while, *T. repens* cv. Haifa, RD84, Huia, *M. polymorpha* cv. Mauguio and, *T. subterraneum* cv. Dalkeit had the lower canopy height (Table 5). Intermediate canopy height, were observed for *L. corniculatus* cv. Leo, *M. polymorpha* cv. Scimitar and, *T. subterraneum* cv. Campeda, Fontanabona, Antas (Table 5).

In May 2019, *L. corniculatus* cv. Leo was the tallest legumes (Table 5). Canopy heights of *T. repens* cv. Huia and *M.polymorpha* cv. scimitar and Anglona were significantly higher than *T. subterraneum* cv. Dalkeit (Table 5).

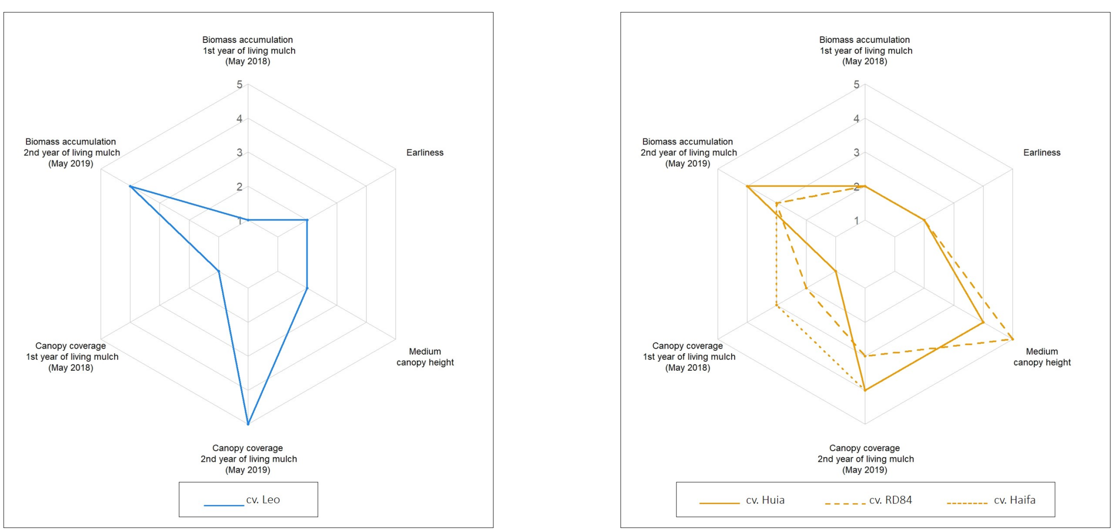
**Table 5.** Canopy height of legumes (h ± SE, cm) in May 2018 and 2019.

| Legume Species | Cultivar | Canopy Height | | | |
|---|---|---|---|---|---|
| | | May 2018 | | May 2019 | |
| *L. corniculatus* L. | Leo | 20.69 ± 2.42 | b | 26.18 ± 2.69 | c |
| *T. repens* L. | Haifa | 9.09 ± 1.83 | a | 13.88 ± 2.01 | ab |
| *T. repens* L. | RD 84 | 10.83 ± 2.04 | a | 17.43 ± 2.27 | ab |
| *T. repens* L. | Huia | 12.03 ± 2.08 | a | 17.74 ± 2.29 | b |
| *M. polymorpha* L. | Mauguio | 11.77 ± 2.07 | a | 15.96 ± 2.22 | ab |
| *M. polymorpha* L. | Scimitar | 24.89 ± 2.62 | b | 18.09 ± 2.30 | b |
| *M. polymorpha* L. | Anglona | 39.16 ± 3.43 | c | 21.12 ± 2.44 | bc |
| *T. subterraneum* L. | Dalkeith * | 8.82 ± 1.83 | a | 9.36 ± 2.00 | a |
| *T. subterraneum* L. | Campeda * | 22.49 ± 2.50 | b | 16.16 ± 2.22 | ab |
| *T. subterraneum* L. | Fontanabona ** | 22.45 ± 2.51 | b | 17.60 ± 2.28 | ab |
| *T. subterraneum* L. | Antas ** | 21.06 ± 2.43 | b | 15.18 ± 2.19 | ab |

Different letters (a–c) within a column indicate significant differences at 0.05 level (Tukey post-hoc test). * subsp. *subterraneum*; ** subsp. *brachycalycinum*.

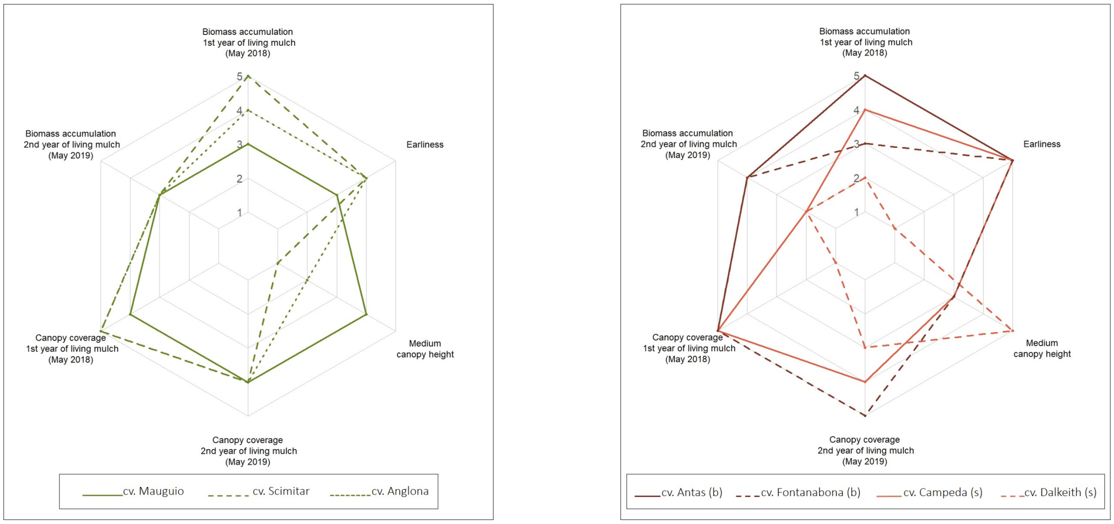
3.1.5. Legume Growth Traits as Indicators for Their Potential Use as Permanent Living Mulch

Data on maximum biomass accumulation, canopy ground cover, maximum canopy height and earliness were summarised in order to provide a general overview of the relevant growth characteristics of legumes used in this research (Figure 4). In the first year of the experiment *T. subterraneum* subsp. *brachycalycinum* cv. Antas was identified as the legume with the best overall performance in terms of biomass production, canopy ground cover, and canopy height. In the second year both cultivars of *T. subterraneum* subsp. *brachycalycinum* (cvs. Antas and Fontanabona) and *T. repens* cv. Haifa showed the most suitable growth characteristics for the target vegetable system. Out of all tested legumes, only *T. repens* cv. Haifa significantly reduced weed biomass in comparison with the control.




(**a**) *Lotus corniculatus* (**b**) *Trifolium repens*

**Figure 4.** *Cont.*




(**c**) *Medicago polymorph* (**d**) *Trifolium subterraneum*

**Figure 4.** Summary of growth characteristics of *Lotus corniculatus* (**a**), *Trifolium repens* (**b**), *Medicago polymorpha* (**c**), *Trifolium subterraneum* (**d**). Scale 1–5 (1 for very unfavourable values and 5 for very favourable values). Maximum biomass accumulation (DW, g·m$^{-2}$): **1**: DW $<$ 49, **2**: 50 $<$DW$<$ 149, **3**: 150 $<$ DW $<$ 199, **4**: 200 $<$ DW $<$ 299, **5**: DW $>$ 300. Canopy coverage (C, %): **1**: C $<$ 24, **2**: 25 $<$ C $<$ 34, **3**: 35 $<$ C $<$ 59, **4**: 60 $<$ C $<$ 89, **5**: C $>$ 90. Maximum canopy height (h, cm): **1**: h $>$ 31, **2**: 26 $<$ h $<$ 30, **3**: 21 $<$ h $<$ 25, **4**: 16 $<$ h $<$ 20, **5**: h $<$ 15. Earliness: **1** = late, **2** = medium-late, **3** = medium, **4** = medium-early, **5** = early.

### *3.2. Evaluation of Medicago Polymorph Ecotypes*

#### 3.2.1. Weed Biomass

Based on the soil cover sampling just before biomass sampling, the most abundant weed species were identified: *P. paradoxa*, *Lolium* spp., *P. annua* among monocotyledonous weeds and *P. echioides*, *P. rhoeas*, *Rumex crispus* and, *S. arvensis* among dicotyledonous weeds.

In May 2018 and 019 weed biomass was negatively correlated with legume biomass (May 2018: R = −0.45, $p$ = 0.003; May 2019: R = −0.5, $p$ = 0.001). In May 2019, *M. polymorpha* ecotypes of Manciano (GR), Talamone (GR), Principina (GR) and San Felice Circeo (LT) significantly reduced weed biomass in comparison with the control. The commercial cultivars and the ecotypes Pitigliano (SI), Villa Salto (SS) and Tarquinia (VT) did not affect weed biomass (Figure 5).

#### 3.2.2. Legume Biomass

In May 2018, the dry biomass of *M. polymorpha* cv. Anglona (455.85 ± 41.11 DW g·m$^{-2}$) was higher than cv. Mauguio and the ecotypes Manciano (GR), Talamone (GR), Principina (GR), San Felice Circeo (LT) and Tarquinia (VT) (Table 6). In May 2019, the ecotypes Principina (GR) and San Felice Circeo (LT) had increased their dry biomass with 127 and 196% respectively compared to the previous year. The commercial cv. Anglona and Scimitar and the ecotypes Tarquinia (VT) and Pitigliano (SI) showed a reduced dry biomass compared to the previous year (Table 6). The variation in dry biomass production between May 2019 and May 2018 (ΔM18–M19) was significantly affected by the interaction between (i) self-seeding capacity of legumes and (ii) biomass production in May 2018 ($p$ = 0.036). Three ecotypes showed an intermediate but stable biomass in the two growing season: Manciano, Talamone and Villa Salto.

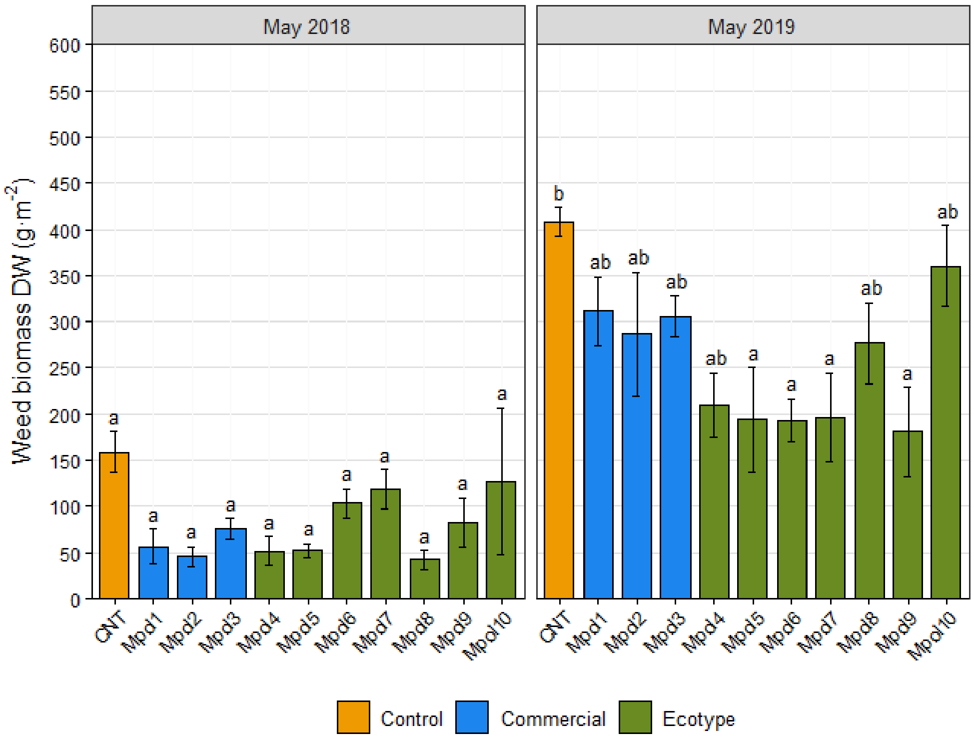



**Figure 5.** Above ground weed biomass (DW g·m$^{-2}$) in May 2018 and 2019. CNT: control. Commercial cultivars: Mpol1: *M. polymorpha* cv. Scimitar; Mpol2: *M. polymorpha* cv. Anglona; Mpol3: *M. polymorpha* cv. Mauguio. Ecotypes: Mpol4: *M. polymorpha* eco. Pitigliano (SI); Mpol5, *M. polymorpha* eco. Manciano (GR); Mpol6, *M. polymorpha* eco. Talamone (GR); Mpol7: *M. polymorpha* eco. Principina (GR); Mpol8: *M. polymorpha* eco. Villa Salto (SU); Mpol9: *M. polymorpha* eco. San Felice Circeo (LT); Mpol10: *M. polymorpha* eco. Tarquinia (VT). Different letters within each sampling time indicate significant differences at the 0.05 level (Tukey post-hoc test). Error bars represent standard error (S.E).

**Table 6.** Above ground biomass of *M. polymorpha* commercial cultivars and ecotypes (DW ± SE, g·m$^{-2}$) in May 2018 and 2019 and difference between years (ΔM18–M19).

| Legume Species | Cultivar | DW | | | | | |
|---|---|---|---|---|---|---|---|
| | | **May 2018** | | **May 2019** | | **ΔM18–M19** | |
| Commercial | | | | | | | |
| *M. polymorpha* | Scimitar | 275.76 ± 45.82 | ab | 129.30 ± 24.68 | a | −146.46 ± 43.41 | *** |
| *M. polymorpha* | Anglona | 455.85 ± 41.11 | b | 137.02 ± 26.78 | a | −318.83 ± 63.20 | *** |
| *M. polymorpha* | Mauguio | 180.16 ± 38.50 | a | 176.52 ± 28.81 | ab | −3.64 ± 38.63 | n.s. |
| Ecotype | | | | | | | |
| *M. polymorpha* | Pitigliano (SI) | 333.31 ± 75.28 | ab | 204.21 ± 28.34 | ab | −129.10 ± 35.26 | * |
| *M. polymorpha* | Manciano (GR) | 203.97 ± 54.15 | a | 240.00 ± 27.88 | ab | +36.03 ± 48.62 | n.s. |
| *M. polymorpha* | Talamone (GR) | 154.59 ± 18.50 | a | 237.94 ± 39.13 | ab | +83.35 ± 40.91 | n.s. |
| *M. polymorpha* | Principina (GR) | 147.42 ± 45.92 | a | 264.69 ± 37.60 | ab | +117.27 ± 43.45 | ** |
| *M. polymorpha* | Villa Salto (SS) | 261.95 ± 34.83 | ab | 199.97 ± 26.93 | ab | −61.98 ± 44.76 | n.s. |
| *M. polymorpha* | San Felice Circeo (LT) | 109.78 ± 9.56 | a | 306.66 ± 46.27 | b | +196.88 ± 48.54 | *** |
| *M. polymorpha* | Tarquinia (VT) | 279.27 ± 60.35 | a | 159.67 ± 30.52 | a | −119.60 ± 47.12 | * |

Different letters (a,b) indicate significant differences at 0.05 level (Tukey post-hoc test). *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$), n.s. ($p > 0.05$).

### 3.2.3. Self-Seeding Capacity (SSC)

*Medicago polymorph* cv. Scimitar and the ecotypes Pitigliano (SI), Talamone (GR), Principina (GR) and, San Felice Circeo (LT) (78.21 ± 8.86 and 57.62 ± 9.25, 64.94 ± 11.43, 63.52 ± 10.77, 63.71 ± 13.78%) had significantly higher self-seeding capacity than cv. Anglona, Mauguio and, the ecotype Manciano (GR) (14.60 ± 3.54%, 10.15 ± 4.85% and 18.73 ± 2.47 %) (Figure 6). The ecotypes Villa Salto (SU) and Tarquinia (VT) showed an intermediate self-seeding capacity (30.45 ± 0.82 and 41.25 ± 7.00 %).

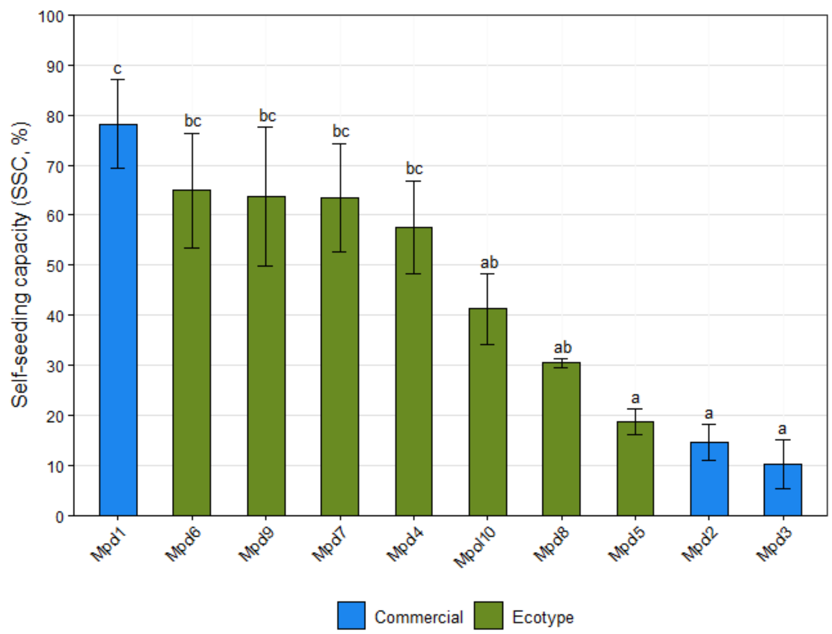



**Figure 6.** Self-seeding capacity of ecotypes and commercial cultivars of *M. polymorpha* (SSC, %). Mpol1: *M. polymorpha* cv. Scimitar; Mpol2: *M. polymorpha* cv. Anglona; Mpol3: *M. polymorpha* cv. Mauguio; Mpol4: *M. polymorpha* eco. Pitigliano (SI); Mpol5: *M. polymorpha* eco. Manciano (GR); Mpol6: *M. polymorpha* eco. Talamone (GR); Mpol7: *M. polymorpha* eco. Principina (GR); Mpol8: *M. polymorpha* eco. Villa Salto (SU); Mpol9: *M. polymorpha* eco. San Felice Circeo (LT); Mpol10: *M. polymorpha* eco. Tarquinia (VT). Different letters (a–c) indicate significant differences at 0.05 (Tukey post-hoc test). Error bars represents standard error (S.E).

## 4. Discussion

In this study, a comparison of various legumes cultivars and ecotypes was made. Particular attention was given to the weed control capacity of legumes and to those growth characteristics that are expected to affect the suitability of legumes as pLM in conservative and organic vegetables system. Since pLM involve the persistence of legumes for more than one growing season, legumes and weeds were monitored for two consecutive years. Studies conducted on this system, rarely investigate weed suppression and legume persistence in time, despite the fact that stable characteristics of pLM over the years and the maintenance of an adequate weed control in time are key factors for a sustainable application of conservative methods in organic systems by the use of pLM.

Overall, perennial legumes tested in this study showed a good weed control capacity, while annual self-seeding legumes showed more suitable characteristics for a rapid and complete establishment of the living mulch. One year after the legume establishment, living mulches were able to keep dicotyledonous weeds under control while they were less efficient in controlling the monocotyledonous weeds. Moreover, the comparison of commercial cultivars and local ecotypes of *Medicago polymorpha* highlighted that ecotypes can be well adapted to the local environmental condition and have good performance in terms of weed control.

It has been reported that biomass accumulation of legumes is an important factor in relation to weed control capacity of the living mulch [49], and maximum canopy height of legumes is largely considered as one of the most relevant characteristic determining competition with the main crop(s) [21,41]. The selection of legumes with a balance between high biomass production and reduced canopy height is therefore recommended to maximise the competitive ability of the living mulch against weeds and limit the above-ground competition with the main crop. Below-ground competition between legumes and the main crop should be also considered for a sustainable application of pLM in vegetable systems. In this context, annual self-seeding legumes seem particularly interesting because they persist in the field as dead mulch during the summer, hence limiting the potential water competition with the vegetable crop during this season. However, in this study dead mulch of annual self-seeding legumes showed less efficiency in weed control than living mulch during the summer.

In this study, *Trifolium subterraneum* subsp. *brachycalycinum* cv. Antas and *Trifolium repens* cv. Haifa showed the best trade-off between biomass production and canopy height respectively during the first and the second year. Nevertheless, even the relatively short canopy of these legumes (respectively 21.06 and 13.8 cm) may still cause inter-specific competition with the main crop [21,41].

Therefore, after this first step of selecting suitable legumes, the next stage is to test various vegetable crops in the most suitable legume permanent living mulches in order to identify the best matches for each crop.

The number of days after legume sowing needed to reach a 50% ground cover was used to evaluate the earliness in soil cover of the legumes. Annual self-seeding legumes generally had a quicker development than perennial legumes, confirming observations from Brandsaeter et al. [22]. Several studies have indicated the importance of early soil cover development of living mulch to improve weed control [33]. In fact, during the first growing stages, weeds are more susceptible with the living mulches competition and early development of legumes may contribute to reduce weed germination and growth [25]. However, in this study there was no clear relationship between earliness and weed control capacity because the slow covering perennials performed best in terms of weed control.

The annual self-seeding legumes used in this study showed favourable growth characteristics for weed control, but they reduced weed biomass only significantly in the first growing season (May 2018), while perennial legumes showed a good weed control in both experimental years (May 2018 and 2019). Differences in weed control capacity could be related to different competitiveness between perennial and annual self-seeding legumes during the summer season. As a consequence of their growth cycle, *L. corniculatus* and cultivars of *T. repens* persisted as living mulch during the summer period while, residues of *M. polymorpha* and *T. subterraneum* formed a dead mulch [24,25]. The cover crop and weed biomass data at the end of the summer (September 2018) confirmed findings from the literature that dead mulches do not suppress weeds as consistently as living mulches [50,51]. In fact, perennial legumes reduced weed biomass significantly by 72% in comparison with the control, while dead mulches of annual self-seeding legumes were not as successful as perennials and only suppressed 12% of the weed biomass. This may have resulted in an increase in the weed seedbank, in particular for monocotyledonous weeds, and subsequently in an increased weed pressure in the following growing season. This ultimately resulted in a less efficient weed suppression capacity of annual self-seeding legumes in May 2019.

Despite the positive effect of perennial legumes and of some annual self-seeding cultivar and ecotypes, weed pressure consistently increased after the first year of the living mulch establishment. The increased weed biomass was particularly relevant for the monocotyledonous weeds representing 77% of the total weed biomass. Notably, legume mulches appeared more effective in dicotyledonous weed control than monocotyledonous weed control. In this perspective, our results confirm the observation of Hiltbrunner et al. [52], who documented that legume living mulch better suppressed dicotyledonous than monocotyledonous weeds and that the establishment of monocotyledonous weeds could even be favoured by living mulches. The selective weed suppressive capacity of legume living mulches remains an unexplored question. The increased presence of weeds over the years, highlighted that the pLM used as sole weed management strategy may not be sufficient to contrast weeds over the years, especially for vegetable system characterized by severe grass weed infestations. Brainard et al. [53] reported that after three years of living mulch, the density of annual weeds can be over ten times higher than in standard herbicide treatments and for this reason, the authors recommended the use of supplementary methods of weed control to prevent accumulation of weed seeds in the soil seed bank. Under organic growing conditions, for instance, the periodic mowing of the pLM in the inter-row may reduced weed seed infestation, and at the same time, restrict the excessive growth of the living mulch.

Results of our study showed that it is necessary to monitor the development of the pLM and weeds after the installation season to determine additional pLM and weed control tactics. In this work, none of the tested legumes performed perfectly during the two consecutive experimental years and in fact none of them demonstrated the perfect trait combination. However, a clear complementarity in growth characteristics between perennial and annual self-seeding legumes was observed between the two years of the experiment. This aspect suggests that the use of these legumes in mixture might be an interesting option in order to optimize the benefits in time. The annual self-seeding legumes guarantee a quick soil cover soon after establishment of the pLM thanks to their fast growth, high biomass accumulation and good canopy ground cover. The perennial component in the mixture would ensure a good persistence over time thanks to the dense and weed suppressive living mulch they form during summer and in the following season. Moreover, the presence of annual self-seeding legumes in mixture with perennial legumes, may improve the weed control capacity of the living mulch by filling the empty spaces left by perennial legumes that could otherwise favour weed growth and weed seed dissemination. Based on the results of this study, *T. subterraneum* cv. Antas combined with *T. repens* cv. Haifa, may be good candidates for a multi-species permanent living mulch.

Screening of legumes for permanent living mulch systems can be extended to ecotypes. In this study, some of the ecotypes of *M. polymorpha* proved to be superior to commercial cultivars in terms of weed control capacity and persistence in time. This aspect was particularly evident during the second year (May 2019), where ecotypes from Manciano (GR), Talamone (GR), Principina (GR), and San Felice Circeo (LT) significantly reduced the weed biomass in comparison with the control, whereas no significant effects were observed for commercial cultivars.

Persistence of commercial cultivars and ecotypes of *M. polymorpha*, expressed as the difference in biomass between the two years (Δ18–19), was significantly affected by the interaction between (i) self-seeding capacity of legumes and; (ii) biomass production in May 2018 which is reasonably related to legume seed production. This confirms that the self-seeding capacity is a relevant factor for the persistence of annual self- seeding legumes. High self-seeding capacity is therefore a recommended characteristic for the selection of suitable annual self-seeding legumes as permanent living mulch. In our study, *M. polymorpha* Scimitar and ecotypes from Talamone (GR), Principina (GR), and San Felice Circeo (LT) showed a good self-seeding capacity (respectively 79% and 63–65%).

The persistence of commercial cultivars of *M. polymorpha* was lower than that of some of the ecotypes. The cvs. Anglona and Mauguio maintained or decreased their biomass in May 2019 and showed very low self-seeding capacity (average of 15%). These legumes were bred respectively in Italy and France for Mediterranean pasture cropping systems [29,31] in which low self-seeding capacity and high levels of hard-seededness are positive factors for a scalar germination of legumes over the time [54]. Legumes with such characteristics are, instead, not suitable for our target cropping system in which a dense and stable living mulch is required.

*M. polymorpha* cv. Scimitar showed an excellent self-seeding capacity (79%). However, despite the good self-seeding capacity, its biomass decreased by more than 50% from the first to the second year. This is likely caused by the significantly lower number of seeds per pod in comparison with other cultivars (2.5 seeds per pods in comparison with 4.5 as average of the others legumes, $p < 0.05$ data not shown) and by the high frost susceptibility of this Australian cultivar [27]. Low temperatures occurred in January 2019 for 10 consecutive days and this may have caused frost damage.

The comparison between the ecotypes and commercial cultivars of *M. polymorpha* confirmed the initial hypothesis that commercial cultivars can be less suitable for use as pLM than local ecotypes because they are selected for traits aimed at optimal fodder production and they are often bred under different environmental conditions. On the contrary, screening of ecotypes proved to be a promising option for the selection of suitable legumes for pLM in vegetable organic and conservative system. In fact, some of the ecotypes tested in this study, showed a good adaptation to the local environmental conditions. In 2018 the winter months were relatively warm and wet, while in 2019 winter and spring were warm and very dry. Climatic conditions are increasingly variable in the Mediterranean region [55], and stability in biomass production is therefore an important trait for permanent legume living mulches. The ecotype Villa Salto (SU) and Tarquinia (VT) and Pitigliano (SI) had a reduced self-seeding capacity and low persistence in time therefore, the most promising ecotypes for further testing are Principina (GR), San Felice Circeo (LT) and Talamone (GR) since they performed well in terms of weed suppression.

However, the decreasing presence of local seed production companies may hinder research and development activities aimed at ecotype selection. For these reasons, a greater understanding of the growth characteristics of available commercial cultivars and ecotypes is necessary to support the selection in the short and long term of legumes with suitable characteristics to be used as permanent living mulch in organic conservation agricultural systems.

## 5. Perspectives

Organic no-till systems can provide an important contribution to support carbon-neutral agriculture. Organic vegetable farmers need innovative tools that allow them to take advantage of the benefits of no-till or reduced tillage systems. These innovative tools need to combine the characteristics of organic farming practices (i.e., non-chemical weed-control, organic fertilisation and crop protection), with the principles of conservation agriculture (i.e., no-till or strip tillage, permanent soil cover with living mulch). In this perspective, the use of pLM is a promising solution. This study highlighted the importance of a preliminary screening to identify the potentially best adapted legumes. One of the critical issues that emerged during the screening was that none of the tested legumes were able to guarantee an adequate weed control over the years, especially for monocotyledonous weeds. Stable characteristics of pLM over the years and the maintenance of an adequate weed control in time are key points for a sustainable application of conservative methods in organic systems by the use of pLM. Therefore, additional integrated weed management strategies need to be selected that are adapted to the characteristics of permanent living mulches and vegetable crops in no- or reduced till organic system. Periodical cutting of the living mulch may be a solution to reduce weed seed dissemination and reduce at the same time excessive legume growth. Furthermore, developing legume mixtures among annual self-seeding and perennial legumes may improve the weed suppressive capacity of the living mulches. Besides the use of suitable commercial cultivars, a more promising approach is the selection and further genetic improvement of ecotypes that are surely more adapted to the local environmental conditions. Following screening of the legumes that are able to quickly establish a dense, low, sward that persists in time, these potential solutions need to be tested in the field together with various vegetable crops in order to develop vegetable crop rotations adapted to the permanent living mulch.

Since this is a costly and time consuming procedure, a first step needs to focus on legume screening based on a set of functional traits (earliness, soil cover capacity, maximum canopy height, weed suppression capacity) as demonstrated in this study. From this study the *T. subterraneum* cv. Antas and *T. repens* cv. Haifa provided the most promising results and some of these cultivars will be tested in vegetable systems in the future. Among the *M. polymorpha* ecotypes, those from Manciano, Talamone and Villa Salto showed the lowest variability among the years and future experiments aimed at testing their performance as pLM in conservative agricultural systems should be promoted.

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