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# Annual, Annual Self-Seeding and Perennial Legume Living Mulches (LLMs) Shape Weed Community Composition and Diversity During the Wheat–Sorghum Fallow Period

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## ABSTRACT

Among the agroecological practices that can be used for sustainable weed management, legume living mulches (LLMs) are receiving increasing attention. This study provides a detailed report on how different LLMs established in durum wheat affected the weed community composition and diversity during the fallow period in a wheat–sorghum crop sequence. We hypothesised that differences in the life cycle of the seven legume species used as LLMs, namely perennial, annual self-seeding and annual species, would result in contrasting soil cover dynamics during the fallow period and, consequently, in distinct effects on weed community composition and diversity. To this end, two complete wheat–sorghum crop sequence cycles were performed. While LLMs did not significantly affect weed community composition during the intercropping with wheat (IC stage), their influence became evident in the post-intercropping fallow period (post-IC stage). In both experimental fields, perennial LLMs (*Hedysarum coronarium* (L.) B. H. Choi & H. Ohashi, *Medicago sativa* L. and *Trifolium repens* L.) and self-seeding legumes (*Medicago polymorpha* L. and Trifolium *subterraneum* L.) establish consistent ground cover during the post-IC stage of the wheat–sorghum crop sequence, shifting weed communities towards perennial species dominance. Perennial/biennial weed species such as *Plantago lanceolata* L., *Cynodon dactylon* (L.) Pers., *Beta vulgaris* L. and *Silene latifolia* subsp. *alba* Poir. thrived due to their underground storage organs, which conferred resilience against LLMs' competition. However, while LLMs hindered most annual weed species, *Papaver rhoeas* L. responded positively to LLMs, producing larger, more vigorous plants compared to control plots. Results showed that, except for the annual LLMs in the first year, all treatments reported a reduction in weed species richness compared to control conditions in both experimental fields. The lower level of weed species richness under LLMs reflects a high capacity for weed suppression that may have reduced the number of weed species germinating and therefore their richness. However, no effect was found for species' evenness, indicating that none of the weeds became dominant despite the lower species' richness. The role of legume species traits for LLMs are discussed, and it is concluded that the adoption of LLMs within diversified crop rotations, which imply alternating soil disturbance practices, can be recommended to maintain a balanced composition between annual and perennial weed species.

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## 1 | Introduction

Weeds are a major challenge for arable cropping systems, and their successful management is a priority for farmers. The exclusive use of herbicides for weed control has long been considered the most efficient strategy for successfully controlling a large number of weeds in a short time, under multiple environmental conditions. However, in the long term, this approach has been demonstrated to tend to select and promote a few resistant and highly specialised weed species, particularly in systems with low crop diversification, such as *Alopecurus myosuroides* Huds. in north-west Europe and Great Britain (Moss and Clarke 1994; Délye et al. 2010), *Amaranthus palmeri* S. *Watson* in southern and central United States (Ward et al. 2013) and *Papaver rhoeas* L., *Lolium rigidum* Gaudin and *Avena sterilis* L. in central-south Europe (Calha et al. 2008; Loureiro et al. 2010; Heap 2025). An alternative to chemical control is soil tillage, but frequent soil disturbance increases the risk of soil erosion and facilitates the spread of certain detrimental weed species. As a result, intensive and specialised agricultural systems that rely solely on herbicides and tillage are often dominated by a few increasingly difficult to control weed species (Mottes et al. 2014; Storkey et al. 2010).

These challenges highlight the urgent need to reassess current weed management strategies by redesigning cropping systems and reducing the exclusive reliance on direct physical and chemical weed control methods (Deguine et al. 2023; Riemens et al. 2022; Wezel et al. 2014). In this context, integrated weed management (IWM) strategies and agroecological preventive practices should be prioritised, as they promote more diversified weed communities, which in turn can reduce competitive pressure on crops (MacLaren et al. 2020). Indeed, recent studies showed that diversified cropping systems (e.g., through diversified crop rotations and the adoption of practices that increase in-field diversity), which involve varying planting dates, growth habits, nutrient requirements and cultural practices, support a broader range of weed species, which may result in a more diverse weed community with a lower overall competitive ability (Weisberger et al. 2019).

Among agroecological preventive practices, living mulches can enhance biodiversity within crop rotations and support IWM. The living mulches involve (relay) intercropping of service crops, often legumes, with the main cash crop and may persist beyond crop harvest. Through early establishment and continuous ground cover, living mulches may control weeds and affect weed community composition and diversity in two phases of the crop sequence: (i) during the intercropping with the main cash crop, by occupying ecological niches otherwise available to weeds, and (ii) in between two cash crops by maintaining soil cover and competitive pressure during fallow periods. The living mulches are already established by the time of crop harvest, and even a small summer rainfall event is sufficient to stimulate vegetation growth, providing effective ground cover immediately afterwards. This represents a clear advantage over cover crops sown in autumn, which require time to establish. The living mulches persist after the cash crop harvest in ways that depend on their growth cycle, either as dead mulch when annual species are used, or as living ground cover when perennial or self-seeding species are employed (Leoni et al. 2022).

It is well known that cultural practices such as tillage, fertilisers, and herbicides shape weed communities by altering resource availability, biotic interactions and habitat stability (Cordeau et al. 2021; Derrouch et al. 2022; Gaba et al. 2017). However, less is known about the filtering effects of living mulches. The living mulches provide continuous ground cover throughout the crop sequence, potentially maintaining constant competition with weeds in a crop sequence. This can reduce the need for herbicides and soil tillage during fallow periods, while also positively influencing weed diversity and the composition of weed communities (Westbrook et al. 2022; Cougnon et al. 2022). The living mulches affect weeds by reducing light availability from the legume canopy and by altering temperature, moisture and nutrient dynamics; they may therefore function as ecological filters, promoting certain weed species while suppressing others, and potentially leading to the dominance of specific functional traits within the weed community (Booth and Swanton 2002). Validating this would offer valuable insights for farmers to adapt their weed management strategies in line with the expected weed community throughout the entire crop rotation.

The living mulches are expected not only to affect weed community structure but also to influence weed community diversity. Recent studies demonstrated that weed communities with high species richness and evenness can simultaneously promote ecosystem services and decrease weed-crop competition (Esposito et al. 2023; MacLaren et al. 2020; Storkey and Cussans 2007). For instance, Adeux et al. (2019) reported that with the same amount of biomass, a more diversified weed community is generally less detrimental than a weed community composed of a low number of very specific species. Moreover, the use of living mulches can also enhance the diversity of other beneficial groups, such as arthropods and weed seed predators, which in turn may contribute to weed control (Schumacher and Gerhards 2022; Nichols et al. 2020; Marshall et al. 2003).

Despite growing interest in living mulches, their effects on weed communities have been primarily studied during the intercropping phase, often focusing on aggregate indicators such as total weed biomass or density. Much less attention has been paid to their role during the inter-crop fallow period, particularly in Mediterranean cereal-based systems, where the fallow phase between two crops can last several months and represents a critical window for weed establishment and seed bank replenishment (Lamichhane et al. 2023).

During wheat cultivation, Barilli et al. (2017) reported that the use of lucerne (*Medicago sativa* L.) as a living mulch significantly reduced total weed abundance while increasing weed diversity, particularly suppressing annual and perennial dicotyledonous species with rosette growth forms. Similarly, Hiltbrunner et al. (2007) demonstrated that white clover (*Trifolium repens* L.), subterranean clover (*Trifolium subterraneum* L.) and birdsfoot trefoil (*Lotus corniculatus* L.), when used under organic farming conditions, effectively reduced the density of both monocotyledonous and dicotyledonous annual weeds such as *Poa annua* L., *Matricaria recutita* L., *Capsella bursa-pastoris* (L.) Medik. and *Stellaria media* (L.). Westbrook et al. (2022) further confirmed that living mulches can suppress highly competitive annual weeds like *Amaranthus retroflexus* L. and *C. album*, primarily through the lower light



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interception due to the presence of living mulches. Notably, Bergkvist (2003) observed a temporal shift in weed community composition in winter wheat grown with white clover LMs: while weed pressure was very low during the first crop cycle, the second wheat crop experienced a significant increase of perennial weeds such as *Elymus repens* Gould and *Lolium perenne* L., along with annual species including *Apera spica-venti* P.B. and *P. rhoeas* L. However, other studies, such as Torra et al. (2018), suggest that without regular soil disturbance, species like *P. rhoeas* may decline over time due to increased seed dormancy.

In Mediterranean cereal-based cropping systems, the fallow period between two crops can be up to 9 months, and in this critical period, weeds can grow and replenish the seed bank. The good persistence of living mulches after crop harvest is important to provide summer and winter soil cover until the sowing of the following summer crop, ensuring a continuous competition with weeds for resources such as light, nutrients and water (Leoni et al. 2024; Vrignon-Brenas et al. 2016; Amossé et al. 2013). However, the effectiveness of living mulches during the fallow period largely depends on the choice of species and their persistence strategy after crop harvest. Annual, annual self-seeding and perennial plants used as living mulches may differ markedly in their ability to maintain soil cover and competitive interactions with weeds during this phase, potentially leading to contrasting effects on weed community composition and diversity. Understanding how these persistence strategies shape weed communities during the fallow period is therefore essential for optimising the use of living mulches within an IWM strategy and represents the main objective of the present study.

In this study, seven legume living mulches (LLMs) with contrasting growth cycles (annual, annual self-seeding and perennial) were established in durum wheat within a crop sequence based on durum wheat and sorghum. A previous paper reported data from this trial on crop yield performance and overall weed control during the intercropping stage (Leoni et al. 2022) and in the subsequent sorghum crop (Leoni et al. 2024). Here, we specifically focus on the effects of LLMs on weed community composition and diversity during the fallow period between durum wheat and sorghum, a critical phase for weed management within Mediterranean crop rotations. This focus allows us to assess how different LLMs' persistence strategies, dead mulch (annual LLMs), perennial living ground cover (perennial LLMs) and mixed persistence (dead mulch in summer and living cover in winter–spring for annual self-seeding species), influence weed communities during the inter-crop period. The specific hypotheses tested are:

- Annual self-seeding and perennial LLMs affect the weed ground cover and weed community composition during the durum wheat–sorghum fallow period, acting as selective filters that reduce annual weed species, which are assumed to be more susceptible to competition from living mulches compared to perennials (hemicryptophyte and geophyte). In contrast, annual LLMs are mostly present during the fallow period as a less persistent dead mulch, with consequently lower expected effects on weed ground cover and community composition. However, the life cycle typology of LLMs is not expected to affect the weed community composition differently during the intercropping phase, as both annual and perennial weed species tend to emerge simultaneously after soil tillage and wheat establishment.
- High-cover LLMs reduce weed species richness during fallow while maintaining species evenness within the weed assemblage, potentially promoting a more balanced community composition without dominance by highly specialised species that are difficult to control.

## 2 | Materials and Methods

### 2.1 | Site Description and Experimental Design

The trial was carried out for two consecutive growing seasons in adjacent fields (Field A and Field B, Figure 1) situated in a rainfed area of 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). The experiment followed a randomised complete block design with four replications. Prior to the establishment of the experiment, composite soil samples (0–30 cm depth) were collected from each block to assess baseline soil properties and soil uniformity. In Field A, the soil texture was composed of 50.6% sand, 26.1% silt and 23.3% clay, with a soil organic matter (SOM) content of 1.77% and a pH of 8.0. In Field B, the soil composition was 39.8% sand, 34.7% silt and 25.5% clay, with 1.18% SOM and a pH of 8.3. Regarding chemical fertility, total nitrogen (Total N) concentrations were 1.20 and 1.23 g/kg in Fields A and B, respectively, while available phosphorus (P) levels were 10.5 mg/kg in Field A and 7.0 mg/kg in Field B. Soil organic matter, total nitrogen and available phosphorus were determined following standard procedures: the Walkley–Black method (FAO 2019) for SOM, the Kjeldahl method (Bremner 1960) for Total N and the Olsen method (Kovar and Pierzynski 2009) for available P.

Each experiment consisted of a two-year crop sequence, including a winter crop (*Triticum durum* Desf. cv. Minosse) intercropped with LLMs and a subsequent spring crop (*Sorghum vulgare* cv. Sugar Graze). Within the durum wheat (IC stage), seven LLMs were established using the relay intercropping technique. LLMs included (i) three perennial legumes, *M. sativa* L. (MEDSA), *T. repens* (TRFRE), *Hedysarum coronarium* (L.) B.H.Choi & H.Ohashi (HESCO); (ii) two annual legumes, *Trifolium incarnatum* L. (TRFIN) and *Trifolium resupinatum L.* (TRFRS); and two annual self-seeding legumes, *T. subterraneum* L. (TRFSU) and *Medicago polymorpha* L. (MEDPO). The control (CNT) consisted of durum wheat sole crop cultivation following the standard low-input management practices applied in the study region. Durum wheat was mechanically harvested on 27 July 2018 (Field A) and 10 July 2019 (Field B), and straw was removed from the field. After durum wheat harvest (post-IC stage), the conventional management practice in Mediterranean cereal-based cropping systems is to leave the field uncultivated until the sowing of the subsequent spring–summer crop (in our case, sorghum). Accordingly, in the control treatment (CNT), the plots remained fallow during the post-C stage with only spontaneous vegetation. In contrast, in the LLM treatments, the post-IC stage was characterised by



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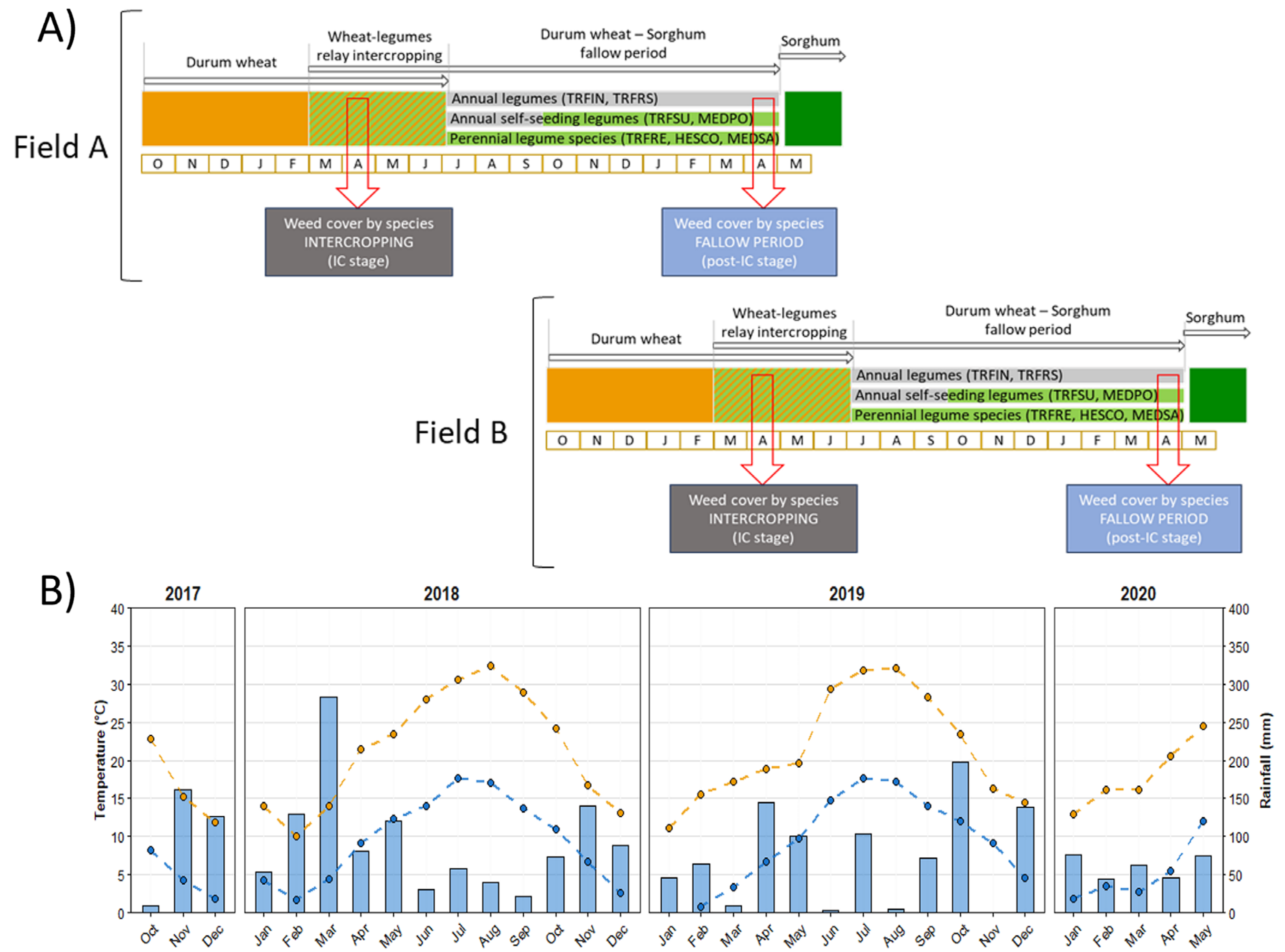


**FIGURE 1** | Experimental timeline showing crop sequence and key sampling times in the two experimental repetitions, namely Field A and Field B (A). After the durum wheat harvest, different colours represent the persistence strategy of each LLMs used in this study: Dead mulch (grey) for annual legumes (TRFIN and TRFRS); dead mulch in summer and living plants in autumn–winter (grey following green) for annual self-seeding legumes (TRFSU and MEDPO); and permanent living ground cover (green) for perennial species (TRFRE and MEDSA). The coverage of LLMs and weed species was assessed at key points in the crop sequence, namely during the intercropping phase (wheat + LLMs: April 2018 in Field A and April 2019 in Field B) and 1 year later during the wheat–sorghum fallow period (April 2019 in Field A and April 2020 in Field B). Climatic conditions throughout the experiment (B). Maximum (orange dots) and minimum (blue dots) monthly mean temperatures (left *y*-axis); and monthly mean rainfall (blue bars, right *y*-axis) from October 2017 to May 2020. Climate conditions are collected by the in situ automatic weather station. Sensors were placed 2 m above ground.

the natural persistence of LLMs in the field, resulting in different vegetation dynamics depending on the growing cycle of legumes (Figure 1). Perennial species persisted in the post-IC stage as living mulch, whereas annual self-reseeding species acted as dead mulch during summer and re-established as living mulch from autumn onward. Annual species without self-reseeding ability persisted only as dead mulch until the subsequent spring.

The LLMs were maintained throughout the post-IC stage until the sowing of the subsequent spring crop (Figure 1), which took place on 14 April 2019 (Field A) and 15 April 2020 (Field B). Plot management was based on the principle of low-input farming, with no application of fertilisers, herbicides and fungicides. This allowed for the detection of the LLM's effect that would otherwise be overridden by herbicide and fertilisation applications.

### 2.2 | Data Collection

During the IC stage at the wheat early flowering (BBCH 51–53), which took place in April for both field experiments, the coverage of weed species was visually assessed (Figure 1). This assessment was conducted in two 54 × 50 cm quadrants (sub-replicates) per plot for a total of eight observations for each treatment. The weed composition was described based on the coverage of weed species. Weed species identification was done according to the Italian flora Pignatti (1982). Each weed species was labelled according to the European and Mediterranean Plant Protection Organization (EPPO) code. To evaluate the impact of each LLM on the weed community diversity and composition during the durum wheat–sorghum fallow period (post-IC stage), assessments of LLMs and weed species ground coverage were repeated within each 54 × 50 cm quadrant per plot in the following spring (April), just before



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ploughing under the LLMs (Figure 1). The subsequent sorghum crop was sown after the LLMs termination at the end of April 2019 for Field A and in 2020 for Field B.

## 2.3 | Statistical Analysis

### 2.3.1 | Weed Community Diversity and Composition

Data on weed species composition and coverage were used to calculate three diversity measures related to the use of each LLM ($\alpha$-diversity), namely the Shannon diversity index ($H$), representing the number of equally common species in an assemblage (Magurran 2013), species richness ($S$), the number of species and Pielou evenness of vegetation ($J$) (Sheldon 1969). For the calculation of the diversity indices $H$ and $S$, only the coverage of each weed species was included, excluding the coverage of the LLMs. These three indices have been analysed with a linear mixed model (LMM) with living mulch species (eight levels: CNTR, HESCO, MEDSA, TRFRE, MEDPO, TRFSU, TRFIN and TRFRS) and experiment repetition, that is, experiment repetition (two levels: April 2019 in Field A and April 2020 in Field B) as fixed factors and sub-replicates (two per plot), nested into replicates (four blocks), were included as a random factor. Following the results of the analysis of variance, for significant explanatory variables, the Sidak post hoc test was performed to separate means ($p \leq 0.05$). Normality and homogeneity of residual variance were confirmed using the Kolmogorov–Smirnov and Levene tests.

The effect of LLMs on weed community composition was tested by means of a permutational analysis of variance (PERMANOVA) using the Bray–Curtis dissimilarity index. This approach partitions multivariate variability in the data according to determined factors on the basis of any distance measure, using permutation methods (Anderson 2001). Living mulch species (eight levels: CNTR, HESCO, MEDSA, TRFRE, MEDPO, TRFSU, TRFIN and TRFRS) were included as a fixed explanatory factor. Permutations were constrained within blocks by specifying the replicate factor as a permutation stratum in the 'adonis2' function in order to account for the nested sampling design. The analyses were performed separately for each experiment repetition (Field A and Field B) and sampling time (IC stage and post-IC stage). The significance of the factors was tested by means of $F$ tests based on sequential sums of squares from permutations of raw data (9999 tries).

The diversity matrix was also used to perform a multivariate ordination through non-metric multidimensional scaling (NMDS) (1000 traces, 20 tries). PERMANOVA and NMDS analyses were run with the vegan package for R (Oksanen et al. 2013). After the global analysis, we tested the pairwise differences between all groups (LLMs) and reported the Bonferroni-corrected $p$ values.

### 2.3.2 | Weeds and Living Mulch Coverage

Total coverage of weeds, calculated as the sum of the cover values of all individual weed species, and coverage of LLMs during the post-IC stage were analysed with generalised linear mixed model (GLMM), using the lme4 package for R (Bates et al. 2015). The considered explanatory (fixed factors) variables were (1) living mulch species (eight levels: CNTR [control], HESCO [*H. coronarium*], MEDSA [*M. sativa*], TRFRE [*T. repens*], MEDPO [*Medicago polymorpha*], TRFSU [*T. subterraneum*], TRFIN [*T. incarnatum*] and TRFRS [*T. resupinatum*]) and (2) experiment repetitions (two levels: April 2019 in Field A and April 2020 in Field B). Sub-replicates (two per plot), nested into replicates (four blocks), were considered as random factors. Following the results of the analysis of variance, for significant explanatory variables, the Sidak post hoc test was performed to separate means ($p \leq 0.05$) using the 'emmeans' package (Lenth et al. 2020). Normality and homogeneity of residual variance have been confirmed respectively with the Kolmogorov–Smirnov and Levene test using 'DHARMa' package (Hartig et al. 2024).

## 3 | Results

### 3.1 | Weed Species

In Field A (Table 1), 30 weed species were identified during the IC stage, and 27 during the durum wheat–sorghum fallow period (post-IC stage). Notable main weed species found during the IC stage included *Ammi majus* L. (AMIMA), *Anagallis arvensis* L. (ANGAR), *Cirsium arvense* (L.) Scop. (CIRAR), *Helminthotheca echioides* (L.) Holub. (PICEC), *Rapistrum rugosum* (L.) All. (RASRU), *R. crispus* L. (RUMCR) and *Sinapis alba* L. (SINAL). Instead, during the post-IC stage, *A. myosuroides* (ALOMY), *C. arvense (CIRAR)*, *Lolium multiflorum* Lam. (LOLMU), *H. echioides* (PICEC) and *R. crispus* (RUMCR) were the most representative species (Table 1). In Field B (Table 1), 19 weed species were observed during the IC stage, while 21 were recorded during the post-IC stage. Key species during the IC stage were *A. retroflexus* (AMARE) and *C. album* (CHEAL) In the subsequent fallow period, *L. multiflorum* (LOLMU), *Oxalis* spp. (OXAAC), *H. echioides* (PICEC), *Sonchus asper* (L.) Hill. (SONAS) and *Veronica persica* Poir. (VERPE) were the most abundant species in the field (Table 1). Only seven species were present in both the IC stage and the post-IC stage, and in both Field A and Field B: *L. multiflorum* (LOLMU), *A. arvensis* (ANGAR), *Cerastium glomeratum* Thuill. (CERGL), *P. rhoeas* L. (PAPRH), *H. echioides* (PICEC), *V. persica* (VERPE) and *Convolvulus arvensis* L. (CONAR). Considering both Field A and Field B, most weed species identified during the IC stage and in the post-IC stage were dicotyledons rather than monocotyledons. Therophyte and hemicryptophyte were the predominant life forms (Table 1), which is in line with the specific pedo-climatic conditions of the studied area.

Considering the weed species present in Field A and Field B, we observe similar behaviour for some species when comparing their presence during the IC stage and the post-IC stage. In both Field A and Field B, certain species, including *A. arvensis* (ANGAR), *Capsella bursa-pastoris* (L.) Medik. (CAPBP), *Kickxia spuria* (L.) Dumort. (KICSP) and *C. album (CHEAL)*, were generally less abundant in the IC stage than in the post-IC stage (Table 1). Instead, other weeds such as *Euphorbia helioscopia* L. (EPHHE), *L. multiflorum (LOLMU)*, *Plantago*



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**TABLE 1** | List of weed species found in the experimental fields (Field A and Field B) during the intercropping stage with wheat (IC stage) and in the subsequent wheat–sorghum fallow period (post-IC stage).

| Plant type | Life form (Raunkiaer) | Genus and species | Code (EPPO) | Field A | | Field B | |
|---|---|---|---|---|---|---|---|
| | | | | IC stage | Post-IC stage | IC stage | Post-IC stage |
| Monocotyledon | Therophyte | *Alopecurus myosuroides* Huds. | ALOMY | +++ | ++++ | 0 | 0 |
| | | *Phalaris paradoxa* L. | PHAPA | ++ | 0 | 0 | 0 |
| | | *Poa annua* L. | POAAN | 0 | + | + | 0 |
| | | *Poa trivialis* L. | POATR | ++ | +++ | + | + |
| | | *Setaria viridis* (L.) P. Beauv. | SETVI | 0 | 0 | ++ | 0 |
| | Hemicryptophytes | *Lolium multiflorum* Lam. | LOLMU | ++ | ++++ | +++ | ++++ |
| | Geophytes | *Cynodon dactylon* (L.) Pers. | CYNDA | +++ | +++ | 0 | + |
| Dicotyledon | Therophyte | *Amaranthus retroflexus* L. | AMARE | 0 | + | ++++ | 0 |
| | | *Ammi majus* L. | AMIMA | ++++ | +++ | 0 | 0 |
| | | *Anagallis arvensis* L. | ANGAR | ++++ | ++ | +++ | + |
| | | *Anthemis arvensis* L. | ANTAR | +++ | + | 0 | 0 |
| | | *Cardamine hirsuta* L. | CARHI | + | + | + | +++ |
| | | *Cerastium glomeratum* Thuill. | CERGL | + | ++ | +++ | ++ |
| | | *Chenopodium album* L. | CHEAL | ++ | + | ++++ | 0 |
| | | *Euphorbia helioscopia* L. | EPHHE | + | ++ | 0 | +++ |
| | | *Fumaria officinalis* L. | FUMOF | + | 0 | 0 | 0 |
| | | *Galium aparine* L. | GALAP | ++ | 0 | 0 | 0 |
| | | *Kickxia spuria* (L.) Dumort. | KICSP | + | 0 | +++ | 0 |
| | | *Lamium purpureum* L. | LAMPU | 0 | 0 | +++ | ++ |
| | | *Papaver rhoeas* L. | PAPRH | ++ | +++ | +++ | ++ |
| | | *Polygonum aviculare* L. | POLAV | +++ | + | 0 | 0 |
| | | *Ranunculus arvensis* L. | RANAR | ++ | 0 | 0 | 0 |
| | | *Rapistrum rugosum* (L.) All. | RASRU | ++++ | 0 | 0 | 0 |
| | | *Senecio vulgaris* L. | SENVU | 0 | 0 | ++ | +++ |
| | | *Sinapis alba* L. | SINAL | ++++ | +++ | 0 | 0 |
| | Hemicryptophytes | *Beta vulgaris* L. | BEAVX | ++ | ++ | 0 | 0 |
| | | *Calystegia sepium* (L.) R. Br. | CAGSE | +++ | 0 | 0 | 0 |
| | | *Capsella bursa-pastoris* (L.) Medik | CAPBP | + | 0 | + | 0 |
| | | *Daucus carota* L. | DAUCA | 0 | 0 | ++ | + |
| | | *Helminthotheca echioides* (L.) Holub | PICEC | ++++ | ++++ | ++ | ++++ |
| | | *Lactuca serriola* L. | LACSE | 0 | 0 | 0 | ++ |
| | | *Plantago lanceolata* L. | PLALA | 0 | ++ | 0 | +++ |
| | | *Rumex crispus* L. | RUMCR | ++++ | ++++ | 0 | 0 |
| | | *Silene latifolia subsp. alba Poir* | MELAL | 0 | 0 | + | ++ |
| | | *Sonchus asper* (L.) Hill | SONAS | ++ | + | 0 | ++++ |
| | | *Verbena officinalis* L. | VEBOF | + | ++ | 0 | +++ |
| | | *Veronica persica* Poir. | VERPE | +++ | ++++ | +++ | ++++ |
| | Geophytes | *Cirsium arvense* (L.) Scop. | CIRAR | ++++ | ++++ | 0 | 0 |
| | | *Convolvulus arvensis* L. | CONAR | +++ | +++ | ++ | + |
| | | *Oxalis* spp. | OXAAC | 0 | 0 | + | ++++ |

*Note:* Relative abundance by species: Absence (0), 0%–25% (+), 25%–50% (++), 50%–75% (+++) and 75%–100% (++++).



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*lanceolata* L. (PLALA), *Verbena officinalis* L. (VEBOF) and *V. persica* (VERPE) tended to increase in abundance (Table 1), suggesting their possible lower susceptibility to competition from the living mulch or a greater adaptation to no-tillage conditions.

### 3.2 | Weed Community Composition

In both experiments, a comparable effect of LLMs has been observed on weed communities following PERMANOVA (Table 2). During the IC stage, LLMs had no significant effect on weed community composition regardless of the legume species used (Field A, $p=0.972$; Field B, $p=0.419$) (Table 2 and SM1 and SM2). In the post-IC stage, the species used as LLMs differently influenced weed community composition (Field A, $p \leq 0.001$; Field B, $p \leq 0.001$) (Table 2 and SM1).

During the post-IC stage, in Field A, weed communities of perennial LLMs of HESCO and TRFRE significantly differed from the control (HESCO vs. CNT, $p=0.006$; TRFRE vs. CNT, $p=0.006$) (Figure 2 and SM1). These LLMs species showed a higher presence of perennial weeds, such as *P. lanceolata* L. (PLALA), *Beta vulgaris* L. (BEAVX) and *C. dactylon* (CYNDA) and annuals such as *P. rhoeas* (PAPRH). The control, instead, was more characterised by annual and ubiquitous species, such as *Poa annua* L. (POAAN), *Bromus sterilis* L. (BROST), *A. majus* (AMIMA) and *A. retroflexus* (AMARE) (Figure 2). Annual self-seeding LLMs such as MEDPO and TRFSU completed their growth cycle by early summer around wheat harvest time, leaving their biomass residues that acted as dead mulch. They subsequently germinated with autumn rains, re-establishing a dense coverage. These species showed different weed community composition compared to the control (MEDPO vs. CNT, $p=0.006$; TRFSU vs. CNT, $p=0.018$) (Figure 2 and SM1). In contrast, LLMs such as TRFIN, TRFRS and MEDSA showed weed communities similar to the control (TRFIN vs. CNT, $p=0.850$; TRFRS vs. CNT, $p=0.225$; MEDSA vs. CNT, $p=0.119$) (Figure 2 and SM1), likely due to insufficient soil cover during the post-IC stage.

In Field B, all perennial LLMs (HESCO, TRFRE and MEDSA) displayed weed communities significantly different from the control ($p=0.006$). Their weed community is characterised by perennial or biennial species such as *S. latifolia* (MELAL) and *Convolvulus arvensis* L. (CONAR), alongside annuals like *P. rhoeas* (PAPRH), *Cardamine hirsuta* L. (CARHI) and *C. album* (CHEAL) (Figure 2). The weed community in the control exhibited a broader diversity of mostly ubiquitous species, including *Daucus carota* L. (DAUCA), *C. dactylon* (CYNDA), *H. echioides* (PICEC), *L. multiflorum* (LOLMU) and *E. helioscopia* (EPHHE). Self-seeding legumes, including MEDPO and TRFSU, alongside annual LLMs such as TRFRS, also showed distinct weed communities compared to the control (MEDPO vs. CNT, $p=0.026$; TRFSU vs. CNT, $p=0.006$) (SM1). Their weed community is characterised by perennial or biennial species such as *S. latifolia* (MELAL), alongside annuals like *Anagallis arvensis* (ANGAR) and *C. album* (CHEAL) (Figure 2).

### 3.3 | Weed Diversity

The Shannon diversity index ($H'$) was not significantly affected by the legume species used as LLMs ($p=0.472$) or by experiment repetition ($p=0.987$), but it was significantly affected by their interaction ($p \leq 0.001$). The reason for this interaction was that during the first experiment repetition (Field A), the Shannon diversity index ($H'$) was similar across LLMs compared to the control, with values ranging between 0.85 (MEDSA) and 1.24 (HESCO) (Table 3). In contrast, during the second experiment repetition (Field B), the $H'$ values for HESCO (0.54), TRFRE, TRFSU and TRFIN were significantly lower than for the control (Table 3).

Species richness ($S$) was significantly affected by the legume species used as LLMs ($p \leq 0.001$), by the experiment repetition ($p=0.014$), and by their interaction ($p \leq 0.001$). In Field A, TRFIN, TRFRS, HESCO and MEDSA showed richness values not significantly different from the control (Table 3). However, other legumes, such as TRFRE, MEDPO and TRFSU, had significantly lower richness compared to the control (Table 3). In Field B, all legume LLMs showed significantly lower species richness (average of 3.16) compared to the control, except for MEDPO. Species evenness ($J$) was not significantly different from the control across all LLMs in both repetitions of the experiment, maintaining a similar weed distribution pattern across legume species (Table 3).

### 3.4 | Total LLMs and Weed Coverage

Legume and weed ground cover was significantly affected by the legume species used as LLMs ($p \leq 0.001$), by the experiment repetition ($p \leq 0.001$), and by their interaction ($p \leq 0.001$). Most LLMs were established well in both fields, except for the annual species TRFIN and TRFRS and MEDSA in Field A and MEDPO in Field B. Weed suppression capacity of the LLMs was generally higher in Field A than in Field B, and our data indicate that the persistence of

**TABLE 2** | Test results of permutational multivariate analysis of variance (PERMANOVA) applied to the weed community analysis (NMDS) in Field A and Field B during the IC stage and post-IC stage.

| Experimental repetition | | Effect | df | $R^2$ | $F$ | $p$ |
|---|---|---|---|---|---|---|
| Field A | IC stage (April 2018) | Legume species | 7 | 0.104 | 0.569 | 0.972 |
| | Post-IC stage (April 2019) | Legume species | 7 | 0.333 | 4.008 | **0.001** |
| Field B | IC stage (April 2019) | Legume species | 7 | 0.234 | 1.048 | 0.419 |
| | Post-IC stage (April 2020) | Legume species | 7 | 0.308 | 3.317 | **0.001** |

*Note:* The bold values indicate statistical significance at $p \leq 0.05$.



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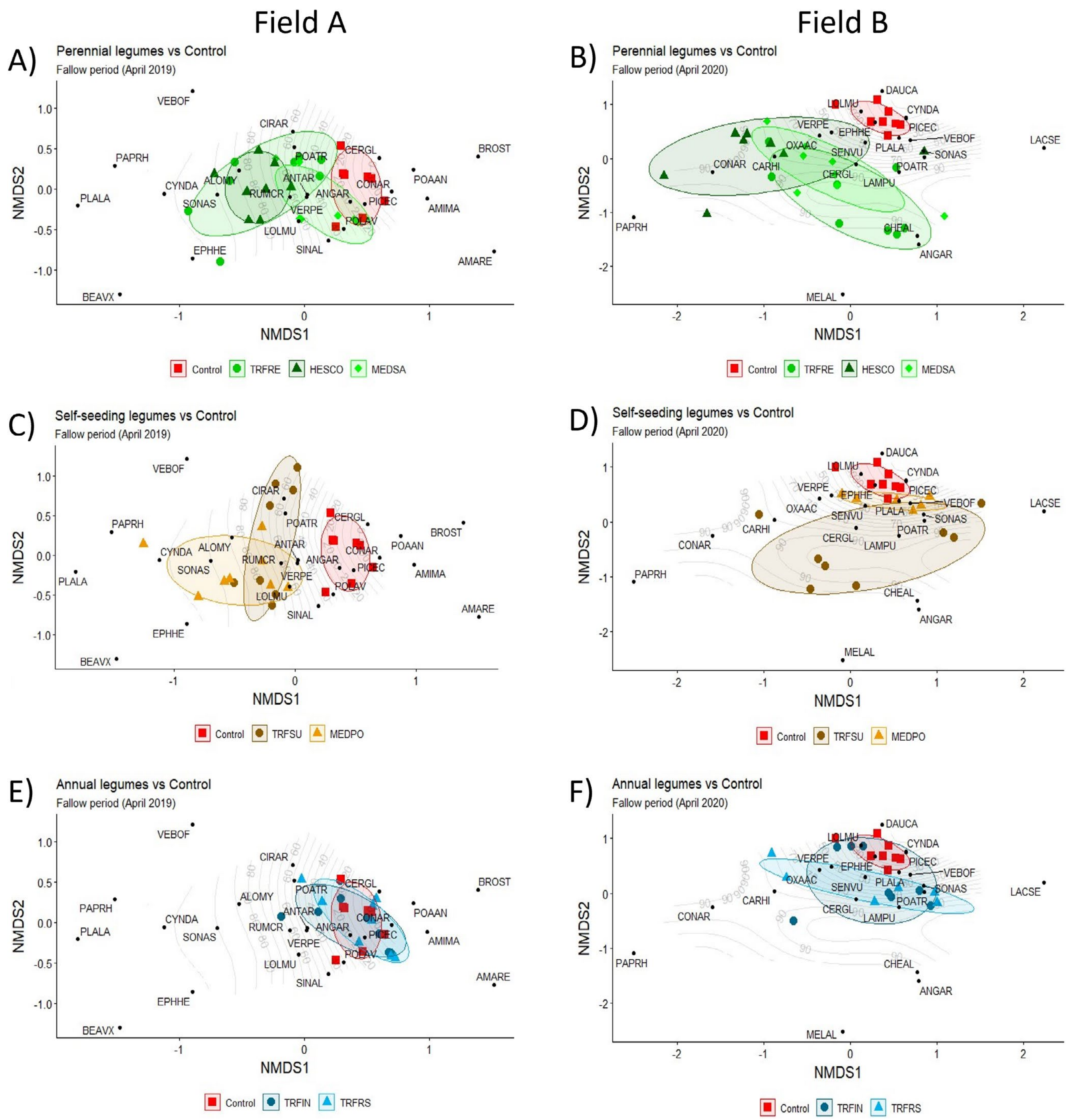


**FIGURE 2** | Two-dimensional NMDS graph illustrating weed community compositions based on Bray-Curtis distances derived from weed species coverage data under different legume living mulches (LLMs) in Field A (A, C, E) and Field B (B, D, F) during the post-IC stage (wheat–sorghum fallow period). The distribution of perennial, annual self-seeding and annual LLMs was graphically presented separately to enhance graph readability. Colours and shapes denote different LLMs (HESCO: *Hedysarum coronarium*; MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; MEDPO: *Medicago polymorpha*; TRFSU: *Trifolium subterraneum*; TRFIN: *Trifolium incarnatum*; TRFRS: *Trifolium resupinatum*). Weed species were labelled according to EPPO coding (see Table 1). The control group (without LLMs, in red) for Field A and Field B was included in all graphs as a reference.

LLMs during the post-IC stage significantly influenced their weed suppression capacity ($p \leq 0.001$). Perennial and self-seeding LLMs effectively suppressed weed growth across both experiment repetitions (Field A and Field B) (Figure 3). Specifically, the perennial species HESCO, MEDSA and TRFRE, as well as the self-seeding species MEDPO and TRFSU, showed a significant reduction in total weed coverage compared to the control (Figure 3). Instead, annual species such as TRFIN and TRFRS produced insufficient biomass in Field A to create an effective dead mulch layer, resulting in weed coverage levels similar to the control (Figure 3). In Field B, annual LLMs regrown in the post-IC stage behaved more similarly to self-seeding legumes and resulted in higher legume cover with correspondingly lower weed presence, comparable to the perennial and self-seeding species (Figure 3).



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**TABLE 3** | Shannon's diversity index ($H'$), species richness ($S$) and species evenness ($J$) calculated for weed communities referred to each of LLMs and experimental repetition (Field A and Field B) at the end of the post-IC stage (April 2019 and April 2020).

| Field (experimental repetition) | Legume species | Diversity indices $H'$ | $S$ | $J$ |
|---|---|---|---|---|
| Field A (Post-IC stage, April 2019) | HESCO | 1.24 (0.13) | 6.50 (0.70) ab | 0.67 (0.08) |
| | MEDSA | 0.85 (0.16) | 6.12 (0.82) abc | 0.47 (0.06) |
| | TRFRE | 1.00 (0.12) | 4.62 (0.45) cd | 0.65 (0.12) |
| | MEDPO | 0.87 (0.11) | 3.75 (0.51) d | 0.69 (0.09) |
| | TRFSU | 0.89 (0.06) | 4.75 (0.74) bcd | 0.60 (0.06) |
| | TRFIN | 1.03 (0.18) | 7.87 (0.72) a | 0.50 (0.09) |
| | TRFRS | 1.16 (0.19) | 6.87 (0.72) a | 0.57 (0.08) |
| | CNT | 1.13 (0.08) | 7.75 (0.74) a | 0.56 (0.05) |
| Field B (Post-IC stage, April 2020) | HESCO | 0.54 (0.10) b | 3.00 (0.46) b | 0.58 (0.13) |
| | MEDSA | 1.03 (0.13) ab | 3.62 (0.25) b | 0.79 (0.12) |
| | TRFRE | 0.60 (0.07) b | 2.25 (0.23) b | 0.73 (0.13) |
| | MEDPO | 1.28 (0.07) a | 5.50 (0.41) ab | 0.76 (0.03) |
| | TRFSU | 0.62 (0.17) b | 3.12 (0.32) b | 0.53 (0.17) |
| | TRFIN | 0.58 (0.15) b | 3.50 (0.88) b | 0.49 (0.13) |
| | TRFRS | 0.80 (0.13) ab | 3.50 (0.41) b | 0.64 (0.12) |
| | CNT | 1.17 (0.17) a | 5.75 (0.74) a | 0.66 (0.11) |
| Analysis of variance | Factors | | | |
| | Legume species (LEG) | $p = 0.472$ | $\mathbf{p \leq 0.001}$ | $p = 0.350$ |
| | Field (F) | $p = 0.987$ | $\mathbf{p = 0.014}$ | $p = 0.366$ |
| | LEG × F | $\mathbf{p \leq 0.001}$ | $\mathbf{p \leq 0.001}$ | $p = 0.185$ |

*Note:* Numbers in parentheses represent ± standard error of the mean. Lowercase letters show significant differences between groups at $p < 0.05$; lettering differs between Field A and Field B. CNT: Control plot (no living mulches); HESCO: *Hedysarum coronarium*; MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; MEDPO: *Medicago polymorpha*; TRFSU: *Trifolium subterraneum*; TRFIN: *Trifolium incarnatum*; TRFRS: *Trifolium resupinatum*. The bold values indicate statistical significance at $p \leq 0.05$.

## 4 | Discussion

### 4.1 | Weed Community Composition

Although Fields A and B were characterised by distinct weed species, LLMs showed comparable effects on weed communities. While LLMs did not significantly affect weed community composition during the IC stage with wheat, their influence became evident in the post-IC stage, where different LLM species significantly altered the weed community composition. During the IC stage, LLMs did not affect the weed community because, in the case of relay intercropping system with wheat, most dominant weeds had already germinated beforehand, and the strongest filtering effect was exerted by wheat itself. Consequently, no short-term shift in weed community composition was expected in response to different types of LLMs. Additionally, annual weed species or new hemicryptophyte weed species were not expected to respond differently during the IC stage, as they germinate before the establishment of LLMs through relay intercropping. Conversely, during the post-IC stage, since leguminous species remained in the field after wheat harvest, their presence may have influenced the emergence of new weed species compared to fallow periods without LLMs. Consistent with findings reported by Westbrook et al. (2022) and Hiltbrunner et al. (2007), these results indicate that living ground cover during fallow periods serves as an important ecological filter, shaping weed community structure. This effect can be even more evident in LLM systems, where the presence of legumes becomes immediately evident after the wheat harvest, without the need for tillage operations that would otherwise be required for the standard sowing of cover crops (Feng et al. 2024). Moreover, some summer rainfall allows the legumes to increase their biomass immediately and provides a summer cover that suppresses summer weeds. In our study, ubiquitous species such as *A. arvensis* (ANGAR), *V. persica* (VERPE) and *C. glomeratum* (CERGL) were annual creeping species that probably will not be able to strongly compete in the winter and summer crops. Their presence at medium abundance in both the IC stage and post-IC stage and both fields suggests that, more than damaging the crops, they could add ecological services to the system, such as filling a niche, avoiding the entrance of other more harmful weed species as suggested by Adeux et al. (2019). Storkey (2006)



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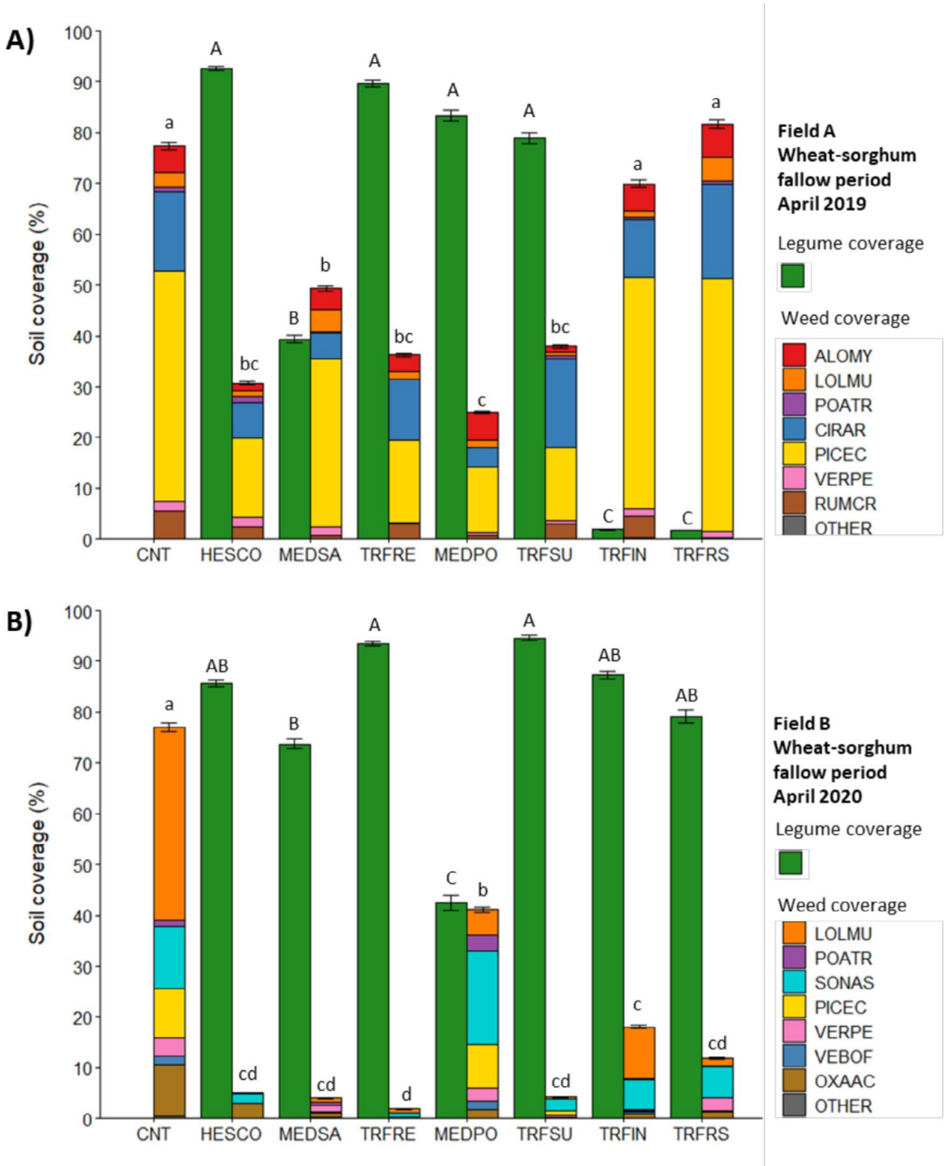


**FIGURE 3** | Total soil coverage (%) of weeds, calculated as the sum of the cover values of all individual weed species, and LLMs in Field A (A) and Field B (B) during the post-IC stage. Bars on weed coverage show detail on the seven most abundant species for (ALOMY, VERPE, CIRAR, POATR, LOLMU, RUMCR and PICEC for the post-IC stage in Field A; LOLMU, VEBOF, SONAS, POATR, OXAAC, VERPE and PICEC for the post-IC stage in Field B). OTHER represents the sum of the rest of the less abundant weed species, respectively, for each experimental repetition. CNT: Control plot (no living mulches); HESCO: *Hedysarum coronarium*; MEDSA: *Medicago sativa*; TRFRE: *Trifolium repens*; MEDPO: *Medicago polymorpha*; TRFSU: *Trifolium subterraneum*; TRFIN: *Trifolium incarnatum*; TRFRS: *Trifolium resupinatum*. Error bars represent ±standard error around the mean total weed or legume coverage. Capital letters refer to legume coverage, and lowercase letters refer to weed coverage. Bars sharing the same letter show no significant differences in terms of total weed or legume coverage at $p < 0.05$.

showed that many of these annual weeds are not very competitive, but they can support invertebrates or provide food for farmland birds. In both Field A and Field B, perennial LLMs such as HESCO, MEDSA and TRFRE, along with self-seeding legumes like TRFSU, successfully persisted in the fallow period, thus establishing a consistent ground cover, which not only suppressed overall weed growth but also shifted the weed community towards a dominance of multi-year weed species (hemicryptophyte + geophyte). Notably, NMDS analysis showed that specific perennial weed species such as *P. lanceolata* (PLALA), *C. dactylon* (CYNDA), *B. vulgaris* (BEAVX) and *S. latifolia* (MELAL) were more prevalent in the presence of these LLMs compared to the control, which was instead characterised by annual and ubiquitarian weed species. As reported by Westbrook et al. (2022) and Gerhards (2018), living mulch systems, like the one described in this study, often tend to select perennial weeds, such as *C. arvense* (CIRAR) and *Sonchus arvensis* (L.) (SONAS). The effectiveness of LLMs and cover crops in suppressing perennial weeds is generally lower compared to their impact on annual weeds. This difference is primarily ascribed to the different survival strategies of perennials, which are equipped with underground storage organs such as rhizomes, tubers, or taproots. These structures store energy reserves, allowing perennial weeds to persist and regenerate even when competition for



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light or nutrients is increased by the presence of LLMs or other cover crops. Unlike annual weeds, which rely heavily on the resource availability from the surrounding environment and germinate from seeds each year, perennials can access these stored resources to sustain growth despite competitive pressures.

In the absence of tillage or soil disturbance, which would, in some cases, help manage perennial weed species, these weeds can remain established in the soil, relatively unaffected by the introduction of LLMs or cover crops. To prevent the progressive accumulation of perennial weeds, living mulch systems should not be implemented as an exclusive management solution but rather integrated into a diverse crop rotation and therefore diversity of disturbance. Crop management within a wide crop rotation can rely on conservative practices like LLMs, with more soil disturbance-intensive interventions, such as tillage, which can be combined with the incorporation of the living mulch biomass before sowing the subsequent cash crop. Another option, as reported by Cougnon et al. (2022), for the control of these weed species in LLMs systems is recommended to cut/mow or to graze the living mulch for improving control or slowing down the development of perennial weeds like *C. arvense* (Favrelière et al. 2020).

For annual weeds such as *C. album*, the competition from cover crops or LLMs has been reported to be much more intense (Ateh and Doll 1996). Cover crops and LLMs form a dense ground cover, reducing light availability, which hinders the germination and early development of annual weeds (Menalled et al. 2022). Additionally, the allelopathic effects or nutrient competition from cover crops and LLMs can further limit the success of annual weeds. However, the results of this study showed that some annual species, such as *P. rhoeas* (PAPRH), also increased in the weed community under perennial and self-seeding LLMs. Although fewer in number, individuals under LLMs were larger and more vigorous compared to those in control plots, indicating that increased soil nutrients favoured their growth despite overall weed suppression. These findings align with Fracchiolla et al. (2018) and Stankiewicz-Kosyl et al. (2020), who reported that *P. rhoeas* benefits from conservation practices and higher soil fertility, conditions created using LLMs. However, other studies, such as Cirujeda et al. (2003), demonstrate that *P. rhoeas* is well-adapted to soil tillage, too, with a mean seed survival rate exceeding 60% 6 years after burial that facilitates adaptation to ploughing (Cirujeda et al. 2006); IWM strategies have been found to be effective both under direct drilling (similar to 95%) and under intensive tillage (similar to 86%) (Torra et al. 2018).

### 4.2 | Weed Diversity

During the wheat–sorghum fallow period (post-IC stage), the effect of LLMs on species richness ($S$), Shannon diversity ($H'$) and Pielou evenness ($J$) appeared highly variable, depending largely on the persistence and coverage of the legume species in the field, as well as on the specific composition of the soil seedbank in each experimental site.

Since no weed control was applied in the control plots during the wheat–sorghum fallow period (neither tillage nor chemical control), we hypothesize that the control plots represent the maximum expression of the weed species available in each experimental field. In Field A, species richness did not significantly differ between treatments and the control, except for plots with TRFSU, MEDPO and TRFRE, which exhibited a significant reduction in the number of species compared to the control. These species were also among the most successful in persisting in the post-IC stage with coverage > 80%. Interestingly, despite the reduction in species richness, the weed community evenness was not significantly affected. We initially expected a similar trend for HESCO, hypothesising that LLM biomass would be the primary factor affecting the number of species. However, in the HESCO treatment, species richness did not differ significantly from the control. This highlights that, besides biomass, other functional traits of the living mulch species may also play an important role in shaping weed community composition, like the growth characteristics of legumes used as living mulches. This is in line with Smith et al. (2015), who highlighted how the species identity in cover crops could be a peculiar biotic filter of weed community and diversity.

LLMs of TRFSU and TRFRE exhibit a prostrate, dense growth habit, forming compact and uniform soil cover, while LLM of HESCO has an erect growth habit, resulting in more heterogeneous ground coverage and potentially allowing more opportunities for weed germination, particularly for species with small seeds and wind-dispersal strategies, such as *H. echioides* (PICEC) and *A. arvensis* (ANGAR), thereby supporting higher weed species richness in the community. These species tend to thrive under reduced cultivation conditions, as also reported by Pardo et al. (2019) and Derrouch et al. (2022). Regarding the annual LLMs (TRFIN and TRFRS), their poor persistence after the previous IC stage was evident, as they achieved very low coverage during the post-IC stage and consequently had no significant effect on weed species richness or diversity, resulting in communities similar to the control. This confirms that in the case of intercropping with annual species, no residual effect on weed composition is expected during the subsequent post-IC stage.

In Field B, the effect of LLMs on weed species richness appeared to be more closely linked to the persistence and soil coverage of the legume species, regardless of the legume species used. Field B exhibited a general decrease in weed species richness across all treatments, except for MEDPO, where species richness remained comparable to the control. Notably, MEDPO was the legume species with the lowest persistence during the post-IC stage, as reflected by its significantly lower coverage and higher proportion of weed coverage. The strong filtering effect of LLMs was evident both in terms of species richness and community structure. Previous studies have shown that high-covering legume-based living mulches can effectively suppress weeds (Den Hollander et al. 2007; Ross et al. 2001). In our study, treatments such as HESCO, TRFSU and TRFRE achieved high soil coverage (> 80%), which corresponded to a significant reduction in weed cover and diversity. This dominance of the LLMs likely limited the germination and establishment of many weed species, resulting in low Shannon diversity values, indicative of communities dominated by a few species.



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### 4.3 | LLMs and Weed Coverage

In accordance with what has been reported by Westbrook et al. (2022), Cougnon et al. (2022) and Osipitan et al. (2019), the results of this study demonstrate that LLMs can be an effective solution for controlling weed growth and shaping weed community composition and diversity during the fallow period in a wheat–sorghum crop sequence, but the effect is dependent on the specific persistence of legumes chosen for LLMs.

The hypothesis that perennial and annual self-seeding LLMs had a higher weed suppression capacity than annual LLMs was confirmed in both experimental replicates (Field A and Field B), likely due to their successful establishment after wheat harvest. Perennial LLMs, including HESCO, MEDSA and TRFRE, as well as self-seeding species such as TRFSU, showed good establishment after the wheat harvest and persisted well in the field (> 80% soil cover). This reduced weed growth with potential beneficial effects in reducing weed dissemination and weed seedbank enrichment (Seefeldt et al. 2023). Notably, the continuous soil coverage provided by LLMs during fallow periods can offer several agronomic advantages beyond weed suppression, such as nitrogen fixation, nitrogen uptake to prevent leaching, soil conservation and improved production in subsequent cash crops, reducing reliance on chemical fertilisers and herbicides (Leoni et al. 2024; Bhaskar et al. 2021; Adetunji et al. 2020; Kim et al. 2020; Hartwig and Ammon 2002).

In contrast, annual legumes, which were expected to serve as dead mulch during the fallow period, were less effective in Field A. This lack of sufficient coverage observed in TRFIN and TRFRS allowed weeds to grow at levels similar to those observed in the control plots and showed similar weed community composition. On the contrary, in Field B, annual legumes manage to regrow from the seedbank, resulting in a weed suppression level comparable to that of perennial and self-seeding species. This outcome underscores the variable performance of annual species during the fallow period, which is highly influenced by seasonal and soil conditions, as already reported by previous studies (Feng et al. 2024; Fracchiolla et al. 2022). Such variability is also related to the lack of direct self-reseeding mechanisms of these legumes, unlike in species such as *T. subterraneum* and *M. polymorpha*, which can readily re-establish in the post-IC stage and therefore have greater efficiency and higher chances of successful regrowth year after year. The lower weed control capacity of annual LLMs observed in Field A may be attributed to suboptimal environmental conditions, which hindered the establishment of annual LLMs. However, the unpredictable spontaneous regrowth of annual LLMs suggests that self-seeding and perennial species, which showed reliable results across both experimental sites, are more suitable because they reduce the variability in the fallow ground cover. To improve the success of living mulch systems, the use of species mixtures is often considered an optimal solution. For instance, within legume mixtures, combining prostrate species like *T. repens* with semi-erect or erect species such as *H. coronarium* can maximise ground coverage and enhance the efficient use of soil resources. Furthermore, including both self-reseeding annual species like *T. subterraneum* and perennial species like *L. corniculatus* can ensure continuous competitiveness against weeds throughout the year. However, it is important to emphasise that when designing living mulch mixtures, species must be selected carefully based on complementary functional traits, particularly their growth habits (Blesh 2018). Using mixtures rather than single species greatly enhances the chances of success and resilience of the living mulch system. Species mixtures can also be created by combining grasses and legumes, rather than relying solely on legume-based mixtures, since the diversification of the botanical families in the mixture can further improve the delivery of ecosystem services, including improved weed suppression and better nitrogen management, as highlighted by Reiss and Drinkwater (2022). Nevertheless, in our study, where the living mulch is established within wheat, the direct inclusion of grasses in the initial mixture is risky. Grasses could compete excessively with wheat during the intercropping phase and might also create future weed problems with seed dispersal. Therefore, a more appropriate strategy would be to rely primarily on legume-based mixtures.

## 5 | Conclusion

This study highlights the importance of using LLMs (comprising either perennial species or annual self-reseeding) as a sustainable tool for weed management, particularly during critical periods such as the fallow phase between the harvest of autumn–winter crops and the sowing of summer crops in Mediterranean cropping systems. No clear relationship was found between the growth cycle type of leguminous species and their suppressive effect on weeds. In general, higher ground cover by living mulches was associated with stronger weed suppression. Instead, effects on weed diversity appeared to be more closely related to growth habits than to the growth cycle per se. Species with dense, prostrate growth habits, such as TRFRE and TRFSU, were associated with a greater reduction in weed species richness. In contrast, species that are more erect and cespitose, such as HESCO and MEDSA, showed similar total weed suppression levels, but in cases where their total soil cover is moderate, they preserved a higher weed species richness. However, species evenness remained largely unaffected in both cases. Overall, the presence of LLMs with high coverage (> 80%) significantly reduced total weed cover, thereby potentially limiting the risk of weed seedbank replenishment. Weed community composition was also influenced. Annual weed species were more effectively suppressed than perennial ones (i.e., hemicryptophytes and geophytes), suggesting that dense living mulches may favour a gradual shift towards weed communities with a higher share of perennial species. This trend has potential long-term implications for weed management, particularly in organic systems where chemical control is not allowed. Annual species such as *P. rhoeas* also seem to benefit from the presence of LLMs; however, their dynamics over time should be assessed through studies on the systematic and repeated use of LLMs within crop rotations. The integration of LLMs within diversified crop rotations, alternating with soil disturbance practices, can be recommended to maintain a balanced composition between annual and perennial weed species.

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## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Supporting Information.



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