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Permanent living mulches based on species mixtures can bridge the gap between organic and conservation agriculture practices in Mediterranean vegetable systems

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ABSTRACT

This study investigated permanent living mulches as a strategy for integrating conservation agriculture practices into organic vegetable systems. Permanent living mulches were established using mixtures of perennial legumes (*Trifolium repens* L., *Lotus corniculatus* L.) and self-reseeding species (*Trifolium subterraneum* L., *Medicago polymorpha* L.) to exploit complementary growth cycles, improve weed suppression, and increase mulch persistence. The system was tested in a three-year rotation of broccoli, aubergine, fennel, and tomato under organic management in central Italy. Vegetables were transplanted into the living mulches using strip tillage without fertilizers, while two controls followed standard organic management with soil tillage, fertilization, and inter-row or inter- and intra-row weeding. The study assessed living mulch development, weed suppression, changes in weed community composition, and crop yield. Permanent living mulches suppressed weeds effectively during the first two years, and self-reseeding species increased total mulch biomass and improved weed suppression. However, mulch biomass declined over time while weed biomass increased, reducing agronomic benefits. Broccoli and aubergine yields were comparable to the fertilized and weeded controls, whereas fennel and tomato yield was substantially lower under living mulches. Weed communities also shifted, with *Polygonum aviculare* L. and *Kickxia elatine* (L.) Dumort. declining, *Picris hieracioides* L. and *Lolium perenne* L. increasing, and *Cyperus rotundus* L. remaining stable. Distinct communities developed between strip-tilled intra-rows, dominated by annuals, and mowed inter-rows, dominated by perennials. Permanent living mulches can therefore support the integration of conservation agriculture practices into organic vegetable systems, although periodic tillage may be needed to manage perennial weeds and maintain long-term system performance.

1. Introduction

Conservation agriculture and organic agriculture are alternative crop management strategies associated with reduced environmental impacts and may enhance functional biodiversity, ecological functioning, and ecosystem service delivery (Wittwer et al., 2021). However, current organic agriculture practices remain debated because organic farmers still largely rely on intensive tillage to incorporate crop residues and organic fertilizers and control weeds (Peigné et al., 2007). Intensive tillage leaves soil vulnerable to wind and water erosion, degrades soil structure, and accelerates organic carbon and nutrient losses through oxidation and leaching (Morris et al., 2004). Conservation agriculture practices such as reduced and minimum tillage have shown potential to mitigate these problems by sustaining soil fertility while reducing greenhouse gas emissions, energy use, and soil erosion (Holland, 2004; Mazzoncini et al., 2011; Soane et al., 2012; Palm et al., 2014). In contrast, conservation agriculture often relies on herbicides for weed control and cover crop termination, increasing selection pressure and the risk of herbicide-resistant weed populations (Melander et al., 2013).

Integrating conservation agriculture principles into organic agriculture may help to overcome limitations of both systems by improving soil quality and resource-use efficiency while reducing reliance on intensive tillage and herbicides (Peigné et al. 2007). This integration is also consistent with the principles of regenerative agriculture (Rhodes,

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2017). However, the adoption of conservation agriculture practices in organic systems remains limited due to the system's complexity and challenges related to weed control and nutrient availability (Peigné et al., 2016; MacLaren et al., 2020). Therefore, integrating conservation agriculture principles into organic systems requires a redesign of the cropping system, with greater reliance on preventive agroecological strategies for weed management and improved internal nutrient cycling, supported by continuous soil cover and greater use of legumes (Mazzoncini et al., 2011; Antichi et al., 2022; Leoni et al., 2024). Cover crops combine these functions and may therefore represent a key practice for such system redesign (Qiu et al., 2024).

In vegetable conservation-organic systems, however, annual cover crops are difficult to integrate because their establishment and termination often require soil disturbance, especially when biomass incorporation is used to maximize nutrient release for the following cash crop (Madsen et al., 2016). Moreover, vegetable systems typically involve frequent crop sequences in rotations, often leaving only short windows for cover crop establishment (Stein et al., 2022). Permanent living mulches (pLMs), which remain established and coexist with vegetables across successive growing cycles, may therefore offer a viable alternative (Hartwig and Ammon, 2002; Cougnon et al., 2022). Legume-based pLMs, in which a legume service crop is maintained throughout the crop rotation, could thus help translate conservation agriculture principles into organic vegetable systems (Wezel et al., 2014). pLMs maintain continuous soil cover over successive crop cycles without the need for repeated sowing, thereby reducing erosion and nitrate leaching, supporting biodiversity, and improving weed control in vegetable systems (Broughton, 2010; Sportelli et al., 2022; Bàrberi et al., 2025).

Although pLMs can provide multiple ecosystem services, their successful implementation in conservation-organic vegetable systems remains challenging because these benefits must be achieved without creating excessive competition with the cash crop. Prostrate, preferably stoloniferous, species such as *Trifolium repens* L. are particularly suitable because they combine rapid soil cover with a low canopy architecture (Den Hollander et al., 2007a, b; Stein et al., 2022). Nevertheless, periodic mowing during intercropping is generally required to limit competitive effects on vegetable crops (Den Hollander et al., 2007b). Species mixtures could further improve pLM stability and multifunctionality by combining complementary traits (Ranaldo et al., 2020). For example, mixtures of perennial and annual self-reseeding legumes could provide not only morphological complementarity but also temporal complementarity across seasons, although this approach has not yet been investigated for pLMs.

Establishment timing and spatial management of pLMs are equally important for their successful use. Studies on living mulches have compared early, simultaneous, and late sowing relative to vegetable transplanting to reduce crop competition (Canali et al., 2015; Montemurro et al., 2017). Earlier establishment can strengthen weed suppression and potentially increase nitrogen availability to the crop, whereas simultaneous or delayed establishment may reduce early competition with the cash crop (Bhaskar et al., 2021; Sportelli et al., 2022). Inter-row management is particularly critical during the first weeks after transplanting, when vegetable crops are most susceptible to competition and mowing may be required to regulate pLM growth.

However, these design and management components have largely been investigated separately, and system-level evidence on how pLM composition and persistence interact with weed dynamics and crop performance across an entire vegetable rotation remains scarce. Moreover, replacing repeated tillage with continuous soil cover, mowing and localized strip tillage is likely to modify weed community assembly by imposing contrasting disturbance filters in inter-row and intra-row areas.

To address these research gaps, this study presents the results of a system experiment in which mixtures of perennial and annual self-reseeding legumes were maintained as pLMs in a 3-year vegetable crop rotation (broccoli–aubergine–fennel–tomato). The study simultaneously assessed pLM persistence and composition, weed suppression and community dynamics, and vegetable crop productivity under reduced soil disturbance (strip tillage) and without external fertilization. This study aims to test the following three hypotheses:

Species mixtures combining annual self-reseeding legumes and a perennial legume are more suitable for achieving stable pLMs over time than pure stand, thanks to their functional trait complementarity. This complementarity includes not only morphological differences among species, but also temporal complementarity between perennial and annual self-reseeding components, ensuring continuous soil cover and biomass production throughout the crop rotation.

Weed community composition in systems with a pLM is different compared with tillage-based management systems. High-intensity soil disturbance associated with tillage is expected to favour fast-colonizing ruderal weeds, whereas continuous pLM presence and regular mowing may select for perennial weed species. Furthermore, weed community composition will differ between inter-row and intra-row areas due to contrasting disturbance types and intensities: inter-row zones are mainly affected by mowing and persistent ground cover, whereas intra-row zones experience localized soil disturbance due to strip tillage before crop establishment.

pLM-based systems can sustain vegetable crop yields across a complete rotation of four typical open-field vegetable crops without external fertilization and repeated tillage. This is expected to occur through functional synergies associated with continuous soil cover, weed suppression and shifts in weed community composition over time.

2. Material and methods

2.1. Experimental design and site description

The experiment was conducted from 2022 to 2024 in a certified organic field at the Centre for Agri-Environmental Research "Enrico Avanzi" (CiRAA), University of Pisa, San Piero a Grado, Italy (43°40′ N, 10°20′ E, 0 m a.s.l.).

The soil was classified as silty loam according to the USDA soil texture triangle (FAO, 2006). Analyses of soil samples (0–0.30 m) from each of the three blocks, composed of a bulk of three sub-samples, showed that the soil consisted of 36% sand, 45.6% silt, and 18.4% clay, with 1.63% soil organic matter (SOM) and a pH of 8.0. Soil chemical fertility parameters included 1.0 g kg^{-1} total nitrogen (N), 33.5 mg kg^{-1} available phosphorus (P_2O_5), and 116.0 mg kg^{-1} exchangeable potassium (K). Organic matter (SOM), total nitrogen (Total N), available phosphorus (P_2O_5), and exchangeable potassium (K) were determined following the Walkley–Black method (FAO, 2020), Kjeldahl method (Bremner, 1960), and Olsen method (Kovar and Pierzynski, 2009), respectively. The preceding crop was durum wheat, managed organically with ploughing, rotary harrowing, and organic fertilization.

2.2. Permanent living mulch and vegetable management

In the field experimental field after ploughing to a depth of 25 cm, seedbed preparation was completed using a rotary harrow operating to a depth of 15 cm. The pLMs were established before transplanting the first vegetable crop of the rotation (broccoli), with legumes sown as pure stands or mixtures in February 2022. Mixtures combined one perennial (*T. repens* cv. Pipolina or *Lotus corniculatus* L. cv. Giada) and one annual self-reseeding legume *(Medicago polymorpha* L. or *Trifolium subterraneum* L. cv. Mintaro) (Table SM1, supplementary material). Species were selected based on their morphological and phenological suitability as pLMs identified in a previous experiment (Leoni et al., 2020). Three ecotypes of *M. polymorpha* from central Italy (eco. Talamone, eco. Pitigliano, and eco. Villa Salto) were selected for high biomass production and a low proportion of hard seeds. *M. polymorpha* cv. Scimitar was included as a commercial reference. The perennial component was sown

at 30 kg ha^{-1} in both mixtures and pure stands; the annual self-reseeding component was sown at 15 kg ha^{-1} in mixtures, half the pure-stand rate (Table SM1, supplementary material). The sowing was carried out with a mechanical row seeder (15 cm inter-row distance) in 18 m^2 plots (3 × 6 m). For mixtures, seeds were combined in the hopper and sown within the same rows.

A typical vegetable rotation was established in the pLMs: broccoli *(Brassica oleracea* L. var. italica Plenck cv. Belstar), aubergine *(Solanum melongena* L. cv. Dalia), fennel *(Foeniculum vulgare* Mill. cv. Leonardo), and tomato *(Solanum lycopersicum* L. cv. Durpeel). The sequence alternated two summer-harvest crops (aubergine and tomato) with two winter-harvest crops (broccoli and fennel). Species and cultivars used for this study were chosen in accordance with the principles of organic farming, maximizing genetic diversity between the species in sequence and prioritizing resistant cultivars to major abiotic stresses.

Before the vegetable transplanting, the pLMs were first mowed and strip tillage was then performed over 20-cm bands to a maximum depth of 15 cm using a strip-tillage unit (model Leonardo, CMA). Certified organic seedlings were subsequently transplanted using a prototype transplanter suitable for tilled and no-till soils (Frasconi et al., 2019; Antichi et al., 2019). Row spacing was 70 cm, with 40 cm between plants (22 cm for fennel). The experiment included three control treatments representing increasing management intensity (Fig. 1). Control 1, the negative control, received strip tillage without fertilization, while spontaneous vegetation was maintained between rows. Control 2 represented standard local organic management, with rotary spading, basal and top-dressing organic fertilization, and mechanical inter-row weed control. Control 3 received the same management as control 2 plus manual intra-row weeding. Drip irrigation was installed after transplanting; irrigation amounts are reported in Table SM2 in supplementary material. Fennel required irrigation only during establishment, with subsequent water requirements supplied by rainfall.

The pLMs were maintained throughout the crop rotation from February 2022 to September 2024. Before the first crop and during intercrop fallow periods, pLMs were left unmanaged. Before transplanting each vegetable (July 2022 for broccoli, April 2023 for aubergine, September 2023 for fennel, and May 2024 for tomato), pLMs were mowed to 3 cm and residues were left on the soil. In pLM plots, seedlings were transplanted into strip-tilled bands. During the vegetable crop cycle, the inter-row living mulch was managed with multiple mowings using a lawn mower that simulates the intervention of an inter-row mower (IRM-X4 Inter-Row Mower) already tested for organic and conservation arable systems (Rowland et al., 2023).

2.3. Weather data

Daily meteorological data were collected throughout 2022–2024 from the weather station located at CiRAA (Fig. 2). The data collected included daily temperature (maximum, minimum, and mean) and precipitation (mm). Three major weather events affected crop development during the experiment.

From August 28th to August 30th, 2023 (3 days), cumulative precipitation reached 151.9 mm (Fig. 2), compared to a historical monthly average of about 50 mm. This intense rainfall affected the aubergine crop, causing severe flower drop. However, this event did not significantly compromise overall production because the plants were at the end of their growth cycle.

From October 18th to November 5th, 2023 (19 days), cumulative precipitation totaled 459.1 mm (Fig. 2). This period of intense precipitation is particularly significant because, over 19 consecutive days of rainfall, cumulative precipitation accounted for 51.7% of the average annual rainfall in the Pisa area (890 mm). Heavy rainfall was accompanied by strong winds (~100 km/h, from southwest to northeast), transporting saline aerosols from the near coastal area. This event severely compromised fennel cultivation in the experiment due to the combined effects of field flooding and leaf burn caused by salty winds.

June 2nd, 2024 (1 day), precipitation of 39.2 mm was recorded (Fig. 2). Although intence, considering the historical June average of about 45 mm, this rainfall caused serious problems related to the propagation of fungal diseases in tomato crops, such as *Phytophthora infestans*. The same problem occurred on August 18th, 2024 (1 day), with intense precipitation of 59.1 mm (Fig. 2), leading to similar issues. However, at this stage of the crop cycle, treatment with copper oxychloride was no longer possible, resulting in direct consequences for the harvest in the following two weeks.



Fig. 1. Control treatments and an example pLM plot. Control 1 (A): strip-tilled crop rows with spontaneous inter-row vegetation; Control 2 (B): mechanically weeded inter-rows with unweeded intra-rows; Control 3 (C): mechanical inter-row and manual intra-row weed control; (D) broccoli grown with a T. repens pLM.

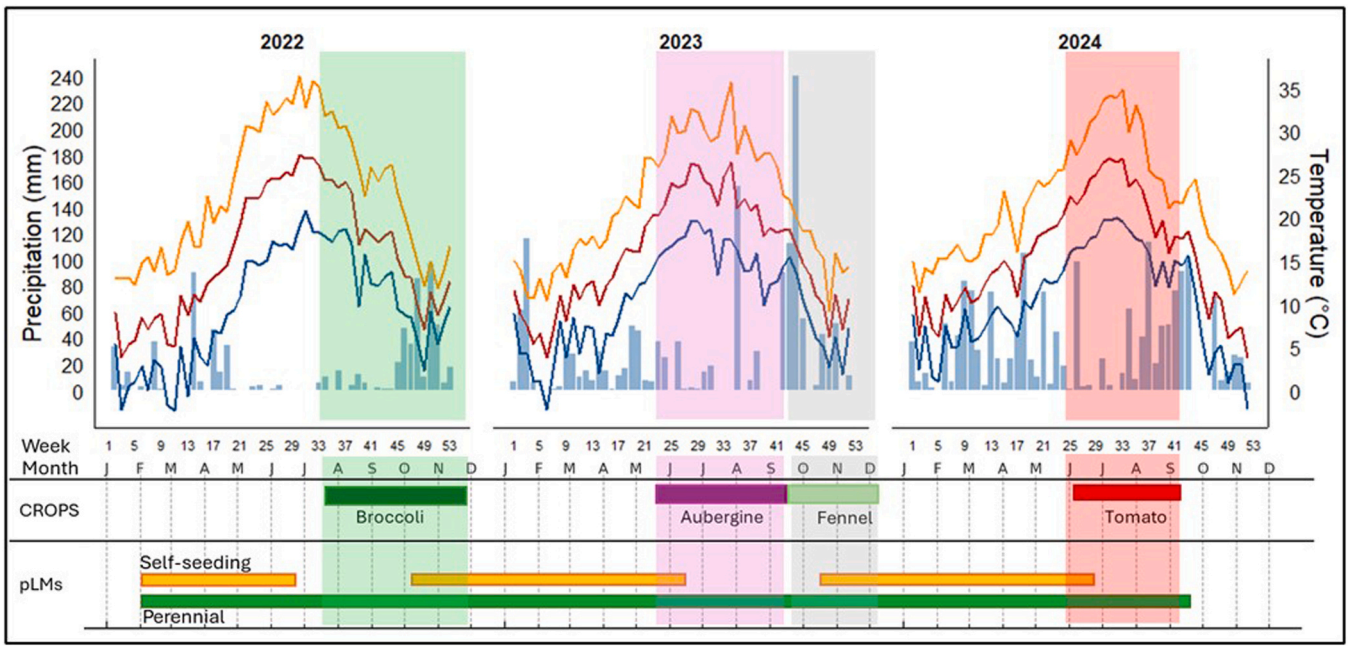


Fig. 2. Weather data (temperature and precipitation) collected throughout the duration of the experiment (2022, 2023, and 2024). Temperature data (right y-axis) are shown as maximum (orange line), minimum (blue line), and mean temperature (red line). Weekly cumulative precipitation (left y-axis) is shown with light blue bars. The crop sequence and cultivation periods are highlighted: broccoli (green background), aubergine (violet background), fennel (grey background), and tomato (red background). In evidence also permanent living mulches (pLMs) consisted of a mixture of perennial species (present throughout the entire experiment) and annual self-seeding species (present from October to June in support of the perennial species). Weather data were collected by an in situ automatic weather station, with sensors positioned 2 m above ground level.

2.4. Data collection

2.4.1. Permanent living mulches and weeds

Biomass of all pLMs components and weeds was monitored throughout the crop rotation, with 13 total samplings: 4 across 2022 (1st June, 10th August, 13th September, 12th October), 6 across 2023 (28th April, 25th May, 10th July, 1st August, 18th September, 12th October), and 3 across 2024 (28th April, 2nd July, 22nd July). Key assessments were conducted before crop transplanting and at mid-crop cycle to characterize pLM composition, weed control, and weed community composition.

The pLM biomass and species-specific weed biomass were assessed both before crop establishment and at mid-crop cycle. Pre-transplant assessments were conducted before broccoli, aubergine, and tomato establishment, whereas mid-cycle assessments were carried out in October 2022 for broccoli, July 2023 for aubergine, October 2023 for fennel, and July 2024 for tomato. Aboveground biomass was cut at soil level and separated into perennial legumes, annual self-reseeding legumes, and individual weed species. At mid-crop cycle, sampling was performed separately in inter-row and intra-row areas, the latter being affected by strip tillage and receiving drip irrigation. Inter-row biomass was sampled in 0.25 m^2 quadrats (50 × 50 cm) positioned centrally between crop rows and separated into perennial legumes, annual self-reseeding legumes, and weed species. Intra-row biomass was sampled using 0.1 m^2 quadrats (20 × 50 cm) following the same procedure. In addition to these key assessments, pLM biomass was sampled during vegetable growth immediately before repeated inter-row mowing. A 0.075 m^2 quadrat (25 × 30 cm) was used, and perennial legumes, annual self-reseeding legumes, and total weeds were separated. All biomass samples were oven-dried at 60 °C to constant weight and expressed as dry biomass per m^2.

2.4.2. Vegetable biomass and yield

Only the two central crop rows were sampled. Two sub-replicates per plot were used, each comprising four plants (two per central row), except for fennel, for which eight plants were sampled because of the narrower within-row spacing. Broccoli was harvested progressively from 17 October (80 days after transplant, DAT) to 30 November 2022 (123 DAT), with harvest readiness assessed every five days based on marketable maturity. Broccoli florets were harvested when fully developed and compact, including the axial stem cut below the third leaf. Fresh weights of broccoli florets and remaining plant biomass were recorded, followed by dry-weight determination as described above.

Aubergines were harvested twice weekly from 4 July (60 DAT) to 18 September 2023 (134 DAT) as fruits reached marketable maturity. Fruits were classified as marketable when uniformly coloured and at least 70 mm in maximum diameter. Fresh weights of marketable and non-marketable fruits were recorded at each harvest, and cumulative yield was calculated. Final aboveground plant biomass was also measured. Fruits and plant biomass were oven-dried at 60 °C to constant weight for dry-weight determination.

Fennel was transplanted on 20 September 2023 after aubergine termination. Because fennel did not reach marketable maturity, biomass was collected once, on 6 December 2023.

Tomato was transplanted on 29 May 2024 and harvested on 2 September 2024 (96 DAT). Plants within the sampling area were collected and fruits were separated into marketable, green, and discarded categories. Fruit number and fresh weight were recorded, and total yield was calculated as the sum of all categories. Fruits and plant residues were oven-dried at 60 °C to constant weight.

2.5. Statistical analysis

Biomass of pLMs over time (Fig. 3) was analysed using generalized linear mixed models (GLMMs) fitted with the glmmTMB package in R (Brooks et al., 2017). Models included pLM type, sampling date (13 sampling times), and their interaction as fixed effects, with block/plot included as a random effect. Models assumed a Tweedie distribution with an identity link function (SM4, supplementary material).

Total weed biomass measured in spring 2022, 2023, and 2024

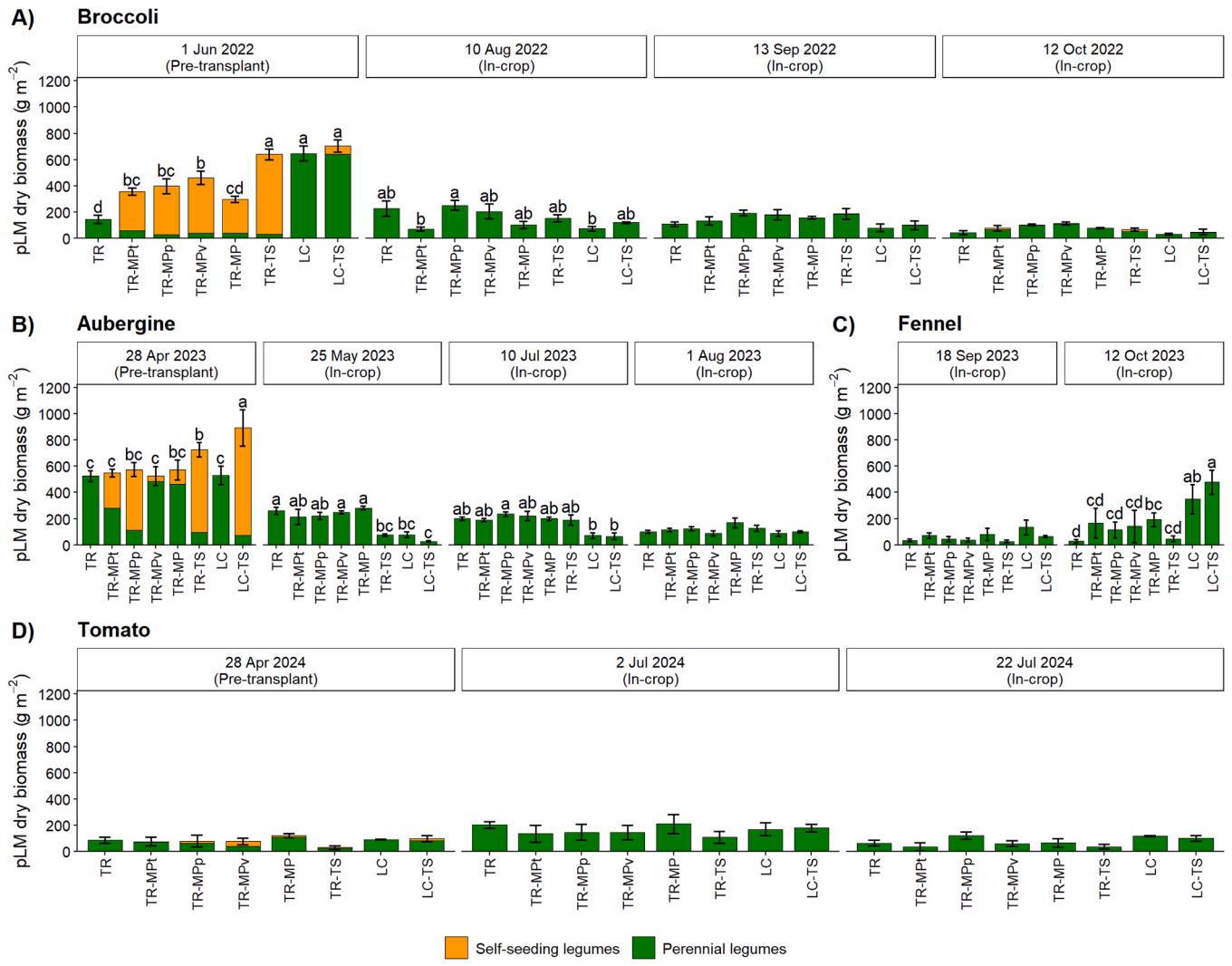


Fig. 3. Dynamics of total aboveground dry biomass (g m^{-2}) of pure legumes and mixtures used as permanent living mulches (pLMs), including their perennial and annual self-reseeding components, throughout the crop sequence. Biomass was assessed before transplanting broccoli (1 June 2022), aubergine (24 April 2023), and tomato (24 April 2024), and during each vegetable growing period before periodic mowing for pLM growth regulation. Treatment abbreviations indicate *T. repens* + *M. polymorpha* eco. Talamone (TR-MPt), *T. repens* + *M. polymorpha* eco. Pitigliano (TR-MPp), *T. repens* + *M. polymorpha* eco. Villa Salto (TR-MPv), *T. repens* + *M. polymorpha* cv. Scimitar (TR-MP), *T. repens* + *T. subterraneum* (TR-TS), *L. corniculatus* + *T. subterraneum* (LC-TS), *T. repens* pure stand (TR), and *L. corniculatus* pure stand (LC). Bars represent means ± SE. Different letters indicate significant differences in total biomass among treatments within each sampling date (Sidak-adjusted pairwise comparisons, $P < 0.05$).

(Fig. 4) was analysed using GLMMs including pLM type, sampling year, and their interaction as fixed effects, with block/plot as a random effect. Models assumed a Tweedie distribution with a log link (SM5, supplementary material).

To investigate temporal dynamics of the main weed species (Fig. 5), biomass was analysed separately for each species using linear mixed-effects models after ln(x + 1) transformation. Models included pLM type (treatment), sampling year, and their interaction as fixed effects. Significant effects were followed by Tukey-adjusted pairwise comparisons of estimated marginal means ($P < 0.05$). Changes in weed community composition among spring 2022, 2023, and 2024 (Fig. 5) were assessed using permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarities, followed by non-metric multidimensional scaling (NMDS). PERMANOVA tested differences among pLM types using 9999 permutations. NMDS ordination was performed using 1000 iterations and 20 random starts. Pairwise comparisons following significant global tests were adjusted using the Bonferroni method. Multivariate analyses were performed with the vegan package in R (Oksanen et al., 2001) (SM6, supplementary material).

Weed biomass in inter-row and intra-row positions (Fig. 6) was analysed separately for broccoli, fennel, aubergine, and tomato. For each crop, GLMMs included pLM type, sampling position, and their interaction as fixed effects, with block as a random effect. Models assumed a Tweedie distribution with a log link function (SM7–SM10, supplementary material).

Weed community composition in inter-row and intra-row positions (Fig. 7) was analysed separately for winter crops (broccoli and fennel) and summer crops (aubergine and tomato) using PERMANOVA and NMDS following the procedure described above. Pairwise comparisons after significant global tests were Bonferroni-adjusted (SM9 and SM12, supplementary material).

Marketable yield (Fig. 8), expressed as fresh and dry weight, was analysed using GLMMs including treatment, crop species, and their interaction as fixed effects. Sub-replicates were nested within blocks, which were included as a random effect. Models assumed a Gamma distribution with a log link function (SM13, supplementary material).

For GLMMs, significant fixed effects were followed by Sidak-adjusted

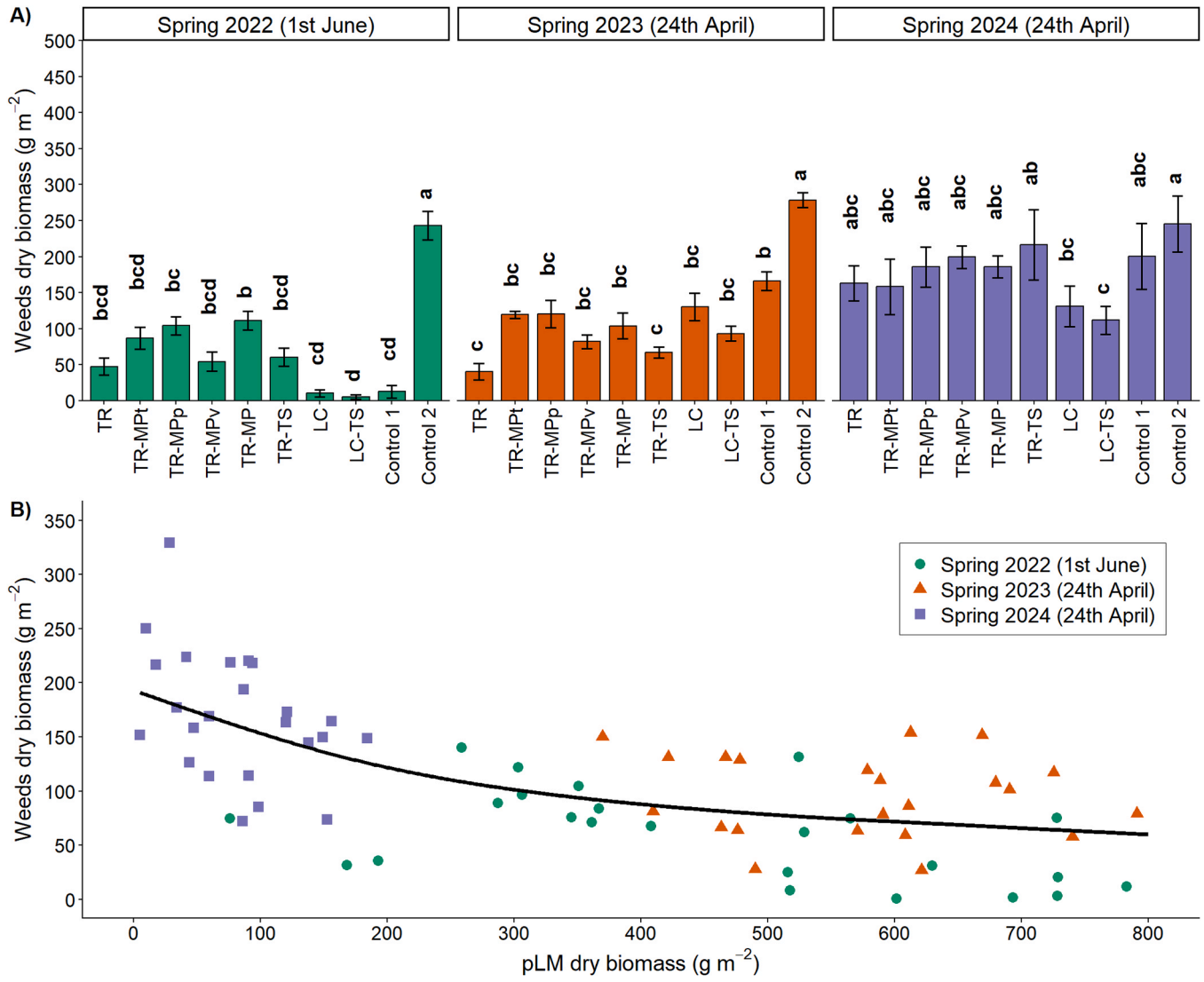


Fig. 4. (A) Dynamics of total weed aboveground dry biomass (g m^{-2}) across treatments in Spring 2022, Spring 2023, and Spring 2024, corresponding to assessments before transplanting broccoli, aubergine, and tomato, respectively. Treatment abbreviations indicate *T. repens* + *M. polymorpha* eco. Talamone (TR-MPt), *T. repens* + *M. polymorpha* eco. Pitigliano (TR-MPp), *T. repens* + *M. polymorpha* eco. Villa Salto (TR-MPv), *T. repens* + *M. polymorpha* cv. Scimitar (TR-MP), *T. repens* + *T. subterraneum* (TR-TS), *L. corniculatus* + *T. subterraneum* (LC-TS), *T. repens* pure stand (TR), and *L. corniculatus* pure stand (LC). Control 2 (CNT2) represents standard organic management based on soil tillage, organic fertilization, and mechanical inter-row weed control. Bars represent means ± SE. Different letters indicate significant differences among treatments within each sampling time (Sidak-adjusted pairwise comparisons, $P < 0.05$). (B) Relationship between weed biomass and pLM biomass in Spring 2022, Spring 2023, and Spring 2024, fitted using a generalized additive model (GAM) with a cubic regression spline. The solid line represents the fitted smooth ($R^2 = 0.42$, $P < 0.0001$).

pairwise comparisons ($P < 0.05$) using the emmeans package (Lenth and Piaskowski, 2017). For the species-level linear mixed-effects models, Tukey-adjusted comparisons were used ($P < 0.05$). Model adequacy was evaluated using residual diagnostics, including simulation-based diagnostics implemented in the DHARMa package. All statistical analyses were conducted in R (R Core Team.).

3. Results

3.1. Biomass dynamics of pLMs

The first biomass assessment after the establishment of the pLMs (June 1st, 2022) revealed differences in behaviour between pLMs composed of mixtures (perennial + annual self-reseeding species) and the respective pLMs composed of pure perennial legume species (Fig. 3 A). In mixtures based on *T. repens*, competitive interactions among the component species were observed, where the self-reseeding component reduced the biomass of *T. repens* on average by 60% compared to *T. repens* pure stand. However, the total biomass of the mixtures was significantly higher than that of the *T. repens* pure stand (Fig. 3 A). This effect was particularly pronounced in the *T. repens* / *T. subterraneum* mixture, which achieved biomass production five times higher than the *T. repens* pure stand. A different trend was observed in the *L. corniculatus* pure stand compared to its corresponding mixture with *T. subterraneum*. In contrast to the pattern observed in *T. repens*-based mixtures, the self-reseeding component experienced competition from the perennial component of the mixture, resulting in no significant advantage in total biomass accumulation for the mixture compared to the *L. corniculatus* pure stands (Fig. 3 A).

Approximately one year later, at the end of the first inter-crop fallow period and prior to aubergine transplanting, pLMs biomass assessment was repeated (Fig. 3 B). The biomass of *T. repens* in pure stand was higher, whereas within the respective mixtures, *T. repens* biomass varied depending on the competitive dynamics with the self-seeding

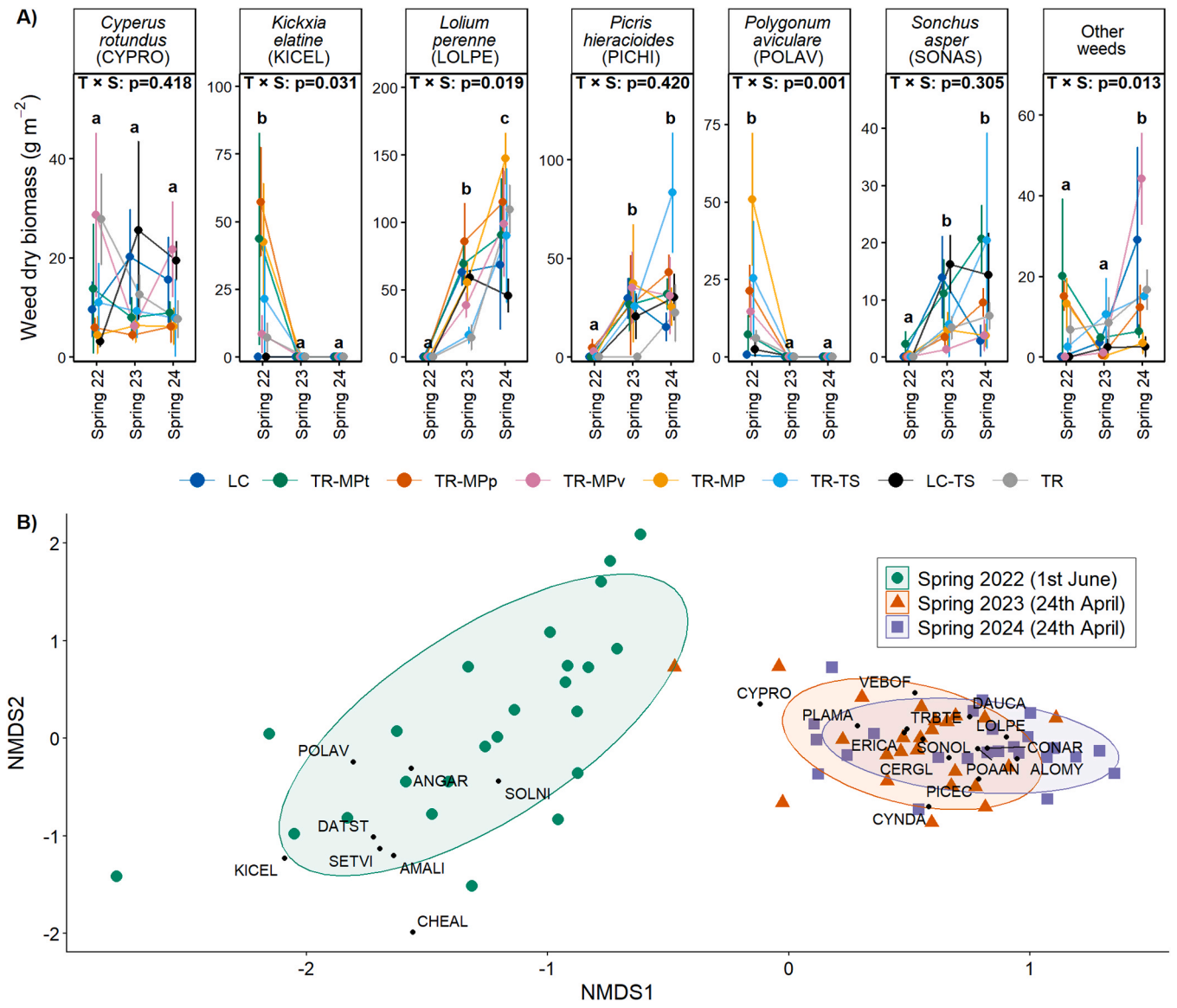


Fig. 5. (A) Dry biomass (g m^{-2}) of the main weed species in spring 2022, 2023, and 2024: *Cyperus rotundus* (CYPRO), *Kickxia elatine* (KICEL), *Lolium perenne* (LOLPE), *Picris hieracioides* (PICHI), *Polygonum aviculare* (POLAV), *Sonchus asper* (SONAS), and "Other weeds" (pooled less abundant species). Coloured points and lines represent treatment means ± SE across the three blocks. Different letters indicate significant differences among sampling years for each species based on Sidak-adjusted pairwise comparisons of estimated marginal means averaged across treatments ($P < 0.05$). T × S indicates the treatment × sampling interaction with the corresponding P-value. Treatment abbreviations indicate *L. corniculatus* pure stand (LC), *T. repens* + *M. polymorpha* eco. Talamone (TR-MPt), *T. repens* + *M. polymorpha* eco. Pitigliano (TR-MPp), *T. repens* + *M. polymorpha* eco. Villa Salto (TR-MPv), *T. repens* + *M. polymorpha* cv. Scimitar (TR-MP), *T. repens* + *T. subterraneum* (TR-TS), *L. corniculatus* + *T. subterraneum* (LC-TS), and *T. repens* pure stand (TR). (B) Two-dimensional NMDS ordination of weed community composition based on Bray–Curtis distances calculated from species biomass data, with samples grouped by sampling year. Ellipses indicate 90% confidence intervals for each sampling year. Weed species are identified by EPPO codes.

component (Fig. 3 B). In the *T. repens / M. polymorpha* eco. Pitigliano mixture, the previously observed trend was confirmed, with *T. repens* negatively affected by competition from *M. polymorpha*. Conversely, in the mixtures *T. repens / M. polymorpha* eco. Villa Salto and *T. repens / M. polymorpha* cv Scimitar, the perennial *T. repens* remained the dominant component. A more balanced situation was observed in the *T. repens / M. polymorpha* eco. Talamone mixture, where the biomass ratio between *T. repens* and *M. polymorpha* eco. Talamone was approximately 50% (Fig. 3 B). Interestingly, despite the differing biomass ratios between *T. repens* and *M. polymorpha* species across mixtures, the total biomass among the mixtures was not statistically different. The mixture *T. repens / T. subterraneum* consistently exhibited the highest total biomass compared to the mixtures based on *M. polymorpha*, although *T. subterraneum* remained highly competitive against *T. repens*. Regarding *L. corniculatus* and its corresponding mixture with *T. subterraneum*, an opposite trend to that observed in the June 2022 pre-transplanting assessment was found. In this case, *L. corniculatus* suffered from competition with *T. subterraneum*. Finally, when comparing *T. repens* and *L. corniculatus* in pure stands, no statistically significant differences were observed in total biomass at this second inter-crop fallow period sampling (Fig. 3 B). During the third sampling, carried out at the end of the inter-crop fallow period prior to tomato transplanting, a significant reduction in biomass was observed in both the mixtures and the pure stands of pLMs. Additionally, the self-seeding component of the mixtures declined markedly in the mixtures (Fig. 3 D).

Regarding the assessments conducted on the biomass of pLMs during the growth cycle of broccoli, aubergine, fennel, and tomato, biomass was affected by repeated mowings, which kept biomass below the levels

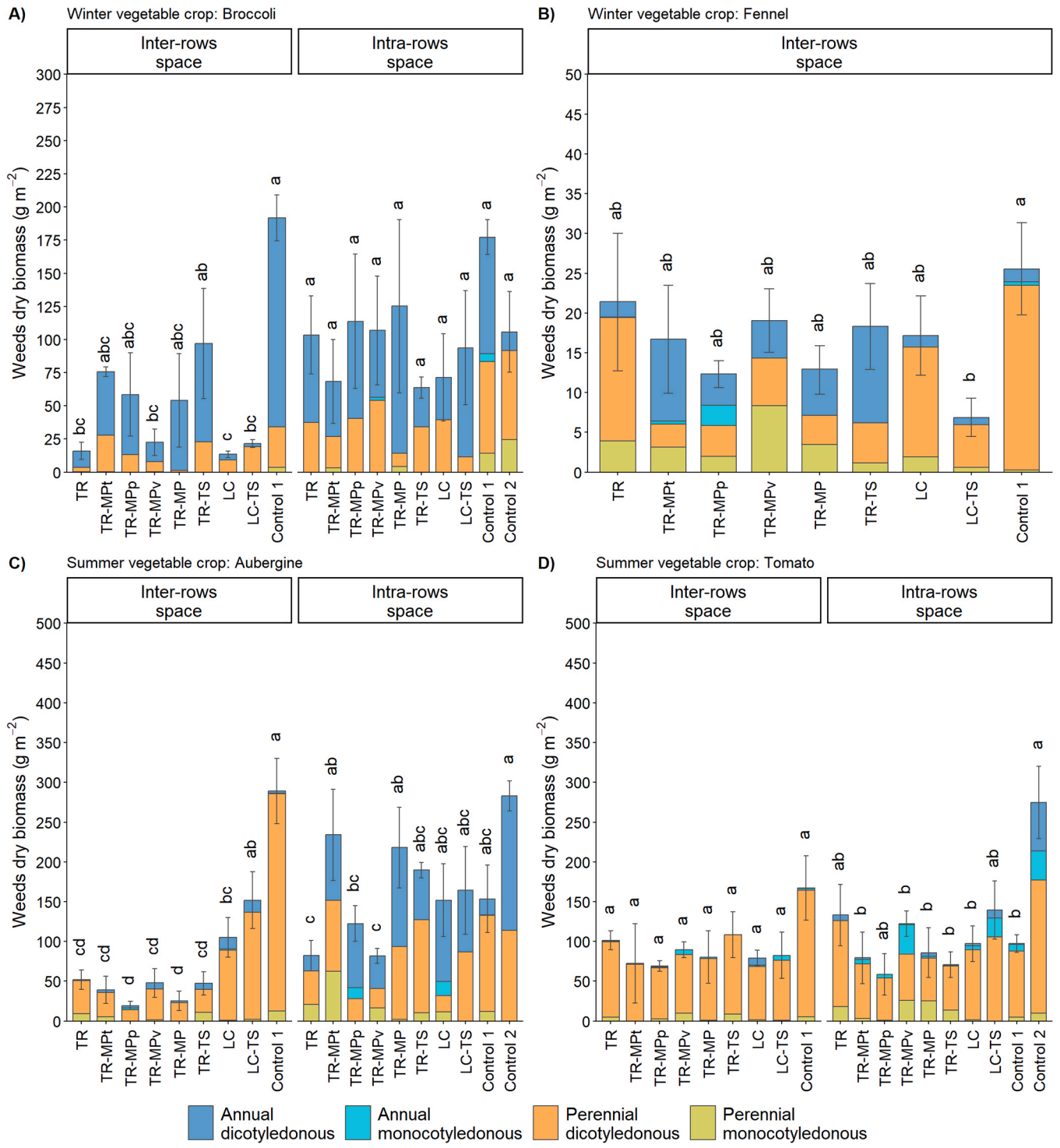


Fig. 6. Total weed aboveground dry biomass (g m^{-2}) and functional-group composition under different permanent living mulch (pLM) treatments at the mid-stage of vegetable crop growth in broccoli (A), fennel (B), aubergine (C), and tomato (D). Weed biomass is shown separately for inter-row and intra-row zones, where available. Treatment abbreviations indicate *T. repens* + *M. polymorpha* eco. Talamone (TR-MPt), *T. repens* + *M. polymorpha* eco. Pitigliano (TR-MPp), *T. repens* + *M. polymorpha* eco. Villa Salto (TR-MPv), *T. repens* + *M. polymorpha* cv. Scimitar (TR-MP), *T. repens* + *T. subterraneum* (TR-TS), *L. corniculatus* + *T. subterraneum* (LC-TS), *T. repens* pure stand (TR), and *L. corniculatus* pure stand (LC). Control 1 received strip tillage without fertilization, with spontaneous vegetation maintained in the inter-row, whereas Control 2 represents standard organic management with soil tillage, organic fertilization, and mechanical inter-row weed control. Stacked bars show the contribution of annual dicotyledonous, annual monocotyledonous, perennial dicotyledonous, and perennial monocotyledonous weeds to total biomass. Bars represent mean total weed biomass ± SE. Different letters indicate significant differences among treatments within each spatial zone (Sidak-adjusted pairwise comparisons, $P < 0.05$).

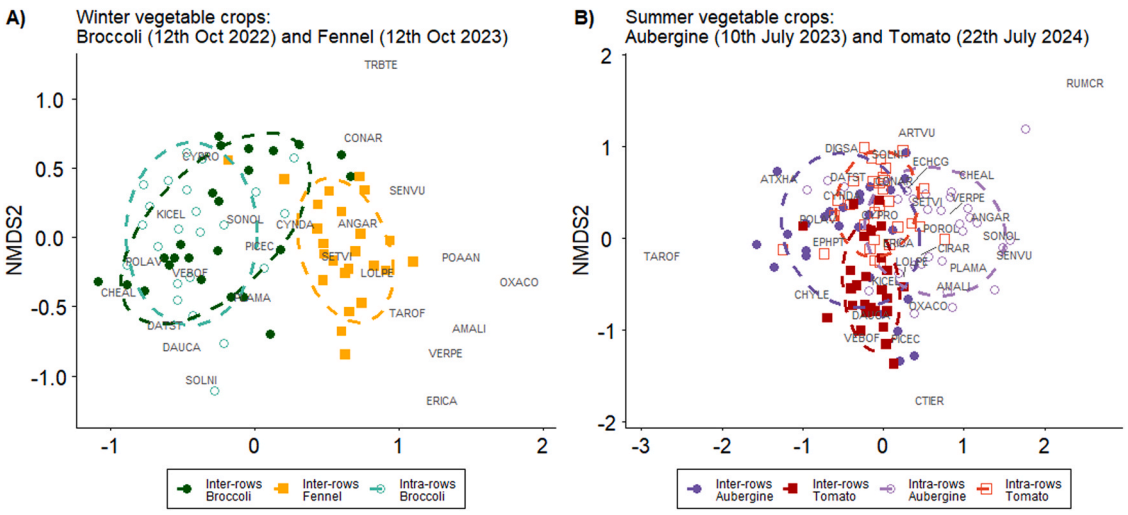


Fig. 7. Two-dimensional NMDS plots illustrating weed community composition based on Bray–Curtis distances derived from weed species biomass data for winter vegetable crops, broccoli and fennel (A), and summer vegetable crops, aubergine and tomato (B). Samples are grouped according to crop type and spatial zone (inter-row vs. intra-row), with ellipses indicating 90% confidence intervals for each crop × spatial zone combination. Weed species are identified by EPPO codes.

recorded before transplanting. In general, in the mixtures, the self-seeding component disappeared due to its vegetative growth cycle that terminated in late spring. Moreover, the perennial components in the mixture reached a biomass level comparable to that of the pLMs pure stands *T. repens* and *L. corniculatus*, despite the differences observed during the pre-cropping period (Fig. 3). During the winter *L. corniculatus* accumulated more biomass, as shown by the assessment conducted on 12th October 2023, near the fennel harvest. In contrast, *T. repens* accumulated more biomass during the summer, as observed in the assessments on 25th May 2023 and 10th July 2023, during aubergine cultivation (Fig. 3).

3.2. Weed biomass and weed community at the establishment of pLMs and in the inter-crops fallow period

In general, weed biomass in control 2, which was managed as standard organic management including tillage and fertilization, remained constant across the three sampling times (Fig. 4 A). In contrast, weed biomass in the pLM treatments was lower than control 2 in Spring 2022 and Spring 2023, but reached levels comparable to control 2 in Spring 2024 (Fig. 4 A). Across pLMs, weed suppression capacity in spring 2022 varied depending on the mixture type used. The treatments based on *L. corniculatus* (*L. corniculatus* pure stand and *L. corniculatus / T. subterraneum*), along with T. *repens / T. subterraneum* mixtures, were the most effective in controlling weeds compared with control 2. In spring 2023, no significant differences were observed between the different pLMs used, whereas in spring 2024, *L. corniculatus* pure stand and *L. corniculatus / T. subterraneum* showed greater effectiveness in weed control compared to control 2 and the other mixtures tested in the study (Fig. 4 A). Overall, a negative relationship was detected between pLM biomass and weed biomass (Fig. 4B). The third year had the lowest pLM biomass and the highest weed biomass, whereas the first two years showed a similar pattern.

Weed community composition also changed over time within the pLM treatments. Species dominant in spring 2022, including *Kickxia elatine* (L.) Dumort. and *Polygonum aviculare* L., declined markedly in spring 2023 and 2024, whereas *Lolium perenne* L., *Picris hieracioides* L., and *Sonchus asper* (L.) Hill increased and became dominant (Fig. 5 A). In contrast, *Cyperus rotundus* L., one of the most abundant species in the experimental field, maintained relatively stable biomass across the three sampling years (Fig. 5 A). NMDS confirmed a shift between the year of pLM establishment and subsequent years (Fig. 5B), with significant differences between spring 2022 and both spring 2023 and spring 2024. Spring 2022 was characterized by annual species such as *P. aviculare*, *K. elatine*, *Chenopodium album* L., *Datura stramonium* L., and *Anagallis arvensis* L. In contrast, spring 2023 and 2024 were dominated by hemicryptophytes and geophytes such as P. hieracioides, L. perenne, *Daucus carota* L., and *Cynodon dactylon* (L.) Pers. (Fig. 5B).

3.3. Weed biomass and community at mid-cycle of vegetable crops

Weed species investigated in the middle of the vegetable growth cycle are reported in Table SM3 of the supplementary material. For the first vegetable crop established on the pLMs, broccoli, weed aboveground dry biomass was generally higher in the intra-row area (103 g/m^2 on average) than in the inter-row area (61 g/m^2 on average), except in control 1, where no differences in weed biomass were observed between the two sampling areas (Fig. 6A). Weeds between rows were effectively controlled by the presence of pLMs; *T. repens*, *L. corniculatus*, and *L. corniculatus / T. subterraneum* mixture showed the lowest weed biomass, significantly lower compared to control 1 (Fig. 6A). Notably, when considering the functional traits of weeds within the community, annual species such as *C. album*, *K. elatine*, *P. aviculare*, and *D. stramonium* represented, on average, the predominant component in both the inter-row (74%) and intra-row (56%) areas (Fig. 6A and Fig. 7A).

The situation changed in the subsequent autumn crop of the rotation, fennel, where the weed community composition shifted toward a prevalence of perennial species, such as *L. perenne*, particularly in the intra-row sampling area (69%) (Fig. 6B and Fig. 7A).

In aubergine, the inter-row area, which was covered by pLMs and managed through repeated mowing, was dominated by perennial weed species (92%), including *C. dactylon*, *P. hieracioides*, and *Taraxacum officinale* F.H.Wigg. (Figs. 6C and 7B). Conversely, the intra-row community was almost equally divided between annual (48%) and perennial (51%) species. Treatments with *T. repens* and its mixtures with *M. polymorpha* eco. Pitigliano and eco. Villa Salto showed lower intra-row weed biomass than control 2 (Fig. 6C). Between rows, all pLM treatments except *L. corniculatus/T. subterraneum* significantly reduced weed biomass compared with control 1.

Differences in weed community composition between the two sampling areas were also observed in tomato, with a clear shift toward perennial weed species in both the inter-row and intra-row areas (96% and 81%, respectively) (Fig. 6 D). Weed control differences were not significant between rows; however, intra-row, some mixtures, such as *T. repens / M. polymorpha* eco. Talamone, *T. repens / M. polymorpha* eco.

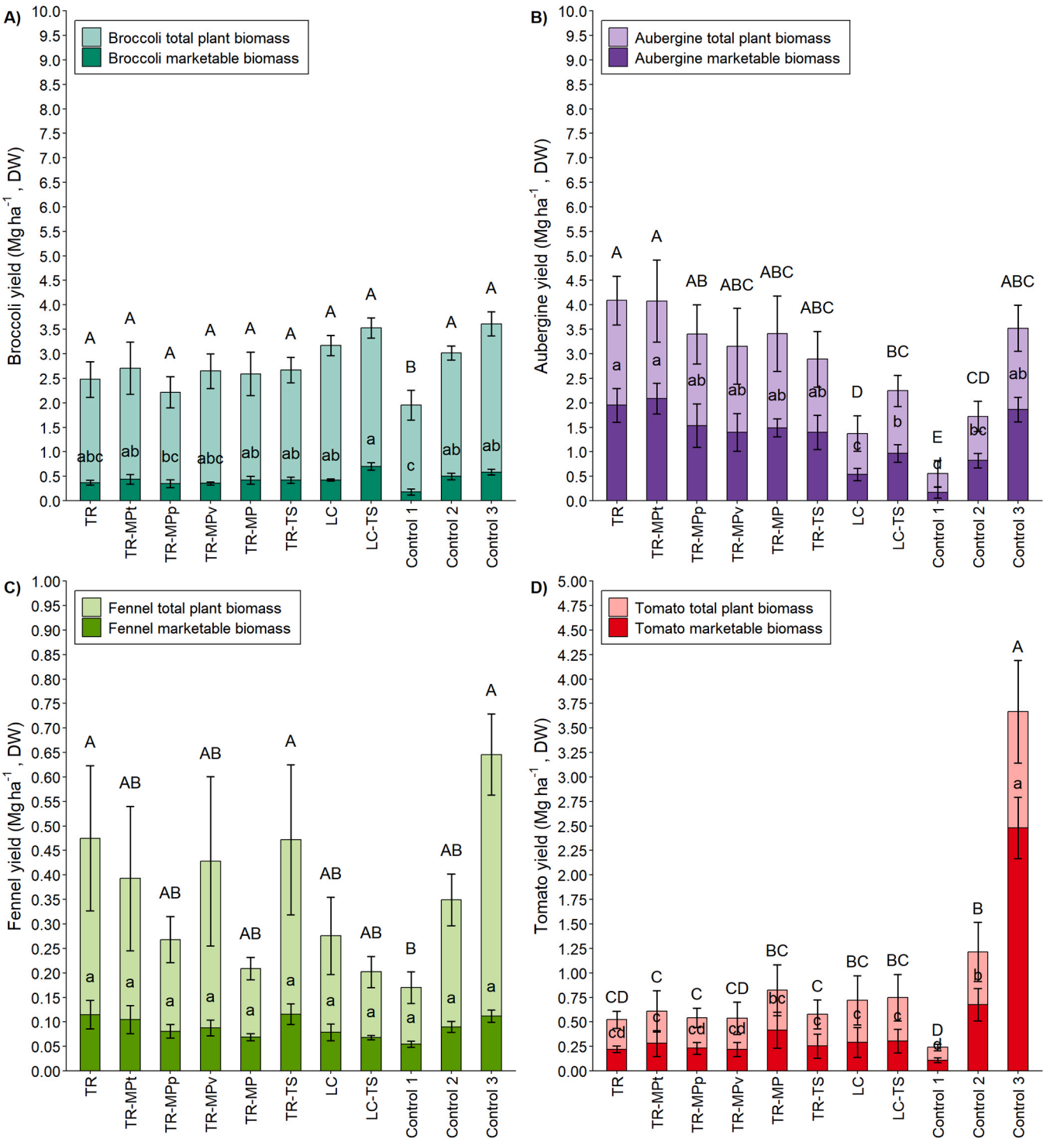


Fig. 8. Total aboveground dry biomass (Mg ha^{-1}) and marketable dry biomass yield (Mg ha^{-1}) of broccoli (A), aubergine (B), fennel (C), and tomato (D). Treatment abbreviations indicate T. repens + M. polymorpha eco. Talamone (TR-MPt), T. repens + M. polymorpha eco. Pitigliano (TR-MPp), T. repens + M. polymorpha eco. Villa Salto (TR-MPv), T. repens + M. polymorpha cv. Scimitar (TR-MP), T. repens + T. subterraneum (TR-TS), L. corniculatus + T. subterraneum (LC-TS), T. repens pure stand (TR), and L. corniculatus pure stand (LC). Control 1 (CNT1) received strip tillage without fertilization, with spontaneous vegetation maintained in the inter-row. Control 2 (CNT2) represents standard organic management with soil tillage, organic fertilization, and mechanical inter-row weed control, whereas Control 3 (CNT3) received the same management as CNT2 plus manual intra-row weeding. Bars represent means ± SE. Total biomass and marketable biomass were analysed separately. Different letters indicate significant differences among treatments within each crop (Sidak-adjusted pairwise comparisons, $P < 0.05$).

Villa Salto, *T. repens / M. polymorpha* cv Scimitar, and *L. corniculatus / T. subterraneum*, showed lower weed biomass than control 2, although not significantly different from control 1 (Fig. 6 D).

3.4. Vegetable biomass production and yield performance

The management-intensity gradient among controls was reflected in crop performance, with the lowest total biomass and marketable yield generally recorded in control 1 and the highest in control 3.

For broccoli (Fig. 8 A), all treatments with pLMs showed total plant biomass and marketable yield that were not statistically different from control 2 and control 3. A similar trend was observed for aubergine (Fig. 8 B), in which all pLM treatments, except for *L. corniculatus*, showed a total biomass similar to control 3. For some pLM treatments, including *T. repens* and the mixture *T. repens / M. polymorpha* eco. Talamone, total biomass and marketable yield were significantly higher than control 2. For fennel (Fig. 8 C), although production levels were much lower than those typically achieved on local farms, total biomass and especially marketable yield were not statistically different between the pLM treatments and control 2 or control 3. For tomato (Fig. 8 D), total plant biomass and marketable yield in the pLM treatments were significantly lower than in control 2 and, in many cases (*T. repens* and its mixture with *M. polymorpha* eco. Talamone, *M. polymorpha* eco. Pitigliano, *M. polymorpha* eco. Villa Salto, *M. polymorpha* cv. Scimitar), significantly lower than in control 3.

4. Discussion

4.1. pLMs performance and stability over time

The choice to use mixtures based on species with different life cycles, namely perennial and annual self-reseeding species, was intended to complement the well-established hypothesis underlying species mixtures, which typically focuses on combining species with complementary functional traits for supporting multifunctionality (Storkey et al., 2015; Blesh, 2018). In this case, given the medium-long term perspective of pLMs, the goal was to enhance complementarity of traits along the temporal dimension, ensuring continuous soil cover throughout the year by using species with complementary growing periods. In Mediterranean environments, for instance, perennial species tend to enter vegetative dormancy during the winter season, while annual self-reseeding species remain active during this period. During summer, instead, perennials reach their maximum growth, while annuals complete their life cycle and die after actively spreading their seeds for regrowth in the following autumn.

The results of this study showed that the self-reseeding component of the mixture successfully established during the first year of pLM establishment and persisted into the mixture also during the second year due to its capacity for self-reseeding. However, the contribution of the self-reseeding species declined over time and by the third spring they were no longer present in the mixtures. The effect was the same for all species and ecotypes tested. The interactions between the self-reseeding and perennial species within the mixtures varied depending on the species involved in the mixture. During the establishment year, in the mixtures with *T. repens,* annual self-reseeding showed rapid growth, which out competed the *T. repens* component. Conversely, in mixtures with *L. corniculatus* as the perennial component, the opposite trend was observed, with *L. corniculatus* dominating over *T. subterraneum*. In the following spring, the dynamics between mixture components changed with the perennial species that became well established and represented the dominant component of the mixtures, while the self-reseeding annuals showed a compensatory function by filling spaces and maintaining uniform soil cover. The *L. corniculatus* mixture was particularly interesting because, while the perennial component was dominant during the first year, the self-reseeding species became prevalent in the second year. The contribution of the self-reseeding species declined over time, independent of legume species or ecotype used, and by the third spring they were no longer present. Nevertheless, the total biomass of the mixtures remained higher than that of perennial pure stands, supporting their greater capacity to maintain soil cover and suppress weeds, consistent with the relationship between cover-crop biomass and weed suppression (Florence et al., 2019). Loss of functional pLM cover was associated with increased weed biomass and very low crop production. New strategies need to be tested that are able to support the establishment of the annual self-seeding species in the pLM, and at the same time, reduce the presence of perennial weeds. Since fertilisation may support annual species that have no storage organs, future research should take into account supportive fertilisation schemes for the self-reseeding component of the pLMs, likely needed from the second year onwards. At the same time, the effect of dead biomass after repeated mowing should be investigated, particularly the trade-off between enhancing weed suppression through mulch residues and potentially hindering the regrowth and re-establishment of self-reseeding pLM components in the mixtures.

4.2. Weed control and weeds community composition dynamics

Weed biomass assessed in spring, after the winter fallow period and before summer-crop transplanting, confirmed the strong initial suppressive capacity of pLMs but also revealed a progressive shift in weed community composition. In the first year, total weed dry biomass in pLM plots was markedly lower (50 g m^{-2}) than in tilled controls (250 g m^{-2}), demonstrating the competitive advantage of pLMs in limiting resource availability for weeds. This effect is consistent with suppression by pLMs through shading and resource competition (Hiltbrunner et al., 2007; Westbrook et al., 2022; Menalled et al., 2022), allelopathic interactions, and enhanced seed predation (Nichols et al., 2015; Schumacher and Gerhards, 2022). Dense pLMs likely acted as an ecological filter favouring low-disturbance tolerant species such as *Veronica persica* Poir., *Plantago major* L., *Atriplex prostrata* Boucher ex DC., and *S. asper* (Booth and Swanton, 2002).

However, as observed in other living mulch systems with cereal crops (Bergkvist, 2003; Gerhards, 2018), weed biomass tended to increase over time, reaching levels comparable to those of the control 2 by the third year. This trend highlights a dynamic in which perennial weeds gradually replace annual species as the main functional group within the community in pLMs systems, as documented by Armengot et al. (2015). The dominance of *L. perenne* and *P. hieracioides* observed in the second and third years highlights this ecological filtering process, as these species tend to thrive under reduced tillage condition (Pardo et al., 2019; Derrouch et al., 2022). Their persistence is favoured by perennial root systems and the advantage they take over other species at low disturbance regimes. Conversely, in this study the reduction of ruderal species such as *P. aviculare* and *K. elatine*, both annuals adapted to soil disturbance, indicates that the absence of tillage, constant cover and repeated mowings, progressively reduced some disturbance-tolerant species (Dorado and López-Fando, 2006; Ruisi et al., 2015). In this experiment, *C. rotundus* was one of the most abundant weed species and showed a distinct temporal response. The biomass of *C. rotundus* remained relatively stable across the three years, whereas species such as *L. perenne* and *P. hieracioides* increased. This is particularly relevant because rotary tillage, a common practice in vegetable production, can favour the spread of *C. rotundus* by fragmenting and redistributing underground propagules that retain a high capacity for regeneration (Tong et al., 2026). The reduced soil disturbance under pLM management may therefore have limited this process, while repeated inter-row mowing may have further restricted vegetative growth (Summerlin et al., 2000).

Inter- and intra-row areas experience contrasting soil disturbance regimes, affecting weed biomass and community composition (Hernández Plaza et al., 2015). In the current experiment, inter-rows were exposed to continuous pLM cover and mowing whereas intra-rows underwent localized strip tillage. These contrasting disturbance regimes interacted with temporal changes in weed composition. Annual species declined over time, particularly in inter-rows where continuous cover and mowing favoured perennials (Armengot et al., 2016). Intra-row strip tillage initially maintained a higher proportion of annuals, although perennial species progressively colonized these areas. In this experiment, it is likely because perennial species, especially those with small seeds and wind-dispersed propagules such as *P. hieracioides*, gradually colonized the area, masking the effects of weed management on plant traits.

4.3. Vegetable yield performance

Crop yields broadly followed pLM performance, remaining relatively high during the first two years before declining in the third year, particularly for tomato. Fennel performance was strongly affected by abiotic stress unrelated to the pLM treatments. The crop was transplanted on 20 September 2023, during a sub-optimal transplanting period, and was subsequently exposed to two days of strong saline winds from the nearby sea, which caused severe damage and complete necrosis of the outer leaves. These events delayed crop development in both control and pLM plots, preventing plants from reaching the minimum marketable size before winter.

Broccoli and aubergine yield under pLMs were comparable to controls 2 and 3 and higher than control 1 despite the absence of fertilization. The mechanisms supporting crop productivity were not directly investigated, but previous studies in similar systems have reported increased nutrient availability, particularly nitrogen and phosphorus (Tittarelli et al., 2018; McAmis et al., 2024), improved soil structure (Romaneckas et al., 2018; Tosti et al., 2019; Timm et al., 2024), and enhanced biological fertility (Trinchera et al., 2017, 2021). These processes may have contributed to crop performance during the first two years. By the third year, however, pLM cover had declined and weed pressure had increased, while tomato yielded similarly to control 1 and remained below controls 2 and 3.

Crop–pLM competition did not appear to be a major constraint during the first two years of the experiment. This was particularly evident for aubergine, when yields in several pLM treatments were comparable to those of controls 2 and 3 despite the more intensive management applied in these controls. Under these conditions, the benefits associated with pLMs appeared to outweigh their competitive effects. However, the experimental design did not allow pLM competition to be fully separated from the effects of weeds and nutrient availability. Although previous studies have reported competition between pLMs and vegetable crops (Den Hollander et al., 2007a, b), the results of this study indicate that suitable management and cultivar choice can help limit these effects. Periodic inter-row mowing, together with the use of species such as *T. repens* and *L. corniculatus*, which tolerate frequent cutting and maintain a relatively low canopy, likely contributed to reducing competition with the crop. Inter-row mowing was required approximately two to three times during each vegetable crop cycle. The feasibility of repeated mowing could be improved through automation, although further adaptation to vegetable row geometry is still required (Sportelli et al., 2022; Averill et al., 2022).

5. Conclusions

Permanent living mulches proved to be a feasible strategy for integrating conservation agriculture into Mediterranean organic vegetable systems. Broccoli and aubergine yields were comparable to fertilized, mechanically weeded controls, while mixtures of perennial and self-reseeding species improved the stability of soil cover over time and supported weed suppression. These benefits declined as pLM biomass decreased over time, showing that their performance should be evaluated across the whole crop rotation rather than on a single crop. The shift toward perennial weeds, particularly *L. perenne* and *P. hieracioides*, indicates that long-term pLM management may require alternating the pLM phase with a crop cycle managed using standard tillage, to reduce perennial weed pressure and avoid weed specialization. Further research should focus on improving mixture persistence, assessing water and economic trade-offs, and developing practical technologies for pLM management in vegetable systems.

CRediT authorship contribution statement

Federico Leoni: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Stefano Carlesi:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Giacomo Nardi:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Daniele Antichi:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **Christian Frasconi:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization. **Anna-Camilla Moonen:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

During the preparation of this work, the authors used GPT 5.6 for English language editing. The authors reviewed and edited the output as needed and take full responsibility for the content of the published article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2026.110763.

Data availability

Data will be made available on request.

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