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Weed suppression and reduced fertilizer requirements in organic durum and bread wheat using forage legume living mulches in Mediterranean systems

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Living mulch is a promising strategy for addressing agricultural challenges in organic wheat-based systems. However, success depends on selecting and managing living mulch species able to reduce interspecific competition with the main crop. This study aimed to assess whether forage legume living mulches can improve weed suppression, reduce nitrogen (N) fertilizer requirements, and enhance wheat performance across two growing seasons (2022/23 and 2023/24) in a Mediterranean organic farming system. Two wheat species, *Triticum turgidum* subsp. *durum* (var. Core) and *Triticum aestivum* (Evolutivo population - Evo) were grown individually with forage legume (*Trifolium subterraneum* or *Medicago polymorpha* or *Lotus corniculatus*) and compared with wheat-sole crops under no, medium, and high N fertilization (F-rates). Compared with wheat sole crops, wheat-living mulches slightly decreased or increased grain yield in Core (-3% to +40%) and Evo (-9% to +20%), and maintained or increased the grain protein content (+1 to +10%). The relationship between kernel dry weight and growing degree days showed that wheat sole crops achieved the greatest kernel weight at the highest F-rate. In contrast, the wheat-living mulch systems maintained stable kernel weights under reduced F-rates. Depending on environmental conditions, Trifolium living mulch provided the highest biological N-fixation (21 to 73 kg N ha^{-1} yr^{-1}), while Medicago living mulch was the most effective in suppressing weed dry biomass (-58 to -94%) as compared with both Core and Evo sole crops. These findings indicate that forage legumes can be safely integrated as living mulches in organic wheat without compromising yield, while improving weed control and reducing dependence on external N, particularly under low nutrient and low-yielding conditions.

KEYWORDS
agroecology, biological N fixation, cover crop, kernel growth, mixed cropping, triticum

1 Introduction

Living mulch is recognized as a promising agroecological practice for addressing global challenges, including the reliance on mineral nitrogen (N) fertilizers and synthetic herbicides in cereal production (Cougnon et al., 2022). Living mulches consist of cover crops established either before or simultaneously sown with the main crop and maintained as living ground cover throughout the growing season (Hartwig and Ammon, 2002). More broadly, cover crops, namely unharvested species sown with or between primary cash crops, are used to provide many provisioning, regulating, and supporting ecosystem services (Lamichhane et al., 2025; von Cossel et al., 2025). When considered for their mulching role, cover crops can serve as living mulches with the companion cash-crop, as dead mulches when their residues remain on the soil surface, or as green manures when their biomass is incorporated into the soil (Scavo et al., 2022).

Although cover crops have been widely studied in perennial systems, such as in vineyards (Calderone et al., 2025), research on annual cropping systems, particularly those involving simultaneous growth with organic wheat, remains comparatively limited (Dzvene et al., 2023). This gap persists despite the rapid expansion of organic agriculture worldwide, which has increased nearly sevenfold over the past two decades, from 11 Mha in 1999 to 76 Mha in 2022 (Willer et al., 2023).

Italy, represents the third-largest organic agricultural area in Europe (Willer et al., 2023), and the most important European producer of organic durum wheat [*Triticum turgidum* subsp. *durum* (Desf.) Husn] (Antichi et al., 2025). Durum wheat is mainly grown in the warmer and drier regions of southern Italy and the islands, whereas bread wheat (*Triticum aestivum* L.) is predominantly cultivated in the cooler and wetter northern and central regions (Istituto Nazionale di Statistica – ISTAT, 2026).

Organic farming largely avoids synthetic inputs such as fertilizers, pesticides, and herbicides, while promoting practices that sustain long-term soil fertility and agrobiodiversity. However, crop nutrition in organic systems often relies on substantial organic external inputs, making these systems dependent on a resource that is limited in availability (Schrama et al., 2018). In addition, crop nutrient uptake is closely tied to the mineralization of organic matter. In Mediterranean climates, erratic spring rainfall can slow or hinder this process, limiting carbohydrate accumulation and translocation to the spikes, reducing grain yield (Pampana et al., 2007). Weed management also remains a major challenge in organic wheat systems. A long-term study reported a progressive increase in weed biomass under organic management in Central Italy, reaching levels 400% higher than in conventional systems by the end of the 15-year experimental period (Antichi et al., 2025).

Therefore, strategies that simultaneously improve weed control and enhance nitrogen (N) availability are required. Forage legume living mulches in wheat-based systems could address these challenges, yet their adoption remains limited. Legumes are well known for their biological N-fixation capacity (BNF), and their use in rotations or intercropping is regarded as an alternative and sustainable way of introducing N into low-input cropping systems (Bedoussac et al., 2015; Zhao et al., 2022). However, demonstrating and quantifying direct N transfer from legumes to companion cereals is challenging (Peoples et al., 2015), and evidence suggests that the in-season N benefit to cereals through legume in intercrops is typically between 0 and 29 kg N ha^{-1} from BNF (Chalk et al., 2014). Increased N uptake in intercropped cereals is therefore more likely due to reduced intraspecific competition and improved access to soil N compared with sole crops (Peoples et al., 2009; Jensen et al., 2020).

Legume living mulches are effective strategies for improving non-chemical weed management in cereal systems (Cougnon et al., 2022). When suitable species are selected, living mulch can suppress weed biomass by 22-75% while maintaining wheat yields and improving soil fertility (Radicetti et al., 2018; Leoni et al., 2022; Devi et al., 2023). Effective perennial legumes, such as *Medicago sativa*, *Trifolium repens, Lotus corniculatus*, and *Sulla coronaria*, among others, have consistently shown strong weed suppression and substantial N contributions in Mediterranean low-input systems (Cougnon et al., 2022; Leoni et al., 2024). However, most advances to date come from perennial cover crops and relay-cropping systems in cereals; nevertheless, high-yielding, tall, and erect perennial legumes may also compete with cereals for light and water, ultimately reducing wheat yield (Carof et al., 2007a,b; Pellegrini et al., 2021; Cougnon et al., 2022; Devi et al., 2023). The simultaneous sowing of annual, short-cycle, and prostrate forage legumes used as living mulches in wheat, rather than relay-cropping, may represent a strategic option for organic farming. A recent trial in southern Italy using *Trifolium subterraneum* and *Medicago polymorpha* as living mulches in durum wheat showed that relay-cropping, in which legume undersowing was delayed until the wheat tillering stage, was less effective for weed suppression than simultaneous wheat/legume sowing (Scordia et al., 2024).

These findings highlight the central role of management, species selection and crop functional traits in designing effective wheat-legume systems (Hiltbrunner et al., 2007; Campiglia et al., 2014; Costanzo and Bàrberi, 2014, CostanzoBàrberi, 2016). Despite increasing interest in agroecological practices for cereal systems, the potential of forage-legume living mulches to simultaneously control weeds and supply N in Mediterranean organic wheat remains insufficiently explored, particularly for bread wheat.

Therefore, this work aimed to assess whether forage-legume living mulch can improve weed suppression, reduce the need for N fertilization, and enhance wheat performance under variable environmental conditions. We evaluated two wheat species, a durum wheat (*Triticum turgidum* subsp. *durum*) and a bread wheat (*T. aestivum*), grown either as sole crops or in mixture with three different cover crop species, namely the annual self-seeding *T. subterraneum* or *M. polymorpha*, or the perennial *L. corniculatus*. Annual self-seeding legumes establish rapidly and senesce relatively early, potentially reducing competition with wheat for light and water during grain filling. In contrast, perennial species establish more slowly but persist beyond wheat maturity and maintain living roots (Scordia et al., 2024), potentially contributing through BNF but increasing competition. We also tested a modern durum wheat and an evolutive bread wheat population (Composite Cross Population), which is considered better suited to organic systems (Ceccarelli and Grando, 2020). Due to their greater genetic

diversity, taller stature, and higher tolerance to biotic stresses, evolutive populations are expected to compete more effectively with weeds and better adapt to living mulch systems than modern cultivars (Bocci et al., 2020). We used an additive intercropping design (i.e., relative total plant density > 1) with a mixed spatial arrangement (full mixtures without row patterns). This configuration generally intensifies interspecific interactions and can enhance weed-suppressive ability (Gu et al., 2025).

Key wheat yield traits and protein content, weed dry biomass, forage-legume dry biomass and BNF, as well as the influence of management and climate on wheat grain-filling development, were evaluated.

2 Materials and methods

2.1 Experimental site

The experimental site was in a certified organic farm in the Mediterranean environmental zone (38°06'N, 14°59'E, 196 m a.s.l., Patti, Messina, Italy), characterized by mild-wet winters and hot-dry summers. The historical climatic data for cool-season crops (i.e., November to July) recorded by the SIAS (Servizio Informativo Agrometeorologico Siciliano, http://www.sias.regione.sicilia.it/) weather station in Patti are as follows: maximum, minimum, and mean air temperatures of 21.4 °C, 11.4 °C, and 16.4 °C, respectively; rainfall of 565.8 mm; and reference evapotranspiration of 676.7 mm.

The soil at the experimental site is classified as a Calcixerepts according to USDA Soil Taxonomy (Soil Survey Staff, 2022), and is characterized by a high calcium carbonate content, clay-rich texture, and low concentrations of organic matter and total nitrogen. Before the experiment started, soil samples were collected from the northern, central, and southern sections of the field at a depth of 0–30 cm for physical and chemical analysis. The primary properties were: sand 29.9 ± 2.11%, silt 12.0 ± 0.85%, clay 58.1 ± 0.87%; pH 7.8 ± 0.06; organic matter 1.3 ± 0.03 g kg^{-1} (Walkley and Black method); total $CaCO_3$ 162 ± 3.9 g kg^{-1} (Scheibler method); total N 0.04 ± 0.01 g kg^{-1} (Kjeldahl method); available P_2O_5 78.7 ± 1.45 mg kg^{-1} (Olsen method); and exchangeable K_2O 45.0 ± 2.52 mg kg^{-1} (ammonium acetate test method).

2.2 Experimental trial set-up

A randomized block design with three replications was implemented over two consecutive growing seasons (2022/23 and 2023/24). Two wheat species, durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn., var. Core) and bread wheat (*Triticum aestivum* L., Evolutivo population), hereafter referred to as Core and Evo, respectively, were evaluated under two agronomic treatments: fertilization rates, cropping systems, and their interaction. Each year, soil tillage included late summer plowing to a depth of 30 cm, followed by autumn disc harrowing to 20 cm. A rotary tiller was used before sowing to incorporate organic fertilizer and prepare the seedbed. The field was fallow the first year, while the experimental crops followed in the second year. In both years, the agronomic treatments were:

i. organic fertilization (Organagro, Choncimer srl, San Severino Marche, Italy), with a pH of 7.05, N (3.10%), P_2O_5 (2.96%) and K_2O (2.0%). The organic fertilizer was applied at the seedbed preparation, at three rates (hereinafter F0, F1, and F2, respectively) to achieve target N at three levels (0, 60, and 120 kg N ha^{-1}); the highest rate is the amount used by local farmers in organic wheat systems;
ii. cropping system, where Core and Evo were sown either as sole-crops (control) or with cover crop legumes: the perennial *Lotus corniculatus* L. (var. Gran San Gabriele), or the annual self-seeding *Medicago polymorpha* L. (var. Scimitar), or the *Trifolium subterraneum* L. var. *subterraneum* (var. Urana), hereinafter Lotus, Medicago, and Trifolium, respectively.

Core was purchased from Centrale Zooagricola srl (Misterbianco, Italy). Evo, a composite cross-population between multiple parent varieties (Bocci et al., 2020), was kindly provided by Simenza Association (Cumpagnia Siciliana Sementi Contadine, Radusa, Italy). Medicago and Trifolium were purchased from Padana Sementi (Tombolo, Italy). Lotus was kindly provided by MAS Seeds (San Pietro di Morubio, Italy).

Broadcast seeding was used for both wheat and cover crops, followed by mechanical soil rolling. The two wheat species were sown alone (sole crops) or individually with each of the forage legumes according to an additive intercropping design, applying the full seed rate of both crops, with a mixed spatial arrangement (full mixtures without row patterns). The seed density (nr. germinable seeds m^{-2}) was 400 for Core and Evo, 450 for Medicago, 300 for Trifolium, and 1000 for Lotus. Each plot measured 30 m^2 (5 × 6 m). Sowing was conducted on 29 December 2022 for the first year and 21 December 2023 for the second year. No top-dressing fertilization or pre- and post-sowing weed, pest, or disease control was carried out in either growing season. These practices are representative of commercial organic farms in the study area.

2.3 Measurements

The SIAS weather station located in Patti, approximately 3 km from the experimental field, provided monthly maximum and minimum air temperatures, rainfall, and reference evapotranspiration data.

Phenological stages of forage legumes and wheat were recorded following the BBCH system (Lancashire et al., 1991) at the plot level, when at least 50% of plants inside each plot reached a specific stage. Growth stages were calculated on different days after sowing (DAS) and growing degree days (McMaster and Wilhelm, 1997):

$$GDD = \left[\frac{T\ \ max + T\ \ min}{2}\right] - Tb$$

where GDD are growing degree days (°Cd), *Tmax* and *Tmin* are the maximum and minimum daily temperature, and *Tb* is the base temperature, 0 °C for both wheat (Jamieson et al., 2008) and forage legumes (Enriquez-Hidalgo et al., 2020).

Starting from the BBCH 71 growth stage, destructive sampling was performed on three representative ears per plot to determine kernel dry weight. Sampling dates in the first growing season were 24 April 2023 (111 DAS), 5 May (122 DAS), 26 May (143 DAS), 9 June (157 DAS), 22 June (170 DAS), and 6 July (184 DAS). In the second growing season, wheat kernel dry weights were sampled on 4 April 2024 (106 DAS), 17 April (119 DAS), 7 May (139 DAS), 20 May (152 DAS), 29 May (161 DAS), and 12 June (175 DAS).

Crops were harvested at wheat grain hardening and complete dryness by collecting wheat, cover crop, and weed species from 0.5×0.5 m quadrats. Harvest dates were 6 July 2023 in the first year and 12 June 2024 in the second year. Fresh legume and weed aboveground samples were dried at 65 °C in a ventilated oven until reaching constant weight for dry matter determination. Harvested wheat grains were dried at 60 °C until constant weight and subsequently corrected to a standard moisture content of 13% for all yield and weight measurements.

The number of wheat ear m^{-2} in each plot, and the number of grains per ear on five representative ears per plot, were counted. A mechanical thresher separated grain and straw for the Core and Evo wheat. The thousand-kernel weight of wheat was measured by weighing three sets of 100 seeds per plot. Grain yield, cover crop and weed dry biomass were expressed per unit land area (Mg ha^{-1}). Wheat grain total N was analyzed using the Kjeldahl method, with grain protein content determined by multiplying Kjeldahl N by 5.7 (AOAC, 2019: method 2001.11). The amounts of N_2 fixed by cover crops (BNF, kg N ha^{-1} yr^{-1}) were estimated from linear relationships with the amount of N in the exported biomass at harvest (N_{output}), as recommended by the EU Nitrogen Expert Panel (2016):

$$BNF = (3 + 0.78 \times N_{output}/HI) \times BGN$$

where BNF is the total N input via biological N_2 fixation (in kg N ha^{-1} yr^{-1}); N_{output} is the amount of N in harvested dry matter (kg N ha^{-1} yr^{-1}); HI is the harvest index, defined as the ratio of the harvested material to the total aboveground production, set to 0.9 for forage legume; BGN is a multiplicative factor to take into account belowground contributions, comprising N associated with roots, nodules and rhizodeposition, set to 1.7 for forage legume (Anglade et al., 2015). Legume N content in dry biomass was determined by the Kjeldahl method (AOAC, 2019: method 2001.11).

2.4 Statistical analysis

Linear mixed models were used to test the effects of fertilization, cropping systems, and their interaction on wheat traits, cover crop, and weed dry biomass for each year and wheat species. Under contrasting climatic conditions, pooling data across years may obscure biologically significant treatment responses. Therefore, following a common practice in multi-year agronomic field experiments, results were analyzed separately for each year to allow a clearer interpretation of treatment effects within each climatic context (Yaldiz et al., 2025). To further assess treatment responses across wheat genotypes, analyses were also performed separately for the modern durum wheat Core and the evolutive bread wheat population Evo.

In all models, the block was considered a random effect, while fertilization and cropping systems were the fixed effects. Repeated measures in time were used to analyze durum and bread wheat phenology each year. The days after sowing (DAS) was the random effect; fertilization and cropping systems the fixed effects (Minitab version 19, Statistical Software Computer. Minitab, Inc., State College, PA, USA).

Observations within each group were randomly sampled according to the experimental design, ensuring independence. The assumptions of normality and homogeneity of variances were assessed using the Shapiro-Wilk test and Bartlett's test, respectively. When the analysis of variance (ANOVA) revealed significant differences among group means, Tukey's HSD *post-hoc* test was applied to identify pairs of means that differed significantly at $P \leq 0.05$.

Logistic functions were fit for dry matter filling in wheat kernels:

$$Y = \frac{a}{1 + exp(-\frac{x - x_0}{b})}$$

where a is the asymptotic or the maximum Y value (kernel dry weight), x_0 is the inflection point at which a is maximized, and b is a coefficient that determines the shape of the curve. The Shapiro-Wilk test was used to test residuals for normality. The goodness of fit was assessed by calculating the R2, and coefficients were considered significant at $P \leq 0.05$ (SigmaPlot 11, Systat Software Inc., San Jose, CA, USA). The 95% confidence intervals (CIs) were calculated to compare kernel asymptotic values:

$$95\,\%\,CI = \frac{X \pm Z}{(S/\sqrt{n})}$$

where X is the asymptotic value, Z is 1.96 for a confidence level of 95%, and $S/\sqrt{n}$ is the standard error of the asymptotic value. Non-overlapping 95%CIs indicate significantly different kernel dry weights.

3 Results

3.1 Meteorological trend and crop phenology

The first growing season was cooler than the second, with average minimum, mean, and maximum air temperatures of 11.3 °C, 16.3 °C, and 21.2 °C, respectively, compared with 12.7 °C, 17.6 °C, and 22.5 °C in the second season (Figure 1). Total rainfall was higher in the first season (409.4 mm) than in the second (294.2 mm). In this latter, most rainfall occurred during winter (229.8 mm), while spring was particularly dry (65.6 mm). In contrast, rainfall in the first season was more evenly distributed between winter (124.6 mm) and spring (275.6 mm). Growing season reference evapotranspiration was similar in both years (480.8 mm and 481.3 mm, respectively).

Despite similar GDD, the warmer and drier season in the second year shortened wheat grain maturity by 9 days, which was achieved at 175 DAS (2796 °Cd) compared to 184 DAS (2784 °Cd)

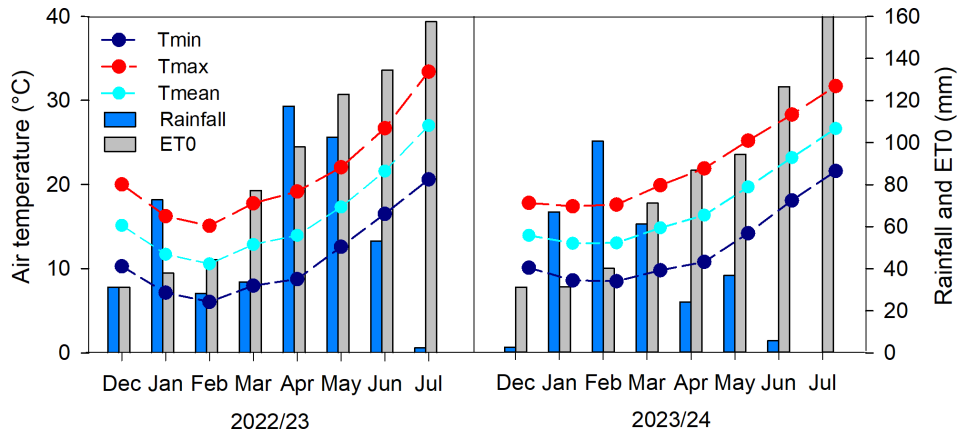

FIGURE 1
Maximum, mean and minimum air temperature (°C), rainfall and reference evapotranspiration at the experimental site (38°06'N, 14°59'E, 196 m a.s.l., Patti, Messina, Italy) in the two growing seasons.

in the first year. The effects of cover crops or the fertilization were not significant on wheat phenological stages in both years (P>0.05; Supplementary Tables 1, 2). Among forage legumes, Medicago was the earliest and approached senescence at wheat ripening stages, whereas Trifolium senescence occurred closer to the wheat harvest in both years. Lotus did not reach senescence at the harvest time of wheat; it was at the flowering stage in the first year and at the inflorescence emergence stage in the second year (Supplementary Tables 3, 4).

3.2 Effects of management practices on durum wheat performance

In the first year, the fertilization × cropping-system showed a significant interaction for thousand-kernel weight (TKW) and ear m^{-2} (Supplementary Table 5). The highest TKW values occurred in Core (Cnt) at F2 and Core(Med) at both F2 and F1, whereas the lowest were recorded in Core(Cnt) and Core(Trif) at F0. For ears m^{-2}, the greatest values were observed in Core(Trif) and Core(Med) at F0, while the lowest occurred in Core(Cnt) and Core(Lot) at F0. In the second year, only the ear m^{-2} was affected: the highest value was found in Core(Lot) at F0, and the lowest in Core(Med) at F0.

The fertilization effect was significant on nearly all traits in the durum wheat Core, except for ear density, cover crop dry biomass (CCB), and biological N fixation (BNF) (Table 1). TKW and kernel number per spike (KN) did not differ between F2 and F1 (on average 49.4 g and 38.5 ears m^{-2}, respectively), while both were higher than F0 (40.1 g and 29.5 ears m^{-2}, respectively). In contrast, grain yield (GY) showed a clear statistical difference (1.72 in F0, 2.74 in F1, and 3.73 Mg ha^{-1} in F2). Grain protein content (GPC) was highest in F2 (12.6%) than F1 and F0 (on average 11.7%). Weed biomass (WB) increased only at the highest F-rate (0.69 Mg ha^{-1} in F2), with no difference between F0 and F1 (on average 0.34 Mg ha^{-1}). The BNF across F-rates averaged 37.7 kg N ha^{-1}. The cropping system (CS) effect was statistically significant for all traits except KN. TKW was higher in Core(Med) and Core(Lot) (on average 48.2 g) than in Core (Cnt) and Core(Trif) (on average 44.6 g). For ear density, Core(Cnt) and Core(Lot) showed the lowest values (on average 260.4 ears m^{-2}), whereas Core(Trif), followed by Core(Med) the highest (on average 329.1 ears m^{-2}). GY was higher in Core(Med) and Core(Lot) (on average 3.31 Mg ha^{-1}), followed by Core(Trif), which did not differ from the former two, and the Core(Cnt), which produced the lowest GY (1.99 Mg ha^{-1}). All legume-based systems had higher GPC than the control (on average 12.3 vs 11.1%), and provided superior weed suppression than the control (on average 0.19 vs 1.33 Mg ha^{-1}). Among cover crops, Trifolium produced the highest dry biomass (3.22 Mg ha^{-1}) and the highest BNF (73.6 kg N ha^{-1}), while Medicago and Lotus the lowest and not different BNF (17.9 and 21.6 kg N ha^{-1}, respectively).

In the second year, fertilization again had significant effects on most traits, except KN, CCB and BNF. TKW increased at F2 (51.4 g), whereas ear m^{-2} was higher in F1 and F2 than F0 (101.6 ears m^{-2}). GY showed a progressive increase (F0<F1<F2), although with a smaller absolute gain than in the previous year. Both F1 and F2 produced significantly higher GPC than F0 (on average 14% vs 13.4%). WB was significantly higher at F2 than at F0 (0.30 and 0.09 Mg ha^{-1}, respectively), with F1 showing intermediate values. The BNF across F-rates averaged 19.1 kg N ha^{-1}. The cropping system effect in the second year remained significant, though TKW and KN did not differ among systems. Ear density was higher in Core(Med) and Core(Cnt) (on average 122.8 ears m^{-2}) than in Core(Lot) and Core(Trif) (on average 99.9 ears m^{-2}). Even in this year, Trifolium showed higher dry biomass than Lotus (0.81 and 0.47 Mg ha^{-1}), but similar to Medicago (0.60 Mg ha^{-1}), and the highest BNF (23.5 kg N ha^{-1}) as compared with Medicago and Lotus, which did not differ (on average 16.8 kg N ha^{-1}).

3.3 Effects of management practices on bread wheat performance

In the bread wheat Evo, significant fertilization × cropping-system interactions emerged for ear density and GPC in the first year (Supplementary Table 6). Evo(Trif) and Evo(Lot) at F0 showed the highest and lowest ears m^{-2}, respectively. The highest GPC was recorded in Evo(Med) at F2, and the lowest in Evo(Lot) and Evo (Cnt) at F0. In the second year, fertilization × cropping-system was

TABLE 1 Main effects and interaction of fertilization (F0, F1, F2), and cropping systems (CS) of durum wheat var. Core [Core(Cnt) – wheat-sole crop; wheat-Trifolium cover crop - Core(Trif); wheat-Medicago cover crop – Core(Med), wheat-Lotus cover crop – Core(Lot)], on wheat yield component (thousand kernel weight – TKW; number of ear per square meter - Ear m^{-2}, and number of kernel per ear - KN), grain yield (GY), grain protein content (GPC), cover crop dry biomass (CCB), weed dry biomass (WB), and biological N fixation (BNF) in the 2022/23 and 2023/24 growing seasons.

2022/2023	Treatments	TKW (g)	Ears (n. m^{-2})	KN (n. ear^{-1})	GY (Mg ha^{-1})	GPC (%)	CCB (Mg ha^{-1})	WB (Mg ha^{-1})	BNF (kg N ha^{-1})
Fertilization (F)	F0	40.1 ± 1.07b	277.5 ± 32.2a	29.5 ± 2.15b	1.72 ± 0.22c	11.4 ± 0.26b	1.06 ± 0.44a	0.37 ± 0.13b	33.6 ± 9.36a
	F1	49.9 ± 0.75a	309.0 ± 12.9a	37.1 ± 3.07a	2.74 ± 0.41b	11.9 ± 0.20b	1.10 ± 0.48a	0.30 ± 0.14b	35.4 ± 11.1a
	F2	48.9 ± 0.89a	297.5 ± 13.8a	39.9 ± 2.44a	3.73 ± 0.28a	12.6 ± 0.16a	1.13 ± 0.29a	0.69 ± 0.40a	44.1 ± 7.17a
Cropping system (CS)	Core(Cnt)	45.5 ± 2.32b	268.7 ± 17.5b	33.6 ± 3.31a	1.99 ± 0.51b	11.1 ± 0.27b	–	1.33 ± 0.46a	–
	Core(Trif)	43.6 ± 1.75b	320.6 ± 28.9a	30.2 ± 2.76a	2.32 ± 0.40ab	12.2 ± 0.28a	3.22 ± 0.31a	0.36 ± 0.16b	73.6 ± 3.77a
	Core(Med)	48.4 ± 1.64a	337.6 ± 16.4a	33.7 ± 3.26a	3.28 ± 0.44a	12.4 ± 0.21a	0.57 ± 0.22b	0.08 ± 0.03b	17.9 ± 4.80b
	Core(Lot)	48.0 ± 0.98a	252.0 ± 24.5b	34.3 ± 2.79a	3.34 ± 0.32a	12.1 ± 0.20a	0.56 ± 0.16b	0.13 ± 0.06b	21.6 ± 4.54b
F × CS		**	***	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
2023/2024									
Fertilization (F)	F0	48.2 ± 0.74b	101.6 ± 6.71b	33.5 ± 2.35a	1.37 ± 0.11b	13.4 ± 0.18b	0.41 ± 0.08a	0.09 ± 0.03b	16.9 ± 1.12a
	F1	47.7 ± 0.75b	118.4 ± 9.90a	35.9 ± 2.90a	1.59 ± 0.07ab	13.9 ± 0.08a	0.45 ± 0.10a	0.21 ± 0.07ab	18.6 ± 1.55a
	F2	51.4 ± 1.10a	123.4 ± 7.14a	36.2 ± 3.36a	1.83 ± 0.11a	14.1 ± 0.24a	0.55 ± 0.14a	0.30 ± 0.06a	21.7 ± 2.95a
Cropping system (CS)	Core(Cnt)	47.7 ± 1.21a	127.4 ± 4.98a	36.7 ± 2.95a	1.49 ± 0.11b	13.5 ± 0.31b	–	0.27 ± 0.05a	–
	Core(Trif)	48.4 ± 1.09a	96.4 ± 6.05b	29.3 ± 4.62a	1.44 ± 0.17b	14.0 ± 0.18a	0.81 ± 0.01a	0.14 ± 0.03b	23.5 ± 2.99a
	Core(Med)	50.2 ± 1.14a	118.2 ± 10.7ab	41.4 ± 2.28a	1.78 ± 0.11a	13.9 ± 0.15a	0.60 ± 0.05ab	0.11 ± 0.04b	16.9 ± 1.08b
	Core(Lot)	50.1 ± 1.05a	103.5 ± 10.4b	33.4 ± 1.38a	1.66 ± 0.07a	13.8 ± 0.17a	0.47 ± 0.13b	0.18 ± 0.02b	16.7 ± 0.46b
F × CS		*ns*	***	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

Means ± standard errors that do not share a letter are significantly different at P ≤ 0.05 according to Tukey's HSD *post-hoc* test. Significant interaction of F × CS at P ≤ 0.001 (***), at P ≤ 0.01 (**), or not significant (ns).

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significant only for the ear density: Evo(Med) at F2 showed the highest, and Evo(Trif) at F2 the lowest ears m^{-2}.

The fertilization effect in the first year significantly enhanced key yield traits, particularly TKW, GY, GPC, and CCB, while ear density, KN, WB, and BNF remained unaffected. In the second year, fertilization effects were weaker: only GY and GPC responded significantly to F-rates (Table 2). On the contrary, the cropping system exerted consistent and pronounced effects across both years. Evo(Med) and Evo(Lot) generally increased TKW (on average 44.2 g in the second year), whereas Evo(Med) and Evo(Trif) increased ear density (on average 261.8 in the first year, and 133.9 ears m^{-2} in the second) and produced higher GY (3.5 and 1.98 Mg ha^{-1} in the first and second year, respectively) and GPC (13.4 and 13.9% the first and second year, respectively) than Evo (Cnt) and Evo(Lot). Trifolium showed the highest dry biomass and BNF. Nonetheless, BNF was comparatively lower in the second (21.3 kg N ha^{-1}) than in the first year (46.6 kg N ha^{-1}). In contrast, Medicago and Lotus showed similar BNF, with lower values at first than in the second year (on average 13.0 vs 16.3 kg N ha^{-1}, respectively). The WB was higher in the Evo(Cnt) and Evo(Lot) as compared with Evo(Trif) in the first year, but similar in the second. Evo(Med) showed the significantly lowest WB in the first and second growing seasons (0.11 and 0.18 Mg ha^{-1}, respectively).

3.4 Comparative grain filling of wheat-cover crops and wheat sole-crops

The relationships between wheat kernel dry weight and growing degree days (GDD, °Cd) are shown in Figure 2. The models demonstrated strong statistical reliability, as evidenced by high R2 values (mostly above 0.95) and significant P-values (Supplementary Tables 7, 8). In 2022/23, favorable growing conditions extended grain-filling to 66 days and the GDD to 2784 °Cd, enabling a prolonged filling period. The kernel dry weight reached the asymptote at 2400 °Cd in both wheat species. In contrast, the 2023/24 season had a shorter grain-filling period (58 days) despite a similar thermal range (1600-2796 °Cd), shifting the plateau earlier (~2200 °Cd) in Core but extending it in Evo (~2500 °Cd). The shorter grain-filling period did not limit kernel growth but altered curve dynamics: the inflection point was delayed, the transition to maximum weight was steeper, and the plateau phase was shorter in both Core and Evo. Higher F-rates (F1 and F2) produced steeper growth curves and higher kernel dry weights than the F0, mainly with wheat-sole crops that showed the lowest overall values (35.6 mg in Core and 38.4 mg in Evo). The living mulch systems at F0 in Core increased the kernel dry weight by 14% with Trifolium, by 17% with Lotus, and by 22% with Medicago. Similarly, the kernel dry weight of Evo-sole crops increased by 16% with Trifolium, 14% with Medicago, and 10% with Lotus living mulch. The 95% confidence intervals (CI) indicate that control treatments experienced a marked year effect, with kernel dry weight in 2023/24 reduced by approximately 5–10 mg relative to 2022/23, mostly in fertilized treatments. In contrast, legume living mulch treatments, particularly Medicago and Trifolium, showed higher interannual stability, with mean year-to-year differences generally ≤3 mg and substantial CI overlap across all F-levels.

4 Discussion

4.1 Environmental conditions on crop phenology

The first growing season showed average air temperatures consistent with the climatic mean of the twenty-year average (Scordia et al., 2024) and rainfall evenly distributed between winter and spring. In contrast, the second season was on average 1.2 °C warmer, with sporadic rainfall and prolonged dry periods from early spring onward. These drier and warmer conditions caused an early and sharp decline in soil moisture, shortening the biological cycle of wheat and legumes, except for Lotus, which maintained its longer cycle (Blackwell et al., 2018).

Despite their early winter establishment, the annual legumes followed the typical winter-annual growth pattern, characterized by vegetative development through spring and natural senescence by late spring or early summer (Radicetti et al., 2018; Cougnon et al., 2022). Medicago was consistently the earliest-maturing species across fertilization levels and growing seasons (Enriquez-Hidalgo et al., 2020), whereas Trifolium senescence aligned with that of wheat (Scordia et al., 2024). The early senescence of Medicago, together with its low dry biomass, indicates relatively weak competition with both wheat, which were still in the early ripening stage when Medicago senesced. In contrast, Trifolium typically senesced around wheat over-ripe, and Lotus remained active until wheat harvest, although its lower dry biomass compared with Trifolium is expected to limit its competitiveness with cereals (Schneider et al., 2015).

Overall, the short growth cycle of Medicago and, to a lesser extent, Trifolium, together with the prostrate growth habit and low biomass yield (Lotus), align well with the living-mulch ideotype proposed by Cougnon et al. (2022).

4.2 Factors affecting wheat, cover crop, and weed

Over the growing seasons, both fertilization and cover cropping showed consistent effects on wheat performance. GY and GPC increased steadily with fertilization, while WB rose primarily under high fertilization levels. The higher WB under the highest F-rate confirms the inefficiency of organic N fertilization, likely due to partial N uptake by weeds. This pattern may indicate a mismatch between crop nutrient demand and the timing or availability of N supplied by organic fertilizers (Little et al., 2021). All cover-cropping systems improved GY and GPC and reduced WB compared with wheat sole-crop. Moreover, forage legumes contributed through BNF, with Trifolium being the most effective among species. No legacy effects were detected in the second year, despite repeated cropping on the same plots. While intercrops are generally expected to generate positive legacy effects, these are not guaranteed when cereals dominate legumes, and should be assessed over longer timeframes (Peltonen-Sainio et al., 2024). The poorer performance observed in the second year (2023/24) is therefore attributable to meteorological conditions rather than legacy effects or disease pressure.

TABLE 2 Main effects and interaction of fertilization (F0, F1, F2), and cropping systems (CS) of bread wheat Evolutivo popolation [Evo(Cnt) – wheat-sole crop; wheat-Trifolium cover crop - Evo(Trif); wheat-Medicago cover crop – Evo(Med), wheat-Lotus cover crop – Evo(Lot)], on wheat yield component (thousand kernel weight – TKW; number of ear per square meter - Ear m^{-2}, and number of kernel per ear - KN), grain yield (GY), grain protein content (GPC), cover crop dry biomass (CCB), weed dry biomass (WB), and biological N fixation (BNF) in the 2022/23 and 2023/24 growing seasons.

2022/2023	Treatments	TKW (g)	Ears (n. m^{-2})	KN (n. ear^{-1})	GY (Mg ha^{-1})	GPC (%)	CCB (Mg ha^{-1})	WB (Mg ha^{-1})	BNF (kg N ha^{-1})
Fertilization (F)	F0	37.9 ± 1.31b	242.0 ± 28.2a	28.8 ± 3.07a	2.64 ± 0.21b	11.2 ± 0.19c	0.34 ± 0.14c	0.31 ± 0.13a	15.2 ± 3.80a
	F1	41.5 ± 1.05ab	265.7 ± 19.5a	30.8 ± 2.83a	3.39 ± 0.18a	12.4 ± 0.10b	0.73 ± 0.23b	0.39 ± 0.12a	33.1 ± 8.78a
	F2	42.2 ± 1.10a	263.5 ± 10.4a	32.7 ± 2.22a	3.63 ± 0.26a	12.9 ± 0.05a	1.08 ± 0.25a	0.79 ± 0.28a	24.2 ± 4.79a
Cropping system (CS)	Evo(Cnt)	42.4 ± 2.16a	206.4 ± 14.0c	33.3 ± 3.31a	2.81 ± 0.27b	11.9 ± 0.30b	–	0.95 ± 0.28a	–
	Evo(Trif)	40.6 ± 0.74a	271.2 ± 9.54a	33.7 ± 3.32a	3.54 ± 0.31a	12.2 ± 0.21ab	2.20 ± 0.34a	0.41 ± 0.14b	46.6 ± 6.27a
	Evo(Med)	41.7 ± 0.91a	252.4 ± 10.5b	33.0 ± 2.70a	3.47 ± 0.26a	12.5 ± 0.13a	0.42 ± 0.08b	0.11 ± 0.01c	13.4 ± 2.10b
	Evo(Lot)	42.6 ± 0.93a	190.7 ± 18.2c	31.2 ± 2.30a	2.57 ± 0.21b	12.0 ± 0.26b	0.27 ± 0.06b	0.62 ± 0.15ab	12.5 ± 1.87b
F × CS		*ns*	*	*ns*	*ns*	**	*ns*	*ns*	*ns*
2023/2024									
Fertilization (F)	F0	42.2 ± 0.98a	123.6 ± 9.80a	32.4 ± 3.07a	1.57 ± 0.09b	13.4 ± 0.17b	0.41 ± 0.08a	0.28 ± 0.07a	16.4 ± 0.59a
	F1	42.4 ± 1.61a	133.7 ± 10.3a	32.2 ± 4.08a	1.82 ± 0.07b	13.8 ± 0.11a	0.47 ± 0.10a	0.33 ± 0.08a	19.1 ± 1.39a
	F2	44.3 ± 1.55a	137.4 ± 13.8a	31.8 ± 4.16a	2.13 ± 0.09a	14.0 ± 0.24a	0.43 ± 0.09a	0.45 ± 0.11a	18.3 ± 1.44a
Cropping system (CS)	Evo(Cnt)	40.1 ± 1.14b	116.0 ± 11.2b	31.2 ± 2.38a	1.68 ± 0.06b	13.3 ± 0.26c	–	0.43 ± 0.08a	–
	Evo(Trif)	41.4 ± 1.42b	127.6 ± 15.3ab	32.0 ± 4.61a	1.94 ± 0.09ab	13.9 ± 0.14a	0.75 ± 0.06a	0.38 ± 0.09a	21.3 ± 1.47a
	Evo(Med)	45.4 ± 0.85a	140.1 ± 8.50a	36.1 ± 3.49a	2.02 ± 0.10a	13.9 ± 0.15a	0.58 ± 0.03ab	0.18 ± 0.04b	16.8 ± 0.29b
	Evo(Lot)	43.1 ± 1.30a	121.3 ± 9.00b	34.4 ± 4.66a	1.72 ± 0.11b	13.7 ± 0.17b	0.40 ± 0.01b	0.42 ± 0.10a	15.7 ± 0.73b
F × CS		*ns*	***	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*

Means ± standard errors that do not share a letter are significantly different at P ≤ 0.05 according to Tukey's HSD *post-hoc* test. Significant interaction of F × CS at P ≤ 0.001 (***), at P ≤ 0.01 (**), at P ≤ 0.05 (*), or not significant (ns).

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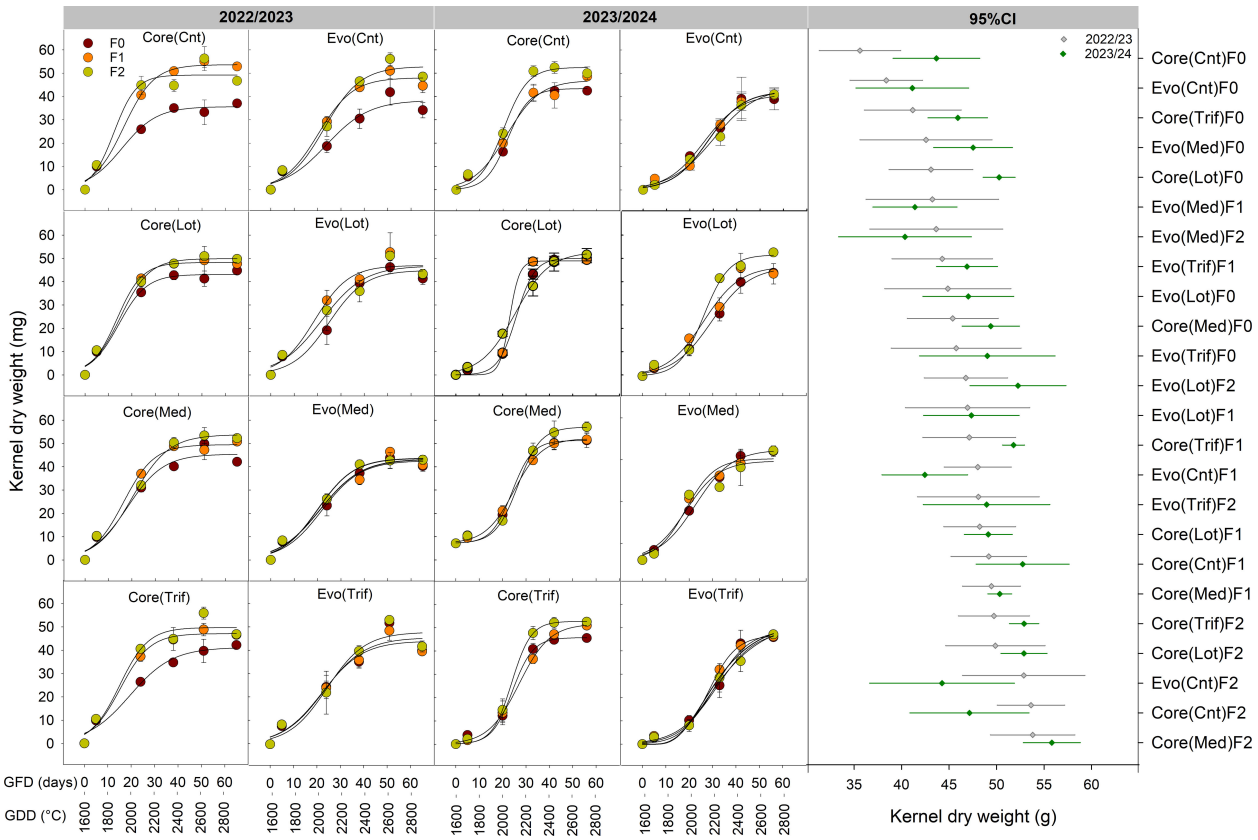

FIGURE 2
Dry matter in wheat kernel during the grain filling (GFD, days) or accumulated thermal time (°Cd) of durum wheat (Core) and bread wheat (Evo) sole crops (Cnt) or with forage legume living mulch (*T. subterraneum* – Trif; *M. polymorpha* – Med; *L. corniculatus* – Lot) under three organic fertilization rates (F0, F1 and F2) in the two growing seasons (2022/23 and 2023/24). The right bar chart represents the kernel asymptotic values and 95% confidence intervals of means (95%CIs). Non-overlapping 95%CIs indicate significantly different kernel dry weights.

In the second growing season, an unprecedented drought in southern Italy caused wheat yield losses of up to 70% in many farms in Sicily, primarily due to poor seed set and reduced KN and weight (Simenza Farmer Association, pers. comm.). At the study site, a pronounced dry period occurred immediately after sowing in December, with only 2.2 mm of rainfall recorded. Although winter conditions were relatively wet and post-anthesis stress was not severe enough to markedly affect yield components, grain yield (GY) declined in the warmer, drier year. This reduction is attributable to lower ear density in both wheat crops, resulting from early drought stress that caused seedling stunting and subsequent desiccation. TKW and KN remained stable in Evo and showed only a slight decline in Core, while GPC increased in both wheat species. These stable kernel weights under heat stress contrast with previous findings (Cosentino et al., 2019; Groli et al., 2024). The observed increase in GPC likely results from the reduced number of filled ears, which concentrate assimilates in the remaining grains (Dinelli et al., 2013; Anastasi et al., 2019). CCB and WB were also reduced in the warmer year, and the average N-fixing capacity of cover crops declined as well. Peoples et al. (2009) reported that for every ton of shoot dry matter produced, symbiotic legume-rhizobia associations fix approximately 30–40 kg N ha^{-1} on a whole-plant basis (including shoots and nodulated roots). The BNF values observed in this study in the warmer year fall within the lower range reported by the EU Nitrogen Expert Panel (2016), which estimates that clovers can fix approximately 20 kg N ha^{-1} yr^{-1} in extensive systems, but up to 250 kg N ha^{-1} yr^{-1} in intensive systems. The superior BNF capacity of *T. subterraneum* relative to other annual forage legumes, including *M. polymorpha*, is consistent with evidence from pot experiments using elite, species-specific rhizobial strains, where it achieved the highest above- and below-ground N yields and overall fixation efficiency (Scavo et al., 2025). Despite some N diversion to weeds at the highest F-rate, increasing N inputs did not enhance BNF. This aligns with established evidence that external N promotes direct N uptake by legumes, while elevated soil N availability suppresses symbiotic fixation by reducing nodule formation (Ladha et al., 2022).

In contrast, fertilizer application consistently enhanced TKW, GY, and GPC, confirming its critical role in optimizing wheat performance. Legume cover crops compensated for reduced fertilization, improving or stabilizing yield components in both Core and Evo. Legume cover crops, particularly Medicago, exerted a strong weed-suppressive effect relative to wheat sole crops, reaching -88% weed dry biomass in Evo(Med) and -94% in Core(Med) in 2022/23. These findings agree with Clark (2007), who emphasized the suitability of *M. polymorpha* for weed management in low-input and organic systems. The strong weed-suppressive effect observed in legume-based treatments was likely driven by competition for space, light, water, and nutrients (Mahé et al., 2022). This effect was amplified by the additive design in a mixed arrangement, where

increased relative seeding density promotes a selection effect arising from species with contrasting weed-suppressive abilities (Bastiaans and van der Werf, 2025). Additive designs may also increase overall crop productivity through enhanced resource capture linked to greater plant density rather than interspecific complementarity (Yu et al., 2016), while mixed arrangements can intensify interspecific interactions, strengthening species dominance over weeds (Gu et al., 2025). In spring, legume living mulches provided continuous soil cover, fostering competitive interactions that limited the emergence of spring-summer weeds. The weed flora was dominated by spring-summer therophytes, a community structure typical of semi-arid agroecosystems (Scavo et al., 2024). Although less effective than Medicago, both Trifolium and Lotus reduced the density of spring-germinating annual weeds by wheat anthesis, consistent with patterns reported by Hiltbrunner et al. (2007). Early weed suppression, likely mediated by shading, may have constrained weed growth, while at later stages, the reduced height of the legumes may have favored wheat over taller weeds. Reduced weed pressure during critical phenological stages, such as flowering and grain filling, is likely a key mechanism underlying the improved wheat performance observed in this organic system with living mulches.

In contrast to other studies, our results did not show significant wheat yield penalties. For instance, temporary intercropping with Persian clover (*Trifolium resupinatum* L.) increased grain protein but often reduced wheat yield by ~20% (Pellegrini et al., 2021). Likewise, subclover (*Trifolium subterraneum* L.) living mulches suppressed weeds by 22-75% across European and Mediterranean environments, but yield reduction (-14 to -16%) occurred where subclover biomass was high, while yields remained unaffected where subclover growth was limited (Radicetti et al., 2018). Even larger wheat yield decreases (-19% to -81%) have been reported for perennial living mulches such as red fescue (*Festuca rubra* L.), sheep's fescue (*Festuca ovina* L.), alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), black medic (*Medicago lupulina* L.), and white clover (*Trifolium repens* L.), mainly due to competition for light linked to high mulch biomass (Carof et al., 2007a; Carof et al., 2007b).

Here, the low cover crop biomass under the additive, fully mixed design likely limited competition with wheat, reflecting the strong dominance of wheat over the companion legume species. This highlights how combined life cycles and management design critically shape competition and yield outcomes in cereal-legume systems. Additionally, the contrasting responses of the two wheat types underscore the importance of species-specific adaptability. The modern, short-statured durum wheat Core and the taller, more heterogeneous bread wheat Evo showed distinct responses when grown with legume cover crops. In fact, living mulches produced positive but species and climate-dependent effects on yield components. In Core, the lowest yielding legumes, Medicago and Lotus living mulches, were the most beneficial in the favorable climatic conditions (2022/23), increasing GY by +39% and +40%, respectively, through higher TKW (+5-6%) and stable or slightly improved KN; in contrast, Trifolium raised GY by +14% mainly via increased ear density (+16%). In 2023/24, the warmer and drier season reduced ear number sharply, especially with Trifolium (-43%), leading to a slight GY decline (-3%) as compared with the control, whereas Medicago still increased GY by +16% and Lotus by +10% due to higher TKW (+5%) and partially of KN. A similar pattern emerged in Evo in 2022/23, where Trifolium and Medicago enhanced GY (+21% and +19%) due to strong increases in ear density (+24% and +18%), despite small reductions in TKW (-4% and -2%). In contrast, Lotus reduced multiple yield components, resulting in a 9% GY loss. Under stress conditions in 2023/24, all legumes improved TKW in Evo (+3% to +12%), with Medicago again showing the highest GY increase (+17%), followed by Trifolium (+13%), while Lotus produced a similar GY to the control. Across both wheat species and seasons, GPC consistently increased (+1% to +10%), reflecting enhanced N availability and, in the stressful year, concentration effects due to reduced sink capacity. Remarkably, unfertilized Medicago living mulch achieved protein concentrations comparable to those of fertilized systems, indicating that it can offset reduced N inputs. These findings are consistent with previous studies showing that the legume component in wheat enhances biological N_2 fixation, soil N uptake, and grain protein content (Rodriguez et al., 2020; Pellegrini et al., 2021), particularly under low-input conditions (Bedoussac et al., 2015).

4.3 Wheat grain filling in mixed and sole-cropping

In the first season, relationships show faster transitions into the exponential growth phase, gradual slopes, and longer plateau phases in most treatments, indicating more favorable growing conditions. The second season shortened grain-filling dynamics, with delayed entry into the exponential phase but steeper transition from low to high dry matter accumulation, and reduced plateau phases, reflecting more challenging environmental conditions during critical grain-filling stages.

Although asymptotic kernel dry weight overlapped (95% CIs) in most treatments and seasons between Core and Evo, the former presents a faster transition to the exponential phase, suggesting an earlier onset of active grain filling in a slightly shorter period. This reflects the inherent characteristics of bread wheat, which thrives under cooler temperatures and higher soil moisture (López-Castañeda and Richards, 1994; Trethowan et al., 2001). The warmer season exacerbated these patterns, delaying the plateau phase in Evo but shortening it in Core.

Medicago living mulch enhanced resource complementarity, accelerating grain filling and achieving higher kernel weights in a shorter period. Trifolium living mulch followed a similar pattern, though with a slightly slower grain-filling rate and a delayed plateau phase, likely due to competitive interactions, particularly with Core wheat (Scordia et al., 2024). In contrast, Lotus living mulch led to only moderate improvements in kernel dry weight relative to the control and living mulch with Evo, possibly due to its slower growth and perennial habit, which may have favored wheat selection effects or shifted intercrop dynamics (Mahmoud et al., 2022).

The unfertilized treatment (F0), particularly in wheat-sole crops, entered the exponential phase later, displayed more gradual growth curves, and produced the lowest kernel dry weights, indicating clear nutrient limitations. Growth rates improved under moderate fertilization (F1) and were highest under F2. Core

showed a stronger response to increasing fertilization, with steeper slopes and higher kernel weights, whereas Evo exhibited only incremental gains, consistent with its reduced ability to exploit high N levels or its better adaptation to low-input systems (Bertholdsson et al., 2016; Bocci et al., 2020). These patterns align with Marti and Slafer (2014), who suggested that bread wheat is generally better suited to sub-optimal Mediterranean environments, likely reflecting a less intensive breeding history compared with durum wheat (Pfeiffer et al., 2001). Furthermore, Evo population tested here underwent Sicilian farmer selection, which evolved into locally adapted sub-populations, with farmers becoming the producers of seed throughout the years. It has been shown that populations resulting from natural selection often outyield commercial varieties in the location in which they evolved (Bocci et al., 2020). In this regard, Evo showed greater stability across cropping systems, while Core benefited mainly from low-selection-effect species, such as the earliest (e.g., Medicago) and poor-yielding ones (e.g., Lotus and Medicago), or high F-rates. Although N fertilization can shift dominance toward cereals (Mahmoud et al., 2022), non-overlapping 95%CIs between F0 or F1 with F2 in most living mulch systems, particularly with Medicago combined with Core or Evo, highlight effective resource complementarity at reduced fertilization while maintaining optimal grain filling dynamics. Correspondingly, CI widths were consistently narrower in legume-based systems, indicating lower variability and enhanced stability of kernel dry weight under changing management and environmental conditions. The meta-analysis of Zhao et al. (2022) on legume-based rotation demonstrated that when N supply already meets crop demand, the added value of legume-derived N levels off. Thus, crop yield benefit is achieved particularly under low-N and low-yielding conditions, such as those of organic systems. While the beneficial legume effect is largely attributed to the N supplied through BNF, non-N mechanisms might also contribute. For example, beyond the reduced weed competition to wheat in legume living mulch systems, hydrogen released from nodules during N_2 fixation can stimulate soil biological activity, further improving crop performance (Peoples et al., 2009), as well as reduced wheat intraspecific competition and improved access to soil N compared with sole crops (Peoples et al., 2009; Jensen et al., 2020).

5 Conclusion

This study addressed a critical research gap in Mediterranean organic wheat systems by evaluating forage legume living mulches for simultaneous weed suppression and nitrogen supply in both bread and durum wheat. The additive design with fully mixed arrangements promoted a selection effect driven by species with contrasting weed-suppressive capacities, enhancing dominance over weeds. Strong weed suppression and, to some extent, biological N fixation, particularly under low fertilization, demonstrated the capacity of living mulches to sustain grain yield and quality.

High fertilization accelerated grain filling and increased kernel weight in wheat sole crops, whereas living mulches mitigated nutrient stress, narrowing the kernel weight gap between low and high fertilization levels. The modern durum wheat (Core) grown with low-yielding forage legume, Lotus or Medicago, and the evolutive bread wheat (Evo) grown with Trifolium or Medicago achieved the highest grain yields, with Evo showing greater kernel weight stability across cropping systems. Grain protein content peaked under medium to high fertilization in wheat sole crops, yet living mulch systems maintained or improved protein levels across fertilization rates in both wheat, as compared with the wheat sole crops.

Overall, these responses were consistent across contrasting climatic conditions, indicating that annual forage legumes can be integrated as living mulches in organic wheat without compromising yield, while improving weed control and reducing reliance on external nitrogen, particularly under low-input conditions. Future research should refine species mixtures, seeding ratios, and spatial arrangements to gain deeper insights into the temporal dynamics of species interactions during the growing season and assess long-term legacy effects under variable climates.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

DS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. FC: Data curation, Investigation, Methodology, Visualization, Writing – review & editing. AM: Data curation, Investigation, Methodology, Visualization, Writing – review & editing. TM: Data curation, Investigation, Methodology, Visualization, Writing – review & editing. MO: Data curation, Investigation, Methodology, Project administration, Resources, Visualization, Writing – review & editing. AS: Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. FG: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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