agronomy

Article

Adoption of Cereal–Legume Double Cropping toward More Sustainable Organic Systems in the Mediterranean Area

Danilo Scordia [1], Paolo Guarnaccia [2,*], Francesca Calderone [1], Aurora Maio [1], Tommaso La Malfa [1], Aurelio Scavo [1] and Fabio Gresta [1]

[1] Department of Veterinary Sciences, University of Messina, Viale G. Palatucci, 98168 Messina, Italy; danilo.scordia@unime.it (D.S.); francesca.calderone1@studenti.unime.it (F.C.); auroramaio18@gmail.com (A.M.); tommasolamalfa@hotmail.it (T.L.M.); aurelio.scavo@unime.it (A.S.); fabio.gresta@unime.it (F.G.)
[2] Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, 95123 Catania, Italy
* Correspondence: paolo.guarnaccia@unict.it

Abstract: Environmental benefits can be achieved by organic farming systems; however, weed pressure and timely crop nutrition remain important drawbacks for many field crops. Agroecological practices, such as double cropping (e.g., intercropping and relay cropping), using forage legume species can provide nitrogen (N) to the companion crop through biological N fixation and tackle weed issues by competing for light, water and land. The present study investigated the effect of intercropping (IC) and relay-cropping (RC) systems of durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn) and forage legumes (*Trifolium subterraneum* L., *Medicago polymorpha* L., and *Lotus corniculatus* L.) by varying organic N fertilization with the aim to reduce N-requirement and weed pressure and increase wheat grain yield and grain protein content in Mediterranean organic farming systems. N fertilizer significantly improved wheat grain yield and grain protein, while a null effect on legume and weed biomass yields was found. Double cropping (*T. durum-M. polymorpha*, and *T. durum-L. corniculatus*) enhanced wheat grain yield as compared to the control and the *T. durum-T. subterraneum*. IC significantly improved legume yield, grain protein and the land equivalent ratio (LER) and reduced weed dry biomass as compared with the RC and the control. Among legume species, *T. subterraneum* outperformed the others and was less affected by the wheat's competitive performance. Nonetheless, *M. polymorpha* was as effective as *T. subterraneum* in controlling weeds. Weed dry biomass was linearly reduced by increasing legume yield; the relationship improved by cumulating wheat grain yield to legume yield. Overall, this study indicated that double cropping, especially IC, can be a suitable agroecological practice to tackle weed issues and reduce N-requirement in Mediterranean organic cereal-based systems.

Keywords: agroecology; mixed-cropping; intercropping; relay-cropping; conservative agriculture; crop yield; land equivalent ratio

Citation: Scordia, D.; Guarnaccia, P.; Calderone, F.; Maio, A.; La Malfa, T.; Scavo, A.; Gresta, F. Adoption of Cereal–Legume Double Cropping toward More Sustainable Organic Systems in the Mediterranean Area. *Agronomy* **2024**, *14*, 772. https://doi.org/10.3390/agronomy14040772

Academic Editor: Christos A. Dordas

Received: 4 March 2024
Revised: 2 April 2024
Accepted: 4 April 2024
Published: 9 April 2024

1. Introduction

The European green transition aims to reach greenhouse gas emissions neutrality by 2050 [1]. In the agricultural sector, the eco-schemes set in the new CAP (2023–2027) target to achieve at least 25% of the EU's agricultural land under organic farming, to reduce nutrient losses by at least 50% and to reduce the use of fertilizers by at least 20% by 2030. Organic production can represent an interesting opportunity for farmers; however, dissecting agronomic practices to reduce the overall energy use per kg of product and increase the land use efficiency is essential [2,3].

Cereal production is of utmost importance for "Made in Italy" products, such as pasta and baked goods. The Italian cereal area is around 3.5 Mha, and 8.7% is represented by organic productions [4]. Organic cereal areas account for 39% of total organic arable land,

and durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn) shares the largest area (41.8% of total cereal area), mainly in southern Italy (Sicily and Apulia) under more semiarid environments [5].

Although the agronomic techniques are quite consolidated for organic systems at a national level, weed pressure and timely crop nutrition remain important drawbacks for many field crops; hence, agroecological practices that make better use of natural resources could represent a possible solution [6]. Intercropping, i.e., growing two or more crops on the same field at the same time [7], and relay cropping, i.e., multiple crops planted on different dates and cultivated together for at least part of their life cycle [8], using forage legume species in double cropping can provide nitrogen to the companion crop through biological nitrogen (N) fixation [9,10] and phosphorus [11], and tackle weed issues by competing for light, water and land [12].

Previous studies have shown that intercropping durum wheat–field beans to produce forage increased dry matter yield, N yields and quality as compared to the sole cropping in Central Italy [13]. In relay cropping, weed density and biomass were negatively correlated with cover crop biomass at wheat harvest in late autumn in France [14]. Furthermore, the presence of a cover crop decreased the density of spring-germinating annual weeds for subsequent spring crops [14]. Also, intercropping oat and forage legumes significantly reduced weed dry biomass, more than the legume monocrop in a continental environment [15]. Nonetheless, it has been pointed out that an appropriate choice of legume species and knowledge of local environmental conditions and management are essential to support the successful implementation of double cropping in Mediterranean cereal-based cropping systems [16]. Interspecies competition in double cropping might affect plant development, productivity, and soil fertility [17]; hence, plant selection and management should be carefully designed to ensure diversity in terms of growth rates, shading ability and morphology, and to minimize competitive effects between the cash crop and the undersown crop [14,16,18–20].

Although the benefits these systems can provide, little research has been tackled in low-input organic farming and suboptimal pedo-climatic conditions (environments prone to high temperature, uneven rainfall distribution, water deficit, and unfertile soils), such as those of southern Italy.

The present study investigated the effect of intercropping and relay-cropping systems of durum wheat–forage legumes by varying the organic fertilization with the aim of reducing N-requirement and weed pressure in semiarid Mediterranean organic farming systems. Three Mediterranean forage legumes (*Trifolium subterraneum* L., *Medicago polymorpha* L., and *Lotus corniculatus* L.), adapted to semiarid climates, were selected based on predominant winter growth, different growth habits and life cycle length to maximize competition to weeds and to provide facilitative effects to the cash crop. *M. polymorpha* is an annual self-seeding species with sprawling or semi-erect growth habit and it matures early in the season [21]. *T. subterraneum* is an annual self-seeding with a medium maturity cycle, which is highly adaptable as living mulch in Mediterranean climates [22]. It has prostrate growth habit and plagiotropic pubescent stems [21]. *L. corniculatus* is a perennial with an erect growth habit, a slow growth rate at the establishment and a medium-to-late maturity cycle [23].

2. Materials and Methods

2.1. Field Trial Set-Up

The field trial was carried out in an organic farm located in Patti (Messina, 38°11′ N, 14°99′ E) in a clay soil in 2022/23. Main soil physical–chemical properties and hydrological constant were as follows: 57.6% clay, 10.1% silt, 32.3% sand; pH 7.8, organic matter 1.2 g kg^{-1} (Walkley and Black method); total $CaCO_3$ 3.0 g kg^{-1} (Scheibler method), total nitrogen 0.40 g kg^{-1} (Kjeldhal method), available P 78.3 mg kg^{-1} (Olsen method), available K 42.6 mg kg^{-1} (ammonium acetate test method); 35% soil field capacity, 15% soil wilting point and 1.3 g cm^{-3} soil bulk density.

The soil was plowed in late summer and dish harrowed in autumn at 30 cm and 20 cm depth, respectively. A light soil milling was performed just before sowing.

In a split–split–plot experimental design with three replications, the main plot was assigned to the organic fertilization distributed at soil milling, which was calculated as targeted N amount in three levels: 0, 60 and 120 kg N ha^{-1} (hereinafter N0, N1 and N2, respectively). The organic fertilizer (Organagro, Choncimer srl, San Severino Marche, Italy) had the following composition: N 3.10%, P_2O_5 2.96%, K_2O 2.0%, S 2.0%, total Ca 5.10%, Fe 0.42%, total Mg 0.74%, Mn 0.05%, Zn 0.03%, Cu 0.035%, and pH 7.05.

Durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn, var. Core) was sown alone (control), or associated with forage legumes (*Trifolium subterraneum* L. var. Urana, *Lotus corniculatus* L. var. Gran San Grabriele, *Medicago polymorpha* L. var. Scimitar) in the sub-plot (Figure 1). The cropping system, namely intercropping and relay cropping (hereinafter IC and RC, respectively), was assigned to the sub-sub-plot. Durum wheat seeds were purchased from Centrale Zoo Agricola S.r.l. (Misterbianco, Catania, Italy), while *Medicago polymorpha* and *Trifolium subterraneum* from Padana sementi (Tombolo, Padua, Italy). *Lotus corniculatus* seed were kindly provided by MAS Seeds (San Pietro di Morubio, Verona, Italy).

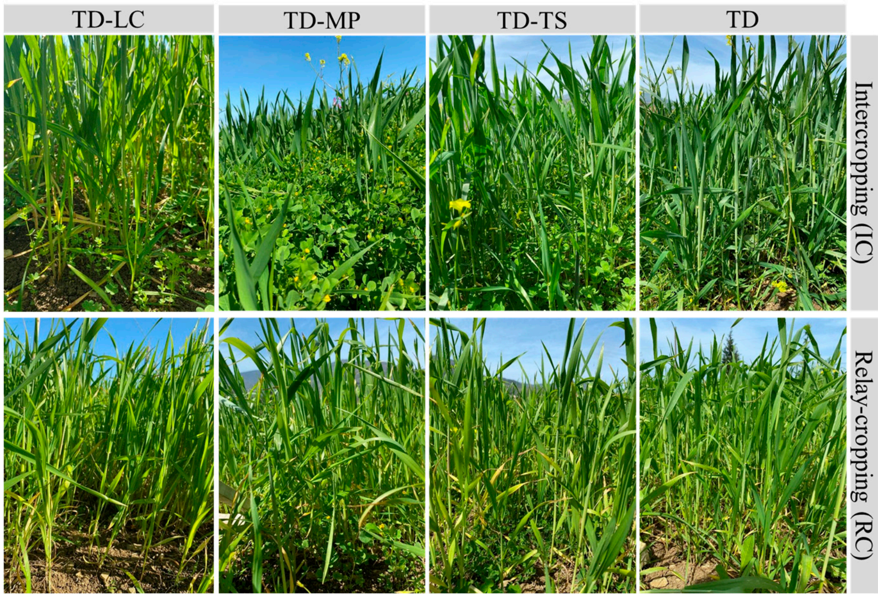

Figure 1. Pictures of *Triticum durum–Lotus corniculatus* (TD-LC), *Triticum durum–Medicago polymorpha* (TD-MP), and *Triticum durum–Trifolium subterraneum* (TD-TS) mixtures, compared to durum wheat sole crop (TD), arranged in intercropping (IC) and relay cropping (RC) in N_2 fertilization plots (120 kg N ha^{-1}).

In the IC system, the sowing of durum wheat and forage legumes was carried out on 3 January 2023. Species were manually broadcasted at about 3–4 cm depth, at a seed density of 250 kg ha^{-1} (*T. durum*), 35 kg ha^{-1} (*T. subterraneum*), and 25 kg ha^{-1} (*L. corniculatus* and *M. polymorpha*), followed by soil rolling.

In the RC, durum wheat was sown at the same date, density, and method, while the seed density of legume species was increased by 50% as compared with the IC, to account for possible seed predation, and manually broadcasted at durum wheat tillering stage (28 February 2023). In both cropping systems, a single plot measured 40 m^2 (5 m × 8 m). In adjacent plots, forage legume monocrops were grown in triplicate in the unfertilized treatment alone (N0) to determine the durum wheat's competitive performance.

Throughout the growing season, neither top dressing fertilization nor pre and post-sowing weed, pest and disease control was performed.

2.2. Measurements

The historical (i.e., twenty-year average) maximum and minimum air temperatures, rainfall and reference evapotranspiration, and the daily meteorological parameters of the current growing season were provided by the weather station of the SIAS (Servizio Informativo Agrometeorologico Siciliano, www.sias.regione.sicilia.it) located about 3 km from the experimental field.

Water deficit (mm) was calculated as the difference between the soil field capacity (35% of dry soil weight) and the soil wilting point (15% of dry soil weight) in a soil bulk density of 1.3 g cm^{-3} and a rooting depth of 0.4 m. This was increased by the rainfall and decreased by the durum wheat potential evapotranspiration. Throughout the growing season, water stress occurred when the soil moisture was lower than 20% of the available water content [24].

The main phenological stages of forage legumes were detected on a decimal scale following the BBCH system [21]. Here, we detected the principal growth stages, from germination to emergence, formation of side shoots to stem elongation, and flowering to senescence, which were equated to the phenology of durum wheat. A stage was considered accomplished when the 50% of plants inside each treatment and replication reached the specific stage.

Harvest was carried out at the durum wheat grain harvest maturity (i.e., 12% moisture content) by collecting durum wheat, legume species and weeds in 0.25 m^2 quadrats in each treatment and replication on 7 July 2023. Fresh samples of legumes and weeds were placed in a ventilated oven at 65 °C, up to constant weight, for the dry matter determination.

Durum wheat grain and straw were separated by a mechanical thresher. The grain yield, legume yield and weed dry biomass were derived and referred to the unit land area (Mg ha^{-1}). Grain total nitrogen was determined according to the Kjeldahl method and grain protein content was calculated (Kjeldahl N × 5.7) on a dry weight basis.

The land equivalent ratio (LER) was calculated as the yield of the double crop in the IC or the RC over the control sole crop [25]. At a LER >1, the amount of land required by the double-crop system is less than that of the sole crop to produce an equal yield. Conversely, at a LER < 1, more land is required for the double crop to be as productive as the sole crop [26].

In both IC and RC, the durum wheat competitive performance (CP) was expressed as the reduction in aboveground dry mass of forage legume monocrop in the unfertilized treatment (N0), as follows [15]:

$$CP_w = \left(\frac{P_{lci} - P_{ldi}}{P_{ldi}} \right) \times 100 \tag{1}$$

where CP_w is the relative competitive ability of the durum wheat; P_{lci} is the aboveground dry biomass of the forage legume *i* grown in a monocrop; and P_{ldi} is the aboveground dry biomass of the forage legume *i* grown in a double crop.

2.3. Statistical Analysis

Data on phenology, wheat grain yield and protein content, legume dry yield and weed dry biomass, and land equivalent ratio were statistically analyzed according to the split–split–plot experimental design, with cropping system, N fertilization and crop species as a fixed effect (CoHorth Software, CoStat version 6.003, Lighthouse Avenue, Monterey, CA, USA). Durum wheat competitive performance (CP_w) was analyzed by the split-plot design since forage legume monocrop yields were determined in the unfertilized treatment only.

Before conducting the ANOVA, the Bartlett's test was run to verify the assumption of homogeneity of variances. Percentage data were arcsine $\sqrt{\%}$ transformed before the analysis. Differences between means were evaluated for significance using the Tukey's HSD test at a 95% confidence level.

Relationships between durum wheat grain yield, legume dry yield and weed dry biomass were calculated by linear models. The Shapiro–Wilk test was used to test residuals for normality. Coefficients were considered significant at $p \leq 0.05$. The goodness of fit was assessed by calculating R^2 (SigmaPlot 11, Systat Software version 11 Inc., San Jose, CA, USA).

3. Results

3.1. Meteorological Trend and Crop Phenology

Figure 2A shows the twenty-year average rainfall, minimum and maximum air temperature of the study area and the daily 2022/23 growing season. The yearly average of historical minimum and maximum air temperature was 12.9 and 22.8 °C, respectively, and very similar to the 2022/23 growing season (13.0 and 23.0 °C, respectively). After sowing, the monthly minimum and maximum air temperatures were slightly warmer than the historical average in January (+0.12 °C and +0.84 °C), March (+0.39 °C and +0.39 °C) and July (+0.84 and +2.69 °C), while they were slightly cooler in February (−0.49 °C and −0.64 °C), April (−1.03 °C and −1.07 °C), May (−0.18 °C and −1.76 °C) and June (−0.36 °C and −1.38 °C). The twenty-year average precipitation was 783.16 mm, while 689.20 mm was registered in 2022/23. November, April, May and June were wetter in 2022/23 (+38.45, +70.52, +75.06 and +37.89 mm, respectively), while January, February, March and July were drier (−21.45, −54.30, −43.13 and −20.47 mm, respectively) than the historical trend.

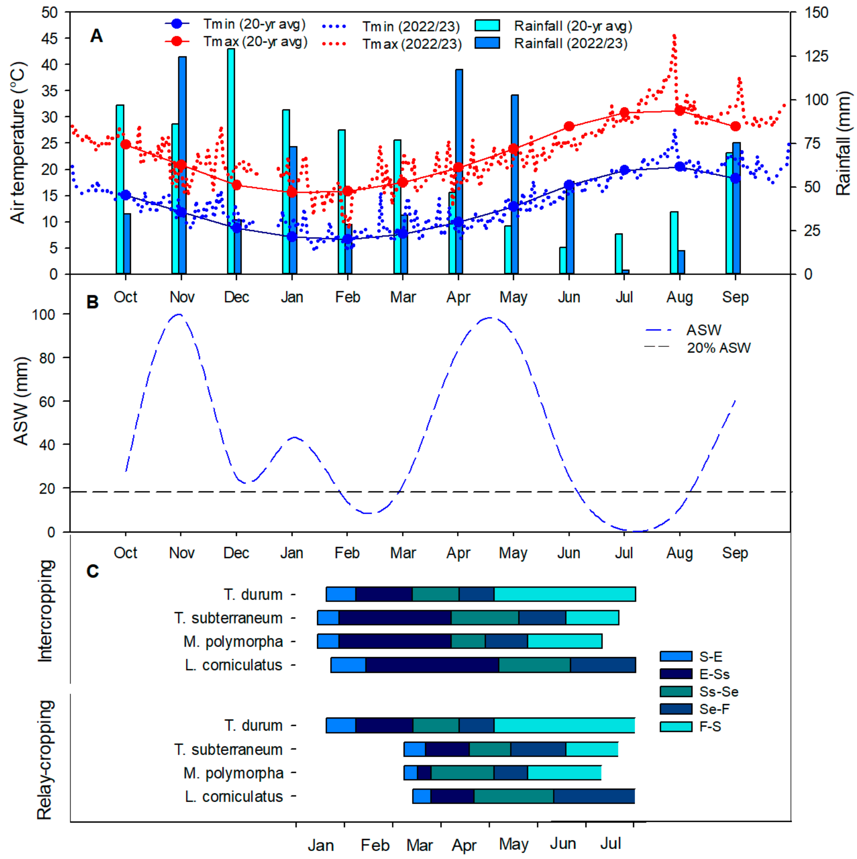

Figure 2. (**A**) Historical (20-year average) and daily meteorological trend of the 2022/23 growing season in Patti (Messina, 38°11′ N, 14°99′ E); (**B**) simulated available soil water (ASW) content at a depth of 0–40 cm throughout the growing season; (**C**) main phenological intervals (sowing–emergence—S-E, emergence–side shoot formation—E-Ss, side shoot–stem elongation—Ss-Se, stem elongation–flowering—Se-F, flowering–senescence—F-S) in the relay-cropping and intercropping systems of *Triticum turgidum* subsp. *durum*, *Trifolium subterraneum*, *Lotus corniculatus* and *Medicago polymorpha*.

The water deficit throughout the durum wheat and legume species growing season was below the 20% available soil water (ASW) content at the first stage of crop development in the IC and continued this trend also in late February and March after sowing legume species in the RC (Figure 2B). Significant rainy events in spring raised the ASW, which peaked in May followed by a remarkable decline in the last phase of crop senescence and up to the harvest in July.

The ANOVA showed a significant effect of crop, cropping system and their interactions on crop phenology, while neither nitrogen nor cropping system in the flowering–senescence stage were significant (Table 1).

Across the average of treatments, the IC system showed longer germination–emergence (16.4 vs. 13.2 days), emergence–side shoot (47.3 vs. 20.1 days), and side shoot–stem elongation stages (42.8 vs. 32.3 days) as compared with the RC (Figure 2C). On the contrary, the stem elongation–flowering stage was longer in the RC (35.9 vs. 29.5 days), while no differences were detected in the flowering–senescence stage (57.7 and 53.2 days in the IC and the RC, respectively). Among species, *T. durum* sole crop had the longest germination–emergence (18.5 days, respectively), *T. subterraneum* the longest emergence–side shoot (41.8 days), and *L. corniculatus* the side shoot–stem elongation and stem elongation–flowering stages (54.4 and 58.2 days, respectively). *T. subterraneum* and *M. polymorpha* showed the longest emergence-side shoot stage (41.8 and 39.1 days). The flowering–senescence stage was the longest in *T. durum* (85.5 days) followed by *M. polymorpha* (56.2 days); this stage was not accomplished in *L. corniculatus*. *T. durum* and *M. polymorpha* showed the shortest side shoot–stem elongation (28.5 and 29.1 days, respectively), and stem elongation–flowering stages (21.5 and 22.6 days, respectively).

Table 1. ANOVA for main effects and interactions on the length of phenological stages (sowing–emergence—S-E; emergence–side shoot formation—E-Ss, side shoot–stem elongation—Ss-Se, stem elongation–flowering—Se-F, flowering–senescence—F-S). Degree of freedom (df), mean square (MS) and significance: not significant (ns), $p \leq 0.001$ (***) and $p \leq 0.01$ (**).

Source	df	S-E	E-Ss	Ss-Se	Se-F	F-S
				MS		
Blocks	2	5.79 ns	48.1 ns	3.76 ns	76.2 ns	358.3 ns
Nitrogen (N)	2	0.09 ns	3.45 ns	1.09 ns	82.2 ns	197.7 ns
Main-plot error	4	2.85	17.7	1.14	47.1	142.8
Crop (C)	3	192.7 ***	1798.2 ***	2590.8 ***	5288.2 ***	11996.7 ***
N × C	6	5.51 ns	29.4 ns	5.63 ns	32.8 ns	83.3 ns
Sub-plot error	18	2.31	16.0	2.62	47.7	110.1
Cropping system (S)	1	176.9 ***	12954.3 ***	1930.1 ***	728.9 ***	274.3 ns
N × S	2	0.10 ns	7.22 ns	2.39 ns	98.1 ns	252.4 ns
C × S	3	80.3 **	3960.5 ***	3889.1 ***	1051.4 ***	1310.4 ***
N × C × S	6	5.07 ns	31.5 ns	5.24 ns	52.4 ns	101.1 ns
Error	24	2.71	18.9	2.49	50.9	139.2

3.2. Crop Yield, Weed Biomass and Grain Protein Content

The ANOVA showed a significant effect of nitrogen fertilizer on durum wheat grain yield, grain protein and land equivalent ratio (LER). The crop effect was significant on durum wheat grain yield, grain protein, legume yield, weed biomass and LER. The cropping system effect was significant on legume yield and weed biomass. Crop × system interaction was significant on durum wheat grain yield, legume yield and LER (Table 2).

Table 2. ANOVA for main effects and interactions on grain yield (GY) and grain protein content (GP), forage legume yield (LY), weed biomass (WB) and land equivalent ratio (LER). Degree of freedom (df), mean square (MS) and significance: not significant (ns), $p \leq 0.001$ (***), $p \leq 0.01$ (**), and $p \leq 0.05$ (*).

Source	df	GY	GP	LY	WB	LER
		MS				
Blocks	2	0.58 ^{ns}	0.84 ^{ns}	0.09 ^{ns}	0.76 ^{ns}	0.77 ^{ns}
Nitrogen (N)	2	20.4 **	12.4 *	0.07 ^{ns}	0.45 ^{ns}	7.57 *
Main-plot error	4	0.44	0.73	0.03	0.30	2.02
Crop (C)	3	2.43 **	9.48 ***	9.65 ***	6.42 **	6.64 **
N × C	6	0.86 ^{ns}	0.03 ^{ns}	0.31 ^{ns}	0.50 ^{ns}	1.27 ^{ns}
Sub-plot error	18	0.32	0.25	0.11	0.99	0.42
Cropping system (S)	1	0.79 ^{ns}	0.56 ^{ns}	17.1 ***	22.6 ***	48.9 ***
N × S	2	0.16 ^{ns}	0.03 ^{ns}	0.02 ^{ns}	0.85 ^{ns}	5.04 ^{ns}
C × S	3	1.96 *	0.07 ^{ns}	7.87 **	1.25 ^{ns}	6.96 *
N × C × S	6	0.88 ^{ns}	0.64 ^{ns}	0.66 ^{ns}	0.34 ^{ns}	1.33 ^{ns}
Error	24	0.64	0.46	0.09	0.61	0.88

Across treatments, durum wheat grain yield was significantly improved by N fertilizer (from 1.70 to 3.50 Mg ha^{-1} in N0 and N2, respectively) and by the association between *T. durum–M. polymorpha* and *T. durum–L. corniculatus* (2.95 and 2.94 Mg ha^{-1}, respectively), as compared with *T. durum–T. subterraneum* and *T. durum* sole crop (2.31 and 2.30 Mg ha^{-1}, respectively). Grain yield was unchanged between IC and RC systems (Figure 3).

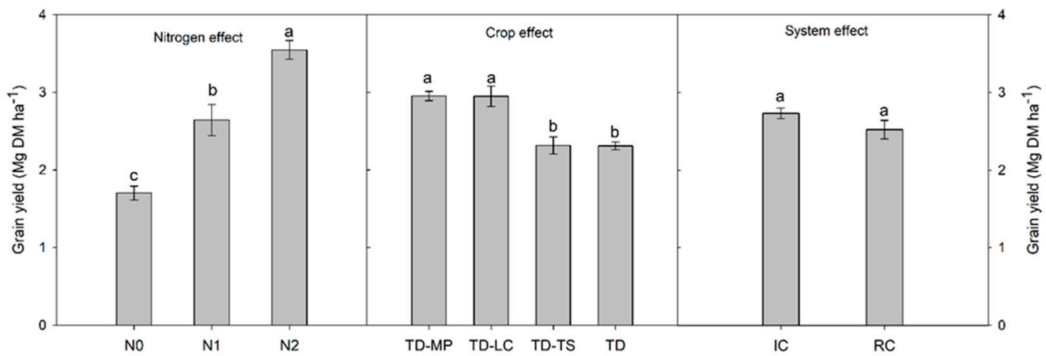

Figure 3. Means ± standard errors of wheat grain yield for main effects: nitrogen (N0, N1, N2) crop (*Triticum durum–Medicago polymorpha*—TD-MP, *Triticum durum–Lotus corniculatus*—TD-LC, and *Triticum durum–Trifolium subterraneum*—TD-TS, and durum wheat sole crop—TD), and cropping system (relay cropping—RC and intercropping—IC). Different letters indicate statistically different means ($p \leq 0.05$).

Also, the IC or the RC system had a null effect on durum wheat grain protein content (Figure 4). On the contrary, grain protein was affected by the N fertilizer (from 11.47 to 12.90% in N0 and N2, respectively) and by the association of *T. durum–M. polymorpha* (12.96%) and *T. durum–T. subterraneum* (12.52%). The lowest grain protein content was found in the *T. durum* sole crop (11.25%).

Legume yield was significantly affected by the cropping systems (1.06 Mg ha^{-1} in the IC and 0.09 Mg ha^{-1} in the RC, respectively). *T. subterraneum* showed a significantly higher yield than *M. polymorpha* and *L. corniculatus* (1.65 Mg ha^{-1} vs. 0.35 and 0.30 Mg ha^{-1}, respectively), while N fertilizer did not change legume yields (Figure 5).

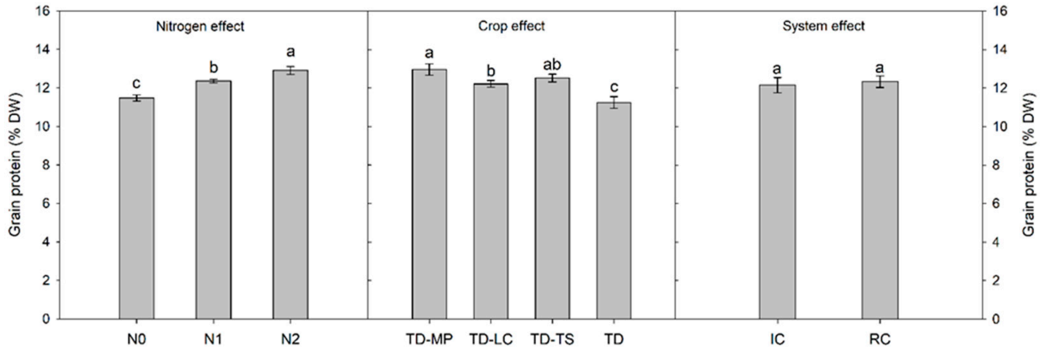

Figure 4. Means ± standard errors of wheat grain protein content for main effects: nitrogen (N0, N1, N2) crop (*Triticum durum–Medicago polymorpha*—TD-MP, *Triticum durum–Lotus corniculatus*—TD-LC and *Triticum durum–Trifolium subterraneum*—TD-TS, and durum wheat sole crop—TD), and cropping system (relay cropping—RC and intercropping—IC). Different letters indicate statistically different means ($p \leq 0.05$).

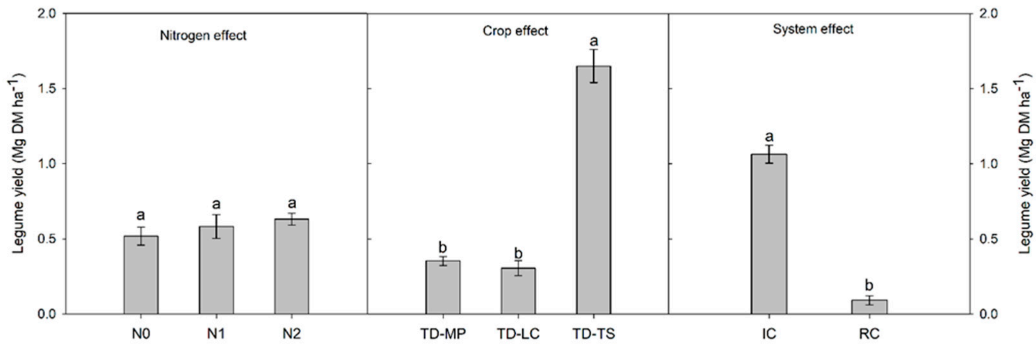

Figure 5. Means ± standard errors of legume dry biomass yield for main effects: nitrogen (N0, N1, N2) crop (*Triticum durum–Medicago polymorpha*—TD-MP, *Triticum durum–Lotus corniculatus*—TD-LC, and *Triticum durum–Trifolium subterraneum*—TD-TS), and cropping system (relay cropping—RC and intercropping—IC). Different letters indicate statistically different means ($p \leq 0.05$).

Also, weed dry biomass was not affected by the N fertilizer. Weed biomass was higher in the RC than the IC system (1.57 vs. 0.45 Mg ha^{-1}, respectively), and in the *T. durum* sole crop compared to *T. durum–L. corniculatus* (1.57 and 1.05 Mg ha^{-1}, respectively). *T. durum–M. polymorpha* and *T. durum–T. subterraneum* showed the lowest weed dry biomass (0.52 and 0.64 Mg ha^{-1}, respectively) (Figure 6).

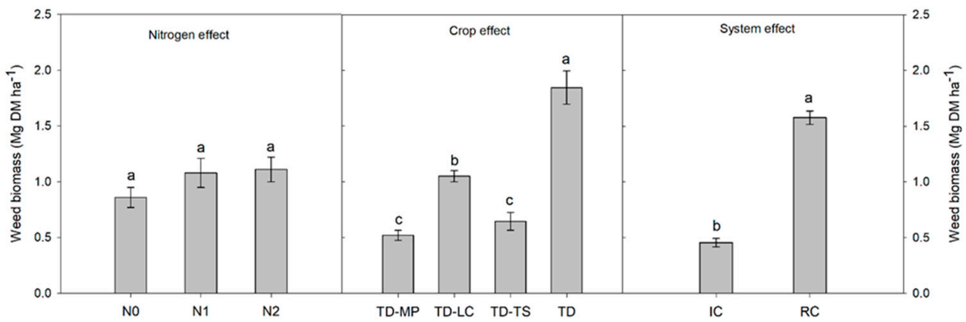

Figure 6. Means ± standard errors of weed dry biomass for main effects: nitrogen (N0, N1, N2) crop (*Triticum durum–Medicago polymorpha*—TD-MP, *Triticum durum–Lotus corniculatus*—TD-LC, and *Triticum durum–Trifolium subterraneum*—TD-TS, and durum wheat sole crop—TD), and cropping system (relay cropping—RC and intercropping—IC). Different letters indicate statistically different means ($p \leq 0.05$).

The land equivalent ratio (LER) was highly enhanced by the IC, while the RC had an almost null effect (2.58 vs. 1.03). All double-cropping systems (*T. durum-M. polymorpha*, *T. durum*-L. *corniculatus* and *T. durum-T. subterraneum*) showed LERs higher than one (i.e., that of the *T. durum* sole crop), particularly under medium and unfertilized treatments (N1 and N0, respectively). At the highest N dose (N2), the LER was similar to the LER of the control (Figure 7).

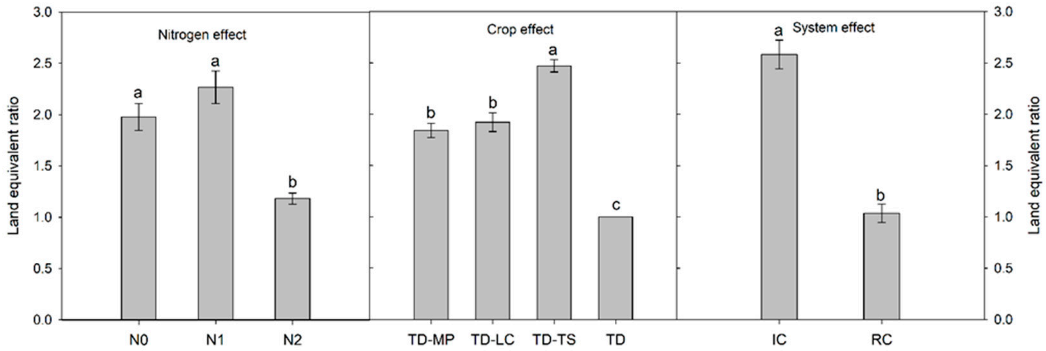

Figure 7. Means ± standard errors of land equivalent ratio for main effects: nitrogen (N0, N1, N2) crop (*Triticum durum–Medicago polymorpha*—TD-MP, *Triticum durum–Lotus corniculatus*—TD-LC, and *Triticum durum–Trifolium subterraneum*—TD-TS, and durum wheat sole crop—TD), and cropping system (relay cropping—RC and intercropping—IC). Different letters indicate statistically different means ($p \leq 0.05$).

The durum wheat competitive performance (CP_w) was calculated for the N0 treatment only. In the RC system, the CP_w was significantly higher than the IC (Table 3). Among legume species, the significantly lowest CP_w was observed in the *T. subterraneum*, while *M. polymorpha* and *L. corniculatus* were subjected to higher and similar CP_w.

Table 3. Main effects and significance on the competitive performance of durum wheat (CP_w). Not significant (ns), $p \leq 0.01$ (**), and $p \leq 0.05$ (*). Different letters within an effect indicate statistically different means according to the Tukey test at $p \leq 0.05$.

Effects		CP_w (%)
Legume (L)	*M. polymorpha*	90.1 a
	T. subterraneum	59.8 b
	L. corniculatus	94.0 a
Cropping system (S)	Relay cropping	93.8 a
	Intercropping	59.6 b
Significance	L	*
	S	**
	L × S	ns

The relationship between weed dry biomass and legume yield showed a negative trend (Figure 8A). The slope of the linear regression predicted that one dry ton of weed biomass was reduced by increasing 0.45 Mg ha^{-1} the legume yield. The goodness of fit of the linear regression was improved by cumulating wheat grain to legume yield (Figure 8B). In this relationship, the rate of change was 0.36 Mg ha^{-1} of double crops to reduce one dry ton of weed biomass.

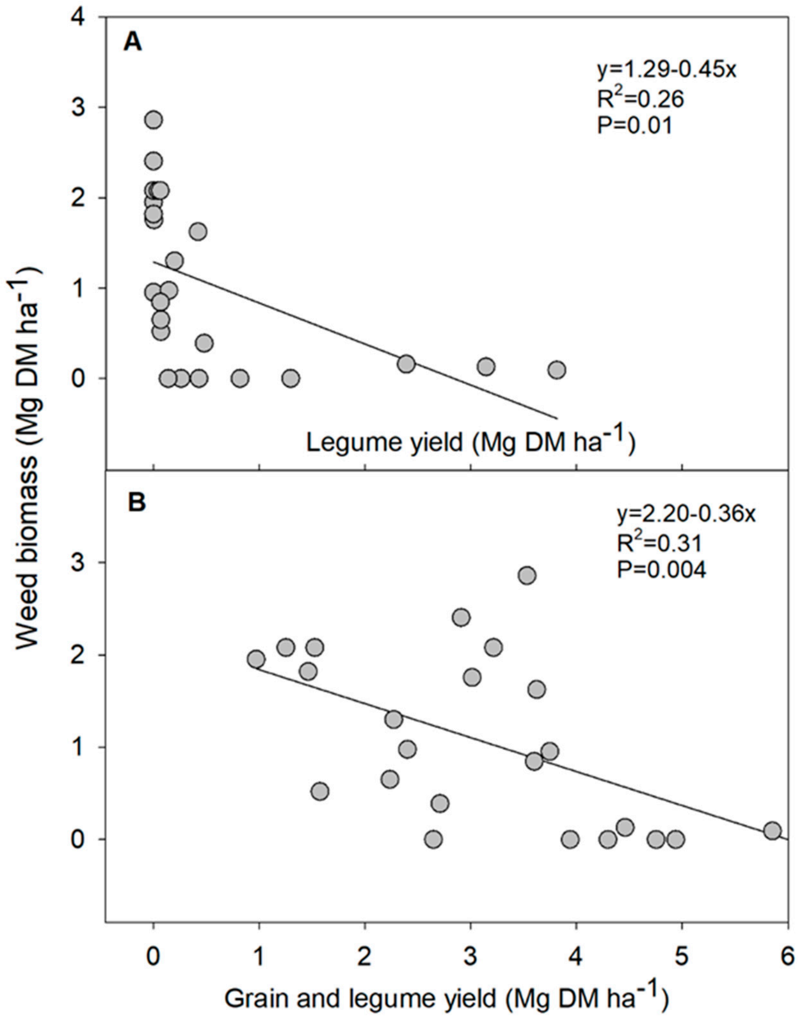

Figure 8. Linear relationship between (**A**) legume dry matter yield and weed dry biomass; (**B**) cumulated wheat grain yield and legume dry matter yield and weed dry biomass. Significance at $p \leq 0.05$.

4. Discussion

Despite the Mediterranean region being indicated as a climate change hot spot for the coming decades [27,28], the minimum and maximum air temperature of the study area in the 2022/23 growing season did not remarkably change as compared with the historical trend (twenty-year average). Contrarily, the rainfall amount was about 94 mm lower, and the winter season was drier than the historical average. Though the spring season was wetter (April, May and June), these months were not combined to an even distribution, particularly in April and May, when about half of the total monthly precipitation was registered in two events, or in June, when almost the total precipitation fell in three events. Nonetheless, soil moisture in the spring season was quite favorable, which accompanied most of the vegetative growth of *T. subterraneum* and *L. corniculatus*, both the vegetative and reproductive stages of *T. durum*, and that of *M. polymorpha* up to senescence, either in the IC or RC system. The previous phenological stage (i.e., emergence to side shoot formation) of *T. durum* in both cropping systems and legume species in the IC system were subjected to water stress as the ASW was constantly below the 20% threshold [24]. In addition, the last part of the flowering to senescence stage for *T. durum* and *T. subterraneum* was rather dry, which is a typical trend in the Mediterranean area for cool season crops [29,30]. *M. polymorpha* escaped this period due to early senescence, while *L. corniculatus* did not reach the senescence phase in both cropping systems due to its longer biological cycle.

The selection of investigated forage legumes was based on different morphological characteristics, growth habits and cycle length to maximize competition with weeds and to provide facilitative effects to the cash crop. Furthermore, to minimize the competitive effects of forage legumes on the cash crop, we also tested the RC system, delaying legume sowings at the wheat tillering stage, and compared it to the complementary cereal–legume sowing in the IC. It is not surprising that the results on durum wheat competitive performance (CP_w) confirmed our hypothesis since forage legumes in RC were more subjected to wheat competition than in the IC. Indeed, delayed sowing of legumes in an already-established wheat stand can reduce the interspecific competition between the cash and the undersown crop [16]. This agrees with studies carried out in other environments and management systems, such as conventional [16,31], organic [14] and low input [16]. Nonetheless, the facilitative effect of the RC system (high CP_w) was combined with a lower weed control ability since the biomass yield of forage legumes was largely reduced as compared to the IC. Among legume species, the *T. subterraneum* was less affected by durum wheat (lower CP_w) and produced the overall highest biomass yield. Despite the lower yield of *M. polymorpha*, weed competition ability was like that of *T. subterraneum.*

The negative relationship between legume yield and weed dry biomass can, in part, explain the competition ability of forage legumes, since traits other than legume yield can play a role in controlling weeds. For instance, legume yield was not significantly different in *M. polymorpha* and *L. corniculatus*; however, the slow growth rate of the latter combined with a low density of leaves and erect stems that characterize this plant limited the control of weeds in double cropping, likely due to a lower efficiency for light interception under shading by the cash crop [32]. A global metanalysis carried out by Gu and co-authors [33] demonstrated a 58% decrease in weed biomass in intercropping systems compared to the less suppressive sole crop, highlighting that mixed arrangement gave stronger weed suppression than row designs. Contrary to our findings, they did not find any effect on weed biomass of simultaneous vs. relay intercropping, and of N fertilization.

Surprisingly, the highest durum wheat grain yield was achieved in association with *M. polymorpha* and *L. corniculatus*, namely the two species with lower biomass yield and subjected to the highest competition by wheat.

The cropping system did not change either grain yield or grain protein content, while the N fertilization had a large effect on these traits. Nitrogen and organic matter are important limiting factors in this soil, as confirmed by the significant effect of fertilization on grain yield and grain protein content. Interestingly, the association of *T. durum* with forage legumes enhanced both traits across the average of N-fertilization as compared to the *T. durum* sole crop (up to 21% for grain yield and up to 13% for grain protein content). These results are consistent with previous studies on RC and IC, which suggested that the beneficial effects of forage legumes on cereals are maximized under low-input or organic systems [16,34], especially in low N availability systems [35]. The complementarity effect in mixtures is generally the main driver of the performance of cereal–legume intercrops, but, when N fertilizers are applied, the choice of a sufficiently competitive legume is of key importance to secure complementarity processes [36].

A recent meta-analysis demonstrated that interspecific complementarity and facilitation between grain legumes and cereals in IC systems stimulate N use by increasing N_2 fixation by legumes and increasing soil N acquisition in cereals [37]. This can explain the higher grain protein content achieved in the double cropping than in the sole crop. This also agrees with the findings of Pellegrini et al. [38], who registered an increase in wheat grain protein content ranging from 15 to 28% in a low-input IC with *T. resupinatum* when compared to the wheat sole crop. However, the same study carried out in Northern Italy, under more favorable environmental conditions, observed a decrease in grain yield from 20 to 59%. On the contrary, Amossé et al. [32] found a null effect in wheat grain yield in an organic RC, but grain protein content decreased from 5 to 11%, particularly with the association of high-yielding *M. lupulina* and *T. pratense* as compared with the wheat sole crop.

Bedoussac et al. [35] suggested that IC facilitation is stronger when the yield of one or both sole crops is quite low and in cases of low grain protein content of the sole cropped cereal. This parallels our results on LER, where the highest N dose (120 kg N ha^{-1}) produced a slightly higher LER value than the wheat sole crop (1.18 vs. 1.0, respectively); conversely, reducing N amount led to a sharp increase in the LER, which was significantly higher in both unfertilized (1.97) and the 60 kg N ha^{-1} (2.27), highlighting the facilitative effect of the IC under low-N availability systems [35].

5. Conclusions

Overall, the N fertilization improved the cash crop yield and quality without supporting weed or forage legume development. The relay-cropping system, delaying legume sowing at the wheat tillering stage, was not as effective as the intercropping in weed control. Among legume species, *M. polymorpha* seems to be more adapted to double cropping due to its short cycle and canopy architecture that enabled good weed control, minimizing competition with the cash crop for natural resources.

In the south Mediterranean environment, subjected to uneven rainfall distribution, a water deficit at critical phenological stages and N-deficient soils, the LER was maximized in the medium or the unfertilized treatment, suggesting that advantages of the double cropping are higher in systems where nutrient levels are significant factors limiting crop yield and quality. While these findings indicated that intercropping can be a suitable and sustainable agroecological practice to tackle weed issues and reduce N-requirement in an organic cereal-based system, which is progress toward the principles of the eco-schemes set in the new CAP, further observations need to be carried out in different agroclimatic conditions and mixtures.

Author Contributions: Conceptualization, D.S. and F.G.; methodology, D.S., P.G., F.C., A.M., T.L.M., A.S. and F.G.; investigation, D.S., P.G., F.C., A.M., T.L.M., A.S. and F.G.; writing—original draft preparation, D.S.; writing—review and editing, D.S., P.G., F.C., A.M., T.L.M., A.S. and F.G.; visualization, D.S., P.G., F.C., A.M., T.L.M., A.S. and F.G.; funding acquisition, D.S. and F.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Italian Ministry of Foreign Affairs and International Cooperation, grant number US23GR18 (RE-FARM project, CUP: J43C23000110001).

Data Availability Statement: The data and material for the current study are included in this published article. There is no other data generated or used. The datasets used and/or analyzed during the present experiments are available from the corresponding author upon reasonable request.

Acknowledgments: The authors warmly acknowledge Enzo Pantano and his team at the "Azienda Agricola La Porticella".

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. European Commission (EC), Directorate-General for Agriculture and Rural Development. List of Potential Agricultural Practices That Eco-Schemes Could Support. January 2021 #EUGreenDeal. 2021. Available online: https://agriculture.ec.europa.eu/system/files/2021-01/factsheet-agri-practices-under-ecoscheme_en_0.pdf (accessed on 31 May 2023).
2. Mondelaers, K.; Aertsens, J.; Van Huylenbroeck, G. A meta-analysis of the differences in environmental impacts between organic and conventional farming. *Brit. Food J.* **2009**, *111*, 1098–1119. [CrossRef]
3. Smith, L.G.; Williams, A.G.; Pearce, B.D. The energy efficiency of organic agriculture: A review. *Renew. Agric. Food Syst.* **2015**, *30*, 280–301. [CrossRef]
4. Istituto Nazionale di Statistica (ISTAT). 2016. Available online: http://www.istat.it (accessed on 31 May 2023).
5. Sistema d'Informazione Nazionale sull'Agricoltura Biologica (SINAB). Bio in Cifre. 2019. Available online: http://www.sinab.it/ (accessed on 31 May 2023).
6. Wezel, A.; Casagrande, M.; Celette, F.; Vian, J.F.; Ferrer, A.; Peigné, J. Agroecological practices for sustainable agriculture. A review. *Agron. Sustain. Dev.* **2014**, *34*, 1–20. [CrossRef]
7. Asseng, S.; Zhu, Y.; Basso, B.; Wilson, T.; Cammarano, D. Simulation Modeling: Applications in Cropping Systems. In *Encyclopedia of Agriculture and Food Systems*; Van Alfen, N.K., Ed.; Academic Press: Cambridge, UK, 2014; pp. 102–212. [CrossRef]

8. Hariharan, G.; Abhayapala, R.; Karunarathna, B. Efficient utilization of rice fallow through pulse cultivation. In *Advances in Legumes for Sustainable Intensification*; Meena, R.S., Kuma, S., Eds.; Academic Press (Elsevier): Amsterdam, The Netherlands, 2022; pp. 71–92. [CrossRef]
9. Sheaffer, C.C.; Seguin, P. Forage legumes for sustainable cropping systems. *J. Crop Prod.* **2003**, *8*, 187–216. [CrossRef]
10. Scholberg, J.M.S.; Dogliotti, S.; Leoni, C.; Cherr, C.M.; Zotarelli, L.; Rossing, W.A.H. Cover crops for sustainable agrosystems in the Americas. In *Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming. Sustainable Agriculture Reviews*; Lichtfouse, E., Ed.; Springer: Dordrecht, The Netherlands, 2010; Volume 4, pp. 23–58. [CrossRef]
11. Raza, M.A.; Zhiqi, W.; Yasin, H.S.; Gul, H.; Qin, R.; Rehman, S.U.; Mahmood, A.; Iqbal, Z.; Ahmed, Z.; Luo, S.; et al. Effect of crop combination on yield performance, nutrient uptake, and land use advantage of cereal/legume intercropping systems. *Field Crops Res.* **2023**, *304*, 109144. [CrossRef]
12. Melander, B.; Rasmussen, I.A.; Bàrberi, P. Integrating physical and cultural methods of weed control-examples from European research. *Weed Sci.* **2005**, *53*, 369–381. [CrossRef]
13. Mariotti, M.; Masoni, A.; Ercoli, L.; Arduini, I. Optimizing forage yield of durum wheat/field bean intercropping through N fertilization and row ratio. *Grass Forage Sci.* **2012**, *67*, 243–254. [CrossRef]
14. Amossé, C.; Jeuffroy, M.H.; Celette, F.; David, C. Relay-intercropped forage legumes help to control weeds in organic grain production. *Eur. J. Agron.* **2013**, *49*, 158–167. [CrossRef]
15. Gecaitė, V.; Arlauskienė, A.; Cesevičienė, J. Competition effects and productivity in oat–forage legume relay intercropping systems under organic farming conditions. *Agriculture* **2021**, *11*, 99. [CrossRef]
16. Leoni, F.; Lazzaro, M.; Ruggeri, M.; Carlesi, S.; Meriggi, P.; Moonen, A.C. Relay intercropping can efficiently support weed management in cereal-based cropping systems when appropriate legume species are chosen. *Agron. Sustain. Dev.* **2022**, *42*, 75. [CrossRef]
17. Schipanski, M.E.; Drinkwater, L.E. Nitrogen fixation of red clover interseeded with winter cereals across a management-induced fertility gradient. *Nutr. Cycling Agroecosyst.* **2011**, *90*, 105–119. [CrossRef]
18. De Notaris, C.; Rasmussen, J.; Sørensen, P.; Melander, B.; Olesena, J.E. Manipulating cover crop growth by adjusting sowing time and cereal interrow spacing to enhance residual nitrogen effects. *Field Crops Res.* **2019**, *234*, 15–25. [CrossRef]
19. Ross, S.M.; King, J.R.; Izaurralde, R.C.; O'Donovan, J.T. Weed suppression by seven clover species. *Agron. J.* **2001**, *93*, 820–827. [CrossRef]
20. Uchino, H.; Iwama, K.; Jitsuyama, Y.; Ichiyama, K.; Sugiura, E.; Yudate, T. Stable characteristics of cover crops for weed suppression in organic farming systems. *Plant Prod. Sci.* **2011**, *14*, 75–85. [CrossRef]
21. Enriquez-Hidalgo, D.; Cruz, T.; Teixeira, D.L.; Steinfort, U. Phenological stages of Mediterranean forage legumes, based on the BBCH scale. *Ann. Appl. Biol.* **2020**, *176*, 357–368. [CrossRef]
22. Scavo, A.; Restuccia, A.; Lombardo, S.; Fontanazza, S.; Abbate, C.; Pandino, G.; Anastasi, U.; Onofri, A.; Mauromicale, G. Improving soil health, weed management and nitrogen dynamics by *Trifolium subterraneum* cover cropping. *Agron. Sustain. Dev.* **2020**, *40*, 18. [CrossRef]
23. Blackwell, M.S.A.; Jarvis, S.C.; Wilkins, R.J. Chapter Four - The importance of sustained grassland and environmental research: A case study from North Wyke Research station, UK, 1982–2017. *Adv. Agron.* **2018**, *149*, 161–235. [CrossRef]
24. Scordia, D.; Testa, G.; Copani, V.; Patanè, C.; Cosentino, S.L. Lignocellulosic biomass production of Mediterranean wild accessions (*Oryzopsis miliacea*, *Cymbopogon hirtus*, *Sorghum halepense* and *Saccharum spontaneum*) in a semi-arid environment. *Field Crops Res.* **2017**, *214*, 56–65. [CrossRef]
25. Mason, S.C.; Leihner, D.E.; Vorst, J.J. Cassava–cowpea and cassava–peanut intercropping. I. Yield and land use efficiency. *Agron. J.* **1986**, *78*, 43–46. [CrossRef]
26. Deb, D.; Dutta, S. The robustness of land equivalent ratio as a measure of yield advantage of multi-crop systems over monocultures. *Exp. Res.* **2022**, *3*, e2. [CrossRef]
27. Scordia, D.; Corinzia, S.A.; Coello, J.; Ventura, R.V.; Jiménez-De-Santiago, D.E.; Just, B.S.; Castaño-Sánchez, O.; Arcarons, C.C.; Tchamitchian, M.; Garreau, L.; et al. Are agroforestry systems more productive than monocultures in Mediterranean countries? A meta-analysis. *Agron. Sustain. Dev.* **2023**, *43*, 73. [CrossRef]
28. Tuel, A.; Eltahir, E.A.B. Why Is the Mediterranean a Climate Change Hot Spot? *J. Clim.* **2020**, *33*, 5829–5843. [CrossRef]
29. Anastasi, U.; Corinzia, S.A.; Cosentino, S.L.; Scordia, D. Performances of durum wheat varieties under conventional and no-chemical input management systems in a semiarid Mediterranean environment. *Agronomy* **2019**, *9*, 788. [CrossRef]
30. Cosentino, S.L.; Sanzone, E.; Testa, G.; Patanè, C.; Anastasi, U.; Scordia, D. Does post-anthesis heat stress affect plant phenology, physiology, grain yield and protein content of durum wheat in a semi-arid Mediterranean environment? *J. Agron. Crop Sci.* **2018**, *205*, 309–323. [CrossRef]
31. Bergkvist, G.; Stenberg, M.; Wetterlind, J.; Båth, B.; Elfstrand, S. Clover cover crops under-sown in winter wheat increase yield of subsequent spring barley—Effect of N dose and companion grass. *Field Crops Res.* **2011**, *120*, 292–298. [CrossRef]
32. Amossé, C.; Jeuffroy, M.H.; David, C. Relay intercropping of legume cover crops in organic winter wheat: Effects on performance and resource availability. *Field Crops Res.* **2013**, *145*, 78–87. [CrossRef]
33. Gu, C.; Bastiaans, L.; Anten, N.P.R.; Makowski, D.; van der Werf, W. Annual intercropping suppresses weeds: A meta-analysis. *Agric. Ecosyst. Environ.* **2021**, *322*, 107658. [CrossRef]

34. Nelson, A.G.; Pswarayi, A.; Quideau, S.; Frick, B.; Spaner, D. Yield and weed suppression of crop mixtures in organic and conventional systems of the western Canadian prairie. *Agron. J.* **2012**, *104*, 756–762. [CrossRef]
35. Bedoussac, L.; Journet, E.-P.; Hauggaard-Nielsen, H.; Naudin, C.; Corre-Hellou, G.; Jensen, E.S.; Prieur, L.; Justes, E. Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agron. Sustain. Dev.* **2015**, *35*, 911–935. [CrossRef]
36. Mahmoud, R.; Casadebaig, P.; Hilgert, N.; Alletto, L.; Freschet, G.T.; de Mazancourt, C.; Gaudio, N. Species choice and N fertilization influence yield gains through complementarity and selection effects in cereal-legume intercrops. *Agron. Sustain. Dev.* **2022**, *42*, 12. [CrossRef]
37. Rodriguez, C.; Carlsson, G.; Englund, J.-E.; Flöhr, A.; Pelzer, E.; Jeuffroy, M.-H.; Makowski, D.; Jensen, E.S. Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. *Eur. J. Agron.* **2020**, *118*, 126077. [CrossRef]
38. Pellegrini, F.; Carlesi, S.; Nardi, G.; Bàrberi, P. Wheat–clover temporary intercropping under Mediterranean conditions affects wheat biomass, plant nitrogen dynamics and grain quality. *Eur. J. Agron.* **2021**, *130*, 126347. [CrossRef]