

Article

Enhancing the Potential Use of Durum Wheat Straw as a Ruminant Feed Resource Through Forage Legume Living Mulch—Part I: Chemical Composition and Nutritional Quality

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Abstract

Durum wheat straw is an abundant by-product in Mediterranean cereal systems, but its low protein and high fiber content limit its use as ruminant feed. Integrating forage legumes as living mulches in durum wheat may enhance its feeding value through the addition of nutrient-rich legume biomass. A two-year field trial evaluated three forage legumes (*Vicia sativa* L., *Trifolium michelianum* Savi, *Trifolium subterraneum* L.) grown as living mulch with organic durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husn.) under two NPK fertilization rates (target 60 and 120 kg N ha^{−1}), with or without rhizobial inoculation. Chemical composition and forage quality indices were determined for sole-wheat straw (SB) and legume biomass (LB) and subsequently used to estimate the nutritional characteristics of wheat straw–legume biomass mixtures (TSB). Biomass production was significantly affected by legume species in the first growing season and by legume species and rhizobial inoculation in the second, with *Vicia sativa* consistently showing the best performances. Correlation analysis showed that increasing LB contribution was significantly associated with all forage quality indices of the resulting TSB. These results indicate that rhizobially inoculated forage legume living mulch may improve the potential use of wheat straw as an on-farm feed resource in Mediterranean organic farming.

Keywords: agroecological intensification; rhizobial inoculation; crude protein; fiber fractions; forage valorization; Mediterranean area



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1. Introduction

The sustainability of ruminant production systems increasingly relies on locally produced forage resources that can reduce the dependence on imported protein feeds while ensuring high forage quality and animal performance. In Mediterranean agroecosystems, rising feed costs, increasing climate variability, and environmental concerns linked to intensive cereal-based farming have highlighted the need for resource-efficient forage production strategies [1–3].

Forage legumes play a key role owing to their symbiotic association with rhizobia, which enables biological nitrogen fixation (BNF). By converting atmospheric nitrogen (N) into plant-available forms, BNF reduces the need for synthetic N fertilizers, enhances soil

fertility, and supports forage productivity [4,5]. Given the species-specificity of legume-rhizobia interactions, selected rhizobial strains are often applied through inoculant formulations to ensure effective nodulation and maximize BNF [6]. Farquharson et al. estimated that forage legumes can fix 3.5 million tonnes of N annually from about 45 million hectares in Australia [7]. Consequently, the integration of forage legumes into cereal-based systems has been proposed as a sustainable strategy to enhance forage self-sufficiency, improve nutrient cycling, and reduce external N inputs in crop–livestock systems.

Living mulch systems based on annual forage legumes can also provide multiple ecosystem services, including weed suppression, soil protection against erosion, and improvements in soil biological activity and nutrient cycling [8–10]. These effects are particularly relevant in organic wheat production, where external inputs for weed and nutrient management are limited. Moreover, living mulch systems can generate additional forage biomass, increasing the availability of on-farm feed resources and strengthening the sustainability of ruminant production.

Beyond agronomic effects, forage legumes are important components of ruminant feeding systems because of their high protein concentration, favorable digestibility, and contribution to animal performance [11,12]. Compared with cereal residues, legume biomass generally shows higher crude protein content and improved digestibility, leading to greater voluntary intake and animal performance [13,14]. Forage nutritional value is largely determined by its chemical composition and fiber fractions, particularly neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), which strongly influence intake and digestibility. Consequently, the incorporation of high-quality legume biomass into low-quality roughage resources may substantially enhance the overall feeding value of the harvested feed resource available to ruminants.

Although forage quality is traditionally assessed through chemical composition (crude protein, ether extract, ash, and fiber fractions), integrative indices, such as total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), and relative forage quality (RFQ) are widely used to provide a more comprehensive evaluation of forage utilization by ruminants [15,16]. In particular, RFQ improves upon RFV by incorporating fiber digestibility and better representing forage variability across species and botanical groups [17]. Consequently, RFQ is currently considered one of the most informative indices for comparing forage quality across different species and management systems.

Wheat straw is a major roughage source in Mediterranean farming systems but is characterized by low crude protein and high fiber content. Intercropping forage legumes as living mulches may improve the nutritional value of wheat straw-based feed resources by providing additional high-quality biomass, thereby enhancing on-farm feed self-sufficiency. Despite the increasing adoption of legume-based living mulch systems in Mediterranean cereal production, most studies have focused primarily on crop productivity, weed suppression, and N dynamics [10,18–23]. Comparatively little information is available regarding the contribution of forage legume biomass to the nutritional characteristics and feeding value of wheat straw-obtained through the integration of legume biomass. Furthermore, the role of rhizobial inoculation in determining biomass production, forage composition, and the feeding value of harvested cereal–legume biomass is lacking under Mediterranean organic farming conditions.

Therefore, the objective of this two-year study was to investigate the potential of annual forage legumes grown as living mulches with organic durum wheat under different rhizobial inoculation and fertilization regimes, in order to improve the nutritional characteristics and feeding value of wheat straw-through the addition of legume biomass. The work was structured as a two-part study: Part I addresses biomass production, chemical compo-

sition, forage quality indices, and feeding value; Part II examines fatty acid composition, polyphenol content, and other bioactive compounds.

Because wheat straw from the legume living mulch system was not chemically characterized, the nutritional characteristics of the harvested wheat straw–legume biomass mixtures were estimated using a mass-balance approach, considering the forage legume chemical composition and the sole-wheat straw chemical composition. Based on the findings of a previous study [24], in which forage legume species and rhizobial inoculation affected legume performance, BNF, nodulation, and soil N availability, the present study hypothesized that legume species and rhizobial inoculation would also modify the chemical composition of the legume biomass and, consequently, the forage quality of the resulting wheat–legume biomass mixture. Specifically, differences in the chemical composition of the legume component were expected to alter the nutritional characteristics of the mixture composed of wheat straw and legume biomass, thereby affecting its overall forage quality.

2. Materials and Methods

2.1. Site, Soil and Climate

A field experiment was conducted in a certified organic farm located in Patti, northeastern Sicily, Italy (38°06' N, 14°59' E, 196 m a.s.l.). Based on the USDA Soil Taxonomy classification [25], this area covers Calcixerept soils. The soil texture is predominantly clay (57% clay, 11% silt, and 32% sand), with low organic matter (0.81 g kg^{−1}), pH 7.8, total N 0.3 g kg^{−1}, and P₂O₅ 79.1 mg kg^{−1}.

The climate of the zone is typical semiarid Mediterranean, characterized by summer droughts and mild winters, with precipitation generally below 500 mm year^{−1} and mainly concentrated in the autumn–winter period. Meteorological data during the experimental period were obtained from the SIAS (Servizio Informativo Agrometeorologico Siciliano, www.sias.regione.sicilia.it) weather station, located about 3 km from the experimental field. The 2023/2024 growing season was hotter and drier than 2024/2025 (Figure 1). Average minimum, mean and maximum air temperatures were 11.4 °C, 16.7 °C, and 21.1 °C in the first season, and 11.1 °C, 15.8 °C, and 20.5 °C in the second, respectively. Cumulative rainfall amounted to 295 mm in 2023/2024 and 456 mm in 2024/2025, highlighting a marked difference between growing seasons.

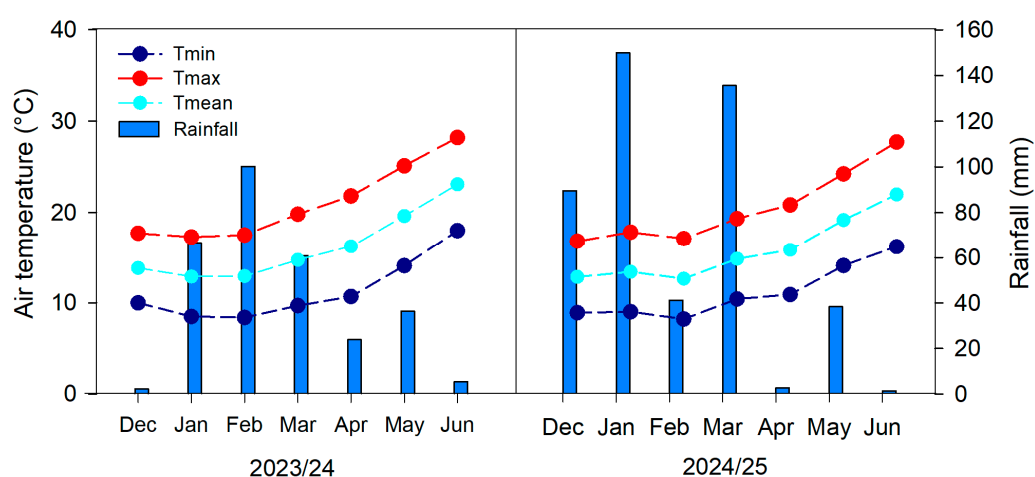


Figure 1. Climatic data (monthly air temperatures and monthly rainfall) from the experimental field in Patti (38°06' N, 14°59' E; 196 m a.s.l.; northeastern Sicily, Italy) over the two growing seasons (2023/2024 and 2024/2025). Tmin, Tmax and Tmean indicate minimum, maximum and mean monthly temperatures, respectively.

2.2. Experimental Design and Agronomic Management

The experiment was arranged as a randomized complete block design with three replicates. Treatments included two fertilization rates, three forage legume species grown as living mulch with durum wheat, and two rhizobial inoculation levels. In addition, sole-durum wheat under both fertilization rates was included as a control treatment. Overall, the experimental design comprised 42 plots, each measuring 2.0×3.5 m (7 m^2). The seedbed was prepared according to standard farm operation: summer ploughing, autumn disk harrowing, and late-autumn rotary tiller before sowing.

Fertilization was applied before sowing, using a commercial organic fertilizer (Organagro, Choncimer srl, San Severino Marche, Italy), containing 3.10% N, 2.96% P_2O_5 , and 2.0% K_2O . Application rates were 1935 and 3871 kg ha^{-1} , corresponding to 60 and 120 kg N ha^{-1} , 57 and 115 $\text{kg P}_2\text{O}_5 \text{ ha}^{-1}$, 39 and 77 $\text{kg K}_2\text{O ha}^{-1}$, hereafter referred to as F60 and F120, respectively. The F120 treatment represented the standard N fertilization rate commonly adopted by local organic wheat farmers.

The three annual forage legumes (*Vicia sativa* L. var. Alexandros, *Trifolium michelianum* Savi var. Bolta, and *Trifolium subterraneum* L. var. Campeda) were selected because of their adaptation to Mediterranean environments and forage quality characteristics [25]. These species were cultivated as living mulch in association with organic durum wheat [*Triticum turgidum* subsp. *durum* (Desf.) Husn. cv. Core].

Forage legume seeds were either inoculated (YES) or not inoculated (NO) with species-specific peat-based rhizobial inoculants (Visjon Biologics, Henrietta, TX, USA). Following the protocol proposed by Scavo et al. [26], inoculants were applied immediately before sowing at twice the manufacturer's recommended rate. The inoculants contained *Rhizobium leguminosarum* bv. *viciae* for *V. sativa*, *R. leguminosarum* bv. *trifolii* for *T. subterraneum*, and a mixture of *Sinorhizobium meliloti* and *R. leguminosarum* bv. *trifolii* for *T. michelianum*. An additive living mulch system with a mixed sowing arrangement was adopted. According to the local practices, seeding rates were 250 kg ha^{-1} for durum wheat, 120 kg ha^{-1} for *V. sativa*, 35 kg ha^{-1} for *T. subterraneum*, and 10 kg ha^{-1} for *T. michelianum*.

Durum wheat and forage legume seeds were hand-sown on 21 December 2023 in the first growing season and on 12 December 2024 in the second.

2.3. Sampling of Forage Biomass and Wheat Straw

Wheat straw and forage legume biomass were collected at full maturity using three 0.5×0.5 m quadrats randomly placed within each plot. Wheat straw and forage legume biomass were cut at ground level, weighed separately, and oven-dried at 65°C to determine dry matter yield.

A mechanical thresher was used to separate wheat grain and straw. Sole-wheat straw dry biomass (SB) and legume dry biomass (LB) were expressed on a land-area basis (Mg ha^{-1}). The total straw dry biomass (TSB), representing the wheat straw–legume biomass mixtures, was calculated as the sum of SB and LB.

All samples were ground to pass a 1 mm sieve using a Cyclotec™ 1093 mill (FOSS, Hillerød, Denmark) and stored until laboratory analyses. For subsequent calculations of the nutritional characteristics of TSB, forage LB from all intercropping treatments and SB from the sole-cropped wheat control were chemically characterized as described below.

2.4. Chemical Analyses

The chemical composition of LB harvested from the living mulch systems and SB, used as the control treatment, was determined in both growing seasons according to the official methods of the Association of Official Analytical Chemists (AOAC) [27].

Dry matter (DM) content was determined according to AOAC method 930.15, whereas ash content was measured by combustion of 2.0 g of sample in a muffle furnace at 550–600 °C according to AOAC method 942.05. Crude protein (CP) concentration was determined using 1.0 g of sample by the Kjeldahl method (AOAC method 2001.11) [27] with a Kjeltec™ system (FOSS, Padua, Italy), and N content was converted to CP using the conversion factor of 6.25. Ether extract (EE) was determined using 5.0 g of sample by Soxhlet extraction with a Soxtec™ 8000 Extraction System (FOSS, Padua, Italy) according to AOAC method 920.39 [27].

Crude fiber (CF) was determined using 2.0 g of sample according to AOAC method 978.10 with a Fibertec™ 2010 system (FOSS, Padua, Italy). Structural carbohydrate fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), were determined using 1.0 g of sample according to the method described by Van Soest et al. [28]. The NDF determination was performed using heat-stable α -amylase. The fiber fractions were quantified gravimetrically.

All analyses were performed in triplicate using independent analytical replicates for each sample, and the results were expressed on a DM basis ($\text{g } 100 \text{ g}^{-1} \text{ DM}$).

The chemical composition of SB was subsequently used as a baseline reference for estimating the nutritional characteristics of wheat straw–legume biomass mixtures through the mass-balance approach described in Section 2.6.

2.5. Forage Quality Indices

To further evaluate the feeding value of LB grown as living mulch with wheat, and SB, several forage quality indices were calculated from fiber composition according to equations derived from NRC [29] and previously published forage evaluation studies [14,15,30].

Total digestible nutrients (TDN) were estimated according to the NRC [29] equation:

$$\text{TDN (\%)} = 87.84 - (0.70 \times \text{ADF\%}) \quad (1)$$

Digestible dry matter (DDM) was calculated according to Rohweder et al. [15] and Jeranyama and Garcia [31]:

$$\text{DDM} = 88.9 - (0.779 \times \text{ADF\%}) \quad (2)$$

Dry matter intake (DMI), expressed as percentage of animal body weight (% BW), was estimated according to:

$$\text{DMI (\% BW)} = 120 / \text{NDF\%} \quad (3)$$

Relative feed value (RFV) was calculated according to Rohweder et al. [15]:

$$\text{RFV} = (\text{DDM} \times \text{DMI}) / 1.29 \quad (4)$$

Relative forage quality (RFQ) was estimated according to the simplified approach proposed by Undersander [30]:

$$\text{RFQ} = (\text{TDN} \times \text{DMI}) / 1.23 \quad (5)$$

where ADF is acid detergent fiber and NDF is neutral detergent fiber.

These indices were used to provide an integrated assessment of forage digestibility, intake potential, and overall feeding value of LB and SB. Subsequently, they were used to evaluate the nutritional characteristics and feeding value of the wheat straw–legume biomass mixtures estimated under the different fertilization and rhizobial inoculation

treatments (Section 2.6). Higher values of TDN, DDM, DMI, RFV, and RFQ indicate greater forage digestibility, intake potential, and overall feeding value for ruminant nutrition.

2.6. Estimation of the Nutritional Characteristics of Wheat Straw–Legume Biomass Mixtures

Sole-wheat straw (SB) obtained from the control treatment under both F60 and F120 fertilization levels was analyzed for chemical composition and forage quality indices (Sections 2.4 and 2.5) and used as a baseline reference feed resource. The chemical composition is reported in Tables 1 and 2.

Table 1. Chemical composition of sole-wheat straw [dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL)] under F60 and F120 (target 60 and 120 kg N ha^{−1}) fertilization rates during the 2023/2024 and 2024/2025 growing seasons. Mean values ± standard errors (n = 3).

2023/2024	DM	CP	EE	CF	Ash	NDF	ADF	ADL
Fertilization	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
F60	90.7 ± 0.05	4.24 ± 0.04	1.42 ± 0.02	41.5 ± 0.17	6.64 ± 0.05	77.7 ± 0.07	50.1 ± 0.12	7.20 ± 0.01
F120	90.6 ± 0.04	4.27 ± 0.02	1.41 ± 0.03	41.3 ± 0.09	6.90 ± 0.04	77.6 ± 0.05	50.0 ± 0.06	7.19 ± 0.05
2024/2025								
F60	91.2 ± 0.08	3.83 ± 0.03	1.26 ± 0.01	41.3 ± 0.14	6.15 ± 0.02	79.2 ± 0.12	51.4 ± 0.16	8.06 ± 0.09
F120	91.1 ± 0.03	3.90 ± 0.04	1.24 ± 0.02	42.1 ± 0.08	6.28 ± 0.03	79.1 ± 0.08	51.2 ± 0.10	8.07 ± 0.07

Table 2. Forage quality indices of sole-wheat straw [total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), relative forage quality (RFQ)] under F60 and F120 (target 60 and 120 kg N ha^{−1}) fertilization rates during the 2023/2024 and 2024/2025 growing seasons. Mean values ± standard errors (n = 3).

2023/2024	TDN	DDM	DMI	RFV	RFQ
Fertilization	(%)	(%)	(%)	(%)	(%)
F60	52.8 ± 0.08	49.9 ± 0.09	1.42 ± 0.02	59.7 ± 0.31	66.3 ± 0.32
F120	52.8 ± 0.04	49.9 ± 0.05	1.41 ± 0.03	59.8 ± 0.22	66.3 ± 0.25
2024/2025					
F60	51.8 ± 0.11	48.8 ± 0.12	1.26 ± 0.01	57.4 ± 0.23	63.9 ± 0.23
F120	51.9 ± 0.07	48.9 ± 0.08	1.24 ± 0.02	57.5 ± 0.14	64.0 ± 0.14

Because wheat straw from the intercropped treatments was not chemically characterized separately, the nutritional characteristics of the harvested TSB were estimated through a mass-balance approach. This combined the measured chemical composition and forage quality characteristics of SB with the measured biomass yield and chemical composition of LB grown as living mulch. This approach assumes (i) additive behavior of the chemical constituents, with no chemical interaction, degradation or nutrient transfer between wheat straw and legume biomass after harvest; (ii) the analytical values measured for each component represent their contribution to the mixture; and (iii) the chemical composition of wheat straw harvested from sole wheat plots is representative of wheat straw produced under intercropping. Although intercropping may alter wheat physiology, N uptake and cell-wall development, potentially affecting straw composition, direct separation and independent chemical analysis of straw from harvested intercrop biomass was not practically feasible. Therefore, the calculated TSB values should be interpreted as model-based estimates.

For each treatment combination, the absolute mass of each nutritional component was first calculated separately for SB and LB. The masses from the two biomass components

were then summed to obtain the TSB of each nutritional parameter within the harvested biomass mixture:

$$TCw = (Sy_i \times Sc_i) + (Ly_{nij} \times Lc_{nij}) \quad (6)$$

where TCw is the total mass (kg) of a given nutritional component in the wheat straw–legume biomass mixture; Sy_i and Sc_i are the straw yield (kg) and composition ($g\ kg^{-1}$), respectively, of SB under fertilization treatment (i); Ly_{nij} and Lc_{nij} are the dry biomass yield (kg) and composition ($g\ kg^{-1}$), respectively, of legume species (n) under fertilization treatment (i) and rhizobium inoculation treatment (j).

The final concentration of each nutritional parameter in the wheat straw–legume biomass mixture (TFc, %) was calculated as:

$$TFc = \left(\frac{TCw}{Sy_i + Ly_{nij}} \right) \times 100 \quad (7)$$

where TCw, Sy_i , and Ly_{nij} are as previously defined.

This procedure allowed estimation of the chemical composition and forage quality of the TSB resource resulting from the integration of SB and LB under each treatment combination.

To evaluate the robustness of this assumption, a sensitivity analysis was conducted by varying the main chemical components of the wheat straw intercropped with forage legume (CP, EE, CF, NDF, ADF, and ADL) relative to the chemically analyzed values of SB. Six scenarios were considered: (i) CP and EE increased by 5%, while CF, NDF, ADF, and ADL decreased by 5%; (ii) CP and EE increased by 10%, while CF, NDF, ADF, and ADL decreased by 10%; (iii) CP and EE increased by 20%, while CF, NDF, ADF, and ADL decreased by 20%; (iv) CP and EE decreased by 5%, while CF, NDF, ADF, and ADL increased by 5%; (v) CP and EE decreased by 10%, while CF, NDF, ADF, and ADL increased by 10%; and (vi) CP and EE decreased by 20%, while CF, NDF, ADF, and ADL increased by 20%. For each scenario, forage-quality parameters were recalculated using the modified wheat straw composition, and the percentage gain or loss relative to the observed baseline was calculated. At 0% variation, the recalculated values corresponded to the baseline values.

2.7. Statistical Analysis

The effects of fertilization, legume species, and rhizobium inoculation on biomass production, chemical composition, and forage quality indices were assessed using linear mixed-effects models. Analyses were performed separately for each growing season to account for treatment responses under contrasting environmental conditions. Combining data across years could mask treatment effects by averaging responses obtained under different climatic conditions [32]. Block was included as a random factor, while fertilization, legume species, rhizobial inoculation, and their interaction were included as fixed factors. Rhizobial inoculation was nested within legume species. When significant effects were detected by analysis of variance (ANOVA), treatment means were separated using Tukey's honestly significant difference (HSD) test at $p \leq 0.05$.

To quantify changes in TSB quality resulting from legume addition, each treatment combination was compared against the control treatment (SB) using Fisher's least significant difference (LSD) test at $p \leq 0.05$. Statistical analyses were performed using Minitab version 19 (Minitab LLC, State College, PA, USA). Pearson correlation coefficients (r) among legume biomass, wheat straw biomass, the chemical composition and forage quality indices of the estimated TSB were calculated using the `rcorr()` function from the `Hmisc` package in R version 4.5.2 (R Core Team, 2025, Vienna, Austria).

3. Results

3.1. Biomass Production

Total system biomass (TSB) production, representing the wheat straw–legume biomass mixtures for feed utilization, was significantly affected by legume species during the 2023/2024 growing season ($F = 4.26$; $p = 0.013$) (Figure 2), whereas neither rhizobial inoculation nor fertilization significantly affected TSB. Among the tested species, *V. sativa* and *T. subterraneum* produced the highest TSB (4.04 and 4.01 Mg ha^{-1} , respectively), whereas *T. michelianum* did not differ from the sole-wheat control (3.04 and 3.25 Mg ha^{-1} , respectively). The contribution of LB to TSB also varied among species, with *V. sativa* providing the greatest biomass contribution ($+1.2$ Mg ha^{-1}).

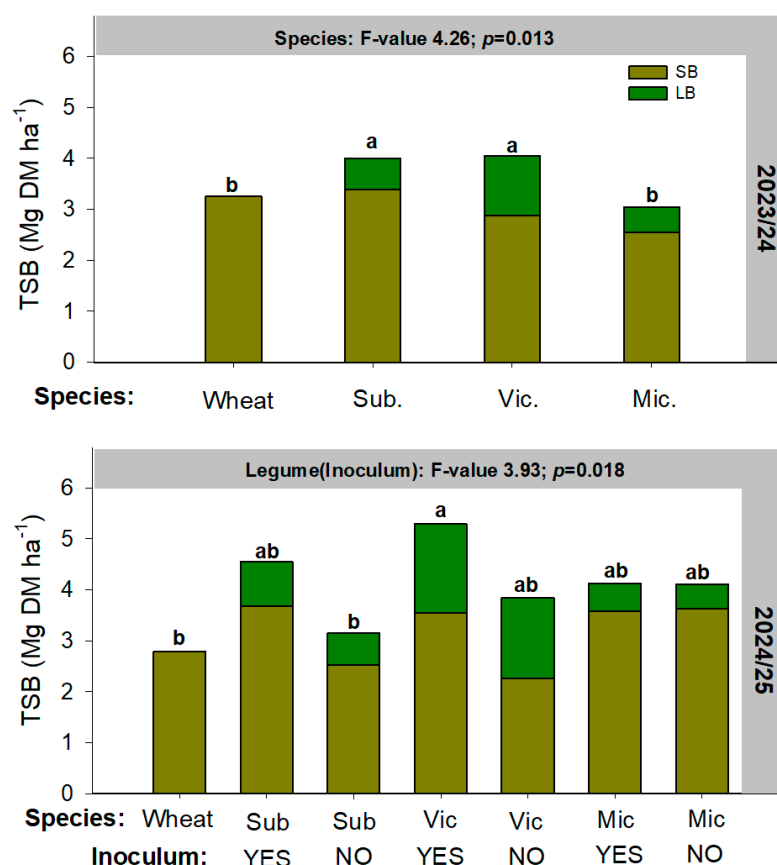


Figure 2. Total system biomass production (TSB) of durum wheat–legume living mulch systems under different legume species, rhizobial inoculation treatments (YES, NO) with species-specific elite rhizobia, and growing seasons (2023/2024 and 2024/2025). TSB includes wheat straw biomass (SB) and legume biomass (LB); Sub: *Trifolium subterraneum*; Vic: *Vicia sativa*; Mic: *Trifolium michelianum*. Means ($n = 3$) followed by different letters within columns are significantly different according to Tukey's HSD test ($p \leq 0.05$).

In the second growing season (2024/2025), a significant legume species and rhizobial inoculation effect was detected ($F = 3.93$; $p = 0.018$), whereas fertilization had no significant effect. Non-inoculated *T. subterraneum* did not differ from the control (3.15 and 2.78 Mg ha^{-1} , respectively). Inoculated *V. sativa* produced the highest TSB (5.3 Mg ha^{-1}). Inoculated legumes outperformed their non-inoculated counterparts: *V. sativa* and *T. subterraneum* increased TSB by 27% and 30% relative to the non-inoculated treatments, while no detectable effects were observed in *T. michelianum*.

3.2. Chemical Composition of Legume Biomass

The LB chemical composition was significantly influenced by species and, to a lesser extent, by fertilization (Table 3). Significant fertilization $\times$ species (inoculation) interactions were detected for CF, NDF, and ADF in 2023/2024 (Supplementary Tables S1–S3), whereas in 2024/2025 significant interactions were observed only for NDF and ADL (Supplementary Tables S4 and S5).

Table 3. Main effects and interaction of fertilization (F60, F120), and legume species intercropped with durum wheat [*Trifolium subterraneum* (Sub), *Trifolium michelianum* (Mic) and *Vicia sativa* (Vic)], either inoculated (Y) or non-inoculated (N), on legume biomass chemical composition during the 2023/2024 and 2024/2025 growing seasons.

2023/2024		DM	CP	EE	CF	Ash	NDF	ADF	ADL
Treatment	Level	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Fertilization (F)	F60	88.5 a	9.13 b	2.84 a	25.1 a	12.9 b	51.1 a	38.2 a	7.23 a
	F120	88.9 a	10.77 a	2.96 a	23.3 b	13.4 a	49.9 a	38.1 a	6.94 a
Species(inoculum) (Si)	Sub(Y)	88.1 b	7.92 bc	3.73 a	25.9 b	14.5 b	59.2 a	45.4 a	5.1 c
	Sub(N)	86.8 b	5.04 d	3.77 ab	21.8 c	16.4 a	54.8 b	41.5 b	4.9 c
	Mic(Y)	87.4 b	9.00 b	3.49 ab	24.3 bc	15.1 ab	56.7 ab	41.6 b	3.8 c
	Mic(N)	89.7 a	6.76 c	3.21 b	28.6 a	12.5 c	58.8 a	43.5 ab	10.7 a
	Vic(Y)	88.9 a	15.6 a	1.63 c	22.3 c	10.1 d	37.2 c	29.1 c	9.3 ab
	Vic(N)	90.2 a	15.3 a	1.57 c	22.1 c	10.5 d	36.2 c	27.9 c	8.6 b
$F \times Si$		ns	ns	ns	*	ns	***	***	ns
2024/2025									
Fertilization (F)	F60	90.8 a	12.7 a	2.62 a	25.33 a	10.7 a	49.1 a	37.3 a	10.7 a
	F120	90.9 a	12.9 a	2.50 a	24.49 b	11.1 a	48.1 a	36.5 a	10.5 a
Species(inoculum) (Si)	Sub(Y)	90.9 a	12.95 b	3.46 a	23.27 bc	12.69 a	54.72 a	37.94 b	10.2 b
	Sub(N)	90.8 a	11.93 bc	3.46 a	25.10 b	12.69 a	51.02 a	38.39 b	9.86 b
	Mic(Y)	90.7 a	11.67 bc	2.48 b	29.21 a	10.56 b	52.15 a	41.19 a	12.3 a
	Mic(N)	90.6 a	10.76 c	2.48 b	28.25 a	10.56 b	53.24 a	42.10 a	12.5 a
	Vic(Y)	90.8 a	14.88 a	1.74 c	21.26 c	9.49 c	39.28 b	29.76 c	9.27 b
	Vic(N)	90.9 a	14.68 a	1.74 c	22.35 c	9.49 c	41.30 b	32.06 c	9.79 b
$F \times Si$		ns	ns	ns	ns	ns	**	ns	*

DM = dry matter; CP = crude protein; EE = ether extract; CF = crude fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin. Means followed by different letters within columns are significantly different according to Tukey's HSD test ($p \leq 0.05$). Significant interaction of $F \times Si$ at $p \leq 0.001$ (***), at $p \leq 0.01$ (**), at $p \leq 0.05$ (*) or not significant (ns). Sub: *Trifolium subterraneum*; Vic: *Vicia sativa*; Mic: *Trifolium michelianum*.

In the 2023/2024 growing season, fertilization did not affect DM, ether extract (EE), ash, NDF, ADF, or ADL concentrations. In contrast, CP concentration was significantly higher under F120 than under F60 (10.77 vs. 9.13%), whereas CF was significantly lower under F120 (23.3 vs. 25.1%). In the 2024/2025 growing season, fertilization effects were limited. DM, CP, EE, ash, NDF, ADF, and ADL were not significantly affected by fertilization. Conversely, CF was significantly lower under F120 than under F60 (24.49 vs. 25.33%).

Species effects were generally more pronounced than fertilization effects. In both growing seasons, *Vicia sativa* consistently exhibited the highest CP concentration and the lowest CF and fiber fractions, whereas the clovers (*T. subterraneum* and *T. michelianum*) showed lower CP concentrations and greater structural fiber contents. Crude protein ranged from 5.04% in Sub(N) to 15.6% in Vic(Y) during 2023/2024 and from 10.76% in Mic(N) to 14.88% in Vic(Y) during 2024/2025.

Ether extract (EE) concentrations were highest in *T. subterraneum* (3.73–3.77% in 2023/2024 and 3.46% in 2024/2025), intermediate in *T. michelianum* (3.21–3.49% in 2023/2024 and 2.48% in 2024/2025), and lowest in *V. sativa* (1.57–1.63% in 2023/2024 and 1.74% in 2024/2025). Similarly, ash content was generally greater in clovers, reaching 16.4% in Sub(N) during 2023/2024, whereas *V. sativa* consistently showed lower values (10.1–10.5% in 2023/2024 and 9.49% in 2024/2025).

V. sativa exhibited also lower CF, NDF, and ADF concentrations than either *T. subterraneum* or *T. michelianum*. In 2023/2024, CF ranged from 22.1–22.3% in *V. sativa* to 21.8–28.6% in the clover species; NDF ranged from 36.2–37.2% in *V. sativa* to 54.8–59.2% in the clovers, and ADF ranged from 27.9–29.1% in *V. sativa* to 41.5–45.4% in the clovers. Similar differences were observed in 2024/2025, when CF ranged from 21.3–22.4% in *V. sativa* to 23.3–29.2% in the clover species, NDF ranged from 39.3–41.3% in *V. sativa* to 51.0–54.7% in the clovers, and ADF ranged from 29.8–32.1% to 37.9–42.1%, respectively.

Species-specific responses to rhizobial inoculation were evident among species–inoculation combinations. In 2023/2024, the highest CF value was recorded in Mic(N) (28.6%), whereas the lowest was observed in Sub(N) (21.8%). The highest NDF and ADF concentrations were found in Sub(Y) (59.2% and 45.4%, respectively), while the lowest values occurred in Vic(N) (36.2% and 27.9%, respectively). In 2024/2025, Sub(Y) exhibited the highest NDF concentration (54.72%), whereas *V. sativa*, irrespective of inoculation treatment, showed the lowest values (average 40.3%).

ADL concentration was significantly affected by species in both growing seasons. In 2023/2024, values ranged from 3.8% in Mic(Y) to 10.7% in Mic(N), whereas *V. sativa* showed intermediate values (8.6–9.3%). In 2024/2025, the highest ADL concentrations were recorded in *T. michelianum* (12.3–12.5%), whereas *V. sativa* exhibited lower values (average 9.5%).

3.3. Legume Biomass Quality Indices

Legume biomass quality indices were primarily influenced by species in both growing seasons (Table 4). During the 2023/2024, fertilization did not exert any significant main effect on TDN, DDM, DMI, RFV, or RFQ. However, significant fertilization × species (inoculation) interactions were detected for all forage quality indices, indicating that the response to fertilization depended on the legume species and inoculation treatment (Supplementary Tables S6–S11).

In the 2024/2025 growing season, fertilization effects were limited and significant only for DDM, which was slightly greater under F120 than under F60 (60.4 vs. 59.9%, respectively). A significant fertilization × species (inoculation) interaction was detected only for DMI (Supplementary Table S12).

Species exerted the strongest influence on legume biomass quality indices in both growing seasons. *V. sativa* consistently showed significantly higher TDN, DDM, DMI, RFV, and RFQ values than either *T. subterraneum* or *T. michelianum*. In 2023/2024, RFQ values ranged from 92.4 in Sub(Y) to 185.0 in Vic(N), whereas in 2024/2025 they ranged from 107.6 in Mic(N) to 166.7 in Vic(Y). Similarly, RFV values were markedly greater in *V. sativa* (165.9–173.5 in 2023/2024 and 144.4–155.9 in 2024/2025) than in the two clover species.

The superior forage quality of *V. sativa* was associated with its lower fiber concentrations and higher CP content (Table 3), which translated into greater digestibility and intake potential in both growing seasons. In contrast, both clover species exhibited lower TDN, DDM, DMI, RFV, and RFQ values, reflecting their higher fiber content (Table 3). Rhizobial inoculation did not alter the overall ranking among species, although its effects on forage quality indices depended on legume species and fertilization level.

Table 4. Main effects and interaction of fertilization (F60, F120), and legume species intercropped with durum wheat [*Trifolium subterraneum* (Sub), *Trifolium michelianum* (Mic) and *Vicia sativa* (Vic)], either inoculated (Y) or non-inoculated (N), on legume biomass quality indices during the 2023/2024 and 2024/2025 growing seasons.

2023/2024		TDN	DDM	DMI	RFV	RFQ
Treatment	Level					
Fertilization (F)	F60	61.1 a	59.1 a	2.47 a	115.7 a	125.0 a
	F120	61.2 a	59.2 a	2.51 a	117.6 a	127.1 a
Species(inoculum) (Si)	Sub(Y)	56.0 c	53.5 c	2.03 b	84.1 b	92.4 b
	Sub(N)	58.8 b	56.6 b	2.19 b	96.1 b	104.8 b
	Mic(Y)	58.7 b	56.5 b	2.12 b	93.2 b	101.5 b
	Mic(N)	57.4 bc	55.1 bc	2.04 b	87.1 b	95.3 b
	Vic(Y)	67.5 a	66.3 a	3.23 a	165.9 a	177.2 a
	Vic(N)	68.3 a	67.1 a	3.33 a	173.5 a	185.0 a
$F \times Si$		***	***	**	*	*
2024/2025						
Fertilization (F)	F60	61.8 a	59.9 a	2.48 a	116.1 a	125.5 a
	F120	62.3 a	60.4 b	2.55 a	120.4 a	129.9 a
Species(inoculum) (Si)	Sub(Y)	61.3 b	59.3 b	2.20 b	101.3 b	109.7 b
	Sub(N)	60.9 b	58.9 b	2.35 b	107.6 b	116.6 b
	Mic(Y)	59.0 c	56.8 c	2.30 b	101.4 b	110.5 b
	Mic(N)	58.4 c	56.1 c	2.26 b	98.6 b	107.6 b
	Vic(Y)	67.0 a	65.7 a	3.06 a	155.9 a	166.7 a
	Vic(N)	65.4 a	63.9 a	2.91 a	144.4 a	154.9 a
$F \times Si$		ns	ns	*	ns	ns

TDN = total digestible nutrients; DDM = digestible dry matter; DMI = dry matter intake; RFV = relative feed value; RFQ = relative forage quality. Means followed by different letters within columns are significantly different according to Tukey's HSD test ($p \leq 0.05$). Significant interaction of $F \times Si$ at $p \leq 0.001$ (***), at $p \leq 0.01$ (**), at $p \leq 0.05$ (*) or not significant (ns). Sub: *Trifolium subterraneum*; Vic: *Vicia sativa*; Mic: *Trifolium michelianum*.

3.4. Nutritional Characteristics of Wheat Straw–Legume Biomass Mixture (TSB)

The integration of LB into SB substantially improved the nutritional characteristics of the estimated wheat straw-legume biomass mixture in both growing seasons (Figure 3). Compared with SB, all treatments, except non-inoculated *T. michelianum*, significantly increased the estimated CP concentration of the TSB. The greatest improvement was observed with inoculated *V. sativa*, which increased CP from baseline values of 4.2% to 8.1% in 2023/2024 and from 3.9% to 6.9% in 2024/2025.

In addition to CP, the incorporation of LB generally increased the estimated EE, ash content, TDN, DDM, DMI, RFV, and RFQ (Figure 3). Among these parameters, RFV and RFQ showed the greatest improvements, indicating a marked improvement in the feeding value of the resulting biomass mixtures. Inoculated *V. sativa* produced the largest increases, raising RFV and RFQ from a baseline value of 59.7% and 66.3% to 96.0% and 104.2%, respectively, in 2023/2024. Similar improvements were observed in 2024/2025, when RFV increased from 57.3% to 97.9% and RFQ from 63.9% to 106.2%. Although less pronounced than those observed for *V. sativa*, significant increases in estimated RFV and RFQ were also obtained with *T. subterraneum* and *T. michelianum*, irrespective of rhizobial inoculation, except for non-inoculated *T. michelianum* 2024/2025, which did not differ from the SB.

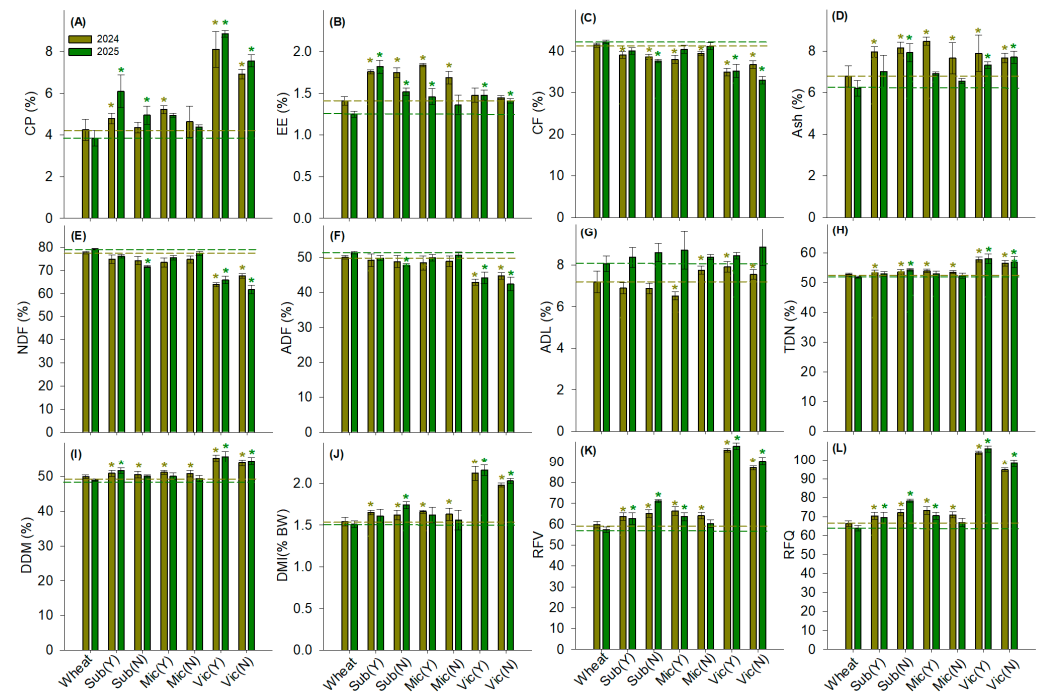


Figure 3. Chemical composition and forage quality indices of estimated wheat straw–legume biomass mixtures compared with sole-wheat straw (wheat) under different legume species [(*Trifolium subterraneum* (Sub), *Trifolium michelianum* (Mic), and *Vicia sativa* (Vic)], rhizobial inoculation treatments (Y or N), across fertilization rates in the two growing seasons (2023/2024 and 2024/2025). All nutritional data are modelled through a mass-balance approach. (A) crude protein (CP), (B) ether extract (EE), (C) crude fiber (CF), (D) ash, (E) neutral detergent fiber (NDF), (F) acid detergent fiber (ADF), (G) acid detergent lignin (ADL), (H) total digestible nutrients (TDN), (I) digestible dry matter (DDM), (J) dry matter intake (DMI), (K) relative feed value (RFV), and (L) relative forage quality (RFQ). Dashed lines (dark yellow for 2023/2024 and dark green for 2024/2025) indicate the corresponding values of sole-wheat straw. Asterisks (dark yellow for 2023/2024 and dark green for 2024/2025) indicate significant differences of treatments from sole-wheat straw according to Fisher's least significant difference (LSD) test ($p \leq 0.05$). Bars are standard error ($n = 3$).

The improvement in forage quality was associated with lower fiber concentrations in the estimated biomass mixtures. In particular, CF, NDF, and ADF decreased following the incorporation of LB, with *V. sativa* producing the greatest reductions irrespective of inoculation treatment. The average baseline CF concentration of SB, ranging from 41.4 to 42.2% in 2023/2024 and 2024/2025, respectively, decreased to 34.9% in Vic(Y), and 33.1–35.2% in the corresponding *V. sativa* treatments. Likewise, average NDF decreased from a baseline value of 78.4% to 64.4–64.8%, while ADF decreased from 50.7% to 43.4–43.5%.

The inclusion of *T. subterraneum* and *T. michelianum* also reduced fiber fractions, although responses were generally smaller and less consistent than those observed for *V. sativa*. Significant reductions in CF were observed for most treatments during 2023/2024, whereas in 2024/2025 significant effects were detected mainly for Sub(N). In the second growing season, Sub(N) also reduced NDF and ADF, whereas no significant effects were observed for the remaining legume–inoculation combinations.

Averaged across fertilization levels, legume species, rhizobial inoculation, and growing season, the sensitivity analysis showed changes in TSB composition across the -20 to $+20\%$ range (Supplementary Figure S1). Relative to the chemically analyzed SB (baseline = 0%), CP increased progressively from $+24\%$ at the -20% scenario to $+50\%$ at the $+20\%$ scenario, while EE and ash increased by approximately 30% and 25%, respectively, at the 20% scenario. CF, NDF, and ADF showed limited changes, increasing by only 5.2%, 5.6% and 7.9%, respectively, at $+20\%$ scenario. ADL increased from $+5\%$ scenario onwards.

Among forage-quality parameters, DDM and TDN decreased only at the +20% scenario (−5.1 and −6.0%, respectively), whereas DMI, RFQ, and RFV remained above baseline across the sensitivity range.

3.5. Correlation Between Legume Biomass, Sole-Wheat Straw Biomass with Nutritional Characteristics of Wheat Straw–Legume Biomass Mixtures (TSB)

The correlation matrix showed that increasing LB contribution to the TSB was associated with substantial improvements in both the chemical composition and forage quality (Figure 4). Legume biomass was strongly and positively correlated with CP ($r = 0.904$), EE ($r = 0.234$), ash content ($r = 0.505$), and the main forage quality indices, including TDN ($r = 0.360$), DDM ($r = 0.899$), DMI ($r = 0.899$), RFV ($r = 0.894$), and RFQ ($r = 0.895$). Conversely, LB was strongly and negatively correlated with CF ($r = -0.915$), NDF ($r = -0.914$), and ADF ($r = -0.899$).

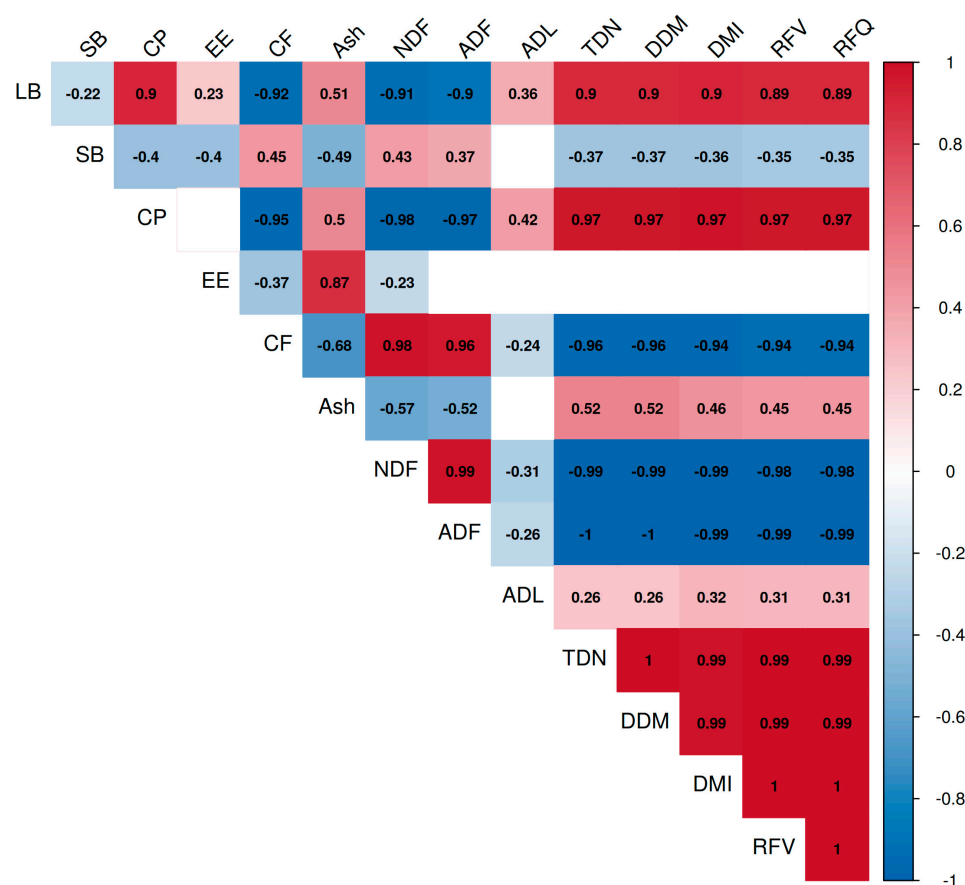


Figure 4. Pearson correlation coefficient matrix of pooled data from the two growing seasons, fertilization rates, legume species and rhizobial inoculation, among legume biomass (LB), sole-wheat straw biomass (SB), chemical composition [crude protein (CP), ether extract (EE), crude fiber (CF), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL)] and forage quality indices [total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), relative forage quality (RFQ)] of the estimated wheat straw–legume biomass mixtures. The value of the correlation coefficient, along with a color (red: positive and blue: negative) gradient, indicates its strength. Only significant correlations ($p \leq 0.05$) are presented.

In contrast, SB was negatively correlated with CP, ash content, and the principal forage quality indices (TDN, DDM, DMI, RFV, and RFQ), while showing positive correlations with the CF and the fiber fractions (NDF and ADF).

Among all variables, the strongest correlations were observed between fiber fractions and forage quality indices. NDF and ADF were strongly and negatively correlated with

DDM, DMI, RFV, and RFQ ($r = -0.96$ to -0.99), while TDN, DDM, DMI, RFV, and RFQ were highly and positively intercorrelated ($r = 0.97$ – 1.00).

4. Discussion

The present study demonstrated that legume species represented the main factor affecting both biomass production and the nutritive value of wheat straw–legume biomass mixtures, whereas fertilization exerted only limited effects. This pattern is consistent with the strong role of species identity in forage-based systems and legume productivity dynamics [22,33,34].

As demonstrated in a previous agronomic study [24], the warmer and drier conditions in 2023/2024 limited legume biomass production and BNF and accelerated wheat development. The yield response in 2024/2025 may reflect better hydroclimatic conditions and partly carry-over effects from the previous year, including residual N availability following N fixation and residue decomposition, as reported for legume cover crops in Mediterranean systems [35–37]. However, these legacy effects were not directly measured in the present study and should therefore be considered a plausible interpretation rather than a demonstrated mechanism.

Among the evaluated species, *V. sativa* showed the greatest biomass contribution to TSB across both growing seasons. In the first growing season, *V. sativa* biomass contribution ($+1.2 \text{ Mg ha}^{-1}$) did not negatively affect durum wheat straw production, as the TSB of common vetch and subterranean clover (4.04 and 4.01 Mg ha^{-1} , respectively) was not statistically different and above the SB. A similar pattern was observed for the second growing season, with no differences in SB between inoculated *T. subterraneum* and *V. sativa*, and between non-inoculated *T. subterraneum* and *V. sativa*, suggesting the absence of competitive effects by *V. sativa* on wheat biomass production.

Moreover, *V. sativa* showed the most favorable nutritional profile, characterized by higher CP concentration and lower structural fiber fractions (NDF and ADF) than both *T. subterraneum* and *T. michelianum*. Consequently, *V. sativa* exhibited the highest DDM, DMI, RFV and RFQ values. Similar results have been reported for annual forage legumes grown under Mediterranean conditions, where common vetch generally provides higher protein concentration and lower fiber content than other annual legumes, resulting in superior forage quality [38–42]. However, it should be highlighted that common vetch generally requires higher seeding rates than clovers and other forage legumes, thus increasing the costs for seeds. A cost–benefit analysis is therefore needed for practical recommendations to farmers.

The superior nutritive value observed in *V. sativa* can largely be attributed to its lower concentration of structural carbohydrates and reduced cell-wall components. Lower NDF and ADF concentrations are directly associated with greater digestibility and voluntary intake, which ultimately determine forage feeding value [13]. Accordingly, the lower fiber concentrations recorded in *V. sativa* translated into consistently higher RFV and RFQ values across both growing seasons.

One key outcome of the present study was the improvement in the nutritional characteristics of the estimated TSB following the integration of forage legume biomass with wheat straw through a mass-balance approach. Although the mass-balance approach provides estimated rather than directly measured TSB nutritional values, the sensitivity analysis supports the robustness of the resulting estimates. Across the -20 to $+20\%$ range of simulated changes in wheat straw composition, CP showed the greatest increase, whereas CF, NDF, ADF, and ADL varied only moderately. Importantly, these changes had limited effects on the main forage-quality parameters: DDM and TDN decreased only under the most extreme ($+20\%$) scenario (5.1 and 6.0% , respectively), while DMI, RFQ, and RFV

remained above the baseline across all scenarios. The direction of the simulated responses was also consistent with available evidence indicating that, under favorable intercropping combinations and spatial arrangements, cereal–legume associations may increase crude protein and reduce fiber-related recalcitrance in the cereal component [43]. This improvement in the nutritional composition (i.e., higher CP, lower fiber fractions, improved RFV and RFQ) of the harvested biomass resource highlights the agro-livestock value of living mulch systems, which not only provide ecosystem services during crop growth but also enhance the feeding value of harvested cereal residue-legume biomass resources [44,45]. Similar improvements in CP concentration and digestibility have been reported in cereal–legume associations under Mediterranean conditions, where legumes increased forage quality and reduced cell wall constituents [40,46].

From a livestock perspective, these results are particularly relevant because wheat straw is traditionally considered a low-quality feed resource owing to its low protein concentration and elevated fiber content. The incorporation of forage legume biomass substantially improved the nutritional profile of the resulting wheat straw–legume biomass mixtures, increasing their potential value as roughage resources in integrated crop-livestock systems. Moreover, legume-based diets have been associated with improved nutrient utilization and greater sustainability of ruminant production systems [47].

From a practical farm-management perspective, legume living mulch may represent an integrated alternative to post-harvest chemical upgrading of cereal straw, such as urea or ammonia treatment. While chemical treatments require additional post-harvest operations, labour and handling of the treated material [48,49], legume living mulch produces additional forage biomass directly in the field and can potentially be harvested using conventional cereal harvesting equipment. The living mulch approach may also be particularly relevant to organic farming systems, where production practices and inputs are subject to specific requirements under EU organic production rules [50]. However, the inclusion of legume biomass may increase the moisture content of the harvested material, making adequate drying essential to limit microbial deterioration, dry-matter losses and reduced storage stability [51]. Because labour requirements and feed shelf-life were not directly measured in the present study, these aspects should be considered as practical considerations rather than experimentally demonstrated differences. Therefore, legume living mulch should be considered an integrated crop–livestock strategy rather than a universal replacement for chemical straw treatment.

From an applied livestock perspective, the estimated CP concentration of the wheat straw–legume mixtures can be considered in relation to the dietary protein requirements of ruminants. For sheep, dietary CP concentrations of approximately 7–9% are generally considered adequate for maintenance, whereas requirements may increase to approximately 14–18% for lactating ewes and growing lambs [52]. Similarly, beef cattle generally require diets containing approximately 7% CP or more under maintenance conditions, with higher concentrations are required during growth, late gestation or other productive stages [53]. The estimated CP concentrations of the *V. sativa*-containing TSB therefore indicate a greater potential dietary protein contribution than sole wheat straw, particularly for sheep and beef cattle maintained under low-input conditions. However, the relatively high fiber concentration of the mixtures and the absence of direct measurements of intake, protein digestibility, ruminal degradation and metabolizable protein supply mean that CP concentration alone cannot establish whether the mixtures would fully satisfy animal requirements. Accordingly, the estimated TSB CP, RFV and RFQ values should be interpreted as indicators of improved forage nutritional potential and as a basis for ration formulation, rather than as evidence of dietary adequacy or animal performance.

A simple farm-scale protein mass balance further illustrates the potential contribution of *V. sativa* living mulch to on-farm protein supply. The estimated CP yield of the *V. sativa*-based TSB was 307 and 371 kg CP ha⁻¹ in the first and second seasons compared with 140 and 107 kg CP ha⁻¹ in the sole-wheat straw. This corresponds to an estimated additional 167 to 264 kg CP ha⁻¹ in the harvested on-farm roughage resource. This represents an upper-bound estimate and should not be interpreted as a demonstrated reduction in purchased or imported protein feed, because protein digestibility, ruminal degradation, nitrogen utilization, animal requirements and diet formulation were not evaluated in the present study. Actual replacement potential would therefore need to be quantified through feeding trials and farm-scale ration and economic analyses.

Fertilization exerted relatively limited effects compared with species, although significant responses were observed for CP and CF concentrations, particularly during the 2023/2024 growing season. This limited response is consistent with the functioning of legume-based systems, in which BNF contributes substantially to plant N nutrition and reduces dependence on external inputs [54,55]. Similar observations have been reported in Mediterranean low-input forage systems, where species composition exerted a greater influence on forage quality than moderate changes in N fertilization [40]. Furthermore, the weaker fertilization effect observed in the second year may be attributable to a legacy effect, as legume living mulches were grown in the same plots in both years. Residual contributions from the previous season could have enhanced soil fertility and N availability, reducing crop responsiveness to fertilizer application [24].

In the present study, year-to-year differences in CP concentration and forage quality indices suggest that environmental conditions affected both biomass accumulation and nutrient partitioning. Similar seasonal effects have been reported in Mediterranean annual forage systems, where rainfall distribution and temperature patterns strongly influence forage yield and quality [56,57], confirming the importance of climatic variability in determining the performance of Mediterranean forage-based systems.

The effects of rhizobial inoculation were species- and season-dependent [58]. Although responses were not consistent across all treatments, inoculation enhanced biomass production, particularly in *V. sativa* and *T. subterraneum*, during the wetter growing season. Significant fertilization × species (inoculation) interactions observed for several fiber fractions and forage quality traits suggest that the response to inoculation varied according to the specific legume–environment combination. Such variability is frequently reported in forage legumes and is largely influenced by host–strain compatibility, the abundance and effectiveness of indigenous rhizobial populations, and environmental conditions [59–61]. In addition, the effectiveness of rhizobial inoculants may depend on the symbiont genotype (commercial elite strains or selected native rhizobia), formulation (peat, granular, liquid, or freeze-dried powders), and application rate [6,7]. Where native rhizobia are already abundant and effective, the benefits of inoculation may therefore be limited. In the present study, the weak response to inoculation observed in *T. michelianum* may be related to the limited host–strain compatibility. This interpretation is consistent with Rodríguez-Navarro et al. [62], who reported greater nodulation of pasture legumes in Spain by *Trifolium*-nodulating rhizobia than by *R. leguminosarum* bv. *trifolii*. Environmental conditions may also have contributed to the stronger inoculation response observed during the 2024/2025 growing season. The cooler and wetter likely increased soil moisture availability and favored rhizobial activity and nodulation, as previously reported by Pérez-Fernández et al. [63]. Given that the native rhizobial communities were presumably similar across seasons and that the positive effects of inoculation were particularly evident in *V. sativa*, our results suggest that inoculation effectiveness was influenced more strongly

by legume species characteristics and environmental conditions than by differences in indigenous rhizobial populations [61].

The forage quality indices obtained for the legume biomass further emphasize the potential value of *V. sativa* living mulch within durum wheat. Relative forage quality (RFQ) values of the legume biomass exceeded 150 in both growing seasons and approached 185 under some treatment combinations. According to the classification proposed by Moore and Undersander [16], RFQ values above 150 are generally associated with premium-quality forage and high voluntary intake. In contrast, both clover species exhibited lower RFQ and RFV values due to their higher fiber concentrations, although their nutritive value remained acceptable for ruminant feeding. The RFQ values observed for *V. sativa* were comparable to or higher than those reported for high-quality annual forage legumes grown under Mediterranean conditions (e.g., vetch and other temperate legumes), supporting its potential value as a companion species in living mulch systems.

The correlation analysis further emphasizes the key role of legume biomass in determining the chemical composition and forage quality of wheat straw-legume biomass mixtures. As the proportion of legume biomass increased, protein concentration and mineral content increased, while the accumulation of structural carbohydrates declined. This shift in chemical composition was accompanied by higher digestibility, greater intake potential, and improved overall feeding value. These findings are consistent with generally lower cell-wall content and higher N concentration of legumes compared with cereal residues [13,39,40]. The strong associations between fiber fractions and the forage quality indices further emphasize the central role of cell-wall components in determining forage utilization by ruminants. In particular, NDF and ADF were closely associated with reductions in digestibility and intake potential, confirming that fiber concentration remains one of the principal constraints to forage nutritive value [13,17]. From a ruminant feeding perspective, high NDF concentrations may restrict voluntary dry matter intake by increasing rumen fill and slowing digesta passage, whereas high ADF concentrations are generally associated with a greater proportion of less digestible cell-wall components, thereby reducing forage digestibility and the supply of digestible nutrients. Conversely, increases in CP and ash content were associated with improvements in all major quality indices.

In contrast, a greater contribution of wheat straw biomass was associated with a decline in forage quality. This outcome reflects the intrinsic characteristics of cereal straw, which is typically rich in structural carbohydrates and poor in protein and minerals. Consequently, estimated mixtures containing higher proportions of wheat straw exhibited lower digestibility and intake potential, resulting in reduced feeding value. Therefore, the positive effects of legume inclusion can be attributed not only to the direct contribution of a more nutritious biomass but also to the dilution of the highly fibrous straw fraction within the harvested forage [13,40].

However, it should also be acknowledged that forage quality indices do not account for all secondary metabolites, some of which may have antinutritional effects depending on their concentration and dietary context. Forage legumes can contain condensed tannins, saponins and other bioactive compounds, although their concentrations and effects vary among species, cultivars, plant tissues and environmental conditions [64]. These compounds were not quantified in the present study; therefore, their potential contribution to differences in voluntary intake or nutrient utilization cannot be directly assessed. Consequently, the estimated RFV and RFQ values should be interpreted as indicators of potential forage quality rather than as direct predictors of voluntary intake or animal performance.

5. Conclusions

The present study demonstrated that (1) forage legume living mulch consistently improved the biomass yield of the resulting wheat straw-legume biomass mixture in the drier year, while inoculation also had a positive effect in the wetter year; (2) legume species selection was the primary factor determining the nutritional value of the estimated wheat straw–legume biomass mixtures derived from legume living mulch systems in durum wheat; (3) fertilization exerted no effects on biomass yield and only minor changes in composition, confirming the suitability of these systems for low-input and organic farming conditions.

Among the evaluated companion legumes, *Vicia sativa* consistently exhibited the highest CP concentration and the lowest fiber fractions, resulting in superior digestibility and forage quality indices.

Incorporating forage legumes into wheat-based living mulch systems enhanced the feeding value of the harvested biomass, mainly through higher CP and improved forage-quality characteristics. This strategy offers a promising means of converting cereal residues into a more valuable on-farm feed resource, with potential to improve feed self-sufficiency and resource efficiency in integrated crop–livestock systems, particularly in organic and low-input mixed crop–livestock farms with sheep and beef production. However, the economic benefits of the system require further evaluation through farm-scale cost–benefit analyses considering seed costs, biomass yield, harvesting and handling requirements, and potential savings from reduced purchased feed inputs.

This study (Part I) assessed biomass production, chemical composition, forage quality indices, and feeding value, whereas Part II focuses on fatty acid composition, polyphenol content, and other bioactive compounds. Collectively, the two studies offer a comprehensive evaluation of the nutritional and functional potential of wheat straw–legume biomass mixtures produced through the integration of forage legume living mulches into durum wheat systems. The mass-balance approach adopted here provides robust estimates of the nutritional characteristics of the harvested biomass resource, with the sensitivity analysis confirming that moderate changes in wheat straw chemical composition associated with forage legume intercropping have limited effects on the estimated nutritional value of the resulting straw–legume biomass mixture.

Future research should validate these findings through direct chemical characterization of harvested wheat straw–legume mixtures, while testing different legume seeding ratios across contrasting Mediterranean environments and under commercial farming conditions. On-farm feeding trials would further help determine effects on feed intake and animal performance.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture16161785/s1>, Table S1: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on fiber content in 2023/24 growing seasons; Table S2: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on neutral detergent fiber (NDF) in 2023/24 growing seasons; Table S3: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on acid detergent fiber (ADF) in 2023/24 growing seasons; Table S4: Grouping information using the Tukey Method and 95% Confidence for the interaction

between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on neutral detergent fiber (NDF) in 2024/25 growing seasons; Table S5: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on acid detergent lignin (ADL) in 2024/25 growing seasons; Table S6: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on total digestible nutrients (TDN) in 2023/24 growing seasons; Table S7: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on digestible dry matter (DDM) in 2023/24 growing seasons; Table S8: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on dry matter intake (DMI) in 2023/24 growing seasons; Table S9: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on relative forage quality (RFQ) in 2023/24 growing seasons; Table S10: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on relative feed value (RFV) in 2023/24 growing seasons; Table S11: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on relative forage quality (RFQ) in 2023/24 growing seasons; Table S12: Grouping information using the Tukey Method and 95% Confidence for the interaction between fertilization (F60, F120), and legume species intercropped with durum wheat [wheat-*Trifolium subterraneum* (Sub); wheat-*Trifolium michelianum* (Mic); wheat-*Vicia sativa* (Vic)], rhizobium inoculated or not (YES or NO), on dry matter intake (DMI) in 2024/25 growing seasons. Figure S1: Sensitivity analysis of TSB forage-quality parameters to changes in the chemical composition of wheat straw. Values represent the percentage gain or loss relative to the observed baseline (0%). Scenarios correspond to simultaneous changes in CP (crude protein) and EE (ether extract) and opposite changes in CF (crude fiber), NDF (neutral detergent fiber), ADF (acid detergent fiber), and ADL (acid detergent lignin) of $\pm 5\%$, $\pm 10\%$, and $\pm 20\%$, and the resulting forage quality indices [total digestible nutrients (TDN), digestible dry matter (DDM), dry matter intake (DMI), relative feed value (RFV), relative forage quality (RFQ)].

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