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Forage legume living mulch and rhizobial inoculation in Mediterranean organic durum wheat

Danilo Scordia[1] | **Francesca Calderone**[1] | **Aurora Maio**[1] | **Tommaso La Malfa**[1] | **Aurelio Scavo**[1] | **Marianna Oteri**[1] | **Calvin Trostle**[2] | **Fabio Gresta**[1]

[1]Department of Veterinary Sciences, University of Messina, Messina, Italy

[2]Department of Soil and Crop Sciences, Texas A&M AgriLife Extension, Lubbock, Texas, USA

Correspondence
Danilo Scordia, Department of Veterinary Sciences, University of Messina, Italy.
Email: danilo.scordia@unime.it

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Abstract

Intercropping rhizobia-inoculated forage legumes in organic wheat systems reduces weed pressure and supplies nitrogen (N) to the companion crop via biological N_2 fixation (BNF). However, adoption of combined intercropping and rhizobial inoculation remains limited. This 2-year field study compared durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), as a sole crop, or intercropped with forage legume (*Trifolium subterraneum* L., *Trifolium michelianum* Savi, or *Vicia sativa* L.) in southern Italy. Legumes were rhizobia-inoculated or non-inoculated. Treatments were evaluated under reduced N fertilization (60 kg N ha^{-1}) or standard N fertilization (120 kg N ha^{-1}). The warmer and drier 2023/2024 season reduced wheat yields but increased grain protein, whereas the wetter 2024/2025 season enhanced yields, BNF (estimated using published empirical relationships linking N_2 fixation to legume biomass yield and shoot N content), and wheat N recovery efficiency. Regardless of environmental conditions or agronomic treatments, wheat–legume systems consistently reduced weed biomass compared with sole wheat. Inoculated *V. sativa* and *T. subterraneum* improved wheat grain yield versus sole wheat, particularly under favorable conditions at reduced N rate. *Vicia sativa* exhibited the highest BNF, especially when inoculated at the low N rate, whereas *T. michelianum* showed smaller benefits. Inoculated *T. subterraneum* showed the highest nodule number and weight, and higher soil nitrate nitrogen (NO_3^--N), particularly at the higher N rate. Although results were context-dependent on environmental conditions, legume living mulches integrated with effective rhizobial inoculation and reduced N fertilization support the agronomic sustainability of organic wheat systems in Mediterranean environments.

Abbreviations: BNF, biological nitrogen fixation; N120, N at 120 kg ha^{-1}; N60, N at 60 kg ha^{-1}; NUEr, N recovery efficiency; NUP, N uptake; Tmax, maximum air temperature; Tmin, minimum air temperature; WB, weed dry biomass; WGP, wheat grain protein; WGY, wheat grain yield.

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Plain Language Summary

Mediterranean agroecosystems face structural constraints (land, water scarcity, and degradation) and agronomic limitations (low crop diversity, limited legume use, and high weed pressure), creating a strong need for resilient agroecological practices: (1) Intercropping forage legume living mulches with rhizobial inoculation improved yield performance and N-use efficiency of organic durum wheat compared with sole cropping or non-inoculated intercrops; (2) all legume living mulches consistently suppressed weeds relative to sole wheat, independent of N rate and inoculation; (3) inoculated common vetch maximized biological N_2 fixation; (4) inoculated subterranean clover showed high nodulation and increased soil nitrogen nitrate availability; and (5) the warmer and drier season reduced wheat yields but increased grain protein, whereas the wetter season enhanced yields, biological N_2 fixation, and N recovery efficiency.

1 | INTRODUCTION

European Mediterranean agroecosystems face multiple structural and environmental constraints, including limited arable land, water scarcity, and land degradation (Antonelli et al., 2022). These challenges are compounded by low crop diversity, infrequent legume breaks in crop rotations, and weed pressure in herbicide-free systems. Despite these challenges, the region also presents several opportunities, such as its exceptional biodiversity (Mittermeier et al., 2004), strong policy incentives supporting sustainable agriculture (e.g., the EU Green Deal, Farm to Fork Strategy, and Common Agriculture Policy), and expansion of organic farming (Willer et al., 2023). In this context, identifying agroecological practices that can enhance the sustainability and resilience of cropping systems is a priority (von Cossel et al., 2025).

Among these practices, intercropping forage legume living mulches in organic wheat systems stands out as a promising strategy (Cougnon et al., 2022). Organic wheat production often suffers from substantial weed pressure and limited nutrient availability. Legume living mulches can help address both constraints by reducing the need for synthetic herbicides and mineral nitrogen (N) fertilizers (Devi et al., 2023). Through biological N_2 fixation (BNF), weed suppression, and the provision of multiple ecosystem services, legume living mulches can provide N to cereals while strengthening the competitive performance of the cash crop (Cougnon et al., 2022; Lamichhane et al., 2025). Nevertheless, their adoption in Mediterranean wheat systems remains limited, often reflecting farmers' concerns about management challenges and the reliability of expected benefits. This practice is even more relevant given the forthcoming "EU carbon tax" (EU Carbon Border Adjustment Mechanism—CBAM; Regulation (EU) 2023/956; European Union, 2023). This likely increases the cost of extra-EU fertilizers through import charges proportional to their N content. Additional price pressure is also arising from geopolitical instability, including the 2026 Middle East conflict and the blockage of the Strait of Hormuz, with a 30%–50% increase in mineral N fertilizer prices, particularly urea.

Another underexplored aspect of wheat–legume systems is legume-specific rhizobial inoculation. Symbiotic BNF, carried out by N_2-fixing bacteria, including *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium* (Kuzmanović et al., 2022), provides legumes with renewable N and enriches soil N for companion or subsequent crops (Drevon et al., 2015; Schwember et al., 2019). Since BNF efficiency depends on compatibility between the legume host and the rhizobial strain, inoculants containing species-specific strains are applied to ensure effective nodulation (Lupwayi et al., 2000).

Many studies on the effects of rhizobial inoculation of cereal/grain legume intercropping have been reported. These include maize (*Zea mays* L.)/soybean (*Glycine max* L.) (Nyoki & Ndakidemi, 2018), maize/faba bean (*Vicia faba* L.) (Mei et al., 2012), maize/common bean (*Phaseolus vulgaris* L.) (Cardoso et al., 2007), and wheat/faba bean (Cheto et al., 2023). In contrast, published evidence on inoculant use in forage legumes grown as living mulches in durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.] is entirely lacking. In grain legumes, most fixed N_2 is removed at harvest because the majority accumulates in the seed at maturity. In contrast, forage legumes typically contribute more to soil N and overall soil quality due to greater rooting biomass and longer field residence (Panagea et al., 2025; Salon et al., 2001).

Research on intercropping or relay-cropping using non-inoculated forage legumes with wheat has reported contrasting outcomes. For example, temporary intercropping with Persian clover (*Trifolium resupinatum* L.) increased wheat grain protein (WGP) content but often reduced yield by ~20% in northern Italy (Pellegrini et al., 2021). Subterranean clover

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(*Trifolium subterraneum* L.) living mulch suppressed weeds by 22%–75% and maintained wheat yield across European and Mediterranean environments when subterranean clover biomass was low but reduced wheat grain yields (WGYs) of 14%–16% when subterranean clover biomass was high (Radicetti et al., 2018). In central Italy, wheat–subterranean clover maintained stable wheat yields and improved weed suppression under narrow row spacing (two rows of wheat and one row of subclover at 10 cm spacing). In contrast, the same-row configurations (wheat and subclover mixed in the same row, with 15 cm row spacing) resulted in wheat yield reductions of about 14% (Campiglia et al., 2014).

Other recent studies in central Italy show a wide range of perennial, annual, and self-seeding legumes can be relay-intercropped with durum wheat without reducing yield, N uptake, or grain protein, while weed suppression reached 90% during wheat growth (Leoni et al., 2022; 2024). Nitrogen inputs were negligible from crimson clover (*Trifolium incarnatum* L.) to >180 kg N ha^{-1} from Italian sainfoin [*Sulla coronaria* (L.) Medik. (syn. *Hedysarum coronarium* L.)]. However, poorly suited legumes, such as Persian clover, hairy vetch (*Vicia villosa* Roth), barrel medic (*Medicago truncatula* Gaertn), and snail medic [*Medicago scutellata* (L.) Mill.], failed to reduce weed pressure (Leoni et al., 2024). Furthermore, management context also made a difference, with alfalfa (*Medicago sativa* L.), Italian sainfoin, and subterranean clover showing superior performance under organic conditions (Leoni et al., 2022). In drier Mediterranean areas of southern Italy, simultaneous intercropping of wheat with annual, low-yielding forage legumes, such as subterranean clover and burr medic (*Medicago polymorpha* L.), was more effective than relay-cropping (Scordia et al., 2024). When legumes are sown later (i.e., relay-cropping), autumn–winter weeds are already established within the wheat stand, reducing legume competitiveness and mulch formation. Hence, species selection and management practices are critical for balancing interspecific competition and resource complementarity.

Objectives of this study were to evaluate the effects of (A) simultaneous sowing of a forage legume for living mulch combined with rhizobial inoculation on organic durum wheat versus (B) sole wheat with and without non-inoculated legumes under two levels of organic N fertilization over two consecutive years in a Mediterranean environment. WGY and protein content, weed biomass, BNF, and wheat N recovery efficiency (NUEr) were assessed. In addition, during the second year, nodule number and weight, and soil nitrate nitrogen, were measured at wheat and legume flowering. The study tested the hypothesis that rhizobial inoculation of a forage legume living mulch strengthens its agroecological functions, enabling concurrent weed suppression and improved N supply while maintaining wheat yield and grain quality, especially in N-reduced conditions.

2 | MATERIALS AND METHODS

2.1 | Experimental design

A 2-year field study was carried out on a certified organic farm in southern Italy (Patti, Messina, Sicily; 38°11′99″ N, 14°98′63″ E; 196 m a.s.l.). The soil was classified as typical Calcixerepts according to USDA Soil Taxonomy (Soil Survey Staff, 2022) and was initially sampled at 0- to 30-cm depth. It is clay (sand 32%, silt 11%, and clay 57%) and calcareous (total $CaCO_3$ 170 g kg^{-1}), with pH 7.8, organic C 0.47% and organic matter 0.81% (Walkley and Black method), total N 0.3 g kg^{-1} (Kjeldahl method), available P 35.6 mg kg^{-1} (Olsen method), exchangeable K 42.0 mg kg^{-1} (ammonium acetate test method), and C/N ratio 15.

The 20-year mean climatic conditions of the area in the cool-season cropping period (December-June) are 10.0°C and 19.7°C for minimum (Tmin) and maximum (Tmax) air temperatures, respectively, and annual rainfall of 439 mm.

Durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn., var. Core] was the cash crop. A randomized block design with three replicates (Figure S1) was implemented over two consecutive growing seasons (2023/2024 and 2024/2025). Each year, soil preparation included late-summer plowing to 30 cm, followed by autumn disc harrowing to 20 cm, and final seedbed preparation with a rotary tiller to 10 cm for organic fertilizer incorporation. The soil was fallow before the first season, and the experimental crop treatments (wheat–legume crops or sole wheat) were continued in the second season in the same plot. After the first season, crop residues were incorporated into the soil through plowing. In both experimental years, the agronomic treatments were (Table 1) as follows:

1. Organic fertilization in pellet form (NPK: 3.1-2.9-2.0) from cattle manure and droppings of domestic birds (Organagro, Choncimer srl) was applied at seedbed preparation to achieve target applied N at 60 and 120 kg N ha^{-1}. This corresponded to 1935 and 3871 kg ha^{-1} of pellets, respectively. The higher N rate is commonly used by local farmers in organic wheat systems. Here, the sole wheat at 120 kg N ha^{-1} is referred to as the control. Unfertilized wheat (0 kg N ha^{-1}) was also established in adjacent plots and used exclusively for the calculation of N uptake and NUEr (see below).
2. Cropping system, where durum wheat was sown either as a sole crop or individually with legumes: subterranean clover (*Trifolium subterraneum* L. var. Campeda), balansa clover (*T. michelianum* Savi var. Bolta), or common vetch (*Vicia sativa* L. var. Alexandros).
3. Inoculum treatment consisted of legume species either inoculated (YES) or not (NO). The three species-specific inoculants were each peat-based formulations ("Exceed,"

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TABLE 1 Description of experimental treatments: (i) organic pelletized fertilizer (pH of 7.05, N of 3.10%, P_2O_5 of 2.96%, and K_2O of 2.0%) applied to achieve two target N rates (60 and 120 kg N ha^{-1}); (ii) species-specific peat-based rhizobial inoculum in colony-forming units per gram (CFU g^{-1})(YES/NO); (iii) cropping system, with wheat grown as a sole crop (control) or intercropped (additive design, mixed layout) with forage legume living mulches: Sub (*Trifolium subterraneum* L.), Mic (*Trifolium michelianum* Savi), or Vic (*Vicia sativa* L.).

Treatment	Level	Dose	Description
Organic fertilizer	N0	0 kg ha^{-1}	Unfertilized wheat, used exclusively for calculating N uptake and N recovery efficiency.
	N60	60 kg ha^{-1}	Half of the N rate commonly applied by local farmers in organic wheat systems.
	N120	120 kg ha^{-1}	N rate commonly applied by local farmers in organic wheat systems.
Rhizobia-inoculum	YES	(2×10^8 CFU g^{-1})	*Rhizobium leguminosarum* biovar *viceae* (for Vic); *R. leguminosarum* biovar *trifolii* (for Sub); *Sinorhizobium meliloti* + *R. leguminosarum* biovar *trifolii* (for Mic).
	NO	0 CFU g^{-1}	Legumes sown without peat-based inoculum.
Cropping system	Control	Sole wheat	Durum wheat sole crop sown at the standard dose.
	Sub	Additive	Durum wheat + Sub living mulch, both sown at the standard dose.
	Mic	Additive	Durum wheat + Mic living mulch, both sown at the standard dose.
	Vic	Additive	Durum wheat + Vic living mulch, both sown at the standard dose.

Visjon Biologics). Seeds were inoculated using the Visjon slurry method at 15 g inoculant per kg of seed for subterranean and balansa clover, and 6.3 g inoculant per kg of seed for common vetch. The inoculant dose used was double the manufacturer's recommendation, based on results from a previous study showing that inoculating Mediterranean forage legumes, especially at a double dose, increases their N_2 fixation ability (Scavo et al., 2025).

Durum wheat variety Core was purchased from Centrale Zooagricola SRL, while forage legumes were from Padana Sementi SRL. Manual broadcasting was used for both wheat and legumes, followed by mechanical soil rolling. Intercropping treatments were established using an additive approach, that is, by adding seed densities to the respective pure stands. Planting arrangements followed a mixed layout. Seed density was 250 kg ha^{-1} for durum wheat, 35 kg ha^{-1} for subterranean clover, 10 kg ha^{-1} for balansa clover, and 120 kg ha^{-1} for common vetch. Each plot was 7 m^2 (2.0 m × 3.5 m). Sowing was conducted on December 21, 2023 and December 12, 2024 for the first and second years, respectively. No top-dressing fertilization or pre- and post-sowing weed, pest, or disease control was applied in either year, reflecting typical management practices of commercial organic farms in the region.

2.2 | Measurements

Daily Tmax and Tmin, and rainfall were obtained from the SIAS weather station located in Patti and reported monthly.

At wheat physiological maturity, harvest was conducted by collecting wheat, legume species, and weeds from three 0.5 m × 0.5 m quadrats within each plot. Quadrats were randomly positioned in the center of each plot, excluding border areas. Harvest dates were June 12, 2024 and June 18, 2025. Fresh legume and weed samples were dried at 65°C in a ventilated oven to constant weight. Wheat grain was dried at 60°C until constant weight and subsequently corrected to a standard moisture content of 13% for all yield calculations. A mechanical thresher separated wheat grain and straw (Cicoria plot 2375, Cicoria SRL). Grain yield, legume dry biomass, and weed dry biomass (WB) were expressed per unit land area (Mg ha^{-1}). Crude protein content in wheat grain and legume biomass was determined according to the AOAC method 2001.11, using the Kjeldahl procedure with a Kjeltec 9 system (FOSS), involving sulfuric acid digestion, ammonia distillation, and titration to quantify total N. This was converted to crude protein using a factor of 5.70 for wheat grain and 6.25 for legume biomass.

Root nodule weight was determined in the second experimental year only, at legume flowering (subclover: BBCH 69-71; balansa clover: BBCH 69-70; common vetch: BBCH 67-68) on May 6, 2025, when wheat was at a stage BBCH 65. In each plot, five whole plants were randomly sampled using 0.5 m × 0.5 m quadrats, excluding border plants, and excavated to a depth of 30 cm. Roots were gently washed with water, air-dried to constant weight, and nodules were then detached, counted, and weighed after air drying. Plant density within each quadrat was recorded. Nodule weight (g m^{-2}) and count (nodules n. m^{-2}) were expressed per unit land area.

At the same time, soil samples were collected at 30-cm depth for soil nitrate nitrogen (NO_3^--N) analysis, approximately 10 months after incorporation of first-year wheat and legume residues into the soil by ploughing. Soil samples were diluted with bidistilled water (1:10), stirred for 2 h, and filtered through a 0.22 μm membrane. The aqueous solutions were analyzed by an ion chromatograph Eco IC (Metrohm, CH-9101), using a Metrosep A Supp 5–250/4.0 column (polyvinyl alcohol with quaternary ammonium groups; 250

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mm × 4.0 mm, 5 μm). Operating conditions were 35°C, 339 mg Na_2CO_3 (3.2 mmol L^{-1}) + 84 mg $NaHCO_3$ (1.0 mmol L^{-1}) as mobile phase, and 0.80 mL min^{-1} as flow rate. Soil NO_3^--N (kg ha^{-1}) in the 30 cm layer was calculated according to Sainju (2017), using a soil bulk density of 1.3 g cm^{-3}.

BNF was estimated using the empirical approach set forth by the EU Nitrogen Expert Panel (2016), based on the model developed by Anglade et al. (2015). Although this approach does not directly distinguish atmospheric N_2 fixation from soil- or fertilizer-derived N uptake, it relies on published linear relationships between N_2 fixation, shoot biomass yield, and shoot N content:

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

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

The wheat grain NUEr was calculated according to You et al. (2023):

$$\mathrm{NUEr} = \frac{\mathrm{NUPf} - \mathrm{NUPunf}}{\mathrm{Nrate}} \times 100$$

The NUP_f (where NUP is N uptake) is the wheat grain N uptake at the N fertilization rate (60 or 120 kg N ha^{-1}), cropping systems (sole wheat or wheat–legumes), and inoculum treatment (YES or NO). The NUP_{unf} is the wheat grain N uptake of the sole wheat unfertilized (0 kg N ha^{-1}), which was 12.3 ± 0.11 and 15.5 ± 0.11 kg N ha^{-1} in 2024 and 2025, respectively. The N_{rate} is the amount of N fertilizer applied. The NUP was calculated as the grain yield by the N content in the grain × 100.

2.3 | Statistical analysis

Linear mixed models were employed to test the effect of fertilization, cropping systems, and legume inoculum on WGY and protein content, WB, BNF, and NUEr for each year (Dong et al., 2025). Analyses were conducted separately for each year to adequately capture treatment-specific responses under contrasting environmental conditions. Pooling data across years might obscure treatment effects by averaging responses across markedly different climatic contexts (Yadiz et al., 2025). The nodule dry weight and number, and soil NO_3^--N were analyzed only in the second year. The block was considered a random effect, while N fertilizer (two-level), cropping system (four-level), and inoculum (two-level) were included as fixed effects. The inoculum treatment was nested in legume species (Minitab version 19).

Observations within each group were randomly sampled according to the experimental design, ensuring independence. The assumptions of normality and homogeneity of variances were assessed using the Shapiro–Wilk test and Bartlett's test, respectively. When the analysis of variance revealed significant differences among group means, Fisher's least significant difference test ($p \leq 0.05$) compared the means between individual treatment combinations and the control (i.e., sole wheat at 120 kg N ha^{-1}). Means without control (i.e., BNF, nodule fresh weight, and count) were compared by Tukey's honestly significant difference at $p \leq 0.05$.

3 | RESULTS

3.1 | Meteorological trend of the two growing seasons

In both seasons (from sowing to harvest), Tmin and Tmax were higher than the long-term means. In 2023/2024, 1°C–3°C higher, particularly from February to June, while in 2024/2025 slightly above from March onward (Table 2). Rainfall differed markedly between years. The 2023/2024 season was substantially drier than the long-term average (295 vs. 439 mm), particularly in December, April, and June. In contrast, 2024/2025 was considerably wetter (456 mm) than 2023/2024 and near the long-term average. However, it was uneven, with intense precipitation events from December to March followed by dry conditions in April and June.

3.2 | Effects of management practices on durum wheat performance

Figure 1A,B shows the interaction between N rate and wheat–legume cropping system (with or without inoculation) on WGY in 2024 and 2025.

Under drier conditions in 2024, WGYs were generally low across treatments. Sole wheat at reduced N rate (N60 [N at 60 kg ha^{-1}]) yielded less than the N120 (N at 120 kg ha^{-1}) control (0.62 vs. 1.01 Mg ha^{-1}). Wheat–subterranean clover (Sub) and wheat–balansa clover (Mic) with inoculation at N120 significantly outyielded the control. Their non-inoculated counterparts at N120 yielded similarly to the control, while Sub at N60 outperformed, and Mic at N60 underperformed the control. Wheat–vetch (Vic) with or without inoculation at N60 yielded like the control, while the non-inoculated Vic at N120 yielded significantly lower. In

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TABLE 2 Monthly minimum and maximum air temperature and rainfall at the wheat–legume intercropping experimental site (Patti, Messina, Italy; 38°06′ N, 14°59′ E; 196 m a.s.l.) in the 2023/2024 and 2024/2025 growing seasons, and long-term (20 years) climatic mean data.

Season	Variable	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Mean or Σ
2023/2024	Tmin (°C)	10.1	8.6	8.5	9.8	10.8	14.2	18.1	11.4
	Tmax (°C)	17.8	17.4	17.6	19.9	21.9	25.2	28.3	21.1
	Rainfall (mm)	2.2	66.6	100.2	60.8	23.8	36.4	5.4	295.4
2024/2025	Tmin (°C)	8.9	9.0	8.2	10.4	10.9	14.1	16.2	11.1
	Tmax (°C)	16.8	17.8	17.1	19.3	20.8	24.2	27.7	20.5
	Rainfall (mm)	89.2	149.6	40.8	135.2	2.2	38.0	1.0	456.0
Long term	Tmin (°C)	10.4	7.3	6.2	8.1	8.9	12.7	16.6	10.0
	Tmax (°C)	20.1	16.4	15.2	17.9	19.3	22.2	26.8	19.7
	Rainfall (mm)	31.2	72.8	28.2	33.6	117.2	102.6	53.2	438.8

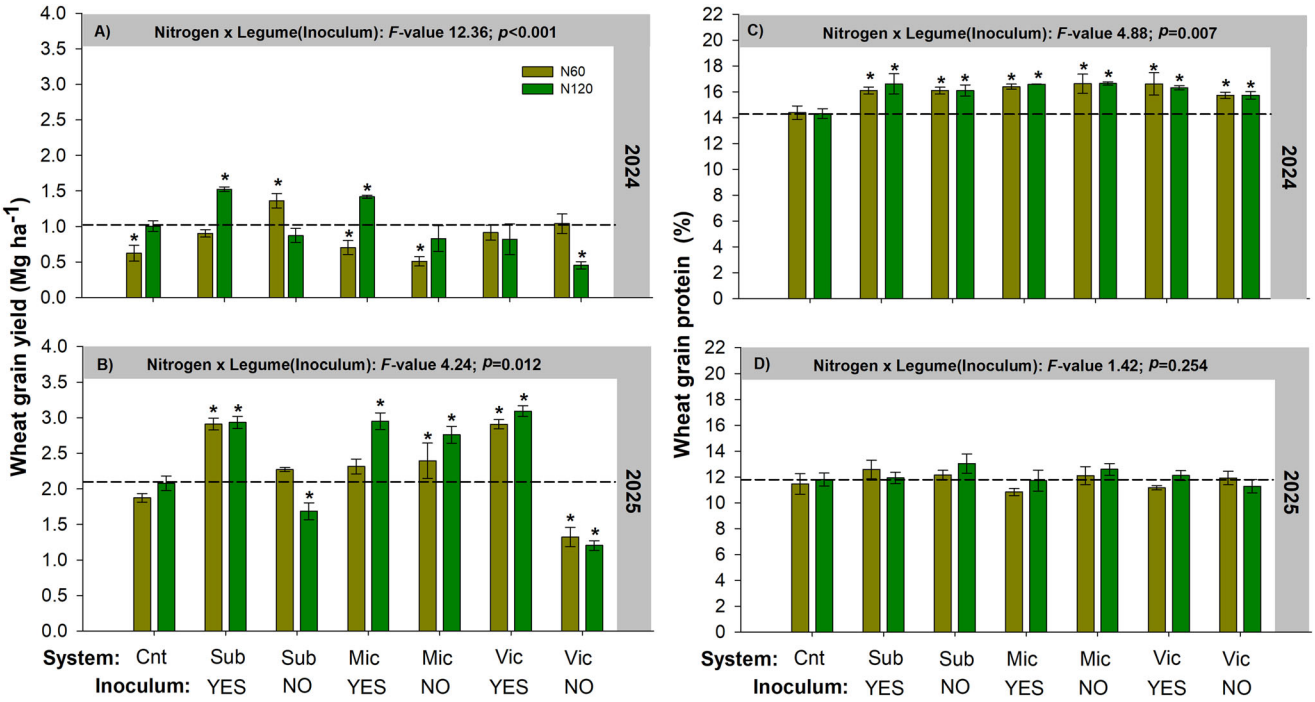


FIGURE 1 (A and B) Multiple comparison of the interaction between N-fertilization (N at 60 kg ha^{-1} [N60] and N at 120 kg ha^{-1} [N120]) and cropping system (wheat–subterranean clover—Sub; wheat–balansa clover—Mic; and wheat–common vetch—Vic), inoculated or not (YES or NO) on wheat grain yield, and on (C and D) wheat grain protein content versus the control (sole wheat at N120—Cnt—horizontal, back, dashed line) or the sole wheat at reduced N rate (N60) in 2024 and 2025 in Patti, Messina (Italy). The asterisks (*) denote statistically different means at $p \leq 0.05$ (Fisher's least significant difference test).

2025, WGY was higher in all treatments versus 2024, reflecting more favorable growing conditions. The sole wheat at N60 remained lower than the control (2.1 Mg ha^{-1}) but not significantly. Inoculated Sub and Vic at any N rate, and Mic only at N120, exceeded the control. Non-inoculated Sub (at N60) and Vic (at N60 and N120) showed lower yields than the control, while non-inoculated Mic, at both N rates, outyielded the control.

The interaction of treatments on WGP content showed a different trend (Figure 1C,D). In 2024, the sole wheat at N60 showed WGP values like the control (14.4%). In contrast, all wheat–legume systems, regardless of species, inoculation, or N rate, increased WGP as compared to the control (average 16.3%). In 2025, the WGP was lower than in 2024 (average 11.9%). Neither the N rate nor the inclusion of legume species (with or without inoculation) showed significant changes in WGP.

3.3 | Effects of management practices on weed control

Nitrogen fertilization and wheat–legume cropping systems, with or without inoculation, significantly reduced WB versus

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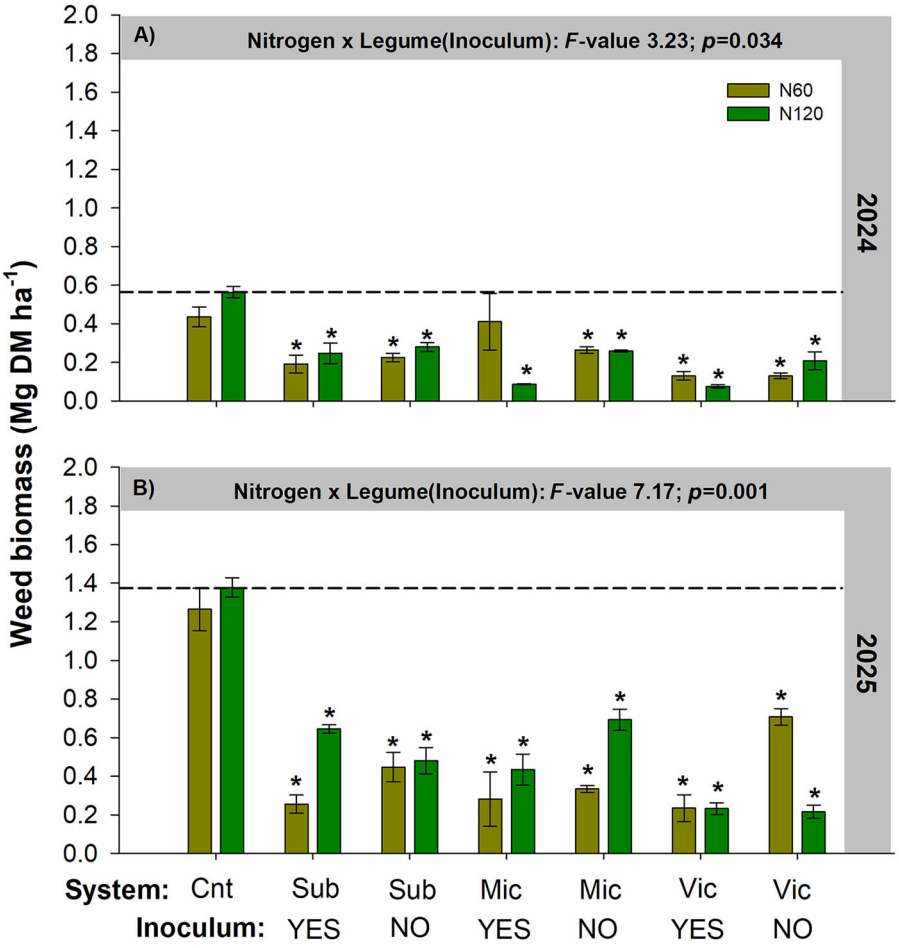


FIGURE 2 (A and B) Multiple comparison of the interaction between N-fertilization (N at 60 kg ha^{-1} [N60] and N at 120 kg ha^{-1} [N120]) and cropping system (wheat–subterranean clover—Sub; wheat–balansa clover—Mic; and wheat–common vetch—Vic), inoculated or not (YES or NO) on weed dry biomass versus the control (wheat-sole crop at N120—Cnt—horizontal, back, dashed line) or the sole wheat at reduced N rate (N60) in 2024 and 2025 in Patti, Messina (Italy). The asterisks (*) denote statistically different means at $p \leq 0.05$ (Fisher's least significant difference test).

sole wheat at both N120 (control) and N60 (Figure 2A,B). In 2024, WB was generally low across all treatments due to dry conditions. The control exhibited the highest WB (0.56 Mg ha^{-1}), whereas sole wheat at N60 showed slightly but not significantly lower values. All wheat–legume-based systems (except Mic inoculated at N60), regardless of inoculation or N rate, had significantly lower WB (average 0.21 Mg ha^{-1}) than sole wheat. In 2025, WB increased substantially across treatments in response to wetter conditions. The sole wheat at both N rates had the highest and not different WB (average 1.32 Mg ha^{-1}). All wheat–legume systems showed significantly lower WB than the control, regardless of inoculation or N rates (average 0.41 Mg ha^{-1}).

3.4 | Effects of management practices on nitrogen use

In 2024, legume biomass and legume protein content were influenced by species, N rate, and inoculation, with Vic showing the highest values, and inoculation benefiting Sub but not Mic. In 2025, only species affected both legume traits, with Vic significantly higher than Sub and Mic (Figure S2A–D).

BNF was significantly affected by the interaction between N rate and wheat–legume cropping system (with or without inoculation) in both years (Figure 3A,B). In 2024, Vic exhibited higher BNF than Sub and Mic, with the highest values observed under N60 with inoculation (59.7 kg fixed N_2 ha^{-1}). Increasing the N rate reduced BNF in Vic with and without inoculation, but not significantly. In contrast, Vic non-inoculated at N60 reduced BNF (38.5 kg fixed N_2 ha^{-1}). However, it was higher than BNF in Sub and Mic, which was the lowest and not significantly influenced by N rate or inoculation (average 13.9 kg fixed N_2 ha^{-1}). In 2025, overall BNF levels increased. Vic again showed higher BNF than other species, particularly at N60 with inoculation (72.3 kg fixed N_2 ha^{-1}). N rate and inoculation were limited for Sub and Mic (average 25.2 and 14.4 kg fixed N_2 ha^{-1}, respectively), which consistently displayed low BNF values.

The NUEr by wheat was influenced by treatments in both years (Figure 4A,B). In 2024, NUEr values were generally low, with non-inoculated Mic at N60 and Vic at N120 under-

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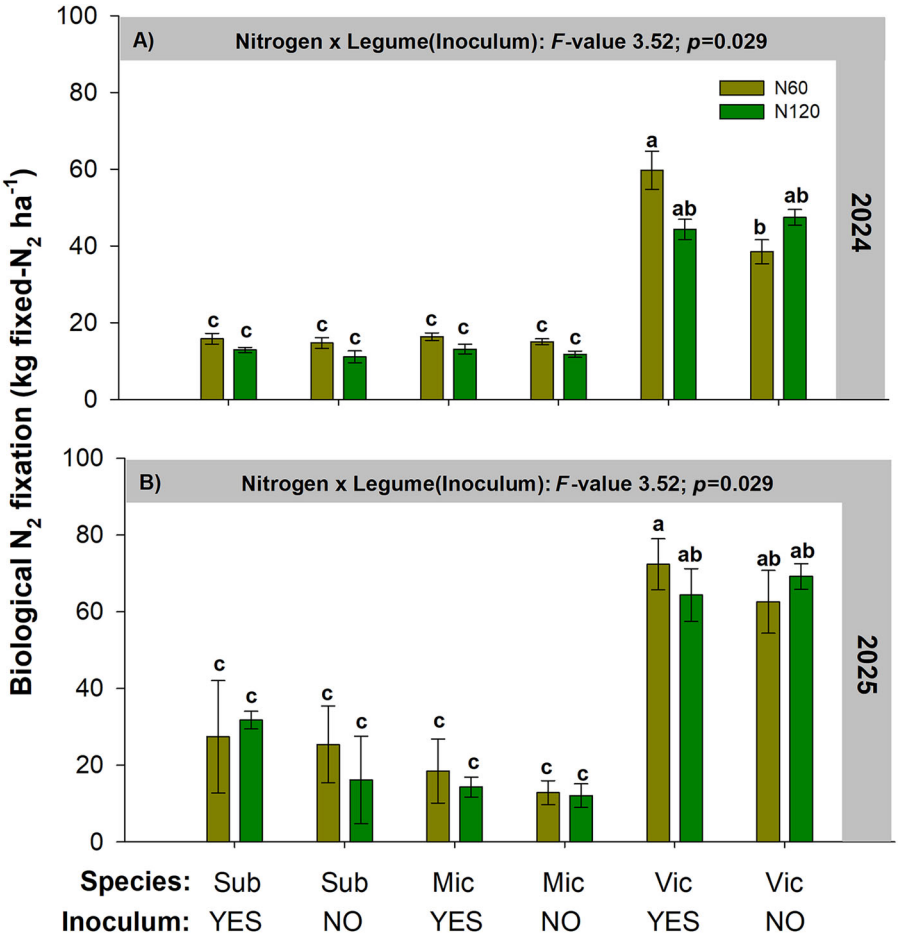


FIGURE 3 (A and B) Pairwise comparison of the interaction between N-fertilization (N at 60 kg ha^{-1} [N60] and N at 120 kg ha^{-1} [N120]) and cropping system (wheat–subterranean clover—Sub; wheat–balansa clover—Mic; and wheat–common vetch—Vic), inoculated or not (YES or NO) on legume biological N_2-fixation in 2024 and 2025 in Patti, Messina (Italy). Different letters denote significantly different means at $p \leq 0.05$ (Tukey's honestly significant difference test).

performing the control (NUEr = 10.8%). Inoculating Sub and Vic (or not) at N60 improved the NUEr significantly (average 22.9%), while Mic did not differ from the control. Sub (non-inoculated) at N60 showed the highest NUEr value (43.6%). In 2025, NUEr markedly increased, particularly under N60 in all inoculated (average 65.9%) and not-inoculated (44.6%) wheat–legume systems, as well as in the sole wheat (36.9%), which outperformed the control (18.4%). Increasing the N rate (N120) resulted in NUEr similar to the control, except for the inoculated Sub and Vic or the non-inoculated Mic, which outperformed the control.

Treatment effects on the nodule dry weight, nodule count, and soil NO_3^--N were sampled in 2025 (Figure 5A–C). The highest nodule weight was recorded in inoculated Sub at N60 and N120, and non-inoculated Sub at N60 (average 4.2 g m^{-2}). Non-inoculated Sub at N120 and the other species, regardless of N rate or inoculum, did not differ (average 1.65 g m^{-2}). The nodule count was higher in inoculated Sub at N60 and N120 (average 1024 nodules m^{-2}) than in non-inoculated Sub, which significantly decreased the nodule number at N120 (532 nodule m^{-2}). In contrast, Mic and Vic showed slightly higher nodule count in N120 than in N60, regardless of inoculation treatment. The soil NO_3^--N at 30-cm depth was generally higher at N120 than at N60, as observed for the sole wheat (48.5 kg ha^{-1} at N120 vs. 36.1 kg ha^{-1} at N60). All wheat–legume systems at N60, except Sub, had significantly lower soil NO_3^--N than the control. Inoculated Sub and Vic at N120 resulted in the highest overall soil NO_3^--N (69.4 and 64.2 kg ha^{-1}). Among legume species, the inoculum had the greatest effect on Vic at N120, as evidenced by the gap with and without inoculum.

4 | DISCUSSION

4.1 | Effect of environment and agronomic treatments on wheat

The two growing seasons were characterized by strong inter-annual climatic variability. The persistence of above-average temperatures in both seasons suggests an ongoing warming trend relative to the historical baseline in the Mediterranean

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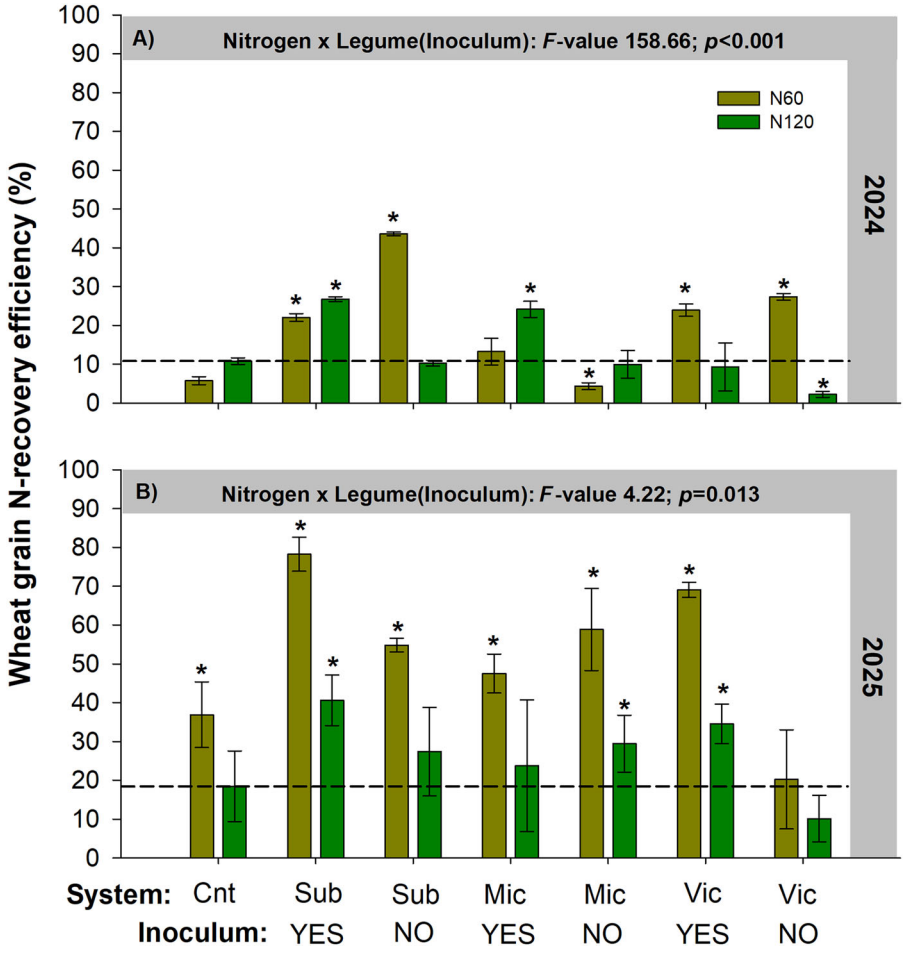


FIGURE 4 (A and B) Multiple comparison of the interaction between N-fertilization (N at 60 kg ha^{-1} [N60] and N at 120 kg ha^{-1} [N120]) and cropping system (wheat–subterranean clover—Sub; wheat–balansa clover—Mic; and wheat–common vetch—Vic), inoculated or not (YES or NO) on wheat grain N recovery efficiency versus the control (sole wheat at N120—Cnt—horizontal, back, dashed line) or the sole wheat at reduced N rate (N60) in 2024 and 2025 in Patti, Messina (Italy). The asterisks (*) denote statistically different means at $p \leq 0.05$ (Fisher's least significant difference test).

(De Luca et al., 2020). The 2023/2024 season was warmer and markedly drier, whereas 2024/2025 showed water availability closer to long-term conditions. At the regional scale, southern Italy experienced severe drought in 2023/2024, causing wheat yield losses up to 70% in parts of Sicily and, in some cases, complete crop failure. Farmer observations indicated yield losses were driven by a dry autumn–early winter (up to December). This impaired germination and early growth, while warm and dry spring constrained reproductive development, reducing both kernel number and weight. Under the harshest conditions, wheat seedlings remained stunted before desiccating (Simenza Farmer Association, personal communication, 2024).

In the study area, the adverse 2023/2024 season did not compromise wheat establishment and early growth, but it affected crop phenology; reduced grain yield, weed pressure, wheat NUEr, and legume BNF; and increased grain protein. The shorter 2023/2024 wheat growing cycle (174 days) is consistent with accelerated development under warm and dry conditions, noted in other Mediterranean wheat systems (Anastasi et al., 2019; Cosentino et al., 2019). In contrast, the longer cycle in 2024/2025 (188 days) is more closely aligned with typical phenological timing for the area (Scordia et al., 2026).

Grain yield responded strongly to interannual variability, with yields in 2024 markedly lower than in 2025 (−52% in the control). Under these conditions, several wheat–legume systems minimized yield losses. Rhizobial inoculation showed inconsistency: only Sub and Mic at N120 outperformed the control wheat yield (+51% and +41%, respectively), while Sub at N60 without inoculation increased yield by 35%. Although higher-than-recommended inoculant rates can enhance nodulation and N_2 fixation under challenging soil conditions (Farquharson et al., 2022; Frame & Laidlaw, 2005), minimal rainfall immediately after sowing in December 2023 likely compromised the peat-based rhizobial formulation. Reduced efficacy of peat-based inoculants under dry conditions, compared with granular formulations, has

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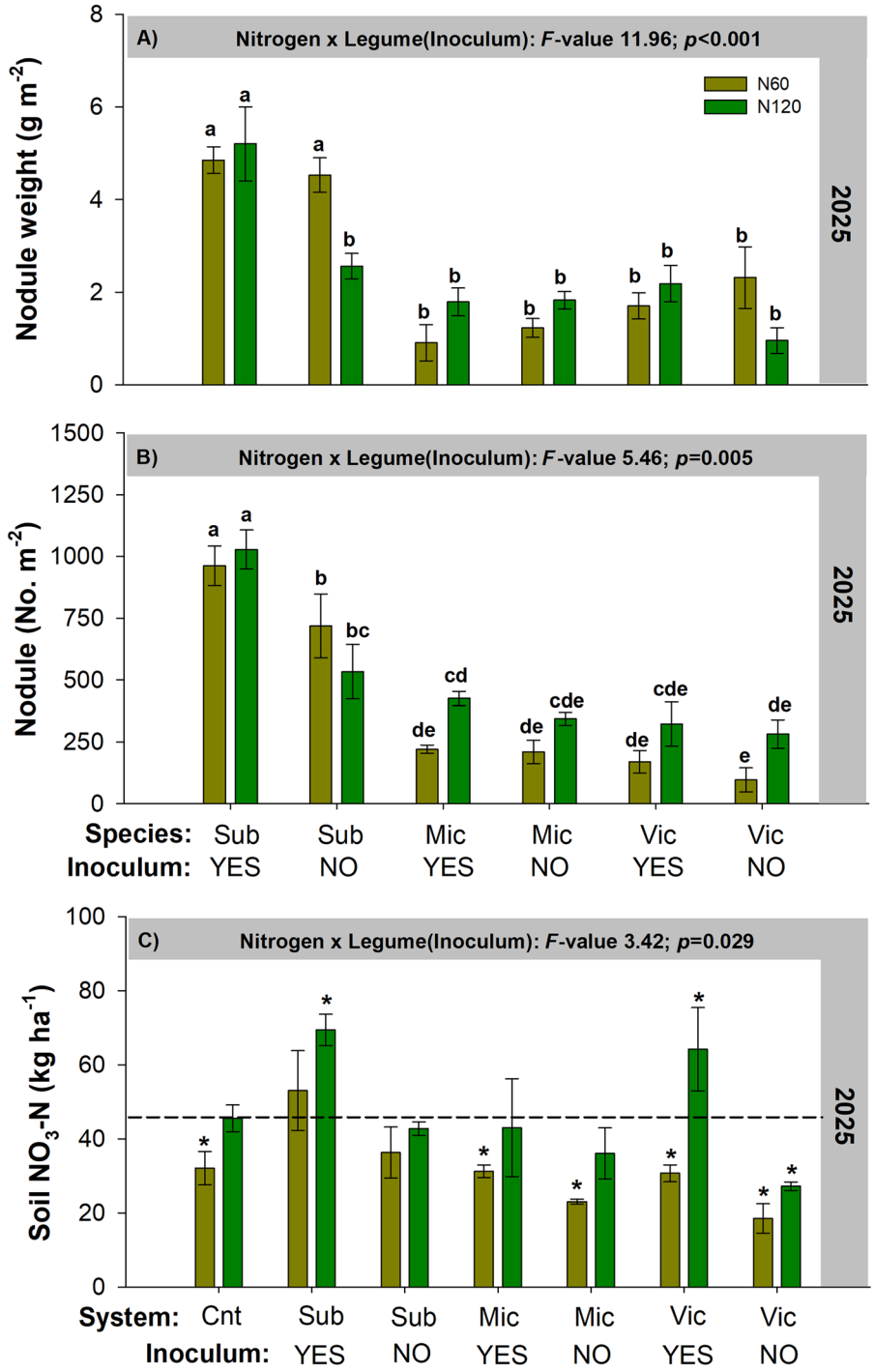


FIGURE 5 (A) Pairwise comparison of the interaction between N-fertilization (N at 60 kg ha^{-1} [N60] and N at 120 kg ha^{-1} [N120]) and cropping system (wheat–subterranean clover—Sub; wheat–balansa clover—Mic; and wheat–common vetch—Vic), inoculated or not (YES or NO) on nodule dry weight per m^2 and (B) on nodule number per m^2. Different letters denote significantly different means at $p \leq 0.05$ (Tukey's honestly significant difference test); (C) multiple comparison of the interaction between N-fertilization (N60 and N120) and the same cropping system (Sub, Mic, and Vic), inoculated or not (YES or NO) on soil nitrate nitrogen in the 30 cm soil layer (NO_3^--N) versus the control (sole wheat at N120—Cnt—horizontal, back, dashed line) or the sole wheat at reduced N rate (N60) in 2025 in Patti, Messina (Italy). The asterisks (*) denote statistically different means at $p \leq 0.05$ (Fisher's least significant difference test).

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been reported for chickpea (Kyei-Boahen et al., 2002), alfalfa (Rice & Olsen, 1992), and arrowleaf clover (Ocumpaugh & Smith, 1991).

Under the more favorable hydroclimate of 2025, overall productivity increased. Inoculation of Vic and Sub improved wheat yield at both N rates, outperforming both the control and their non-inoculated counterparts. Balansa clover (Mic) inoculation showed yield increases only at N120. Non-inoculated Mic outperformed the control at both N rates. The poor performance of non-inoculated Sub and Vic indicates that without effective rhizobial activity, legume inclusion may fail to supply sufficient N and may exacerbate interspecific competition (Mahmoud et al., 2022).

The potential for N_2 fixation is directly related to rhizobia survival, the extent of effective infection on the roots and subsequent nodulation, and plant growth factors (Allito et al., 2021). Nitrogen fertilization generally suppresses nodulation and N_2 fixation in legumes, although responses vary with the host–*Rhizobium* association. Legumes characterized by slow nodulation or low fixation capacity may benefit from moderate N inputs. On the contrary, species with high N_2-fixation potential during establishment typically exhibit reduced nodulation and N_2 fixation (Seguin et al., 2001). Consistent with this framework, our results revealed clear species-specific responses: Sub showed strong nodulation responses to inoculation, especially at N60, while nodulation declined under higher N supply when not inoculated. Conversely, Vic and Mic increased nodule numbers and weights at N120 regardless of inoculation, though this did not translate into higher BNF, which was more closely linked to legume biomass and N content.

Nodule numbers and weights were lower than those reported in a previous pot study using the same peat-based inoculum, which was effective for Sub but not for Mic (Scavo et al., 2025). For Mic, the manufacturer recommended the use of the same rhizobial inoculant applied to Sub (*Rhizobium leguminosarum* bv. *trifolii* in combination with *Sinorhizobium meliloti*), as no specific commercial strain for Mic was then available. Although Mic and Sub might nodulate with the same *Rhizobium* Group C strains (J. Hall, personal communication, 2026), the use of a non-species-specific inoculant may not represent optimal symbiotic pairing. Consequently, some uncertainty remains regarding whether the inoculant fully maximized nodulation efficiency and N fixation in Mic.

Furthermore, no positive inoculation effect on Vic was detected under the present field conditions. The high clay soil (57%) at the experimental site, combined with the inherent constraints of field root sampling, likely reduced the accuracy of nodule recovery and quantification. In fact, in a controlled experiment across multiple common vetch genotypes, Lopez-Roman et al. (2025) reported substantially higher nodulation levels (30–95 nodules plant^{-1} and 40–160 mg dry nodule biomass plant^{-1}) than observed here.

Soil NO_3^--N was primarily driven by fertilizer application. Although a fraction of fixed N_2 may also be transferred during the growing season through nodule decomposition and rhizodeposition (Ashworth et al., 2015; Thilakarathna et al., 2016), higher soil NO_3^--N levels under N120 were likely due to enhanced mineralization of soil organic matter stimulated by organic fertilization. Moreover, wheat and legume residues were incorporated into the soil after full maturity, nearly 10 months before soil NO_3–N sampling. Thus, their contribution to NO_3^--N content was probably limited because of their relatively high C/N ratio. An exception was observed in inoculated Sub and Vic, likely related to the greater root biomass of Sub (data not shown) and the higher biomass production of Vic. This may have promoted N accumulation in soil organic matter pools from the preceding year (Suter et al., 2015).

Regardless of climatic conditions, BNF was consistently higher in Vic than Sub or Mic, particularly under the lower N rate, with this response further enhanced by inoculation. In contrast, both Sub and Mic showed limited BNF, largely unaffected by either N rate or inoculation. Consistent with our findings, Enrico et al. (2020) evaluated common vetch with and without inoculation and its residual effects on maize in the Pampean region, Argentina. They reported average BNF of 70 kg fixed N_2 ha^{-1} and a significant 62% BNF increase following inoculation. Here, inoculation enhanced BNF in Vic under N60 (+35% in 2024 and +15% in 2025), whereas no effect was detected at N120, confirming the positive role of rhizobial inoculation under reduced N.

Overall, the BNF values in this study are lower than reported by Parr et al. (2011) for common vetch, balansa clover, and subterranean clover in North Carolina. These differences likely reflect both contrasting climatic conditions and experimental management. In Parr et al. (2011), legumes were grown as sole cover crops and terminated before sowing the cash crop, whereas here legumes were grown in biculture with wheat and self-terminated at maturity. Consequently, interspecific competition with wheat and the later growth stage likely constrained legume growth and nodulation, reducing overall BNF potential.

Furthermore, BNF is also influenced by soil N availability. In the present study, organic fertilization at 60 and 120 kg N ha^{-1} likely increased mineral N availability during early growth stages, potentially suppressing nodulation, as legumes preferentially use available soil nitrate rather than investing carbon and energy in symbiotic N_2 fixation (Liu et al., 2019). High soil N availability activates nitrate-responsive signaling pathways involving NIN-like proteins, CLAVATA3/ESR-related peptides, and autoregulation of nodulation, which repress nodulation-related gene expression and limit rhizobial symbiosis (Nishida et al., 2018). In wheat–legume systems, however, the strong nitrate uptake capacity of wheat may progressively reduce rhizosphere mineral N availability, partially alleviating nitrate-mediated repression of nodulation

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and increasing the relative contribution of atmospheric N_2 fixation (Jensen et al., 2020). At the same time, competition for light from the wheat canopy may reduce photosynthate allocation to nodules, constraining N_2 fixation and legume biomass production. This balance between mineral N availability, nitrate signaling, and interspecific competition may explain the variable response of forage legumes and rhizobial inoculation observed in this study.

NUEr was highest at N60 in both wheat–legume systems and the sole wheat, indicating improved synchrony between N supply and crop demand at moderate fertilization rates. Sub and Vic at N60 increased NUEr regardless of inoculation, whereas in Mic this response was inconsistent, occurring only under the more favorable conditions in 2025 or at higher N rate in 2024 with inoculation.

However, this improved NUEr in the second year did not translate into higher grain protein content, which followed an opposite pattern to yield across seasons. In 2024, all wheat–legume systems increased grain protein relative to the control, regardless of N rate or inoculation, consistent with a dilution effect under low yields (Cosentino et al., 2019; Dinelli et al., 2013). In 2025, lower grain proteins were associated with higher grain yields, and neither N rate nor intercropping significantly affected grain protein. These results align with previous findings showing that legumes enhance grain protein content in cereals, particularly under low-input or yield-limiting conditions (Bedoussac et al., 2015; Rodriguez et al., 2020; Zhao et al., 2022).

Weed pressure reflected both management and environmental conditions. Typical of organic systems, weed biomass was substantial in sole wheat, although in 2024 it remained generally low due to severe drought compared with the more favorable conditions of 2025. The most common weeds were annual therophytes (*Anagallis arvensis* L., *Anethum graveolens* L., *Avena* sp., and *Centaurea napifolia* L.), biennial hemicryptophytes (*Daucus carota L.* and *Sulla coronaria* L.), and perennial hemicryptophytes (*Ferula communis* L., *Lolium perenne* L., *Malva sylvestris* L., and *Sonchus oleraceus* L.). All wheat–legume systems (except Mic inoculated at N60 in 2024) significantly reduced WB relative to sole wheat, regardless of inoculation or N rate. Simultaneous sowing of wheat with legumes provided strong early-season weed suppression, with average WB reductions ranging from 27% in Mic inoculated at N60 to 86% in Vic inoculated at N120. The negligible differences in weed suppression between inoculated and non-inoculated legumes suggest the mechanism is predominantly physical-ecological (i.e., early canopy closure and rapid resource uptake) rather than microbial.

4.2 | Limits of study and future applications

Although this study focused on legume living mulches, that is, unharvested, noncash crops intended to deliver ecosystem services (Calderone et al., 2025; Lamichhane et al., 2025), the absence of pure legume sole crops as a reference represents a key limitation. This precluded the calculation of intercropping performance metrics such as land equivalent ratio, transgressive overyielding index, net effect ratio, among others, and related N-based indicators (Li et al., 2023), limiting comparability with studies using standardized intercropping indices.

An additional source of uncertainty likely arises from the reduced precision of root and nodule assessments at the experimental site due to the predominance of hard clay soil. High clay and spring moist soil constrained complete root recovery. This increased nodule loss during sampling, potentially attenuating treatment effects.

Uncertainty also remains as to whether the inoculant used fully optimized nodulation efficiency and N_2 fixation in Mic.

Another limitation of this study is that BNF was estimated using the empirical approach proposed by the EU Nitrogen Expert Panel (2016) rather than being directly quantified. Consequently, the reported BNF values should be considered approximations, as this method does not distinguish atmospheric N_2 fixation from soil or fertilizer-derived N uptake, which requires ^{15}N-based techniques. Although ^{15}N labeling is the gold standard for BNF quantification, its application in the present organic system would have been challenging. The use of ^{15}N-enriched fertilizers is complicated by the predominance of organically bound N and would require accounting for common complexities in field studies (Follett, 2001). Direct labeling with ^{15}N-enriched N_2 gas would be even more demanding, requiring specialized enclosure systems and prolonged exposure periods to ensure diffusion into the rhizosphere and uptake by symbiotic rhizobia (Queiroz et al., 2025). Such approaches are rarely feasible under field experiments with several treatment combinations (Follett, 2001), particularly in the presence of N fertilizer treatments (Peoples et al., 2015). Therefore, the estimation method adopted here can represent a practical alternative for assessing BNF and comparing relative differences among legume-based cropping systems.

Nevertheless, the study has several potential future applications. Though experimental plots were harvested and managed manually, the simultaneous intercropping of these forage legumes with wheat represents a highly practical and easily adaptable agronomic practice. The system is fully compatible with standard mechanized field operations and does not interfere with wheat harvesting, as these forage legumes are predominantly low-statured and typically senescent before wheat maturity. Consequently, no modifications to conventional harvesting equipment or procedures are required. The only notable exception is Vic, which in some cases may exhibit climbing behavior and partially interfere with wheat spikes.

This study is particularly relevant for Sicily (Italy), where soil organic matter is progressively declining. Crop

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residue burning, still practiced for short-term benefits such as increased phosphorus (P) and potassium (K) availability (Erenstein, 2002), imposes substantial long-term costs, including losses of soil fertility, soil microbial communities, and pollutant emissions (Jamali et al., 2021). Conversely, residue retention and incorporation improve system performance, such as yield (C. Liu et al., 2014), fertilizer-derived N recovery in biomass, and overall soil-crop N recovery (Murphy et al., 2016). Thus, incorporating wheat stubble and legume dead mulch, either by tillage or leaving crop residues on the surface, represents a viable strategy to enhance nutrient availability for subsequent crops and build soil organic matter in the long term.

These benefits are expected to become increasingly important in the face of growing external uncertainties, including geopolitical conflicts (e.g., Russia's invasion of Ukraine in 2022 and the Middle East conflict in 2026) and evolving policy frameworks such as the EU carbon tax. Such factors can trigger disruptions in N fertilizer markets, which remain heavily dependent on fossil-fuel-based ammonia production, thereby exposing farmers to energy price volatility, supply-chain disruptions, and geopolitical risks.

Under these scenarios, additive intercropping with fully mixed forage legumes represents a practical and scalable solution for Mediterranean organic cereal-based systems. The multi-year regenerative cost of rhizobial inoculants, together with the marginal cost of additional legume seed, is relatively low compared with the costs and impacts of mineral N and herbicide inputs.

5 | CONCLUSION

This study demonstrates that the simultaneous sowing of forage legume living mulches and the use of rhizobial inoculation can enhance the performance and N efficiency of organic durum wheat systems compared with sole wheat cropping or non-inoculated legume intercrops. However, responses were strongly shaped by weather conditions. Dry and warm conditions limited yield but increased grain protein, and wetter conditions favored higher yields, biological N_2 fixation, and N-recovery efficiency. Regardless of N rates and inoculation in both years, weeds were strongly suppressed by all legume living mulches versus the sole wheat.

Benefits were species-specific, with inoculated common vetch the most effective for estimated BNF, while inoculated subterranean clover achieved high nodulation, which was associated in part with increased soil nitrate nitrogen, especially at the higher N rate.

Taken together, these results indicate that forage legume living mulches, when combined with appropriate rhizobial inoculation and reduced N fertilization, represent a viable strategy for reducing external N input and herbicide use in Mediterranean environments.

Future research should evaluate newly developed rhizobial formulations, including additional species-specific strains and high bacterial-density liquid inoculants, which may offer improved ease of application and adhesion for broadcast-sown forage legumes compared with traditional peat-based inoculants. In addition, to fully evaluate the assimilation of biologically fixed N_2 into the wheat (plus free fixed N_2 in the soil), ^{15}N isotopic techniques would require use of $^{15}N_2$ gas in controlled conditions. This would necessitate a season-long tent over the legume-wheat intercrop infused with trace isotopic $^{15}N_2$. This is a challenging approach to improve the accuracy of BNF and better characterize N dynamics in wheat–legume systems. Otherwise, indirect methods of measuring assimilation of biologically fixed N_2, as conducted here, provide an intermediate means of assessment.

AUTHOR CONTRIBUTIONS

Danilo Scordia: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review and editing. **Francesca Calderone**: Data curation; investigation; methodology; visualization; writing—review and editing. **Aurora Maio**: Data curation; investigation; methodology; visualization; writing—review and editing. **Tommaso La Malfa**: Data curation; investigation; methodology; visualization; writing—review and editing. **Aurelio Scavo**: Data curation; investigation; methodology; validation; visualization; writing—review and editing. **Marianna Oteri**: Funding acquisition; investigation; project administration; resources; writing—review and editing. **Calvin Trostle**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; validation; visualization; writing—review and editing. **Fabio Gresta**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; validation; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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ORCID

Danilo Scordia https://orcid.org/0000-0002-3822-788X
Francesca Calderone https://orcid.org/0009-0005-7462-5059
Aurora Maio https://orcid.org/0009-0001-2458-1247
Tommaso La Malfa https://orcid.org/0009-0003-8266-6980
Aurelio Scavo https://orcid.org/0000-0002-9762-9141
Marianna Oteri https://orcid.org/0000-0003-1940-2600
Calvin Trostle https://orcid.org/0000-0003-0600-9180
Fabio Gresta https://orcid.org/0000-0002-4527-2136

REFERENCES

Allito, B. B., Ewusi-Mensah, N., Logah, V., & Hunegnaw, D. K. (2021). Legume-rhizobium specificity effect on nodulation, biomass production and partitioning of faba bean (*Vicia faba* L.). *Scientific Reports*, *11*, Article 3678. https://doi.org/10.1038/s41598-021-83235-8

Anastasi, U., Corinzia, S. A., Cosentino, S. L., & Scordia, D. (2019). Performances of durum wheat varieties under conventional and no-chemical input management systems in a semiarid Mediterranean environment. *Agronomy*, *9*, 788. https://doi.org/10.3390/agronomy9120788

Anglade, J., Billen, G., & Garnier, J. (2015). Relationships for estimating N_2 fixation in legumes: Incidence for N balance of legume-based cropping systems in Europe. *Ecosphere*, *6*, 1–24. https://doi.org/10.1890/ES14-00353.1

Antonelli, M., Basile, L., Gagliardi, F., & Isernia, P. (2022). The future of the Mediterranean agri-food systems: Trends and perspectives from a Delphi survey. *Land Use Policy*, *120*, 106263. https://doi.org/10.1016/j.landusepol.2022.106263

AOAC. (2019). *Official methods of analysis* (21st ed.). Association of Official Analytical Chemists International.

Ashworth, A. J., West, C. P., Allen, F. L., Keyser, P. D., Weiss, S. A., Tyler, D. D., Taylor, A. M., Warwick, K. L., & Beamer, K. P. (2015). Biologically fixed nitrogen in legume intercropped systems: Comparison of nitrogen-difference and nitrogen-15 enrichment techniques. *Agronomy Journal*, *107*, 2419–2430. https://doi.org/10.2134/agronj14.0639

Bedoussac, L., Journet, E. P., Hauggaard-Nielsen, H., Naudin, C., Corre-Hellou, G., Jensen, E. S., Prieur, L., & Justes, E. (2015). Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. *Agronomy for Sustainable Development*, *35*, 911–935. https://doi.org/10.1007/s13593-014-0277-7

Calderone, F., Scavo, A., Gresta, F., Ferlito, F., Dimauro, C., & Scordia, D. (2025). Cover crops promote regulating and supporting ecosystem services without compromising grape yield in temperate vineyards. A meta-analysis. *Agronomy for Sustainable Development*, *45*, Article 70. https://doi.org/10.1007/s13593-025-01074-7

Campiglia, E., Mancinelli, R., Radicetti, E., & Baresel, J. P. (2014). Evaluating spatial arrangement for durum wheat (*Triticum durum* Desf.) and subclover (*Trifolium subterraneum* L.) intercropping systems. *Field Crops Research*, *169*, 49–57. https://doi.org/10.1016/j.fcr.2014.09.003

Cardoso, E. J. B. N., Nogueira, M. A., & Ferraz, S. M. G. (2007). Biological N_2 fixation and mineral N in common bean–maize intercropping or sole cropping in southeastern Brazil. *Experimental Agriculture*, *43*(3), 319–330. https://doi.org/10.1017/S0014479707005029

Cheto, S., Oukaltouma, K., Chamkhi, I., Ibn Yasser, A., Benmrid, B., Qaddoury, A., Kouisni, L., Geistlinger, J., Zeroual, Y., Bargaz, A., & Ghoulam, C. (2023). Inoculation with rhizobacterial consortia alleviates combined water and phosphorus deficit stress in intercropped faba bean and wheat. *Frontiers in Sustainable Food Systems*, *7*, 1147939. https://doi.org/10.3389/fsufs.2023.1147939

Cosentino, S. L., Sanzone, E., Testa, G., Patanè, C., Anastasi, U., & Scordia, D. (2019). Does post-anthesis heat stress affect plant phenology, physiology, grain yield and protein content of durum wheat in a semi-arid Mediterranean environment? *Journal of Agronomy and Crop Science*, *205*(3), 309–323. https://doi.org/10.1111/jac.12323

Cougnon, M., Durand, J. L., Julier, B., Barre, P., & Litrico, I. (2022). Using perennial plant varieties for use as living mulch for winter cereals. A review. *Agronomy for Sustainable Development*, *42*, Article 110. https://doi.org/10.1007/s13593-022-00844-x

De Luca, P., Messori, G., Faranda, D., Ward, P. J., & Coumou, D. (2020). Compound warm–dry and cold–wet events over the Mediterranean. *Earth System Dynamics*, *11*, 793–805. https://doi.org/10.5194/esd-11-793-2020

Devi, V. V. S., Raj, S. K., Sreekumar, A., & Sherafudeen, H. (2023). A review on live mulch for better agriculture. *Environment and Ecology*, *41*(4A), 2452–2459. https://doi.org/10.60151/envec/ZQQJ1938

Dinelli, G., Marotti, I., Di Silvestro, R., Bosi, S., Bregola, V., Accorsi, M., Di Loreto, A., Benedettelli, S., Ghiselli, L., & Catizone, P. (2013). Agronomic, nutritional and nutraceutical aspects of durum wheat (*Triticum durum* Desf.) cultivars under low input agricultural management. *Italian Journal of Agronomy*, *8*(2), e12. https://doi.org/10.4081/ija.2013.e12

Dong, B., Wang, Z., Stomph, T. J., Evers, J. B., van der Putten, P. E. L., & van der Werf, E. (2025). Temporal complementarity drives nitrogen uptake in strip intercropping with or without legumes. *Agronomy for Sustainable Development*, *45*, Article 59. https://doi.org/10.1007/s13593-025-01052-z

Drevon, J. J., Alkama, N., Bargaz, A., Rodiño, A. P., Sungthongwises, K., & Zaman-Allah, M. (2015). The legume–rhizobia symbiosis. In A. M. De Ron (Ed.), *Grain legumes* (pp. 267–290). Springer Science + Business Media. https://doi.org/10.1007/978-1-4939-2797-5_9

Enrico, J. M., Piccinetti, C. F., Barraco, M. R., Agosti, M. B., Eclesia, R. P., & Salvagiotti, F. (2020). Biological nitrogen fixation in field pea and vetch: Response to inoculation and residual effect on maize in the Pampean region. *European Journal of Agronomy*, *115*, 126016. https://doi.org/10.1016/j.eja.2020.126016

Erenstein, O. (2002). Crop residue mulching in tropical and semi-tropical countries: An evaluation of residue availability and other technological implications. *Soil and Tillage Research*, *67*, 115–133.

EU Nitrogen Expert Panel. (2016). *Nitrogen use efficiency (NUE)—Guidance document for assessing NUE at farm level*. Wageningen University, Alterra.

European Union. (2023). Regulation (EU) 2023/956 of the European Parliament and of the Council of 10 May 2023 establishing a carbon border adjustment mechanism. *Official Journal of the European Union*, *130*(25), 1–53.

Farquharson, E. A., Ballard, R. A., Herridge, D. F., Ryder, M. H., Denton, M. D., Webster, A., Yates, R. J., Seymour, N. P., Deaker, R. J., Hartley, E., Gemmel, L. G., Hackney, B., & O'Hara, G. W. (2022). *Inoculating legumes: Practice and science*. Grains Research and Development Corporation.

Follett, R. F. (2001). Innovative ^{15}N microplot research techniques to study nitrogen use efficiency under different ecosystems. *Communi-*

14350645, 2026, 4, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/agj2.70515 by CochraneItalia, Wiley Online Library on [05/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

cations in Soil Science and Plant Analysis, *32*(7–8), 951–979. https://doi.org/10.1081/CSS-100104099

Frame, J., & Laidlaw, A. J. (2005). Prospects for temperate forage legumes. In S. G. Reynolds & J. Frame (Eds.), *Grasslands: Developments, opportunities, perspectives* (pp. 1–28). Science Publishers, Inc..

Jamali, M., Bakhshandeh, E., Yaghoubi Khanghahi, M., & Crecchio, C. (2021). Metadata analysis to evaluate environmental impacts of wheat residues burning on soil quality in developing and developed countries. *Sustainability*, *13*(11), 6356. https://doi.org/10.3390/su13116356

Jensen, E. S., Carlsson, G., & Hauggaard-Nielsen, H. (2020). Intercropping of grain legumes and cereals improves the use of soil N resources and reduces the requirement for synthetic fertilizer N: A global-scale analysis. *Agronomy for Sustainable Development*, *40*, Article 5. https://doi.org/10.1007/s13593-020-0607-x

Kuzmanović, N., Fagorzi, C., Mengoni, A., Lassalle, F., & DiCenzo, G. C. (2022). Taxonomy of *Rhizobiaceae* revisited: Proposal of a new framework for genus delimitation. *International Journal of Systematic and Evolutionary Microbiology*, *72*, 5243. https://doi.org/10.1099/ijsem.0.005243

Kyei-Boahen, S., Slinkard, A. E., & Walley, F. L. (2002). Evaluation of rhizobial inoculation methods for chickpea. *Agronomy Journal*, *94*, 851–859. https://doi.org/10.2134/agronj2002.8510

Lamichhane, J. R., van der Worf, W., Alletto, L., & Blanco-Canqui, H. (2025). A call toward a consistent terminology of "cover crops" in agroecological literature. *Agronomy Journal*, *117*, e70237. https://doi.org/10.1002/agj2.70237

Leoni, F., Lazzaro, M., Carlesi, S., & Moonen, A. C. (2024). Screening suitable legumes for living mulches to support nitrogen dynamics and weed control in a durum wheat-forage sorghum crop sequence. *Field Crops Research*, *307*, 109246. https://doi.org/10.1016/j.fcr.2023.109246

Leoni, F., Lazzaro, M., Ruggeri, M., Carlesi, S., Meriggi, P., & Moonen, A. C. (2022). Relay intercropping can efficiently support weed management in cereal-based cropping systems when appropriate legume species are chosen. *Agronomy for Sustainable Development*, *42*, Article 75. https://doi.org/10.1007/s13593-022-00787-3

Li, C., Stomph, T., Makowski, D., Li, H., Zhang, C., Zhang, F., & van der Werf, W. (2023). The productive performance of intercropping. *Proceedings of the National Academy of Sciences of the USA*, *120*(2), e2201886120. https://doi.org/10.1073/pnas.2201886120

Liu, L., Knight, J. D., Lemke, R. L., & Farrell, R. E. (2019). A side-by-side comparison of biological nitrogen fixation and yield of four legume crops. *Plant and Soil*, *442*(1–2), 169–182. https://doi.org/10.1007/s11104-019-04167-x

Liu, C., Lu, M., Cui, J., Li, B., & Fang, C. (2014). Effects of straw carbon input on carbon dynamics in agricultural soils: A meta-analysis. *Global Change Biology*, *20*, 1366–1381. https://doi.org/10.1111/gcb.12517

López-Román, M. I., Castaño-Herrero, C., De la Rosa, L., & Ramírez-Parra, E. (2025). Optimizing nitrogen fixation in *Vicia sativa*: The role of host genetic diversity. *Agronomy*, *15*, 1479. https://doi.org/10.3390/agronomy15061479

Lupwayi, N. Z., Olsen, P. E., Sande, E. S., Keyser, H. H., Collins, M. M., Singleton, P. W., & Rice, W. A. (2000). Inoculant quality and its evaluation. *Field Crops Research*, *65*(2-3), 259–270. https://doi.org/10.1016/S0378-4290(99)00091-X

Mahmoud, R., Casadebaig, P., Hilgert, N., Alletto, L., Freschet, G. T., de Mazancourt, C., & Gaudio, N. (2022). Species choice and N fertilization influence yield gains through complementarity and selection effects in cereal-legume intercrops. *Agronomy for Sustainable Development*, *42*, Article 12. https://doi.org/10.1007/s13593-022-00754-y

Mei, P. P., Gui, L. G., Wang, P., Huang, J. C., Long, H. Y., Christie, P., & Li, L. (2012). Maize/faba bean intercropping with rhizobia inoculation enhances productivity and recovery of fertilizer P in a reclaimed desert soil. *Field Crops Research*, *130*, 19–27. https://doi.org/10.1016/j.fcr.2012.02.007

Mittermeier, R., Robles, G. P., Hoffmann, M., Pilgrim, J., Brooks, T., Mittermeier, C., Lamoreux, J., & daFonseca, G. (2004). *Hotspots revisited: Earth's biologically richest and most endangered ecoregions*. Cemex.

Murphy, R. P., Montes-Molina, J. A., Govaerts, B., Six, J., van Kessel, C., & Fonte, S. J. (2016). Crop residue retention enhances soil properties and nitrogen cycling in smallholder maize systems of Chiapas, Mexico. *Applied Soil Ecology*, *103*, 110–116. https://doi.org/10.1016/j.apsoil.2016.03.014

Nishida, H., Tanaka, S., Handa, Y., Ito, M., Sakamoto, Y., Matsunaga, S., Betsuyaku, S., Miura, K., Soyano, T., Kawaguchi, M., & Suzaki, T. (2018). A NIN-like protein mediates nitrate-induced control of root nodule symbiosis in *Lotus japonicus*. *Nature Communications*, *9*(1), Article 499. https://doi.org/10.1038/s41467-018-02831-x

Nyoki, D., & Ndakidemi, P. A. (2018). Yield response of intercropped soybean and maize under rhizobia (*Bradyrhizobium japonicum*) inoculation and P and K fertilization. *Communications in Soil Science and Plant Analysis*, *49*(10), 1168–1185. https://doi.org/10.1080/00103624.2018.1455846

Ocumpaugh, W. R., & Smith, G. R. (1991). Granular inoculum enhances establishment and forage production of arrowleaf clover. *Journal of Production Agriculture*, *4*, 219–224. https://doi.org/10.2134/jpa1991.0219

Panagea, I. S., Quataert, P., Alonso-Ayuso, M., Bárcena, T. G., De Boever, M., Diacono, M., Jacobs, A., Jensen, J. L., Seidel, F., Seitz, D., Spiegel, H., Vanden Nest, T., Don, A., & Ruysschaert, G. (2025). Forage vs. grain legumes: Contrasting effects on soil organic carbon stocks—Evidence from 30 European field experiments. *European Journal of Soil Science*, *76*, e70086. https://doi.org/10.1111/ejss.70086

Parr, M., Grossman, J. M., Reberg-Horton, S. C., Brinton, C., & Crozier, C. (2011). Nitrogen delivery from legume cover crops in no-till organic corn production. *Agronomy Journal*, *103*(6), 1578–1590. https://doi.org/10.2134/agronj2011.0007

Pellegrini, F., Carlesi, S., Nardi, G., & Bàrberi, P. (2021). Wheat–clover temporary intercropping under Mediterranean conditions affects wheat biomass, plant nitrogen dynamics and grain quality. *European Journal of Agronomy*, *130*, 126347. https://doi.org/10.1016/j.eja.2021.126347

Peoples, M. B., Chalk, P. M., Unkovich, M. J., & Boddey, R. B. (2015). Can differences in 15N natural abundance be used to quantify the transfer of nitrogen from legumes to neighbouring non-legume plant species? *Soil Biology and Biochemistry*, *87*, 97–109. https://doi.org/10.1016/j.soilbio.2015.04.010

Queiroz, L. M. D., Dubeux, J. C. B., Sollenberger, L. E., Vendramini, J. M. B., Liao, H. L., Jaramillo, D. M., Santos, E. R. S., Abreu, D. S., Lira, M. A., Casagrande, D. R., Mackowiak, C. L., & Ruiz-Moreno, M. (2025). A novel technique utilizing enriched $^{15}N_2$ to trace nitrogen

14350645, 2026, 4, Downloaded from https://acsess.onlinelibrary.wiley.com/doi/10.1002/agj2.70515 by CochraneItalia, Wiley Online Library on [05/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

transfer in grass and legume mixtures. *Scientific Reports*, *15*, Article 24514. https://doi.org/10.1038/s41598-025-08988-y

Radicetti, E., Baresel, J. P., El-Haddoury, E. J., Finckh, M. R., Mancinelli, R., Schmidt, J. H., Thami Alami, I., Udupa, S. M., van der Heijden, M. G. A., Wittwer, R., & Campiglia, E. (2018). Wheat performance with subclover living mulch in different agro-environmental conditions depends on crop management. *European Journal of Agronomy*, *94*, 36–45. https://doi.org/10.1016/j.eja.2018.01.011

Rice, W. A., & Olsen, P. E. (1992). Effects of inoculation method and size of *Rhizobium meliloti* population in the soil on nodulation of alfalfa. *Canadian Journal of Soil Science*, *72*, 57–67. https://doi.org/10.4141/cjss92-006

Rodriguez, C., Carlsson, G., Englund, J.-E., Flöhr, A., Pelzer, E., Jeuffroy, M.-H., Makowski, D., & Jensen, E. S. (2020). Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate agroecosystems. A meta-analysis. *European Journal of Agronomy*, *118*, 126077. https://doi.org/10.1016/j.eja.2020.126077

Sainju, U. M. (2017). Determination of nitrogen balance in agroecosystems. *MethodsX*, *4*, 199–208. https://doi.org/10.1016/j.mex.2017.06.001

Salon, C., Munier-Jolain, N. G., Duc, G., Voisin, A. S., Grandgirard, D., Larmure, A., Emery, R. J. N., & Ney, B. (2001). Grain legume seed filling in relation to nitrogen acquisition: A review and prospects with particular reference to pea. *Agronomie*, *21*(6–7), 539–552. https://doi.org/10.1051/agro:2001143

Scavo, A., Maio, A., Calderone, F., La Malfa, T., Trostle, C., Yates, R. J., Toscano, S., Cavallo, C., Oteri, M., Furfaro, M. E., Virga, A. N., Scordia, D., & Gresta, F. (2025). Enhancing Mediterranean forage legume production through inoculation with elite rhizobia. *Frontiers in Agronomy*, *7*, 1551176. https://doi.org/10.3389/fagro.2025.1551176

Schwember, A. R., Schulze, J., del Pozo, A., & Cabeza, R. A. (2019). Regulation of symbiotic nitrogen fixation in legume root nodules. *Plants*, *8*, 333. https://doi.org/10.3390/plants8090333

Scordia, D., Calderone, F., Maio, A., La Malfa, T., Oteri, M., Scavo, A., & Gresta, F. (2026). Weed suppression and reduced fertilizer requirements in organic durum and bread wheat using forage legume living mulches in Mediterranean systems. *Frontiers in Agronomy*, *8*, 1801099. https://doi.org/10.3389/fagro.2026.1801099

Scordia, D., Guarnaccia, P., Calderone, F., Maio, A., La Malfa, T., Scavo, A., & Gresta, F. (2024). Adoption of cereal–legume double cropping toward more sustainable organic systems in the Mediterranean area. *Agronomy*, *14*, 772. https://doi.org/10.3390/agronomy14040772

Seguin, P., Sheaffer, C. C., Ehlke, N. J., Russelle, M. P., & Graham, P. H. (2001). Nitrogen fertilization and rhizobial inoculation effects on kura clover growth. *Agronomy Journal*, *93*, 1262–1268. https://doi.org/10.2134/agronj2001.1262

Soil Survey Staff. (2022). *Keys to soil taxonomy* (13th ed.). USDA Natural Resources Conservation Service. https://www.nrcs.usda.gov/sites/default/files/2022-09/Keys-to-Soil-Taxonomy.pdf

Suter, M., Connolly, J., Finn, J. A., Loges, R., Kirwan, L., Sebastià, M. T., & Lüscher, A. (2015). Nitrogen yield advantage from grass–legume mixtures is robust over a wide range of legume proportions and environmental conditions. *Global Change Biology*, *21*, 2424–2438. https://doi.org/10.1111/gcb.12880

Thilakarathna, M. S., McElroy, M. S., Chapagain, T., Papadopoulos, Y. A., & Raizada, M. N. (2016). Belowground nitrogen transfer from legumes to non-legumes under managed herbaceous cropping systems. A review. *Agronomy for Sustainable Development*, *36*, Article 58. https://doi.org/10.1007/s13593-016-0396-4

von Cossel, M., Scordia, D., Altieri, M., & Gresta, F. (2025). Spotlight on agroecological cropping practices to improve the resilience of farming systems: A qualitative review of meta-analytic studies. *Frontiers in Agronomy*, *7*, 1495846. https://doi.org/10.3389/fagro.2025.1495846

Willer, H., Schlatter, B., & Trávníček, J. (Eds.). (2023). *The world of organic agriculture. Statistics and emerging trends 2023* (Online Version 2). Research Institute of Organic Agriculture FiBL, Frick, and IFOAM–Organics International.

Yaldiz, G., Camlica, M., & Apaydın, H. (2025). Genotypic variation in morphological traits, yield, essential oil profiles, and mineral composition of fennel (*Foeniculum vulgare* L.) across two growing seasons. *Scientific Reports*, *15*, Article 34989. https://doi.org/10.1038/s41598-025-18991-y

You, L., Ros, G. H., Chen, Y., Shao, Q., Young, M. D., Zhang, F., & de Vries, W. (2023). Global mean nitrogen recovery efficiency in croplands can be enhanced by optimal nutrient, crop and soil management practices. *Nature Communications*, *14*, Article 5747. https://doi.org/10.1038/s41467-023-41504-2

Zhao, J., Chen, J., Beillouin, D., Lambers, H., Yang, Y., Smith, P., Zeng, Z., Olesen, J. E., & Zang, H. (2022). Global systematic review with meta-analysis reveals yield advantage of legume-based rotations and its drivers. *Nature Communications*, *13*, Article 4926. https://doi.org/10.1038/s41467-022-32464-0

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