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Physiological Responses and Industrial Oil Quality of Camelina sativa under Camelina–Vicia faba Intercropping and Contrasting Water Regimes

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Abstract

Industrial oilseed crops provide renewable feedstocks for the bioeconomy, but water deficits in semi-arid regions can adversely affect physiological performance and seed-oil quality. This three-year field study evaluated the effects of *Camelina sativa–Vicia faba* intercropping under irrigated and rainfed conditions, with particular emphasis on physiological performance, nitrogen acquisition, and seed-oil quality characteristics relevant to industrial applications. A split-plot experiment compared replacement intercropping ratios (75:25, 50:50, and 25:75) and additive systems with sole Camelina cropping. Relative water content, membrane permeability, gas exchange, leaf pigments, nitrogen acquisition patterns, seed oil content, fatty acid composition, oxidative stability, and total tocopherol concentration were evaluated. Under irrigation, the balanced 50:50 replacement system generally showed the most favourable physiological responses, including higher photosynthetic rates and relative water content and lower membrane permeability. These responses were accompanied by high seed oil content (39.0%), the numerically highest Rancimat oxidative-stability induction period (7.3 h), and the highest total tocopherol concentration (768 mg kg^{-1} oil), together with changes in fatty acid composition and an estimated cetane number of 48.2. The concurrent responses of physiological traits, tocopherol concentration, and oxidative stability indicate associations among these characteristics under favourable intercropping and water conditions, but do not establish direct causal relationships. Likewise, the observed nitrogen-acquisition patterns based on ^{15}N dilution do not provide quantitative evidence of biological nitrogen fixation or direct nitrogen transfer between the component crops. Overall, balanced Camelina–Vicia intercropping, particularly under irrigation, was associated with favourable physiological performance and selected seed-oil quality attributes, suggesting potential value as a low-input cropping strategy for producing Camelina feedstock with characteristics relevant to industrial applications.

1. Introduction

Industrial oilseed crops are central to the emerging bioeconomy, supplying renewable feedstocks for biofuels, bioplastics, lubricants, and specialty polymers [61]. Among these crops, *Camelina sativa* (L.) Crantz is increasingly recognised as a low-input industrial oilseed whose seed oil, typically accounting for 30–40% of seed weight, is rich in polyunsaturated fatty acids, particularly α-linolenic acid (30–40%). This distinctive oil composition, together with its short growing season, adaptability to marginal environments, and relatively high drought tolerance, makes camelina a promising feedstock for diverse bio-based applications [1, 30, 44, 67]. Its compatibility with diversified cropping systems further increases its potential contribution to sustainable agricultural production and rural economic development while reducing direct competition with conventional food crops [18, 48, 47, 49, 59].

For industrial utilisation, including biodiesel and other bio-based applications, the quality of camelina oil is determined by a combination of fatty-acid composition, oxidative stability, antioxidant constituents, and processing characteristics [55]. High oxidative stability can improve resistance to lipid oxidation during storage and processing, while tocopherols contribute to the antioxidant capacity of vegetable oils. In semi-arid environments such as central Iran, however, irregular rainfall and water deficits can modify

fatty-acid composition and other seed-quality attributes, potentially affecting the suitability of camelina oil for different industrial applications [23, 32, 46]. In addition to oil content and fatty-acid composition, glucosinolates represent an important component of camelina seed quality because their accumulation can respond to environmental and nutritional conditions and has implications for the utilization of both the seed and the resulting meal [25]. Thus, evaluation of camelina for industrial production requires consideration of multiple quality attributes rather than reliance on a single compositional indicator.

Water limitation and repeated cultivation can further constrain camelina productivity in semi-arid environments. Under water-deficit conditions, reduced plant water status, photosynthetic activity, and carbon assimilation may limit biomass and seed production and can modify the accumulation of storage lipids and secondary metabolites [53]. Diversification through intercropping may provide an alternative strategy for improving resource capture and stabilising crop performance under such conditions [22, 45]. Intercropping camelina with nitrogen-fixing legumes such as *Vicia faba* may enhance complementary acquisition of light, water, and nutrients and improve resource-use efficiency at the cropping-system level [19, 31, 50]. However, the magnitude and direction of these interactions depend on companion-crop identity, relative crop density, environmental conditions, and the design of the intercropping system [8, 16, 24, 34, 37, 66].

Recent studies have increasingly examined camelina in association with pulse crops, highlighting the potential of these systems to improve land-use efficiency while maintaining the productivity of the component crops. Pagani et al. (2024), for example, evaluated camelina intercropped with field pea and lentil and showed that the response of camelina depended on the companion crop and cropping configuration. These findings indicate that the benefits of camelina–legume intercropping cannot be generalised across systems and that crop identity, relative density, and environmental conditions should be considered simultaneously [60]. In particular, replacement and additive designs represent different resource-sharing strategies and may therefore produce contrasting effects on camelina growth, yield, and seed composition.

Despite increasing interest in camelina–legume intercropping, systematic field-scale comparisons of replacement and additive configurations under contrasting rainfed and irrigated conditions remain limited, particularly in semi-arid environments. Moreover, the relationships among plant water status, photosynthetic performance, pigment characteristics, nitrogen acquisition, and changes in seed-quality traits have not been sufficiently resolved. It remains uncertain whether favourable physiological responses under intercropping are consistently accompanied by changes in oil content, fatty-acid composition, tocopherol concentration, oxidative stability, and glucosinolate accumulation when water availability varies among seasons. The potential contribution of intercropping to nitrogen acquisition also requires careful interpretation because relative nitrogen acquisition or isotopic dilution does not by itself provide a quantitative estimate of biological nitrogen fixation or direct nitrogen transfer between component crops.

The industrial relevance of camelina further extends beyond conventional biodiesel applications to other bio-based and renewable-fuel pathways. Camelina oil has attracted attention as a potential biodiesel feedstock because of its fatty-acid composition and suitability for low-input production systems. However, biodiesel quality cannot be inferred directly from seed-oil composition alone. Fatty-acid composition, oxidative stability, cetane-related characteristics, oil extraction, transesterification, and subsequent fuel processing all contribute to the properties of the final biodiesel. Therefore, changes in camelina seed composition induced by field management should be interpreted as changes in biodiesel-relevant feedstock characteristics rather than as direct evidence of improved properties of the finished fuel [11]. Camelina also has potential for renewable aviation-fuel pathways, for which crop productivity, input requirements, and downstream processing are important determinants of overall sustainability [54].

A key novelty of the present study is the integration of crop configuration, water regime, plant physiological performance, nitrogen acquisition, and multiple seed-quality attributes within a single three-year field experiment. Specifically, we compared replacement and additive Camelina–*Vicia faba* intercropping configurations under both irrigated and rainfed conditions in a semi-arid environment. Rather than evaluating intercropping solely in terms of agronomic performance, we examined whether differences in plant water relations, photosynthetic performance, and pigment status were associated with changes in seed oil content, fatty-acid composition, total tocopherol concentration, glucosinolate accumulation, oxidative stability, and estimated cetane number. This integrated approach provides an opportunity to evaluate how intercropping configuration and water availability jointly influence the physiological performance and industrially relevant characteristics of camelina oil.

The present three-year field study therefore evaluated replacement (75:25, 50:50, and 25:75 Camelina:*Vicia faba*) and additive (100% camelina combined with 25–100% *Vicia faba*) intercropping patterns under irrigated and rainfed conditions in a semi-arid environment. We hypothesised that balanced intercropping configurations would favour resource acquisition and physiological performance under adequate water availability and would be associated with favourable changes in selected seed-quality traits, including oil content, tocopherol concentration, oxidative stability, and fatty-acid composition. We further hypothesised that intercropping would modify relative nitrogen acquisition and that these responses would vary according to cropping configuration and water regime. The specific objectives were to (i) quantify physiological and relative nitrogen-acquisition responses across water regimes, (ii) compare replacement and additive intercropping configurations using statistical and multivariate analyses, and (iii) determine how intercropping and water availability affect camelina seed composition and oil-quality attributes relevant to industrial and biodiesel-feedstock applications.

2. Materials and Methods

To assess the influence of intercropping patterns and irrigation regimes on physiological performance,, seed oil content, and fatty acid composition of *Camelina sativa*, a three-year field experiment was conducted during the 2022–2023, 2023–2024, and 2024–2025 growing seasons at the experimental station of Feridounshahr, Isfahan Province, Iran (32° 56' N, 50° 07' E, 2490 m a.s.l.). This location

represents a typical semi-arid highland climate with limited and variable water availability, making it suitable for evaluating low-input industrial oilseed systems [46, 67].

2.1. Site Characteristics

Soil samples were collected from the 0–30 cm depth before planting in each season and analyzed for physical and chemical properties (Table 1). The soil was classified as clay loam, slightly alkaline, with low electrical conductivity and moderate nutrient levels characteristic of semi-arid rainfed agriculture. No agronomic mineral or organic nitrogen fertilizer was applied as a treatment during the experiment. However, uniformly applied ^{15}N-enriched ammonium sulfate (10 atom% ^{15}N; equivalent to 50 kg N ha^{-1}) was used at sowing as an isotopic tracer to characterize treatment-related differences in nitrogen acquisition. The same amount of labeled N was applied uniformly to all experimental plots; therefore, it was not included as an experimental nitrogen-fertilization factor. Nevertheless, because the tracer supplied mineral N to the soil, its potential contribution to the soil mineral-N pool is acknowledged and the results are interpreted as relative differences in ^{15}N enrichment and nitrogen acquisition rather than as evidence of absolute nitrogen fixation or nitrogen transfer [5, 12, 20, 21, 33]. Monthly meteorological data, including air temperature, precipitation, and frost occurrence, for the three growing seasons (2022–2023, 2023–2024, and 2024–2025) are presented in Fig. 1. Based on the monthly precipitation data shown in the figure, total precipitation during the growing season was approximately 345, 450, and 530 mm in 2022–2023, 2023–2024, and 2024–2025, respectively. The corresponding average air temperatures over the November–May period were approximately 6.4, 6.8, and 7.7°C, respectively. The 2024–2025 season received the greatest precipitation, whereas the 2022–2023 season had the lowest precipitation. Seasonal temperature patterns also differed among years, with the lowest temperatures occurring during January and February and a gradual increase from March toward May. Overall, these meteorological conditions provide an important environmental context for interpreting seasonal variation in *Camelina sativa* responses to irrigation and intercropping.

2.2. Experimental Design and Crop Management

The experiment was conducted over three consecutive growing seasons using a split-plot arrangement based on a randomized complete block design with three replications. Irrigation regime (irrigated and rainfed) was assigned to main plots, and cropping system was assigned to subplots. The camelina cultivar ‘Soheil’ was used in this study. The cultivar is owned by Dr. Danial Kahrizi and was provided by Bistoon Shafa Medicinal Plants Cultivation and Development Company, Iran. The faba bean (*Vicia faba* L.) cultivar ‘Shadan’ (G-Faba-133; F6/Latt/338/08), a locally adapted cultivar commonly grown in the study region, was used in the experiment. Cropping treatments included sole *Camelina sativa* and sole *Vicia faba*, three replacement intercropping ratios (75:25, 50:50, and 25:75, based on the recommended sole-crop plant densities of approximately 150–200 plants m^{-2} for camelina and 30–40 plants m^{-2} for Vicia), and four additive intercropping treatments consisting of 100% Camelina combined with 25%, 50%, 75%, and 100% of the recommended Vicia density.

Each subplot consisted of six rows, 4 m in length, with a row spacing of 25 cm. The gross plot area was 6.0 m². Camelina and *Vicia faba* were sown manually on 4 November and 4 November, respectively, during the 2022–2023 growing season, on 8 November and 8 November during 2023–2024, and on 5 November and 5 November during 2024–2025. Standard agronomic practices, including weed and pest control, were applied uniformly across all treatments to avoid confounding effects. For seed-yield determination, the central four rows were harvested over a length of 3 m, corresponding to a net harvested area of 3.0 m² per subplot. The harvested plants were dried, threshed, cleaned, and weighed to determine seed yield, which was expressed as kg ha^{-1}.

Under irrigated conditions, irrigation was applied when soil moisture in the top 30 cm, determined gravimetrically, declined to approximately 60% of field capacity. The amount of water applied at each irrigation event was calculated to restore soil moisture to field capacity, corresponding to approximately 25–35 mm per irrigation depending on seasonal conditions. Rainfed plots received no supplemental irrigation and relied solely on natural precipitation.

No additional nitrogen fertilizer was applied during the growing season to avoid interference with nitrogen dynamics among treatments. Harvest was conducted at physiological maturity. Border rows were excluded to minimize edge effects, and the central rows of each plot were used for measurements and sampling.

2.3. Measurements

For each measurement, five randomly selected plants per plot were sampled and averaged for statistical analysis. The dates and corresponding developmental stages of physiological measurements and seed harvesting across the three growing seasons are provided in Table S1.

Leaf pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were quantified at full flowering using spectrophotometric analysis following 80% acetone extraction, with pigment concentrations calculated from absorbance measurements according to established equations [9]. Absorbance readings at 663, 645, and 470 nm were used to calculate concentrations with standard equations. Membrane permeability was assessed by measuring electrolyte leakage from leaf discs following Kaya (2025), with electrolyte leakage used as an indicator of cell membrane damage and stability, and relative water content (RWC) was measured using the standard fresh weight–turgid weight–dry weight method at midday. Gas exchange parameters (net photosynthesis, transpiration, intercellular CO_2 concentration) were recorded at flowering under ambient light (PAR 1200–1500 µmol m^{-2} s^{-1}) with a LI-6400XT portable photosynthesis system (LI-COR, USA). Leaf temperature was measured at midday on the youngest fully expanded leaves using a handheld infrared thermometer immediately before gas-exchange measurements under the same environmental conditions [51].

Seed oil content was estimated non-destructively by near-infrared spectroscopy (NIR) calibrated against Soxhlet extraction (R^2 = 0.96). For fatty acid composition, oxidative stability, and tocopherol analyses, seed oil was extracted from cleaned seeds using the Soxhlet method with n-hexane as solvent,

consistent with the NIR calibration protocol. Fatty acid methyl esters (FAMEs) were prepared and analyzed by gas chromatography–flame ionization detection (GC-FID) on a CP-Sil 88 column with temperature programming. Peaks were identified using authentic FAME standards. The oven temperature was programmed from 140°C to 220°C at 4°C min^{-1}. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The injection volume was 1 µL with a split ratio of 1:50. The concentrations of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), α-linolenic (C18:3), eicosenoic (C20:1), and erucic (C22:1) acids were quantified and expressed as percentages of the total identified fatty acids according to the chromatographic peak area normalization procedure [3]. Protein content was determined by the Kjeldahl method and calculated as total nitrogen × 6.25 [2]. Cetane number was estimated from fatty acid composition using the empirical formula $CN = \Sigma (w_i \times CN_i)$ (1), where w_i is the weight fraction of each fatty acid, and CN_i is its literature-derived cetane value.

Glucosinolate concentration was determined following methanolic extraction using the spectrophotometric procedure described by AOCS (2017), and results were expressed as µmol g^{-1} dry weight.

Oxidative stability was determined using a Rancimat apparatus at 110°C with an airflow of 20 L h^{-1}; the induction period (h) was used as the oxidative stability index [29].

Tocopherol composition (α-, γ-, and δ-tocopherols) was determined by high-performance liquid chromatography (HPLC) according to AOCS Official Method Ce 8–89. Oil samples were dissolved in n-hexane and injected into an HPLC system equipped with a fluorescence detector (excitation 290 nm, emission 330 nm). Separation was achieved on a silica column using n-hexane: isopropanol (99:1, v/v) as the mobile phase at a flow rate of 1.0 mL min^{-1}. Tocopherols were identified and quantified using authentic external standards. Total tocopherol content was calculated as the sum of α-, γ-, and δ-tocopherols and expressed as mg kg^{-1} oil.

2.4. Nitrogen Tracer Approach

To assess treatment-specific variations in nitrogen uptake patterns and possible interspecific relative nitrogen acquisition, the ^{15}N tracer technique was implemented. At sowing in each growing season, ^{15}N-enriched ammonium sulfate (10 atom% ^{15}N) was uniformly applied to all experimental plots at a rate equivalent to 50 kg N ha^{-1}. The labeled ammonium sulfate was used as an isotopic tracer to assess relative nitrogen acquisition among the Camelina–Vicia cropping systems. Because the same tracer amount was applied to every treatment, it was not considered an experimental nitrogen-fertilization factor. However, the application necessarily introduced a measurable amount of mineral N into the soil and therefore represents a common N input across all treatments. The tracer was hand-broadcast over each subplot and immediately incorporated into the upper 0–20 cm soil layer to promote uniform distribution within the active rooting zone. No additional mineral or organic N fertilizer was applied during the growing season.

At physiological maturity, the aboveground biomass of *Camelina sativa* and *Vicia faba* was harvested independently, oven-dried, pulverized, and subjected to analysis for total nitrogen concentration by the Kjeldahl method as well as for ^{15}N atom% via isotope ratio mass spectrometry (IRMS). Atom percent excess values were determined by subtraction from natural abundance.

Owing to the absence of a non-fixing reference crop and the uniform application of the tracer to all treatments, absolute biological nitrogen fixation (%Ndfa) as well as direct nitrogen transfer could not be determined quantitatively. Rather, isotopic enrichment values were evaluated on a relative basis to elucidate treatment-related differences in dependence on soil nitrogen sources.

A relative ^{15}N dilution index (RDI) for Camelina was calculated as:

RDI = (Atom%^{15}N□□□□ − Atom%$^{15}N_i$□□□$_r$c$_r$□□) / Atom%^{15}N□□□□ × 100 (2)

This index served as a comparative measure of relative ^{15}N dilution index (RDI) across various intercropping setups. Furthermore, nodulation in *Vicia faba* was quantified by determining the nodule dry weight per plant, thereby providing additional insight into legume nitrogen dynamics. Isotopic analysis was performed using an isotope ratio mass spectrometer (IRMS). International reference standards were used to ensure analytical accuracy.

2.5. Statistical Analysis

Land equivalent ratio (LER) was calculated according to the following equation:

LER = (Y_Ci / Y_Cs) + (Y_Vi / Y_Vs) (3)

where Y_Ci and Y_Vi represent the yields of *Camelina sativa* and *Vicia faba*, respectively, under intercropping, whereas Y_Cs and Y_Vs represent the corresponding yields under sole cropping. The land equivalent ratio (LER) was calculated as the sum of the partial land equivalent ratios of the two component crops, a metric that remains widely used for evaluating land-use efficiency in intercropping systems [41].

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using a combined analysis of variance appropriate for the split-plot experimental design conducted across three growing seasons. Year, irrigation regime, intercropping pattern, and their interactions were included in the model. Replications were nested within year, with irrigation regime assigned to the main plots and intercropping pattern assigned to the subplots. The appropriate main-plot and subplot error terms were used to test the corresponding effects and interactions. Variance components were estimated using restricted maximum likelihood (REML), and denominator degrees of freedom were adjusted using the Kenward–Roger approximation. Model assumptions were assessed using residual diagnostics, including the Shapiro–Wilk test for residual normality and Levene's test for homogeneity of variance. When necessary, response variables were transformed before analysis. Least-squares means were compared using the Tukey– HSD adjustment, with statistical significance declared at $P < 0.05$. Unless otherwise

indicated, means presented in the tables represent values pooled across the three growing seasons. LER values were tested against the theoretical reference value of 1.0 using a one-sample t-test, and Pearson correlation analyses were performed to evaluate associations between RDI and selected oil-quality traits. Oil yield was calculated as seed yield × seed oil content / 100 and expressed as kg ha^{-1}. The mixed-model framework accounted for the hierarchical structure of the split-plot experiment and variation associated with year and replication within year.

3. Results

Combined ANOVA showed that irrigation and intercropping pattern were the main factors affecting the physiological traits, photosynthetic pigments, seed quality, fatty acid composition, and glucosinolate concentration of *Camelina sativa*. Irrigation significantly influenced water status, gas exchange, chlorophyll pigments, seed protein and oil contents, cetane number, oxidative stability, total tocopherol content, and several fatty acids and glucosinolates. Intercropping significantly affected all major trait groups, while significant irrigation × intercropping interactions were observed for several physiological, biochemical, and seed-quality traits. Year effects were generally non-significant, indicating relatively stable responses across the three growing seasons. Overall, irrigation regime and intercropping pattern, together with their interaction, were the primary determinants of camelina performance and seed composition (Supplementary Tables S2–S6).

3.1. Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoid Concentrations

Irrigation × intercropping interactions significantly affected chlorophyll a, total chlorophyll, and carotenoid concentrations (Table 2). The irrigated 50:50 replacement treatment produced the highest values for all three pigments (Chl a = 0.84 ± 0.02 mg g^{-1} FW; Total Chl = 1.12 ± 0.01 mg g^{-1} FW; Carotenoids = 0.51 ± 0.02 mg g^{-1} FW), significantly exceeding sole camelina under both irrigation regimes. The lowest pigment concentrations occurred in rainfed sole camelina (Chl a = 0.35 ± 0.02; Total Chl = 0.45 ± 0.01; Carotenoids = 0.23 ± 0.01) and in the densest rainfed additive treatments (100 + 75, 100 + 100), which did not differ significantly from rainfed sole cropping. Chlorophyll b, evaluated as a simple (main) effect (Table 3), followed a similar pattern: irrigation increased chlorophyll b by 36% relative to rainfed conditions (0.53 ± 0.02 vs. 0.39 ± 0.01 mg g^{-1} FW), and the 50:50 replacement and 100 + 75/100 + 100 additive treatments produced the highest pooled values (0.47–0.52 mg g^{-1} FW), while sole camelina produced the lowest (0.37 ± 0.02 mg g^{-1} FW). Across all pigment traits, the balanced replacement (50:50) system consistently outperformed both sole cropping and the denser additive configurations, particularly under irrigation.

3.2. Relative Water Content, Membrane Permeability, Leaf Temperature, Transpiration, Net Photosynthesis, and Intercellular CO_2 Concentration

Water status and membrane integrity. Irrigation increased relative water content (RWC) by 14.4% and decreased foliar temperature by 14.1% relative to rainfed conditions (Table 3). Intercropping further improved RWC, with the highest pooled value recorded in the additive 100 + 75 treatment (81.4 ± 0.8%) and the lowest in the 75:25 replacement treatment (76.1 ± 0.9%); sole camelina had the lowest overall RWC (73.7 ± 1.0%). Foliar temperature was highest in sole camelina (32.5 ± 0.5°C) and lowest in the denser additive treatments (29.5–29.8°C). Irrigation × intercropping significantly affected membrane permeability (ionic leakage) (Table 2). The highest ionic leakage occurred in rainfed dense additive treatments (100 + 75: 59.4 ± 1.2%; 100 + 100: 59.3 ± 1.0%), while the lowest values were recorded in irrigated sole camelina (22.3 ± 0.6%) and irrigated balanced intercropped plots (22.5–22.7%), which did not differ significantly from one another. Gas exchange. No three-way (year × irrigation × intercropping) interactions were detected for photosynthetic rate (P□), intercellular CO_2 concentration (C_i), or transpiration. Irrigation × intercropping significantly influenced P□ and C_i (Table 2). The highest P□ occurred in the irrigated 50:50 replacement treatment (9.7 ± 0.3 μmol CO_2 m^{-2} s^{-1}), statistically similar to the irrigated 100 + 25 additive treatment (9.5 ± 0.2), while the lowest P□ was recorded in the rainfed 100 + 100 additive treatment (3.2 ± 0.3). C_i was highest in the rainfed 100 + 50 and 100 + 75 additive treatments (563 ± 8 and 569 ± 7 μmol $C0^2$ mol^{-1} air, respectively) and lowest in irrigated replacement/low-density additive treatments (485 μmol $C0^2$ mol^{-1} air). Transpiration (Table 3) was 36.1% higher under irrigation than under rainfed conditions (5.92 ± 0.12 vs. 4.35 ± 0.10 mmol H_2O m^{-2} s^{-1}), with the maximum pooled value observed in the additive 100 + 75 treatment (5.55 ± 0.10) and the minimum in sole camelina (4.48 ± 0.14).

3.3. Protein Content, Seed Oil Content, and Glucosinolate Concentration

Irrigation × intercropping significantly affected both protein and seed oil content (Table 2). Seed oil content was highest in the irrigated 50:50 replacement treatment (39.0 ± 0.5%), statistically comparable to the irrigated 100 + 25 additive treatment (38.8 ± 0.4%), while the lowest oil contents occurred in rainfed dense additive treatments (100 + 75: 29.7 ± 0.7%; 100 + 100: 29.6 ± 0.6%). Protein content showed an inverse pattern relative to oil content between irrigation regimes, with the highest value recorded in the rainfed 50:50 replacement treatment (32.5 ± 0.5%) and the lowest in the irrigated 100 + 100 additive treatment (23.6 ± 0.6%). Glucosinolate concentration (Table 4) also varied significantly among treatments. The highest concentrations occurred in irrigated low-density replacement and additive treatments (75:25: 146 ± 1; 50:50: 148 ± 2; 100 + 25: 146 ± 2; 100 + 50: 148 ± 1 μmol g^{-1} DW), whereas the lowest values were recorded in irrigated sole camelina (102 ± 2) and the irrigated 100 + 100 additive treatment (103 ± 1 μmol g^{-1} DW).

3.4. Land Equivalent Ratio (LER)

Land equivalent ratios exceeded 1.0 in all intercropping systems, indicating a consistent land-use advantage over sole cropping (Fig. 2). The maximum LER (1.34) was achieved in the irrigated 50:50 replacement treatment, while the dense additive 100 + 100 system produced the lowest LER among

intercropped treatments (≈ 1.05–1.12), still significantly above the reference value of 1.0 (one-sample t-test, $P < 0.05$ in all cases). LER values were calculated from seed yield data collected at physiological maturity, as described in Section 2.5.

3.5. ^{15}N Enrichment Patterns and Relative Dilution Indices

Clear differences in ^{15}N enrichment were observed among cropping systems under both irrigation regimes (Fig. 3; Supplementary Table S7). Under irrigated conditions, *Vicia faba* in the 50:50 replacement system showed the lowest atom% ^{15}N enrichment among intercropped treatments, while camelina in the same configuration showed the greatest reduction in atom% ^{15}N relative to sole cropping, corresponding to the highest relative ^{15}N dilution index (RDI = 40.5%). Under rainfed conditions, atom% ^{15}N values were generally higher in sole camelina than in any intercropped treatment. Relative dilution indices remained positive across all intercropping systems but were lowest in the 100 + 100 additive treatment. Across both irrigation regimes, intercropped camelina consistently showed lower atom% ^{15}N enrichment than sole cropping, consistent with differences in the relative acquisition of labelled soil nitrogen among cropping systems.

3.6. Fatty Acid Composition of Camelina sativa Oil

Irrigation × intercropping significantly affected the fatty acid composition of *Camelina sativa* oil (Table 4). Palmitic acid was highest in the irrigated 25:75 replacement treatment (5.7 ± 0.06%) and lowest in the irrigated 100 + 75 additive treatment (2.1 ± 0.04%), with low-density irrigated additive treatments generally showing lower palmitic acid concentrations than sole cropping. Stearic acid was highest in rainfed sole Camelina (2.5 ± 0.02%) and the rainfed 100 + 100 additive treatment (2.6 ± 0.02%), whereas the lowest values were observed in irrigated 100 + 50 and rainfed 50:50 and 25:75 replacement treatments (1.3 ± 0.02%).

Oleic acid, reported as a pooled main effect (Table 3), was highest in the 50:50 replacement treatment (19.8 ± 0.26%) and lowest in sole Camelina (16.8 ± 0.20%). Irrigation increased oleic acid concentration by approximately 12% compared with rainfed conditions (19.2 ± 0.25% vs. 17.1 ± 0.22%). Linoleic acid was highest in the irrigated 25:75 replacement treatment (31.6 ± 0.2%) and lowest in rainfed 100 + 75 (27.1 ± 0.3%). α-Linolenic acid reached its highest values (38.8–38.9%) in rainfed sole Camelina, rainfed 100 + 25 additive, and rainfed 100 + 100 additive treatments, whereas the lowest value occurred in the irrigated 25:75 replacement treatment (31.7 ± 0.1%).

Eicosenoic acid was highest in the irrigated 25:75 replacement and 100 + 25 additive treatments (1.3%) and lowest in the rainfed dense additive treatments (100 + 75 and 100 + 100; 0.2–0.3%). Erucic acid ranged from 0.5% in the irrigated 100 + 25 additive treatment to 2.8% in irrigated and rainfed sole Camelina. Overall, erucic acid concentrations tended to be lower in intercropped treatments than in sole cropping, particularly under irrigation.

3.7. Cetane Number, Oxidative Stability, and Total Tocopherol Content of Camelina sativa Oil

Cetane number (Table 2) ranged from 40.5 to 48.2, with the highest value recorded in the irrigated 50:50 replacement treatment (48.2) and the lowest in rainfed sole camelina (40.5). Oxidative stability and total tocopherol content (Tables 2 and 4) were significantly affected by irrigation regime and intercropping pattern ($P < 0.001$). The highest oxidative stability (7.3 ± 0.14 h) and total tocopherol content (768 ± 10 mg kg^{-1} oil) were recorded in the irrigated 50:50 replacement treatment. For total tocopherol content, the irrigated 50:50 treatment was statistically comparable to the irrigated 100 + 25 additive treatment (754 ± 13 mg kg^{-1} oil), whereas it was significantly higher than the irrigated 25:75 replacement treatment (735 ± 11 mg kg^{-1} oil). Under irrigation, sole camelina and the denser additive treatments (100 + 75 and 100 + 100) showed the lowest oxidative stability and tocopherol contents. Under irrigation, sole camelina and the denser additive treatments (100 + 75, 100 + 100) showed the lowest oxidative stability (5.6–5.9 h) and tocopherol contents (694–710 mg kg^{-1}). Under rainfed conditions, both traits declined across all treatments relative to irrigation. The highest rainfed values occurred in the 50:50 replacement system (5.9 ± 0.12 h; 721 ± 10 mg kg^{-1}), while the lowest were observed in rainfed sole camelina (4.6 ± 0.10 h; 654 ± 12 mg kg^{-1}) and the rainfed 100 + 100 additive treatment (4.5 ± 0.13 h; 643 ± 12 mg kg^{-1}).

3.8. Associations between relative ^{15}N dilution and oil-quality traits

Pearson correlation analysis revealed positive associations between the relative ^{15}N dilution index (RDI) and several oil-quality traits (Table 5). RDI was positively correlated with total tocopherol concentration ($r = 0.62$, $P < 0.05$) and oxidative stability ($r = 0.55$, $P < 0.05$), indicating that treatments characterized by greater relative ^{15}N dilution tended to have higher tocopherol concentrations and longer oxidative-stability induction periods. Positive but non-significant correlations were also observed between RDI and seed oil content ($r = 0.38$, $P > 0.05$) and between RDI and oil yield ($r = 0.44$, $P > 0.05$). Total tocopherol concentration showed a stronger positive correlation with oxidative stability ($r = 0.71$, $P < 0.01$). These associations suggest that variation in relative nitrogen acquisition was accompanied by variation in selected oil-quality characteristics; however, the correlation analysis does not establish causal relationships between nitrogen-acquisition patterns and oil-quality responses.

3.9. Seed yield of Camelina sativa and Vicia faba

Seed yield differed significantly among irrigation regimes and intercropping configurations (Table 6). Under irrigated conditions, camelina seed yield was highest in sole cropping, whereas intercropping reduced the individual camelina yield as the proportion of *Vicia faba* increased. However, the reduction in camelina yield was partly compensated by the seed yield of V*icia* faba, resulting in higher combined seed production in several intercropping configurations. The 50:50 replacement treatment produced a favourable balance between camelina and *Vicia faba* yields, whereas the additive treatments maintained relatively high camelina yield while providing additional *Vicia faba* production. Under rainfed conditions,

seed yields of both species were generally lower than under irrigation, although the relative advantage of intercropping was maintained in several configurations.

3.10. Principal Component Analysis Integrating Physiological Traits and Oil Quality Parameters

Principal component analysis was performed on nine physiological and oil-quality traits. The first two principal components explained 96.6% of total variance (PC1 = 89.0%, PC2 = 7.6%; Fig. 4). PC1 was strongly associated with improved physiological performance and enhanced industrial oil quality: treatments on the negative side of PC1 exhibited higher net photosynthetic rate, total chlorophyll, carotenoids, oil content, total tocopherols, and oxidative stability, together with lower membrane permeability and intercellular CO_2 concentration. Variable contributions to PC1 were dominated by photosynthetic rate (18.2%), total tocopherols (16.7%), oxidative stability (15.9%), and oil content (14.3%). The irrigated 50:50 replacement treatment clustered at the extreme negative end of PC1, reflecting superior physiological status coupled with the highest total tocopherol content (768 mg kg^{-1}) and oxidative stability (7.3 h). Rainfed sole camelina was positioned at the positive extreme of PC1, reflecting combined drought effects on both physiological performance and oil-quality parameters. These results demonstrate a clear positive association between improved physiological performance under optimal intercropping and irrigation and elevated total tocopherols and oxidative stability in camelina oil.

4. Discussion

The results of this three-year field study indicate that intercropping configuration and water availability were associated with variation in the physiological performance and seed and oil-quality attributes of *Camelina sativa*. Under irrigated conditions, the 50:50 replacement system generally exhibited favourable physiological responses, including higher relative water content, lower membrane permeability, greater photosynthetic rates, and higher pigment concentrations. These responses suggest improved plant water status and photosynthetic performance under balanced intercropping, which may have favoured assimilate availability during seed development and contributed to the observed variation in oil and antioxidant characteristics [61]. However, these concurrent responses should be interpreted as associations rather than evidence of a single causal physiological pathway linking intercropping with changes in industrially relevant oil characteristics.

Under irrigated conditions, the 50:50 replacement system produced the strongest physiological responses, including elevated relative water content, reduced membrane permeability, higher photosynthetic rates, and increased pigment concentrations. Improved plant water status and photosynthetic performance can favour carbon assimilation and resource allocation during reproductive development, potentially supporting the accumulation of storage lipids and antioxidant compounds such as tocopherols [8, 13, 16, 20]. The lower ionic leakage observed under favourable intercropping conditions is also consistent with improved membrane integrity and reduced cellular stress. Such responses may

arise from complementary resource acquisition and below-ground interactions between component crops, including modifications in root activity and soil biological processes [26, 34, 37, 62, 64. 65]. These physiological responses coincided with the highest total tocopherol concentration (768 mg kg^{-1} oil) and the numerically highest Rancimat oxidative-stability induction period of Camelina oil (7.3 h) in the irrigated 50:50 treatment.

Tocopherols are important endogenous antioxidants in Camelina oil and can contribute to protection against lipid oxidation. In the present study, the highest tocopherol concentration occurred in the same treatment that exhibited the numerically highest oxidative-stability induction period. This concurrent response is consistent with the antioxidant role of tocopherols, but the present experiment did not manipulate tocopherol concentration experimentally; therefore, the observed association should not be interpreted as direct proof that the increase in tocopherols caused the longer induction period. The fatty-acid profile of the 50:50 treatment also showed a relatively favourable composition, including higher oleic acid and differences in polyunsaturated fatty acids. Because unsaturated fatty acids differ in their susceptibility to oxidation, changes in fatty-acid composition may have contributed to the observed oxidative-stability response alongside endogenous antioxidants [15, 40, 57]. Thus, the results suggest that several oil-composition characteristics changed concurrently under favourable intercropping conditions rather than demonstrating a single mechanism controlling oxidative stability.

An additional aspect of seed quality that deserves consideration is glucosinolate accumulation. Under irrigation, glucosinolate concentration was highest in the 50:50 replacement treatment (148 ± 2 μmol g^{-1} DW), compared with 102 ± 2 μmol g^{-1} DW in irrigated sole Camelina. The response indicates that intercropping influenced not only primary storage compounds such as oil and protein but also secondary seed metabolites. Jiang et al. (2016) demonstrated that glucosinolate accumulation in Camelina can respond to nutrient management, particularly nitrogen and sulfur availability, and can therefore vary with crop nutritional status. In the present experiment, however, no agronomic nitrogen fertilizer was applied and sulfur availability was not experimentally manipulated. Consequently, the higher glucosinolate concentration observed under selected intercropping configurations cannot be attributed to a specific nutrient mechanism. Differences in resource acquisition, plant nutritional status, and crop competition associated with intercropping may instead have contributed to the observed variation. This finding is important because glucosinolates represent an additional quality attribute when considering the utilization of Camelina seed and meal for food, feed, and broader biorefinery applications [25].

The present findings are also consistent with the growing literature on Camelina–legume intercropping. Pagani et al. (2024) evaluated Camelina in association with field pea and lentil and demonstrated that the response of Camelina depended on the companion pulse species and intercropping configuration. Their results highlight that intercropping does not produce a uniform benefit across all crop combinations, but rather that the relative performance of component crops depends on crop identity, density, and resource availability. The present three-year experiment extends this perspective by comparing both replacement and additive Camelina–*Vicia faba* configurations under irrigated and rainfed conditions. The favourable response of the 50:50 replacement system under irrigation suggests

that balanced replacement may provide a more favourable compromise between interspecific complementarity and competition than dense additive configurations. Conversely, the weaker responses observed in some high-density additive systems indicate that increasing total plant density does not necessarily translate into proportional improvements in Camelina physiological performance or oil quality.

The yield implications of these responses are particularly important when considering Camelina as an industrial feedstock. Blume et al. (2022) reported that Camelina oil yield is strongly influenced by seed yield, indicating that improvements in oil concentration alone do not necessarily translate into greater oil productivity at the field scale. In the present study, the irrigated 50:50 replacement treatment combined favourable seed-oil characteristics with high overall system productivity, suggesting that intercropping configuration should be evaluated using both yield and quality criteria. This distinction is important for industrial applications because a cropping system that increases oil concentration but substantially reduces seed yield may not necessarily provide a greater quantity of recoverable oil per unit land area. Therefore, the present results support evaluating balanced intercropping as a system-level strategy rather than identifying a treatment solely on the basis of a single oil-quality trait [6].

The industrial value of Camelina also depends on post-harvest processing and oil recovery. Field management determines the composition of the harvested seed, but extraction and subsequent processing determine the quantity and characteristics of the oil available for downstream applications. Veljković et al. (2022) highlighted the importance of seed pretreatment, mechanical pressing, solvent extraction, and subsequent processing steps in determining Camelina oil recovery and its potential industrial utilisation. Accordingly, the favourable oil-quality characteristics observed in the irrigated 50:50 system should be regarded as feedstock-level advantages rather than direct evidence of superior performance of a final industrial product. Further optimization of extraction and downstream processing would be required to determine whether the field-induced differences in oil composition translate into economically meaningful improvements in industrial oil recovery and utilization.

These quality responses are relevant to biodiesel production, but they should be interpreted carefully. Oxidative stability is an important property of biodiesel because it influences resistance to oxidation during storage and handling. In the present study, the highest Rancimat induction period was 7.3 h in Camelina oil from the irrigated 50:50 replacement treatment. This value was numerically higher than those observed in sole Camelina and several dense additive systems; however, it was not statistically different from the values recorded in the irrigated 100 + 25 additive and 25:75 replacement treatments. Therefore, the 50:50 treatment should be described as having the numerically highest oxidative stability rather than statistically superior oxidative stability.

The estimated cetane number showed a similar pattern. The highest estimated value (48.2) occurred in the irrigated 50:50 replacement treatment, whereas the lowest value (40.5) occurred in rainfed sole Camelina. Importantly, these values were estimated from fatty-acid composition and were not measured directly in a finished biodiesel fuel. Ciubota-Rosie et al. (2013) demonstrated the relevance of Camelina

oil as a biodiesel feedstock and emphasized the importance of fatty-acid composition and fuel properties in determining biodiesel performance. Nevertheless, the estimated cetane values in the present study should be interpreted as biodiesel-relevant indicators of the feedstock rather than as direct measurements of biodiesel ignition quality.

This distinction is particularly important when comparing the present results with fuel standards. EN 14214:2012 + A2:2019 specifies requirements and test methods for fatty acid methyl esters (FAME) used as diesel fuel or heating fuel, whereas ASTM D6751-24 specifies requirements for biodiesel fuel blendstock (B100). Both standards apply to finished biodiesel/FAME fuels rather than extracted Camelina oil. Consequently, the 7.3-h induction period and the estimated cetane number of 48.2 observed in the present study cannot be interpreted as demonstrating compliance with either standard [4, 14]. The comparison presented in Table 7 is therefore provided for contextual interpretation only.

The comparison indicates that the values obtained in the present study should be interpreted as feedstock characteristics rather than direct measurements of finished biodiesel quality. In particular, the 7.3-h oxidative-stability induction period and the estimated cetane number of 48.2 do not demonstrate compliance with biodiesel fuel specifications.

Under rainfed conditions, physiological performance was generally lower than under irrigation, although low-density additive intercropping (100:25) mitigated some stress responses, as indicated by reduced foliar temperature and membrane leakage. The associated changes in fatty-acid composition, including the relative increase in α-linolenic acid, may reflect adjustments in membrane lipid metabolism under water limitation. However, the lower oxidative stability and tocopherol concentration observed under rainfed conditions indicate that environmental water availability remained an important determinant of seed-quality characteristics [16, 23, 30, 36, 42, 43, 46, 58]. These findings further demonstrate that the effects of intercropping cannot be separated from the prevailing water regime and that the same cropping configuration may not provide equivalent physiological or oil-quality responses under contrasting environmental conditions [56].

The ^{15}N tracer data provide complementary information on relative nitrogen acquisition among cropping systems. Lower atom% ^{15}N enrichment in intercropped Camelina relative to sole cropping indicates greater dilution of the applied isotopic label and therefore differences in relative acquisition of labelled nitrogen. The strongest dilution occurred in the irrigated 50:50 treatment. However, these patterns do not provide a quantitative estimate of biological nitrogen fixation or prove direct nitrogen transfer from *Vicia faba* to Camelina. Because no agronomic nitrogen fertilizer was applied, the observed isotopic patterns may reflect differences in soil-N acquisition, crop competition, and potential legume-associated nitrogen inputs. Thus, the concurrence of stronger ^{15}N dilution with higher tocopherol concentration and oxidative stability in the 50:50 treatment should be interpreted as an association rather than evidence that nitrogen fixation directly caused the observed oil-quality responses [7, 27, 35, 37,]. In contrast, dense additive systems showed weaker dilution, which may be consistent with stronger competition for available resources and the weaker physiological responses observed in these treatments [5, 10, 23, 39, 52].

The broader industrial relevance of Camelina extends beyond conventional biodiesel to renewable aviation-fuel pathways. Resurreccion et al. (2021) demonstrated that Camelina-based aviation biofuel can have favourable sustainability characteristics, while also showing that crop productivity, agricultural inputs, and downstream processing strongly influence the overall environmental performance of the production pathway. In this context, the present results suggest that intercropping may have relevance beyond individual seed-quality traits by potentially influencing both crop productivity and the composition of the harvested feedstock. Nevertheless, a complete assessment of the sustainability or life-cycle performance of the present intercropping systems would require additional information on energy inputs, greenhouse-gas emissions, land-use efficiency, oil extraction, and downstream fuel conversion.

Collectively, these findings indicate that balanced intercropping was associated with favourable physiological performance and several desirable Camelina seed-quality characteristics, particularly under adequate water availability. The response involved concurrent changes in plant water status, photosynthetic performance, oil composition, tocopherol concentration, glucosinolate accumulation, and oxidative stability. However, the present study does not establish that a single physiological mechanism caused the observed changes in industrial oil quality. Similarly, the results do not demonstrate that nitrogen fixation directly increased tocopherol accumulation or that higher tocopherol concentration directly improved biodiesel quality. Instead, the findings support a more cautious interpretation in which intercropping configuration and water availability jointly modify the physiological environment and seed composition of Camelina, with consequences for several properties relevant to downstream industrial utilization.

From an applied perspective, the irrigated 50:50 replacement system emerged as a promising configuration because it combined favourable physiological performance with high oil content, total tocopherol concentration, and oxidative stability, while also showing a relatively high estimated cetane number. Nevertheless, these advantages should be considered at the feedstock level, because the present experiment did not directly evaluate biodiesel production, standardized fuel properties, or industrial processing performance. Further studies combining direct measurements of biological nitrogen fixation, quantitative N transfer, oil extraction efficiency, transesterification, and standardized biodiesel testing are therefore required to determine whether the field-level improvements observed under balanced Camelina–*Vicia faba* intercropping translate into measurable advantages in final industrial products.

5. Conclusion

This three-year field study showed that Camelina–*Vicia faba* intercropping configuration and water availability were associated with substantial variation in the physiological performance and seed-oil quality of *Camelina sativa*. Under irrigated conditions, the balanced 50:50 replacement system generally exhibited favourable plant water status, photosynthetic performance, and pigment characteristics, together with high seed oil content, total tocopherol concentration (768 mg kg^{-1} oil), and the numerically

highest oxidative-stability induction period (7.3 h). However, the concurrent responses of these traits should be interpreted as associations rather than evidence of a single causal physiological mechanism.

The results further indicate that intercropping affected several components of Camelina seed quality, including fatty-acid composition, tocopherol concentration, glucosinolate accumulation, and oxidative stability. The stronger ^{15}N dilution observed in selected intercropping treatments provides evidence of differences in relative nitrogen acquisition, but it does not establish quantitative biological nitrogen fixation or direct nitrogen transfer from *Vicia faba* to Camelina. Likewise, the observed association between higher tocopherol concentration and oxidative stability does not demonstrate a direct causal effect of tocopherols on oil stability or biodiesel quality.

From an industrial perspective, the irrigated 50:50 replacement system appears to be a promising configuration for producing Camelina feedstock with favourable combinations of physiological performance and selected oil-quality attributes under the conditions of this study. The estimated cetane number and oxidative-stability values should, however, be considered as biodiesel-relevant feedstock characteristics rather than evidence of compliance with biodiesel fuel standards, because biodiesel was not produced or directly tested. Overall, the findings suggest that balanced Camelina–*Vicia faba* intercropping may provide a useful low-input strategy for modifying industrially relevant seed characteristics in semi-arid environments, while further multi-environment studies, direct measurements of nitrogen fixation and transfer, and standardized biodiesel testing are needed to determine the underlying mechanisms and their implications for final fuel performance.

Declarations

Data availability

All data included in this study are available upon request by contacting the corresponding author.

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Author contributions

Zohrab Adavi: Writing – original draft, Resources, Investigation, Supervision, Data curation. Mehdi Ghanavati: Writing – review & editing, Formal analysis, Software, Resources. Peyman Aghaie: Methodology, Investigation, Formal analysis. Negar Nourmahnad: review & editing

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The authors declare no competing interests.

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References

1. Alberghini, B., Zanetti, F., Corso, M., Boutet, S., Lepiniec, L., Vecchi, A., Monti, A., 2022. Camelina (*Camelina sativa* (L.) Crantz) seeds are a multi-purpose feedstock for bio-based applications. Ind. Crops Prod. 182, 114944. https://doi.org/10.1016/j.indcrop.2022.114944.
2. AOAC International, 2019. Official Methods of Analysis of AOAC INTERNATIONAL, 21st ed. AOAC International, Rockville, MD, USA.
3. AOCS, 2017. Official Methods and Recommended Practices of the AOCS, 7th ed. AOCS Press, Urbana, IL, USA.
4. ASTM International, 2024. ASTM D6751-24: Standard Specification for Biodiesel Fuel Blendstock (B100) for Middle Distillate Fuels. ASTM International, West Conshohocken, PA, USA. https://doi.org/10.1520/D6751-24.
5. Bing, B., Shi, W., Wang, Y., 2025a. Assessment of nitrogen status in a maize–soybean intercropping system by establishing a novel critical nitrogen dilution curve under different irrigation amounts. Field Crops Res. 333, 110081. https://doi.org/10.1016/j.fcr.2025.110081.
6. Blume, R.Y., Rakhmetov, D.B., Blume, Y.B., 2022. Evaluation of Ukrainian *Camelina sativa* germplasm productivity and analysis of its amenability for efficient biodiesel production. Ind. Crops Prod. 187, 115477. https://doi.org/10.1016/j.indcrop.2022.115477
7. Bo, Y., Xing, Y., Liu, C., Shi, X., Liu, C., Zhou, Y., 2025. Unveiling drivers and optimizing strategies for enhanced land and nitrogen fertilizer use efficiency of sorghum intercropping: A meta-analysis. Field Crops Res. 332, 110033. https://doi.org/10.1016/j.fcr.2025.110033.
8. Breitenmoser, S., Steinger, T., Baux, A., Hiltpold, I., 2022. Intercropping winter oilseed rape (*Brassica napus* L.) has the potential to lessen the impact of the insect pest complex. Agronomy 12, 723. https://doi.org/10.3390/agronomy12030723.
9. Chazaux, M., Schiphorst, C., Lazzari, G., Caffarri, S., 2022. Precise estimation of chlorophyll a, b and carotenoid content by deconvolution of the absorption spectrum and new simultaneous equations for Chl determination. Plant J. 109, 1630–1648. https://doi.org/10.1111/tpj.15643
10. Chen, P., Xian, G., Pu, T., Gong, W., Li, J., Yang, F., Wang, X., Yong, T., Li, Y., Yan, Y., Liu, J., Yang, W., Wu, Y., 2025. Soybean–maize intercropping combined with water management enhances leaf traits to obtain yield benefits in semi-arid regions. Field Crops Res. 327, 109869. https://doi.org/10.1016/j.fcr.2025.109869.
11. Ciubota-Rosie, C., Ruiz, J.R., Ramos, M.J., Pérez, Á., 2013. Biodiesel from *Camelina sativa*: A comprehensive characterisation. Fuel 105, 572–577. https://doi.org/10.1016/j.fuel.2012.09.062.

12. Demie, D.T., Seidel, S.J., Wallach, D., Döring, T.F., Ewert, F., Gaiser, T., Paul, M., Hernández-Ochoa, I.M., 2025. Resource acquisition and interactions in spring wheat/faba bean intercropping under diverse environments. Field Crops Res. 325, 109817. https://doi.org/10.1016/j.fcr.2025.109817.
13. Dong, B., Wang, Z., Stomph, T.J., van der Werf, W., Zhang, C., Zhang, F., Li, L., 2025. Temporal complementarity drives nitrogen uptake in strip intercropping with or without legumes. Agron. Sustain. Dev. 45, 59. https://doi.org/10.1007/s13593-025-01052-z.
14. European Committee for Standardization (CEN), 2019. EN 14214:2012+A2:2019: Liquid petroleum products—Fatty acid methyl esters (FAME) for use in diesel engines and heating applications—Requirements and test methods. CEN, Brussels, Belgium.
15. Fan, Z., Qiang, B., Lin, Y., Liu, P., Li, Z., Cheng, M., Zhao, X., Ren, X., Chen, X., 2026. Leaf senescence and source–sink dynamics in soybean: Insights from maize–soybean intercropping systems. Field Crops Res. 335, 110130. https://doi.org/10.1016/j.fcr.2025.110130.
16. Gao, Y., Lv, Z.-C., Jiang, Y., Wu, H.-Z., Chen, M., Kim, D.-S., Lim, S.-H., Yan, X., Zhang, C.-J., 2025a. Agronomic performance of camelina (*Camelina sativa* (L.) Crantz) intercropped in temperate orchard-based agroforestry systems in eastern China. Eur. J. Agron. 170, 127747. https://doi.org/10.1016/j.eja.2025.127747.
17. Gao, Y., Zhang, Y., Yu, J., Kim, D.-S., Lim, S.-H., Zhang, H., Yan, X., Zhang, C.-J., 2024b. *Medicago polymorpha* and *M. sativa*–camelina intercropping increases land use efficiency, productivity, and economic benefits in eastern China. Eur. J. Agron. 159, 127283. https://doi.org/10.1016/j.eja.2024.127283.
18. Gesch, R.W., Eberle, C.A., Berti, M.T., Ott, M., Anderson, J.V., 2025. Productivity and seasonal water use of double-cropped dry bean, proso millet, and sunflower after early-maturing winter camelina. Ind. Crops Prod. 229, 120953. https://doi.org/10.1016/j.indcrop.2025.120953.
19. Griffin, A.J., Jungers, J.M., 2025. Effects of intercropping perennial legumes on intermediate wheatgrass productivity. Field Crops Res. 330, 109954. https://doi.org/10.1016/j.fcr.2025.109954.
20. Gui, D., Zhang, K., Sha, Z., 2025. Response of reactive nitrogen losses and nitrogen fate in the soil–crop system to intercropping regimes. Field Crops Res. 326, 109870. https://doi.org/10.1016/j.fcr.2025.109870.
21. Han, D., Zhang, L., Yang, W., 2025. Sowing and fertilizing machine in maize–soybean strip intercropping, in: Yang, W., Yang, F., Wu, Y. (Eds.), Theories and Application in Maize–Soybean Strip Intercropping System. Springer, Singapore, pp. 187–207. https://doi.org/10.1007/978-981-97-8723-4_10.
22. Haug, B., Messmer, M.M., Enjalbert, J., Goldringer, I., Flutre, T., Mary-Huard, T., Hohmann, P., 2023. New insights towards breeding for mixed cropping of spring pea and barley to increase yield and yield stability. Field Crops Res. 297, 108923. https://doi.org/10.1016/j.fcr.2023.108923.
23. Heineck, G.C., Smith, K.L., Neely, H.L., Casanova, J., Salguero, D., Gonzalez-Tapia, F., Peery, S.R., Carpenter-Boggs, L.A., Reganold, J.P., Huggins, D.R., 2025. Yield and water use of grain legumes

intercropped with spring canola in eastern Washington. Agrosyst. Geosci. Environ. 8, e70167. https://doi.org/10.1002/agg2.70167.

24. Hu, B., Zheng, Y., Wang, D., Guo, Y., Dong, Y., 2024. Intercropping wheat with appropriate nitrogen supply alleviates faba bean wilt disease by reshaping soil microbial community structure. Ind. Crops Prod. 222, 119538. https://doi.org/10.1016/j.indcrop.2024.119538.
25. Jiang, Y., Li, J., Caldwell, C.D., 2016. Glucosinolate content of Camelina genotypes as affected by applied nitrogen and sulphur. Crop Sci. 56, 3250–3262. https://doi.org/10.2135/cropsci2016.01.0018
26. Jing, B., Shi, W., Chen, T., Song, J., 2025. Underground interaction reduces nitrogen input by driving nitrogen fixation and transfer in maize–soybean intercropping. Field Crops Res. 334, 110150. https://doi.org/10.1016/j.fcr.2025.110150.
27. Kawasaki, N., Stomph, T.J., Woittiez, L.S., Laksono, W.M., Simanihuruk, D.M.P., Fitriani, D., Duhita, N., van der Werf, W., 2025. Performance of maize, soybean, and mung bean intercropping systems in East Java, Indonesia. Field Crops Res. 333, 110015. https://doi.org/10.1016/j.fcr.2025.110015.
28. Kaya, G., 2025. A plant-derived biostimulant Aminolom Enzimatico® application stimulates chlorophyll content, electrolyte leakage, stomata density and root yield of radishes under salinity stress. PeerJ 13, e18804. https://doi.org/10.7717/peerj.18804.
29. Keivanfar, L., Nateghi, L., Rashidi, L., Pourahmad, R., 2025. Effect of *Rosmarinus officinalis*, *Lavandula sublepidota*, and *Ferulago contracta* essential oils on oxidative stability of camelina oil. J. Food Qual. 2025, 1781599. https://doi.org/10.1155/jfq/1781599.
30. Kidane, B., Hunde, W., Urugo, M.M., Tessema, F., Teka, T.A., 2025. Camelina oil as Ethiopia's next edible oil: Insights into chemical composition and agro-ecological adaptability—A review. J. Oleo Sci. 74, 655–666. https://doi.org/10.5650/jos.ess25078.
31. Kong, J., Zhou, Z., Wang, J., Zhang, S., 2025. Suitable intercropping patterns effectively increase woody oil crop production by enriching soil nutrient-acquiring microbiota. Ind. Crops Prod. 236, 122082. https://doi.org/10.1016/j.indcrop.2025.122082.
32. Krzyżaniak, M., Warmiński, K., Olba-Zięty, E., Białoskórski, P., Stolarski, M.J., 2025. Life cycle assessment of camelina and spelt wheat in organic intercropping systems. Ind. Crops Prod. 231, 121198. https://doi.org/10.1016/j.indcrop.2025.121198.
33. Kumar, S., Singh, M., Kumar, S., Rajeev, 2025. Changes in root nodule dynamics under mixed cropping with varying nutrient management of berseem and succeeding fodder cowpea. J. Plant Nutr. 48, 617–638. https://doi.org/10.1080/01904167.2024.2410818.
34. Lan, Y., Zhang, H., He, Y., Jiang, C., Yang, M., Ye, S., 2023. Legume–bacteria–soil interaction networks linked to improved plant productivity and soil fertility in intercropping systems. Ind. Crops Prod. 196, 116504. https://doi.org/10.1016/j.indcrop.2023.116504.
35. Lassoued, F., Slade, P., Dyck, A., 2024. Crop rotations and canola yields: Evidence from field-level data in western Canada. Agron. J. 117, e21739. https://doi.org/10.1002/agj2.21739.

36. Li, H., Fan, Z., Wang, G., Wang, Y., Hu, F., Yin, W., Wang, Q., Cao, W., Chai, Q., Yao, T., 2025. Root plasticity of maize intercropped with a legume green manure maintains grain yield under reduced nitrogen inputs. Field Crops Res. 334, 110151. https://doi.org/10.1016/j.fcr.2025.110151.
37. Liu, Y., Fan, Y., Kuzyakov, Y., Wang, Y., Zhang, X., Li, Z., Song, Y., Chen, J., Wang, X., 2025. Effects of maize/soybean intercropping on nitrogen mineralization and fungal communities in soil. Plant Soil. In press. https://doi.org/10.1007/s11104-025-07824-6.
38. Liu, Z., Shangguan, X., Hu, H., Wang, X., Qiao, M., Liu, Y., Liang, Z., Zong, R., Li, Q., 2026. Maize–soybean intercropping improves grain yield by modifying water uptake strategies and dry matter accumulation and translocation mechanisms. Field Crops Res. 335, 110191. https://doi.org/10.1016/j.fcr.2025.110191.
39. Magnin, L., Hiltpold, I., Jullien, A., Baux, A., 2025. Intercropping mitigates the incidence of the oilseed rape insect pest complex. Pest Manag. Sci. In press. https://doi.org/10.1002/ps.8835.
40. Marcheva, M., Petkova, M., Slavova, V., Popov, V., 2024. Positive effect of camelina intercropping with legumes on soil microbial diversity assessed by NGS analysis and mobile fluorescence spectroscopy. Appl. Sci. 14, 9046. https://doi.org/10.3390/app14199046.
41. McAuley, W.P., Bourgault, M., Congreves, K.A., 2025. Productivity, soil fertility and soil health benefits associated with intercropping small grains and oilseeds with legumes in the semi-arid environment of the Canadian Prairies. Agric. Ecosyst. Environ. 389, 109694. https://doi.org/10.1016/j.agee.2025.109694.
42. McDonald, G.K., Minkey, D., Desbiolles, J., Clarke, G., Allen, R., Noack, S., Schmitt, S., Amougis, A., 2024. Responses to precision planting in canola and grain legume crops. Field Crops Res. 315, 109451. https://doi.org/10.1016/j.fcr.2024.109451.
43. Mirdoraghi, M., Maleki Farahani, S., Rezazadeh, A., 2025. Optimizing chickpea (*Cicer arietinum* L.) and dragon's head (*Lallemantia iberica*) intercropping in a semi-arid region. Ind. Crops Prod. 236, 121932. https://doi.org/10.1016/j.indcrop.2025.121932.
44. Mondor, M., Hernández-Álvarez, A.J., 2021. *Camelina sativa*: Composition, attributes, and applications—A review. Eur. J. Lipid Sci. Technol. 124, 2100035. https://doi.org/10.1002/ejlt.202100035.
45. Nurgia, N., Tana, T., Dechassa, N., Alemayehu, Y., Tesso, B., 2023. Effects of planting density and variety on productivity of maize–faba bean intercropping system. Heliyon. 9, e12967. https://doi.org/10.1016/j.heliyon.2023.e12967.
46. Obeng, E., Obour, A.K., Nelson, N.O., Ciampitti, I.A., Wang, D., 2024. Cropping sequence influences crop yield, soil water, and soil properties in the wheat–camelina cropping system. Farm. Syst. 2, 100066. https://doi.org/10.1016/j.farsys.2023.100066.
47. Olba-Zięty, E., Krzyżaniak, M., Stolarski, J., Stolarski, M.J., 2025. Economic efficiency of camelina and spelt wheat production in organic intercropping systems. Ind. Crops Prod. 237, 122190. https://doi.org/10.1016/j.indcrop.2025.122190.

48. Ouda, S., Zohry, A.E.H., 2025. Legume-based intercropping systems mitigate climate change, in: Zohry, A.E.H., Ouda, S. (Eds.), Resilient Agroecosystems. Springer, Singapore, pp. 261–283. https://doi.org/10.1007/978-981-96-9630-7_12.
49. Pagani, E., Zanetti, F., Ferioli, F., Facciolla, E., Monti, A., 2024. Camelina intercropping with pulses a sustainable approach for land competition between food and non-food crops. Agronomy 14, 1200. https://doi.org/10.3390/agronomy14061200.
50. Patel, S., Lenssen, A.W., Moore, K.J., Mohammed, Y.A., Gesch, R.W., Wells, M.S., Johnson, B.L., Berti, M.T., Matthees, H.L., 2021. Interseeded pennycress and camelina: Yield performance and influence on row crops. Agron. J. 113, 2626–2641. https://doi.org/10.1002/agj2.20655.
51. Prashar, A., Jones, H.G., 2016. Infra-red thermography as a high-throughput tool for field phenotyping. Agronomy 6, 50. https://doi.org/10.3390/agronomy6040050.
52. Raboin, L.-M., Ouedraogo, N., Kabore, M., Zabre, Y., Ganeme, A., Bonnal, L., Assouma, M.H., Gozé, E., Dusserre, J., 2025. Sorghum genetic variability for adaptation to intercropping with cowpea under Sudano–Sahelian conditions. Field Crops Res. 330, 109984. https://doi.org/10.1016/j.fcr.2025.109984.
53. Raseduzzaman, M., Ali, M.R., Dong, W., Aluoch, S.O., Li, X., Gaudel, G., Zhang, Y., Hu, C., 2025. Soil greenhouse gas emissions, yield, and water-use efficiency under maize–soybean intercropping with long-term nitrogen fertilization. Field Crops Res. 326, 109861. https://doi.org/10.1016/j.fcr.2025.109861.
54. Resurreccion, E.P., Roostaei, J., Martin, M.J., Maglinao, R.L., et al., 2021. The case for camelina-derived aviation biofuel: Sustainability underpinnings from a holistic assessment approach. Ind. Crops Prod. 170, 113777. https://doi.org/10.1016/j.indcrop.2021.113777.
55. Rodríguez-Rodríguez, M.F., Moreno-Pérez, A.J., Makni, S., Troncoso-Ponce, M.A., Acket, S., Thomasset, B., Sánchez, R., Venegas-Calerón, M., Garcés, R., Martínez-Force, E., Salas, J.J., 2021. Lipid profiling and oil properties of *Camelina sativa* seeds engineered to enhance saturated and omega-7 fatty acid production. Ind. Crops Prod. 170, 113765. https://doi.org/10.1016/j.indcrop.2021.113765.
56. Saha, A., Ghosh, S., Kumar, S., Meena, R.S., 2026. Crop diversification for enhancing carbon sequestration in cropping systems, in: Meena, R.S., Kumar, S., Pradhan, G., Jhariya, M.K., Srinivasarao, C. (Eds.), Carbon-Negative Agriculture. Springer, Singapore, pp. 109–136. https://doi.org/10.1007/978-981-95-1483-0_5.
57. Smychkovich, A., Glaze-Corcoran, S., Keiser, A., Hashemi, M., 2025. Root and shoot composition, decomposition, carbon contribution, and nitrogen mineralization of single and mixed cover crops. Field Crops Res. 327, 109902. https://doi.org/10.1016/j.fcr.2025.109902.
58. Song, Y., Sun, J., Li, Z., 2025. Intercropping increases land use efficiency and reduces soil salinity in salt-affected soils with minimal effect on pH. Field Crops Res. 332, 110028. https://doi.org/10.1016/j.fcr.2025.110028.

59. Stolarski, M.J., Krzyżaniak, M., Olba-Zięty, E., Stolarski, J., 2025. Energy efficiency of spring camelina production under organic sole-cropping and intercropping systems. Ind. Crops Prod. 228, 120909. https://doi.org/10.1016/j.indcrop.2025.120909.
60. Sun, L., Li, X., Shen, J., Wang, S., Xu, X., Fan, K., Wang, Y., Bi, C., Ding, Z., 2025. Optimizing tea plantations through rapeseed intercropping: Ecological and pest-resistant benefits. Ind. Crops Prod. 227, 120821. https://doi.org/10.1016/j.indcrop.2025.120821.
61. Terpinc, P., Schreiner, M., Abramovič, H., 2026. Kinetic and thermodynamic evaluation of the oxidative stability of camelina oil and its blends with olive oil using the Rancimat test. Food Chem. 501, 147571. https://doi.org/10.1016/j.foodchem.2025.147571.
62. Thers, H., Jensen, J.L., Rasmussen, J., Eriksen, J., 2024. Increasing cattle slurry application to grass–clover leys of different ages did not affect nitrate leaching but increased legacy effects in mixed organic crop rotations. Field Crops Res. 306, 109233. https://doi.org/10.1016/j.fcr.2023.109233.
63. Veljković, V.B., Kostić, M.D., Stamenković, O.S., 2022. Camelina seed harvesting, storing, pretreating, and processing to recover oil: A review. Ind. Crops Prod. 178, 114539. https://doi.org/10.1016/j.indcrop.2022.114539.
64. Wang, C., Peng, F., Chen, S., Zhao, Q., Gao, J., Zhang, G., Zhou, L., Shao, M., 2025. Nitrogen fertilizer fate and plant nitrogen uptake characteristics in a waxy sorghum–soybean intercropping system. Field Crops Res. 326, 109862. https://doi.org/10.1016/j.fcr.2025.109862.
65. Wu, J., Cao, Y., Chen, Z., Wu, F., 2025. Mixed *Triticum aestivum* and *Vicia sativa* cover crops with biofertilizers improve the growth of continuously cropped *Apium graveolens* by altering soil chemistry and rhizosphere bacterial communities. Ind. Crops Prod. 237, 122106. https://doi.org/10.1016/j.indcrop.2025.122106.
66. Xiao, J.-X., Zhu, Y.-A., Bai, W.-L., Liu, Z.-Y., Tang, L., Zheng, Y., 2021. Yield performance and optimal nitrogen and phosphorus application rates in wheat–faba bean intercropping. J. Integr. Agric. 20, 3012–3025. https://doi.org/10.1016/S2095-3119(20)63489-X.
67. Zanetti, F., Alberghini, B., Marjanović Jeromela, A., Grabowska, A., Monti, A., 2021. Camelina is an ancient oilseed crop actively contributing to the rural renaissance in Europe: A review. Agron. Sustain. Dev. 41, 2. https://doi.org/10.1007/s13593-020-00663-y.

Tables

Tables 1 to 7 are available in the supplementary files section

Figures

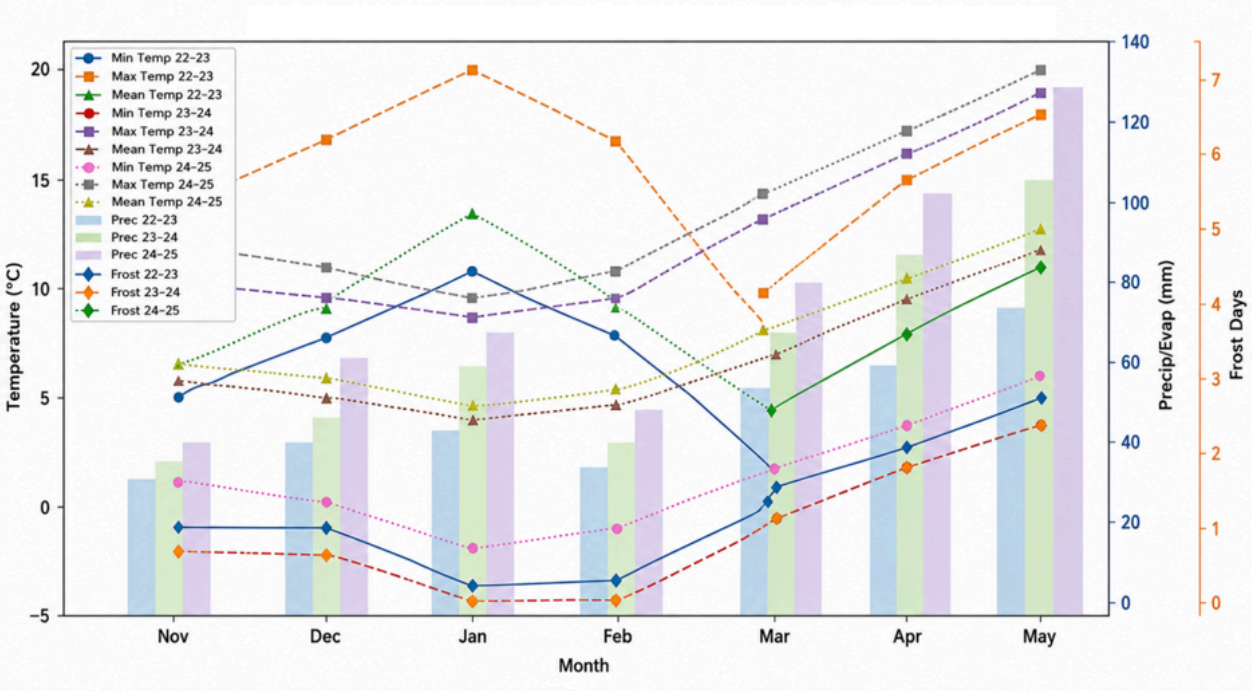


Figure 1

Monthly meteorological conditions, including minimum, maximum, and mean air temperature, precipitation, evaporation, and frost days, during the three Camelina growing seasons (2022–2023, 2023–2024, and 2024–2025).

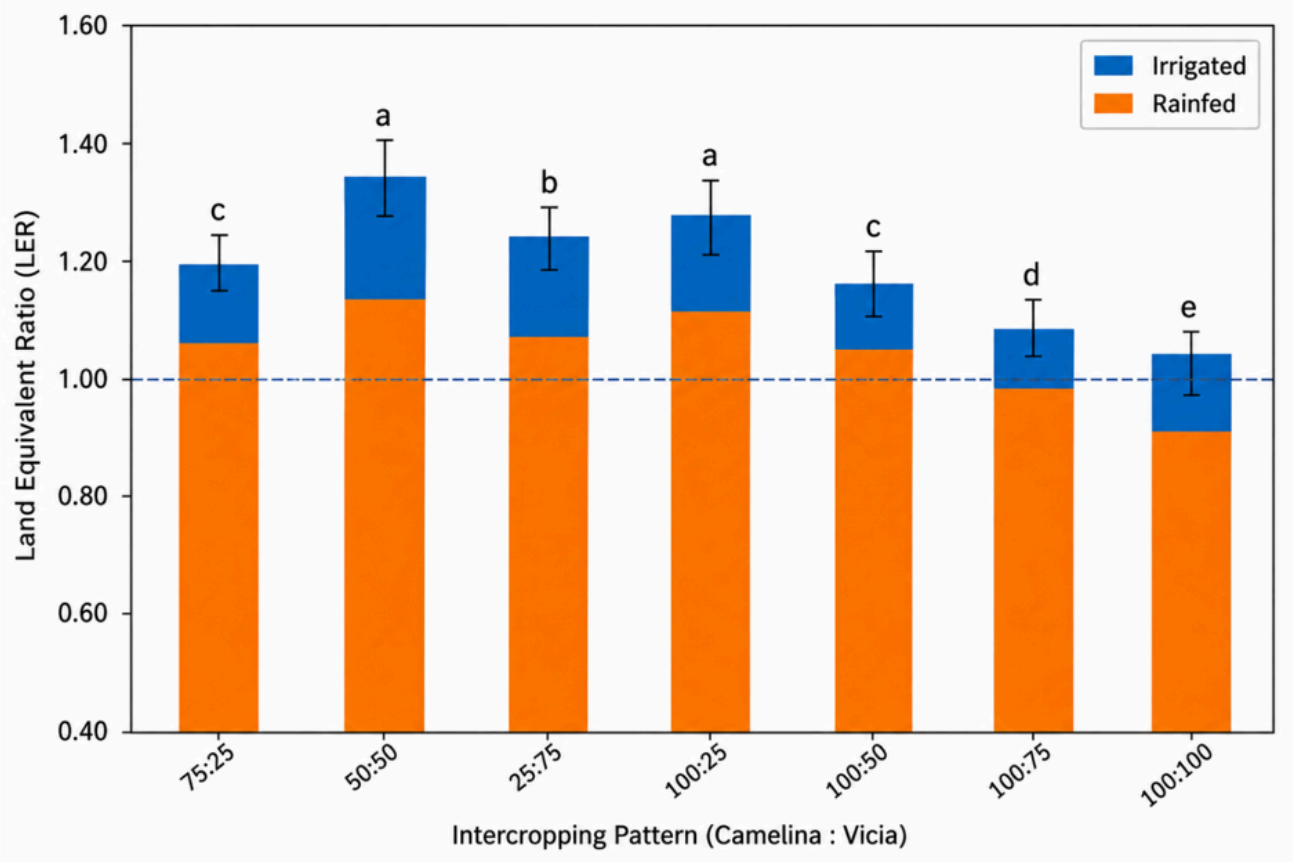


Figure 2

Land equivalent ratio (LER) of *Camelina sativa–Vicia faba* intercropping systems under irrigated and rainfed conditions. Bars represent mean LER values ± SE for different replacement and additive intercropping patterns. The horizontal dashed line at LER = 1 indicates the threshold at which intercropping equals sole cropping in land-use efficiency. Values above unity demonstrate a yield advantage. Data are means pooled across three growing seasons (2022–2025). Error bars represent the standard error of the mean.

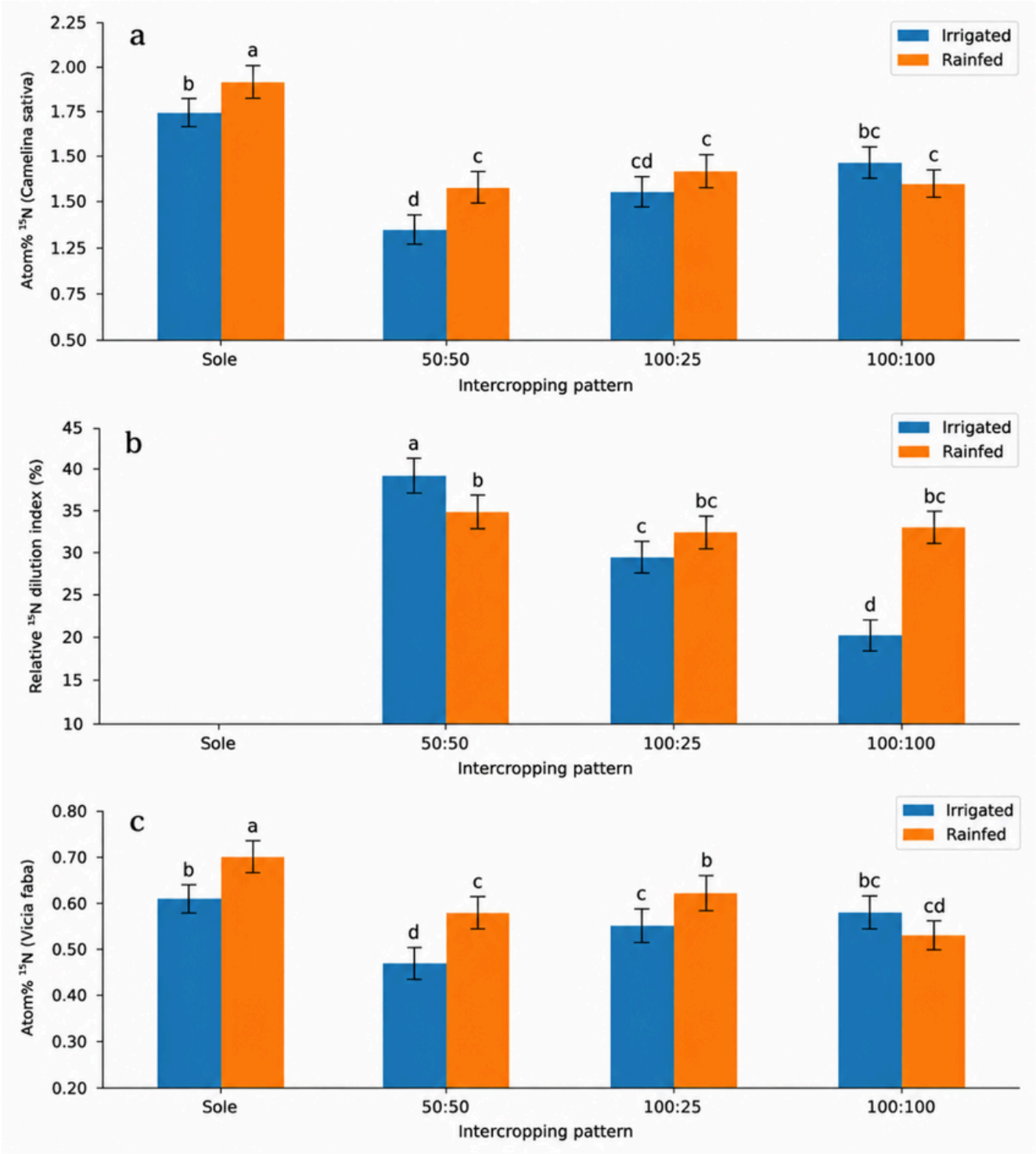


Figure 3

Atom% ¹⁵N enrichment and relative nitrogen dilution of *Camelina sativa* and *Vicia faba* under sole and intercropped conditions across contrasting irrigation regimes. (a) Atom% ¹⁵N enrichment of *Camelina sativa*. (b) Relative ¹⁵N dilution index (RDI) of *Camelina sativa*, calculated relative to sole cropping within each irrigation regime (RDI = 0 for sole Camelina by definition). (c) Atom% ¹⁵N enrichment of *Vicia faba*. Bars represent mean values ± SE measured at physiological maturity. Error bars represent the standard error of the mean.

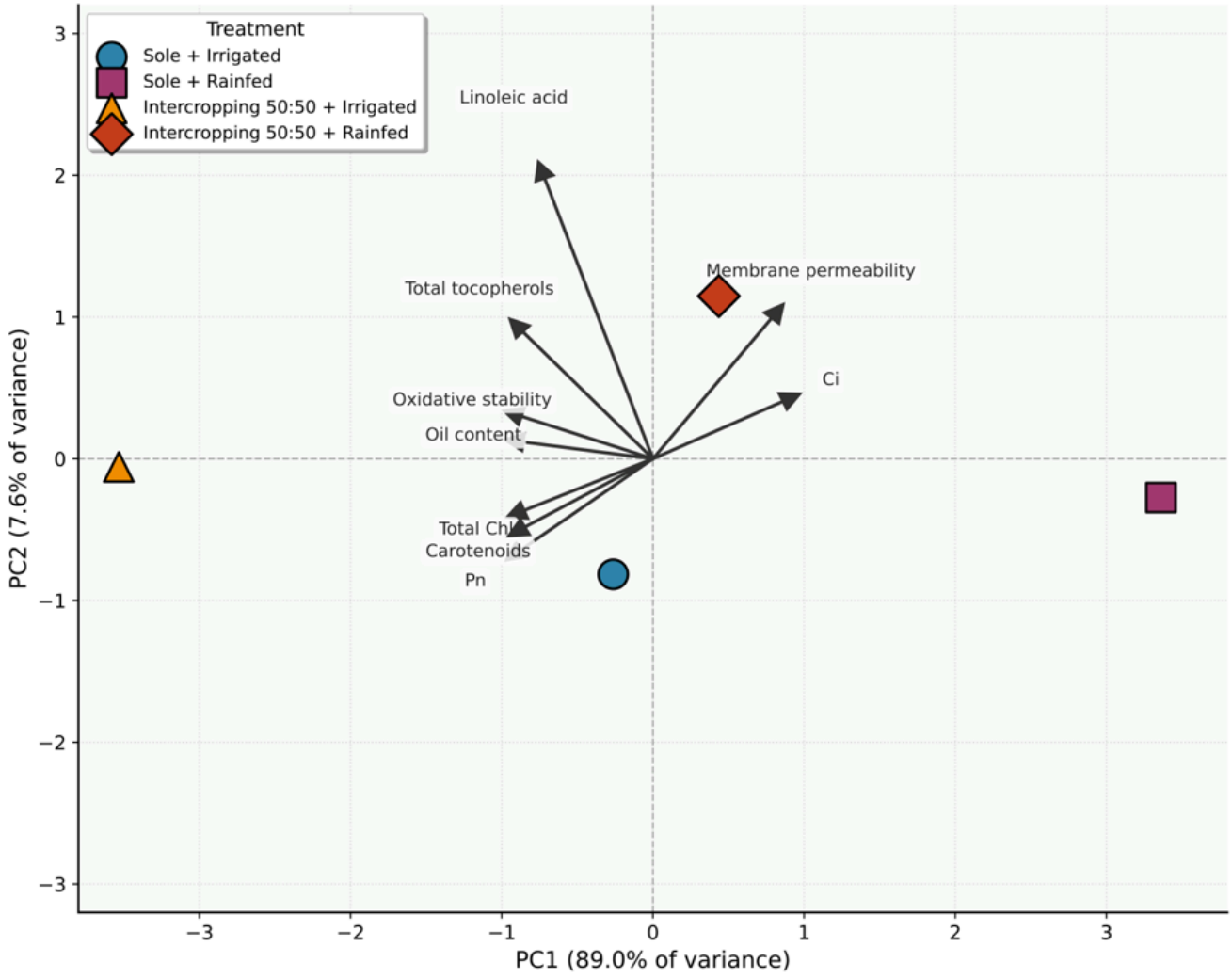


Figure 4

Principal component analysis (PCA) biplot showing relationships among physiological traits and oil quality parameters of *Camelina sativa* under different intercropping systems and water regimes. Points represent treatment centroids. Arrows indicate variable loadings. PC1 (89.0 %) primarily separates treatments along a gradient of physiological performance and oil quality, with the irrigated 50:50 replacement intercropping associated with higher tocopherols and oxidative stability. Data pooled across three growing seasons.

Supplementary Files

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