

Organ-Level Nutrient Partitioning in Legume Green Manures Grown under Calcareous Mediterranean Field

Meric BALCI^{1*} 

¹ Akdeniz University, Manavgat Vocational School, Department of Food Processing, Antalya

* Corresponding author: mericbalci@akdeniz.edu.tr

Abstract

Green manuring with legumes is widely used to enhance nutrient recycling in Mediterranean agroecosystems, especially under calcareous soil conditions where nutrient availability is commonly constrained. This study examined growth performance and organ level macro and micronutrient distribution in four legume species chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), forage pea (*Pisum sativum* L.), and vetch (*Vicia sativa* L.) grown as sole green manure crops under field conditions in a calcareous Mediterranean field soil. Growth parameters, nodulation traits, and nutrient concentrations were quantified in roots, stems, leaves, flowers, and nodules. Plant organ had a highly significant effect on all macro- and micronutrients analyzed, whereas species effects were limited and significant only for N, Fe, and B. Significant species × organ interactions were observed for Ca, Mg, Fe, Cu, Mn, and B, indicating context-dependent nutrient allocation among organs. Nitrogen was preferentially concentrated in nodules (measurable in chickpea and faba bean, but weak in forage pea and vetch) and flowers, consistent with symbiotic N fixation in nodules and high metabolic demand in reproductive tissues. In contrast, Fe and Mn were enriched primarily in roots and nodules and, for Mn, also in photosynthetically active leaves, whereas B was predominantly enriched in leaves and, in some species, roots under calcareous, alkaline soil conditions. Overall, the results indicate that organ-level nutrient partitioning is the primary determinant of nutrient distribution in legumes grown under calcareous Mediterranean conditions (soil pH=7.49), whereas species-specific effects are secondary and element-dependent. These findings show that organ composition and biomass distribution, together with soil constraints, should be considered when evaluating legume species for green manure use.

Research Article

Article History

Received	:05.12.2025
Accepted	:20.01.2026

Keywords

Mediterranean conditions
green manure
nutrient partitioning
calcareous soil
organ-level allocation

Citation: Balci, M., 2026. Organ-Level Nutrient Partitioning in Legume Green Manures Grown under Calcareous Mediterranean Field. *ISPEC Journal of Agricultural Sciences*, 10(1): 208-222.
DOI: <https://doi.org/10.5281/zenodo.18502528>

© This study was published by *ISPEC Journal of Agricultural Sciences* under a Creative Commons License (<https://creativecommons.org/licenses/by-nc/4.0/>).

1. Introduction

Green manuring is widely applied to support nutrient recycling and organic matter inputs in agricultural soils. Leguminous species are commonly preferred as green manure crops because of their ability to fix atmospheric nitrogen symbiotically and to accumulate both macro- and micronutrients during vegetative and reproductive growth stages (Dutta et al., 2022). The performance of legume-based green manures mainly depends on species-specific traits, including growth habit, biomass production, nodulation, and organ-level nutrient distribution at incorporation (Allito et al., 2021; Aydi Ben Abdallah et al., 2024). Previous studies show that nutrients in legumes are unevenly distributed among roots, nodules, stems, leaves, and reproductive organs. Metabolically active organs, particularly nodules and photosynthetically active tissues, tend to accumulate higher levels of nitrogen and certain mineral elements than structural tissues (Dovrat et al., 2020; Tang et al., 2024). In contrast, most research has concentrated on total aboveground biomass or single organs, and detailed organ-level analyses under field conditions, particularly those simultaneously considering multiple plant organs and nutrients, remain limited (Hansen et al., 2021). Mediterranean agroecosystems are generally characterized by calcareous soils with high lime content, neutral to slightly alkaline pH, and low organic matter (Durán et al., 2024). These conditions may alter nutrient uptake and internal allocation in legumes compared with non-calcareous soils, making organ-level nutrient partitioning a key factor in evaluating their suitability as green manure crops (Bolan et al., 2023; Stein et al., 2023). Chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), forage pea (*Pisum sativum* L.), and vetch (*Vicia sativa* L.) are widely cultivated across Mediterranean regions and are frequently used as sole green manure crops under field conditions (Yeremko et al., 2025). Their

contrasting growth habits, biomass allocation patterns, and nodulation traits allow robust comparisons of organ-specific nutrient distribution under comparable environmental conditions (Allito et al., 2021; de Freitas et al., 2023). Accordingly, this study aimed to evaluate growth traits and macro- and micronutrient distribution among plant organs in four legume species grown as green manure under field conditions in a calcareous Mediterranean soil. We hypothesized that (i) nutrient concentrations differ among organs and (ii) legume species exhibit distinct organ-level nutrient distribution patterns relevant to green manure use.

2. Material and Methods

2.1 Site preparation and growth conditions

2.1.1. Study area and field preparation

The experiment was carried out in a coastal area of Manavgat, Antalya (Türkiye) (36°48'30" N, 31°23'18" E), at an elevation of about 25 m above sea level. The field had been left fallow for four years with no cropping or chemical inputs. Before sowing, soil samples were taken from two layers (0–30 and 30–60 cm) to assess initial soil conditions, after which the field was ploughed and prepared using conventional tillage practices.

2.1.2. Initial soil characterization

Initial soil physicochemical and nutrient properties of the experimental field were determined prior to plot establishment, and the results are presented in Table 1. The soil had a clay loam texture and was non-saline, with neutral to slightly alkaline pH and very high lime content in both soil layers. Organic matter and total N levels were low throughout the soil profile, indicating limited native fertility. Available P and Zn contents were very low, whereas K and Ca levels ranged from medium to high. These characteristics describe the baseline soil conditions under which the field experiment was conducted.

Table 1. Initial physicochemical and nutrient properties of the soil before green manuring.

Parameter	Unit	0–30 cm	Classification	30–60 cm	Classification
Texture	–	Clay loam	Fine-textured soil	Clay loam	Fine-textured soil
Field capacity	%	58.89	High	55.37	High
Saturation (%)	%	44.22	–	42.24	–
pH	–	7.49	Neutral	7.75	Slightly alkaline
Total salt	%	0.01	Non-saline	0.01	Non-saline
Lime	%	45.92	Very calcareous	45.30	Very calcareous
Organic matter	%	1.11	Low	0.70	Very low
Total organic C	%	0.64	–	0.40	–
Total N	%	0.06	Low	0.05	Low
Extractable S	mg kg ⁻¹	11.35	–	11.35	–
Available Ca	mg kg ⁻¹	4893.90	High	4612.50	High
Available Mg	mg kg ⁻¹	168.80	Medium	187.00	Medium
Available P (P ₂ O ₅)	kg da ⁻¹	1.46	Very low	0.56	Very low
Available K (K ₂ O)	kg da ⁻¹	25.56	Medium	21.12	Medium
Available B	mg kg ⁻¹	0.34	Very low	0.30	Very low
Fe	mg kg ⁻¹	8.30	High	6.90	High
Cu	mg kg ⁻¹	1.80	Sufficient	1.08	Sufficient
Zn	mg kg ⁻¹	0.25	Low	0.15	Very low
Mn	mg kg ⁻¹	8.24	Low	6.80	Low
Available Mo	ppb	17.70	–	12.84	–
Available Co	mg kg ⁻¹	9.12	–	8.80	–

2.1.3. Meteorological conditions

Meteorological data covering the experimental period from December 2022 to October 2023 were obtained from the Turkish State Meteorological Service (Anonymous, 2024). The region has a typical Mediterranean climate, with mild and rainy winters and hot, dry summers. During the growing and decomposition periods, temperature and precipitation conditions were within the long-term regional averages, providing suitable climatic conditions for the growth of the studied legume species and subsequent green manure decomposition.

2.1.4. Crop management practices

No irrigation was applied during the experiment, as sowing was carried out in the winter season and crop water requirements were met by natural precipitation. No mineral or organic fertilizers were applied throughout the experimental period. Weed control was performed regularly using mechanical methods when necessary.

2.2. Experimental design and treatments

The experiment was arranged in a randomized complete block design with four plant species used as green manure—chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), forage pea (*Pisum sativum* L.), and vetch (*Vicia sativa* L.)—and three replications. Each species was randomly assigned within each block, yielding a total of 12 experimental plots. Plot size was 5 × 2 m, with buffer zones of 0.5 m between adjacent plots within a block and 1 m between blocks to minimize potential cross-plot interference. Sowing was carried out on 6 December 2022. Certified seeds of all species were obtained from a local seed producer. Plant species was the sole experimental factor.

2.3. Sampling and analytical procedures

2.3.1. Plant

At harvest (29 April 2023), plants from each plot were randomly sampled and combined into a composite sample. Before excavation, soil moisture was increased, and plants were removed by shovel and hand to limit root damage, with care taken to preserve roots and nodules. After sampling, plant materials were first washed with tap water and then with distilled water to remove adhering soil

particles. Samples were then separated into roots, fine roots, nodules, stems, leaves, and reproductive organs. Prior to drying, fresh weights were recorded for physical measurements. The plant samples were air-dried on absorbent paper and then oven-dried at 65 °C until constant weight. The dried samples were prepared for laboratory analyses. Plant samples were analyzed for total N, P, K, and selected macro- and micronutrients. Total N was determined using the Kjeldahl method (Bremner, 1965). Total P was measured colorimetrically using the vanadomolybdophosphoric yellow method (Murphy and Riley, 1962), and K was determined following standard procedures (Jackson, 2005). Macro- and micronutrient concentrations were determined after wet digestion and quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) following standard instrumental protocols (Senilä, 2024).

2.3.2. Soil

Immediately after harvest, the entire above- and belowground biomass of each species was returned to the soil in the same plots to minimize N losses. Incorporation was carried out manually, without mechanical mixing, leaving no plant residues on the soil surface. Biomass was buried within the effective soil fertility layer. After a decomposition period of about 7–8 months, soil samples were collected from each plot on 18 October 2023 for chemical analyses. Because the experimental plots were small (5 × 2 m), sampling was carried out from the plot center at a single depth (0–30 cm) to minimize edge effects and spatial variability. Soil samples were air-dried in the shade, gently crushed, and sieved through a 2-mm mesh prior to chemical analyses. Care was taken to avoid contamination during sampling and preparation, particularly for micronutrient analyses. Physicochemical and nutrient properties were determined using standard laboratory methods. Total N was measured by the Kjeldahl method (Bremner, 1965). Soil pH and electrical conductivity (EC) were measured potentiometrically in a 1:2.5 (w:v)

soil-to-water suspension. Soil organic matter and total organic carbon (TOC) were determined using the Walkley–Black wet oxidation method (Walkley and Black, 1934). Available phosphorus (P_2O_5) was determined using the Olsen method (Olsen, 1954). Exchangeable potassium (K_2O), Ca, and Mg were extracted with ammonium acetate and quantified by ICP-OES (Thomas, 1982). Available micronutrients (Fe, Cu, Zn, and Mn) were determined using DTPA extraction (Lindsay and Norvell, 1978). Soil texture was determined using the Bouyoucos hydrometer method (Bouyoucos, 1962), and saturation percentage was determined gravimetrically. Detailed soil physicochemical properties are presented in Table 1.

2.4. Statistical analysis

Statistical analyses were conducted using SPSS software. Data were analyzed by analysis of variance (ANOVA) under a randomized complete block design, with plant species and plant organ treated as fixed factors and their interaction (species × organ) included in the model. Statistical significance was assessed at $p \leq 0.05$.

3. Result

3.1 Growth and nodulation characteristics

Growth and nodulation characteristics of the four legume green manure species are presented in Table 2. Shoot dry matter showed marked variation among species, ranging from approximately 26 g plant⁻¹ in forage pea to about 452 g plant⁻¹ in faba bean, while chickpea and vetch had intermediate values. Root dry matter also varied among species, with the highest mean recorded in faba bean (≈ 19 g plant⁻¹); lower values were observed in forage pea and vetch (approximately 0.6–1.0 g plant⁻¹). These large interspecific differences reflect inherent differences in growth habit and biomass allocation among species. Plant height ranged from approximately 78 to 141 cm among species, with greater values in forage pea and vetch than in chickpea and faba bean. Root length and fine root length showed moderate interspecific variation, ranging from about 19 to 28 cm and from approximately 15

to 27 cm, respectively. Nodule number per plant ranged from approximately 12 to 24, with higher means in forage pea and vetch. Nodule dry weight was measured only in chickpea and faba bean and averaged approximately 6.4 and 5.4 g plant⁻¹, respectively. Nodules of forage pea and vetch did not reach sufficient mass for dry weight determination and were therefore

not included in nodule dry weight measurements. The growth and nodulation data presented in Table 2 are descriptive (mean $\pm$ SD) and were not subjected to formal statistical testing; therefore, observed differences among species are reported as trends rather than statistically significant effects.

Table 2. Growth and nodulation of 4 legume green manure species grown under field conditions (mean $\pm$ SD, n = 3).

Species	Shoot dry matter (g plant ⁻¹)	Root dry matter (g plant ⁻¹)	Plant height (cm)	Root length (cm)	Fine root length (cm)	Nodule number (plant ⁻¹)	Nodule dry weight (g plant ⁻¹)
Chickpea	231.00 $\pm$	5.01 $\pm$	78.50 $\pm$ 16.04	21.17 $\pm$	18.83 $\pm$ 1.26	20.32 $\pm$	6.35 $\pm$
	268.89	0.87		8.39		7.73	1.74
Faba bean	452.00 $\pm$	19.17 $\pm$	77.58 $\pm$ 15.97	27.83 $\pm$	27.42 $\pm$ 4.50	11.53 $\pm$	5.42 $\pm$
	108.39	3.55		6.66		0.50	0.52
Forage Pea	26.07 $\pm$ 10.39	0.60 $\pm$	140.56 $\pm$	19.08 $\pm$	15.08 $\pm$ 0.38	23.03 $\pm$	NA
		0.12	38.63	3.41		7.56	
Vetch	372.67 $\pm$ 32.46	1.00 $\pm$	114.78 $\pm$	23.83 $\pm$	20.56 $\pm$ 2.41	23.56 $\pm$	NA
		0.28	19.25	4.21		4.34	

3.2. Distribution of macronutrients among plant organs and species

The distribution of macronutrients among plant organs of the four legume green manure species is presented in Table 3. TOC values were relatively consistent across species and organs ($\approx$ 50–56%), while nodules exhibited consistently lower TOC values ($\approx$ 41–45%) in chickpea and faba bean. N concentrations varied markedly among organs and species. In chickpea and faba bean, nodules and flowers showed the highest N concentrations, with nodule N ranging from approximately 3.8 to 4.9% and flower N from approximately 3.6 to 4.2%. In forage pea and vetch, flowers also had the highest N concentrations ($\approx$ 4.7 and 4.9%, respectively), whereas root and stem N concentrations across all species generally remained at or below approximately 1.7%. P concentrations varied clearly among plant organs. Stem P concentrations remained below 0.10% across species, while root P concentrations ranged from approximately 0.05 to 0.14%. In contrast, leaves, nodules (when present), and flowers showed higher P concentrations, with the highest value found in forage pea flowers ($\approx$ 0.49%). K concentrations

showed moderate variation among species and organs ($\approx$ 0.7–2.0%). Higher K concentrations were commonly observed in nodules (where present), leaves, and flowers. Elevated K values were recorded in nodules of chickpea and faba bean and in flowers of forage pea, whereas stem K concentrations showed greater interspecific variability, ranging from approximately 0.7 to 1.9%. Ca concentrations ranged widely among plant organs and species ($\approx$ 0.15–3.56%). The highest Ca concentration was recorded in chickpea nodules ($>$ 3.5%). Elevated Ca concentrations were also found in leaves of faba bean and forage pea and in roots of forage pea, whereas stem and flower Ca concentrations were consistently lower across species. Under calcareous soil conditions characterized by high CaCO₃ content, Ca availability is generally high, and plant Ca distribution is therefore largely governed by limited phloem mobility and tissue-specific structural demand rather than uptake limitation, reinforcing accumulation in nodules, leaves, and roots. Mg concentrations showed relatively limited variation compared to other macronutrients, ranging from approximately 0.09 to 0.50%. Slightly higher

Mg concentrations were observed in nodules and leaves across species, whereas stem and flower Mg concentrations generally remained below approximately 0.20%. In forage pea and

vetch, nodule dry weight could not be determined due to insufficient nodule biomass, and therefore these values were excluded from further quantitative analyses.

Table 3. Distribution of macronutrients (TOC, N, P, K, Ca, Mg) among plant organs of 4 legume green manure species under field conditions (mean $\pm$ SD, n = 3)

Species	Organ	TOC (%)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Chick pea	Root	51.68 $\pm$ 0.57	0.87 $\pm$ 0.27	0.05 $\pm$ 0.015	1.02 $\pm$ 0.31	1.20 $\pm$ 0.58	0.27 $\pm$ 0.17
	Nodule	40.93 $\pm$ 7.06	4.88 $\pm$ 0.57	0.23 $\pm$ 0.066	1.95 $\pm$ 0.59	3.56 $\pm$ 0.97	0.50 $\pm$ 0.12
	Stem	55.08 $\pm$ 0.75	1.29 $\pm$ 0.54	0.09 $\pm$ 0.031	1.41 $\pm$ 0.47	0.58 $\pm$ 0.20	0.09 $\pm$ 0.04
	Leaf	52.13 $\pm$ 0.24	2.81 $\pm$ 0.28	0.17 $\pm$ 0.040	1.25 $\pm$ 0.32	2.44 $\pm$ 0.38	0.21 $\pm$ 0.03
Faba bean	Flower	55.70 $\pm$ 1.41	3.64 $\pm$ 0.44	0.36 $\pm$ 0.062	1.57 $\pm$ 0.41	0.30 $\pm$ 0.07	0.16 $\pm$ 0.02
	Root	51.87 $\pm$ 0.87	1.06 $\pm$ 0.10	0.07 $\pm$ 0.015	0.86 $\pm$ 0.24	1.26 $\pm$ 0.07	0.13 $\pm$ 0.01
	Nodule	44.85 $\pm$ 2.54	3.76 $\pm$ 0.57	0.26 $\pm$ 0.122	1.71 $\pm$ 0.79	2.12 $\pm$ 1.18	0.23 $\pm$ 0.04
	Stem	54.22 $\pm$ 0.35	0.99 $\pm$ 0.17	0.06 $\pm$ 0.010	0.73 $\pm$ 0.12	1.43 $\pm$ 0.44	0.14 $\pm$ 0.04
	Leaf	50.68 $\pm$ 0.90	2.50 $\pm$ 0.28	0.10 $\pm$ 0.021	1.24 $\pm$ 0.06	2.98 $\pm$ 0.54	0.34 $\pm$ 0.03
Forage Pea	Flower	55.75 $\pm$ 0.10	4.21 $\pm$ 0.34	0.32 $\pm$ 0.053	1.30 $\pm$ 0.06	0.15 $\pm$ 0.01	0.10 $\pm$ 0.01
	Root	50.13 $\pm$ 3.23	1.67 $\pm$ 0.07	0.14 $\pm$ 0.087	1.81 $\pm$ 1.02	2.50 $\pm$ 0.79	0.44 $\pm$ 0.25
	Stem	55.14 $\pm$ 0.63	1.17 $\pm$ 0.41	0.07 $\pm$ 0.015	1.43 $\pm$ 0.35	0.58 $\pm$ 0.11	0.10 $\pm$ 0.04
	Leaf	53.22 $\pm$ 1.65	3.24 $\pm$ 0.13	0.17 $\pm$ 0.044	1.45 $\pm$ 0.22	1.97 $\pm$ 0.24	0.30 $\pm$ 0.05
Vetch	Flower	54.47 $\pm$ 0.62	4.70 $\pm$ 0.34	0.49 $\pm$ 0.076	1.95 $\pm$ 0.21	0.36 $\pm$ 0.18	0.18 $\pm$ 0.01
	Root	52.65 $\pm$ 0.28	1.49 $\pm$ 0.16	0.09 $\pm$ 0.006	1.12 $\pm$ 0.21	1.36 $\pm$ 0.17	0.16 $\pm$ 0.00
	Stem	53.94 $\pm$ 0.44	1.48 $\pm$ 0.35	0.09 $\pm$ 0.023	1.88 $\pm$ 0.11	0.82 $\pm$ 0.12	0.15 $\pm$ 0.02
	Leaf	52.41 $\pm$ 0.47	3.32 $\pm$ 0.07	0.18 $\pm$ 0.015	1.75 $\pm$ 0.14	1.88 $\pm$ 0.29	0.24 $\pm$ 0.02
	Flower	54.40 $\pm$ 1.87	4.86 $\pm$ 1.40	0.37 $\pm$ 0.124	1.64 $\pm$ 0.42	0.42 $\pm$ 0.51	0.16 $\pm$ 0.03

3.3. Distribution of micronutrients among plant organs and species

The distribution of micronutrients (Fe, Cu, Zn, Mn and B) among plant organs of the four legume green manure species is presented in Table 4. Fe concentrations showed pronounced variation among both plant organs and species. Across species, the highest Fe concentrations were consistently observed in roots and nodules (where present), whereas stem, leaf, and flower Fe concentrations remained substantially lower. In chickpea and faba bean, nodule Fe concentrations ranged from approximately 2447 to 3743 mg kg⁻¹, whereas root Fe concentrations reached around 3569 mg kg⁻¹ in forage pea and 1921 mg kg⁻¹ in vetch. Fe concentrations in stems, leaves, and flowers were generally $\leq$ 450 mg kg⁻¹ across species. Accordingly, high Fe concentrations were mainly restricted to roots and nodules, with much lower levels in aboveground organs. Cu concentrations differed among species and organs. Root Cu concentrations were highest in forage pea ($\approx$ 17.4 mg kg⁻¹) and vetch ($\approx$ 14.7 mg kg⁻¹), whereas leaves and flowers of chickpea and faba bean also showed

elevated Cu levels ($\approx$ 6–9 mg kg⁻¹). Stem Cu concentrations remained low across species, generally below $\approx$ 3 mg kg⁻¹. Zn concentrations differed markedly among plant organs and peaked in forage pea flowers ($\approx$ 102.8 mg kg⁻¹). Elevated Zn concentrations were also found in vetch flowers and leaves ($\approx$ 33–40 mg kg⁻¹), leaves of faba bean and forage pea ($\approx$ 30–40 mg kg⁻¹), and nodules of chickpea and faba bean ($\approx$ 26–30 mg kg⁻¹), whereas stem Zn concentrations generally remained below $\approx$ 13 mg kg⁻¹ across species. Mn concentrations showed clear organ-specific patterns. The highest Mn concentration was observed in chickpea nodules ($\approx$ 80.6 mg kg⁻¹). Elevated Mn concentrations were also recorded in roots of forage pea ($\approx$ 46.9 mg kg⁻¹) and vetch ($\approx$ 40.4 mg kg⁻¹), as well as in leaves of each species ($\approx$ 32–47 mg kg⁻¹). In contrast, stem Mn concentrations generally remained below approximately 20 mg kg⁻¹, whereas flower Mn concentrations showed greater variability. B concentrations varied substantially among organs and species. The highest B concentrations were observed in chickpea leaves ($\approx$ 82.4 mg kg⁻¹) and in roots and leaves

of forage pea and vetch ($\approx 60\text{--}78 \text{ mg kg}^{-1}$). Moderate B concentrations were recorded in forage pea flowers ($\approx 56.8 \text{ mg kg}^{-1}$), whereas

stem and flower B concentrations in chickpea and faba bean generally remained below approximately 40 mg kg^{-1} .

Table 4. Distribution of micronutrients (Fe, Cu, Zn, Mn and B) among plant organs of four legume green manure species under field conditions (mean $\pm$ SD, $n = 3$).

Species	Organ	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})
Chick-pea	Root	1226.07 $\pm$ 601.78	5.93 $\pm$ 3.44	8.90 $\pm$ 4.34	22.73 $\pm$ 8.81	32.61 $\pm$ 14.03
	Nodule	3743.27 $\pm$ 949.48	3.96 $\pm$ 0.98	29.70 $\pm$ 15.67	80.58 $\pm$ 20.14	24.76 $\pm$ 2.77
	Stem	91.55 $\pm$ 40.98	1.98 $\pm$ 1.00	9.25 $\pm$ 4.14	8.92 $\pm$ 3.42	25.42 $\pm$ 8.28
	Leaf	329.21 $\pm$ 107.81	5.27 $\pm$ 1.50	17.46 $\pm$ 3.90	32.27 $\pm$ 7.23	82.40 $\pm$ 2.28
	Flower	181.41 $\pm$ 32.29	5.91 $\pm$ 2.54	36.84 $\pm$ 6.93	36.52 $\pm$ 7.67	14.70 $\pm$ 3.95
Faba bean	Root	1159.85 $\pm$ 81.76	4.95 $\pm$ 1.02	10.23 $\pm$ 2.02	27.72 $\pm$ 1.81	19.14 $\pm$ 4.77
	Nodule	2446.56 $\pm$ 1272.34	4.69 $\pm$ 2.25	26.64 $\pm$ 3.04	64.15 $\pm$ 14.30	32.29 $\pm$ 1.52
	Stem	149.62 $\pm$ 33.62	2.65 $\pm$ 1.52	11.62 $\pm$ 5.54	9.62 $\pm$ 1.19	22.89 $\pm$ 2.72
	Leaf	454.09 $\pm$ 260.64	6.60 $\pm$ 0.53	29.72 $\pm$ 6.14	46.87 $\pm$ 3.35	35.61 $\pm$ 2.37
	Flower	50.15 $\pm$ 6.03	8.91 $\pm$ 1.00	30.35 $\pm$ 3.52	9.57 $\pm$ 0.59	9.88 $\pm$ 1.01
Forage Pea	Root	3569.50 $\pm$ 718.88	17.39 $\pm$ 1.45	23.32 $\pm$ 4.28	46.87 $\pm$ 34.94	69.56 $\pm$ 37.58
	Stem	89.21 $\pm$ 33.94	2.31 $\pm$ 1.15	12.56 $\pm$ 5.50	8.92 $\pm$ 1.02	36.31 $\pm$ 5.34
	Leaf	407.15 $\pm$ 117.18	5.64 $\pm$ 1.52	39.50 $\pm$ 9.64	35.19 $\pm$ 2.78	65.10 $\pm$ 16.36
	Flower	130.96 $\pm$ 41.37	3.05 $\pm$ 1.28	102.75 $\pm$ 70.77	17.24 $\pm$ 2.96	56.78 $\pm$ 12.68
Vetch	Root	1921.05 $\pm$ 304.93	14.74 $\pm$ 6.29	16.03 $\pm$ 2.69	40.35 $\pm$ 4.07	77.18 $\pm$ 11.02
	Stem	117.94 $\pm$ 3.63	2.65 $\pm$ 0.58	12.59 $\pm$ 5.11	9.94 $\pm$ 1.71	25.17 $\pm$ 3.79
	Leaf	305.08 $\pm$ 148.38	4.30 $\pm$ 1.15	33.43 $\pm$ 8.27	33.43 $\pm$ 3.16	60.89 $\pm$ 14.84
	Flower	58.09 $\pm$ 1.49	6.86 $\pm$ 0.93	40.24 $\pm$ 9.48	18.66 $\pm$ 3.42	13.97 $\pm$ 10.59

Table 5. Summary of univariate ANOVA results for the effects of species, organ, block and species $\times$ organ interaction on measured chemical variables

Variable	Species (F,p)	Organ (F,p)	Species $\times$ Organ (F,p)	Block (F,p)
TOC	0.161, 0.919	20.045, <0.001	1.442, 0.233	0.710, 0.534
N	8.953, 0.012	172.282, <0.001	1.270, 0.310	1.873, 0.729
P	2.519, 0.155	108.096, <0.001	1.375, 0.260	0.995, 0.466
K	3.430, 0.093	4.610, 0.032	1.902, 0.106	2.191, 0.179
Ca	0.263, 0.850	43.673, <0.001	5.177, 0.001	0.455, 0.656
Mg	3.849, 0.075	18.094, <0.001	3.458, 0.009	1.807, 0.413
Fe	7.691, 0.018	91.103, <0.001	4.800, 0.001	2.553, 0.396
Cu	4.411, 0.058	15.858, 0.001	13.639, <0.001	1.563, 0.273
Zn	4.641, 0.053	11.875, 0.002	2.069, 0.080	1.409, 0.363
Mn	0.671, 0.601	21.554, <0.001	3.574, 0.007	0.536, 0.607
B	28.621, 0.001	28.615, <0.001	4.430, 0.002	–

3.4. Statistical effects of species and organ

Univariate ANOVA results indicated that organ effects were significant for all macro- and micronutrients analyzed (Table 5). Species effects were significant for N, Fe and B, whereas no significant species effect was detected for TOC, P, K, Ca, Mg, Cu, Zn or Mn. Significant species $\times$ organ interactions were observed for Ca, Mg, Fe, Cu, Mn and B, indicating that organ-level variation differed among species for these elements. In contrast,

interaction effects were not significant for TOC, N, P, K and Zn. Block effects were not significant for any variable, except that the block effect for boron could not be estimated due to negligible residual variance.

4. Discussion

4.1. Organ-level nutrient partitioning in a calcareous mediterranean soil

4.1.1 Macronutrients

This study demonstrated pronounced differences in macronutrient concentrations among plant organs across all four legume green manure species, confirming the first hypothesis. The strong and consistent organ-level responses observed for all macronutrients show that nutrient allocation within the plant is mainly governed by organ-specific functional demands rather than species identity (Tang et al., 2025; Zhao et al., 2020). Since external nutrient and water inputs were absent, these patterns are interpreted as reflecting inherent physiological allocation strategies associated with organ function. TOC was generally stable across most above- and belowground organs. However, nodules consistently showed lower TOC values in chickpea and faba bean. This is linked to the biochemical nature of nodules, which are richer in mineral and protein fractions associated with symbiotic N fixation than in structural C (Suliman and Tran, 2014; Zhang et al., 2025). Nitrogen showed clear organ-level differentiation. The highest concentrations occurred in nodules (where present) and flowers, whereas the lowest were consistently observed in roots and stems. In forage pea and vetch, where nodule dry weight could not be quantified, flowers and leaves functioned as the primary N-enriched organs. This distribution reflects the key role of nodules and reproductive tissues in N fixation, transport, and metabolic demand, whereas stems and roots primarily fulfill structural and transport functions (Ma et al., 2024; Melino et al., 2022). P differed clearly among plant organs across species. Low P concentrations were observed in stems, moderate levels in roots, and higher concentrations in leaves, nodules, and flowers, with flowers showing the highest values, especially in forage pea. This distribution reflects the preferential allocation of P to metabolically active tissues associated with energy transfer, nucleic acid synthesis, and reproductive development (Lambers, 2022). Potassium exhibited moderate yet distinct organ-level differences, with higher concentrations typically found in nodules (where present), leaves, and flowers, while stems showed lower concentrations despite some interspecific variation. This pattern is

consistent with the role of K in osmotic regulation, enzyme activation, and phloem transport, particularly in active and reproductive organs (Wegner et al., 2025). Ca varied more among plant organs than other macronutrients. The highest Ca levels were found in chickpea nodules, with higher concentrations also observed in leaves and roots depending on species. Stem and flower concentrations were consistently low. This is related to the low phloem mobility of Ca and its accumulation in tissues important for structural stability and cell wall development (Wdowiak et al., 2024). Under calcareous soil conditions characterized by high CaCO_3 content, Ca availability is generally high, and plant Ca distribution is therefore largely governed by limited phloem mobility and tissue-specific structural demand rather than uptake limitation, reinforcing accumulation in nodules, leaves, and roots. Mg varied within a narrower range than Ca and K but still showed clear organ-level differences. Higher Mg concentrations were generally found in nodules, leaves, and roots, whereas stems and flowers consistently showed lower levels. This pattern reflects the role of Mg in chlorophyll structure and enzyme activity, especially in photosynthetically active tissues (Ishfaq et al., 2022). Overall, the results show that macronutrient distribution under calcareous Mediterranean field conditions is primarily determined by organ identity. The consistent enrichment of nodules, leaves, and reproductive organs in N, P, K, Ca, and Mg emphasizes the role of internal nutrient partitioning in governing nutrient dynamics of legume green manures, regardless of species differences. Reports from studies conducted under contrasting environmental conditions show that organ-specific macronutrient distribution in legumes is a common feature, indicating that the relevance of these findings extends beyond the specific conditions of this study (Lambers, 2022; Zhao et al., 2020).

4.1.2. Micronutrients

Clear organ-level differentiation in micronutrients further indicates that internal nutrient regulation extends beyond

macronutrients (Table 4). Because the study was conducted under non-fertilized and non-irrigated conditions, these patterns are interpreted as reflecting intrinsic physiological allocation and internal regulation rather than short-term management effects. Fe concentrations were highest in roots and nodules where present. Leaves nonetheless showed relatively higher Fe levels than stems and flowers, indicating that Fe distribution is not confined to belowground or symbiotic tissues. This pattern reflects Fe retention in roots together with selective translocation to metabolically active aerial organs under calcareous conditions, where Fe availability is chemically constrained (Bhat et al., 2024; Ning et al., 2023). Comparable Fe accumulation patterns in roots and nodules have been reported for legumes grown on calcareous and alkaline soils, where restricted Fe mobility enhances tissue-specific retention rather than uniform distribution (Ning et al., 2023; Vélez-Bermúdez and Schmidt, 2023). Importantly, very high Fe concentrations were restricted to roots and nodules, while aboveground organs showed much lower levels, indicating organ-specific regulation rather than soil contamination. Mn showed clear organ-level specificity, but its distribution differed from that of Fe. Although nodules and roots contained high Mn concentrations in some species, leaves also represented an important Mn sink, especially in faba bean, forage pea, and vetch, indicating greater demand in photosynthetically active tissues rather than uniform accumulation in belowground organs (Alejandro et al., 2020). Such contrasting Fe–Mn allocation patterns highlight element-specific regulatory mechanisms operating within plant organs under calcareous soil conditions. Cu concentrations showed clear species- and organ-dependent patterns: they were highest in roots for forage pea and vetch, whereas in chickpea Cu concentrations were comparable between roots and flowers, and in faba bean the highest Cu concentrations were observed in flowers and leaves. This pattern suggests species-dependent differences in Cu uptake efficiency and internal redistribution rather than strict organ dominance, consistent

with known mechanisms of Cu transport and tissue-specific homeostasis (Wairich et al., 2022). Similar species- and organ-specific Cu allocation strategies have been reported in legumes grown under contrasting soil chemical constraints, supporting the broader relevance of these findings. Zn distribution among plant organs reflected differences in tissue-specific uptake and transport. Higher Zn concentrations were typically observed in metabolically active tissues, including leaves and reproductive organs, as well as in nodules, in agreement with reported Zn allocation patterns (Rasheed et al., 2024). Preferential Zn enrichment in reproductive and symbiotic tissues has been interpreted as an adaptive strategy that supports enzymatic activity and reproductive success under Zn-limiting conditions. B showed clear organ-level variation, with highest concentrations mainly in leaves and, in some species, roots. This distribution reflects the close link between B, cell wall structure, and actively growing tissues, and aligns with established concepts of B transport and homeostasis (Miwa and Fujiwara, 2010). Considering the very low available B content of the experimental soil (Table 1), these organ-specific patterns emphasize the role of internal allocation mechanisms in sustaining tissue-level B sufficiency (Fatnassi et al., 2022; Miwa and Fujiwara, 2010). These micronutrient patterns are especially relevant under the soil conditions of this study, characterized by very high CaCO_3 content (>45%), neutral to slightly alkaline pH, and low organic matter (Table 1). In calcareous Mediterranean soils, the availability of several nutrients—especially Fe, Zn, and B—is limited by chemical fixation and low solubility (Jamal et al., 2023; Martínez-Ríos et al., 2024; Vélez-Bermúdez and Schmidt, 2023; Vera-Maldonado et al., 2024). In this context, the pronounced organ-level effects observed for micronutrients suggest that functional internal regulation and selective organ allocation largely determine nutrient concentrations, even when soil availability is strongly constrained.

4.2. Species effects: limited but meaningful differentiation

Although organ effects were pronounced, species effects were limited and significant only for N, Fe, and B (Table 5), thus partially supporting the second hypothesis. Overall, the four legume species showed broadly similar nutrient allocation patterns, with species-dependent variation confined to a few elements (Li et al., 2022; Zhao et al., 2020). Species-level differences in N concentration were consistent with variation in growth and nodulation traits (Table 2) (Allito et al., 2021; Koutroubas et al., 2023). Clear interspecific differences were also evident in shoot and root dry matter: faba bean produced substantially higher above- and belowground biomass, forage pea showed very low biomass despite greater plant height, and chickpea and vetch had intermediate values. The low biomass observed in forage pea reflects a species-specific growth strategy marked by rapid vertical growth and reduced investment in structural biomass. Such a pattern is common in fast-growing legumes that favor early canopy formation and reproductive development over overall biomass accumulation under non-fertilized field conditions, rather than stress or experimental limitation (de Deus et al., 2022; Koutroubas et al., 2023). These contrasting growth strategies are critical for green manure systems because biomass production governs the return of organic matter and nutrients to the soil. In this context, forage pea acts as a more qualitative than quantitative green manure, where higher nutrient concentrations, rapid tissue turnover, and early decomposition may partly offset lower biomass, whereas faba bean contributes primarily through greater biomass production. Evidence from legume-based systems likewise shows that biomass quality and allocation influence N and P cycling beyond total biomass alone (Gao et al., 2024). Nodulation traits further explained species-dependent N dynamics: chickpea and faba bean, which produced measurable nodule biomass, showed higher N concentrations in nodules and reproductive organs, reflecting effective symbiotic fixation and internal allocation (Allito et al., 2021; Ma et al., 2024). In contrast, forage pea and vetch formed many

small nodules with insufficient mass for dry weight determination; therefore, comparisons of nodule-derived N were limited to chickpea and faba bean, emphasizing that species differences in N concentration should be interpreted in relation to measurable nodule biomass rather than nodule number alone (Iqbal et al., 2022; Koutroubas et al., 2023).

4.3. Species × organ interactions and context-dependent nutrient allocation

Significant species × organ interactions were detected for Ca, Mg, Fe, Cu, Mn, and B (Table 5), indicating that organ-level nutrient differentiation for these elements varied among species. Rather than indicating superior nutrient accumulation by any single species, these interactions point to context-dependent allocation patterns shaped by organ function and species-specific traits (Liu et al., 2024a; Wang et al., 2022). Across species, Fe concentrations were consistently highest in roots and nodules (where present), whereas Mn concentrations were highest in nodules and were elevated in roots or leaves depending on species. Variation in the degree of enrichment among species resulted in significant interaction effects. These patterns are consistent with the limited mobility of Fe and Mn and their preferential retention in root-associated and symbiotic tissues, particularly under high-pH, calcareous soil conditions where availability is chemically constrained (Alejandro et al., 2020; Ning et al., 2023; Vélez-Bermúdez and Schmidt, 2023; Saleem et al., 2023). Similarly, species × organ interactions for Ca and Mg likely reflect differences in tissue-specific structural demand and ion partitioning rather than differences in total nutrient uptake (de Bang et al., 2021; Wdowiak et al., 2024). These results indicate that nutrient distribution cannot be adequately described by species means alone and that organ-specific responses must be considered when evaluating nutrient dynamics in green manure systems, particularly for elements exhibiting significant interaction terms (Tou et al., 2025; Zhang et al., 2024). From a green manure perspective, nutrient return to the soil is therefore more strongly

influenced by the relative contribution of different plant organs than by species identity per se (Cai et al., 2025; Ji et al., 2022; Tripolskaja et al., 2023). Organs enriched in N and P (e.g., leaves, flowers, and nodules), together with micronutrient-specific hotspots (e.g., Fe in roots and nodules, Zn in leaves and flowers, and B in leaves or roots), are likely to play a disproportionate role in nutrient cycling following soil incorporation (Aydi Ben Abdallah et al., 2024; Cai et al., 2025). Because nutrient return is a function of both concentration and biomass proportion, organs with high nutrient density may contribute less to total nutrient inputs if their biomass fraction is small at incorporation. Accordingly, selecting legumes for green manure use should consider biomass production, growth traits, and adaptation to calcareous Mediterranean soils, as well as organ-level nutrient distribution (Attia et al., 2024; de Deus et al., 2022; Durán et al., 2024; Liu et al., 2024b).

5. Conclusion and Recommendations

Under the conditions of this field study, nutrient distribution in legume green manures grown on calcareous Mediterranean soils was governed mainly by plant organ rather than by species identity. Across the four species examined, internal nutrient partitioning repeatedly favored nodules, leaves, and reproductive tissues, indicating that organ composition plays a central role in determining nutrient return potential. Species-specific differences were observed for only a limited number of elements and were strongly element dependent, with their expression largely shaped by organ-level allocation. In this context, species identity alone provides an incomplete basis for evaluating green manure performance, and species effects are better interpreted through organ-specific responses than through whole-plant averages. From a practical agronomic perspective, these results suggest that green manure management should move beyond total biomass production. Biomass distribution and organ composition at the time of incorporation are also critical, as species producing lower biomass may still contribute to soil fertility when enriched in

rapidly decomposing, nutrient-dense organs, particularly in low-input systems without fertilization or irrigation. Although this experiment was conducted at a single site with limited replication ($n = 3$) over one growing season, and nodule dry weight could not be determined for forage pea and vetch due to insufficient biomass, the consistency of organ-level nutrient patterns across species

Funding

This study received no financial support.

Ethical Committee Approval

This study did not involve human or animal subjects; therefore, ethical approval was not required.

References

- Alejandro, S., Höller, S., Meier, B., Peiter, E., 2020. Manganese in plants: from acquisition to subcellular allocation. *Frontiers in Plant Science*, 11: 300.
- 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(1): 3678.
- Anonymous, 2024. Official climate statistics: General statistical data for Antalya province. Turkish State Meteorological Service, Ankara. <https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=Aandm=ANTALYA> (Accessed: 26.09.2025)
- Attia, A., Marohn, C., Shawon, A.R., de Kock, A., Strassmeyer, J., Feike, T., 2024. Do rotations with cover crops increase yield and soil organic carbon? A modeling study in southwest Germany. *Agriculture, Ecosystems and Environment*, 375: 109167.
- Aydi Ben Abdallah, R., Chaieb, N., Abdelkrim, S., Jabnoun-Khiareddine, H., Daami-Remadi, M., 2024. A three-year field experiment of faba bean var. minor effects on soil health and production of pepper grown under conventional farming

- system. *Soil Use and Management*, 40(4): e13157.
- Bhat, M.A., Mishra, A.K., Shah, S.N., Jan, S., Rahman, S., Jan, A.T., 2024. Soil and mineral nutrients in plant health: a prospective study of iron and phosphorus in the growth and development of plants. *Current Issues in Molecular Biology*, 46(6): 5194–5222.
- Bolan, N., Srivastava, P., Rao, C.S., Satyanaraya, P.V., Anderson, G.C., Bolan, S., Kirkham, M.B., 2023. Distribution, characteristics and management of calcareous soils. *Advances in Agronomy*, 182: 81–130.
- Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal*, 54(5): 464–465.
- Bremner, J.M., 1965. Nitrogen—total. In: Black, C.A. (Ed.), *Methods of Soil Analysis. Part II. Chemical and Microbiological Properties*, Vol. 9. American Society of Agronomy, Madison, pp. 595–624.
- Cai, M., Chen, H., Tan, H., Chen, J., He, S., Long, M., 2025. Temporal dynamics of nutrient release from mulching of legume roots and shoots litter driven by microbial community during decomposition in organic orchards. *BMC Plant Biology*, 25(1): 374.
- de Bang, T.C., Husted, S., Laursen, K.H., Persson, D.P., Schjoerring, J.K., 2021. The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. *New Phytologist*, 229(5): 2446–2469.
- de Deus, T.R.V., Giongo, V., Salviano, A.M., da Silva Santana, M., da Silva, V.C., dos Santos, T.C., 2022. Selection of green manures to provide ecosystem services in a semi-arid environment. *Revista Brasileira de Ciência do Solo*, 46: e0210105.
- de Freitas, I.C., Ferreira, E.A., Alves, M.A., de Oliveira, J.C., Frazão, L.A., 2023. Growth, nodulation, production, and physiology of leguminous plants in integrated production systems. *Agrosystems, Geosciences and Environment*, 6(1): e20343.
- Dovrat, G., Bakhshian, H., Masci, T., Sheffer, E., 2020. The nitrogen economic spectrum of legume stoichiometry and fixation strategy. *New Phytologist*, 227(2): 365–375.
- Durán, G.A., Sacristán, D., Farrús, E., Vadell, J., 2024. Towards defining soil quality of Mediterranean calcareous agricultural soils: reference values and potential core indicator set. *International Soil and Water Conservation Research*, 12(1): 145–155.
- Dutta, A., Trivedi, A., Nath, C.P., Gupta, D.S., Hazra, K.K., 2022. A comprehensive review on grain legumes as climate-smart crops: challenges and prospects. *Environmental Challenges*, 7: 100479.
- Fatnassi, S., Candel, A., Pedreño, J.N., Lucas, I.G., Hachicha, M., 2022. Boron adsorption in semiarid Mediterranean soils under the influence of background electrolytes. *Global Journal of Environmental Science and Management*, 8(4): 519–532.
- Gao, X., He, Y., Chen, Y., Wang, M., 2024. Leguminous green manure amendments improve maize yield by increasing N and P fertilizer use efficiency in yellow soil of the Yunnan–Guizhou Plateau. *Frontiers in Sustainable Food Systems*, 8: 1369571.
- Hansen, V., Eriksen, J., Jensen, L.S., Thorup-Kristensen, K., Magid, J., 2021. Towards integrated cover crop management: N, P and S release from aboveground and belowground residues. *Agriculture, Ecosystems and Environment*, 313: 107392.
- Iqbal, N., Sadras, V.O., Denison, R.F., Zhou, Y., Denton, M.D., 2022. Clade-dependent effects of drought on nitrogen fixation and its components: number, size and activity of nodules in legumes. *Field Crops Research*, 284: 108586.

- Ishfaq, M., Wang, Y., Yan, M., Wang, Z., Wu, L., Li, C., Li, X., 2022. Physiological essence of magnesium in plants and its widespread deficiency in the farming system of China. *Frontiers in Plant Science*, 13: 802274.
- Jackson, M.L., 2005. Soil chemical analysis: advanced course. A manual of methods useful for instruction and research in soil chemistry, physical chemistry of soils, soil fertility, and soil genesis. UW-Madison Libraries Parallel Press, Madison, WI.
- Jamal, A., Saeed, M.F., Mihoub, A., Hopkins, B.G., Ahmad, I., Naeem, A., 2023. Integrated use of phosphorus fertilizer and farmyard manure improves wheat productivity by improving soil quality and P availability in calcareous soil under subhumid conditions. *Frontiers in Plant Science*, 14: 1034421.
- Ji, D., Ding, F., Dijkstra, F.A., Jia, Z., Li, S., Wang, J., 2022. Crop residue decomposition and nutrient release are independently affected by nitrogen fertilization, plastic film mulching, and residue type. *European Journal of Agronomy*, 138: 126535.
- Koutroubas, S.D., Damalas, C.A., Fotiadis, S., Markopoulos, T., 2023. Species, cultivar and seasonal effects on nodulation and nitrogen utilization of spring Mediterranean grain legumes. *Journal of Soil Science and Plant Nutrition*, 23(3): 4463–4473.
- Lambers, H., 2022. Phosphorus acquisition and utilization in plants. *Annual Review of Plant Biology*, 73(1): 17–42.
- Li, L., Liu, K.H., Sheen, J., 2021. Dynamic nutrient signaling networks in plants. *Annual Review of Cell and Developmental Biology*, 37(1): 341–367.
- Li, X., Li, M., Xu, L., Liu, C., Zhao, W., Cheng, C., He, N., 2022. Allometry and distribution of nitrogen in natural plant communities of the Tibetan Plateau. *Frontiers in Plant Science*, 13: 845813.
- Lindsay, W.L., Norvell, W.A., 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42(3): 421–428.
- Liu, J.Y., Zhang, X.L., Jin, X.Y., Wang, M.T., Zhang, Y.Y., Wang, X.Y., 2024a. Nutrient allocation patterns in different aboveground organs at different reproductive stages of four introduced *Calligonum* species in a common garden in northwestern China. *Frontiers in Plant Science*, 15: 1504216.
- Liu, M., Hu, Z., Fan, Y., Hua, B., Yang, W., Pang, S., Zhang, X., 2024b. Effects of leguminous green manure–crop rotation on soil enzyme activity and stoichiometry. *Journal of Plant Ecology*, 17(6): rtac065.
- Ma, Y., Xiao, C., Liu, J., Ren, G., 2025. Nutrient-dependent regulation of symbiotic nitrogen fixation in legumes. *Horticulture Research*, 12(3): uhae321.
- Martínez-Ríos, O., Bravo-Vinaja, Á., San-Martín-Hernández, C., Hidalgo-Moreno, C.I., Sánchez-de-Jesús, M.A., Llampallas-Díaz, J.D., García-Preciado, J.C., 2024. Zinc deficiency in calcareous soils: a bibliometric analysis from 1989 to 2024. *Agriculture*, 14(12): 2285.
- Melino, V.J., Tester, M.A., Okamoto, M., 2022. Strategies for engineering improved nitrogen use efficiency in crop plants via redistribution and recycling of organic nitrogen. *Current Opinion in Biotechnology*, 73: 263–269.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27: 31–36.
- Miwa, K., Fujiwara, T., 2010. Boron transport in plants: co-ordinated regulation of transporters. *Annals of Botany*, 105(7): 1103–1108.
- Ning, X., Lin, M., Huang, G., Mao, J., Gao, Z., Wang, X., 2023. Research progress on iron absorption, transport, and molecular regulation strategy in plants. *Frontiers in Plant Science*, 14: 1190768.

- Olsen, S.R., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate (No. 939). US Department of Agriculture.
- Rasheed, N., Maqsood, M.A., Aziz, T., Ashraf, M.I., Saleem, I., Ehsan, S., Xu, M., 2024. Zinc partitioning and allocation patterns among various tissues confer variations in Zn use efficiency and bioavailability in lentil genotypes. *Frontiers in Plant Science*, 14: 1325370.
- Saleem, A., Zulfiqar, A., Saleem, M.Z., Ali, B., Saleem, M.H., Ali, S., Mostafa, R.M., 2023. Alkaline and acidic soil constraints on iron accumulation by rice cultivars in relation to several physio-biochemical parameters. *BMC Plant Biology*, 23(1): 397.
- Senilă, M., 2024. Recent advances in the determination of major and trace elements in plants using inductively coupled plasma optical emission spectrometry. *Molecules*, 29: 3169.
- Stein, S., Hartung, J., Perkons, U., Möller, K., Zikeli, S., 2023. Plant and soil N of different winter cover crops as green manure for subsequent organic white cabbage. *Nutrient Cycling in Agroecosystems*, 127(2): 285–298.
- Suliman, S., Tran, L.S.P., 2014. Symbiotic nitrogen fixation in legume nodules: metabolism and regulatory mechanisms. *International Journal of Molecular Sciences*, 15(11): 19389–19393.
- Tang, J., Li, W., Wei, T., Huang, R., Zeng, Z., 2024. Patterns and mechanisms of legume responses to nitrogen enrichment: a global meta-analysis. *Plants*, 13(22): 3244.
- Tang, X., Zhang, Y., Li, C., Zhi, X., Wang, C., 2025. Concentration-dependent NP interactions cause organ-specific responses and nutrient allocation in poplar seedlings. *Plants*, 14(19): 3037.
- Thomas, G.W., 1982. Exchangeable cations. In: *Methods of soil analysis: Part 2 chemical and microbiological properties*, 9: 159–165.
- Tou, C., Liu, X., Gan, J., Wanek, W., Jones, D.L., Wu, L., Ma, Q., 2025. Plant residue quality regulates its incorporation into soil aggregates and organic carbon stabilisation. *Geoderma*, 460: 117407.
- Tripolskaja, L., Kazlauskaite-Jadzevice, A., Razukas, A., 2023. Organic carbon, nitrogen accumulation and nitrogen leaching as affected by legume crop residues on sandy loam in the eastern Baltic region. *Plants*, 12(13): 2478.
- Vera-Maldonado, P., Aquea, F., Reyes-Díaz, M., Cárcamo-Fincheira, P., Soto-Cerda, B., Nunes-Nesi, A., Inostroza-Blancheteau, C., 2024. Role of boron and its interaction with other elements in plants. *Frontiers in Plant Science*, 15: 1332459.
- Vélez-Bermúdez, I.C., Schmidt, W., 2023. Plant strategies to mine iron from alkaline substrates. *Plant and Soil*, 483(1): 1–25.
- Wairich, A., De Conti, L., Lamb, T.I., Keil, R., Neves, L.O., Brunetto, G., Ricachenevsky, F.K., 2022. Throwing copper around: how plants control uptake, distribution, and accumulation of copper. *Agronomy*, 12(5): 994.
- Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37: 29–38.
- Wang, Z., Gong, H., Sardans, J., Zhou, Q., Deng, J., Niklas, K.J., Peñuelas, J., 2022. Divergent nitrogen and phosphorus allocation strategies in terrestrial plant leaves and fine roots: a global meta-analysis. *Journal of Ecology*, 110(11): 2745–2758.
- Wegner, L.H., Pottosin, I., Dreyer, I., Shabala, S., 2025. Potassium homeostasis and signalling: from the whole plant to the subcellular level. *Quantitative Plant Biology*, 6: e13.

- Wdowiak, A., Podgórska, A., Szal, B., 2024. Calcium in plants: an important element of cell physiology and structure, signaling, and stress responses. *Acta Physiologiae Plantarum*, 46(12): 108.
- Yeremko, L., Czopek, K., Staniak, M., Marenich, M., Hanhur, V., 2025. Role of environmental factors in legume–rhizobium symbiosis: a review. *Biomolecules*, 15(1): 118.
- Zhang, J., He, W., Wei, Z., Chen, Y., Gao, W., 2025. Integrating green manure and fertilizer reduction strategies to enhance soil carbon sequestration and crop yield: evidence from a two-season pot experiment. *Frontiers in Sustainable Food Systems*, 8: 1514409.
- Zhang, Z., Wang, J., Huang, W., Han, Y., Wang, G., Feng, L., Wang, Z., 2024. Respective advantages of growing different green manure with nitrogen fertilization in cotton-based cropping systems: insights from a three-year field study. *Food and Energy Security*, 13(6): e70015.
- Zhao, N., Yu, G., Wang, Q., Wang, R., Zhang, J., Liu, C., He, N., 2020. Conservative allocation strategy of multiple nutrients among major plant organs: from species to community. *Journal of Ecology*, 108(1): 267–278.