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Increased uptake and accumulation of phosphorus and other nutrients by legumes enhance their bioavailability for non-legume species

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Abstract: Legumes are promoted in agroecosystems for their ability to fix atmospheric nitrogen (N), thereby reducing or eliminating the need for N fertilisation while also contributing N-rich organic residues, which non-legume species can subsequently utilise. In phosphorus (P)-poor soils, certain legumes appear to access less available forms of P, converting them into organic P and facilitating its use by non-legume species. This study evaluated seven legume species/cultivars and one grass species (as a control) in a trial conducted in low-fertility soils under four different growing conditions (location × year). The objective was to investigate the role of legumes in P and other nutrient uptake and accumulation in plant tissues. Some lupins and broad beans accumulated up to 30 kg/ha of P in their biomass, even without accounting for P in the roots. Calcium (Ca) and magnesium (Mg) concentrations in plant tissues were also significantly higher in legumes than in grass. In addition to concentrating certain nutrients in their tissues, legumes produced substantially more biomass due to their access to atmospheric N, resulting in considerably higher nutrient accumulation. Ca and Mg in some legumes exceeded 100 and 40 kg/ha in aboveground biomass, respectively, whereas in grasses, they remained below 4 kg/ha. Thus, when legumes are cultivated as green manure, these nutrients are returned to the soil in organic form, which can subsequently become available to non-legume crops through the mineralisation process of the organic substrate. Therefore, cultivating legumes not only enhances N availability for other species but also improves the cycling of other essential nutrients.

Keywords: nutrient recovery; nutrient cycling; white lupin; yellow lupin; narrow-leaf lupin; macronutrient

The cultivation of legumes has been increasingly encouraged worldwide due to their symbiotic association with nitrogen (N)-fixing microorganisms. Biological N fixation represents a natural mechanism for introducing N into agroecosystems, thereby reducing reliance on industrially synthesised fertilisers (Weil and Brady 2017). Legumes can be integrated into agricultural systems through various approaches, including crop rotation, intercropping with non-leguminous species, serving as cover crops in perennial orchards, or using green manure incorporated into the soil.

Legumes contribute to subsequent crops in rotation systems by leaving behind organic residues rich in N. These residues gradually mineralise, enhancing nutrient availability for the following crops (Jensen et al. 2004, Mesfin et al. 2023). Additionally, studies have demonstrated that integrating legumes into intercropping systems facilitates N transfer to companion crops (Rodriguez et al. 2020, Homulle et al. 2022).

In perennial tree crops, interrow vegetation is often maintained using cover crops, and when legumes are included, they enhance soil N availability (Rodrigues et al. 2013, Amassaghrou et al. 2023). Using legumes as green manure is a targeted agronomic practice designed to supply N to the system. These legumes are cultivated exclusively for incorporation into the soil, without any commercial purpose, to enrich N availability for the subsequent cash crop (Li et al. 2020, Aguiar et al. 2024).

Legumes benefit not only from their ability to access atmospheric N but also from their capacity to utilise poorly soluble forms of phosphorus (P) in the soil (Tang et al. 2021, Kiær et al. 2022). This provides an additional advantage over non-leguminous species, particularly in P-deficient soils. P is a critical limiting factor in vast world regions, and crops often exhibit positive responses to P fertilisation (Roy 2017, Lambers 2022). However, phosphate rock reserves, the primary source of P fertilisers, are finite resources that may be significantly depleted within the 21st century (Roy 2017, Hawkesford et al. 2023). This impending scarcity highlights the challenges of ensuring a sustainable P supply for agricultural production in the near future.

Some legumes could access sparingly soluble P, thereby reducing their dependence on external P inputs. In P-deficient soils, species such as white lupin (*Lupinus albus* L.) have been observed to develop a variety of morphological, biochemical, and metabolic adaptations. Notably, the formation of cluster roots and the exudation of organic acids and phenolic compounds into the rhizosphere play a crucial role in mobilising P (Buoso et al. 2022, Lambers 2022). As a result, cultivating these species enhances P uptake from the soil. Once assimilated into plant tissues, this P remains within the system in organic form and can become available to subsequent crops as the plant debris decomposes by soil phosphatase enzymes (Cesco et al. 2010, Buoso et al. 2022).

The functioning of cluster roots and the modification of the rhizosphere microenvironment through the secretion of carboxylates and phenolic compounds can significantly influence the bioavailability of various nutrients, such as calcium (Ca), iron (Fe), and manganese (Mn), thereby enhancing their uptake (Cesco et al. 2010, Buoso et al. 2022, Lambers 2022). Legumes and dicot species, in general, tend to accumulate higher amounts of essential elements such as Ca, Mg, and boron (B) in their tissues than grasses (Bryson et al. 2014). Furthermore, the ability of legumes to access atmospheric N allows them to produce significantly greater biomass than non-N-fixing species, particularly in nutrient-poor soils (Rodrigues et al. 2013, Natera et al. 2023, Aguiar et al. 2024). Increased biomass production inherently leads to greater nutrient uptake, with these nutrients subsequently returning to the soil in organic form, thereby enhancing their availability to other plants.

The collateral effects of atmospheric N fixation and the resulting increase in biomass production by legumes on nutrient cycling provided the rationale for this study. The relatively limited emphasis on this aspect in the international literature represents a gap in current knowledge. Therefore, this research was based on four field trials conducted over three years, involving seven legume species/cultivars grown to be used as green manure. Additionally, a grass species, oats (*Avena sativa* L.), was included as a control. Data on dry matter yield (DMY) and N concentration in plant tissues have already been published. The present study focuses on assessing the potential benefits of legume cultivation for subsequent crops, incorporating data on the uptake of other essential macronutrients, P, potassium (K), Ca, and Mg, as well as micronutrients, including B, Fe, Mn, zinc (Zn), and copper (Cu).

MATERIAL AND METHODS

General experimental conditions. Four field trials were conducted: one in 2021/22, two in 2022/23, and one in 2023/24. Three of the trials took place in the municipality of Bragança (Br), but in three different plots, while one of the 2022/23 trials was conducted in the municipality of Mirandela (Mi). The cycle of these crops begins in autumn and ends in the following spring. However, from this point forward, each experiment is identified by the year it concluded. Thus, the four experiments referenced above were designated as Br2022, Br2023, Br2024, and Mi2023. All trials were established in the interrows of young olive orchards.

The climate of the Bragança region is typically Mediterranean, classified as Csb according to the Köppen classification (IPMA 2025). The annual average temperature is 12.6 °C, and the annual precipitation totals 773 mm. Mirandela also has a Mediterranean climate, but it is warmer and drier, classified as Csa. The annual average temperature and cumulative precipitation are 14.3 °C and 509 mm, respectively. Table 1 presents the climatological

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Table 1. Meteorological normals for Bragança and Mirandela, and monthly air temperature and precipitation records during the trial period

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Set	Oct	Nov	Dec
						Bragança						
Temperature (°C)												
Normal	4.5	6.2	9.2	10.7	14.0	18.8	21.7	21.6	18.4	13.1	8.3	5.5
2021									17.5	14.2	6.9	7.9
2022	4.6	7.6	8.9	10.2	17.1	19.2	24.8	24.1	17.6	15.2	9.1	8.2
2023	4.3	4.9	9.6	13.5	15.0	19.3	21.2	22.6	17.0	14.7	10.2	4.5
2024	6.3	8.1	8.6	11.8	13.4	18.4						
Rainfall (mm)												
Normal	92.8	64.2	53.5	65.2	65.0	35.4	15.4	17.4	47.7	102.2	92.4	121.6
2021									68.1	30.7	9.4	63.3
2022	8.6	9.4	49.6	45.6	27.6	12.2	14.6	6.0	19.6	98.6	89.8	191.8
2023	99.8	2.8	55.0	5.8	85.4	63.0	0.0	0.4	87.6	212.8	126.8	70.0
2024	107.0	86.2	122.2	57.4	35.6	85.8						
						Mirandela						
Temperature (°C)												
Normal	5.5	7.9	10.4	12.4	15.9	20.4	23.7	23.4	20.3	15.0	9.8	7.1
2022									20.1	16.7	10.5	10.5
2023	5.6	5.6	11.1	14.6	18.0	21.9						
Rainfall (mm)												
Normal	59.2	47.0	30.2	44.4	49.9	31.7	17.8	13.4	28.8	60.3	53.9	72.0
2022									42.8	107.2	70.4	111.6
2023	92.4	5.6	21.4	13.8	54.0	57.0						

normals for Bragança and Mirandela and the recorded monthly temperature and precipitation values observed during the trial periods in each location.

In the Br2022 and Mi2023 trials, the soils are classified as dystric Leptosols, whereas in the Br2023 and Br2024 trials, they are eutric Regosols (WRB 2022). Table 2 presents some soil properties at the beginning of the experimental setup.

Experimental designs and trial installation. All trials were organised in a completely randomised design with three replicates. Each experimental unit (plot) covered an area of 49 m^2, defined by the space surrounding four adjacent trees in two rows, following the olive orchards' 7 × 7 m planting grid.

Seven legume species/cultivars were sown: broad bean (*Vicia faba* L., cvs. Favel and Vesuvio), white lupin (*Lupinus albus* L., cv. Estoril), yellow lupin (*Lupinus luteus* L., cv. Nacional), narrow-leaf lupin (*Lupinus angustifolius* L., cv. Karo), crimson clover (*Trifolium incarnatum* L., cv. Diogene), and subterranean clover (*Trifolium subterraneum* L. ssp. *subterraneum* Katzn. and Morley, cv. Dalkeith). Additionally, a grass species, oat (*Avena sativa* L., cv. Boa Fé), was sown to contrast with the legumes and serve as a control.

The sowing rates were as follows: 300 kg/ha for broad bean (cv. Favel), 200 kg/ha for white lupin and broad bean (cv. Vesuvio), 180 kg/ha for yellow and narrow-leaf lupins, 120 kg/ha for oats, 25 kg/ha for subterranean clover, and 10 kg/ha for crimson clover. The sowing dates were October 10, 2021, November 11, 2022, November 12, 2022, and September 20, 2023, respectively, for the Br2022, Mi2023, Br2023, and Br2024 trials.

The soil was prepared using a cultivator before sowing. Larger seeds were incorporated into the soil at a depth of 8 cm using a cultivator, while clover seeds were sown at a depth of 5 cm using a smooth wooden bar.

Sample collection and laboratory analyses. Soil samples were collected from each plot at 0–20 cm depth to characterise the soil before trial establishment. Composite samples were collected by randomly sampling multiple points within each plot in three

Table 2. Soil properties (mean ± standard deviation) at the beginning of the experimental trials across different sites and years [Br2022 (41°39'21.7"N, 6°33'30.9"W), Mi2023 (41°30'50.8"N, 7°11'14.4"W), Br2023 (41°48'27.9"N, 6°43'59.9"W), Br2024 (41°48'28.1"N, 6°44'00.8"W)], based on composite soil samples (*n* = 3) collected at a depth of 0–20 cm

	Br2022	Mi2023	Br2023	Br2024
Clay (g/kg)[1]	121.3 ± 9.5	61.0 ± 7.9	145.3 ± 11.5	146.0 ± 10.0
Silt (g/kg)[1]	221.7 ± 21.2	173.3 ± 13.7	277.0 ± 16.8	292.3 ± 9.1
Sand (g/kg)[1]	657.0 ± 18.3	766.7 ± 20.1	577.7 ± 32.2	561.7 ± 24.7
Texture[2]	Sandy loam	Loamy sand	Sandy loam	Sandy loam
$\text{pH}_{\text{H}_2\text{O}}$[3]	4.9 ± 0.12	5.9 ± 0.11	5.8 ± 0.17	5.6 ± 0.32
Organic carbon (g/kg)[4]	3.1 ± 0.37	8.5 ± 0.59	27.4 ± 3.26	26.5 ± 2.42
Extr. phosphorus (mg/kg)[5]	6.2 ± 1.54	41.0 ± 4.18	38.9 ± 5.03	40.4 ± 4.62
Extr. potassium (mg/kg)[5]	98.8 ± 6.59	130.8 ± 14.53	81.0 ± 7.84	90.4 ± 9.14
Exch. calcium (cmol_+/kg)[6]	1.3 ± 0.31	3.9 ± 0.62	7.3 ± 0.20	6.6 ± 0.57
Exch. magnesium (cmol_+/kg)[6]	0.6 ± 0.16	0.7 ± 0.13	2.2 ± 0.35	2.4 ± 0.17
Exch. potassium (cmol_+/kg)[6]	0.3 ± 0.01	0.3 ± 0.05	0.2 ± 0.03	0.3 ± 0.10
Exch. sodium (cmol_+/kg)[6]	0.6 ± 0.02	0.8 ± 0.11	0.4 ± 0.05	0.4 ± 0.04
Exch. acidity (cmol_+/kg)[6]	1.2 ± 0.06	0.1 ± 0.02	0.1 ± 0.06	0.1 ± 0.06
CEC (cmol_+/kg)[7]	3.9 ± 0.48	5.6 ± 1.50	10.1 ± 0.14	9.7 ± 0.36
Extr. boron (mg/kg)[8]	0.05 ± 0.02	1.0 ± 0.20	0.5 ± 0.12	0.6 ± 0.10

[1]Robinson pipette method; [2](USDA, United States Department of Agriculture); [3]Potentiometry; [4]Wet digestion (Walkley-Black); [5]Egnér-Riehm; [6]Ammonium acetate; [7]Cation exchange capacity; [8]Hot-water, azomethine-H

replicates. The soil samples were air-dried at 40 °C and sieved through a 2-mm mesh before analysis.

The following parameters were analysed: texture (Robinson pipette method), organic carbon content (wet digestion, Walkley-Black method), $\text{pH}_{\text{H}_2\text{O}}$ (soil:solution ratio of 1:2.5), extractable P and K contents (ammonium lactate extract, Egner-Riehm method), extractable B (Hot-water, azomethine-H), exchangeable bases, and cation exchange capacity (ammonium acetate, pH 7.0). Further details on all these analytical methods can be found in van Reeuwijk (2002).

Biomass samples were collected regularly from the end of winter until the plants reached full bloom. In 2022 and 2024, four vegetation cuts were made, while in 2023, only three cuts were performed. A 50 cm square grid was used, with the biomass collected corresponding to samples of 0.25 m^2. The sampling was conducted in the central part of each individual plot (experimental unit) corresponding to the three replicates of each treatment. The plants were cut using an electric shear at a height less than 5 cm from the soil surface. The samples were initially weighed fresh. From each sample, a subsample of approximately 300 to 400 g was collected and reweighed while still fresh.

In the laboratory, the subsamples were dried in a forced-air oven at 70 °C and subsequently weighed dry. This allows the results to be expressed as DMY per hectare. Thereafter, the subsamples were ground to pass through a 1-mm mesh and digested with nitric acid using microwave-assisted digestion. In these plant tissue samples, the concentration of P was determined by colourimetry using the blue ammonium molybdate method with ascorbic acid as a reducing agent. B was also determined by colourimetry using the azomethine-H method. After extraction with ammonium acetate and EDTA, the concentrations of K, Ca, Mg, Fe, Mn, Cu, and Zn were measured by atomic absorption spectrophotometry. A more detailed description of all these methodologies can be found in Temminghoff and Houba (2004).

Data analysis. Results were tested for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett's tests, respectively. Considering that four trials were conducted across different site/year combinations and 14 harvests carried out across these trials, and 21 response variables were generated (1 for DMY, 10 for nutrient concentration and 10 for nutrient recovery), a total of 294 ANOVAs were performed. However, the application of analysis of variance (one-way ANOVA) to the results was not particularly useful, as the differences in DMY, nutrient concentrations in the tissues and nutrient recovery of the different species/cultivars were always highly significant ($P < 0.001$). Therefore, the results were presented graphically, using means and standard deviations, along with the evolution of the measured variables over time. On the *X*-axis, the time variable was presented as days after sowing, expressing it as a continuous variable rather than a discrete one associated with specific biomass collection dates. This method of presenting the results facilitates their interpretation by the reader, as fixing the same value on the *Y*-axis provides a reference point for comparing the values of different species/cultivars over time.

RESULTS

An overall increase in DMY was observed over time in most species/cultivars, consistent with their phenological development (Figure 1). Nonetheless, values

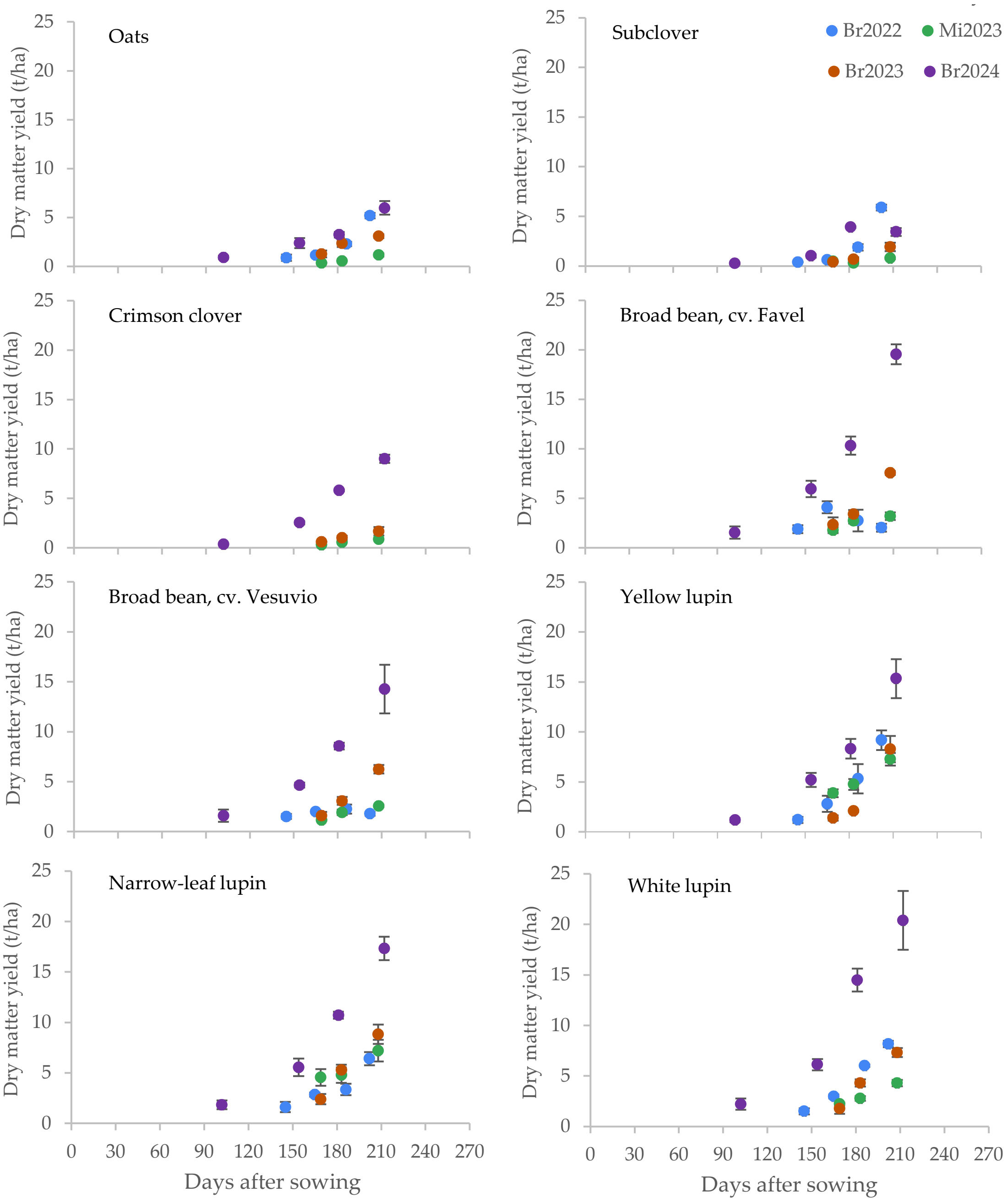

Figure 1. Dry matter yield in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation ($n = 3$)

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varied markedly depending on the species/cultivar and the specific location/year combination. The Br2024 combination resulted in the highest DMYs across all legume species. Conversely, some legumes recorded their lowest yields at the final sampling under the Mi2023 and Br2022 conditions, likely due to increased sensitivity to drought and soil acidity, respectively. Oats consistently exhibited lower DMYs than the legumes, except in the case of clovers, where yields were of a similar magnitude.

For most location/year combinations and species/cultivars, a decreasing P concentration trend in plant tissues was observed over time (Figure 2). It is also evident that location consistently influenced tissue P concentration. For instance, the Mi2023 combination increased P concentration in tissues compared to Br2022. However, the effect of species was less pronounced. P levels were close to or slightly below 2 g/kg in the final sampling dates.

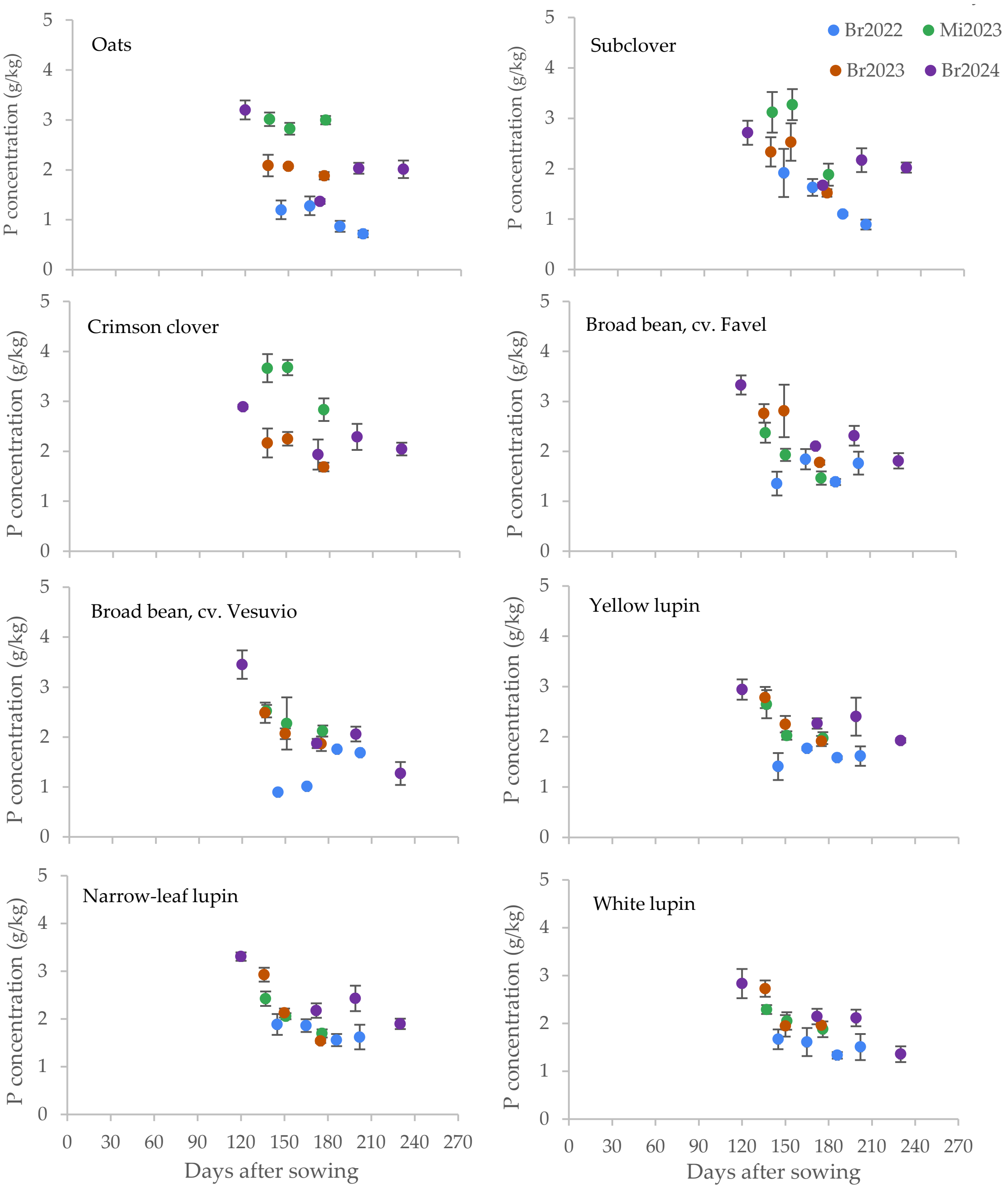

Figure 2. Phosphorus (P) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (*n* = 3)

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A rising trend in P recovered in aerial biomass was observed over time, regardless of the cultivated species (Figure 3). Clear differences between locations were also evident within each species. For example, in broad bean (cv. Favel), P recovery reached 35 kg/ha at Br2024 in the final harvest, whereas only 3 kg/ha was recorded at Br2022. Substantial differences were also observed between species; oats and subclover generally exhibited values below 5 kg/ha, except at Br2024. In contrast, yellow lupin showed P recovery levels close to 15 kg/ha, reaching approximately 30 kg/ha at location Br2024.

Plant tissue K concentration decreased over time, with a marked decline in the final harvest, regardless of location/year and species (Figure 4). While consistent temporal differences were observed, variations between species were less pronounced. In the final harvests, tissue K levels generally ranged between 5 and 15 g/kg.

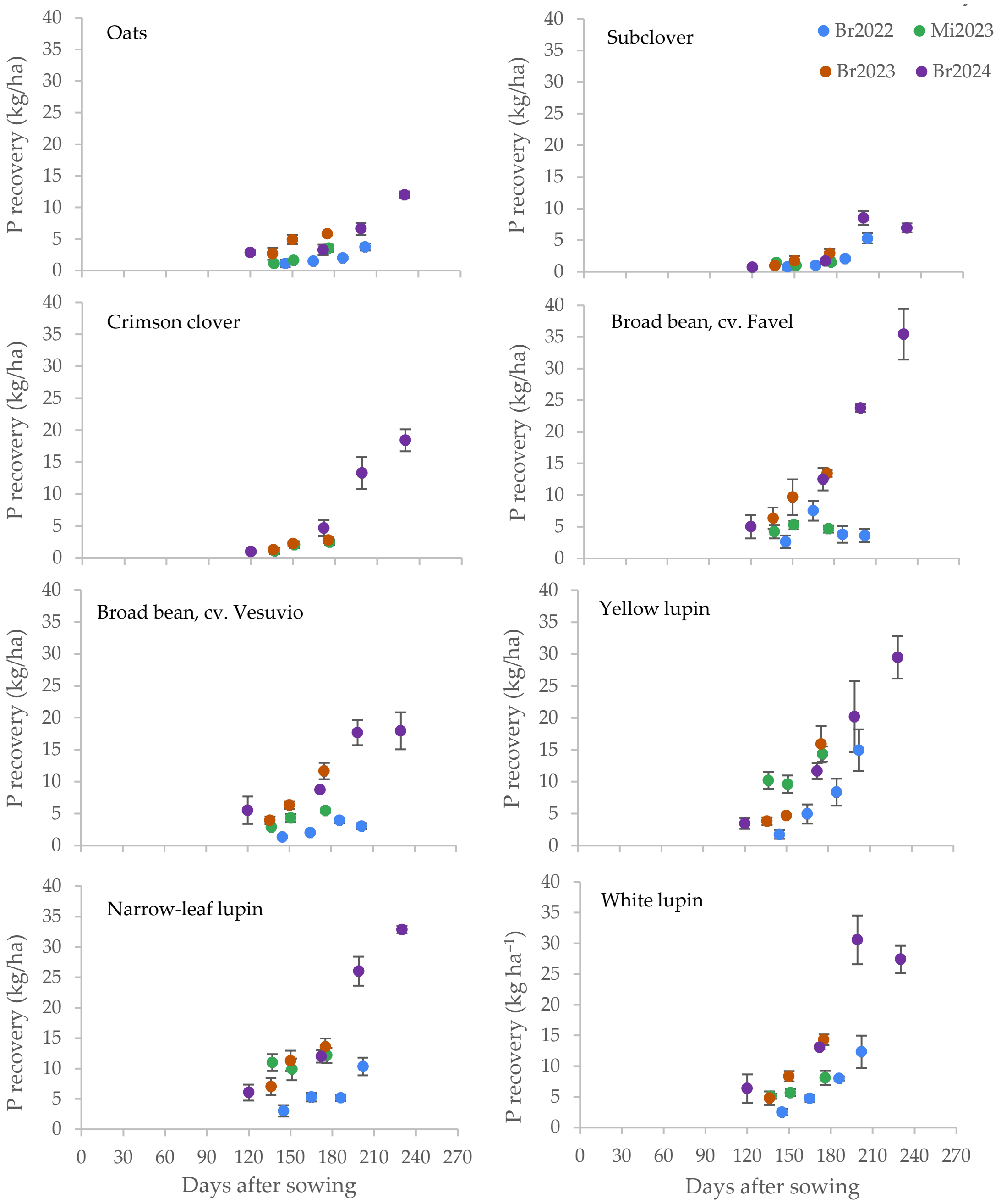

Figure 3. Phosphorus (P) recovery in the aboveground biomass of eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (*n* = 3)

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The Ca (Figure 5) and Mg (Figure 6) concentrations in plant tissues did not exhibit a consistent temporal pattern. However, substantial differences were observed across location/year combinations. The Br2023 combination generally showed higher Ca and Mg levels in tissues, while Br2022 tended to have lower values. Notable differences were also found between species, with grass displaying lower concentrations. Among legumes, white and yellow lupin stood out for having comparatively lower Ca and Mg levels than the other species.

There is no clear trend in the evolution of B concentration in plant tissues over time, with both increasing and decreasing patterns observed depending on the location/year combination and species (Figure 7). However, there were significant differences among location/year combinations, with Mi2023 exhibiting higher B concentrations than the others. Among

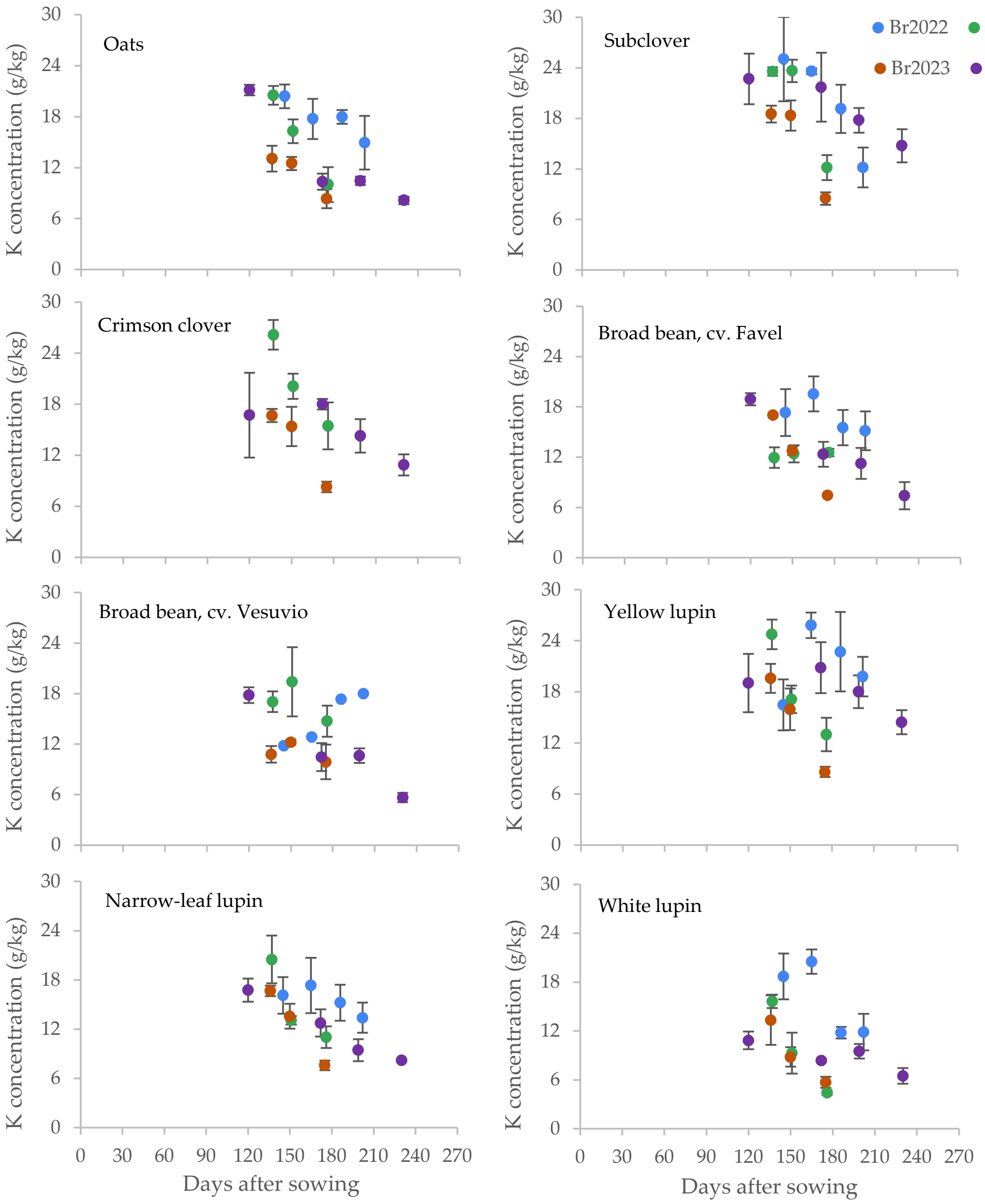

Figure 4. Potassium (K) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (n = 3)

species, the monocot oats consistently showed lower B levels.

In the case of Fe, no clear increasing or decreasing trend over time was observed, nor was there a consistent pattern among species (Figure 8). The most notable aspect was the consistent differences across location/year conditions. The Br2022 combination tended to exhibit higher Fe concentrations.

The concentration of Mn in plant tissues did not follow a consistently increasing or decreasing trend over time, seemingly influenced by multiple interactions with location/year and species/cultivar (Figure 9). Location/year had a pronounced effect, with the Br2022 combination generally associated with higher Mn levels, except for the consistent exception of white lupin. Notable differences were observed among species, with lupins, particularly narrow-leaf and

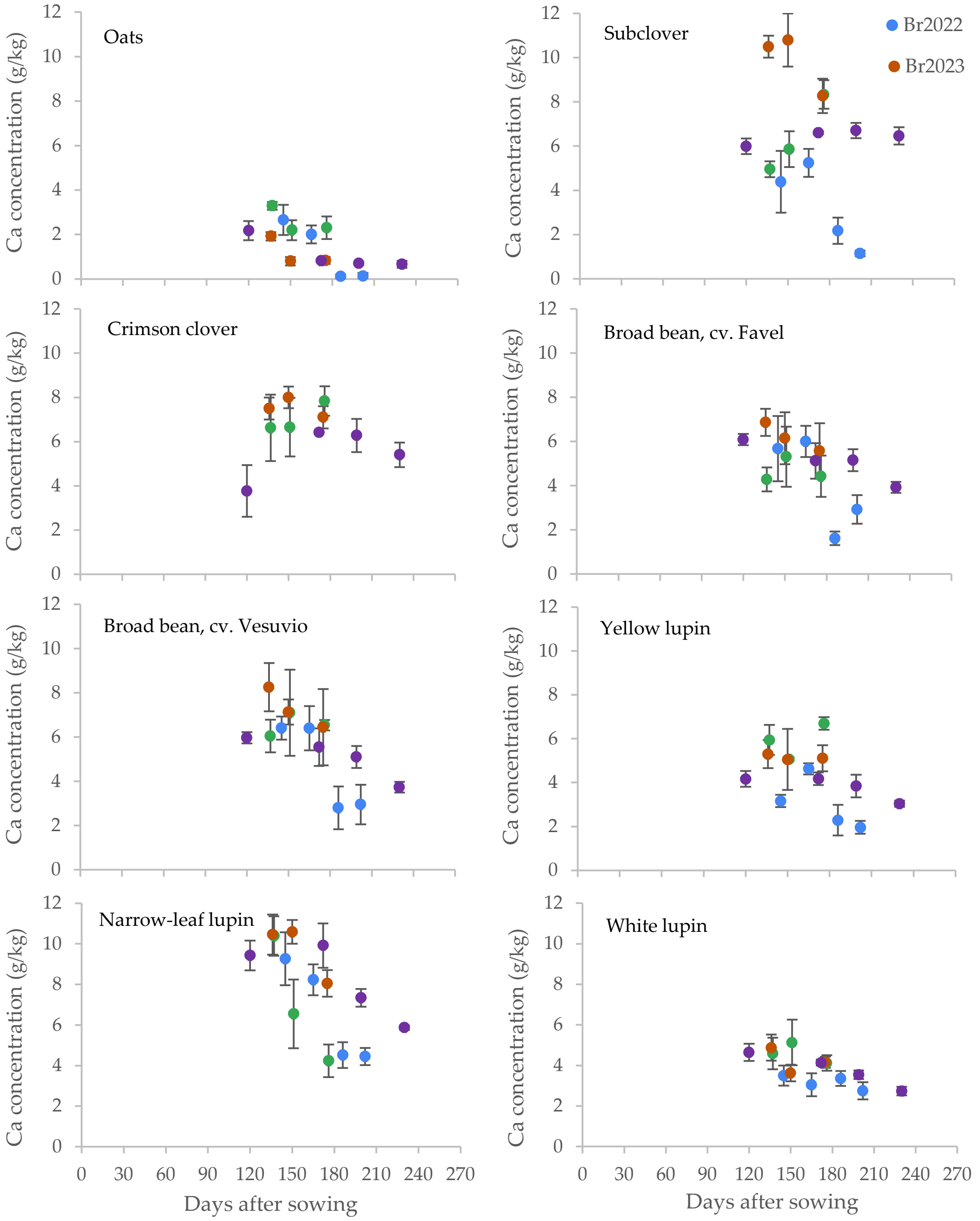

Figure 5. Calcium (Ca) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (*n* = 3)

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white lupins, showing significantly higher Mn concentrations than other species/cultivars.

The concentrations of Zn and Cu in plant tissues did not exhibit a consistent trend over time (data not shown). Similarly, the effect of location/year on these elements was not highly consistent, although Br2022 tended to show higher values, as observed for Fe and Mn. Among species, oats generally had lower Zn and Cu concentrations than legumes.

The results of nutrient accumulation in above-ground biomass revealed that the differences between legumes and grasses became even more pronounced, highlighting the combined effect of higher nutrient concentrations in dry matter and greater biomass production (Figure 10). For Ca and Mg, the average values in the grass species remained below 4 kg/ha, whereas narrow-leaf lupin recorded values exceeding 100 and 40 kg/ha, respectively. Similarly, the differ-

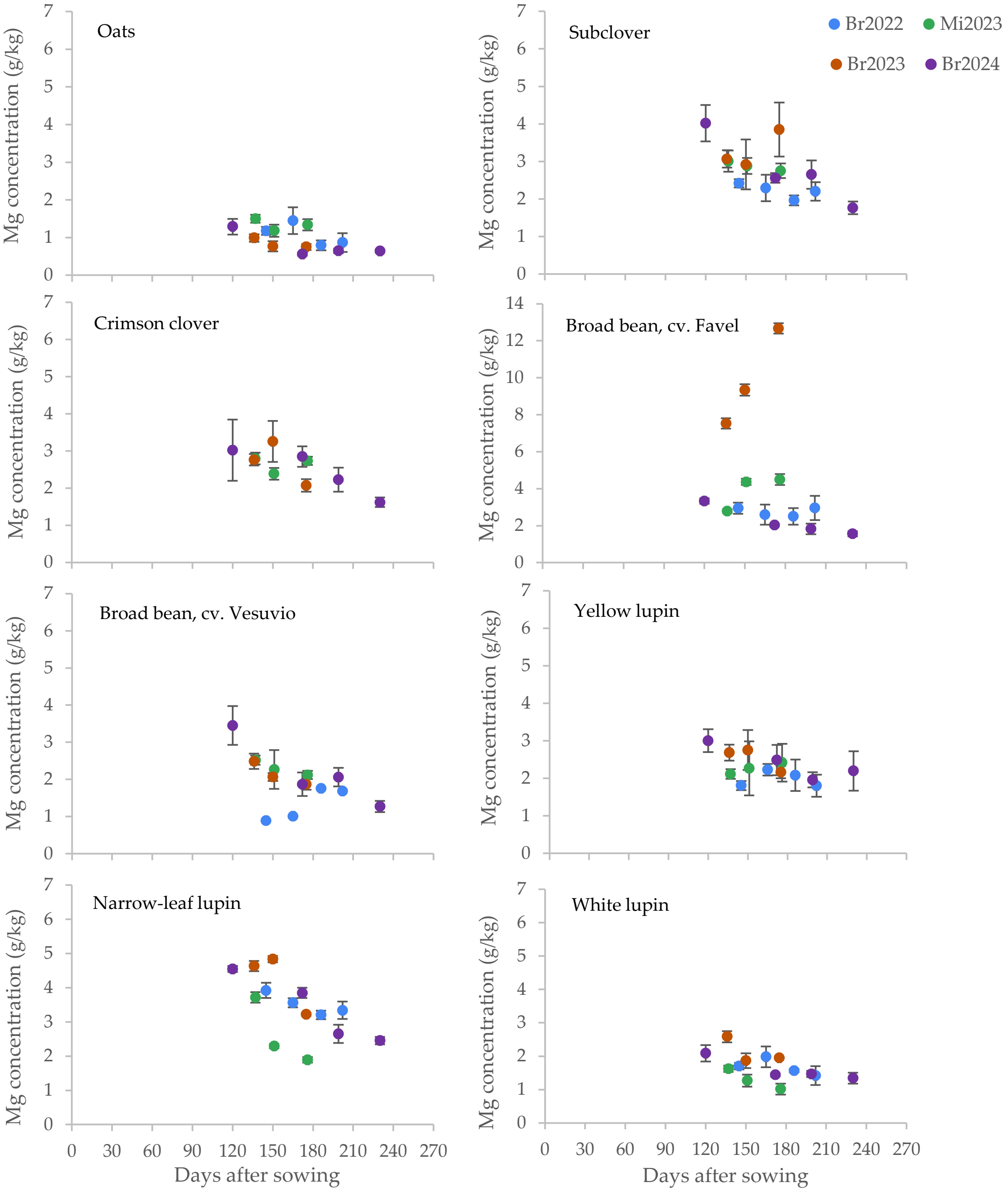

Figure 6. Magnesium (Mg) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (n = 3)

ences were striking for B, with average values in grass species remaining below 40 g/ha, while narrow-leaf lupin exhibited average values surpassing 240 g/ha.

DISCUSSION

Phosphorus uptake and return to the soil by legumes. A slight decreasing trend in P concentration in plant tissues was observed throughout the growing seasons. A dilution or concentration effect of a nutrient in plant tissues occurs when the rate of nutrient uptake is lower or higher than the accumulation of dry matter, respectively (Bell 2023). In this case, the declining trend was likely due to a relative increase in the proportion of structural components, such as cell walls and lignin, which have lower P concentrations (Bryson et al. 2014, Bell 2023). However, the results do not indicate a pronounced P dilution

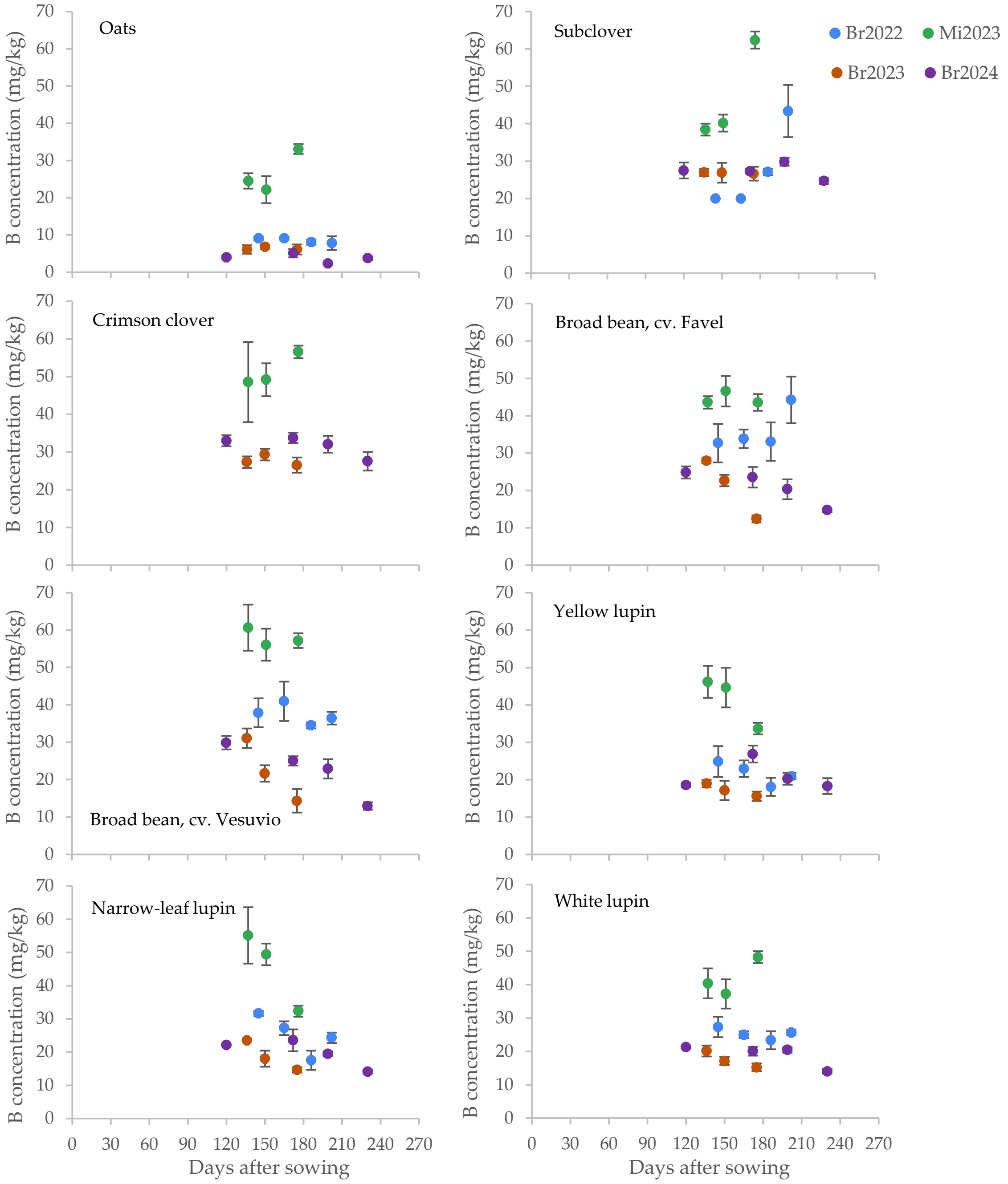

Figure 7. Boron (B) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (n = 3)

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effect with increasing biomass, suggesting that the plant continued to uptake P until the final harvest.

The effect of the plot on P concentration in plant tissues was notable. In Br2022, lower P concentrations were generally observed, whereas in Mi2023, the values tended to be higher. The soil at Br2022 is particularly acidic, with very low P levels, as determined by the Egner-Riehm method. P is less available to plants in highly acidic soils due to its reaction with Fe and Al oxides, leading to precipitation as $AlPO_4$ and $FePO_4$ (Havlin et al. 2017). This process reduces P availability for root uptake, which explains the observed values.

The effect of species on P concentration in plant tissues was less pronounced. In general, tissue P concentrations ranged between 1 and 3 g/kg (Bryson et al. 2014). However, it was evident that legumes (except for subterranean clover) tended to exhibit

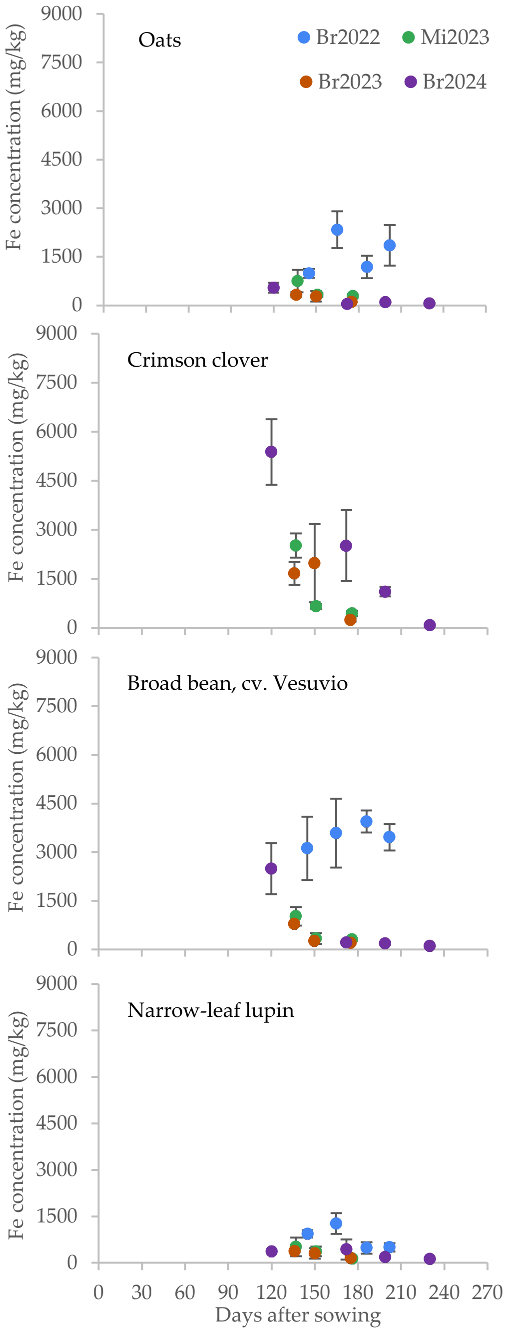

Figure 8. Iron (Fe) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (*n* = 3)

higher P levels in their tissues than grass when grown in the more acidic soil, suggesting that P nutrition in legumes is less reliant on the readily available P in the soil. Many legumes have developed strategies to access sparingly soluble P that other plants cannot, involving morphological, biochemical, and metabolic adaptations that enhance P acquisition (Buoso et al. 2022, Chen et al. 2023, Hawkesford et al. 2023). The most well-documented mechanisms include the formation of proteoid or cluster roots and the exudation of organic acids and acid phosphatases, which increase P solubilisation (Buoso et al. 2022, Hawkesford et al. 2023).

The total accumulation of P in the plant increased over time, maintaining an almost direct relationship with DMY, as its concentration declined only slightly. Consequently, if legumes are incorporated into the soil as green manure, they introduce large amounts

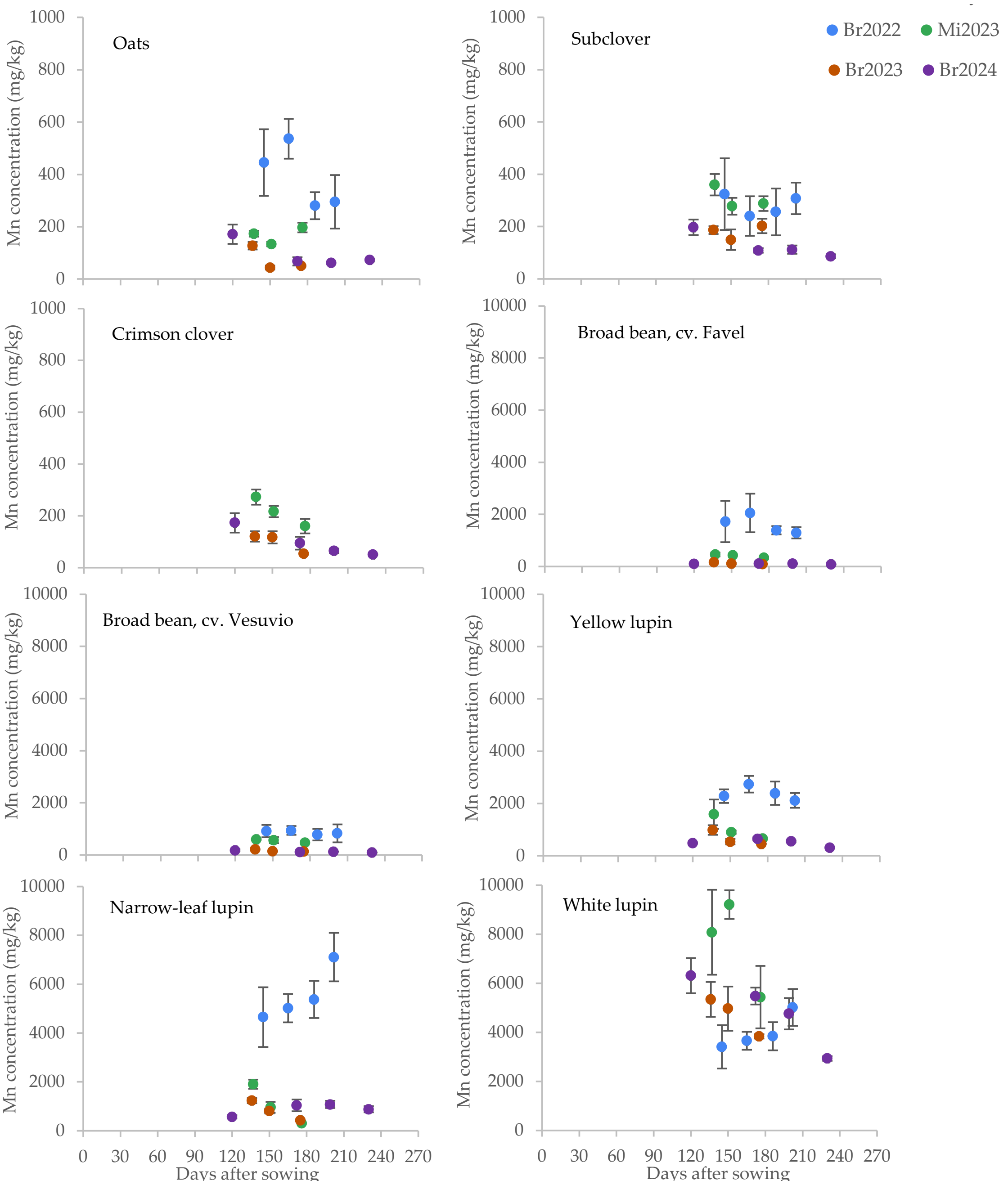

Figure 9. Manganese (Mn) concentration in eight species/cultivars grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (*n* = 3)

of organic P, which may become more bioavailable than other soil P forms following the mineralisation of organic substrates by phosphatase activity. Thus, legumes contribute not only to increasing soil N availability through biological N fixation (Barbieri et al. 2023, Aguiar et al. 2024) but also to enhancing soil P availability (Buoso et al. 2022, Chen et al. 2023, Gómez-Gallego et al. 2025). The P contained in the aboveground plant parts may even underestimate the total amount of organic P returned to the soil, as P stored in the root system was not accounted for in this study. Legumes can store more than 50% of their total N in their roots (Herridge et al. 2008) and may similarly retain significant amounts of P. Nodules serve as key sites for P accumulation due to the high number of metabolic reactions occurring there, which require energy-rich phosphates (Hungria and Nogueira 2023).

As a summary, in favourable years for crop growth, lupins and broad beans contained nearly 30 kg/ha of P in their biomass at the final harvest, excluding P in the roots. When incorporated into the soil as green manure, this P, primarily in organic forms, becomes available to subsequent crops through mineralisation by soil phosphatases. The role of legumes in P cycling may be particularly significant in P-deficient soils, as some legumes can access P forms largely unavailable to most other species and convert them into organic forms (Buoso et al. 2022, Hawkesford et al. 2023).

Macronutrient cations. The K concentration in plant tissues decreased over time, regardless of location, year, or species, due to a dilution effect caused by biomass accumulation. K appears to play a limited role in managing legume species as green manure, as its uptake depends primarily on soil availability. In the soil, K is found within the structure of primary

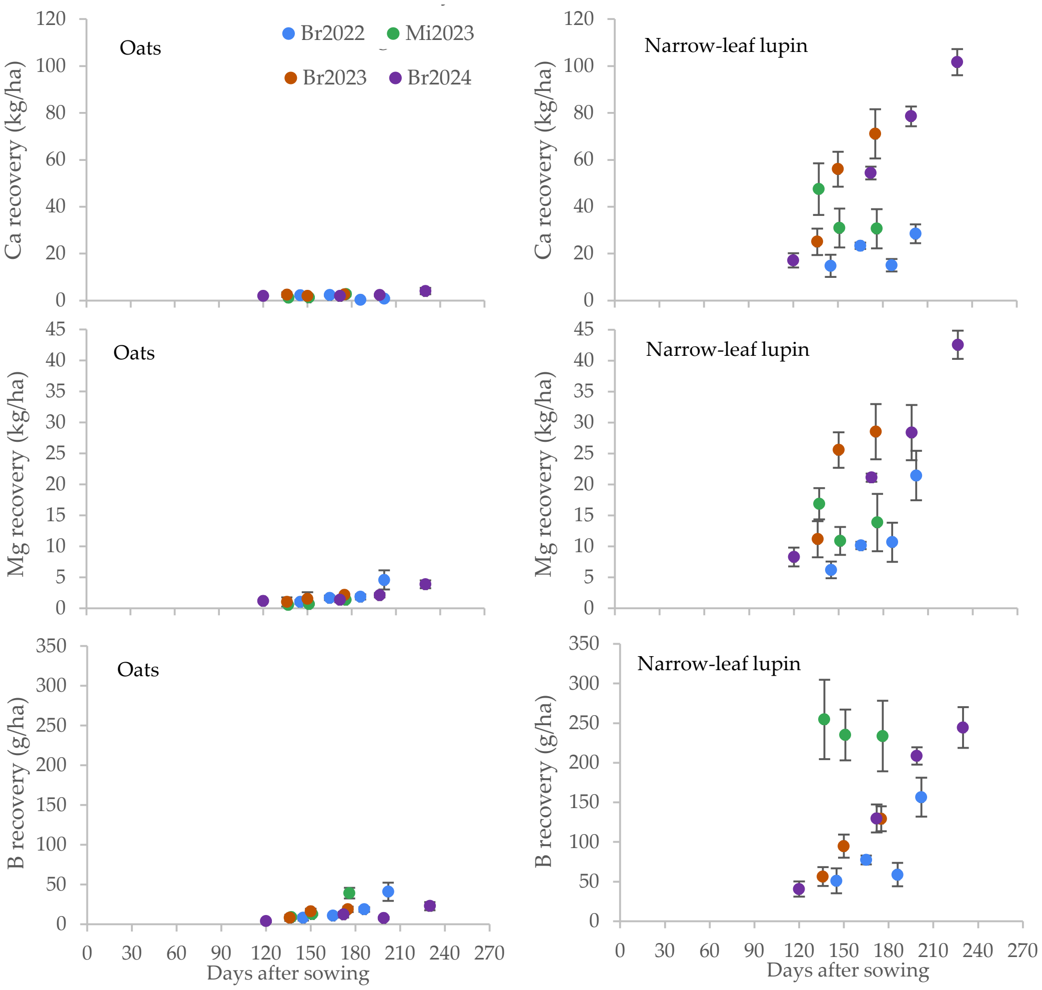

Figure 10. Recovery of calcium (Ca), magnesium (Mg), and boron (B) in aboveground biomass of oats and narrow-leaf lupin grown in four different plots, resulting from the combination of locality (Br – Bragança; Mi – Mirandela) and year (2022–2024). Error bars represent the standard deviation (n = 3)

minerals (such as feldspars and micas), fixed in 2:1 clay minerals, adsorbed onto the exchange complex, and dissolved in the soil solution, with the latter two fractions being the most bioavailable (Havlin et al. 2017, Weil and Brady 2017). Since K does not integrate into organic structures in plant tissues (Hawkesford et al. 2023), it is readily returned to the soil with plant biomass after uptake, remaining available for reabsorption without requiring mineralisation. Consequently, K cycling is largely independent of cultivation processes.

Calcium concentrations were notably lower in grass tissues compared to legumes. Monocots have lower Ca requirements than most other plant species, as a significant proportion of total Ca in plant tissues is located in cell walls. This is attributed to their lower pectate concentrations and reduced cation exchange capacity (Hawkesford et al. 2023). No pronounced decreasing trend in Ca concentration was observed, which is expected for elements with low mobility in plants, such as Ca (Bryson et al. 2014, Hawkesford et al. 2023). However, a clear decline in Ca concentration in plant tissues was evident in highly acidic soils over time. This underscores the importance of liming as a common agricultural practice to raise soil pH and supply Ca to crops (Havlin et al. 2017). This practice is particularly critical for legumes, as nodulation is highly sensitive to soil acidity and Ca deficiency (Yang et al. 2021, Hungria and Nogueira 2023). Among legumes, white lupin exhibited lower Ca concentrations in its tissues compared to most other species. As this plant is particularly well adapted to acidic soils (Jensen et al. 2004, Rodrigues et al. 2013), it may have developed high physiological efficiency in Ca utilisation as an evolutionary strategy for thriving in acidic environments.

The grass also exhibited lower Mg levels in its tissues than the legumes. The critical Mg concentration in plant tissues typically exceeds 1 g/kg, with dicots generally displaying higher concentrations (Bryson et al. 2014). Among legumes, white lupin had the lowest Mg concentrations in its tissues relative to other species. Like Ca, this may indicate an adaptation to acidic soils. Acidic soils also tend to have low Mg availability in both the exchange complex and the soil solution (Havlin et al. 2017, Weil and Brady 2017).

In summary, this study demonstrated that legumes can uptake and accumulate substantial amounts of other nutrients. This absorption process is enhanced by increased biomass production, as legumes have access to atmospheric N (Hungria and Nogueira 2023). When used as green manure, these nutrients are returned to the soil in organic form, becoming available to non-leguminous crops following the mineralisation of the organic substrate (Li et al. 2020, Mesfin et al. 2023). Therefore, cultivating legumes not only increases N availability for other species through biological N fixation but also improves the cycling and bioavailability of other essential soil nutrients. However, this process can accelerate nutrient mining, as continuous nutrient removal from the soil without proper replenishment may lead to long-term depletion. Therefore, legume cultivation should consider this aspect to ensure sustainable soil fertility management.

Micronutrients. No clear trend in B concentration in plant tissues over time was observed. As an element largely immobile in the phloem in most species (Cakmak et al. 2023), B is minimally affected by the phenological stage of the plants. A marked effect of location on B concentration in the tissues was observed, with the Mi2023 location consistently yielding plants with higher levels than the other locations due to higher B concentrations in the soil (Aguiar et al. 2024). The grass exhibited lower B concentrations in its tissues compared to the legumes. A significant portion of B in plants is complexed with cis-diol esters associated with pectins in the cell walls. The higher B requirement in dicots is related to the more significant proportion of compounds with cis-diol configurations in the cell walls, as compared to monocots (Cakmak et al. 2023). In the region where this study was conducted, B has been identified as a significant issue for dicot plants due to the low availability of B in the soil (Portela et al. 2015, Arrobas et al. 2024).

Higher Fe concentrations in plant tissues were observed in the more acidic soil (Br2022). As soil acidity increases, Fe solubility also rises. Insoluble Fe compounds, such as hydroxides, oxyhydroxides, and oxides, dissolve into their ionic forms, ferrous (Fe^{2+}) or ferric (Fe^{3+}) (Havlin et al. 2017, Weil and Brady 2017). Fe concentrations in plant tissues were remarkably high, often exceeding the typical sufficiency range of 50 to 150 mg/kg for most cultivated species (Bryson et al. 2014, Cakmak et al. 2023). Values reached up to ten times this range in the more acidic soil (Br2022). However, the different species and cultivars used in this study exhibited varying Fe concentrations when grown in the same soil. Narrow-leaf lupin had the lowest Fe concentrations in its tissues, whereas clovers and broad beans displayed higher levels, though this did not appear to affect DMY. Tolerance mechanisms to high Fe levels in soil and plant tissues can be diverse. Some plants have developed exclusion strategies, such as reducing Fe uptake and restricting xylem transport through root oxidation, thereby lowering Fe concentrations in aerial parts (Bell 2023, Ullah et al. 2023). Additionally, plants have evolved tolerance mechanisms for high Fe levels in tissues, including intracellular Fe compartmentalisation, sequestration of active Fe in Fe-binding proteins, scavenging of free radicals, and gene regulation (Bell 2023, Ullah et al. 2023). These mechanisms explain why different species exhibit distinct Fe concentrations despite being cultivated in the same soil.

As for Fe, Mn concentrations in plant tissues were higher in the more acidic soil. In acidic soils, soluble forms of Mn, such as Mn^{2+}, increase due to the dissolution of insoluble Mn oxides and hydroxides (Havlin et al. 2017, Weil and Brady 2017). Mn concentrations in the tissues reached exceptionally high levels, especially in narrow-leaf lupin and white lupin, with values exceeding those found in grasses and clovers by more than tenfold. It is well known that Mn concentrations in plant tissues, including toxic levels, depend on species and cultivar (Alejandro et al. 2020, Cakmak et al. 2023). Although, like Fe, plants possess physiological mechanisms for Mn tolerance (Alejandro et al. 2020), the species in this study appear to employ distinct strategies for each element. For example, narrow-leaf lupin exhibited the lowest Fe concentrations in its tissues but the highest Mn levels, suggesting it utilises exclusion mechanisms for Fe and accumulation mechanisms for Mn.

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