

Liming alone is not enough: The role of phosphorus and boron in supporting legume pasture growth

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Abstract. Soil fertility management is a critical factor for ensuring the persistence of the legume component in improved pastures in Mediterranean regions. To promote legume growth, the most common practices include the application of lime to correct Soil acidity and phosphate fertilizers to address phosphorus (P) deficiencies. This study examined two acidic soils and evaluated the need for P and boron (B) supplementation following liming. A factorial pot experiment (Soil type × vegetation type × fertilizer treatment) was conducted using subterranean clover (*Trifolium subterraneum* L.), annual ryegrass (*Lolium multiflorum* Lam.), and their mixture. Results revealed significantly higher dry matter yield (DMY) for the legume (18.1 g pot⁻¹) compared to the grass (5.9 g pot⁻¹), due to the legume's ability to access atmospheric nitrogen (N). Soil 2, which had higher initial P availability than Soil 1, and P fertilization both enhanced N accumulation in legume tissues, the percentage of N derived from the atmosphere (%Ndfa), and DMY. These outcomes indicate that liming alone was insufficient to increase P availability and eliminate the need for P fertilization. For instance, DMY increased from 15.0 g pot⁻¹ with liming alone to 19.4 g pot⁻¹ when P was supplemented. Additionally, B application increased N uptake and %Ndfa in the legume, with the latter rising from 48.9% in the liming-only control to 77.5% with B supplementation. These results suggest that liming, by increasing Soil pH, may reduce B bioavailability, highlighting the need for B supplementation following lime application.

Key words: annual ryegrass, biological nitrogen fixation, dry matter yield, Mediterranean pastures, subterranean clover, %Ndfa.

INTRODUCTION

Grasslands play a central role in animal nutrition and form the foundation of livestock production systems across vast regions of the world. In recent decades, improved pastures have been expanding globally as a response to the growing need for more productive agricultural systems, both in intensive and extensive forms of pasture-based livestock farming (Hayes et al., 2017; Badgery et al., 2024). The concept of improved pastures is primarily based on the introduction of high-yielding species, with particular emphasis on legumes, due to their capacity to access atmospheric N, thereby reducing reliance on synthetic fertilizers (Ovalle et al., 2006; Teixeira et al., 2015; Monjardino et al., 2022).

The N introduced into the system by legumes can also benefit associated non-leguminous species, thus enhancing the overall productivity of the pasture (Riday & Albrecht, 2012; Monjardino et al., 2022). However, ensuring the persistence of legume species remains a challenge, requiring grazing management strategies that support successful seed production and natural reseeding (Nyfeler et al., 2009; Hayes et al., 2017). In addition to grazing strategies, several Soil management practices have also been recommended to enhance legume persistence, with liming and P application being the most prominent (Moir et al., 2016; Morton, 2020; McLachlan et al., 2024). Nonetheless, under specific conditions, the role of other nutrients should not be underestimated (Bellaloui et al., 2014; Hamilton et al., 2015; Zhou et al., 2023).

Soil acidity represents a major constraint on plant productivity, given the widespread prevalence of acidic soils globally, with liming being a widely adopted corrective practice (Upjohn et al., 2005; Holland et al., 2018; Hackney et al., 2019). Soil acidity impairs plant growth primarily due to the toxicity of aluminum (Al^{3+}), and in some cases, manganese (Mn^{2+}), which are present in high concentrations in the Soil solution. Additionally, deficiencies in essential cations such as calcium (Ca^{2+}), and in some cases magnesium (Mg^{2+}), also contribute to reduced plant performance under acidic conditions (Kochian et al., 2004; Weil & Brady, 2017). Once applied, agricultural lime reacts with carbon (C) dioxide in the Soil atmosphere and with water, forming calcium (Ca) and magnesium (Mg) bicarbonates. These compounds neutralize both exchangeable and residual acidity. Acidic ions in the Soil solution and adsorbed onto Soil colloids, namely H^+ , Al^{3+} , and Mn^{2+} , are displaced by basic cations (Ca^{2+} and Mg^{2+}), leading to the precipitation of aluminum (Al) and manganese (Mn) as their respective hydroxides [$\text{Al}(\text{OH})_3$ and $\text{Mn}(\text{OH})_2$], thereby increasing Soil pH (Weil & Brady, 2017). In improved pastures enriched with legumes, liming, in conjunction with appropriate grazing management, is a key strategy to enhance both productivity and the persistence of the legume component (Moir et al., 2016; Morton, 2020; McLachlan et al., 2024). Legumes are particularly sensitive to Soil acidity, as they tend to have higher Ca requirements than grasses, and nodulation is highly susceptible to Al toxicity and Ca deficiency (Morton, 2020; Yang et al., 2021; Hungria & Nogueira, 2023).

In legume-rich pastures, P application is a key management practice, as liming may correct Soil acidity for several years, while P typically needs to be applied annually (Olson-Rutz & Jones, 2015; Teixeira et al., 2015; McLachlan et al., 2024). P availability in the Soil is crucial not only for plant growth, but also for the development and

functioning of root nodules, thereby facilitating biological N fixation and providing legumes with a competitive advantage over grasses (Maxwell et al., 2013; Mitran et al., 2018). Acidic soils are often associated with low levels of available P (Upjohn et al., 2005; Arrobas et al., 2023a). P availability in highly acidic soils is reduced due to its strong affinity for iron (Fe) and Al oxides, leading to its precipitation as insoluble compounds such as $AlPO_4$ and $FePO_4$ (Weil & Brady, 2017). Thus, alleviating Soil acidity through liming may be sufficient to mobilize previously precipitated P, potentially rendering additional P fertilization redundant in the years following lime application.

B has been reported as one of the most significant plant nutritional disorders in inland regions of Portugal (Portela et al., 2015; Arrobas et al., 2024). This issue is most prevalent in dicot species, which generally have higher B requirements than monocots. B plays a critical role in cell wall biosynthesis, particularly through its binding with cis-diol-containing compounds, which are more prevalent in dicots than in monocots (Behera et al., 2023; Cakmak et al., 2023). Since pasture systems often aim to maintain a balance between legumes (dicots) and grasses (monocots), B may represent a key factor in effective pasture management. The importance of B in the growth and development of legume root nodules, and thus in biological N fixation, has also been well documented (Bellaloui et al., 2014; Hamilton et al., 2015; Hackney et al., 2019). Conversely, lime application can induce B deficiency in plants (Arrobas et al., 2023b), as at higher pH levels, B becomes more tightly bound to Soil particles, reducing its availability. Furthermore, plants may exhibit an increased demand for B when Ca is abundant (Weil & Brady, 2017), a situation that may compromise the persistence and productivity of the legume component in pasture systems.

Since liming is generally regarded as a routine practice in acidic soils, this study seeks to determine whether liming is sufficient to enhance P availability to the point of eliminating the need for its direct application. Additionally, the study examines whether liming interferes with the bioavailability of other nutrients, particularly B, as previous research in other crops (Arrobas et al., 2023b) has shown that liming can induce severe B deficiency. Thus, the experimental design included liming as the control treatment, with three additional treatments: lime with P addition, lime with B addition, and lime with both P and B additions. The experiment was conducted under controlled conditions using pots and included three vegetation types: subterranean clover, annual ryegrass, and a mixture of both species. Two acidic soils of different origins were tested.

The main working hypotheses were as follows: i) the addition of P is not necessary when Soil is limed, due to increased P availability; and ii) the supply of B is essential, particularly for the legume component, due to the risk of B deficiency induced by liming.

MATERIALS AND METHODS

General experimental conditions

The pot experiment was conducted at the agricultural experimentation unit of the Polytechnic Institute of Bragança, located at coordinates 41°47'49.96" N, 6°45'42.90" W, at an altitude of 670 m above sea level. Sowing was done on September 19, 2023, and harvest was on April 29, 2024. The region is characterized by a Mediterranean climate with Atlantic influence. According to the Köppen-Geiger climate classification, the region is categorized as Csb, which denotes cold, rainy winters and hot, dry summers

(IPMA, 2025). The mean annual temperature is 12.9 °C, and the average annual precipitation amounts to 790.0 mm. The monthly average temperature and precipitation values recorded during the experimental period are presented in Table 1.

Table 1. Monthly mean air temperature and precipitation values during the experimental period, alongside the regional climatological normal (1991–2020) (IPMA, 2025)

		Temperature (°C)		Precipitation (mm)	
		Normal	2023/2024	Normal	2023/2024
2023	September	18.3	17.0	43.9	87.6
	October	13.4	14.7	106.9	212.8
	November	8.2	10.2	91.8	126.8
	December	5.4	4.5	112.3	70.0
2024	January	4.8	6.3	106.6	107.0
	February	6.3	8.1	62.4	86.2
	March	9.3	8.6	68.6	122.2
	April	11.3	11.8	68.0	57.4
	May	14.8	13.4	63.4	35.6
	June	19.0	18.4	32.3	85.8

Experimental design, description, and trial management

The experiment was arranged as a factorial design comprising three factors: Soil type, vegetation type, and fertilisation treatments. Each combination of factors was replicated three times. Two Soil types were used: Soil 1, collected from a seminatural lowland pasture in central Portugal, and Soil 2, sourced from a seminatural mountain pasture in northern Portugal. The plant species selected for this study were subterranean clover (*Trifolium subterraneum* ssp. *subterraneum*, cv. Denmark), annual ryegrass (*Lolium multiflorum* Lam., cv. Falladino), and a mixture of the two species. Calcium carbonate was applied as a liming treatment (Lime) to address the high Soil acidity, and in this experiment, it was considered the control treatment. The additional treatments included: Lime plus P (Lime + P), Lime plus B (Lime + B), and Lime combined with both P and B (Lime + P + B). In total, the experiment comprised 72 pots, resulting from the combination of two soils, three vegetation types, four treatments, and three replicates.

Soil 1 is classified as a Dystric Leptosol, while Soil 2 corresponds to an Umbric Dystric Leptosol (WRB, 2022). Selected physical and chemical properties of the soils, determined from samples collected at a depth of 0–15 cm shortly before the onset of the trial, are presented in Table 2.

Laboratory-grade calcium carbonate (neutralizing value 100% as $\text{CaCO}_3 \approx 56\%$ as CaO) was applied to each Soil at rates adjusted according to initial pH to reach pH 6.5. This value is considered optimal for maximizing the bioavailability of key Soil nutrients, including P (Weil & Brady, 2017). The estimation of the required calcium carbonate dose was based on Soil pH, texture, and organic matter content, following the standardized procedures outlined by Veloso et al. (2022). For Soil 1, the application rate was estimated at $4,000 \text{ kg ha}^{-1}$, whereas for Soil 2 it was $7,000 \text{ kg ha}^{-1}$. To translate these hectare-based values into the amounts required for pot experiments, it was considered that each pot contained 3 kg of oven-dried fine soil, while one hectare corresponds to 2,000 t of soil. Accordingly, the final application rates per pot were determined as 7 g for Soil 1 and 12 g for Soil 2, respectively. P was applied at a rate equivalent to $80 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$

(37.4 kg P ha⁻¹). Considering the surface area of each pot (0.022 m²), this corresponded to 0.076 g of P (0.174 g P₂O₅) per pot, supplied as 1 g pot⁻¹ of single superphosphate (18% P₂O₅). Using a similar calculation, B was applied at a rate of 1.5 kg ha⁻¹, corresponding to 0.02 g pot⁻¹ of Neobor (14.9% B as disodium tetraborate pentahydrate).

Table 2. Selected physical and chemical Soil properties (mean ± standard deviation, *n* = 3) of Soil samples collected at a depth of 0–15 cm shortly before the onset of the trial, from the lowland pasture (Soil 1) and the mountain pasture (Soil 2)

	Soil 1	Soil 2
¹ Clay (g kg ⁻¹)	12.4 ± 0.08	14.8 ± 0.27
¹ Silt (g kg ⁻¹)	12.4 ± 0.23	10.7 ± 2.07
¹ Sand (g kg ⁻¹)	75.3 ± 0.31	74.5 ± 1.89
Texture (USDA)	Sandy loam	Sandy loam
² pH (H ₂ O)	5.4 ± 0.06	5.0 ± 0.10
³ Organic carbon (g kg ⁻¹)	17.7 ± 0.29	50.8 ± 3.32
⁴ Total nitrogen (g kg ⁻¹)	1.4 ± 0.13	3.04 ± 0.25
⁵ Extractable phosphorus (mg kg ⁻¹)	33.4 ± 1.54	106.4 ± 9.32
⁵ Extractable potassium (mg kg ⁻¹)	108.4 ± 2.94	312.5 ± 78.59
⁶ Exchangeable calcium (cmol _c kg ⁻¹)	1.87 ± 0.14	4.40 ± 0.49
⁶ Exchangeable magnesium (cmol _c kg ⁻¹)	0.61 ± 0.03	1.25 ± 0.12
⁶ Exchangeable potassium (cmol _c kg ⁻¹)	0.45 ± 0.02	1.20 ± 0.24
⁶ Exchangeable sodium (cmol _c kg ⁻¹)	0.13 ± 0.01	0.25 ± 0.11
⁶ Exchangeable acidity (cmol _c kg ⁻¹)	0.70 ± 0.00	0.43 ± 0.12
⁷ CEC (cmol _c kg ⁻¹)	3.76 ± 0.15	7.54 ± 0.79
⁸ Extractable boron (mg kg ⁻¹)	0.72 ± 0.47	1.05 ± 0.22

¹Robinson pipette method; ²Potentiometry; ³Walkley-Black; ⁴Kjeldahl; ⁵Ammonium lactate (Egner- Riehm); ⁶Ammonium acetate; ⁷Cation exchange capacity; ⁸Hot water, azomethine-H.

The fertilizers corresponding to each treatment were thoroughly mixed with 3 kg of air-dried fine Soil (< 2 mm) per pot. The seeding rate for each pot was based on the recommended field application rates for the selected species, namely 20 kg ha⁻¹ for subterranean clover and 40 kg ha⁻¹ for annual ryegrass. The seed quantities per pot were estimated based on the surface area of the pots, resulting in the application of 44 mg and 88 mg of subterranean clover and annual ryegrass seeds, respectively. In the mixed-species treatment, half of each seed dose was used. Seeds were sown at a depth of 1 cm.

Irrigation was provided whenever rainfall was insufficient to support regular plant growth. During the initial weeks, weeds were manually removed whenever distinguishable from the sown species, to maintain uniformity across the vegetation samples to be analysed. Pots were surrounded by wooden stakes to shield their sides from direct sunlight and prevent overheating. To ensure uniform exposure to solar radiation, pot positions were rotated weekly.

Sampling and laboratory analyses

Plants were harvested near ground level at the advanced flowering stage of the legume and the early heading stage of the grass, 212 days after sowing. After harvesting, the samples were briefly washed in clean water to remove any Soil or mineral debris attached to the plants. Subsequently, the samples were dried in a forced-air oven set to 70 °C, weighed, and ground using a 1 mm mesh sieve.

The samples were subjected to elemental analysis. N was determined by the Kjeldahl method, while B and P were determined by colorimetry. Potassium (K), Ca, Mg, Mn, Fe, copper (Cu), and zinc (Zn) were determined by atomic absorption spectrophotometry following digestion of the samples with nitric acid in a microwave oven (Temminghoff & Houba, 2004).

Initial Soil samples were air-dried and passed through a 2 mm sieve. Soil texture was analysed using the Robinson pipette method. Soil pH was determined potentiometrically in a 1:2.5 soil-to-solution ratio. Organic C content was assessed by wet oxidation following the Walkley-Black method. Soil N was determined using the Kjeldahl method. Extractable P and K were determined using the ammonium lactate method (Egner-Riehm), while exchangeable bases were accessed with the ammonium acetate method. B was determined using the hot-water extraction method followed by azomethine-H colorimetry. All these analytical procedures are fully described in van Reeuwijk (2002).

Data analysis

The data analysis was conducted using the statistical software SPSS Statistics (version 25, IBM SPSS, Armonk, NY, USA). Normality and homogeneity of variances were initially assessed using the Shapiro-Wilk test and Levene's test, respectively. The effects of the treatments were compared using a three-way analysis of variance (ANOVA), with factors Soil type, vegetation type, and fertilisation treatment. When significant interactions were observed for relevant results, a two-way ANOVA was applied separately for each vegetation type, considering only the factorial combination of Soil type \times fertiliser treatment. When significant differences between treatments were found ($P < 0.05$), means were separated using the Tukey *HSD* test ($\alpha = 0.05$).

The %Ndfa was estimated using the difference method, which compares the N content in legume tissues to that found in the non-legume species (Unkovich et al., 2008):

$$\%Ndfa = \frac{N_{legume} - N_{non-legume}}{N_{legume}} \cdot 100 \quad (1)$$

RESULTS

Dry matter yield

The results showed a significant interaction among most of the factors under study, although within each factor, significant differences were also observed (Fig. 1). DMY was significantly higher in Soil 2 (14.8 g pot⁻¹) compared to Soil 1 (10.7 g pot⁻¹). Regarding vegetation types, the legume exhibited the highest DMY (18.1 g pot⁻¹), significantly surpassing that of the mixture (14.3 g pot⁻¹), which in turn was significantly greater than that of the grass (5.9 g pot⁻¹). Among treatments, those that received P showed significantly higher values compared to the control (11.2 g pot⁻¹), particularly the Lime + P (13.2 g pot⁻¹) and Lime + P + B (14.2 g pot⁻¹) treatments.

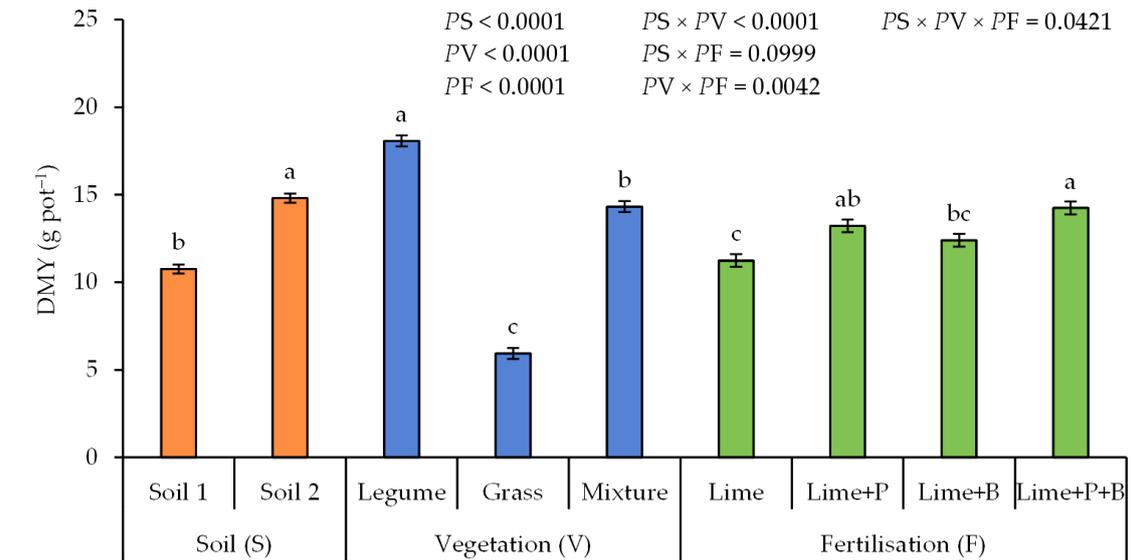


Figure 1. Dry matter yield (DMY) as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

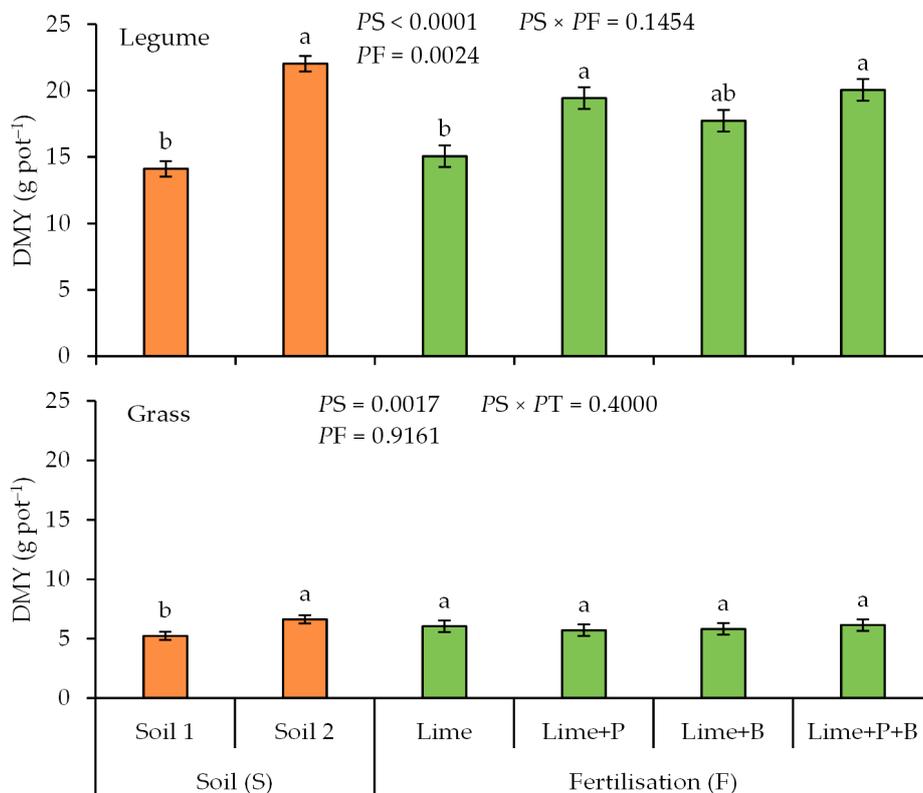


Figure 2. Dry matter yield (DMY) of legume and grass as a function of Soil type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P+B]. The probability (*P*) values from a two-way ANOVA and interactions between factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

Nitrogen concentration and uptake in plant tissues

The analysis of N concentration in plant tissues also revealed significant interactions among some of the factors studied, although not in the three-way combination (Fig. 3). Soil type did not influence tissue N concentration. Legume exhibited the highest N concentration (24.1 g kg⁻¹), followed by the mixture (22.2 g kg⁻¹), and grass (19.0 g kg⁻¹). This finding suggests that vegetation type had a greater impact on N acquisition than Soil type, likely due to the legume's ability to access atmospheric N. Fertilizer treatments significantly affected tissue N concentration, with the control showing the lowest values

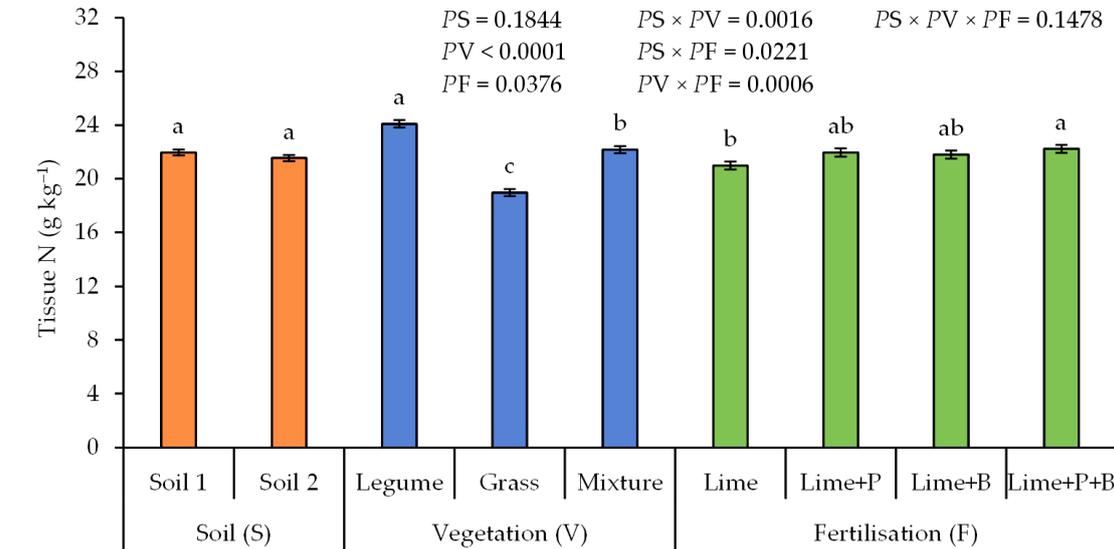


Figure 3. Tissue nitrogen (N) concentration as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

Due to the significant interaction observed and to allow for a clearer discrimination among factors and factor levels, a two-way ANOVA was conducted separately for each vegetation type (Fig. 4). Soil type did not significantly influence N concentration in the tissues of either species. In legume, fertilizer treatments that included P, B, or both nutrients led to significantly higher tissue N concentrations compared to the control. In grass, no significant effect of fertilizer treatments on N concentration was observed

The amount of N recovered in the aboveground plant biomass exhibited a response pattern distinct from that observed for tissue N concentration (Fig. 5). Soil had a marked effect on N uptake, with Soil 2 showing the highest mean value, possibly due to the greater DMY observed under cultivation in this soil. Differences among vegetation types were further accentuated, reflecting the combined and congruent effects of both tissue N concentration and DMY. Regarding fertilizer treatments, the contrast with the control became more pronounced, with treatments receiving P standing out as having the highest N uptake.

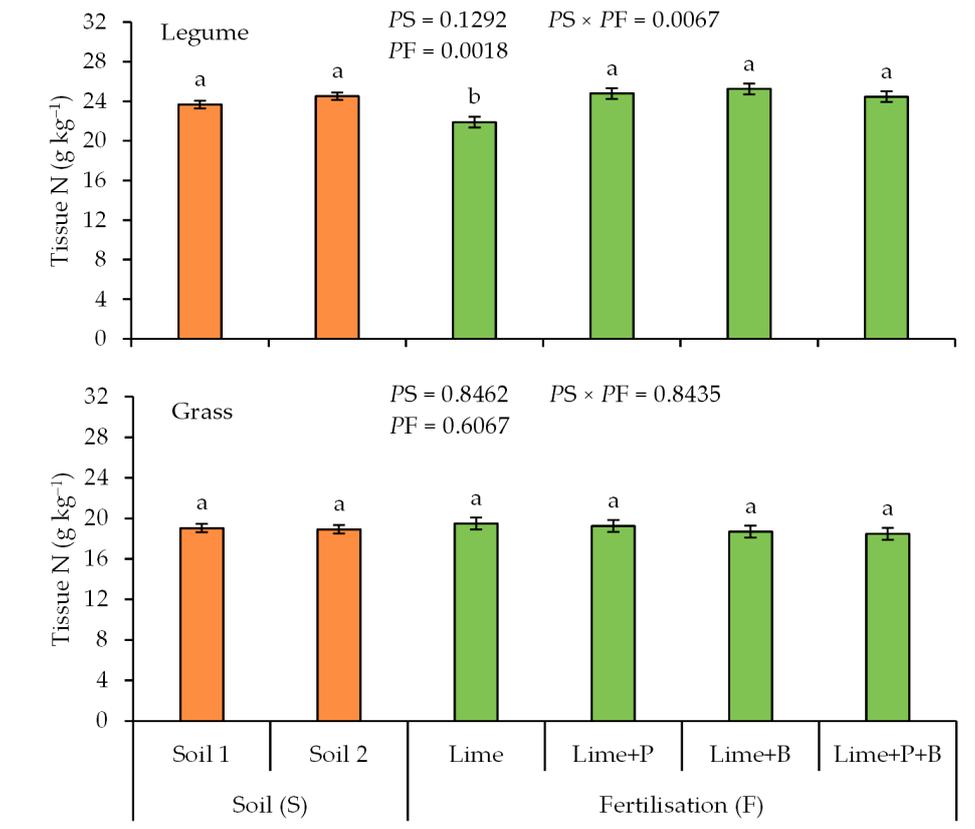


Figure 4. Tissue nitrogen (N) concentration in legume and grass as a function of Soil type and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a two-way ANOVA and interactions between factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

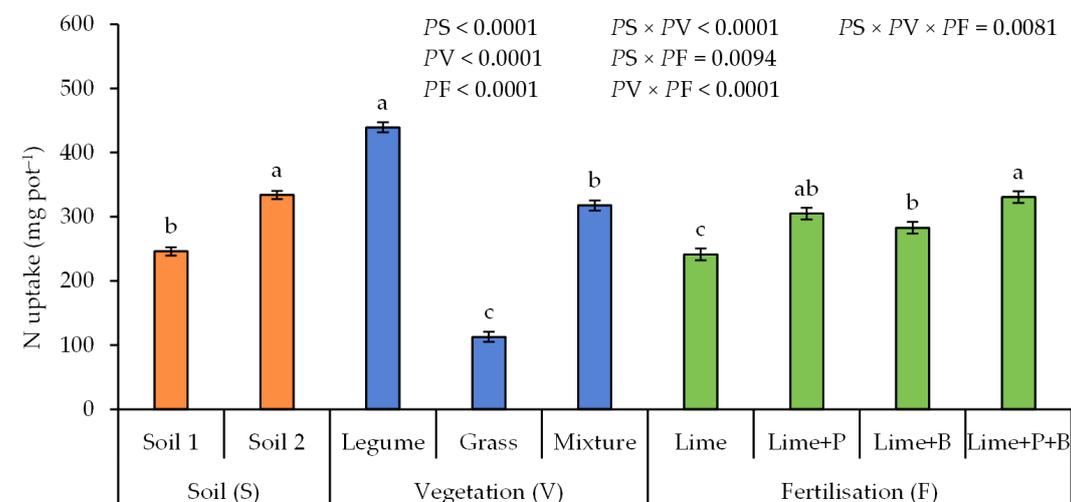


Figure 5. Nitrogen (N) uptake as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

The %Ndfa was higher in Soil 2 compared to Soil 1 when the results were expressed in mg pot^{-1} (Table 3). Treatments that received P, B, or both nutrients also showed higher values of the %Ndfa compared to the control. Specifically, in the case of B, the %Ndfa values were particularly higher in Soil 1, which initially had lower levels of B and organic matter.

Table 3. Nitrogen derived from the atmosphere in legume pots subjected to various fertiliser treatments [Lime, Lime + Phosphorus (P), Lime + Boron (B), and Lime + P + B], expressed in mg per pot (mg pot^{-1}) and as a percentage (%)

	Lime	Lime + P	Lime + B	Lime + P + B	Average
	mg pot^{-1}				
Soil 1	109.0	225.9	299.7	308.8	235.8
Soil 2	324.2	533.7	371.1	438.6	416.9
	%				
Soil 1	48.9	68.6	77.5	75.7	70.0
Soil 2	72.5	82.0	73.6	77.5	76.9

Phosphorus concentration and uptake in plant tissues

No significant interaction among factors was observed for tissue P concentration (Fig. 6). P concentration in plant tissues was higher in Soil 1 than in Soil 2. Among vegetation types, the grass exhibited the highest P concentration, followed by the mixture, and then the legume. Fertilizer treatments did not significantly influence tissue P concentration.

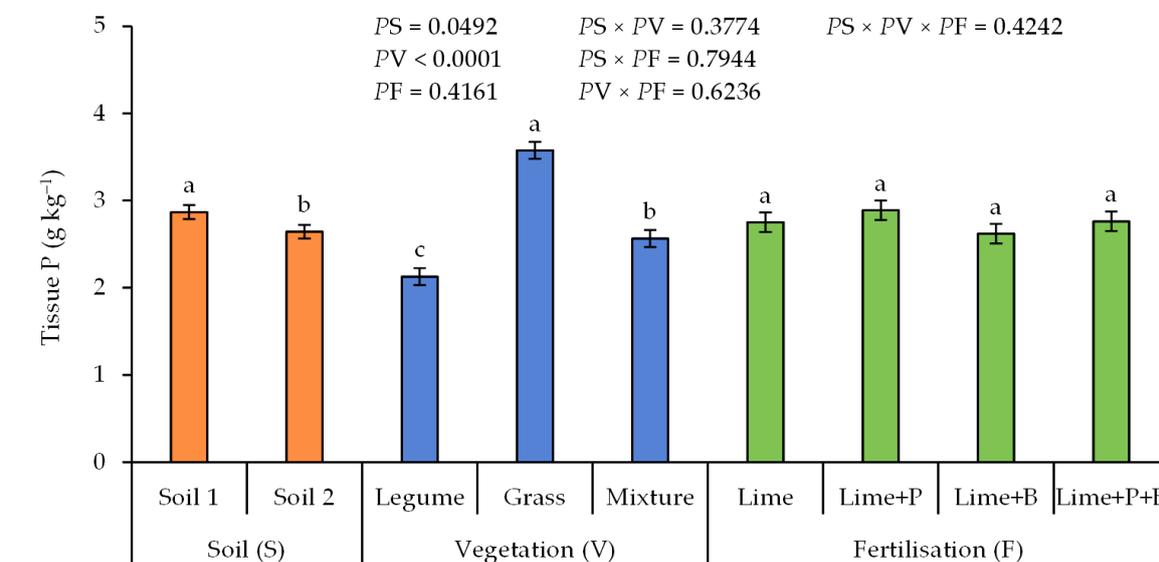


Figure 6. Tissue phosphorus (P) concentration as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (P) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

The amount of P recovered in the aboveground plant biomass was higher in Soil 2, displaying an inverse trend relative to tissue P concentration (Fig. 7). This was attributed to the greater DMY observed in Soil 2. P uptake was also higher in the legume than in the grass, again contrasting with the pattern of tissue P concentration, due to the higher DMY of the legume. Among fertilizer treatments, P application, with or without B, stood out as having the most pronounced effect on P recovery in plant biomass.

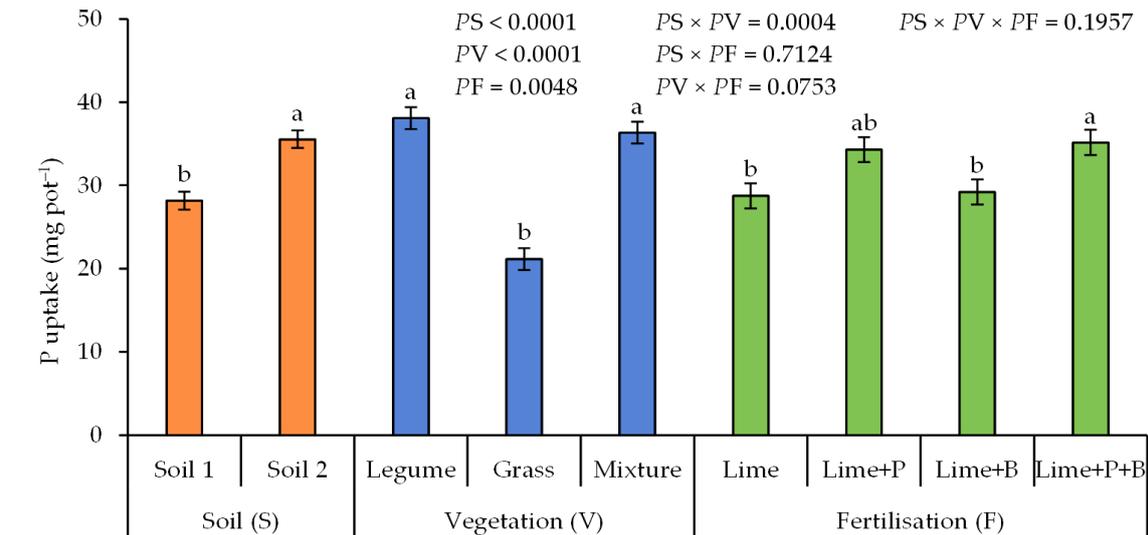


Figure 7. Phosphorus (P) uptake as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

Boron concentration and uptake in plant tissues

Tissue B concentration was significantly higher in plants grown in Soil 1 compared to those grown in Soil 2 (Fig. 8). The legume exhibited significantly higher tissue B levels than the mixture, which in turn had higher levels than grass. Treatments that included B resulted in higher tissue B concentrations than those that did not receive B.

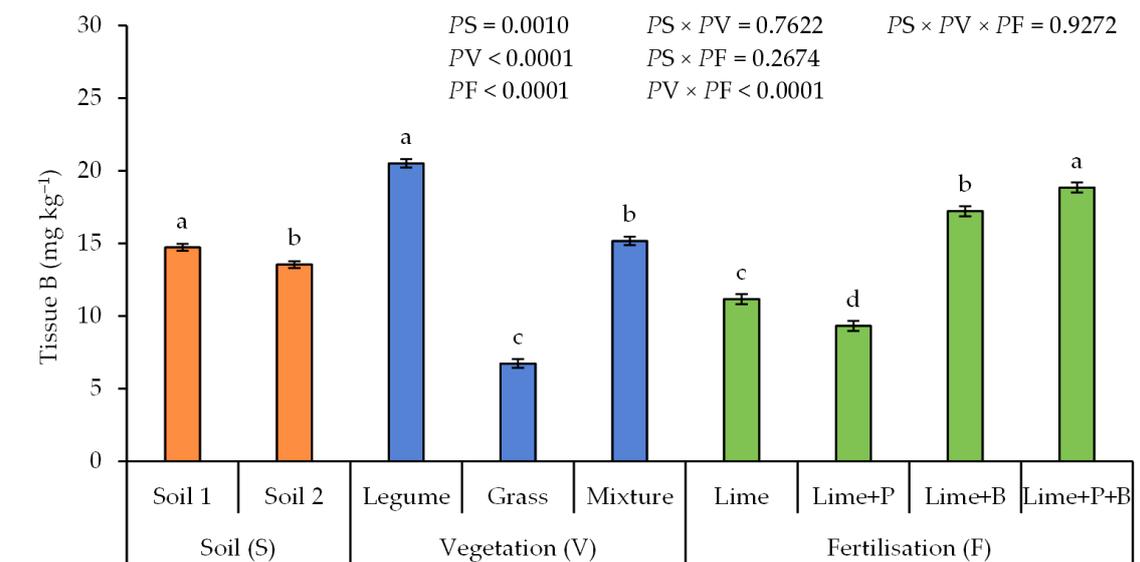


Figure 8. Tissue boron (B) concentration as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

The total amount of B recovered by the vegetation was higher in Soil 2 compared to Soil 1 (Fig. 9), contrary to the pattern observed for tissue B concentration. In contrast, the patterns observed for vegetation type and fertilizer treatments were maintained; however, the differences among factors were amplified due to their combined effect with DMY.

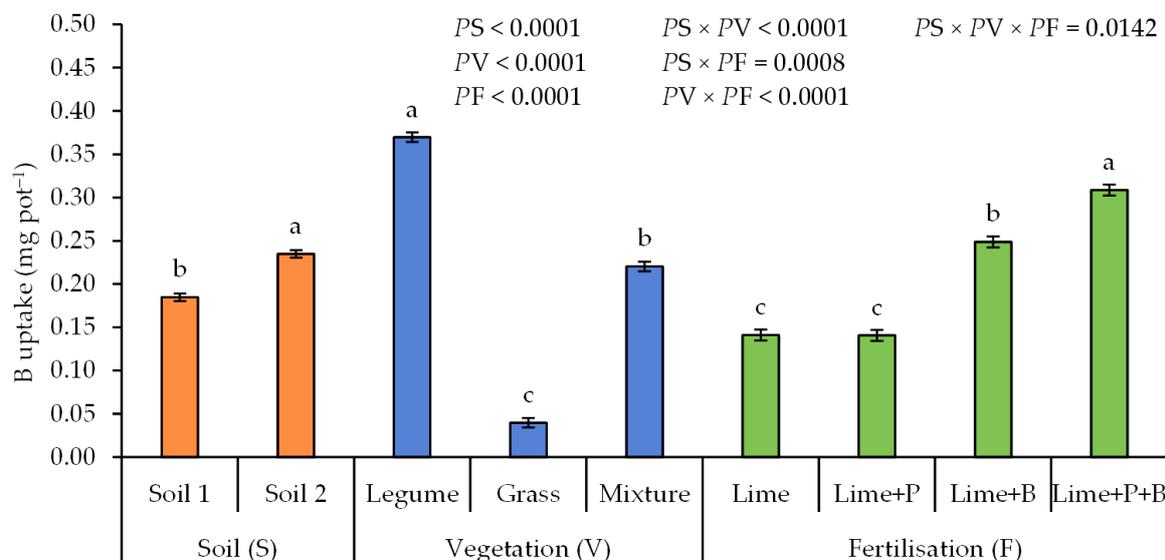


Figure 9. Boron (B) uptake as a function of Soil type, vegetation type, and fertilisation [lime, lime + phosphorus (P), lime + boron (B), and lime + P + B]. The probability (*P*) values from a three-way ANOVA and interactions between all factors are reported. Within each factor, means followed by the same letter are not significantly different according to the Tukey *HSD* test ($\alpha = 0.05$). Error bars represent standard errors.

DISCUSSION

Effect of Soil type and plant species on dry matter yield

Soil type had a significant effect on the DMY of both grass and legume species, with consistently higher yields recorded in Soil 2 (Fig. 2). At the start of the experiment, Soil 2 exhibited superior fertility properties known to support plant productivity, including higher levels of organic matter, P, K, Ca, and Mg. Although Soil 2 was more acidic than Soil 1, the application of lime across all pots as a baseline (and control treatment) likely minimized pH-related differences. Thus, nutrient availability, rather than Soil pH, appears to have been the dominant factor influencing plant growth. Plants grown in Soil 2 recovered more N than those in Soil 1, likely reflecting the higher total N and organic matter contents, which may have enhanced N availability through mineralization. The soils used in the experiment were sieved, a process that improves aeration but also disrupts Soil aggregates, often increasing mineralization rates (Weil & Brady, 2017). The increased N release from organic matter in Soil 2 likely explains the greater DMY observed in the grass species, which typically depend more heavily on soil-available N (Dordas et al., 2019; Dimande et al., 2024).

For the legume species, a higher %Ndfa was observed when grown in Soil 2 (Table 3), which may indicate enhanced biological N fixation in this soil. Both species produced more biomass in Soil 2 than in Soil 1; however, the legume substantially outperformed the grass in terms of DMY. These findings highlight the importance of N availability as a key limiting factor for plant productivity, an established constraint in many agricultural soils (Weil & Brady, 2017; Hawkesford et al., 2023). Legumes, through their symbiotic association with N-fixing microorganisms of the Rhizobiaceae family, often produce greater biomass than non-legume species under comparable growth conditions, as consistently demonstrated in intercropping systems (Dordas et al., 2019; Dimande et al., 2024). Generally, the lower the availability of mineral N in the soil, the greater the competitive advantage of legumes over grasses, provided that essential conditions for nodulation and nodule activity are met (Aguiar et al., 2024; Akshit et al., 2024). This ability to access atmospheric N enables legumes to grow and achieve high productivity even in the absence of N fertilization (Rodrigues et al., 2015; Enriquez-Hidalgo et al., 2018; Aguiar et al., 2024).

Plant response to Soil phosphorus and phosphorus fertilization

P application increased DMY exclusively in the legume species (Fig. 2). Furthermore, P supplementation led to higher N concentrations in legume tissues, whereas no such effect was observed in the grass. Notably, P application also enhanced the %Ndfa in the legume across both Soil types when compared to the control (Table 3). When comparing soils, legumes cultivated in Soil 2 exhibited a higher %Ndfa. Overall, these results suggest that both native Soil P and P applied as fertilizer may have influenced nodule formation and functionality, thereby enhancing biological N fixation. This, in turn, indirectly contributed to the observed increase in DMY of the legume component. The fact that grass DMY did not respond to P application reinforces this interpretation. It is well-established that nodulated legumes have significantly higher P requirements than non-symbiotic plants due to P's critical role in various stages of the symbiotic N fixation process (Chen et al., 2023).

P is essential for maintaining energy metabolism within root nodules, supporting the energetically demanding reduction of atmospheric dinitrogen to ammonia and the subsequent assimilation into amino acids and ureides (Hawkesford et al., 2023; Hungria & Nogueira, 2023). Moreover, P is crucial for the biosynthesis of nucleic acids and phospholipids (O'Hara, 2001; Hungria & Nogueira, 2023). As a result, nodules function as strong P sinks and often contain P concentrations up to three times higher than those found in roots or shoots, particularly under P-limited conditions (Chen et al., 2023).

In response to P deficiency, many legumes have evolved adaptive strategies to access sparingly soluble P pools, including a range of morphological, biochemical, and metabolic adaptations that collectively enhance P acquisition (Buoso et al., 2022; Lambers, 2022). Remarkably, rhizobia mirror their host plant's response by upregulating genes involved in P uptake and metabolism (Sadowsky, 2005; Hungria & Nogueira, 2023).

Although Soil acidity was corrected in this study, a factor known to enhance P bioavailability (Moir et al., 2016; Morton, 2020; Bouray et al., 2022), and both soils, particularly Soil 2, were initially well-supplied with P, the legume still responded positively to P fertilization in terms of increased DMY. This finding underscores the

essential role of P fertilisation and corroborates previous studies that highlight its importance in promoting legume growth, an effect typically attributed to enhanced biological N fixation (Moir et al., 2016; Somavilla et al., 2021; McLachlan et al., 2024).

Plant response to boron application

B application had a modest effect on DMY, although in the case of the legume, the increase was consistent (Fig. 2). Supporting this observation, B supplementation significantly increased N concentration in legume tissues, but not in the grass. Furthermore, B application clearly enhanced the %Ndfa compared to the control, with this effect being particularly pronounced in Soil 1, which had lower initial B content and lower organic matter levels. Although nodule counts were not performed and nodule activity was not assessed, this sequence of results provides strong evidence that B favored nodule formation and/or functionality, thereby enhancing the plant's access to atmospheric N and subsequently promoting vegetative growth, a relationship that has been well documented (Cakmak et al., 2023; Hungria & Nogueira, 2023).

B plays a critical role in the establishment and functionality of legume-rhizobia symbiosis. It is essential for the formation of infection threads and the successful invasion of root cortical cells by rhizobia. Under B-deficient conditions, the adhesion of rhizobial cells to the infection thread wall is impaired, hindering bacterial progression through the thread and ultimately preventing colonization of the host plant (Cakmak et al., 2023; Hungria & Nogueira, 2023). B is also vital for maintaining proper nodule architecture. Legumes require B concentrations that are four to five times higher for nodule development than for root elongation (Carpena et al., 2000). Furthermore, studies have shown that nitrogenase activity is significantly greater in B-fertilized plants compared to unfertilized controls (Santachiara et al., 2019). Consequently, B deficiency leads to reduced nodulation and compromised nodule function (Reguera et al., 2010; Cakmak et al., 2023; Hungria & Nogueira, 2023).

Given that initial B levels in the soils ranged from medium (Soil 1) to high (Soil 2), according to the classification by Veloso et al. (2022), a significant response to B fertilization was not expected, despite the observed increases in B concentration in plant tissues following its application. Indeed, several studies have reported that in soils with B concentrations exceeding 0.5 mg kg^{-1} , DMY responses to B fertilization are typically negligible (Castagnara et al., 2012; Das et al., 2020). Furthermore, the relatively high levels of organic matter in both soils, known to enhance B availability (Weil & Brady, 2017), further complicate the interpretation of the positive effects observed in this study on both DMY and biological N fixation.

However, when these findings are considered alongside the significant differences in tissue B concentrations between the non-supplemented treatments (Lime and Lime + P) and the B-supplemented ones (Lime + B and Lime + B + P), a plausible explanation emerges. It is likely that liming reduced B bioavailability, thereby inducing a functional B deficiency in treatments without B supplementation. This effect would be particularly pronounced in legumes, which have a substantially higher B requirement (Cakmak et al., 2023; Hungria & Nogueira, 2023). A similar phenomenon was reported by Arrobas et al., (2023b) in olive. At elevated Soil pH, B tends to become less available due to increased adsorption onto inorganic and organic colloids (Weil & Brady, 2017).

Moreover, B demand in plants tends to increase in Ca-rich environments (Weil & Brady, 2017). Therefore, joined together, these results suggest that liming may reduce B bioavailability, underscoring the necessity of B supplementation following lime application

CONCLUSIONS

The combined results for dry matter yield (DMY), total nitrogen (N) uptake, and the percentage of N derived from the atmosphere (%Ndfa) across different soils and treatments demonstrated that the legume accessed atmospheric N, which resulted in substantially higher DMY compared to the grass. This combination of findings, particularly the differences between treatments with and without phosphorus (P), also indicated that both the initial Soil P and the P applied as fertilizer enhanced biological N fixation. These results support the hypothesis that, even when lime is applied, an amendment known to increase P availability, P fertilization remains necessary to increase N fixation and DMY in legumes. Moreover, the data suggest that liming increases the need for boron (B) supplementation. Even in soils with initially adequate B levels and organic matter content, B application led to increased N uptake, higher %Ndfa, and a slight increase in DMY, underscoring its importance following liming.

These findings have important implications for pasture management in acid soils. They highlight the agronomic advantage of including legumes in mixed swards to enhance N availability and reduce reliance on synthetic N fertilizers. Fertilizer strategies should be tailored to meet the specific nutritional demands of legumes, particularly following lime application, with attention to potential B limitation. To build on these findings, future studies should quantify nodulation and assess nodule function directly, as well as investigate Soil N dynamics over time. Such data would improve understanding of how P and B availability, in interaction with liming, influence N fixation and long-term pasture productivity under field conditions.

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REFERENCES

- Aguiar, P., Arrobas, M., Nharreluga, E.A. & Rodrigues, M.Â. 2024. Different species and cultivars of broad beans, lupins, and clovers demonstrated varying environmental adaptability and nitrogen fixation potential when cultivated as green manures in Northeastern Portugal. *Sustainability* **16**, 10725. <https://doi.org/10.3390/su16231072512>
- Akshit, S.K., Sheoran, N., Devi, P., Sharma, K., Kamboj, E. & Kumar, P. 2024. Legumes in cropping systems: a way toward agricultural sustainability and diversification. *Commun. Soil Sci. Plant Anal.* **55**(4), 596–608. <https://doi.org/10.1080/00103624.2023.2274035>

- Arrobas, M., Raimundo, S., Correia, C.M. & Rodrigues, M.Â. 2024. Omitting the application of nitrogen or potassium reduced the growth of young chestnut (*Castanea sativa*) trees, while a lack of boron decreased fruit yield. *Soil Syst.* **8**, 104. doi: 10.3390/soilsystems8040104
- Arrobas, M., Conceição, N., Pereira, E., Martins, S., Raimundo, S., Brito, C., Correia, C.M. & Rodrigues, M.Â. (2023a). Dolomitic limestone was more effective than calcitic limestone in increasing Soil pH in an untilled olive orchard. *Soil Use Manage.* **39**, 1437–1452. <https://doi.org/10.1111/sum.12948>
- Arrobas, M., Raimundo, S., Conceição, N., Moutinho-Pereira, J., Correia, C.M. & Rodrigues, M.Â. 2023b. On sandy, boron-poor soils, liming induced severe boron deficiency and drastically reduced the dry matter yield of young olive Trees. *Plants* **12**, 4161. doi: 10.3390/plants12244161
- Badgery, W., Broadfoot, K., Millar, G., Harris, C. & Boschma, S. 2024. Tropical pastures add value to grazing systems in temperate environments of Eastern Australia. *Field Crops Res.* **312**, 109402. <https://doi.org/10.1016/j.fcr.2024.109402>
- Behera, B., Kancheti, M., Raza, M.B., Shiv, A., Mangal, V., Rathod, G., Altaf, M.A., Kumar, A., Aftab, T., Kumar, R., Tiwari, R.K., Lal, M.K. & Singh, B. 2023. Mechanistic insight on boron-mediated toxicity in plants vis-à-vis its mitigation strategies: A review. *Int. J. Phytoremediation* **25**(1), 9–26. <https://doi.org/10.1080/15226514.2022.2049694>
- Bellaloui, N., Mengistu, A., Abdelmajid, M.A., Abel, C. & Zobiolo, L.H.S. 2014. Role of boron nutrient in nodules growth and nitrogen fixation in soybean genotypes under water stress conditions. In: Price, A. (Ed.). *Improving Crop Productivity in Sustainable Agriculture* (Chapter 10, pp. 237–258). InTech. <https://doi.org/10.5772/56994>
- Bouray, M., Moir, J.L., Condrón, L.M. & Paramashivam, D. 2022. Early effects of surface liming on Soil P biochemistry and dynamics in extensive grassland. *Nutr. Cycl. Agroecosys.* **124**, 173–187. <https://doi.org/10.1007/s10705-021-10163-4>
- Buoso, S., Zamboni, A., Franco, A., Commisso, M., Guzzo, F., Varanini, Z., Pinton, R., Tomasi, N. & Zanin, L. 2022. Nodulating white lupins take advantage of the reciprocal interplay between N and P nutritional responses. *Physiol. Plant.* **174**(1), 13607. doi: 10.1111/ppl.13607
- Cakmak, I., Brown, P., Colmenero-Flores, J.M., Husted, S., Kutman, B.Y., Nikolic, M., Rengel, Z., Schmidt, S.B. & Zhao, F.J. 2023. Micronutrients. In: Rengel, Z., Cakmak, I. & White, P.J. (Eds.), *Marschner's Mineral Nutrition of Plants*. 4th Ed., pp. 283–385. Chennai Ltd., India: Elsevier. <https://doi.org/10.1016/B978-0-12-819773-8.00006-X>
- Carpena, R.O., Esteban, E., Sarro, M.J., Peñalosa, J., Gárate, A., Lucena, J.J. & Zornoza, P. 2000. Boron and calcium distribution in nitrogen-fixing pea plants. *Ciênc. Veg.* **151**(2), 163–170. [https://doi.org/10.1016/S0168-9452\(99\)00210-1](https://doi.org/10.1016/S0168-9452(99)00210-1)
- Castagnara, D.D., Krutzmann, A., Zoz, T., Steiner, F., Conte, E., Castro, A.M., Neres, M.A. & Oliveira, P.S.R.D. 2012. Effect of boron and zinc fertilization on white oats grown in Soil with average content of these nutrients. *Rev. Bras. Zootec.* **41**(7), 1598–1607. <https://doi.org/10.1590/S1516-35982012000700007>
- Chen, Z., Wang, L., Cardoso, J.A., Zhu, S., Liu, G., Rao, I.M. & Lin, Y. 2023. Improving phosphorus acquisition efficiency through modification of root growth responses to phosphate starvation in legumes. *Front. Plant Sci.* **14**, 1094157. doi: 10.3389/fpls.2023.1094157
- Das, H., Devi, N.S., Singh, R.K., Singh, A.H., Singh, L.N. & Singh, N.G. 2020. Influence of boron and lime on boron concentration and dry matter yield of green gram (*Vigna radiata* L.). *Int. J. Chem. Stud.* **8**(6), 631–634. <https://doi.org/10.22271/chemi.2020.v8.i6i.10843>
- Dimande, P., Arrobas, M. & Rodrigues, M.Â. 2024. Intercropped maize and cowpea increased the land equivalent ratio and enhanced crop access to more nitrogen and phosphorus compared to cultivation as sole crops. *Sustainability* **16**, 1440. doi: 10.3390/su16041440

- Dordas, C.A., Lithourgidis, A.S. & Galanopoulou, K. 2019. Intercropping of faba bean with barley at various spatial arrangements affects dry matter and N yield, nitrogen nutrition index, and interspecific competition. *Not. Bot. Horti Agrobot. Cluj-Napoca* **47**(4), 1116–1127. <https://doi.org/10.15835/nbha47411520>
- Enriquez-Hidalgo, D., Gilliland, T.J., Egan, M. & Hennessy, D. 2018. Production and quality benefits of white clover inclusion into ryegrass swards at different nitrogen fertilizer rates. *J. Agric. Sci.* **156**(3), 378–386. <https://doi.org/10.1017/S0021859618000370>
- Hackney, B., Jenkins, J., Powells, J., Edwards, C., Meyer, S.D.D., Howieson, J., Yates, R. & Orgill, S. 2019. Soil acidity and nutrient deficiency cause poor legume nodulation in the permanent pasture and mixed farming zones of south-eastern Australia. *Crop Pasture Sci.* **70**, 1128–1140. <https://doi.org/10.1071/CP19039>
- Hamilton, L.J., Reed, K.F.M., Leach, E.M.A. & Brockwell, J. 2015. Boron deficiency in pasture based on subterranean clover (*Trifolium subterraneum* L.) is linked to symbiotic malfunction. *Crop Pasture Sci.* **66**(11), 1197–1212. <https://doi.org/10.1071/CP14300>
- Hawkesford, M.J., Cakmak, I., Coskun, D., De Kok, L.J., Lambers, H., Schjoerring, J.K. & White, P.J. 2023. Functions of macronutrients. In: Rengel, Z., Cakmak, I. & White, P.J. (Eds.), *Marschner's Mineral Nutrition of Plants*. 4th Ed., Elsevier, Ltd., Chennai, India, pp. 201–281. doi: 10.1016/B978-0-12-819773-8.00019-8.
- Hayes, R.C., Li, G.D., Sandral, G.A., Swan, T.D., Price, A., Hildebrand, S., Goward, L., Fuller, C. & Peoples, M.B. 2017. Enhancing composition and persistence of mixed pasture swards in southern New South Wales through alternative spatial configurations and improved legume performance. *Crop Pasture Sci.* **68**(12), 1112–1130. doi: 10.1071/CP16395
- Holland, J.E., Bennett, A.E., Newton, A.C., White, P.J., McKenzie, B.M., George, T.S., Pakeman, R.J., Bailey, J.S., Fornara, D.A. & Hayes, R.C. 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. *Sci. Total Environ.* **610–611**, 316–332. <https://doi.org/10.1016/j.scitotenv.2017.08.020>
- Hungria, M. & Nogueira, M.A. 2023. Nitrogen fixation. In: Rengel, Z., Cakmak, I. & White, P.J. (Eds.), *Marschner's Mineral Nutrition of Plants*. 4th Ed. India, Chennai, pp 615–650. Elsevier, Ltd. DOI: <https://doi.org/10.1016/B978-0-12-819773-8.00006-X>.
- IPMA (Instituto Português do Mar e da Atmosfera). 2025. *Normais Climatológicas* (in Portuguese) [Climate Normals]. Available online: <https://www.ipma.pt/pt/oclima/normais.clima/> (accessed on 15 May 2025).
- Kochian, L.V., Hoekenga, O.A. & Piñeros, M.A. 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. *Annu. Rev. Plant Biol.* **55**, 459–493. <https://doi.org/10.1146/annurev.arplant.55.031903.141655>
- Lambers, H. 2022. Phosphorus acquisition and utilization in plants. *Annu. Rev. Plant Biol.* **73**, 17–42. DOI: <https://doi.org/10.1146/annurev-arplant-102720-125738>.
- Maxwell, T.M.R., Moir, J.L. & Edwards, G.R. 2013. Phosphorus response and efficiency of four adventive annual clovers grown in a New Zealand high country Soil under glasshouse conditions. *N. Z. J. Agric. Res.* **56**, 203–214. doi: 10.1080/00288233.2013.809775
- McLachlan, J.W., Flavel, R.J. & Guppy, C.N. 2024. Preferential phosphorus placement improves the productivity and competitiveness of tropical pasture legumes. *Legum. Sci.* **6**(2), e235. <https://doi.org/10.1002/leg3.235>
- Mitran, T., Meena, R.S., Lal, R., Layek, J., Kumar, S. & Datta, R. 2018. Role of Soil phosphorus on legume production. In: Meena, R., Das, A., Yadav, G., Lal, R. (eds), *Legumes for Soil Health and Sustainable Management*, pp. 487–510. Springer. doi:10.1007/978-981-13-0253-4_15
- Moir, J., Jordan, P., Moot, D. & Lucas, R. 2016. Phosphorus response and optimum pH ranges of twelve pasture legumes grown in an acid upland New Zealand Soil under glasshouse conditions. *J. Soil Sci. Plant Nutr.* **16**, 438–460. doi: 10.4067/S0718-95162016005000038

- Monjardino, M., Loi, A., Thomas, D.T., Revell, C.K., Flohr, B.M., Llewellyn, R.S. & Norman, H.C. 2022. Improved legume pastures increase economic value, resilience and sustainability of crop-livestock systems. *Agric. Syst.* **203**, 103519. doi: 10.1016/j.agsy.2022.103519
- Morton, J.D. 2020. A review of research on the effect of lime on New Zealand soils and pastures. *N. Z. J. Agric. Res.* **63**(2), 189–201. <https://doi.org/10.1080/00288233.2018.1537293>
- Nyfelner, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J. & Lüscher, A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *J. Appl. Ecol.* **46**(3), 683–691. doi: 10.1111/j.1365-2664.2009.01653.x
- O'Hara, G.W. 2001. Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: A review. *Aust. J. Exp. Agric.* **41**, 417–433. DOI: 10.1071/EA00087
- Olson-Rutz, K. & Jones, C. 2015. *Soil Nutrient Management for Forages: Phosphorus, Potassium, Sulfur and Micronutrients*. Department of Land Resources and Environmental Sciences, Montana State University. Bozeman, MT 59717
- Ovalle, C., Urquiaga, S., Pozo, A.D., Zagal, E. & Arredondo, S. 2006. Nitrogen fixation in six forage legumes in Mediterranean central Chile. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **56**(4), 277–283. <https://doi.org/10.1080/09064710500310246>
- Portela, E., Ferreira-Cardoso, J., Louzada, J. & Gomes-Laranjo, J. 2015. Assessment of boron application in chestnuts: nut yield and quality. *J. Plant Nutr.* **38**(6), 973–987. doi: 10.1080/01904167.2014.963116
- Reguera, M., Bonilla, I. & Bolanos, L., 2010. Boron deficiency results in induction of pathogenesis-related proteins from the PR-10 family during the legume-rhizobia interaction. *J. Plant Physiol.* **167**(8), 625–632.
- Riday, H. & Albrecht, K.A. 2012. Combining Kura clover with forage legumes and grasses to optimize pasture forage legume content. *Agron. J.* **104**(2), 353–362. doi: 10.2134/agronj2011.0088
- Rodrigues, M.Â., Dimande, P., Pereira, E.L., Ferreira, I.Q., Freitas, S., Correia, C.M., Moutinho-Pereira, J. & Arrobas, M. 2015. Early-maturing annual legumes: An option for cover cropping in rainfed olive orchards. *Nutr. Cycl. Agroecosys.* **103**(2), 153–166. <https://doi.org/10.1007/s10705-015-9730-5>
- Sadowsky, M.J. 2005. Soil stress factors influencing symbiotic nitrogen fixation. In: Werner, D. & Newton, W.E. (Eds.), *Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment*. Springer. Dordrecht, The Netherlands, pp. 89–112
- Santachiara, G., Salvagiotti, F. & Rotundo, J.L. 2019. Nutritional and environmental effects on biological nitrogen fixation in soybean: A meta-analysis. *Field Crops Res.* **240**, 106–115.
- Somavilla, A., Ramos Marques, A.C., Caner, L., Oliveira, L.B., Quadros, F.L.F., Chabbi, A., Tiecher, T. & Santos, D.R. 2021. Phosphate fertilization and liming in a trial conducted over 21 years: A survey for greater forage production and Pampa pasture conservation. *Eur. J. Agron.* **125**, 126259. <https://doi.org/10.1016/j.eja.2021.126259>
- Teixeira, R.F., Proença, V., Crespo, D., Valada, T. & Domingos, T. 2015. A conceptual framework for the analysis of engineered biodiverse pastures. *Ecol. Eng.* **77**, 85–97. doi: 10.1016/j.ecoleng.2015.01.002
- Temminghoff, E.E. & Houba, V.J. 2004. *Plant Analysis Procedures*, 2nd ed.; Kluwer Academic Publishers: London, UK. <https://doi.org/10.1007/978-1-4020-2976-9>
- Unkovich, M., Herridge, D., Peoples, M., Cadish, G., Boddey, B., Giller, K., Alves, B. & Chalk, P. 2008. *Measuring Plant-Associated Nitrogen Fixation in Agricultural Systems* (ACIAR Monograph **136**, 258 pp.). Australian Center for International Agricultural Research.

- Upjohn, B., Fenton, G. & Conyers, M. 2005. *Soil Acidity and Liming*, 3rd ed.; Agfact AC.19. Department of Primary Industries, Wellington, Australia, 24 pp.
- Van Reeuwijk, L.P. 2002. *Procedures for Soil Analysis*, 6th ed., Technical Paper 9. International Soil Reference and Information Centre: Wageningen, The Netherlands, 101 pp.
- Veloso, A., Sempiterno, C., Calouro, F., Rebelo, F., Pedra, F., Castro, I.V., Gonçalves, M.C., Marcelo, M.E., Pereira, P., Fareleira, P., Jordão, P., Mano, R. & Fernandes, R. 2022. *Manual Fertilização das Culturas* [Crop Fertilization Manual]. INIAV-Instituto Nacional de Investigação Agrária e Veterinária, I.P. Oeiras, Portugal, 468 pp. (in Portuguese).
- Weil, R.R. & Brady, N.C. 2017. *Nature and Properties of Soils*, 15th ed.; Pearson: London, UK, 1086 pp.
- WRB (World Reference Base for Soil Resources). 2022. *International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*. (4th ed.). International Union of Soil Sciences (IUSS), Working Group. Austria, Vienna, 236 pp.
- Yang, Q., Lin, Y., Jin, L., Ren, X., He, C. & Liu, Q. 2021. Responses of mineral nutrient contents and transport in red clover under aluminum stress. *Legum. Sci.* **3**(4), 94. <https://doi.org/10.1002/leg3.94>
- Zhou, J., Sun, X., Chen, C. & Chen, J. 2023. The effect of molybdenum fertilizer on the growth of grass-legume mixtures related to symbiotic rhizobium. *Agronomy* **13**, 495. <https://doi.org/10.3390/agronomy13020495>