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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

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Abstract: The success of growing legumes as green manure depends on their spatial and temporal integration within agroecosystems, which minimizes competition with cash crops, and on their nitrogen (N) fixation potential. This study evaluated seven legume species for biomass production, N fixation, and suitability for use in cropping systems in northern Portugal. Oats (*Avena sativa* L.) were grown to estimate the N fixation using the difference method, as a non-legume reference crop is required for this purpose, and oats are widely grown in the region. The study was conducted over four cropping cycles (2021–2024) in two climate zones across four land plots. The results indicated that the biomass production and N fixation varied by the species/cultivar and cropping cycle, which was significantly influenced by spring precipitation. Broad beans (*Vicia faba* L.) failed to develop in one cycle on highly acidic soil (pH 4.9), showing negative N fixation values when calculated by the difference method. Conversely, the lupins maintained a relatively high level of N fixation across all the conditions, demonstrating strong environmental adaptability. Thus, the N fixation values across the four cycles ranged from −5.4 to 419.4 kg ha^{-1} for broad bean (cv. Favel), while yellow lupin (*Lupinus luteus* L.) exhibited average values between 204.0 and 274.0 kg ha^{-1}. The percentage of N derived from the atmosphere (%Ndfa) ranged from −13.3 to 91.6, −39.4 to 85.8, 83.8 to 94.7, 74.9 to 94.3, 72.8 to 92.2, 23.1 to 75.8, and 11.7 to 21.7 for these species/cultivars. Due to their environmental adaptability, biomass production, and N fixation capacity, these legumes could be used as green manure in inter-rows of woody crops or in summer annual crops like tomatoes and maize, grown in winter as an alternative to fallow land. The lupins showed strong promise due to their environmental resilience.

Keywords: *Vicia faba*; *Lupinus luteus*; *Lupinus albus*; *Lupinus angustifolius*; *Trifolium incarnatum*; *Trifolium subterraneum*; biological nitrogen fixation; nitrogen derived from atmosphere

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Citation: Aguiar, P.; Arrobas, M.; Nharreluga, E.A.; Rodrigues, M.Â. 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* **2024**, *16*, 10725. https://doi.org/10.3390/su162310725

Academic Editor: Jan Hopmans

Received: 13 November 2024
Revised: 28 November 2024
Accepted: 4 December 2024
Published: 6 December 2024

1. Introduction

Agricultural productivity has significantly increased in recent decades due to the high intensification of cropping systems driven by external inputs [1,2]. Commercial fertilizers, particularly N, have boosted crop productivity [3–5]. Soils under continuous cultivation cannot naturally replenish the N in the soil solution to meet the substantial amounts extracted by crops [6,7]. Consequently, N fertilizers are applied to offset the nutrient depletion caused by harvests. In 2022, 108.1 × 10^6 t N was used globally in agriculture [8].

Despite the significant role of N fertilizer in the Green Revolution that followed the Second World War, the N use efficiency by crops tends to be low. In major crops, no more than 50% of the N applied as a fertilizer is recovered by plants [6,9], with the remaining portion being lost to watercourses and the atmosphere. The eutrophication of rivers, lakes, and oceans due to nitrate leaching from agricultural fields [10,11], as well as the emission of greenhouse gases such as N oxides into the atmosphere due to denitrification [12,13], are major environmental problems associated with agricultural activity mostly linked to the excessive use of N.

Organic amendments are often seen as a good alternative to industrial synthetic fertilizers. They improve soils' physical, chemical, and biological properties [14–16] and are commonly recognized for their specific manuring effect that cannot be replaced by adding mineral fertilizers [7,17]. However, organic amendments are currently unavailable in sufficient quantities to meet the needs of major crops. In vast regions of the world, they even have poor availability due to the specialization of agriculture, with intense mechanization that has reduced mixed farming systems [4,15].

One possible alternative to bringing external N into agricultural systems through a natural process is to increase the cultivation of legumes. Most species in the Fabaceae family can establish symbiotic relationships with microorganisms of the Rhizobiaceae family that can fix N from the atmosphere. N fixation associated with legumes can vary greatly depending on the species and cultivation conditions [18,19]. Russelle [18] summarized the wide range of values reported in the international literature, which can be as low as 20 kg ha^{-1} yr^{-1} or exceed 500 kg ha^{-1} yr^{-1}, as observed in crops such as alfalfa (*Medicago sativa* L.).

Farmers often use legumes in intercropping with species from other botanical groups in the expectation that they will use atmospheric N for their growth and provide some to the companion species [20,21]. Using legumes as cover crops is also common in perennial woody crops, considering that legumes provide N to the main crop [22–24]. In northern Portugal, for example, it is common to cultivate white lupin (*Lupinus albus* L.) in low-intensity olive groves to reduce the need for N fertilizers. These cover crops are often incorporated into the soil in spring or, less frequently, left on the surface as mulch. Furthermore, legumes are recommended as strategic species in crop rotations because they leave a N-rich residue for the following crop, reducing the need for N fertilization [25–27]. However, the greatest potential of legumes to supply N to subsequent crops is realized as green manure, where they are grown not for food but to be incorporated into the soil [28,29]. All these forms of using legumes have been promoted in recent years in the search for more sustainable agricultural practices and to support organic farming systems [24,28,30].

Despite all of the recognized benefits of using legumes in agricultural systems, their expansion has not been as widespread as the supposed advantages might suggest. It is fundamental to consider that legumes compete for space and time with other cultivated species. On the other hand, they cannot replace major crops such as rice, corn, wheat, potatoes, and many others in human nutrition. Therefore, for their cultivation to expand, it is necessary to assess how they can enter cropping systems as a complementary component rather than in competition with other cultivated plants. The environmental adaptability, cycle length, and N-fixing capacity are also crucial for their successful inclusion in agricultural systems. These represent the most significant gaps in previous studies involving legumes, warranting the efforts of researchers worldwide to promote the cultivation of legumes in agroecosystems. These gaps also form the rationale for conducting this study.

Thus, this study assessed the potential of seven annual legumes for use as green manure. The dry matter yield (DMY) and N fixation capacity were evaluated, with these variables being related to the phenological stage of the plants. Oats, a species unable to access atmospheric N, were also cultivated to enable comparison and estimate N fixation by legumes using the N difference method. The study was conducted in northeastern Portugal and involved four experimental field trials divided over three growing seasons (2021/2022–2023/2024), with 2022/2023 involving two field plots.

2. Materials and Methods

2.1. General Characterization of the Experimental Plots

Four field trials were conducted over three years in three rainfed managed olive groves from October 2021 to June 2024. The first trial, hereafter referred to as L1/22 (location 1, year 2022), was set up in the inter-row of a young six-year-old olive grove located in Bragança (41°39′21.7″ N 6°33′30.9″ W). In the second year, two trials were established: one in Mirandela (41°30′50.8″ N 7°11′14.4″ W) in the inter-row of an olive grove over 50 years old, designated as L2/23 (location 2, year 2023), and another in Bragança (41°48′27.9″ N 6°43′59.9″ W) in a 10-year-old olive grove, designated as L3/23 (location 3, year 2023). The trial in the third year was conducted in Bragança (41°48′28.1″ N 6°44′00.8″ W) in a second plot of the same olive grove from the previous year, designated as L4/24 (location 4, year 2024).

The climate in the regions of Bragança and Mirandela is typically Mediterranean. According to the Köppen classification, Bragança has a Csb climate, while Mirandela has a Csa climate [31], reflecting temperature and precipitation differences between the two regions. In Bragança, the annual precipitation is 772.7 mm, and the average annual temperature is 12.6 °C. In Mirandela, the annual precipitation is 509 mm, and the average annual temperature is 14.3 °C. The climatological normal and meteorological records for the periods under study are presented in Figure 1.

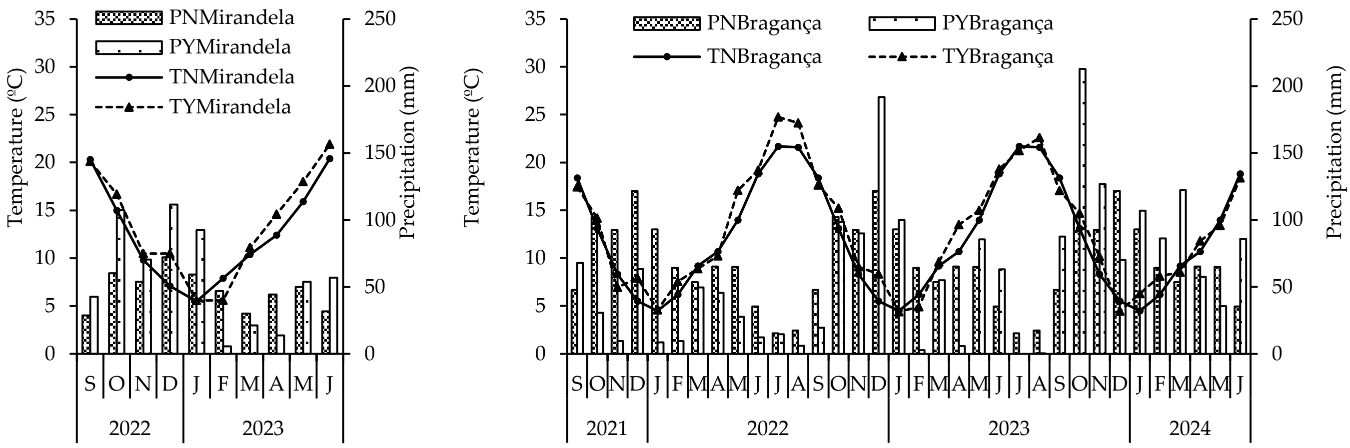


Figure 1. Climatological normal (N) and annual values (Y) of monthly temperature (T) and precipitation (P) in Mirandela (**left**) and Bragança (**right**) for the respective study periods.

The soil in which the first trial (L1/22) was conducted is a dystric Leptosol with a sandy loam texture (121 g kg^{-1} clay, 222 g kg^{-1} silt, and 657 g kg^{-1} sand), characterized by high acidity (pH 4.9), low organic carbon (C) content (3.1 g kg^{-1}), and low phosphorus (P) availability (14.1 g kg^{-1} P_2O_5). The soil for the L2/23 experiment is also a dystric Leptosol but with a loamy sand texture (61, 173, and 766 g kg^{-1} clay, silt, and sand, respectively), similarly low in organic C (8.5 g kg^{-1}). The soils for the L3/23 and L4/24 experiments are eutric Regosols with a sandy loam texture. For L3/23, the soil separates were 145 g kg^{-1} clay, 277 g kg^{-1} silt, and 578 g kg^{-1} sand, while for L4/24, they were 146 g kg^{-1} clay, 292 g kg^{-1} silt, and 562 g kg^{-1} sand. Other soil properties, determined before the installation of the trials from composite soil samples collected at a depth of 0 to 0.20 m, are presented in Table 1.

Table 1. Soil properties (mean ± standard deviation) at the time of experiment installation at different locations and years (L1/22, L2/23, L3/23, and L4/24), based on composite soil samples collected at 0 to 0.20 m depth.

	L1/22	L2/23	L3/23	L4/24
[1] pH(H_2O)	4.9 ± 0.12	5.9 ± 0.11	5.8 ± 0.17	5.6 ± 0.32
[2] Organic carbon (g kg^{-1})	3.1 ± 0.37	8.5 ± 0.59	27.4 ± 3.26	26.5 ± 2.42
[3] Extr. phosphorus (mg kg^{-1}, P_2O_5)	14.1 ± 3.50	93.1 ± 9.5	88.3 ± 11.44	91.8 ± 10.51
[3] Extr. potassium (mg kg^{-1}, K_2O)	119.0 ± 7.94	157.6 ± 17.5	97.6 ± 9.45	108.9 ± 11.01
[4] Exch. calcium ($cmol_+$ kg^{-1})	1.3 ± 0.31	3.9 ± 0.62	7.3 ± 0.20	6.6 ± 0.57
[4] Exch. magnesium ($cmol_+$ kg^{-1})	0.6 ± 0.16	0.7 ± 0.13	2.2 ± 0.35	2.4 ± 0.17
[4] Exch. potassium ($cmol_+$ kg^{-1})	0.3 ± 0.01	0.3 ± 0.05	0.2 ± 0.03	0.3 ± 0.10
[4] Exch. sodium ($cmol_+$ kg^{-1})	0.6 ± 0.02	0.8 ± 0.11	0.4 ± 0.05	0.4 ± 0.04
[4] Exch. acidity ($cmol_+$ kg^{-1})	1.2 ± 0.06	0.1 ± 0.02	0.1 ± 0.06	0.1 ± 0.06
[5] Cation exch. capacity ($cmol_+$ kg^{-1})	3.9 ± 0.48	5.6 ± 1.50	10.1 ± 0.14	9.7 ± 0.36
[6] Extr. boron (mg kg^{-1})	0.05± 0.02	1.0 ± 0.20	0.5 ± 0.12	0.6 ± 0.10

[1] Potentiometry; [2] wet digestion (Walkley–Black); [3] Egnér–Riehm; [4] ammonium acetate; [5] cation exchange capacity; [6] hot-water azomethine-H.

2.2. Experimental Design and Installation of the Trials

The plants used in the study have an autumn/winter cycle, which, in the northern hemisphere, corresponds to autumn sowing (September through November), with crop development continuing until the end of the following Spring (May through July).

In this study, seven legumes and one grass species were used to determine the amount of N fixed by the N difference method [32]. The grass species used was oat (*A. sativa* L., cv. Boa Fé), a widely cultivated and highly adaptable plant in the region. The legumes included subterranean clover (*Trifolium subterraneum*, L. ssp. *subterraneum* Katzn. and Morley, cv. Dalkeith); crimson clover (*Trifolium incarnatum* L., cv. Diogene); yellow lupin (*L. luteus* L., cv. Nacional); white lupin (*L. albus* L., cv. Estoril); narrow-leaf lupin (*Lupinus angustifolius* L., cv. Karo); and two broad bean varieties (*V. faba* L., cvs. Favel and Vesuvio). Each species/cultivar was seeded in a plot defined by four trees in two contiguous rows (49 m^2, 7 m × 7 m), replicated three times in a completely randomized design. The seed rates used were as follows: crimson clover, 10 kg ha^{-1}; subterranean clover, 25 kg ha^{-1}; oats, 120 kg ha^{-1}; yellow and narrow-leaf lupins, 180 kg ha^{-1}; white lupin and broad bean, cv. Vesuvio, 200 kg ha^{-1}; and broad bean, cv. Favel, 300 kg ha^{-1}.

Sowing occurred on 10 October 2021, 11 November 2022, 12 November 2022, and 20 September 2023, for the L1/22, L2/23, L3/23, and L4/24 experiments, respectively. The olive groves where the trials were conducted are typically managed using conventional tillage practices. Before sowing, a cultivator was used to prepare the soil, followed by manual broadcast sowing. Only a smooth wooden bar attached to the cultivator was used to minimize the risk of planting seeds too deep for the plots designated for subterranean clover and crimson clover. For the plots of the other species, the cultivator was used superficially, aiming to incorporate the seeds at a depth of 5 to 8 cm.

2.3. Soil and Plant Sampling and Pre-Treatments

At the beginning of each trial, composite soil samples (10 random cores per experimental unit) were collected for plot characterization. The samples were oven-dried at 40 °C and then sieved through a 2 mm mesh.

During the growing season, the phenological stages were evaluated according to the BBCH scale (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) [33]. The aboveground parts of the plants were cut 3 to 4 times during the growing season, from early winter to late spring, up to the full bloom/first pods visible, to capture the most active phase of biomass growth and N fixation, which occurs as air temperatures rise. A square metal grid, measuring 0.5 m on each side (0.25 m^2 samples), and electric shear were used, trying to cut as close to ground level as possible. The harvested biomass was oven-dried at 70 °C until a constant weight was achieved. The biomass was then ground in a mill until it passed through a 1 mm mesh.

2.4. Laboratory Analyses

The soil samples were analyzed for pH (H_2O and KCl) (soil/solution, 1:2.5), cation exchange capacity (ammonium acetate, pH 7.0), organic carbon (wet digestion, Walkley–Black method), and extractable P and potassium (ammonium lactate extract, Egnér–Riehm method). Soil boron was extracted using hot water and determined by the azomethine-H method. For full details on these analytical procedures, refer to van Reeuwijk [34].

The analysis of N in plant tissues involved the use of the Kjeldahl method, which consists of sample mineralization with sulfuric acid and a selenium-based catalyst, followed by distillation with sodium hydroxide and measurement of the NH_3 carried in the steam stream by titration [35].

2.5. Data Analysis

The DMY, N concentration in plant tissues, and total N in the aboveground parts of the plants varied greatly among species/cultivars, rendering the analysis of variance redundant. Thus, most data were presented as means with their standard deviations.

The N fixed by legumes was estimated using the N difference method based on the difference in N content between the legumes and a non-fixing crop [32]. In this study, oats were used as a non-fixing crop. The method assumes that legumes uptake soil N similarly to non-legumes, with the difference attributed to N fixed from the atmosphere.

3. Results

3.1. Dry Matter Yield

The DMY increased over time in most of the species/cultivars from the first to the last samplings (Figure 2). However, the values varied significantly depending on the species/cultivar and the location/year of cultivation. The location/year combination L4/24 resulted in the highest DMY across all the legumes except for subterranean clover. In L4/24, subterranean clover reached 3.9 t ha^{-1} (compared to 5.9 t ha^{-1} in L1/22), oats reached 6.0 t ha^{-1}, crimson clover 9.0 t ha^{-1}, broad bean (cv. Vesuvio) produced 14.3 t ha^{-1}, yellow lupin 15.3 t ha^{-1}, narrow-leaf lupin 17.3 t ha^{-1}, broad bean (cv. Favel) 19.5 t ha^{-1}, and white lupin 20.4 t ha^{-1}. Some legumes recorded their lowest values in the final sampling under the location/year combination L2/23, namely, subterranean clover with 0.8 t ha^{-1}, crimson clover with 0.9 t ha^{-1}, oats with 1.2 t ha^{-1}, and white lupin with 4.3 t ha^{-1}. For other species/cultivars, the lowest values were recorded under the location/year combination L1/22, including broad beans (cv. Vesuvio) with 2.3 t ha^{-1}, broad beans (cv. Favel) with 4.1 t ha^{-1}, and narrow-leaf lupin with 6.4 t ha^{-1}. In 2023, the experiment was conducted in two locations, with the highest DMY for all species observed in the L3/23 combination, i.e., in the trial conducted in Bragança.

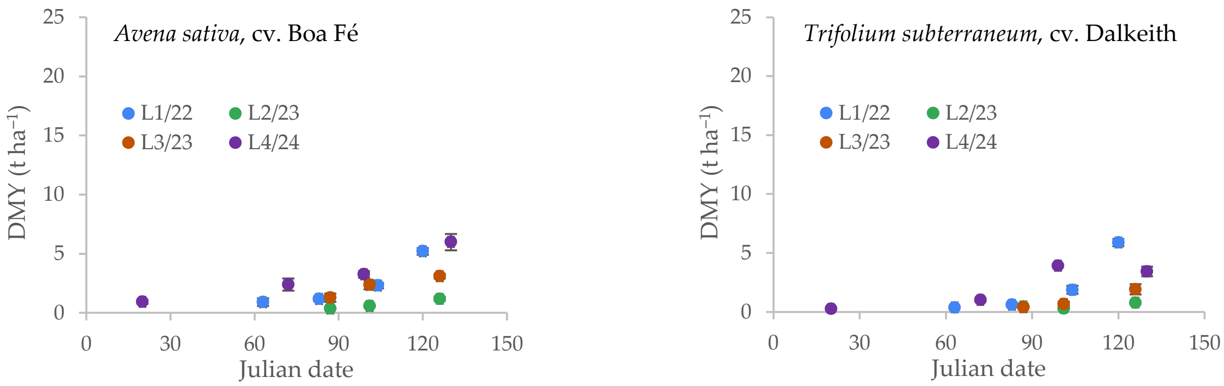


Figure 2. *Cont.*

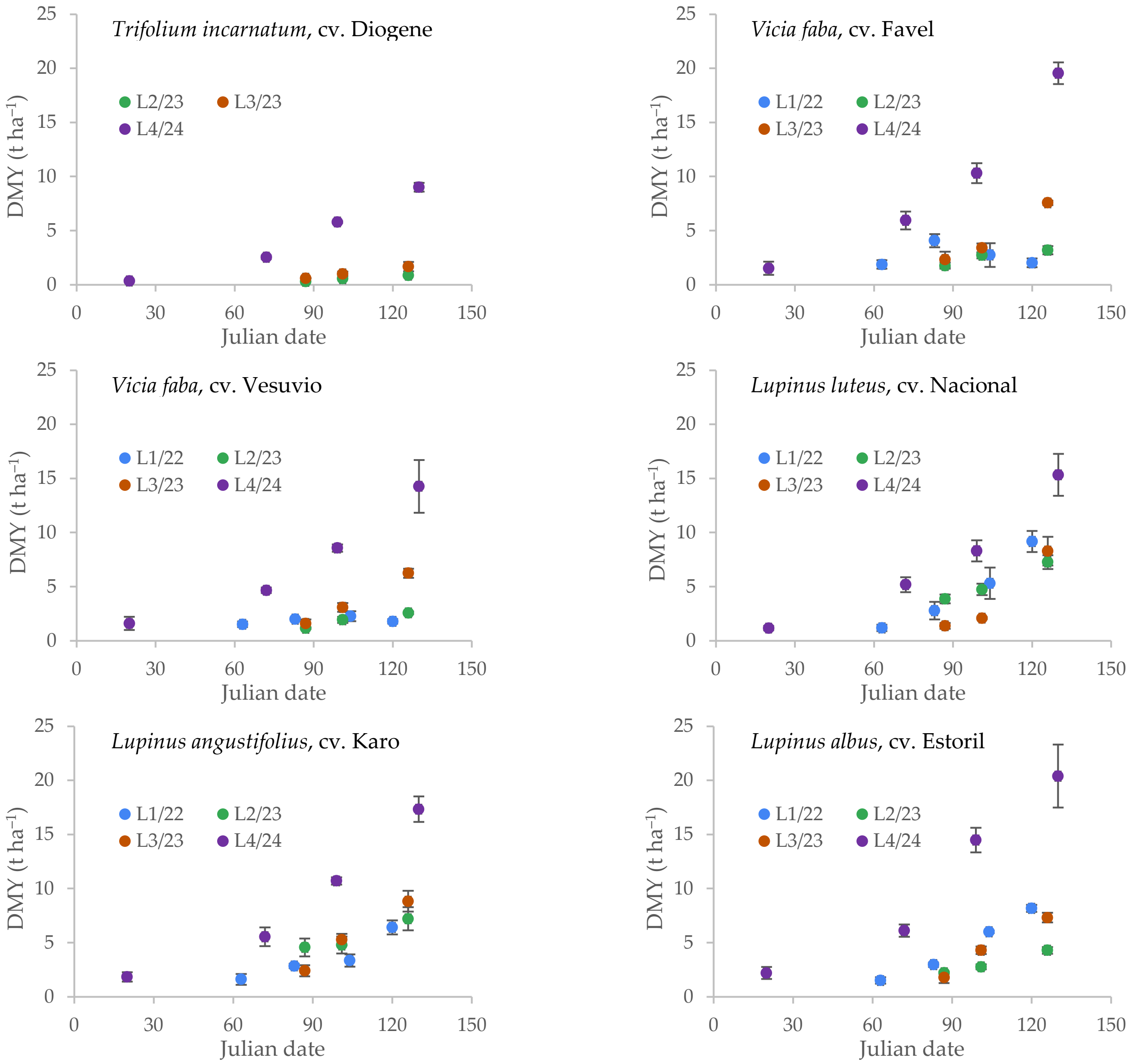


Figure 2. Dry matter yield (DMY) of eight species/cultivars grown under four location/year combinations [Bragança 2022 (L1/22), Mirandela 2023 (L2/23), Bragança 2023 (L3/23), and Bragança 2024 (L4/24)]. The *X*-axis represents Julian dates (starting from 1 January) to express the dates as a continuous variable. Error bars represent standard deviations (n = 3).

3.2. Nitrogen Concentration in Plant Tissues

In contrast to the DMY, the N concentration in the plant tissues decreased throughout the growing seasons across all the cultivated species. However, this decline was more pronounced in some species than in others (Figure 3). In certain species/cultivars, differences between the location/year combinations were not particularly significant, whereas in others, these effects were very pronounced. Subterranean and crimson clovers are notable examples of the first group, where the differences were less marked. In contrast, broad beans stand out in the second group, exhibiting a more evident response to the location/year variation.

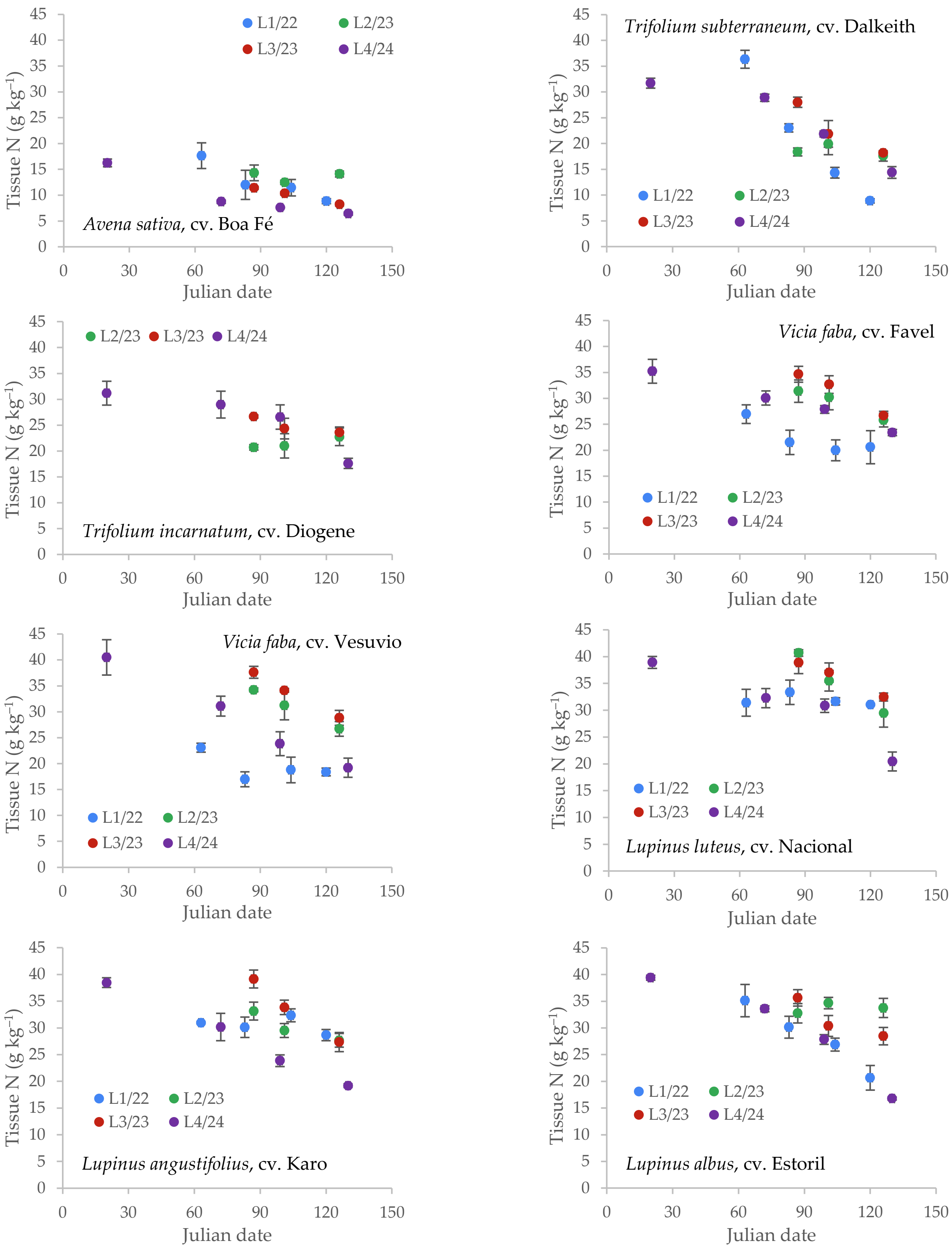


Figure 3. Tissue nitrogen (N) concentrations in eight species/cultivars grown under four location/year combinations [Bragança 2022 (L1/22), Mirandela 2023 (L2/23), Bragança 2023 (L3/23), and Bragança 2024 (L4/24)]. The *X*-axis represents Julian dates (starting from 1 January) to express the dates as a continuous variable. Error bars represent standard deviations (n = 3).

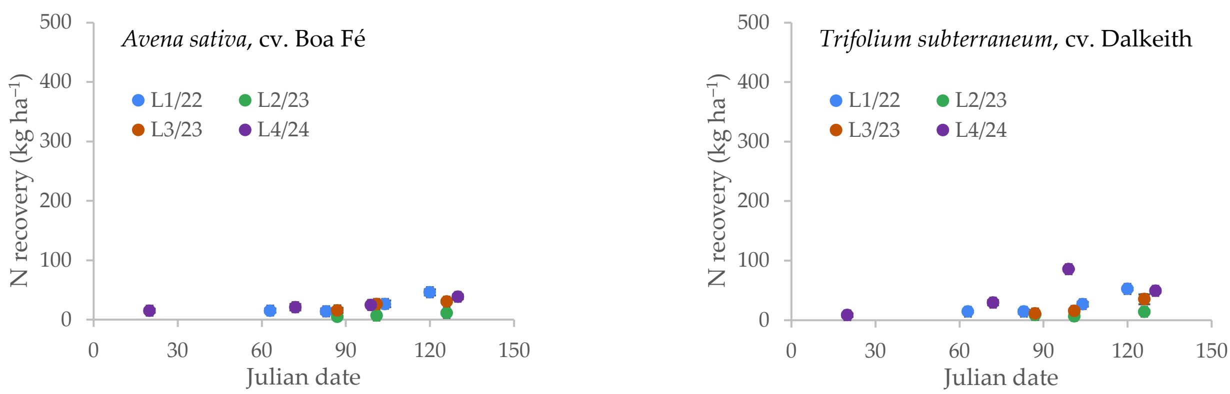
Considering all of the growing cycles and sampling dates, the oats exhibited average N concentrations ranging from 17.6 to 6.4 g kg^{-1}, showing a slightly decreasing trend over the growing season, with no significant impact of the location/year combination. In subterranean clover, the average values varied from 36.3 to 14.4 g kg^{-1}, with a pronounced downward trend throughout the growing season and minimal influence from location or year. Crimson clover showed average values ranging from 31.2 to 17.6 g kg^{-1}, with no relevant differences across the location/year combinations. The broad bean, cv. Vesuvio, recorded a maximum average value of 40.5 g kg^{-1} and a minimum of 18.4 g kg^{-1}, with notably lower values observed in the L1/22 growing condition. Similarly, the Favel cultivar maintained the pattern of low values in the L1/22 combination, with maximum and minimum average values of 35.2 and 20.0 g kg^{-1}, respectively, across all the location/year combinations. Yellow lupin registered maximum and minimum average values of 40.7 and 20.4 g kg^{-1}, displaying a similar decreasing pattern across all the growing cycles. Narrow-leaf lupin recorded the highest and lowest average values at 39.2 g kg^{-1} and 19.2 g kg^{-1}, respectively, while in white lupin, these values were 39.4 and 16.8 g kg^{-1}.

3.3. Nitrogen Recovery in Aboveground Biomass

The N recovered in the aboveground biomass was calculated as the product of the DMY and the N concentration in the tissues. The results are presented in Figure 4. The multiplicative nature of this estimation amplifies the differences between the species/cultivars and location/year combinations. However, the general trend of the increasing N content in the aboveground biomass throughout the growing season remains evident, with most of the species/cultivars and location/year combinations showing their highest values in the final sampling.

In the samplings with the highest values for each growing cycle, the oats showed average values ranging from 11.3 kg ha^{-1} (L2/23) to 46.0 kg ha^{-1} (L4/24). For subterranean clover, the values ranged from 14.0 kg ha^{-1} (L2/23) to 52.2 kg ha^{-1} (L4/24), and for crimson clover, they ranged from 19.7 kg ha^{-1} (L2/23) to 158.7 kg ha^{-1} (L4/24). The broad bean, cultivar Favel, recorded values between 82.2 kg ha^{-1} (L2/23) and 457.8 kg ha^{-1} (L4/24), while the cultivar Vesuvio showed values from 42.3 kg ha^{-1} (L1/22) to 271.3 kg ha^{-1} (L4/24). Yellow lupin registered values between 215.3 kg ha^{-1} (L2/23) and 312.4 kg ha^{-1} (L4/24), narrow-leaf lupin ranged from 183.6 kg ha^{-1} (L1/22) to 333.1 kg ha^{-1} (L4/24), and white lupin ranged from 144.8 kg ha^{-1} (L2/23) to 341.6 kg ha^{-1} (L4/24).

These results highlight substantial differences between the species regarding the highest recorded values, with the oats (46.0 kg ha^{-1}) and the broad bean, cultivar Favel (457.8 kg ha^{-1}), representing opposite extremes. The Favel cultivar also exhibited the most pronounced variation across the location/year combinations, ranging from 82.2 kg ha^{-1} in L2/23 to 457.8 kg ha^{-1} in L4/24.



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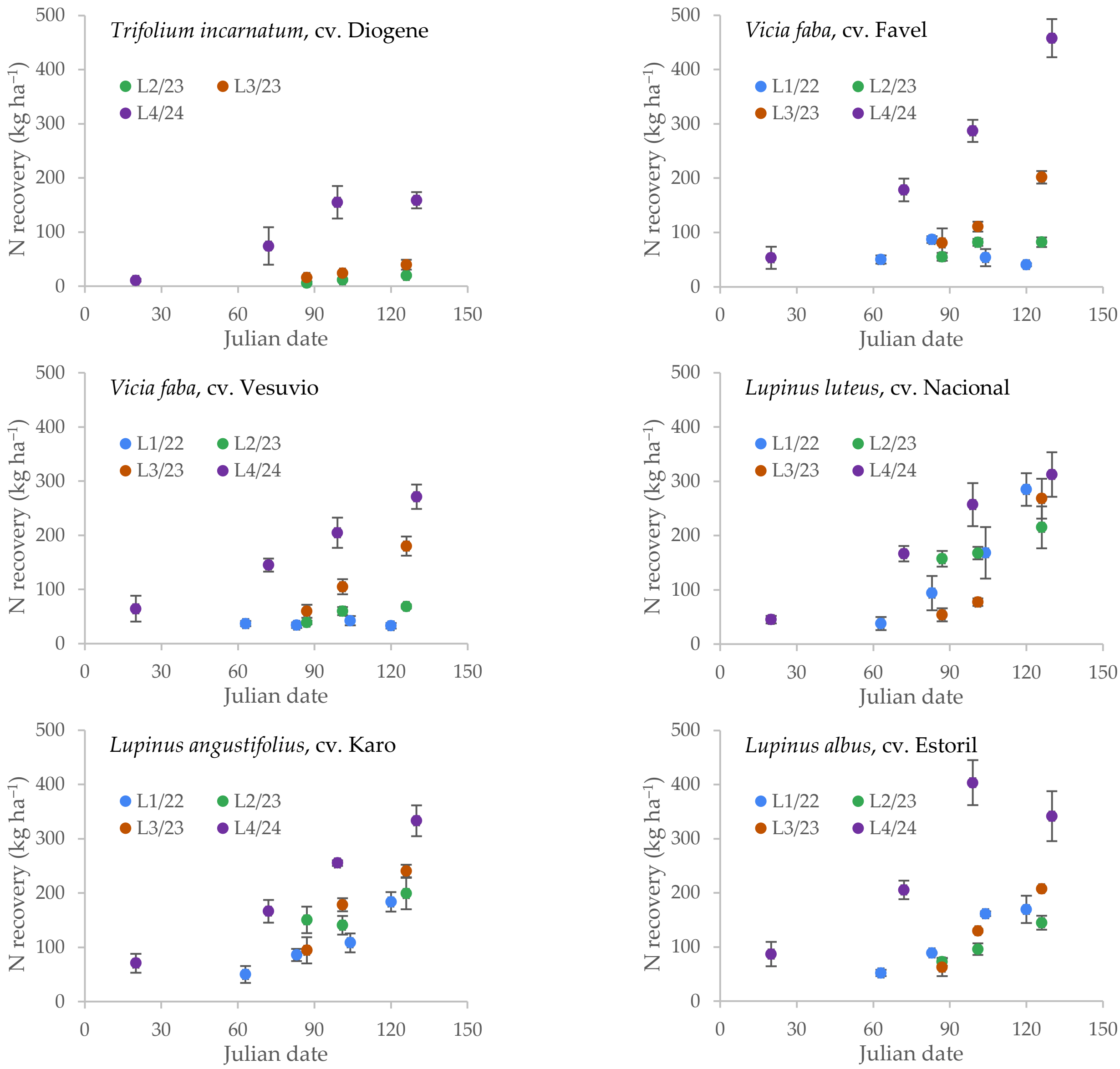

Figure 4. Nitrogen (N) recovery in aboveground biomass in eight species/cultivars grown under four location/year combinations [Bragança 2022 (L1/22), Mirandela 2023 (L2/23), Bragança 2023 (L3/23), and Bragança 2024 (L4/24)]. The *X*-axis represents Julian dates (starting from January 1) to express the dates as a continuous variable. Error bars represent standard deviations (n = 3).

3.4. Nitrogen Fixation by Legumes

The values in Table 2 represent the estimates of N fixed by the legumes. These estimates were calculated by subtracting the N recovered by the legumes at their peak N content from the N recovered by oats. Table 2 also indicates the timing of the maximum N fixation across the four growing conditions.

Subterranean clover did not fix significant amounts of N. The average values were found to be between 2.6 and 10.7 kg ha^{-1}. Crimson clover also showed modest results in L2/23 and L3/23, with considerably higher values recorded in L4/24 (120.4 kg ha^{-1}). The broad beans exhibited highly contrasting N fixation values across different location/year combinations. The Favel cultivar showed a negative value in L1/22 but achieved a remarkably high peak of 419.4 kg ha^{-1} in L4/24. The Vesuvio cultivar also showed a negative value in L1/22, reaching 232.9 kg ha^{-1} in L4/24. Yellow lupin exhibited consistent N fixation values across all the cultivation conditions, with average values ranging between 204.0 and 274.0 kg ha^{-1}. Narrow-leaf lupin recorded relatively high values in all the cultivation conditions, with the lowest average in L1/22 (137.6 kg ha^{-1}) and the highest in L4/24 (294.8 kg ha^{-1}). White lupin displayed a similar pattern to that of narrow-leaf lupin, with the lowest average value in L1/22 (123.2 kg ha^{-1}) and the highest in L4/24 (303.2 kg ha^{-1}).

Table 2. Nitrogen (N) fixation (kg ha^{-1}) by the legumes cultivated under the four location/year combinations [Bragança 2022 (L1/22), Mirandela 2023 (L2/23), Bragança 2023 (L3/23), and Bragança 2024 (L4/24)], estimated by the difference method (N in legume shoots − N in oat shoots).

	L1/22	L2/23	L3/23	L4/24	Full Flowering
Trifolium subterraneum, cv. Dalkeith	6.2	2.6	4.6	10.7	30 March–15 April
Trifolium incarnatum, cv. Diogene	---	9.4	9.2	120.4	30 April–20 May
Vicia faba, cv. Favel	−5.4	70.8	171.3	419.4	20 April–5 May
Vicia faba, cv. Vesuvio	−13.0	57.4	149.7	232.9	18 April–2 May
Lupinus luteus, cv. Nacional	238.8	204.0	237.6	274.0	25 April–10 May
Lupinus angustifolium, cv. Karo	137.6	187.8	210.0	294.8	10–30 April
Lupinus albus, cv. Estoril	123.2	133.5	177.3	303.2	30 April–20 May

Some differences were also observed regarding earliness, with the subterranean clover and the narrow-leaf lupin being early-maturing, typically reaching full flowering until April 15 and 30, respectively. The differences between the broad bean cultivars and yellow lupin were minor, with flowering between late April and early May. The crimson clover and white lupin were slightly late-maturing than the others, with full bloom between April 30 and May 20.

The percentage of N derived from the atmosphere (%Ndfa) was notably low in subterranean clover, with average values ranging between 11.7% and 21.7% (Table 3). Similarly, crimson clover exhibited low %Ndfa values, ranging from 23.1% to 42.5%, except for the L4/24 cultivation condition, which reached 75.8%. The broad beans showed negative values under the L1/22 growing condition but later displayed consistently high values, always exceeding 80%. Lupins demonstrated high and consistent values across all growing conditions, with %Ndfa always above 70% and occasionally surpassing 90%.

Table 3. Percentage of nitrogen derived from atmosphere (%Ndfa) in the legumes cultivated under the four location/year combinations [Bragança 2022 (L1/22), Mirandela 2023 (L2/23), Bragança 2023 (L3/23), and Bragança 2024 (L4/24)], estimated by the difference method [100 × (N in legume shoots − N in oat shoots)/N in legume shoots].

	L1/22	L2/23	L3/23	L4/24	Full Flowering
Trifolium subterraneum, cv. Dalkeith	11.7	18.8	13.1	21.7	30 March–15 April
Trifolium incarnatum, cv. Diogene	---	42.5	23.1	75.8	30 April–20 May
Vicia faba, cv. Favel	−13.3	86.2	84.9	91.6	20 April–5 May
Vicia faba, cv. Vesuvio	−39.4	83.5	83.1	85.8	18 April–2 May
Lupinus luteus, cv. Nacional	83.8	94.7	88.6	87.7	25 April–10 May
Lupinus angustifolium, cv. Karo	74.9	94.3	87.3	88.5	10–30 April
Lupinus albus, cv. Estoril	72.8	92.2	85.3	88.8	30 April–20 May

4. Discussion

4.1. Crop Growth and Dry Matter Yield

In general, DMY increased from the first to the final cut, except for the two broad bean cultivars, particularly cv. Favel in the L1/22 combination and the subclover in the L4/24 combination. The increase in the DMY over the growth cycle is a direct consequence of the plants' phenological development and growth, which continued to progress until the final biomass cut, close to full flowering/first pod visible. This outcome aligns with typical findings in studies evaluating the DMY over time [36–38]. Under the L1/22 growing condition, the broad beans displayed a low DMY. They exhibited symptoms of leaf burn, possibly due to high soil acidity, with a pH level (4.9) below the recommended range for this species [39,40]. Although limited information is available on the pH adaptability of the cultivars used in this study, the DMY was markedly compromised, with no other apparent cause. It appears advisable for future research to consider this variable, particularly given that soils in Portugal [4,22] and globally [6,7] are predominantly acidic. The subclover used in this study (Dalkeith) is an early-maturing cultivar [41]; therefore, unlike other legumes, it did not fully benefit from the wetter spring of 2024. This year, its peak productivity was reached by the penultimate cut.

Overall, the L4/24 combination resulted in the highest DMY across all the legume species. Conversely, the L2/23 combination generally led to low yields across the species and cultivars. In 2024, the precipitation levels remained high throughout the spring (Figure 1), promoting plant growth. In Mediterranean climates, the legumes used in this study are typically grown as autumn/winter crops [23,38,39]. Because they are cold-resistant, they are sown in the autumn to benefit from winter and early spring rainfall. Consequently, their productivity heavily depends on spring precipitation as vegetative growth expands [22,23,42]. The very low yields recorded in the L2/23 combination support this hypothesis, as the trial was conducted in a significantly drier region where spring precipitation was incomparably lower (Figure 1). As noted by Berger and Ludwig [43], reproductive strategies become increasingly conservative as rainfall decreases or becomes more variable, accelerating reproduction and senescence at the expense of biomass production. Therefore, it appears that climatic conditions, rather than soil differences, were the primary drivers of the biomass production disparities observed between the L2/23 and L4/24 combinations.

In the most favourable location/year combination (L4/24) for most species, white lupin achieved the highest DMY (20.4 t ha^{-1}), followed by broad bean (cv. Favel) (19.5 t ha^{-1}), narrow-leaf lupin (17.3 t ha^{-1}), yellow lupin (15.3 t ha^{-1}), and broad bean (cv. Vesuvio) (14.3 t ha^{-1}). These values greatly surpass the typical biomass yields reported in the literature for these species, which generally range from 5 to 10 t ha^{-1} [36,38,44], as well as those observed in the other cultivation conditions (L1/22, L2/23, and L3/23). This indicates that the environmental conditions in L4/24 were particularly favourable. The lupins and broad beans used in this study are relatively tall plants with a high biomass production potential [22,39,40]. Although crimson clover and subclover did not achieve biomass levels as high as those of the taller species, they still reached magnitudes like those reported in other studies under optimal growing conditions [41,45]. The oats yielded a substantially lower biomass (6.0 t ha^{-1}), surpassing only subclover (3.9 t ha^{-1}). As a non-legume species, oats lack access to atmospheric N and cannot fully benefit from spring precipitation. These soils are notably N-poor, with low organic matter content, limited depth, and clay levels (Table 1). Organic matter is typically the primary N reserve in soil [6,7], as is the clay content, particularly 2:1 clay, in which ammonium N can be fixed, contributing to soil structure stabilization by balancing the negative charges arising from the isomorphic substitution of silicon by aluminum [7,46].

Across the four cultivation conditions, there was significant variation in the DMY for all the species. Such variation is frequently observed in field studies, although identifying cause–effect relationships can be challenging due to the influence of various pedoclimatic and biotic factors [38,47,48]. Nevertheless, the species within the *Lupinus* genus consistently exhibited relatively high biomass production, although *L. albus* performed less effectively under the L2/23 condition. Plants in the *Lupinus* genus generally display a high tolerance to acidic soils [38,49]. For instance, *L. albus* has produced high biomass yields when cultivated in the region, even in highly acidic soils with a pH below 5 [22,50]. The relatively high productivity under the L1/22 condition (pH 4.9) further supports this observation. The yield reduction observed in the L2/23 condition likely resulted from lower precipitation levels, as white lupin tends to begin flowering slightly later than other *Lupinus* species [49], making it more susceptible to reduced spring rainfall. On the other hand, *L. luteus* evolved in sandy soils with a low water retention capacity, which supports its adaptation to intermittent water deficits, thereby maintaining more stable biomass production [43].

4.2. Nitrogen Concentration in Plant Tissues

The N concentration in plant tissues followed a general reduction pattern over time across all the species and years, with only rare exceptions. In Mediterranean climates, vegetative growth during winter is modest, limited by low temperatures [51,52]. With the arrival of spring, rising temperatures trigger rapid vegetative expansion, leading to a dilution effect in nutrient concentrations within plant tissues, particularly for highly mobile nutrients like N. The phenomenon of nutrient dilution/concentration in plant tissues has long been scientifically established [53]. It occurs when biomass accumulation fluctuates for a given level of nutrient availability in the soil [53,54]. As flowering begins, the plant growth intensifies significantly. Flowers, and, later, seeds, become a priority sink, competing for photosynthates with other parts of the plant, including nodules, which tends to reduce biological N fixation [18,48,55], further accentuating nutrient dilution in the tissues.

Exceptions to the pronounced decline in the N concentration within plant tissues were observed in white lupin under the L2/23 growing conditions and in broad bean under the L1/22 conditions. In the case of white lupin, limited branching and a low biomass yield were noted, attributed to drought stress during the final phase of the growth cycle. While the productivity was diminished due to the high soil acidity for the broad beans, this factor did not exacerbate N dilution within the plant tissues. Conversely, during the years of higher DMY across most of the species, a marked reduction in the N concentration in the tissues was evident. This decline occurred as N fixation processes could not keep pace with the rapid biomass expansion, aligning with the findings from previous studies [38,45,56].

4.3. Nitrogen Recovery in Aboveground Biomass

The total N accumulated in the aboveground biomass results from the multiplicative combination of the DMY and N concentration in the plant tissues. Generally, the total amount of N in the plant tissues increased due to a higher DMY, despite the decreased tissue N concentration. This pattern indicates that N uptake and/or fixation was sustained throughout the plants' biological cycle until the final harvest for most species and cultivars. Notably, N fixation typically only declines when there is heightened competition for photosynthates between the nodules and reproductive structures [18,55].

Different legume species displayed markedly varied capacities for N accumulation. For instance, broad bean (cv. Favel) reached 457.8 kg N ha^{-1} under the L4/24 cultivation conditions, whereas subclover recorded only 52.2 kg N ha^{-1}. Such a large variability between species and varieties is common and is primarily linked to their biomass production potential [38,57]. Generally, it was established that in legumes, the N content in the shoot ranges from 15 to 25 kg per Mg of shoot dry matter (DM), with an average value of approximately 20 kg N per Mg DM [58]. In this study, the only non-leguminous species examined were oats, which exhibited average N values between 6.4 and 9.8 kg N per Mg DM at the final harvest. At the same time, the legumes displayed a range of 8.9–18.2, 17.6–23.7, 20.1–26.7, 18.4–28.9, 20.4–32.4, 19.2–28.6, and 16.8–33.7 kg N per Mg DM for the varieties Dalkeith, Diogene, Favel, Vesuvio, Nacional, Karo, and Estoril, respectively. Besides the clovers, the other legumes presented values within or exceeding the 15–25 kg N range. However, the N values reported in the literature typically refer to measurements taken at the grain filling or harvest stages [48,49,56]. Between flowering and seed ripening, some N is remobilized from the shoots to the seeds as plant senescence progresses. It is known that N losses via NH_3 also occur during this process, which is a phenomenon called N loss from the canopy [59,60]. Therefore, measurements taken at full flowering or at the onset of pod formation are expected to show a higher plant N content than values recorded at harvest. This factor may help explain the high N levels observed in most legume species across various cultivation conditions. This study also did not consider the N contained in the root system. Studies that evaluated this component have shown that it can represent between 22% and 68% of the total N in the plant for pulses, including faba bean and narrow-leaf lupin, and 32% to 68% for pasture/fodder legumes, including subterranean clover and alfalfa [58]. This is also a non-negligible contribution of legumes to the increase in available N in the soil.

The various legume species also exhibited substantial differences in N accumulation in their aerial biomass depending on the local/year combinations, indicating differing sensitivities to cultivation conditions. Yellow and narrow-leaf lupin were among the species with the lowest variation in N accumulation across the different local/year combinations, consistently maintaining relatively high N levels. Broad beans, for example, showed a very high N accumulation in one year but notably low values in another, highlighting their high sensitivity to cultivation conditions. When a species encounters favourable growth conditions, these conditions also tend to enhance N fixation due to the increased availability of photosynthates [18,55]. This relationship generally correlates positively with the biomass production and total N content in plant tissues [48,57,58]. Adverse environmental conditions, however, can influence plant growth by reducing the availability of photosynthates, including those allocated to nodules, and may also impact the nodulation process, which is sometimes even more sensitive than the overall plant growth [18,55,57]. Various studies have demonstrated significant variation in the total N content within the same species when grown under different environmental conditions [47,57]. In this study, a low soil pH and drought stress were likely the primary environmental factors contributing to the performance variations among the species across the four cultivation conditions.

Oat, the only non-leguminous species and one used as a control in this experiment, accumulated relatively low N levels in its tissues. This suggests low N availability in the soil, as previously noted. It also indicates that cultivating non-leguminous species relies heavily on external N inputs [6,7], underscoring the importance of including legumes in cropping systems.

4.4. Nitrogen Fixed by Legumes

The total apparent N fixation varied significantly across the four cultivation conditions for most of the species. For broad bean (cv. Favel), the year-to-year difference exceeded 400 kg N ha^{-1}, with the N levels in the plants under the L1/22 condition falling below those of oat, resulting in a negative N fixation value. Similarly, the Vesuvio cultivar showed negative N fixation values in the L1/22 trial, though the highest observed value was less pronounced under the L4/24 conditions. These results collectively illustrate the varying sensitivities of species and cultivars to cultivation conditions, aligning with previously reported findings [18,55,57]. The complete failure of the broad bean observed under the L1/22 cultivation conditions is attributed to the high soil acidity. The soil acidity can affect plant growth in various ways, primarily through the risks of aluminum toxicity but also due to deficiencies in Ca and/or P [6,7]. Aluminum toxicity contributes to poor nodulation, affecting all stages of the infection process, including the exchange of molecular signals between the symbiotic partners and the inhibition of root hair formation [6,55]. This soil also exhibits a very low cation exchange capacity, with extremely low exchangeable Ca and extractable P levels [61]. These are also adverse environmental factors for the nodulation process and N fixation. Ca affects the synthesis of *nod* gene-inducing flavonoids, the recognition of Nod factors by the host plant, and the structure of the nodule [55]. Nodulated legumes also have higher P requirements than non-symbiotic plants. P is responsible for early signaling in nodulation and supplying ATP to nitrogenase and energy metabolism in nodules [18,55].

The difference in the individual N fixation potential among the species was also pronounced. The highest recorded value for subclover was 10.7 kg N ha^{-1} of fixed N, while for broad bean (cv. Favel), it was 419.4 kg N ha^{-1}. This result stems from differences in plant phenology and biomass production potential and the ecological adaptation of each species to the cultivation conditions. Conversely, some legumes managed to maintain relatively stable and high N fixation values across different cultivation conditions. Among this group, lupins stand out, with yellow lupin varying between 204.0 and 274.0 kg N ha^{-1}, narrow-leaf lupin ranging from 137.6 to 294.8 kg N ha^{-1}, and white lupin showing values between

123.2 and 303.2 kg N ha^{-1}. On the other hand, while capable of fixing high amounts of N in certain years, the broad beans displayed negative values in others, indicating significant sensitivity to cultivation conditions. Overall, these results align with findings from other studies documenting variability among species, across locations, and in the interaction of these two factors [38,56,57,62]. Furthermore, these findings underscore the need for regional information to assist producers in making optimal choices suited to their specific cultivation conditions.

The data presented as %Ndfa indicate a similar trend in the failure of subclover, with %Ndfa values ranging from 11.7% to 21.7%. Generally, the results for a specific legume tend to exhibit consistency across years [63], as observed in this study with lupins. Conversely, fava beans in the L1/22 combination showed negative values, indicating that, at the last harvest, they contained less N in their tissues compared to oats. In other cultivation conditions, the values exceeded 80%, typically surpassing those reported in the literature for various legume species, which generally range from 65% to 75% [48,56–58,62]. Lupins consistently demonstrated high values, frequently approaching or exceeding 90%. Natera et al. [38] reported average percentages of %Ndfa of 81% across six varieties of narrow-leaf lupin, with values ranging from 72% to 93%. These findings are consistent with the results observed in this study.

High levels of %Ndfa depend on effective nodulation processes and the plant's ability to supply photosynthates to the bacteroids within the nodules. However, they are also influenced by the availability of soil N. Elevated soil N levels can inhibit %Ndfa by reducing nodulation due to decreased bacterial attachment to infection sites and impaired root infection. High soil N concentrations can also hinder leghemoglobin synthesis and nitrogenase activity while accelerating nodule senescence [55]. In this study, most species exhibited a high potential for N fixation, supported by adequate nodulation, suggesting rhizobia in the environment. Increased soil N availability reduces %Ndfa [55,62], while limited soil N availability negatively impacts the productivity of non-leguminous species [4,5,64]. Thus, this condition limited the productivity of the control plant, oats, while not adversely affecting the legumes.

5. Conclusions

Oats produced a relatively low biomass under N-poor soil conditions. However, some leguminous species exhibited a high DMY and N content in their shoots, demonstrating a substantial N fixation potential even in nutrient-deficient soils. Subterranean clover showed limited suitability as a green manure crop due to its low DMY and N fixation capacity. Similarly, crimson clover displayed inconsistent performance, with notable biomass and N fixation only in the years with high spring rainfall. The broad beans (cvs. Favel and Vesuvio) recorded a high biomass and N fixation in three out of the four growing conditions but were adversely affected by highly acidic soils. These cultivars may thus be recommended for green manure applications, provided the soil is not overly acidic or has been previously limed to increase the pH. Lupins, in general, demonstrated robust environmental adaptability, maintaining a high DMY and N fixation across various conditions, presenting significant potential as green manure crops. White lupin, however, encountered challenges during drier springs, which may limit its suitability in areas with low spring precipitation. Under favourable growth conditions, the %Ndfa was high, indicating the presence of effective rhizobia inoculants in the soil and low natural N availability.

Overall, these leguminous species show promise as green manures for use in perennial woody systems, such as orchards and vineyards. During winter and early spring, before the dry season, they contribute N to the system with minimal competition for water with the main crop. Additionally, these species could be incorporated into herbaceous farming systems before spring–summer cash crops, like maize and tomatoes, since winter is typically fallow and crops are rarely cultivated.

Author Contributions: P.A.: investigation, data curation, and writing—original draft preparation; M.A.: funding acquisition, investigation, methodology, and writing—review and editing; E.A.N.: investigation, and data curation; M.Â.R.: conceptualisation, funding acquisition, project administration, data curation, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for the financial support from national funds FCT/MCTES, to CIMO (UIDB/AGR/00690/2020), and SusTEC (LA/P/0007/2020), and for the Doctoral scholarship PRT/BD/154361/2023 to Peltier Rossi Lino de Aguiar.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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