agronomy

MDPI

Article

Lupine Cultivation Affects Soil's P Availability and Nutrient Uptake in Four Contrasting Soils

Cristina Mori Alvez [1,*], Carlos Perdomo Varela [1,†], Pablo González Barrios [2], Andrea Bentos Guimaraes [1] and Amabelia del Pino Machado [1,†]

[1] Soil and Water Department, Agronomy College, University of the Republic, Av. Garzon 780, CP, Montevideo 12900, Uruguay; chperdom@fagro.edu.uy (C.P.V.); andreabentosguimaraes@gmail.com (A.B.G.); amabelia@fagro.edu.uy (A.d.P.M.)
[2] Biometrics, Statistics and Computing Department, Agronomy College, University of the Republic, Av. Garzón 780, CP, Montevideo 12900, Uruguay; pablog@fagro.edu.uy
* Correspondence: cristinamori@fagro.edu.uy
† These authors contributed equally to this work.

Abstract: A substantial amount of phosphorus (P) in the soil is not readily available for plant uptake. Certain species may enhance P availability from poorly soluble P forms. This study focused on improving our comprehension of the effect of two lupine species (*L. albus* and *L. angustifolius*) on soil's P mobilization and its link with soil acidity variations, comparing the response of the lupine species in terms of plant traits (i.e., aboveground biomass and nutrient uptake) with that of oats (*Avena strigosa* L.) in four contrasting soils (i.e., available P in soil, soil acidity, soil fertility, and texture). The phosphorus solubilization capacity was assessed on variations of P availability (PBray1) at four points in time, comparing soils with lupine to oat-containing soils and their baseline values. Compared to soils containing oats, at harvest, lupine soils had significantly increased PBray1 concentrations; the maximum average increment was around 5.3 mg kg^{-1}, with *L. albus* in Sites 1 and 2, which presented higher organic matter (OM) contents than the other two sites. Lupine-induced soil acidification did not fully explain that P increase. Oats exhibited the highest increase in shoot dry weight in response to soil's P availability, while lupine was the least affected. Nevertheless, *L. albus* showed similar or higher nutrient uptake than oats across all soils. The manganese (Mn) concentration was high in both lupine species' shoot biomass; however, within each lupine species, across all soil types tested, these legumes had different Mn accumulation levels depending on the soil acidity. *Lupinus albus* had a higher ability to mobilize non-labile P in the light-textured soil with a high OM content, achieving comparable and higher plant P status than oats and providing N through biological N fixation (BNF), positioning it as a suitable crop for diversifying Uruguay's agricultural crop rotation systems.

Keywords: lupine; soil–plant interactions; soil P availability; acidification; nutrient uptake

check for updates

Citation: Mori Alvez, C.; Perdomo Varela, C.; González Barrios, P.; Bentos Guimaraes, A.; del Pino Machado, A. Lupine Cultivation Affects Soil's P Availability and Nutrient Uptake in Four Contrasting Soils. *Agronomy* **2024**, *14*, 389. https://doi.org/10.3390/agronomy14020389

Academic Editors: Oqba Basal, Jozef Sowinski and Éva Domokos-Szabolcsy

Received: 16 January 2024
Revised: 9 February 2024
Accepted: 13 February 2024
Published: 18 February 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The adoption of continuous cropping (CC), primarily soybean (*Glycine max* L. Merr.), has increased in Uruguayan agricultural systems since the early 2000s [1], replacing the traditional crop–pasture rotation [2,3]. While grazing persists in agricultural systems, its duration within the rotation has been significantly reduced. According to the Ministry of Livestock, Agriculture and Fisheries of Uruguay, the 2021 agricultural survey [4], estimated that pastures associated with wheat cropping constituted less than 8% of the total area that was planted with this winter crop. In the past 15 years, the proportion of pastures associated with winter crops has significantly decreased, from 28% in 2005 to 4% in 2021. Despite the increase in grain production due to pasture phase exclusion, recent studies have confirmed persistent wheat yield gaps in Uruguay [3], which cannot be explained by nutrient deficiency and remain unaddressed by correcting nutrient deficiencies. The issue

is exacerbated because the soil quality has been compromised, which affects the sustainability of the system [2,5].

In this scenario, there is significant concern regarding the impact of a permanent negative nitrogen (N) balance in the CC rotation; thus, balancing N within this system becomes necessary [6]. In contrast to crop–pasture rotation, the CC systems have experienced an increase in the quantities of both N and P fertilizers due to agricultural intensification. However, for P in particular, due to the accumulated yield's increased extraction of P from the soil, the amount of remaining P decreased, especially when highly extractive crops were the main components of the sequences in the rotation, such as soybean and corn [7]. Moreover, applying P fertilizer above crop requirements leads to a slightly positive soil P balance, as P gradually accumulates in the soil over time [7]. This accumulation is affected by the low P use efficiency in most crops, which typically ranges from 15 to 30% [8].

Although the majority of residual P is scarcely assimilable by plants, certain species may be able to make this P available [9,10]. The lupine genus may be able to mobilize residual P through root exudates, releasing phosphatases [11], acidifying [12], and chelating compounds or carboxylates [13–15], which can enhance their growth and possibly that of subsequent crops [16,17]. However, this genus has not yet been incorporated into crop rotations in Uruguay, which typically favor winter cereal crops like wheat or barley and summer crops such as soybean or maize. Additionally, further investigations [18] on lupine cultivation and its effects on soil nutrient dynamics must to be conducted.

Accordingly, for competitive crop production, the integration and diversification of functional groups—such as annual winter legumes like lupine—may be a key strategy to reverse soil deterioration processes [19–21]. This lupine genus is recognized for its high potential as a pulse crop in Australia, Chile, and other countries for a variety of production purposes [17,22–24]. Integrating crops that are capable of N fixation from the atmosphere and P solubilization from unavailable forms provides an alternative to excessive or improper fertilizer applications. Moreover, the root activity of this annual winter legume contributes to soil OM as a carbon source and enhances soil's N content through its N-fixing ability, while also improving physical, chemical, and biological soil properties.

Given the growing interest in diversifying crops within agricultural systems and reducing fertilizer use, there is a timely opportunity to evaluate lupine cultivation. Species such as white lupine (*L. albus*) and narrow-leaf lupine (*L. angustifolius*) are renowned in regions of Australia and Chile as N fixers and P solubilizers, particularly in mildly acidic or neutral soils of light-to-medium texture [25]. Consequently, we hypothesize that the availability of plant P in contrasting soils (pH, texture, OM, and soil P concentration) increases by acidification of the soil surrounding roots by those lupine species, enhancing the solubility of P from sparingly available soil P sources. This enhanced plant P availability is expected to improve the dry matter (DM) yield and the P and N biomass content of lupine, potentially having a residual effect on subsequent crops.

This study focused on improving our comprehension of the impacts of two lupine species (*L. albus* and *L. angustifolius)* on the mobilization of P in soil and its association with soil acidification, comparing the response of aboveground biomass productivity and nutrient (i.e., P, N, base cation, Mn, and Fe) uptake effectiveness to these lupine species with the response to oats (*Avena strigosa*) in four contrasting (i.e., available P in soil, soil acidity, soil fertility, texture) soil types. Hence, the aims were to assess the changes in soil's P availability and its link with soil acidification and to establish which type of lupine has a more substantial P solubilization capacity and which soil–lupine combination yields the most effective P mobilization effect.

2. Material and Methods

2.1. Soil Collection and Preparation Prior to Experiment Installation

Soil samples were collected from the topsoil layer (0–20 cm depth) from four Uruguayan agroecological areas under different soil uses with the following geographic coordinates: agricultural—Site 1 (33°59′05.4″ S and 57°43′42.7″ E); livestock grassland—Site

2 (32°49′1.20″ S and S 54°25′28.27″ O); grassland—Site 3 (34°50′15.61″ S and 56°13′21.62″ O); forestry—Site 4 (31°23′43.44″ S and 55°41′39.37″ O). The sites have contrasting soil textures, pH values, organic matter (OM) levels, and P concentrations (PBray1). Table 1 displays the collection sites and USDA soil classification [26] for each of the four soils analyzed in this study; these references (Site 1 to Site 4) were used throughout the text to identify each soil treatment.

Table 1. Site location, soil type, dominant geological material, physical characterization, and soil texture of the soil samples collected from four sites in Uruguay.

Site (Location)	Soil Type (USDA Classification System) †	Geological Material	OM	Sand	Silt	Clay	Texture ‡
			%				
Site 1 (Colonia)	Pachic Argiudoll	Clay silt sediment/Crystalline basement	4.8	16.3	38.8	44.9	C
Site 2 (T. and Tres)	Typic Dystrutepts	Crystalline basement	3.7	23.5	44.2	32.3	CL
Site 3 (Montevideo)	Typic Argiudolls	Libertad clayey silt sediment	1.7	19.3	52.3	28.4	SiCL
Site 4 (Rivera)	Typic Hapludults	Colluvial material (Sandy soils)/Tacuarembó sandstones	1.4	84.7	1.4	13.9	SL

† USDA source: Keys to soil taxonomy. Soil Conservation Service. 2014. ‡ Texture: C = clay; SiCL = silty clay loam; SL = sandy loam; CL = clay loam.

Before planting, each soil was sieved through a 1 cm mesh sieve to homogenize the size of the aggregates and discard coarse plant material. After this screening, for each soil type, the granulometric composition (texture) and the chemical characterization (i.e., PBray1, inorganic N forms, exchangeable bases, exchangeable Al, and soil pH) were determined (Supplementary Materials, Table S1).

2.2. Greenhouse Experiment

2.2.1. Experimental Design and Plant Growth Conditions

A pot experiment was conducted under natural light conditions in a greenhouse at the Agronomy College in Montevideo (34°50′18.20″ S, 56°13′16.36″ W) to evaluate the efficacy of two lupine species in enhancing soil's P availability. A total of 48 three-liter pots (15 cm in diameter) were filled with the different soil types. The treatments were arranged in a factorial design with two factors, four soil types and three species, in a completely randomized design (CRD) with four replications.

The evaluated species were *Lupinus angustifolius* L. var. Lavalle, a non-cluster root-forming lupine (narrow leaf lupine or blue lupine), and *L. albus* L. Blu25, a cluster root-forming legume (white lupin). Additionally, an annual grass, black oat (*Avena strigosa* L., var. Agroplanalto), was included in the study as a comparative measure to assess the increase in soil P that was induced by lupines and to evaluate BNF. The seed company Fadisol S.A. (Montevideo, Uruguay) supplied all seed species that were utilized in this study, constituting those of lupine, the single plant material available by Fadisol S.A. during the research period of the current work. The white lupine accession was provided by Seeds Baer (Temuco, Chile), a breeder who conducted formal identification and permitted using it for research purposes; blue lupine and black oat were developed and protected by Fadisol S.A. Oats were chosen as the control in our study because they are the most widely cultivated and regionally adapted cover crops in Uruguay due to their precocity and high growth rate during the winter season [27]. Furthermore, according to Wang et al. [28], this genus would be a good candidate for evaluating soil's P utilization, given its extraordinary cover's fibrous root system promoting key roles, such as preventing erosion and scavenging excess nutrients [29]. Both lupine species were inoculated with a non-specific

commercial inoculant (Bradyrhizobium sp., strains U-612, and U620), known to be effective for these species were provided by Lage y Cía Company (Montevideo, Uruguay). The planting date was 10 July 2019, with two or three lupine seeds per pot, leaving only one plant per pot after emergence. There were five oat plants per pot.

Plants were irrigated using potable water, supplemented once a week during the first month of the experiment with a nutrient solution that contained (µM): KCl, 200; CaCl, 150; $MgSO_4$, 100; H_3BO_3, 2.0; $MnSO_4$, 0.4; $ZnSO_4$, 0.4; and $CuSO_4$, 0.2, Na_2MoO_4, 0.05; Fe Edta, 0.005, free of N and P and using 200 mL per pot. Throughout the trial, each pot was maintained around 60% of its field capacity (FC), estimated by gravimetric determination and considering the water content at FC (based on mass) of each soil type (35, 30, 28, and 13% for Sites 1 to 4, respectively). The experiment was conducted from June to October 2019, encompassing the mid-winter and early spring seasons. The average temperatures during this period varied between 8 and 25 °C. There was no need to control weeds and pests during the experiment.

2.2.2. Soil and Plant Sampling

Soil samples were collected at four different time points: 48, 76, 87, and 103 days after planting (dap). The initial soil samples were obtained on 9 September 2019, two months after the start of the experiment, when the crop was in the vegetative stage. The second sampling took place on 2 October 2019, during flowering stage, the third sampling on 14 October 2019, at the beginning of the lupine grain filling, which was the harvest time, and the final sampling on 30 October 2019. The soil pH was measured on 9 September and 14 October 2019. The soil's exchangeable acidity was assessed to confirm any connection between changes in the solubility of inorganic P or Al and soil acidity. A hand drill, 1.9 cm in diameter, was used to collect a single soil sample from each pot, reaching a depth of 0 to 10 cm.

The plants were harvested at 87 dap at ground level using pruning shears to remove all aerial biomass per pot. At this sampling time, the oat plants had reached full maturity, while *L. angustifolius* was in the initial phase of grain filling, and *L. albus* exhibited pods in an early stage of development. Compared to *L. albus*, *L. angustifolius* had more mature pods, which accounted for around ten percent of the total P absorbed in the aboveground biomass. Consequently, the study separated the analytical determination of *L. angustifolius* pods from the remaining aerial plant parts. However, except for P concentration, the other analyses on these pod components were not achievable due to the sample size being insufficient for accomplishing this. The shoot dry weight was expressed per pot (g pot^{-1}), and in some instances, it was converted to its equivalent per hectare (kg ha^{-1}) based on the pot's surface area.

2.3. Sample Processing and Analytical Determinations

2.3.1. Soil Measurements

The soil samples were dried in a forced-air oven at 40 °C for at least 48 h and were ground to a 2 mm sieve size. For the initial characterization, the determined parameters were granulometric composition, organic matter (OM), pH, labile P, mineral nitrogen (ammonium and nitrate), exchangeable cations (Ca, Mg, K, and Na), and exchangeable acidity. OM was determined by the Walkley–Black method [30], while exchangeable cations Ca and Mg were determined by atomic absorption and K and Na by flame spectrophotometry, following extraction with 1 M ammonium acetate at pH 7 [31]. Soil pH was measured in deionized water (1:2.5 soil/deionized water ratio) using a pH probe (Orion Research 701 pH electrode), while exchangeable acidity was determined using the potassium chloride method [31]. The labile P or available P content was measured using the Bray N° 1 (henceforth PBray1), method extraction system [32], which is the most widely used method in Uruguay for evaluating plants' P availability in most agricultural soils in the country [33]. Nitrate–N (NO_3-N) concentration was determined using the Griess–Ilosvany method [34], and ammonium–N (NH_4-N) was determined using the colorimetric method [35]. Phos-

phorus, ammonium, and nitrate readings were taken in a spectrophotometer at 880, 650, and 540 nm, respectively, using the MRC microplate reader for the first two elements and the UNICAM spectrophotometer for nitrate. The hydrometer method [36] was employed to analyze the granulometric soil composition. During the growing season, soil samples were analyzed for PBray1 and pH using the same techniques described for soil characterization analysis.

2.3.2. Plant Measurements

The harvested plants were oven-dried at 65 °C for a minimum of 48 h until the mass remained constant. Then, initially, dried plant materials were ground with a stationary and mobile knife mill (Marconi MA-580) until the particulate size was less than 2 mm. Plant samples of this granulometric size were analyzed for total P and K contents. Afterward, previously ground subsamples were newly ground by a rotary mill (SampleTek 200 vial Rotator) to a fine powder (typically a consistency approaching that of the talcum powder), which was necessary for ^{15}N analysis by mass spectrometry. Total C and N concentration and $^{15}N/^{14}N$ of the samples (at the natural abundance) were determined by mass spectrometry in a US laboratory "https://csi.unm.edu, accessed on 10 November 2020"). The following formula by Shearer and Kohl [37] was used to calculate the proportion of N fixed from the air (% Ndfa) for each lupine species:

$$\mathbf{Ndfa}(\%) = \left(\frac{\boldsymbol{\delta^{15}N_{ref}} - \boldsymbol{\delta^{15}N_{fix}}}{\boldsymbol{\delta^{15}N_{ref}} - \mathbf{B}} \right) \times \mathbf{100}$$

where the following abbreviations are used:

Ndfa is the proportion of plant N derived from BNF;
δ^{15}Nref is the $\delta^{15}N$ value of the reference plant (non-fixing);
δ^{15}Nfix is the $\delta^{15}N$ value of the fixing plant (lupine);
B is the $\delta^{15}N$ value of a fixing plant growing in a medium without N.

The B value was estimated as the mean $\delta^{15}N$ value from pure lupine growing in the sand, with a value of +1.6‰ and −0.6‰ of $\delta^{15}N$ for *L. albus* and *L. angustifolius*, respectively. The reference plant used was oat, and all $\delta^{15}N$ values were determined under the same conditions as lupine legumes.

The total concentrations of P and potassium (K), calcium (Ca), magnesium (Mg), Mn, and iron (Fe) were also determined after calcination at 550 °C for 5 h. Phosphorus concentration was determined using the ascorbic acid method [38] after extraction with diluted HCl (20% *v*/*v*) [39]. In the ash extracts, the remaining elements (Ca, Mg, K, Fe, and Mn) were determined by spectrometry as described for soil samples. Shoot's P and N contents per pot (mg pot^{-1}) were calculated by P or N concentrations (mg g^{-1} dw) × shoot dry weight (g pot^{-1}), respectively. In certain instances, the plant nutrient content was also expressed per hectare, considering the pot's surface area.

2.4. Statistics

The changes in soil PBray1, at four points in time, were evaluated by repeated measures analysis of variance using a MIXED procedure of SAS (between-subject factors were species and soil type, and within-subject factor was sampling time). Additionally, two-way ANOVAs performed at two time points (i.e., at 87 and 103 dap) were used to study the factors of interest (i.e., soil, species, and their interaction as fixed effects) on the evaluated soil and plant variables. Datasets that did not follow assumptions of normality of residuals and homogeneity of variance were log10 transformed. Two additional soil variables were estimated: 1: the difference between PBray1 (and pH) at a specific sampling time and the corresponding parameter, measured at the beginning of the experiment, is denoted as ΔPBray1−initial and ΔpH−initial, respectively; 2: for each soil parameter (PBray1 and

pH values), the difference between the soil samples containing lupine and those containing oats (ΔPBray1$_{_Lup\text{-}Oat}$ and ΔpH$_{_Lup\text{-}Oat}$) was estimated.

The further analyses included orthogonal contrasts (C1: lupine vs. oat, C2: *L. albus* vs. *L. angustifolius*) that were performed to identify differences between groups of treatments and Pearson correlation and linear regression to describe and explain the association between soil and plant variables. A Tukey's test with a confidence level of 5% was used to compare the means of treatments between species, across soils, and within each soil type. All analyses were conducted using SAS statistical software version 9.04 (SAS Institute) and R software (version 4.0.4) [40].

3. Results

3.1. Species, Soil, and Sampling Time Effects on PBray1 Concentration

The effects of species and soil type were highly significant (p = 0.001) on the soil's PBray1, but their interaction was not (Table 2). Considering the effect of species, *L. albus* had the highest mean values (Figure 1), while concerning the soil factor, its effect on PBray1 reflected, on average, the initial P concentrations of each soil, and this was observed as early as 76 dap, after which the concentration tended to stabilize.

Table 2. Repeated measures analysis of variance model for soil PBray1 concentration.

Treatment Effect	DF	F Value	$p > F$ †
Species	2	23.05	**<0.0001**
Soil type	3	101.56	**<0.0001**
Species x soil type	6	1.36	0.2564
Sampling time	3	91.85	**<0.0001**
Species × sampling time	6	2.29	0.0518
Soil type × sampling time	9	6.66	**<0.0001**
Species × soil type × sampling time	18	2.17	**0.0133**

† Significant effects ($p < 0.05$) are in bold. Note: The within-subject factor was sampling time. Results are from 48, 76, 87, and 103 days after planting, and the between-subject factors were species and soil type.

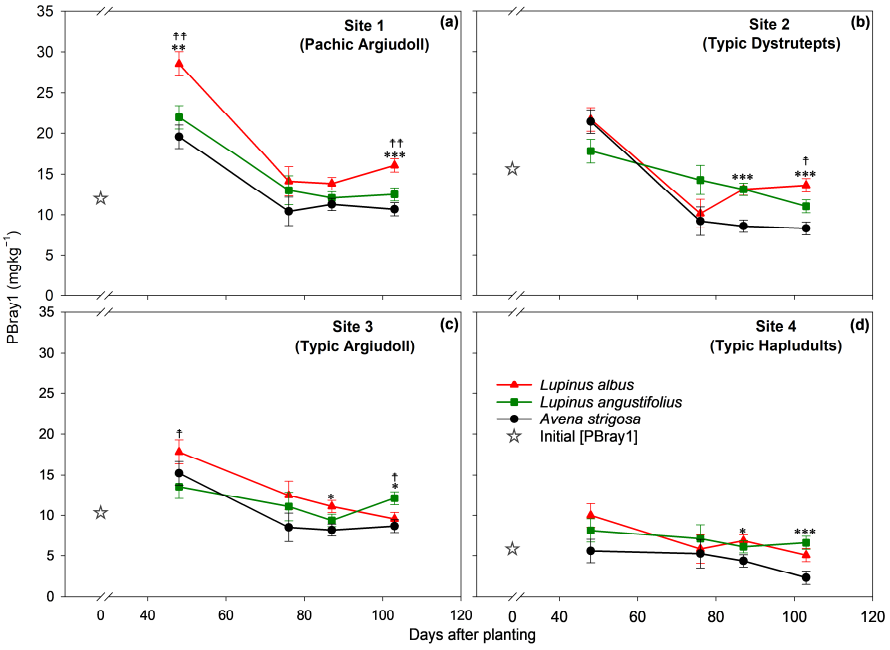

Figure 1. Soil PBray1 concentrations under two legumes (*L. albus* and *L. angustifolius*) and one grass (*Avena strigosa*) across Sites (soil type) 1 (**a**), 2 (**b**), 3 (**c**), and 4 (**d**). The vertical bars indicate the standard error, and the symbols "*" and "†" indicate the sampling time in which Contrast 1 (lupine vs. oat) and Contrast 2 (*L. albus* vs. *L. angustifolius*) were significant (* † $p < 0.05$; ** †† $p < 0.01$; *** $p < 0.001$), respectively.

The C1 (lupine vs. oat) was significant at 48 dap, but only in the soil of Site 1 ($p = 0.0029$), whereas C2 (*L. albus* vs. *L. angustifolius*) was significant in Site 1 and Site 3 ($p = 0.0028$ and $p = 0.0445$, respectively). At 76 dap, both contrasts were no longer statistically significant in any of the soils, indicating that the differences between species diminished. At harvest (87 dap), C1 was significant in Site 3 ($p = 0.0331$), Site 4 ($p = 0.029$), and Site 2 ($p = 0.0001$), but differences between lupine species were not detected. Fourteen days after harvesting (103 dap), C1 was highly significant in all the soils, while C2 was significant in all soils except Site 4, where no differences in PBray1 between the lupine species were observed.

At harvest time (87 dap), the two-way ANOVA showed significant effects of the main factors, species and soil, but not of their interaction (Supplementary Materials, Table S2). The contrast analysis between lupine vs. oats (C1) was significant, the estimated difference being 2.6 mg kg^{-1}, while the contrast between the lupine species (C2) was not significant on this sampling date. Multiple comparisons of means using the Tukey method revealed a significant difference between the lupine species, with higher values for *L. albus*. For this sampling time, the difference in PBray1 compared to the initial values was almost null in the soils with lupine; with oats, meanwhile, there was a mean decrease of 2.8 mg kg^{-1}. At 87 dap, there were also statistically significant differences in the concentrations of PBray1 between soils, primarily reflecting the P analysis values of the tested soils before the start of the experiment. The concentration of PBray1 did not differ statistically between Site 1 and Site 2. However, the P levels in these soils were substantially higher and statistically different from those of the other soils ($p = 0.05$). In the heavy-textured soils and Site 4, the ΔPBray1-initial was minimal in this sampling period; however, Site 2 had a mean decrease of 4.0 mg kg^{-1}. Additionally, a significant percentage increase in PBray1 of 57% was observed in the loamy soil containing lupine relative to this soil containing oat, reaching 4.6 mg kg^{-1}.

At 103 dap, a significant effect of the main factors ($p < 0.0001$) but also of the species x soil type interaction ($p = 0.0048$) was detected (Figure 2; Supplementary Materials, Table S3). The C1 was significant in all the soils, the difference being 4.0, 3.6, 3.6, and 2.2 mg kg^{-1} in Site 2, Site 1, Site 4, and Site 3, respectively. The C2 was also significant in three soils, especially for *L. albus* in Site 1 and Site 2, with a higher P content. Site 3 was the other soil with a significant difference between the lupine species. In this soil, however, *L. angustifolius* stood out over *L. albus*, with a difference from the oat of 3.4 mg kg^{-1}; meanwhile, the difference between *L. albus* and oats was statistically non-significant and estimated at 0.9 mg kg^{-1}. In summary, the $\Delta PBray1_{\_Lup\text{-}Oat}$, depending on the lupine species and the soil, ranged from 0.9 (non-significant difference) to 5.4 mg kg^{-1} ($p < 0.0001$). In addition, a significant difference was found between both species of lupine to oats only in light-textured soils (Site 2 and Site 4). In the other soils, meanwhile, that difference was significant only in one species of lupine. This was *L. albus* in Site 1 and *L. angustifolius* in Site 3.

3.2. Species and Soil Type Effects on Soil pH

The pH of the soil at harvest differed considerably between soils with lupine and those with oats, by 0.5 pH units on average (Figure 3). Compared to the pH values at 48 dap, the values were lower at harvest, although this decrease was only observed in soils with lupine (0.1 pH unit) and not in soils with oat. When the soil's pH at harvest was compared with the initial pH of each soil, it was found that oats caused a mean 0.25 units of pH increase, while both species of lupine caused a decrease in pH, but without statistically significant differences. The effect of soil on pH changes was also highly significant, with an average increase of 0.57 units in Site 4 and a decrease of a similar magnitude (−0.62 units) in Site 2. The species x soil type interaction on soil pH measured at harvest was significant ($p = 0.0365$; Supplementary Materials, Table S4). As shown in Figure 3, oat-containing soils consistently recorded the lowest acidity levels. In addition, the soil pH of this species differed significantly from that of lupine in all soils except Site 1, where the differences between species were not statistically significant. Based on the statistical differences between

soils across species, Figure 3 reveals that Site 2 had the lowest soil pH at harvest, while Site 3 had the highest (coincidentally with the highest initial soil pH).

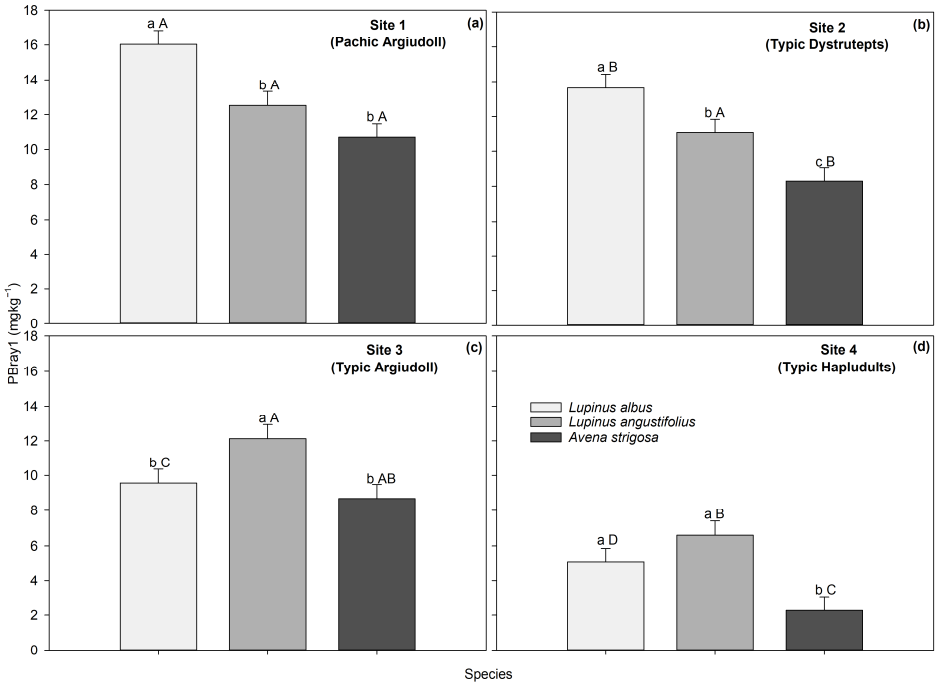

Figure 2. PBray1 concentration according to species across Sites (soil types) 1 (**a**), 2 (**b**), 3 (**c**), and 4 (**d**) at 103 days after planting. The vertical bars indicate the standard error. Different lowercase letters indicate significant differences between species within each soil type, and different capital letters indicate differences between sites across species, according to Tukey's test, with a $p \leq 0.05$.

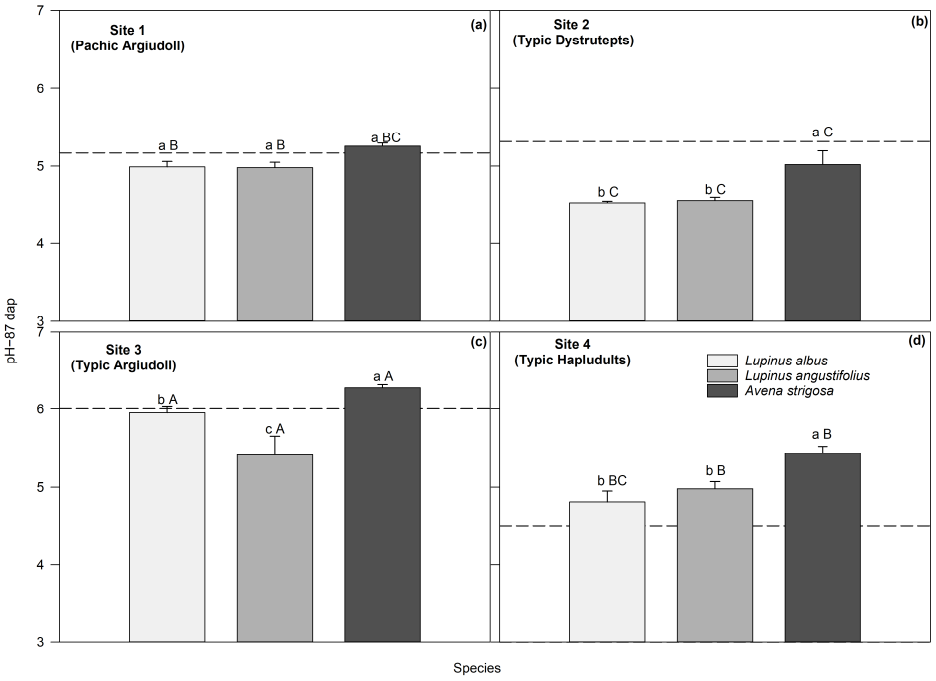

Figure 3. Soil pH measured 87 days after planting of the two legumes (*L. albus* and *L. angustifolius*) and the grass (*Avena strigosa*) across Sites (soil type) 1 (**a**), 2 (**b**), 3 (**c**), and 4 (**d**). The dotted line represents the soil's pH value at the beginning of the experiment. Different lowercase letters indicate significant differences between species within each soil type, and different capital letters indicate differences between sites across species, according to Tukey's test, with a $p \leq 0.05$.

At 87 dap, in Site 3, Site 4, Site 2, and Site 1, the $\Delta pH_{\_Lup\text{-}Oat}$ was −1.2, −1.1, −1.0, and −0.6 units, respectively. According to the contrast analysis, the difference between the pH values of lupine and oat (C1) was statistically significant in all soils. The C2 was only significant in Site 3, with *L. angustifolius* standing out with a decrease in pH of −0.6 units from the initial value. Figure 3 also shows that the ΔpH−initial fluctuated within a more negative range for soils with lupine (−0.8 to 0.5) and a more positive range for soils with oat (−0.3 to 0.9). Likewise, the exchangeable acidity values corresponded to those of the pH; when the pH decreased, the soil acidity increased. However, this increase in acidity was not associated with exchangeable aluminum but rather with an increase in the hydrogen ion concentration. On average, the highest increase in exchangeable aluminum was 0.14 $cmol_c\ kg^{-1}$ at 87 dap in Site 2; this concentration would not impose production limitations (Table 3).

Table 3. Soil's exchangeable acidity and exchangeable aluminum according to species and soil type. Values are means ± standard error.

Site	Species	Exchangeable Acidity		Exchangeable Aluminum	
		$cmol_c\ kg^{-1}$			
1	*Lupinus albus*	0.07 ± 0.01	ns C	0.01 ± 0.03	ns C
	Lupinus angustifolius	0.08 ± 0.02	ns C	0.04 ± 0.03	ns ns
	Avena strigosa	0.07 ± 0.01	ns BC	0.04 ± 0.01	ns AB
2	*Lupinus albus*	0.69 ± 0.04	a A	0.18 ± 0.03	ns A
	Lupinus angustifolius	0.56 ± 0.09	a A	0.10 ± 0.03	ns ns
	Avena strigosa	0.33 ± 0.04	b A	0.13 ± 0.03	ns A
3	*Lupinus albus*	0.08 ± 0.03	ns C	0.06 ± 0.02	ns BC
	Lupinus angustifolius	0.05 ± 0.00	ns C	0.06 ± 0.02	ns ns
	Avena strigosa	0.03 ± 0.01	ns C	0.03 ± 0.03	ns B
4	*Lupinus albus*	0.32 ± 0.08	a B	0.11 ± 0.03	ns AB
	Lupinus angustifolius	0.23 ± 0.04	ab B	0.10 ± 0.03	ns ns
	Avena strigosa	0.17 ± 0.07	b B	0.08 ± 0.03	ns AB
Significance of treatment effect					
Species		**0.0008**		0.6089	
Soil type		**<0.0001**		**0.0008**	
Species × soil type		**0.0179**		0.6339	

Significant effects ($p < 0.05$) are in bold. Different lowercase letters within a column indicate differences between species within each soil type, and different capital letters within a column indicate differences between soils across species being significant at a *p*-level of 0.05.; ns means no significant difference.

Additionally, sandy soils at Sites 2 and 4 displayed more significant pH fluctuations. At Site 2, where the ΔpH−initial was the most pronounced, the difference in exchangeable acidity between soil holding lupine and soil holding oats was most noticeable and statistically significant. In contrast, at Site 4, the exchangeable acidity with oat-bearing soil was only statistically distinct from soil containing *L. albus*. On the other hand, the exchangeable acidity between species was not statistically significant at Sites 1 and 3 (Table 3).

3.3. Aboveground Biomass and Nutritional Status: Effects of Species and Soil Type

3.3.1. Shoots' Dry Weight and P and N Content

As shown in Table 4, the species, soil, and their interaction significantly influenced the shoots' dry weight and P content. The maximum shoot dry weight was produced by oat, followed by *L. albus* and *L. angustifolius*. Regarding soils, Site 4 had the lowest yield, heavier soils offered an intermediate yield, and Site 2 presented the highest yield, which was three times that of Site 4. The yield of each species was also differentially affected by the soil type (soil x species interaction; $p < 0.0001$). Except for Site 3, oats outyielded lupines, while *L. albus* produced roughly twice as much as *L. angustifolius* across all soil types.

Table 4. Shoots' dry weight, plants' P and N concentration, and plants' P and N content of different species, as affected by soil type. Values are means ± standard error.

Site	Species	Shoots' Dry Weight	Plants' P Concentration	Plants' N Concentration	Plants' P Content	Plants' N Content
		g pot^{-1}	mg g^{-1} Dry Weight		mg pot^{-1}	
1	*Lupinus albus*	12.5 ± 1.5 a A	1.3 ± 0.1 a AB	29.3 ± 1.0 a ns	15.5 ± 1.7 a A	366.5 ± 46.5 a A
	Lupinus angustifolius	6.7 ± 0.3 b A	1.4 ± 0.1 a A	26.1 ± 1.6 a ns	9.0 ± 0.6 b A	174.4 ± 15.3 b A
	Avena strigosa	14.5 ± 1.1 a B	1.2 ± 0.1 b A	5.7 ± 0.2 b B	16.8 ± 1.3 a B	81.9 ± 4.8 c B
2	*Lupinus albus*	13.9 ± 1.3 b A	1.4 ± 0.1 a A	31.9 ± 1.3 a ns	19.5 ± 1.3 a A	436.9 ± 24.7 a A
	Lupinus angustifolius	7.8 ± 1.1 c A	1.4 ± 0.1 a A	24.5 ± 0.4 b ns	10.6 ± 1.6 b A	189.8 ± 25.8 b A
	Avena strigosa	27.0 ± 2.7 a A	0.9 ± 0.0 b B	8.4 ± 1.0 c AB	23.5 ± 1.4 a A	222.1 ± 19.1 b A
3	*Lupinus albus*	14.0 ± 1.3 a A	1.1 ± 0.1 c B	28.8 ± 1.9 a ns	15.4 ± 2.1 a A	402.4 ± 37.0 a A
	Lupinus angustifolius	7.0 ± 1.9 b A	1.5 ± 0.1 a A	29.4 ± 1.4 a ns	10.8 ± 2.9 b A	203.6 ± 52.9 b A
	Avena strigosa	8.1 ± 0.4 b C	1.3 ± 0.1 b A	6.5 ± 0.3 b B	10.2 ± 0.9 b C	52.9 ± 3.01 c B
4	*Lupinus albus*	6.5 ± 1.1 b B	0.9 ± 0.1 a C	27.5 ± 2.4 a ns	5.6 ± 1.2 ns B	180.7 ± 38.6 a B
	Lupinus angustifolius	1.7 ± 0.4 c B	0.6 ± 0.2 b B	30.2 ± 0.0 a ns	1.1 ± 0.6 ns B	38.1 ± 0.0 b B
	Avena strigosa	11.0 ± 0.4 a BC	0.5 ± 0.0 b C	10.2 ± 0.6 b A	5.3 ± 0.3 ns D	111.8 ± 2.8 ab B
Significance of treatment effect						
Species		**<0.0001**	**0.0004**	**<0.0001**	**<0.0001**	**<0.0001**
Soil type		**<0.0001**	**<0.0001**	0.3286	**<0.0001**	**<0.0001**
Species × soil type		**<0.0001**	**0.0017**	**0.0088**	**0.0062**	**0.0009**

Significant effects ($p < 0.05$) are in bold. Different lowercase letters within a column indicate differences between species within each soil type, and different capital letters within a column indicate differences between sites across species being significant at a p-level of 0.05; ns means no significant difference.

The plants' P content in oats and *L. albus* was not statistically different, except in Site 3, where *L. albus* absorbed more P than oats (Table 4). The absorption of P and the yield of the aboveground biomass varied substantially between the lupine species, with the lowest values detected in *L. angustifolius*. In terms of P concentrations, there were fewer differences within each species across sites, because they were already similar in all soils except for Site 4, where the concentrations were half of those that were registered in the other sites, whatever the species considered. Oats displayed the most noticeable variation in P content between sites. In soils with lupine, such differences between soils diminished, although in Site 4, the P content of lupine was substantially lower than in the other sites. The contrast analysis between lupine and oat (C1) was significant only in Site 1 and Site 2, where the plant P content in oats was markedly higher than that of lupines (Supplementary Materials, Table S5). Contrast 2 was not significant in Site 4; the remaining soils had higher P contents in *L. albus* than in *L. angustifolius*. All data that are displayed in Table 4 exclude the pod components of *L. angustifolius*. However, upon their inclusion, the effect of species became not significant, eliminating the difference between the P content that was reached by both lupine species in Sites 1, 2, and 4. In contrast, *L. albus* continued to attain a higher plant P content than *L. angustifolius* in Site 3. On average, the P concentration of *L. angustifolius* plants in Sites 1, 2, and 3, both with and without pods, was 1.6 and 1.4 mg g^{-1} dry weight, respectively.

Similar to the shoots' dry weight, the content of N in the biomass significantly differed between species and soils, with the lowest values in Site 4 for both lupines and in Sites 1 and 3 for oats. In contrast to the lupines' P content, the values of N content differed less between sites, as the soil type effect was not statistically significant in terms of N concentration (Table 4). The ANOVA results revealed no significant difference between lupine species in Ndfa (Supplementary Materials, Table S6). However, there were statistically significant differences in the N biomass and the amount of N that was fixed, with average values of 347 and 237 mg N pot^{-1}, respectively, registering the highest values in *L. albus*. These variables, expressed as total N and N fixed content in shoots' dry weight per hectare to determine their agronomic significance to N entering the soil system, represent approximately 190 kg N ha^{-1} and 130 kg ha^{-1} of N, respectively.

3.3.2. Plants' Ca, Mg, K, and Micronutrient (Fe and Mn) Concentrations in Aboveground Biomass

The ANOVA of the plant nutrient concentration revealed that only the K concentration was similar between species. The K concentration in *L. albus* showed differences between soils, with the highest concentration values found in Site 3 and the lowest in Site 4. On the other hand, there were significant variations in Ca and Mg levels among species, with *L. angustifolius* consistently exhibiting the highest concentrations across all soil types (Table 5).

The impact of different species on the concentration of micronutrients in plants varied depending on the soil type (notably, a significant interaction effect was found between soil type x species). Across most soils, *L. angustifolius* exhibited the highest concentrations of Mn and Fe, with two exceptions. Specifically, *L. albus* had statistically higher Mn content at Site 3 compared to oats, and oats displayed the highest concentrations of Fe at Site 4 compared to lupines (Table 5). In all soils, the Mn concentration was substantially higher in lupines than in oats. The concentrations of Mn and Fe in the aboveground biomass also differed among soils within each species, with oats showing no significant differences in their Mn concentration across soils. Similarly, *L. angustifolius* did not show significant differences in their Fe concentration. When examining the Fe concentrations of each species across soils, both lupine species consistently exhibited higher values than oats in all soils except in Site 4, as previously mentioned.

Table 5. Plants' Ca, Mg, K, Mn, and Fe concentration of different species, as affected by soil type. Values are means ± standard error.

Site	Species	Plants' Ca Concentration	Plants' Mg Concentration	Plants' K Concentration	Plants' Mn Concentration	Plants' Fe Concentration
		mg g^{-1}			mg kg^{-1}	
1	*Lupinus albus*	5.6 ± 0.4 b AB	1.4 ± 0.1 b AB	14.3 ± 1.7 ns B	482.6 ± 51.2 b B	97.2 ± 12.7 b BC
	Lupinus angustifolius	26.5 ± 1.6 a ns	4.7 ± 0.3 a ns	16.2 ± 0.2 ns ns	1067.0 ± 150.2 a B	214.2 ± 23.5 a ns
	Avena strigosa	3.1 ± 0.2 c B	1.2 ± 0.1 c B	15.5 ± 0.6 ns ns	216.7 ± 27.6 b ns	36.9 ± 8.6 b B
2	*Lupinus albus*	3.9 ± 0.4 b B	1.1 ± 0.1 b B	13.7 ± 1.1 ns B	537.6 ± 218.2 b B	54.0 ± 5.5 ab C
	Lupinus angustifolius	22.5 ± 1.1 a ns	4.4 ± 0.2 a ns	14.7 ± 1.9 ns ns	3161.0 ± 259.1 a A	142.1 ± 4.8 a ns
	Avena strigosa	2.9 ± 0.3 b B	1.5 ± 0.1 b B	13.0 ± 0.9 ns ns	214.9 ± 21.66 b ns	28.7 ± 2.6 b B
3	*Lupinus albus*	7.2 ± 0.1 b A	2.0 ± 0.3 b A	17.1 ± 0.7 ns A	1191.2 ± 21.51 a A	165.3 ± 21.5 a AB
	Lupinus angustifolius	26.7 ± 1.3 a ns	4.5 ± 0.2 a ns	17.4 ± 1.1 ns ns	791.9 ± 184.0 a B	214.6 ± 36.6 a ns
	Avena strigosa	4.4 ± 0.6 b AB	1.0 ± 0.2 b B	15.1 ± 0.2 ns ns	209.8 ± 96.0 b ns	47.7 ± 18.7 b B
4	*Lupinus albus*	4.3 ± 0.1 b B	1.2 ± 0.2 b B	13.2 ± 1.3 ns B	628.6 ± 210.0 ab B	189.0 ± 28.5 b A
	Lupinus angustifolius	12.0 ± 0.0 a ns	3.1 ± 0.0 a ns	10.8 ± 0.0 ns ns	1021.5 ± 0.0 a B	218.6 ± 0.0 b ns
	Avena strigosa	4.1 ± 0.2 a A	2.9 ± 0.2 a A	14.7 ± 0.2 ns ns	403.0 ± 47.3 b ns	440.1 ± 88.7 a A
Significance of treatment effect						
Species		**<0.0001**	**<0.0001**	0.7014	**<0.0001**	**0.0342**
Soil type		0.1651	0.4564	**0.0453**	**<0.0001**	**<0.0001**
Species × soil type		**0.0237**	**0.0026**	0.4818	**<0.0001**	**<0.0001**

Significant effects ($p < 0.05$) are in bold. Different lowercase letters within a column indicate differences between species within each soil type, and different capital letters within a column indicate differences between sites across species being significant at a *p*-level of 0.05. ns means no significant difference.

3.4. Relationships between Soil and Plant Parameters

The correlations between the soil and plant variables revealed a moderate, yet significant (p = 0.0001) positive relationship between plants' N and P contents (Table 6). This correlation increased to 0.90 (p = 0.0001) and 0.65 (p = 0.0068) when the legumes and oats datasets were separated. The shoots' dry weight and shoots' P and N contents were also positively related to the soil's PBray1 availability at 48 dap and negatively related to the ΔpH-initial (Table 6). This last finding suggests that a pH drop, relative to its initial value, increased the P and N uptake. In addition, the relationships between ΔpH−initial and ΔPBray1−initial, depicted in Figure 4, displayed a positive association for each species, showing that when the pH decreased (soil acidification), the PBray1 at harvest diminished compared to its initial value. This outcome can be linked to the significant negative correlation (−0.59, $p < 0.001$) between plants' P content and ΔpH−initial across all combinations of species and soils (Table 6) and within each species across all four soils types (Supplementary Materials, Table S7).

Table 6. Matrix of Pearson correlation coefficients (r) for the relationships between soil (PBray1 and pH) and plant variables (P and N content, Fe and Mn concentration) for all combinations of species (lupines and oats) and soils at the harvest time of the experiment (87dap).

	ΔpH− Initial †	**PBray1− 48dap**	**PBray1− 87dap**	**Shoots' Dry Weight**	**Plants' N Content**	**Plants' P Content**	**Plants' Fe Concentration**
PBray1−48dap	−0.56 ***						
PBray1−87dap	−0.61 ***	0.76 ***					
Shoots' dry weight	−0.24	0.39 **	0.06				
Plants' N content	−0.56 ***	0.49 ***	0.50 ***	0.32 *			
Plants' P content	−0.59 ***	0.66 ***	0.45 **	0.83 ***	0.54 ***		
Plants' Fe concentration	0.53 ***	−0.57 ***	−0.42 **	−0.36 *	−0.17	−0.61 ***	
Plants' Mn concentration	−0.36 *	0.02	0.36 *	−0.28	0.14	−0.13	0.15

† ΔpH-initial: difference between soil pH at harvest and soil pH at the beginning of the experiment. Significant at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

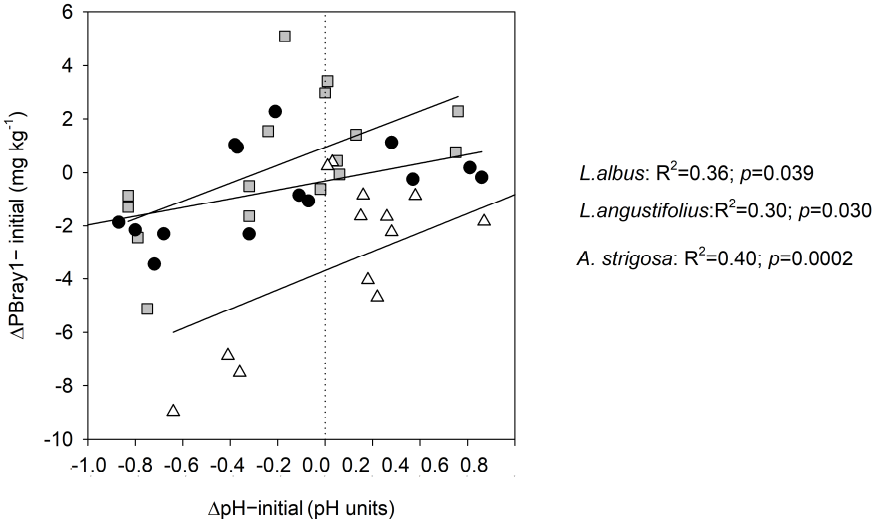

Figure 4. Relationships between ΔpH−initial (difference between soil pH at harvest and soil pH at the beginning of the experiment) and ΔPBray1−initial (difference between soil PBray1 at harvest and soil PBray1 at the beginning of the experiment). The dotted line splits the data from soil acidification to alkalinization relative to the initial pH. Gray squares, black circles, and white triangles represent *Lupinus albus*, *Lupinus angustifolius*, and *Avena strigosa* species, respectively.

This study also revealed a notable inverse relationship between plants' Fe concentration and PBray1 and a direct relationship between PBray1 and plants' P content. Addition-

ally, a significant positive association was detected between plants' Fe concentration and the soil's ΔpH−initial. The first two associations establish a positive relationship between the soil's P availability and the plants' P content, while indicating a negative relationship between plants' Fe concentration and the soil's P availability. Conversely, the final association suggests that an increase in the soil pH was associated with an increase in plants' Fe concentration. The concentration of Mn in plants, on the other hand, exhibited a weak and inverse correlation with the soil's ΔpH-initial, suggesting that as the soil becomes more acidic within the range of the examined pH values (4.5–6.4 units), the soil's Mn solubility will increase (Table 6). Nevertheless, when analyzing within each species (Supplementary Materials, Table S7), the correlation was statistically significant and positive just for oats (r = 0.52, p = 0.037). Meanwhile, the soil pH at harvest only correlated with plants' Mn concentrations for both lupines, but it was positive (r = 0.66, p = 0.006) for *L. albus* and negative (−0.76, p = 0.011) for *L. angustifolius*. In the case of oats, the significant correlations that were observed between plants' Mn concentrations and PBray1−48 dap (r = −0.58) or plants' Mn concentrations and plant's P concentrations (r = −0.59) might be due to the influence of data from Site 4, in which the soil's plant Mn concentration was more than double compared to oats that were cultivated in the other soils (Table 5). For *L. albus*, there was a negative trend (r = −0.5, p = 0.1, excluding Site 4) between the Mn concentration (as an index of the organic anion concentration) and plant's P concentrations (Supplementary Materials, Table S8). In contrast, there was a significant correlation at a higher soil PBray1 at 48 dap (r = −0.63, p = 0.048, excluding Site 4, Supplementary Materials, Table S8). These correlations were not significant for *L. angustifolius* (Supplementary Materials, Table S8).

4. Discussion

4.1. Effects of Species and Soil Types: Variations in Soil's PBray1 Concentration and Soil pH

The changes in the soil's PBray1 relative to its initial concentration were agronomically negligible in soils that were planted with lupine. These results suggest that this species could solubilize plant-unavailable residual P or mobilize organic P forms, consequently maintaining an equilibrium level and not being depleted by plant absorption. In contrast, the absorption by oats reduced the amount of available P. When analyzing $\Delta PBray_{\_Lup\text{-}Oat}$, the values ranged from 0.9 (not a significant difference) to 5.4 mg kg^{-1}, and assuming an equivalent fertilizer value of 10 kg ha^{-1} of P_2O_5 (the average value used as equivalent fertilizer for Uruguayan soils according to Hernández and Zamalvide [41]), the highest increase in P availability due to lupines occurred in Site 2, which represents an equivalent of 54 kg ha^{-1} of P_2O_5. This increase in soil's PBray1 availability would significantly contribute to systems with a high proportion of P-extractive crops, leading to a considerable reduction in the application of P fertilizers. However, the species effect was also affected by the soil type, as lupine plants grew better in sites with higher PBray1 availability (Sites 1, 2, and 3) and greater soil fertility (high OM and high exchangeable cations).

The increase in PBray1 at 48 dap may be primarily attributed to the mineralization of organic P. The OM content would explain the extent of this increase, with the most significant increase in Site 1 and Site 2, which had higher OM contents than the other two sites. Interestingly, by 76 dap, the available P in the soil began to stabilize, i.e., returning to pre-experimental levels. This finding suggests that the mineralized organic P was either absorbed by the plants, retrograded in the soil, or immobilized by microorganisms [42]. The soil's biological activity may positively influence the mobilization (accessibility) of organic P forms [43,44] or negatively hamper the P mobilization efficiency of carboxylates by way of microbial degradation [13] and biochemical factors, such as the hydrolysis rates of extracellular phosphatase enzymes [11,43,45], which play a role in plant P acquisition [46]. Other physical factors, such as variations in the soil moisture, drying conditions, and aggregate stability, may have facilitated the mineralization of organic P [46].

The higher soil buffering capacity in Site 1 and Site 3 (higher clay content, OM, and cation exchange capacity) may account for the lower PBray1 variation compared with their baseline values at 87 and 103 dap. Conversely, the minimal change in Site 4 could be due to

the generally poor performance of the lupine, particularly *L. angustifolius*, which exhibited the lowest shoot dry weight and P uptake. The soil of Site 2, however, demonstrated the most remarkable differences in $\Delta PBray_{\_Lup\text{-}Oat}$, which could be attributed to the higher P biomass that was attained by oats. The loamy texture, moderate clay, and OM content of Site 2 suggest a lower P adsorption capacity, leading to a higher equilibrium concentration of labile P forms in this soil compared to Sites 1 and 3, which possibly boosts the efficiency of organic anions in solubilizing inorganic P [42]. Moreover, this site had the highest PBray1 concentration at the start of the experiment, which can be partially attributed to organic P sources, ensuring a more consistent and substantial P supply for plants.

Even though lupine increased the PBray1 availability in all soils, the species effect alone could not entirely explain the increase in P by soil acidification, suggesting that other factors associated with the species could account for the differences [12]. It is crucial to recognize that variations in a species' capacity to obtain P can be attributed to the inherent traits of each species. Furthermore, morphological modifications in the root architecture and the development of specialized structures such as proteoid or cluster roots can also contribute to these differences [14,47]. While our study did not directly measure the contribution and composition of the root exudates of organic acids, we consider this trait indirectly based on the Mn concentration, which is proposed as an index of the carboxylate concentration in the rhizosphere under low P availability conditions in soil [21,48]. The subsequent section of this paper will delve further into this matter.

As demonstrated in our study, the BNF process contributed to variations in the soil pH during the experiment, leading to higher plant Mn concentrations in lupines than in oats. Legumes also absorb more cations than anions, resulting in the rhizosphere's exudation of protons and acidification [49]. It was further reported that P deficiency in *L. albus* stimulates proton release and citrate root exudation by the proteoid roots of this species, along with the inhibition of nitrate uptake [50,51]. Pearse et al. [14] showed that seven lupine species had a greater acidification capacity of the soil rhizosphere than grasses and even than other legume crops, arguing that this could be an adaptation to increase the solubility of acid-soluble Ca-phosphates. In the present study, both lupine species contributed to pH decreases in different soil types, showing comparable trends in acidification levels, except for in Site 3, where *L. albus* had a minimal influence on the soil pH. This discrepancy could be attributed to differences in the inherent characteristics of the species, as mentioned above. *Lupinus albus* is a cluster root-forming legume and grows well in strongly acidic soils and mildly acid to neutral soils; *L. angustifolius*, on the contrary, does not form cluster roots and is sensitive to calcareous soil, displaying a preference for acidic soil conditions. Consequently, the growth of *L. angustifolius* in Site 3 may have induced changes in the rhizosphere that resulted in a decrease in the soil pH by an average of 0.6 units. This decline in pH, caused by a net release of protons, may have occurred as a compensatory mechanism to counterbalance the elevated uptake of cations. The high concentration of cations that was observed in the aboveground biomass of *L. angustifolius* in this study was consistent with previous studies [52,53].

When oats were cultivated, all soils (except Site 2) experienced an increase in pH compared to the initial values, with the Site 4 soil experiencing an increase of nearly one pH unit. Wang et al. [28], using the rhizotron technique with *Avena sativa*, also found that the soil pH increased compared to the control (without plants). In that study, despite having the lowest acid phosphatase activity, oats absorbed the same amount of P as other species, a result that was attributed to the extensive root mass and high mycorrhizal colonization, which contributed to the high P absorption [28]. We did not investigate the morphology or other aspects of the roots in our study, but this explanation would be valid for our experiment. Additionally, we can partially explain the pH change trends in oat-bearing soils based on an imbalance of the cation–anion uptake (mainly influenced by the N source). In Site 2, ammonium was the predominant form of N, and its absorption by oats would account for the soil acidification in this case [54]. However, the increased uptake of N in the form of nitrate would increase the absorption of H+, consequently promoting soil alka-

linization [54]; this would explain the increase in the soil pH with oats in the acidic soil of Site 4.

4.2. Effects of Species and Soil Types: Plant Growth and Nutrient Uptake

While the availability of PBray1 differed among Sites 1, 2, and 3, there were no significant differences in the shoot biomass within each lupine species across these soils. However, the P concentrations were comparable to or higher than those of oats across these soils. In addition, these sites exhibited significant differences in aboveground biomass and plants' N and P contents for oats, indicating that the P supply and an additional factor (N availability) impacted the performance of this species across soil types differently. These findings align with a prior study that indicated that *L. albus* was the least impacted species by P supply, but that it exhibited the greatest P concentration in its shoots among all plant species [11].

Regarding P concentrations, whatever the species considered, there were differences across sites, particularly for Site 4, where the concentrations were, on average, half of those registered in the other sites. Previous studies have reported that the critical threshold for optimum development for *L. albus* and *L. angustifolius* is 2.2 and 2.3 mg g^{-1} of P, respectively [55]. In our work, the shoots' P concentrations of lupine species was below 2.0 mg g^{-1}, a level that typically stimulates cluster root development [56]. Concerning oats, the plant's P concentration would be adequate for their mature stage, which oscillates between 1.0 and 1.5 mg g^{-1} [57]. Furthermore, all concentrations of P, regardless of the species, were lower than 2.0 mg P g^{-1} dry weight, implying that the shoot biomass decay will probably lead to limited P availability in the soil due to P immobilization in the microbial biomass, as Hallama et al. noted [21].

The lowest plant growth, P uptake, and BNF values were observed at Site 4, suggesting that even though lupine species have adapted to acidic soils, Site 4 had several growth-limiting factors that prevented them from thriving as well as they did at the other sites. *Lupinus angustifolius* exhibited reduced growth and accelerated senescence of leaves, and both lupine species showed lower P and K concentrations in plants in Site 4 compared to other sites with higher soil PBray1, OM, and K concentration. The poor performance of lupines in Site 4, which experienced severe P deficiency, can be linked to the disruption in their ability to balance the carbon costs that are involved in cluster root development and nodulation [58,59].

The proportion and amount of N in the lupine's aerial biomass that are derived from the air fall within the reported ranges of 44 to 95% and 147 to 400 kg ha^{-1}, respectively [60]. The amount of N that is fixed by *L. albus* is, in most cases, higher than that of *L. angustifolius*, as evidenced by our research [23,61]. The wide variation between soils in the quantity of fixed N (60–240 mg pot^{-1}, or approximately 30–140 kg N ha^{-1}) reflects the varying levels of natural fertility among soils. The N that is fixed in biomass also varies between species (105–240 mg pot^{-1}, approximately 60–130 kg N ha^{-1}; $p = 0.0001$), indicating that lupines have different soil requirements that mainly affect the shoots' dry weight. Besides solubilizing non-labile forms of P, the N that is derived from the BNF process is an additional benefit of the lupine genus, because this contribution of N to the system might facilitate the balancing of N losses that occur during agricultural cycles [62].

4.3. Effects of Species and Soil Types: Links between Traits of Soil and Plants

The high PBray1 at harvest in soil containing *L. albus* might suggest a P-sparing effect of this species. However, during the growing phase, the amount of P that was absorbed by *L. albus* was, as was previously mentioned, comparable to and even higher than those taken up by oats. Although higher amounts of PBray1 were present at harvest in soils under *L. albus* than under oats, the differences are probably not due to variations in P uptake but instead to the "rhizosphere effect" that is induced *L. albus*. Neumann et al. [63] stated that *L.albus* employs diverse mechanisms to fulfill its P requirements in nutrient-deficient environments. This species exudes organic acids (citric and malic) that are sufficient to

mobilize scarcely available P sources (Ca, Al, and Fe phosphates), primarily via chelation of the bound cations to P or by competition for P adsorption sites in the soil matrix [64]. However, the role of these carboxylates was not consistent in several studies that have reported that in other species, and even *L. albus*, the carboxylate concentration was not explained by any variation in soil or plant traits [11,15,48], leading to the conclusion in those studies that organic anions play a minor role in P acquisition strategies.

The concentration of PBray1 at harvest showed a negative correlation ($r = -0.61$) with the difference in pH from the initial pH across all combinations of species and soils. This result suggests that the increase in the acidification level within the tested soil pH range (4.5 to 6.4 units) was more pronounced in soils with high PBray availability. This result was unexpected in acidic soils, given that it has been reported that the P adsorption capacity of Fe and Al oxides increases with a decreasing pH [49]. However, other studies have shown that lowering the pH of acidic soils can also increase the solubility of soil P [65]. The proposed explanation is that competitive adsorption of sulfate ions would increase with a decreasing pH, leading to a higher equilibrium P concentration [49]. In the same way, the negative relationship between the amount of P biomass and the ΔpH−initial indicated that the P absorption increased with the degree of acidification, which is consistent with other studies [12,65,66]. On average, the plant P content was higher in *L. albus* and oats (14 mg pot^{-1}) than in *L. angustifolius* (7.9 mg pot^{-1}), mainly due to the low biomass yield of the latter species. The positive association of accumulated N and P amounts with the availability of PBray1 ($r = 0.50$ and 0.45, respectively) at harvest (87 dap) and the negative association with the ΔpH−initial ($r = -0.56$ and -0.59, respectively) show that N and P absorption increased with the available soil P and with soil acidification. This last association explains the negative correlation between the ΔpH−initial and the PBray1 that is available in the soil ($r = -0.61$).

Lupinus angustifolius reached higher levels of base cation than *L. albus*, as observed by other researchers [53], although, for K, there were no statistically significant differences between species at each site. The total cation concentrations in plants within each species also differ across soils. These differences were exclusively observed in *L. albus*, showing the lowest concentrations in plants that were cultivated in Sites 2 and 4, whose sites corresponded to soils that were strongly desaturated in base cations. Nevertheless, these last two soils differed widely in their pH and OM content. For the plant cation concentration in the lupine genus in our experiment, and based on previous research [53,67], this would indicate that plant nutrition was adequate in Sites 1, 2, and 3, and insufficient to cover lupine's requirements in Site 4.

The lupine-induced soil acidification remarkably impacted plants' micronutrient concentrations such as Mn and Fe. The Mn concentrations in *L. albus* and *L. angustifolius* reached mean values of 710 and 1510 mg kg^{-1}, respectively, which represent approximately three and seven times the concentration in oats (228 mg kg^{-1}), as was reported in previous studies [47,68]. These high concentrations of foliar Mn may be deleterious to other organisms or species, but the lupine genus tolerates them [48]. It has been found that plants using a P mobilization strategy based on the release of carboxylates have elevated Mn concentrations in their leaves [68], because carboxylates mobilize both inorganic and organic P from the soil, as well as micronutrients [69]. Modifications in soil acidity or the oxidation/reduction conditions of the rhizosphere can also enhance the increased uptake of Mn [68]. It was further confirmed that lupines' exudation of organic acid anions may or may not be linked to soil acidification, as these processes self-regulate independently [70]. Further, a negative correlation between citrate exudation and plants' P status has been established, which has been perceived clearly with *L. albus* species [71]. Pearse et al. [14] and Wang et al. [72] also observed the variable response among lupine species, who found variation in both their P uptake and sensitivity to external P supply on the formation and development of proteid roots. These authors highlighted that the variations across species within the lupine genus open the possibility of selecting species with high plasticity regarding P supply [14]. In those studies, under pot experiments and using river

sand as a growth medium, it was also reported that *L. albus* developed the most cluster roots with the lowest P concentration in shoots. Considering this context and under similar plant cultivation conditions, but using soils as a growth medium, in the present study, the Mn concentration (as an index of the organic anion concentration) in *L. albus* was down-regulated at a greater P concentration in plants. However, this association was barely a trend ($r = -0.5$, $p = 0.1$, excluding Site 4). In addition, there was a significant correlation at a higher soil PBray1—48 dap ($r = -0.63$, $p = 0.048$, excluding Site 4), suggesting that the organic anion exudation increased when the growth medium had a low PBray1 concentration. These results are in accordance with previous studies [15,28], which found no simple linear relationships between plant and soil traits. In our work, *L.albus* showed a low Mn concentration in plants under severe P stress (conditions under Site 4), maximum under moderate stress (conditions under Site 3), and low again at a high P availability (conditions under Sites 1 and 2). In contrast, the *L. angustifolius* plants with the highest concentration of Mn were those growing in the soil of Site 2, which exhibited the highest initial PBray1 concentration. These findings suggest that the exudation of carboxylates by *L. angustifolius* in the soil is constitutive, similar to what was reported by Pang et al. [73] for chickpea species (*Cicer arietinum* L.), a non-cluster root-forming crop. In contrast, the exudation of carboxylates by *L. albus* would be inducible, because the plants' Mn concentration changed in response to the soil's PBray1 availability [72]. Monei et al. [74], who also noted a divergent response in these lupine species, found that *L. albus* exhibited a high release of carboxylates in conditions of P deficiency, whereas *L. angustifolius* responded with the highest release of carboxylates when the soil had a high P supply. Although there was no clear relationship between Mn concentrations and plants' P contents or soil's P availability in both lupines across all sites in the current study, there was a significant association between the soil acidity at harvest and the Mn concentration; however, it was noticeably different between the lupine species. The correlation between these last variables was statistically significant, but positive ($r = 0.66$) for *L. albus* and negative ($r = -0.76$) for *L. angustifolius*, suggesting that the exudation of organic acid anions was concomitant with proton extrusion in soils containing *L. angustifolius*, while in *L.albus*, this was the case with base cations [48].

5. Conclusions

This research contributes to understanding how the species and soil type affect P mobilization and nutrient uptake and helped us inquire about the relationships between plant and soil factors that might explain or give us a clue to increase the comprehension of the processes of controlling P demand relative to its availability in the soil. *Lupinus albus* had a more substantial P solubilization capacity in the light-textured soil with a high OM content. However, the plant growth and nutrient uptake of *L. albus* in this site were comparable to those in the heavy-textured soils. The degree of soil acidity had a direct relationship with the P and N uptake within each species, across all soil types tested. The soil pH differed among species, with the lowest values found in soils that were cultivated with lupine species. In contrast, the soils under oats tended to maintain or even increase their pH compared to the original values. Our investigation revealed a significant variation in Mn accumulation (used as an index of the organic anion concentration) within species across the tested soils. The soil acidity and changes in P supply that were induced by lupines could explain such variation, particularly in soils with *L. albus.* Comparing these results with those obtained with these species in the field would be necessary to determine the robustness of the identified patterns in the soil's available P by the presence of lupine and to analyze them from the perspective of long-term stability, because the interaction and feedback with other soil–plant factors and microbial activity processes could hamper the increase in soil's P availability that is facilitated by lupine species. Hence, field research should be carried out for at least two growing seasons to ascertain whether the advantageous impacts of lupine last with the same magnitude over time and whether these variations result in enhanced soil health and crop production. Additionally, the future recommendations that emerge from the findings of our study include the need to measure

soil's phosphatase activity, as this is an essential indicator for evaluating the effectiveness of P that is taken out by plants and analyzing the associated changes in organic P sources, such as the immobilized P within the living soil microbial biomass.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy14020389/s1: Table S1: Initial soil properties before the establishment of the experiment; Table S2: ANOVA for soil PBray1 concentration at 87 days after planting; Table S3: ANOVA for soil PBray1 concentration at 103 days after planting; Table S4: ANOVA for soil pH at 87 days after planting; Table S5: Orthogonal contrasts for biomass measurements; Table S6: Mean values of BNF proportion (% BNF) and N fixed content of lupines as affected by species and soil type; Table S7: Pearson correlation coefficients (r) within each species across all four soil types; Table S8: Pearson correlation coefficients (r) within each species across three soils types (excluding Site 4).

Author Contributions: Conceptualization, C.M.A. and C.P.V.; methodology, C.M.A. and A.B.G.; formal analysis, C.M.A., C.P.V., P.G.B. and A.d.P.M.; investigation, C.M.A. and A.B.G.; resources, C.M.A.; data curation, C.M.A., C.P.V., P.G.B. and A.d.P.M.; writing—original draft preparation, C.M.A., C.P.V., P.G.B., A.B.G. and A.d.P.M.; writing—review and editing, C.M.A., C.P.V., P.G.B. and A.d.P.M.; visualization, C.M.A. and A.d.P.M.; supervision, A.d.P.M. and C.P.V.; project administration, C.M.A.; funding acquisition, C.M.A., C.P.V. and A.d.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research and Innovation Agency of Uruguay, with the following funds: María Viñas, grant number ANII: FMV_1_2017_1 135487. The research was also supported by C.M.A., a recipient of a doctoral fellowship from CAP (ComisiónAcadémica de Posgrado), Universidad de la República (UdelaR). The APC was funded by the National Research of our Institution University of the Republic (UdelaR, Uruguay). This work is part of the Doctoral Dissertation of the senior author at the Doctoral Program in Agricultural Sciences, Agronomy College, UdelaR, Uruguay.

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

Acknowledgments: The authors express their gratitude to N. Di Muro and B. Romano for their assistance in conducting the greenhouse experiment and laboratory work.

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

References

1. Vassallo, M. Dinámica y competencia intrasectorial en la agricultura uruguaya: Los cambios en la última década. *Agrociencia* **2013**, *17*, 170–179. [CrossRef]
2. Ernst, O.R.; Dogliotti, S.; Cadenazzi, M.; Kemanian, A.R. Shifting crop-pasture rotations to no-till annual cropping reduces soil quality and wheat yield. *Field Crops Res.* **2018**, *217*, 180–187. [CrossRef]
3. Ernst, O.R.; Kemanian, A.R.; Mazzilli, S.R.; Cadenazzi, M.; Dogliotti, S. Depressed attainable wheat yields under continuous annual no-till agriculture suggest declining soil productivity. *Field Crops Res.* **2016**, *186*, 107–116. [CrossRef]
4. Ministerio de Ganadería, Agricultura y Pesca. DIEA presenta los resultados de la Encuesta Agrícola "Invierno 2021". Available online: https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/datos-y-estadisticas/estadisticas/diea-presenta-resultados-encuesta-agricola-invierno-2021 (accessed on 7 October 2021).
5. Beretta-Blanco, A.; Pérez, O.; Carrasco-Letelier, L. Soil quality decrease over 13 years of agricultural production. *Nutr. Cycl. Agroecosystems* **2019**, *114*, 45–55. [CrossRef]
6. Ernst, O.; Siri-Prieto, G. Impact of perennial pasture and tillage systems on carbon input and soil quality indicators. *Soil Tillage Res.* **2009**, *105*, 260–268. [CrossRef]
7. Cano, J.D.; Ernst, O. Balance aparente de fósforo en rotaciones agrícolas del litoral oeste del Uruguay 1. *Inf. Agronómicas* **2005**, *32*, 8–11.
8. Syers, J.K.; Johnston, A.E.; Curtin, D. Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soil phosphorus behaviour with agronomic information. In *FAO Fertilizer and Plant Nutrition Bulletin*; FAO: Rome, Italy, 2008; p. 128.
9. Menezes-Blackburn, D.; Giles, C.; Darch, T.; George, T.S.; Blackwell, M.; Stutter, M.; Shand, C.; Lumsdon, D.; Cooper, P.; Wendler, R.; et al. Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: A review. *Plant Soil* **2018**, *427*, 5–16. [CrossRef]

10. Richardson, A.E.; Barea, J.M.; McNeill, A.M.; Prigent-Combaret, C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* **2009**, *321*, 305–339. [CrossRef]
11. Touhami, D.; McDowell, R.W.; Condron, L.M. Role of organic anions and phosphatase enzymes in phosphorus acquisition in the rhizospheres of legumes and grasses grown in a low phosphorus pasture soil. *Plants* **2020**, *9*, 1185. [CrossRef]
12. Barrow, N.J.; Hartemink, A.E. The effects of pH on nutrient availability depend on both soils and plants. *Plant Soil* **2023**, *487*, 21–37. [CrossRef]
13. Schack-Kirchner, H.; Loew, C.A.; Lang, F. The Cumulative Amount of Exuded Citrate Controls Its Efficiency to Mobilize Soil Phosphorus. *Front. For. Glob. Change* **2020**, *3*, 550884. [CrossRef]
14. Pearse, S.J.; Veneklaas, E.J.; Cawthray, G.R.; Bolland, M.D.A.; Lambers, H. Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant Soil* **2006**, *288*, 127–139. [CrossRef]
15. Veneklaas, E.J.; Stevens, J.; Cawthray, G.R.; Turner, S.; Grigg, A.M.; Lambers, H. Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. *Plant Soil* **2003**, *248*, 187–197. [CrossRef]
16. Kamh, M.; Abdou, M.; Chude, V.; Wiesler, F.; Horst, W.J. Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. *J. Plant Nutr. Soil Sci.* **2002**, *165*, 566–572. [CrossRef]
17. Lambers, H.; Clements, J.C.; Nelson, M.N. How aphosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, *Fabaceae*). *Am. J. Bot.* **2013**, *100*, 263–288. [CrossRef] [PubMed]
18. Ayala, W.; Barrios, E.; Macedo, I.; Sawchik, J.; Terra, J.A. Scouting benefits and developing innovations in temperate grassland to sustainable agriculture production. In Proceedings of the 23rd International Grassland Congress on Sustainable Use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection, New Delhi, India, 20–24 November 2015; pp. 208–214.
19. Fontaine, S.; Abbadie, L.; Aubert, M.; Barot, S.; Bloor, J.M.G.; Derrien, D.; Duchene, O.; Gross, N.; Henneron, L.; Le Roux, X.; et al. Plant–soil synchrony in nutrient cycles: Learning from ecosystems to design sustainable agrosystems. *Glob. Change Biol.* **2023**, *30*, e17034. [CrossRef] [PubMed]
20. Kirkegaard, J.; Christen, O.; Krupinsky, J.; Layzell, D. Break crop benefits in temperate wheat production. *Field Crops Res.* **2008**, *107*, 185–195. [CrossRef]
21. Hallama, M.; Pekrun, C.; Lambers, H.; Kandeler, E. Hidden miners—The roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* **2019**, *434*, 7–45. [CrossRef]
22. Mera, M.; Lizana, X.C.; Calderini, D.F. Cropping systems in environments with high yield potential of southern Chile. In *Crop Physiology: Applications for Genetic Improvement and Agronomy*, 2nd ed.; Sadras, V., Calderini, D., Eds.; Academic Press: Cambridge, MA, USA, 2015; pp. 111–140. [CrossRef]
23. Espinoza, S.; Ovalle, C.; Zagal, E.; Matus, I.; Tay, J.; Peoples, M.B.; del Pozo, A. Contribution of legumes to wheat productivity in Mediterranean environments of central Chile. *Field Crops Res.* **2012**, *133*, 150–159. [CrossRef]
24. Nuruzzaman, M.; Lambers, H.; Bolland, M.D.A.; Veneklaas, E.J. Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. *Aust. J. Agric. Res.* **2005**, *56*, 1041–1047. [CrossRef]
25. Wolko, B.; Clements, J.C.; Naganowska, B.; Nelson, M.N.; Yang, H. *Lupinus*; Kole, C., Ed.; Springer: Berlin/Heidelberg, Germany, 2011. [CrossRef]
26. Soil Survey Staff. *Claves para la Taxonomía de Suelos*, 12th ed.; USDA-Natural Resources Conservation Service: Washington, DC, USA, 2014; p. 399. Available online: https://www.nrcs.usda.gov/resources/guides-and-instructions/keys-to-soil-taxonomy (accessed on 15 September 2018).
27. Sawchik, J.; Siri, G.; Ayala, W.; Barrios, E.; Bustamante, M.; Ceriani, M.; Gutiérrez, F.; Mosqueira, J.; Otaño, C.; Perez, M.; et al. El sistema agrícola bajo amenaza: ¿qué aportan los cultivos de cobertura y/o laspasturas cortas? In Proceedings of the IV Simposio Nacional de Agricultura, Paysandú, Uruguay, 28–29 October 2015; Ribeiro, A., Barbazán, M., Eds.; Editorial Hemisferio Sur: Montevideo, Uruguay, 2015; pp. 149–168. Available online: http://www.ainfo.inia.uy/digital/bitstream/item/5164/1/Simposio-Nacional-Agricultura-2015-Sawchik-p.149-168.pdf (accessed on 12 February 2024).
28. Wang, Y.; Krogstad, T.; Clarke, J.L.; Hallama, M.; Øgaard, A.F.; Eich-Greatorex, S.; Kandeler, E.; Clarke, N. Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. *Front. Plant Sci.* **2016**, *7*, 1664. [CrossRef] [PubMed]
29. Suzuki, L.E.A.S.; Amaral, R.D.L.D.; Almeida, W.R.D.S.; Ramos, M.F.; Nunes, M.R. Oat Straw Mulching Reduces Interril Erosion and Nutrient Losses Caused by Runoff in a Newly Planted Peach Orchard. *Soil Syst.* **2023**, *7*, 8. [CrossRef]
30. Nelson, D.W.; Sommers, L.E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis*; Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Eds.; Soil Science Society of America and American Society of Agronomy: Madison, WI, USA, 1996. [CrossRef]
31. Thomas, G.W. Exchangeable Cations. In *Methods of Soil Analysis*; Page, A., Ed.; Wiley: Hoboken, NJ, USA, 1983; pp. 159–165. [CrossRef]
32. Lajtha, K.; Driscoll, C.T.; Jarrell, W.M.; Elliott, E.T. Soil phosphorus: Characterization and total element analysis. In *Standard Soil Methods for Long Term Ecological Research*; Oxford University Press: New York, NY, USA, 1999; pp. 115–143.
33. Rabuffetti, A. *La Fertilidad del Suelo y Su Manejo*; Hemisferio Sur: Montevideo, Uruguay, 2017.
34. Mulvaney, R. Nitrogen-inorganic forms. In *Methods of Soil Analysis: Chemical Methods—Part 3*; Sparks, D.L., Ed.; Soil Science Society of America, Inc.: Madison, WI, USA, 1996.

35. Rhine, E.D.; Mulvaney, R.L.; Pratt, E.J.; Sims, G.K. Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. *Soil Sci. Soc. Am. J.* **1998**, *62*, 473–480. [CrossRef]
36. Beretta, A.N.; Silbermann, A.V.; Paladino, L.; Torres, D.; Bassahun, D.; Musselli, R.; García-Lamohte, A. Soil texture analyses using a hydrometer: Modification of the Bouyoucos method. *Cienc. Investig. Agrar.* **2014**, *41*, 25–26. [CrossRef]
37. Shearer, G.; Kohl, D.H. N2-fixation in field settings: Estimations based on natural 15N abundance. *Aust. J. Plant Physiol.* **1987**, *13*, 699–756. [CrossRef]
38. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* **1962**, *27*, 31–36. [CrossRef]
39. Jones, J.B.; Case, V.W. Sampling, handling, and analyzing plant tissue samples. In *Soil Testing and Plant Analysis*; Westerman, R.L., Ed.; SSSA: Madison, WI, USA, 1990; pp. 389–427. [CrossRef]
40. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019; Available online: http://www.R-project.org (accessed on 15 February 2021).
41. Hernández, J.; Zamalvide, J. Procesos de retención de fosforo por los suelos evaluados a través de parámetros de suelo y planta. *Agrociencia* **1998**, *2*, 48–63. [CrossRef]
42. Oburger, E.; Jones, D.L.; Wenzel, W.W. Phosphorus saturation and pH differentially regulate the efficiency of organic acid anion-mediated P solubilization mechanisms in soil. *Plant Soil* **2011**, *341*, 363–382. [CrossRef]
43. Renella, G.; Landi, L.; Valori, F.; Nannipieri, P. Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil. *Appl. Soil Ecol.* **2007**, *36*, 124–129. [CrossRef]
44. Ai, J.; Banfield, C.C.; Shao, G.; Zamanian, K.; Stürzebecher, T.; Shi, L.; Fan, L.; Liu, X.; Spielvogel, S.; Dippold, M.A. What controls the availability of organic and inorganic P sources in top- and subsoils? A 33P isotopic labeling study with root exudate addition. *Soil Biol. Biochem.* **2023**, *185*, 109129. [CrossRef]
45. Janes-Bassett, V.; Blackwell, M.S.A.; Blair, G.; Davies, J.; Haygarth, P.M.; Mezeli, M.M.; Stewart, G. A meta-analysis of phosphatase activity in agricultural settings in response to phosphorus deficiency. *Soil Biol. Biochem.* **2021**, *165*, 108537. [CrossRef]
46. Condron, L.; Turner, B.; Cade-Menun, B. Chemistry and Dynamics of Soil Organic Phosphorus. In *Phosphorus: Agriculture and the Environment*; Thomas Sims, J., Sharpley, A.N., Westermann, D.T., Eds.; Wiley: Hoboken, NJ, USA, 2005. [CrossRef]
47. Watt, M.; Evans, J.R. Phosphorus acquisition from soil by white lupin (*Lupinus albus* L.) and soybean (*Glycine max* L.), species with contrasting root development. *Plant Soil* **2003**, *248*, 271–283. [CrossRef]
48. Wang, Y.; Lambers, H. Root-released organic anions in response to low phosphorus availability: Recent progress, challenges and future perspectives. *Plant Soil* **2020**, *447*, 135–156. [CrossRef]
49. Hinsinger, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. *Plant Soil* **2001**, *237*, 173–195. [CrossRef]
50. Neumann, G.; Römheld, V. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. *Plant Soil* **1999**, *211*, 121–130. [CrossRef]
51. Shen, J.; Li, H.; Neumann, G.; Zhang, F. Nutrient uptake, cluster root formation and exudation of protons and citrate in *Lupinus albus* as affected by localized supply of phosphorus in a split-root system. *Plant Sci.* **2005**, *168*, 837–845. [CrossRef]
52. Brand, J.D.; Tang, C.; Rathjen, A.J. Adaptation of *Lupinus angustifolius* L. and *L. pilosus* Murr. to calcareous soils. *Aust. J. Agric. Res.* **1999**, *50*, 1027–1033. [CrossRef]
53. Kerley, S.J.; Shield, I.F.; Huyghe, C. Specific and genotypic variation in the nutrient content of lupin species in soils of neutral and alkaline pH. *Aust. J. Agric. Res.* **2001**, *52*, 93–102. [CrossRef]
54. Kirkby, E.A.; Le Bot, J.; Adamowicz, S.; Römheld, V. Nitrogen in physiology—An agronomic perspective and implications for the use of different nitrogen forms. *Proc. Int. Fertil. Soc.* **2009**, *653*, 1–48.
55. Bolland, M.D.A.; Brennan, R.F. Comparing the phosphorus requirements of wheat, lupin, and canola. *Aust. J. Agric. Res.* **2008**, *59*, 983–998. [CrossRef]
56. Li, H.; Shen, J.; Zhang, F.; Tang, C.; Lambers, H. Is there a critical level of shoot phosphorus concentration for cluster-root formation in *Lupinus albus*? *Funct. Plant Biol.* **2008**, *35*, 328–336. [CrossRef] [PubMed]
57. Mengel, K.; Kirkby, E.A.; Kosegarten, H.; Appel, T. *Principles of Plant Nutrition*; Mengel, K., Kirkby, E.A., Kosegarten, H., Appel, T., Eds.; Springer: Dordrecht, The Netherlands, 2001. [CrossRef]
58. Uhde-Stone, C. White Lupin: A Model System for Understanding Plant Adaptation to Low Phosphorus Availability. In *Legume Nitrogen Fixation in Soils with Low Phosphorus Availability: Adaptation and Regulatory Implication*; Sulieman, S., Tran, L.S., Eds.; Springer: Cham, Switzerland, 2017; pp. 243–280. [CrossRef]
59. Pueyo, J.J.; Quiñones, M.A.; Coba de la Peña, T.; Fedorova, E.E.; Lucas, M.M. Nitrogen and Phosphorus Interplay in Lupin Root Nodules and Cluster Roots. *Front. Plant Sci.* **2021**, *12*, 184. [CrossRef] [PubMed]
60. Unkovich, M.J.; Baldock, J.; Peoples, M.B. Prospects and problems of simple linear models for estimating symbiotic N2 fixation by crop and pasture legumes. *Plant Soil* **2010**, *329*, 75–89. [CrossRef]
61. Unkovich, M.J.; Pate, J.S. An appraisal of recent field measurements of symbiotic N2 fixation by annual legumes. *Field Crops Res.* **2000**, *65*, 211–228. [CrossRef]
62. McLauchlan, K. The nature and longevity of agricultural impacts on soil carbon and nutrients: A review. *Ecosystems* **2006**, *9*, 1364–1382. [CrossRef]

63. Neumann, G.; Massonneau, A.; Martinoia, E.; Römheld, V. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. *Planta* **1999**, *208*, 373–382. [CrossRef]
64. Fink, J.R.; Inda, A.V.; Bavaresco, J.; Barrón, V.; Torrent, J.; Bayer, C. Adsorption and desorption of phosphorus in subtropical soils as affected by management system and mineralogy. *Soil Tillage Res.* **2016**, *155*, 62–68. [CrossRef]
65. Barrow, N.J. The effects of pH on phosphate uptake from the soil. *Plant Soil* **2017**, *410*, 401–410. [CrossRef]
66. Bouray, M.; Moir, J.L.; Lehto, N.J.; Condron, L.M.; Touhami, D.; Hummel, C. Soil pH effects on phosphorus mobilization in the rhizosphere of *Lupinus angustifolius*. *Plant Soil* **2021**, *469*, 387–407. [CrossRef]
67. Martínez-Alcalá, I.; Clemente, R.; Bernal, M.P. Metal availability and chemical properties in the rhizosphere of *Lupinus albus* L. growing in a high-metal calcareous soil. *Water Air Soil Pollut.* **2009**, *201*, 283–293. [CrossRef]
68. Lambers, H.; Hayes, P.E.; Laliberté, E.; Oliveira, R.S.; Turner, B.L. Leaf manganese accumulation and phosphorus-acquisition efficiency. In *Trends in Plant Science*; Elsevier: Amsterdam, The Netherlands, 2015; Volume 20, pp. 83–90. [CrossRef]
69. Ding, W.; Cong, W.F.; Lambers, H. Plant phosphorus-acquisition and -use strategies affect soil carbon cycling. *Trends Ecol. Evol.* **2021**, *36*, 899–906. [CrossRef]
70. Lambers, H.; Shane, M.W.; Cramer, M.D.; Pearse, S.J.; Veneklaas, E.J. Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Ann. Bot.* **2006**, *98*, 693–713. [CrossRef]
71. Dessureault-Rompré, J.; Nowack, B.; Schulin, R.; Luster, J. Spatial and temporal variation in organic acid anion exudation and nutrient anion uptake in the rhizosphere of *Lupinus albus* L. *Plant Soil* **2007**, *301*, 123–134. [CrossRef]
72. Wang, X.; Pearse, S.J.; Lambers, H. Cluster-root formation and carboxylate release in three Lupinus species as dependent on phosphorus supply, internal phosphorus concentration and relative growth rate. *Ann. Bot.* **2013**, *112*, 1449–1459. [CrossRef]
73. Pang, J.; Bansal, R.; Zhao, H.; Bohuon, E.; Lambers, H.; Ryan, M.H.; Ranathunge, K.; Siddique, K.H.M. The carboxylate-releasing phosphorus-mobilizing strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. *New Phytol.* **2018**, *219*, 518–529. [CrossRef]
74. Monei, N.; Hitch, M.; Heim, J.; Pourret, O.; Heilmeier, H.; Wiche, O. Effect of substrate properties and phosphorus supply on facilitating the uptake of rare earth elements (REE) in mixed culture cropping systems of *Hordeum vulgare*, *Lupinus albus* and *Lupinus angustifolius*. *Environ. Sci. Pollut. Res.* **2022**, *29*, 57172–57189. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.