agronomy

MDPI

Article

Unveiling the Effects of Phosphorus on the Mineral Nutrient Content and Quality of Alfalfa (*Medicago sativa* L.) in Acidic Soils

Zhou Li [1], Yunfei Hao [1], Xiaowen Wang [1], Jin He [2], Xuechun Zhao [1], Jihui Chen [1], Xinyao Gu [1], Mingjun Zhang [3], Feng Yang [4] and Rui Dong [1,5,*]

1 College of Animal Science, Guizhou University, Guiyang 550025, China; guxy@gzu.edu.cn (X.G.)
2 College of Agriculture, Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), Guizhou University, Guiyang 550025, China
3 Livestock and Poultry Genetic Resources Management Station of Guizhou Province, Guiyang 550025, China
4 Grassland Technology Experiment and Extension Station, Guiyang 550025, China; yangf0508@163.com
5 School of Natural Sciences, Massey University, Palmerston North 4410, New Zealand
* Correspondence: rdong@gzu.edu.cn

Abstract: Alfalfa (*Medicago sativa* L.) grown in acidic soils is often affected by phosphorus (P) deficiency, which results in reduced mineral nutrient content and forage quality. In this context, the effects of phosphorus (P) fertiliser remain unclear. In this study, we analysed the effects of P application on mineral nutrient content and forage quality in aluminium (Al)-sensitive (Longzhong) and Al-tolerant (Trifecta) alfalfa cultivars cultivated in two acidic soil environments. Mineral nutrient content and quality were affected by genotype, soil type, and P treatment concentration ($p < 0.001$). In limestone soil, for Longzhong and Trifecta, the optimal potassium (K), calcium (Ca), and magnesium (Mg) contents as well as crude protein content (CP) and ether extract (EE) values were observed at 20 mg P kg^{-1}, that of the P content was observed at 40 mg P kg^{-1}, and the minimum neutral detergent fibre (NDF) acid detergent lignin (ADL) values were observed at 40 mg P kg^{-1}. In yellow soil, the maximum K, Ca, Mg, and P contents in Longzhong and Trifecta were observed at 40 mg P kg^{-1}, whereas the maximum CP, EE, and ADL values were observed at 20 mg P kg^{-1}. Our study provides an empirically based framework for optimising alfalfa fertilisation programmes in acidic soils.

Keywords: acidic soil; forage quality; *Medicago sativa* L.; mineral nutrient; phosphorus

Citation: Li, Z.; Hao, Y.; Wang, X.; He, J.; Zhao, X.; Chen, J.; Gu, X.; Zhang, M.; Yang, F.; Dong, R. Unveiling the Effects of Phosphorus on the Mineral Nutrient Content and Quality of Alfalfa (*Medicago sativa* L.) in Acidic Soils. *Agronomy* **2024**, *14*, 2271. https://doi.org/10.3390/agronomy14102271

Academic Editor: Jianjun Yang

Received: 14 July 2024
Revised: 17 September 2024
Accepted: 29 September 2024
Published: 2 October 2024

1. Introduction

Alfalfa (*Medicago sativa* L.) is the most extensively cultivated leguminous forage crop globally due to its high nutritional value and palatability [1]. The total global area under alfalfa cultivation is ~32 million hectares, with China's cultivation area surpassing 4.72 million hectares in 2015. Despite the area increasing annually, it still cannot meet the demands for global animal husbandry [2]. From 2012 to 2020, China's imports of alfalfa hay increased from 0.44 million tonnes to 1.36 million tonnes, while the average self-sufficiency rate of high-quality alfalfa was only 64% [3]. One important explanation is that most alfalfa in China can only be planted on marginal lands, which typically have poor soil fertility [4].

Crop yield and quality are of great concern in alfalfa production [5]. The optimal growth pH of alfalfa is 6.7–7.5, and it grows well in alkaline soils; however, its yield diminishes with decreasing soil pH [1,6]. This is because, at low pH levels, aluminium (Al) in the soil exists in the form of $[Al(H_2O)_6]^{3+}$ or Al^{3+}, which inhibits root growth and leads to severe aluminium toxicity in plants [6]. Meanwhile, in acidic soils, phosphorus (P) tends to bind with Al^{3+} to form stable, insoluble complexes that cannot be absorbed by plant roots [1]. As a result, P deficiency and Al toxicity are considered the main factors limiting crop yields in acidic soils, seriously hindering the cultivation and expansion of alfalfa in

China [7]. Notably, ~50% of potentially arable soil worldwide is acidic, which underscores the substantial production potential of tolerant alfalfa cultivars in these areas [8].

Increased soil P concentrations may ameliorate plant Al toxicity in acidic soils, possibly through improved root development and nutrient uptake [9]. Moreover, as the P content in plant roots increased, the toxic effects of Al ions gradually weakened. Therefore, fertilisation is a direct and effective management strategy for improving the yield and quality of alfalfa, especially in low-fertility acidic soils [4].

Phosphorus, an essential nutrient for plant growth and development, improves alfalfa photosynthesis and increases dry matter yield [10]. Phosphorus application can significantly increase the shoot phosphorus concentration, phosphorus uptake, and forage yield of young and old alfalfa while also increasing the crude protein (CP) content and regulating the acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents, ultimately affecting plant nutritional quality [10,11]. Phosphorus application also affects the uptake and utilisation of other mineral nutrients. Nitrogen (N), P, potassium (K), calcium (Ca), and magnesium (Mg) are essential macronutrients in plant growth and production [12]. Under high phosphorus concentrations, plants absorb more phosphorus and simultaneously increase their uptake of some cations such as K, Ca, Mg, and manganese (Mn) [13]. Soil phosphorus concentration and hay quality (CP and ADF) are closely related to plant growth and quality [14]. However, in reality, phosphorus is usually applied once to the soil as a base fertiliser, resulting in a large amount of available phosphorus being adsorbed by metal ions in the soil or converted into an insoluble form, thereby reducing the absorption of phosphorus by plants and promoting phosphorus pollution [10,14]. The rational application of phosphate fertiliser not only improves alfalfa hay yield and nutritional quality but also promotes the absorption and transformation of nitrogen and other mineral nutrients [13].

We previously analysed genotypic variation in various agronomic traits and the relationship between the yield components of forty-four alfalfa varieties in two acidic soil environments [1]. We also analysed the differences in phosphorus uptake strategies between Al stress-sensitive and AI-tolerant cultivars. The tolerant cultivars exhibited significantly higher shoot biomass, root morphology, and organic acid secretion from the roots compared to the sensitive cultivars. However, the relationships between alfalfa yield, nutritional quality, and mineral nutrient uptake at different phosphorus levels in acidic soils remain unclear. Therefore, this study aimed to characterise the effects of phosphorus fertiliser in acidic soil on the nutritional quality and absorption of other mineral nutrients in different genotypes of alfalfa (Al-sensitive and Al-tolerant) and determine the relationship between the associated drivers.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Two previously evaluated and screened alfalfa cultivars, Trifecta (Al-tolerant) and Longzhong (Al-sensitive), were used in this study [1]. We used limestone (26°50′02″ N, 105°15′47″ E) and yellow soil (26°25′39.62″ N, 106°40′5.81″ E), the two typical soil types in the karst area of Guizhou, as the substrate for pot experiments. Both soils were collected from the top 15 cm layers of undisturbed sites, air-dried, and sieved through a 2 mm sieve. The physicochemical properties of the soils are presented in Table 1. This study was conducted in a greenhouse at the College of Animal Science, Guizhou University, China, from August to December 2021. The experimental groups were set up with five P concentrations [$Ca(H_2PO_4)_2 \times H_2O$ (Fang Ke, Xi'an, China) at 10, 20, 40, 80, and 120 mg P kg^{-1}] or no added P as the control (0 mg P kg^{-1}), with three replications. Plastic pots (upper diameter: 19 cm; lower diameter: 15 cm; depth: 17 cm; Huan Qiu, Yangzhou, China) were used for potting, each holding 2 kg of soil.

Table 1. Physicochemical properties of the limestone and yellow soils.

Parameter	Limestone Soil	Yellow Soil
pH (H_2O 1:5)	6.01	5.46
Total N (g/kg)	2.94	1.99
Total P (g/kg)	0.56	0.35
Total K (g/kg)	24.08	7.25
Total Mg (g/kg)	6.82	2.31
Total Ca (g/kg)	20.51	5.28
NH^{4+}-N (mg/kg)	31.57	19.67
NO^{3-}-N (mg/kg)	4.75	1.41
Available P (mg/kg)	3.02	7.99
Available N (mg/kg)	132.72	33.05
Available K (g/kg)	0.31	0.17
Exchangeable Al content (mg/kg)	397	832

Note: Values are means of three replicates.

Alfalfa seeds were sterilised in a 30% (v/v) hydrogen peroxide (H_2O_2; Huan Tai, Hang zhou, China) solution for 5 min and rinsed five times with sterile water. Germination was conducted for 3 d in an incubator at 20 °C (day, 16 h)/15 °C (night, 8 h). We sowed 20 seeds in each pot, and after 14 days, the seedlings were thinned to nine plants per pot. During plant growth, weeding was performed regularly, both soil types were watered daily to 60% of their field capacity, and pots were randomly moved every 5 d. A total of 72 pots (two cultivars × two soil types × six levels of P application × three replicates) were used.

2.2. Measurement of Mineral Nutrient Contents in Plants

The dried shoots and roots were finely ground. For each sample, ~0.1 g was weighed and digested with aqua regia and H_2O_2. The concentrations of K, Ca, Mg, and P were measured using an inductively coupled plasma mass spectrometer (Nexion 300D ICP-MS, PerkinElmer, Waltham, MA, USA) [13].

2.3. Forage Quality Analysis

The dried buds were finely ground, and NDF, acid detergent lignin (ADL), crude protein (CP), and ether extract (EE) levels were measured using a near-infrared reflectance spectrometer (SupNIR-2700, JiTian Instruments, Beijing, China) [15].

2.4. Statistical Analysis

The experiment used a three-factor randomised complete block design (variety, phosphorus application, and soil type). All variables were analysed by three-way ANOVA, t-test, and multiple comparisons using GenStat 21.0 software (VSN International, Rothamsted, Harpenden, UK). No data transformations were required to satisfy the assumptions of ANOVA. Pearson correlation analysis was used to analyse the correlation between the traits.

3. Results

3.1. Effects of P Application on Mineral Nutrient Contents in Alfalfa

Our experiment demonstrated that P application strongly influenced the plant concentrations of K, Ca, Mg, P, and shoot dry mass, with P, genotype, soil type, and their interactions playing significant roles (Table 2). For alfalfa in limestone soil, mineral nutrient contents differed between the P treatments ($p < 0.05$, Figure 1). Under each treatment, the K, Ca, Mg, and P contents in Trifecta were higher than those in Longzhong ($p < 0.05$), with the highest K, Ca, and Mg contents observed at 20 mg P kg^{-1}. The maximum K, Ca, and Mg contents in Trifecta were 5.62 mg g^{-1}, 8.00 mg g^{-1}, and 0.90 mg g^{-1}, respectively, and those in Longzhong were 3.80 mg g^{-1}, 5.02 mg g^{-1}, and 0.66 mg g^{-1}, respectively. The maximum P content in Trifecta was 0.77 mg g^{-1} under the 40 mg P kg^{-1} treatment, which was significantly higher ($p < 0.05$) than that in the other treatments. For Longzhong, the maximum P content was 0.40 mg g^{-1} under the 80 mg P kg^{-1} treatment, which was significantly higher ($p < 0.05$) than that in all treatments except the 40 mg P kg^{-1} treatment. For the shoot dry mass, both Trifecta and Longzhong showed significantly higher values under the 20 mg P kg^{-1} and 40 mg P kg^{-1} treatments compared to the other treatments (Table 3).

Table 2. Effects of genotype, phosphorus, and soils and their interactions on mineral content and quality of alfalfa.

Character	Significance of Sources of Variability							
	df	G	P	S	G × P	G × S	P × S	G × P × S
Plant K content (mg)	1.48	447.8 ***	652.70 ***	895.7 ***	180.2 ***	37.8 ***	121.4 ***	121.4 ***
Plant Ca content (mg)	1.48	2201.5 ***	405.32 ***	645.5 ***	18.1 ***	427.1 ***	78.1 ***	78.1 ***
Plant Mg content (mg)	1.48	224.6 ***	87.2 ***	642.9 ***	6 ***	33.9 ***	15.8 ***	15.8 ***
Plant P content (mg)	1.48	1684.9 ***	219.2 ***	723.8 ***	24 ***	335.1 ***	33.2 ***	33.2 ***
Ether extract (%)	1.48	809.1 ***	296.3 ***	107.4 ***	38.3 ***	49.8 ***	103.3 ***	103.3 ***
Crude protein	1.48	432.2 ***	303 ***	822.3 ***	14.5 ***	n.s	60.8 ***	60.8 ***
Neutral detergent fibre	1.48	343.4 ***	150.3 ***	921 ***	9.1 ***	32.9 ***	10.31 ***	10.31 ***
Acid Detergent Lignin	1.48	158.2 ***	722.7 ***	115.5 ***	192.3 ***	330 ***	330 ***	210.6 ***
Shoot dry mass (g)	1.48	104.1 ***	48.1 ***	290.3 ***	18 ***	93.9 ***	29.2 ***	18.1 ***

Significant effects are indicated for genes (G), phosphorus application (P), soil types (S), and their interactions (n.s. not significant, *** $p < 0.001$). Numbers in parentheses are the least significant differences (LSD) at $p = 0.05$.

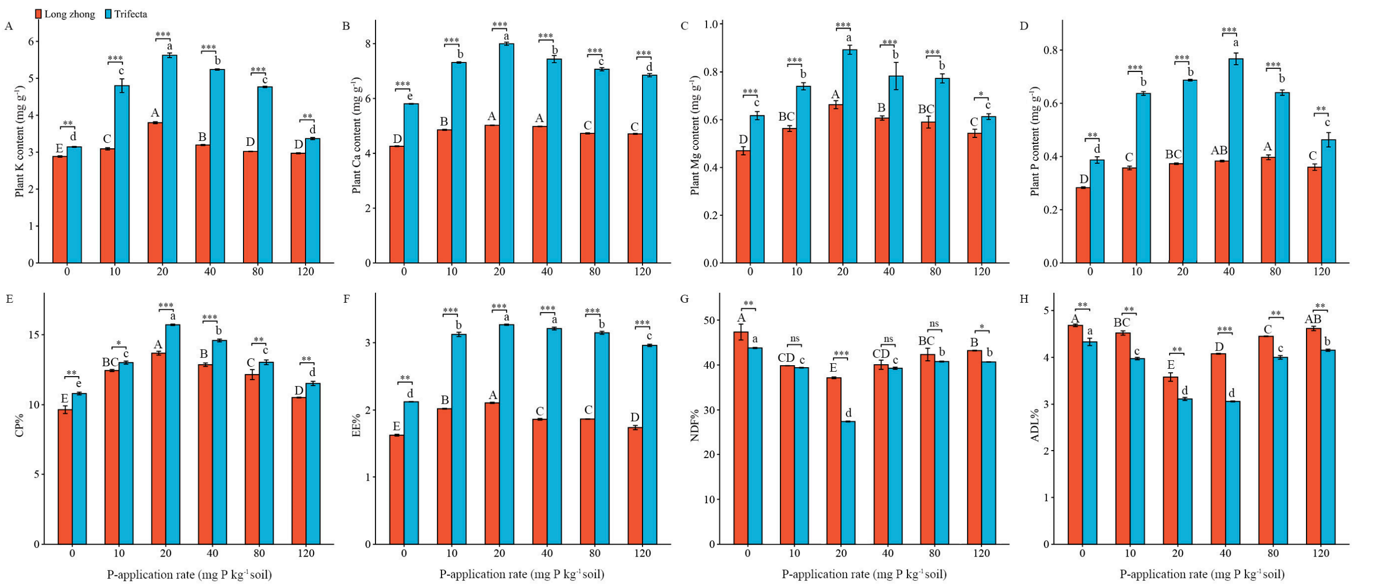

Figure 1. Plant potassium (**A**), calcium (**B**), magnesium (**C**), phosphorus content (**D**), crude protein (**E**), ether extract (**F**), neutral detergent fibre (**G**), and acid detergent lignin level (**H**) in two alfalfa cultivars (Trifecta and Longzhong) grown in limestone soil under six P treatment levels. Data are presented as the mean + standard error of the mean (SE, $n = 3$). Different lowercase and capital letters represent significant differences among the six P treatment levels for Trifecta and Longzhong at $p < 0.05$. ns: non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 3. The effect of phosphorus application on the shoot dry mass of different alfalfa genotypes.

Soil Type	**Cultivar**	**mg P kg^{-1} Soil**					
		0	**10**	**20**	**40**	**80**	**120**
Limestone soil	Longzhong	1.09 ± 0.12 Db	1.64 ± 0.18 Cb	2.28 ± 0.13 Ab	2.19 ± 0.11 Ab	1.96 ± 0.08 Ba	1.78 ± 0.11 Cb
	Trifecta	1.24 ± 0.16 Da	1.81 ± 0.17 Ca	2.85 ± 0.22 Aa	2.57 ± 0.21 Aa	2.27 ± 0.15 Ba	2.19 ± 0.13 Ca
yellow soil	Longzhong	0.19 ± 0.03 Db	0.34 ± 0.04 Cb	0.53 ± 0.06 Aa	0.48 ± 0.07 Bb	0.42 ± 0.02 Bb	0.40 ± 0.06 Bb
	Trifecta	0.25 ± 0.02 Da	0.42 ± 0.03 Ca	0.60 ± 0.07 Ba	0.70 ± 0.04 Aa	0.64 ± 0.09 Ba	0.52 ± 0.07 Ba

Uppercase letters indicate significant differences within the same cultivar under different P applications in the same soil type ($p < 0.05$). Lowercase letters indicate significant differences between different cultivars under the same P application in the same soil type ($p < 0.05$).

In yellow soil, except for the Mg content in the CK treatment and P content in the 0 and 10 mg P kg^{-1} treatments, the K, Ca, Mg, and P contents were higher in Trifecta than those in Longzhong ($p < 0.05$, Figure 2). Under the treatments of 40 mg P kg^{-1} and 20 mg P kg^{-1}, the K content of Trifecta was significantly higher than the other treatments, while the Ca content under the 40 mg P kg^{-1} treatment was significantly higher than the other treatments. For Longzhong, the K content under the 20 mg P kg^{-1} treatment was significantly higher than the other treatments, and the Ca contents under both the 20 mg P kg^{-1} and 40 mg P kg^{-1} treatments were significantly higher than the other treatments. The maximum K and Ca contents in Trifecta were 4.67 mg g^{-1} and 5.75 mg g^{-1}, respectively, while those in Longzhong were 3.95 mg g^{-1} and 4.45 mg g^{-1}, respectively. The maximum Mg contents were observed at 40 mg P kg^{-1} in both Trifecta (0.67 mg) and Longzhong (0.54 mg g^{-1}). The maximum P content in Trifecta (0.69 mg g^{-1}) was observed at 40 mg P kg^{-1} and that in Longzhong (0.46 mg g^{-1}) was observed at 80 mg P kg^{-1}. For Longzhong, the shoot dry mass under the 20 mg P kg^{-1} treatment was significantly higher than the other treatments, while for Trifecta, the shoot dry mass was significantly higher under the 40 mg P kg^{-1} treatment compared to the other treatments (Table 3).

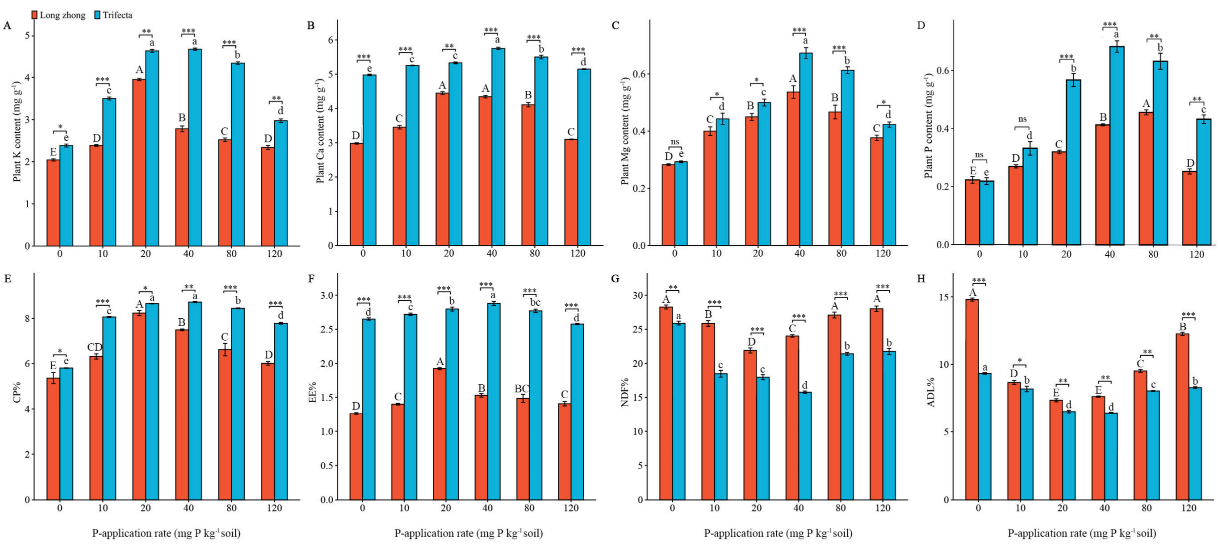

Figure 2. Plant potassium (**A**), calcium (**B**), magnesium (**C**), phosphorus content (**D**), crude protein (**E**), ether extract (**F**), neutral detergent fibre (**G**), and acid detergent lignin level (**H**) in Trifecta and Longzhong grown in yellow soil under six P treatment levels. Data are presented as the mean + SE ($n = 3$). Different lowercase and capital letters represent significant differences among the six P treatment levels for Trifecta and Longzhong at $p < 0.05$. ns: non-significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

In Trifecta cultivated in limestone soil, K, Ca, Mg, and P contents were positively correlated with each other ($p < 0.05$, Figure 3). However, in Longzhong, there were no correlations between K, Ca, Mg, and P contents, while the shoot dry mass was significantly correlated with Ca and P contents ($p > 0.05$, Figure 4). In yellow soil, except for a non-significant correlation between Longzhong's K and P contents, the K, Ca, Mg, and P contents showed positive correlations with each other in the two cultivars ($p < 0.05$, Figures 5 and 6). The shoot dry mass was significantly correlated with the K, Ca, Mg, and P contents in both cultivars ($p < 0.01$, Figures 5 and 6).

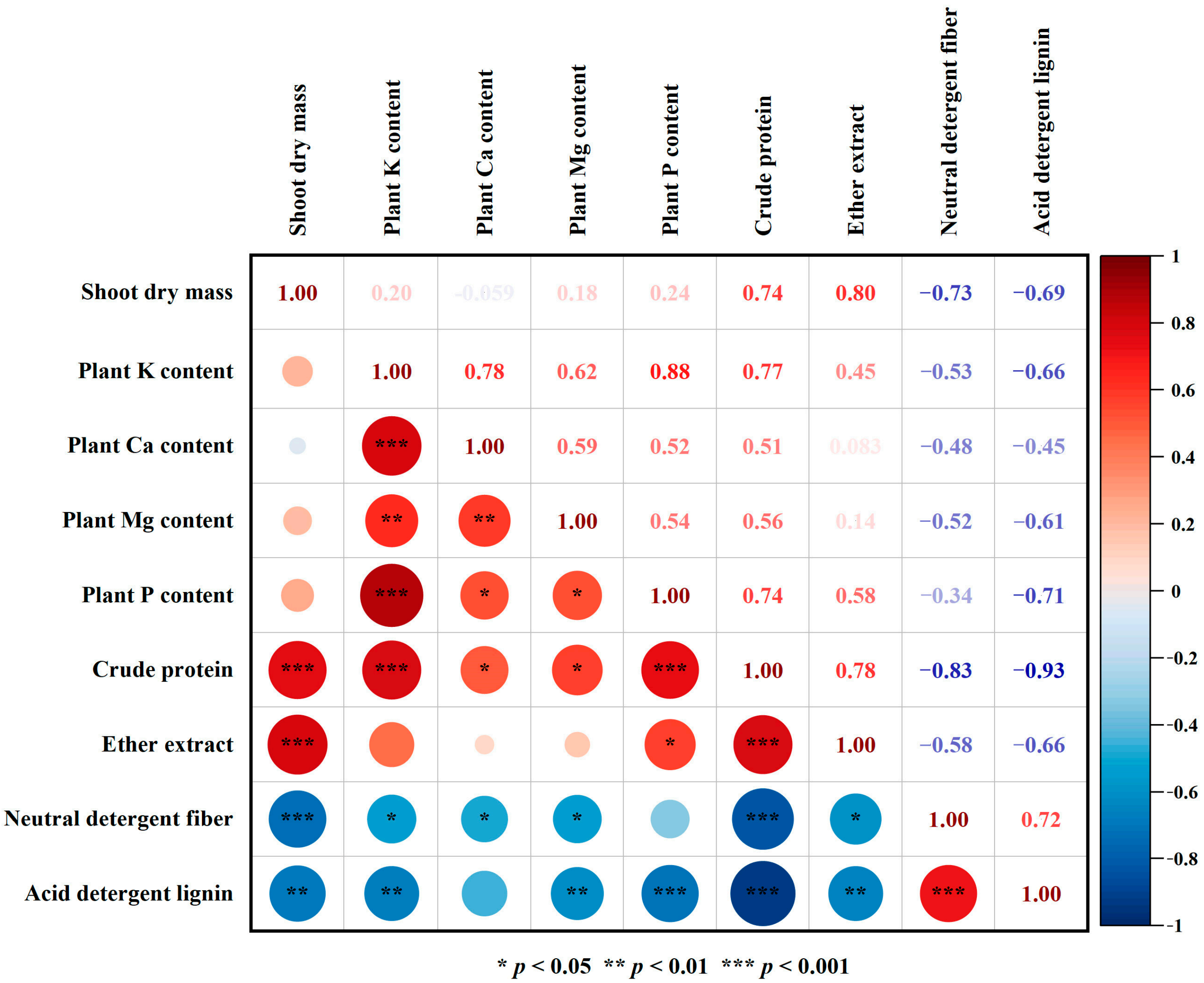

Figure 3. Pearson correlation analysis between individual traits of Trifecta grown in limestone soil supplied with different P levels. Grey colour indicates lack of significance, whereas blue and red colours indicate significant negative and positive correlations at $p < 0.05$, respectively.

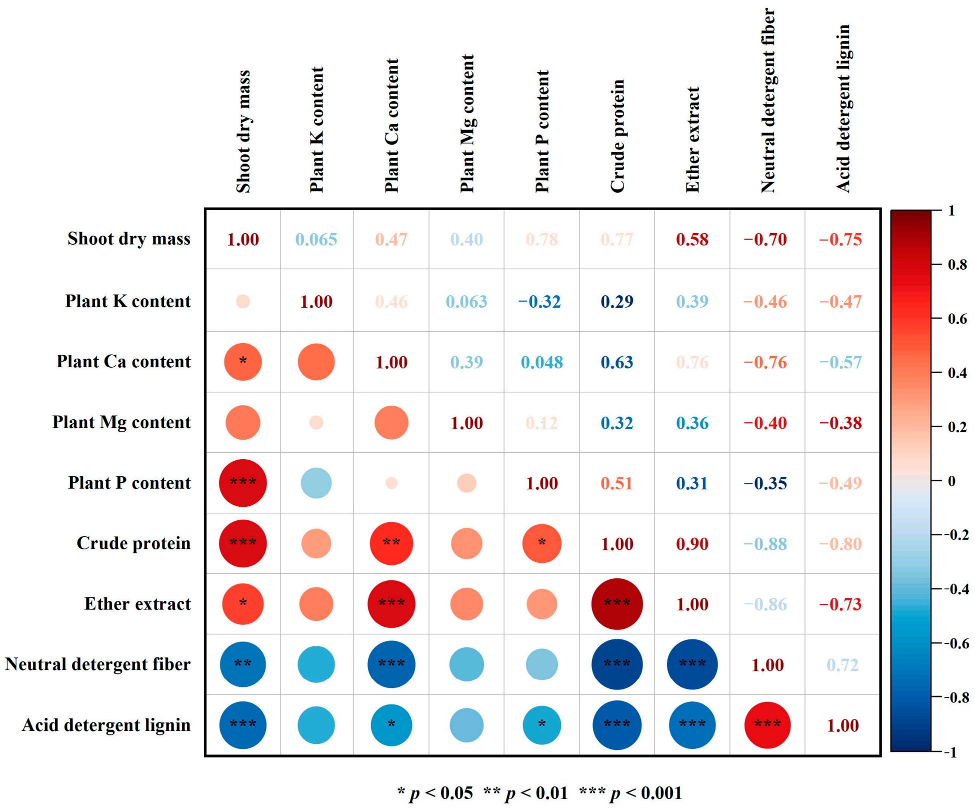

Figure 4. Pearson correlation analysis between individual traits of Longzhong grown in limestone soil supplied with different P levels. Grey colour indicates lack of significance, whereas blue and red colours indicate significant negative and positive correlations at $p < 0.05$, respectively.

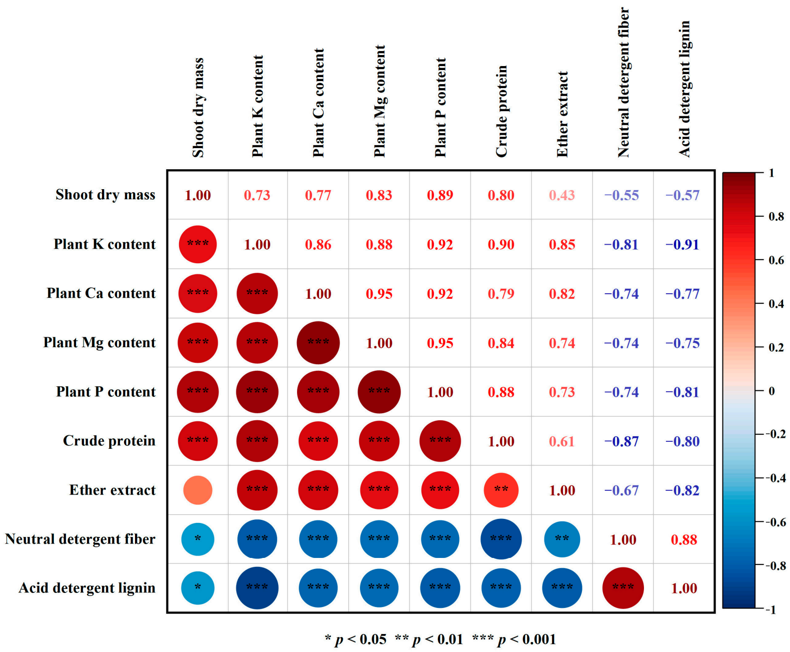

Figure 5. Pearson correlation analysis between individual traits of Trifecta grown in yellow soil supplied with different P levels. Grey colour indicates lack of significance, whereas blue and red colours indicate significant negative and positive correlations at $p < 0.05$, respectively.

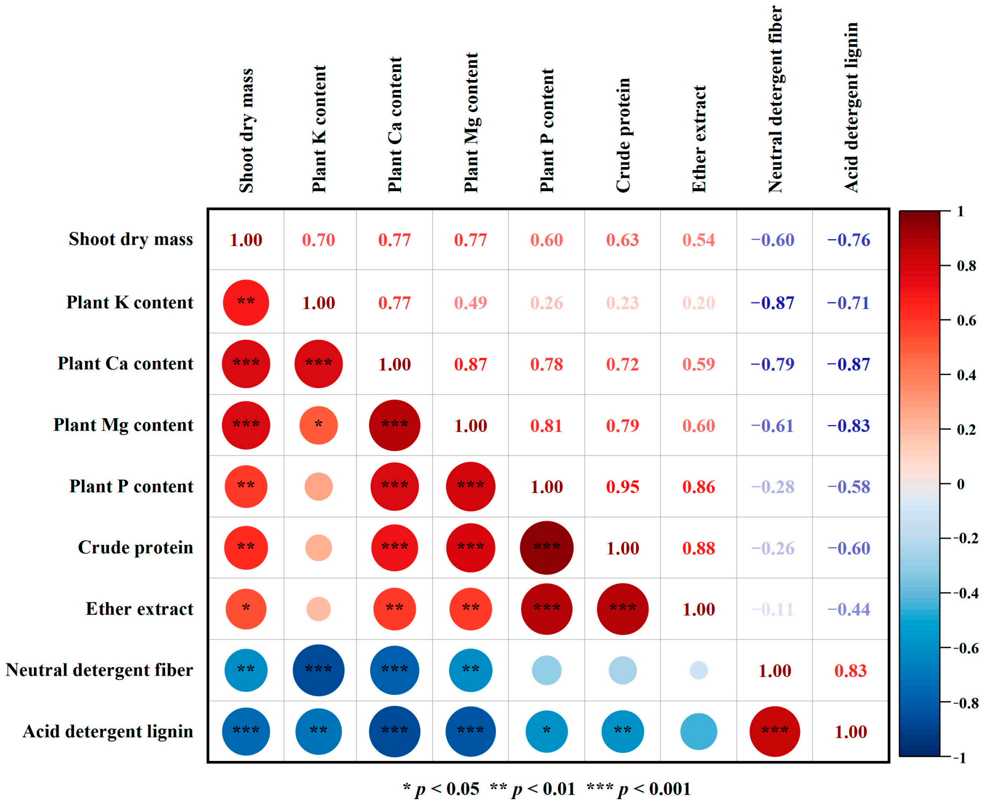

Figure 6. Pearson correlation analysis between individual traits of Longzhong grown in yellow soil supplied with different P levels. Grey colour indicates lack of significance, whereas blue and red colours indicate significant negative and positive correlations at $p < 0.05$, respectively.

3.2. Effect of P Application on Alfalfa Quality

P, genotype, soil type, and their interactions greatly affected CP, EE, NDF, and ADL levels, except for the interaction between genotype and soil type on CP (Table 2). In limestone soil, CP and EE showed similar trends in both alfalfa cultivars. Under each P treatment, Trifecta had higher values than Longzhong, and the CP of Trifecta under the 20 mg P kg^{-1} treatment was significantly higher than the other treatments, and the EE under both the 20 mg P kg^{-1} and 40 mg P kg^{-1} treatments was significantly higher than the other treatments ($p < 0.05$, Figure 1). The maximum CP values were 15.71% (Trifecta) and 13.68% (Longzhong), and the maximum EE values were 3.27% (Trifecta) and 2.11% (Longzhong, Figure 1E,F). The NDF value in Longzhong was higher than that in Trifecta at 0 mg P kg^{-1}, 20 mg P kg^{-1}, and 120 mg P kg^{-1} ($p < 0.05$), with the minimum values observed at 20 mg P kg^{-1} (27.43%, Trifecta; 37.15%, Longzhong; Figure 1G). The ADL value was higher in Longzhong than in Trifecta under each P treatment ($p < 0.01$), with the minimum values observed at 20 mg P kg^{-1} (3.58%, Longzhong) and 40 mg P kg^{-1} (3.06%, Trifecta) (Figure 1H). In Trifecta, CP had a positive correlation with K, Ca, Mg, and P contents ($p < 0.05$); EE only had a positive correlation with P content ($p < 0.05$); NDF had a negative correlation with K, Ca, and Mg contents ($p < 0.05$); and ADL had a positive correlation with K, Mg, and P contents ($p < 0.05$, Figure 3). The shoot dry mass was significantly correlated with CP, EE, NDF, and ADL ($p < 0.01$, Figure 3). In Longzhong, CP and EE were positively correlated with Ca content ($p < 0.01$), with which NDF and ADL were negatively correlated ($p < 0.05$). CP was positively correlated and ADL was negatively correlated with P content (both $p < 0.05$, Figure 4). The shoot dry mass was significantly correlated with CP, EE, NDF, and ADL ($p < 0.05$, Figure 4).

In yellow loam soil, the CP and EE values were higher ($p < 0.05$) in Trifecta than in Longzhong for all P treatments. The CP of Trifecta under the 20 mg P kg^{-1} and 40 mg P kg^{-1} treatments was significantly higher than the other treatments ($p < 0.05$,

Figure 2E). The maximum EE values in Trifecta were observed at 40 mg P kg^{-1} (2.77%, Figure 2F), while those in Longzhong were observed at 20 mg P kg^{-1} (8.10% and 1.90%, respectively). The NDF and ADL values in Longzhong were higher than those in Trifecta for all P treatments ($p < 0.05$, Figure 2G,H). The minimum NDF values in Longzhong were observed at 20 mg P kg^{-1} (28.67%), while those in Trifecta were observed at 40 mg P kg^{-1} (15.87%). For ADL, both Trifecta and Longzhong had significantly lower values under the 20 mg P kg^{-1} and 40 mg P kg^{-1} treatments compared to the other treatments ($p < 0.05$, Figure 2H). In Trifecta, CP and EE were positively correlated with K, Ca, Mg, and P contents ($p < 0.001$), with which NDF and ADL were negatively correlated ($p < 0.001$, Figure 5). The shoot dry mass was significantly correlated with CP, NDF, and ADL ($p < 0.05$, Figure 5). In Longzhong, CP and EE were positively correlated with Ca, Mg, and P contents ($p < 0.01$); NDF was negatively correlated with K, Ca, and Mg contents ($p < 0.01$); and ADL was negatively correlated with K, Ca, Mg, and P contents ($p < 0.05$, Figure 6). The shoot dry mass was significantly correlated with CP, EE, NDF, and ADL ($p < 0.05$, Figure 6).

4. Discussion

P deficiencies are a widespread issue in agricultural production globally, with more than 30% of arable soils having P levels below those required for optimal crop productivity [16]. P is a limiting nutrient in both natural and agricultural ecosystems because of its strong adsorption by, particularly, Fe and Al in acidic soils, which readily form stable complexes with P, thereby restricting its absorption and utilisation by plants [17,18]. The application of phosphate fertiliser is a direct and effective strategy to alleviate Al toxicity in acidic soil and improve alfalfa yield and quality [4].

Under a high P supply, the accumulation of P usually affects the uptake of other elements, which seems to be an intrinsic mechanism for balancing P levels in plants [13]. When the P anion is absorbed in large amounts, there is a corresponding increase in the uptake of cations, such as K, Ca, and Mg, to maintain charge balance [16]. In the present study, the K, Ca, Mg, and P contents of alfalfa were strongly affected by genotype, soil type, and P treatment. In limestone soil, Trifecta's P content was positively correlated with K, Ca, and Mg contents, whereas that of Longzhong was not. In yellow soil, Trifecta's P content showed a similar correlation with the contents of other mineral elements as in limestone soil, whereas Longzhong's P content showed a highly positive correlation with Ca and Mg contents. In acidic soils, the acquisition of P, S, Mg, Ca, and K by chickpea (Cicer arietinum) was enhanced by different P sources, especially when using phosphate rock compared to KH_2PO_4 [19]. In aeolian sandy and loessial soils, previous studies have shown that the total plant mineral nutrient content in alfalfa was strongly affected by soil P supply; however, there was no correlation with soil available P but a strong positive correlation with the total P content of plants [13]. Consistent with the findings of their results, we showed that when more P is absorbed, the uptake of other mineral nutrients increases. Moreover, except for the non-significant correlation between the shoot dry mass and P contents in the tolerant alfalfa genotype grown in limestone soil, the shoot dry mass of alfalfa under all other treatments was significantly correlated with P contents. In addition, the extent of P utilisation by plants depends on many factors, such as soil texture, plant species, P concentration, and P fertiliser type [20]. This explains why Trifecta and Longzhong performed differently in different soils.

The CP, EE, NDF, and ADL levels in alfalfa directly reflect its nutritional quality [10]. Excellent nutritional value is an ideal goal in alfalfa production because it directly affects the profitability of feed and livestock producers [21]. In this study, fertiliser application strongly affected the CP, EE, NDF, and ADL levels in alfalfa, regardless of the soil P supply level. In both limestone and yellow soil, CP and EE in both cultivars were positively correlated with K, Ca, Mg, and P contents, whereas NDF and ADL were negatively correlated. The CP and EE levels of the two cultivars first increased and then decreased with increasing phosphorus application, consistent with the results of Wan et al. [4]. This may be associated with the increase in root growth, nitrogen utilisation, and metabolic rates with increasing phosphorus input, which enhance nitrogen fixation, photosynthesis, and leaf area [10]. However, the respiration rates of alfalfa are exacerbated above a certain threshold for P uptake, which negatively affects growth and development [4]. Excess P reduces the transpiration and photosynthesis rates of alfalfa leaves, inhibiting their absorption and utilisation of nitrogen, thereby increasing the fibre content and reducing the CP content [10]. The changing trends in NDF and ADL contents in this study reflect this phenomenon. In the present study, increasing P content in the plants was associated with increasing K content. Notably, high K levels increase plant lignification, leading to a decrease in plant CP content [22]. Ca is essential for plant growth and development [23]. Although Ca deficiency in nature is rare, it is more common in acidic soils [24]. An increase in Ca^{2+} can improve the adaptability of plants to abiotic and biotic stresses and restore growth, dry matter production, and leaf photosynthetic capacity [25]. In the present study, as the amount of P applied increased, the Ca content of the plants first increased and then decreased. These changes in Ca content may affect the nutritional quality of alfalfa by altering photosynthesis rates. Mg plays an important role in plant chlorophyll and protein biosynthesis, and Mg deficiency leads to leaf interveinal chlorosis and impaired primary (energy) metabolism, which negatively affect crop yield and quality parameters [26]. In this study, the changing trends in Mg and Ca contents were consistent, and nutritional quality traits were positively correlated with Mg content. The important roles of Mg in plant protein formation and amino acid transport underlie its positive effects on plant protein yield. Vrataric et al. showed that the application of a 5% $MgSO_4 \cdot 7H_2O$ solution during vegetative growth increased soybean seed yield along with protein and oil concentrations [27]. Collectively, our findings indicated that P application strongly affected the absorption of mineral elements in alfalfa and regulated nutritional quality.

5. Conclusions

P application strongly increased K, Ca, Mg, and P contents and CP and EE levels in alfalfa, while reducing NDF and ADL levels. However, P supplied at >40 mg P kg^{-1} had no greater or lesser effects. Compared with Longzhong, Trifecta exhibited higher K, Ca, Mg, P, CP, and EE levels. Moreover, limestone soil was associated with more optimal traits in both cultivars than yellow soil. This study provides a useful reference for optimising P fertiliser management for alfalfa production in acidic soils.

Author Contributions: R.D. and Z.L. conceived the experiments; Y.H., X.W., X.Z., X.G., M.Z. and F.Y. performed the experiments; Y.H., J.H. and J.C. analysed the data; and R.D. and Z.L. wrote this paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (32060392 and 32160337), Qian Ke He Cheng Guo ([2022] Zhong Dian 005), and GZMARS-Forage Industry Technology System of Guizhou Province.

Data Availability Statement: The original contributions presented in this study are included in the article; further enquiries can be directed to the corresponding author.

Acknowledgments: We thank Jing Zhou (Guizhou University, China) for their assistance with the experiments.

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

References

1. Tian, Z.; Yang, Z.; Lu, Z.; Luo, B.; Hao, Y.; Wang, X.; Yang, F.; Wang, S.; Chen, C.; Dong, R. Effect of genotype and environment on agronomical characters of alfalfa (*Medicago sativa* L.) in a typical acidic soil environment in southwest China. *Front. Sustain. Food Syst.* **2023**, *7*, 1144061. [CrossRef]
2. Guo, Z.; Zhang, T.; Chen, Z.; Niu, J.; Cui, X.; Mao, Y.; Hassan, M.U.; Kareem, H.A.; Xu, N.; Sui, X.; et al. Occurrence, distribution, and genetic diversity of alfalfa (*Medicago sativa* L.) viruses in four major alfalfa-producing provinces of China. *Front. Microbiol.* **2022**, *12*, 771361. [CrossRef]
3. Feng, Y.; Shi, Y.; Zhao, M.; Shen, H.; Xu, L.; Luo, Y.; Liu, Y.; Xing, A.; Kang, J.; Jing, H.; et al. Yield and quality properties of alfalfa (*Medicago sativa* L.) and their influencing factors in China. *Eur. J. Agron.* **2022**, *141*, 126637. [CrossRef]

4. Wan, W.F.; Li, Y.J.; Li, H.G. Yield and quality of alfalfa (*Medicago sativa* L.) in response to fertilizer application in China: A meta-analysis. Front. *Plant Sci.* **2022**, *13*, 1051725. [CrossRef] [PubMed]
5. Yang, C.; Zhang, F.; Jiang, X.; Yang, X.; He, F.; Wang, Z.; Long, R.; Chen, L.; Yang, T.; Wang, C.; et al. Identification of genetic loci associated with crude protein content and fiber composition in alfalfa (*Medicago sativa* L.) using QTL mapping. *Front. Plant Sci.* **2021**, *12*, 608940. [CrossRef]
6. Lu, Z.J.; Tian, Z.; Yang, Z.Y.; Yin, X.Y.; Dong, R. Comparative transcriptomic analysis reveals coordinated mechanisms of different genotypes of common vetch root in response to Al stress. *Environ. Exp. Bot.* **2023**, *213*, 105450. [CrossRef]
7. Su, L.; Lv, A.; Wen, W.; Fan, N.; Li, J.; Gao, L.; Zhou, P.; An, Y. *MsMYB741* is involved in alfalfa resistance to aluminum stress by regulating flavonoid biosynthesis. *Plant J.* **2022**, *112*, 756–771. [CrossRef]
8. Walworth, J.L.; Sumner, M.E. Alfalfa response to lime, phosphorus, potassium, magnesium, and molybdenum on acid ultisols. *Fert. Res.* **1990**, *24*, 167–172. [CrossRef]
9. Sun, Q.B.; Shen, R.F.; Zhao, X.Q.; Chen, R.F.; Dong, X.Y. Phosphorus enhances Al resistance in Al-resistant *Lespedeza bicolor* but not in Al-sensitive *L. cuneata* under relatively high Al stress. *Ann. Bot.* **2008**, *102*, 795–804. [CrossRef]
10. Zhang, Q.; Liu, J.; Liu, X.; Sun, Y.; Li, S.; Lu, W.; Ma, C. Optimizing the nutritional quality and phosphorus use efficiency of alfalfa under drip irrigation with nitrogen and phosphorus fertilization. *Agron. J.* **2020**, *112*, 3129–3139. [CrossRef]
11. Gu, Y.J.; Han, C.L.; Kong, M.; Shi, X.Y.; Zdruli, P.; Li, F.M. Plastic film mulch promotes high alfalfa production with phosphorus-saving and low risk of soil nitrogen loss. *Field Crop. Res.* **2018**, *229*, 44–54. [CrossRef]
12. Cole, J.C.; Smith, M.W.; Penn, C.J.; Cheary, B.S.; Conaghan, K.J. Nitrogen, phosphorus, calcium, and magnesium applied individually or as a slow release or controlled release fertilizer increase growth and yield and affect macronutrient and micronutrient concentration and content of field-grown tomato plants. *Sci. Hortic.* **2016**, *211*, 420–430. [CrossRef]
13. He, H.; Peng, Q.; Wang, X.; Fan, C.; Pang, J.; Lambers, H.; Zhang, X. Growth, morphological and physiological responses of alfalfa (*Medicago sativa*) to phosphorus supply in two alkaline soils. *Plant Soil* **2017**, *416*, 565–584. [CrossRef]
14. Li, S.; Liu, X.; Sun, Y.; Ma, C.; Gu, X.; Zhang, Q. Yield, nutrient quality and water and phosphorus recovery efficiencies of alfalfa under different drip irrigation and phosphorus levels in Northern Xinjiang, China. *Grass Forage Sci.* **2022**, *77*, 189–200. [CrossRef]
15. Mainetti, A.; Ravetto Enri, S.; Pittarello, M.; Lombardi, G.; Lonati, M. Main ecological and environmental factors affecting forage yield and quality in alpine summer pastures (NW-Italy, Gran Paradiso National Park). *Grass Forage Sci.* **2023**, *78*, 254–267. [CrossRef]
16. Aziz, T.; Lambers, H.; Nicol, D.; Ryan, M.H. Mechanisms for tolerance of very high tissue phosphorus concentrations in P tilotus polystachyus. *Plant Cell Environ.* **2015**, *38*, 790–799. [CrossRef]
17. Mardamootoo, T.; Du Preez, C.C.; Barnard, J.H. Phosphorus management issues for crop production: A review. *Afr. J. Agr. Res.* **2021**, *17*, 939–952. [CrossRef]
18. Amadou, I.; Faucon, M.P.; Houben, D. Role of soil minerals on organic phosphorus availability and phosphorus uptake by plants. *Geoderma* **2022**, *428*, 116125. [CrossRef]
19. Alloush, G.A.; Zeto, S.K.; Clark, R.B. Phosphorus source, organic matter, and arbuscular mycorrhiza effects on growth and mineral acquisition of chickpea grown in acidic soil. *J. Plant Nutr.* **2000**, *23*, 1351–1369. [CrossRef]
20. Kassir, L.N.; Darwish, T.; Shaban, A.; Olivier, G.; Ouaini, N. Mobility and bioavailability of selected trace elements in Mediterranean red soil amended with phosphate fertilizers: Experimental study. *Geoderma* **2012**, *189*, 357–368. [CrossRef]
21. Kamran, M.; Yan, Z.; Jia, Q.; Chang, S.; Ahmad, I.; Ghani, M.U.; Hou, F. Irrigation and nitrogen fertilization influence on alfalfa yield, nutritive value, and resource use efficiency in an arid environment. *Field Crop. Res.* **2022**, *284*, 108587. [CrossRef]
22. Barker, D.J.; Culman, S.W. Fertilization and Nutrient Management. In *Forages: The Science of Grassland Agriculture*, 7th ed.; The Ohio State University: Columbus, OH, USA, 2020; Volume II.
23. Huang, D.; Gong, X.; Liu, Y.; Zeng, G.; Lai, C.; Bashir, H.; Zhou, L.; Wang, D.; Xu, P.; Cheng, M.; et al. Effects of calcium at toxic concentrations of cadmium in plants. *Planta* **2017**, *245*, 863–873. [CrossRef]
24. White, P.J.; Broadley, M.R. Calcium in plants. *Ann. Bot.* **2003**, *92*, 487–511. [CrossRef] [PubMed]
25. Song, Q.; Liu, Y.; Pang, J.; Yong, J.W.H.; Chen, Y.; Bai, C.; Gille, C.; Shi, Q.; Wu, D.; Han, X.; et al. Supplementary calcium restores peanut (*Arachis hypogaea*) growth and photosynthetic capacity under low nocturnal temperature. *Front. Plant Sci.* **2020**, *10*, 1637. [CrossRef] [PubMed]
26. Gerendás, J.; Führs, H. The significance of magnesium for crop quality. *Plant Soil* **2013**, *368*, 101–128. [CrossRef]
27. Vrataric, M.; Sudaric, A.; Kovacevic, V.; Duvnjak, T.; Krizmanic, M.; Mijic, A. Response of soybean to foliar fertilization with magnesium sulfate. *Cereal. Res. Commun.* **2006**, *34*, 709–712. [CrossRef]