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Alfalfa adapts to soil nutrient surplus and deficiency by adjusting the stoichiometric characteristics of main organs and nutrient reabsorption

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Abstract

Accurate nutrient diagnosis is essential for simulating alfalfa (*Medicago sativa* L.) yield and optimizing resource-use efficiency under diverse soil nutrient conditions. However, limited knowledge exists about how fertilization impacts soil–plant nutrient stoichiometric constraints, especially in nutrient-deficient gray desert soils. This study conducted a field experiment with four nitrogen (N) application rates: 0, 60, 120, and 180 kg N·ha^{-1} and four phosphorus (P) application rates: 0, 50, 100, and 150 kg P_2O_5·ha^{-1}. We assessed changes in the nutrient limitation characteristics of alfalfa and identified its primary driving factors, focusing on soil nutrient perspectives, nutrient distribution in main organs (leaves, shoots, and roots) and nutrient resorption. The results demonstrated that fertilization increased N and P concentrations in various alfalfa organs while reducing carbon (C) content. A strong synergy was observed in nutrient concentrations across the different alfalfa organs. With increasing application of single-nutrient fertilizers, the C:N and C:P ratios in alfalfa organs decreased, while the N:P ratio stabilized under conditions of sufficient or co-limiting soil N and P. Alfalfa N:P ratios under different fertilization treatments were 4.89–5.46 in roots, 6.19–8.45 in stems, and 9.10–15.16 in leaves. The C:N and C:P ratios were significantly negatively correlated with alfalfa yield, but the relationship between the N:P ratio and yield was not statistically significant. Soil nutrient status positively influenced N and P concentrations in leaves, stems, and roots, however, their effect on stoichiometric ratios was primarily mediated through indirect effects on corresponding organ-level nutrients. Moreover, soil nutrients directly or indirectly explained 98% of the variation in nutrient resorption in leaves. In conclusion, fertilization indirectly affects the stoichiometric characteristics of alfalfa organs via soil nutrients. Adjusting fertilizer nutrient ratios can mitigate nutrient limitations in both soil and alfalfa, providing valuable insights for fertilizer formulation, timing of fertilizer application, and fertilization application strategies. Highlights 1.Fertilization alters the C-N-P stoichiometry

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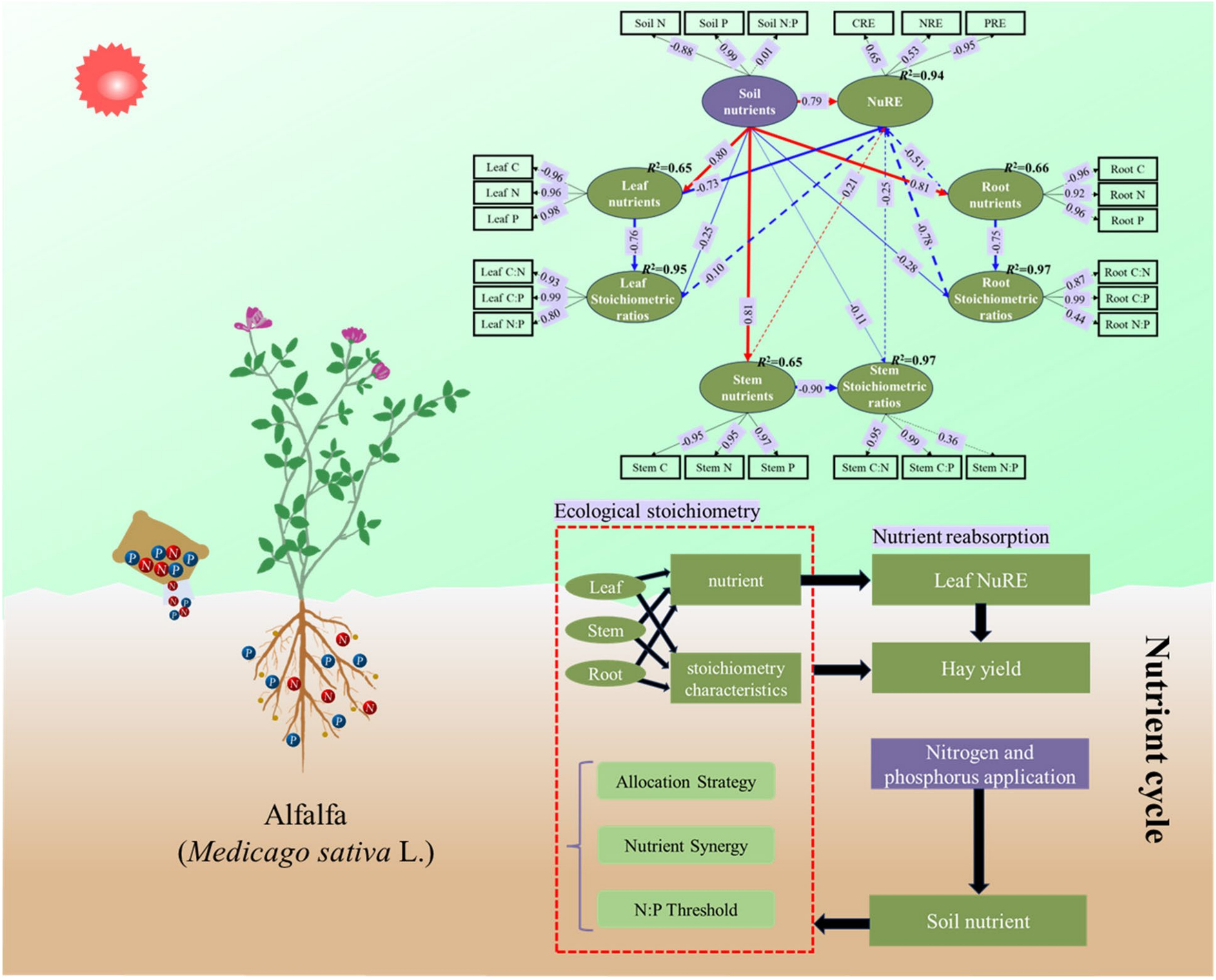
of the soil–plant system. 2.The stoichiometric characteristics and ratios of different organs exhibit a certain degree of synergy. 3.Stoichiometric ratios can represent nutrient limitation to a certain extent. 4.Soil nutrient changes affect the stoichiometric characteristics and ratios of alfalfa.

Highlights

1. Fertilization alters the C-N-P stoichiometry of the soil-plant system.
2. The stoichiometric characteristics and ratios of different organs exhibit a certain degree of synergy.
3. Stoichiometric ratios can represent nutrient limitation to a certain extent.
4. Soil nutrient changes affect the stoichiometric characteristics and ratios of alfalfa.

Keywords Fertilization management, Soil–plant interaction, Nutrient cycling, Nutrient reabsorption, Stoichiometric characteristic

Graphical Abstract



Introduction

The gray desert soil area in Xinjiang covers ~ 178.95×10^4 ha, of which ~ 62×10^4 ha is currently cultivated, accounting for 80% of China's cultivated land area [1]. Gray desert soil is the predominant soil type in northern Xinjiang, and is characterized by poor fertility, high salinity and alkalinity, and poor water retention. These regional soil conditions make alfalfa (*Medicago sativa* L.) production challenging, as there is insufficient soil organic matter and nutrients to sustain long-term, high-efficiency crop growth and yield [2]. Hannaway and Shuler [3] demonstrated that alfalfa can fix nitrogen from the atmosphere through rhizobia, eliminating the need for additional nitrogen. However, various studies have found that in poor soils, adding nitrogen can promote alfalfa growth and increase yield, especially during the establishment year and each year's regreening period [4, 5]. We have observed that on the gray desert soil in northern Xinjiang N application increased alfalfa dry matter (DM) yield by 8.9%–19.6% in the first year of planting, and although the yield increase in the second year was lower than that in the first year, it still increased alfalfa DM yield by 1.8%–14.8% [6].

The demand for phosphorus (P) fertilizer in alfalfa is different from that of N fertilizer, and research has consistently shown that alfalfa is a plant that requires a large amount of P [5]. Phosphorus plays an important role in alfalfa growth, metabolism, yield formation, and nutritional quality [7, 8]. In order to maintain a minimum sustainable yield in grain crops within Xinjiang, alfalfa is primarily cultivated on nutrient-poor fields, where insufficient nutrient availability is the primary constraint on alfalfa production. Furthermore, alfalfa is a perennial legume forage crop, and in continuous multi-year cultivation, soil frequency become deficient in key soil nutrients, which may lead to poor alfalfa growth and yield [9].

Plant ecological stoichiometry is a key indicator for understanding plant nutrient limitation, and focuses on the quantitative characteristics and content/ratio of chemical elements in plant organs (leaf, stem, roots, etc.), and their relationship with environmental factors and ecosystem functions [10]. Currently, carbon (C), N, and P have been the main research focus for plant ecological stoichiometry. Carbon is the basis of the organic skeleton, while N and P are important functional elements in plants, which play key roles in plant growth and development, and in a variety of physiological metabolic processes [11]. Studies have shown that the stoichiometric ratios of alfalfa leaves changed with the growth years and cutting times, and leaf N and P content were negatively correlated with the growth year, but had no significant effect on the change of organic C content [12, 13]. Short-term N addition significantly increased the N content of alfalfa fine roots and significantly decreased the C content, and C:N ratio, but had no significant effect on the Mg and Ca content of fine roots [14]. Alfalfa ecological stoichiometry research has mainly focused aboveground on shoots and leaves, with some research reported on fine roots [12, 13, 15], however, little is known about nutrient reabsorption and inter-organ stoichiometry characteristics. Element ratios are key indicators for understanding nutrient cycling and interactions. Plant stoichiometry ratios typically remain in a steady state, however, the soil C, N, and P content, climatic conditions, growth stages, and elemental stoichiometry may influence plant responses [16]. The C:N and C:P ratios indicate the coordinated capacity of plants to absorb nitrogen and phosphorus through roots while fixing carbon via leaf photosynthesis, and N:P ratio reflects the dynamic balance between soil nutrients and plant nutrient uptake [17–19]. By analyzing N, P, and N:P ratio, it is possible to effectively identify the main limiting nutritional elements for plant growth and development. Therefore, understanding plant stoichiometric characteristics is an important management tool, which can enhance our comprehension of plant growth, development, yield, and adaptation to environmental and cultural stresses.

Nutrient resorption is the process by which elements from senescent leaves are transferred to younger tissues, serving as a crucial internal nutrient utilization mechanism which can reduce the dependence of plants on external nutrients [20]. This study focuses on alfalfa N and P source-sink relationships during field production at different N and P fertilization rates. Based on this, we propose three hypotheses: (1) Nitrogen and phosphorus fertilization significantly affect the C, N, and P contents and their stoichiometric ratios among different organs of alfalfa, with coordinated nutrient changes across organs. (2) When soil fertility changes, alfalfa adapts by modifying its plant nutrient stoichiometric characteristics and enhancing leaf nutrient resorption capacity to cope with soil nutrient fluctuations. (3) Within the same cutting cycle, the stoichiometric ratios of alfalfa can serve as indicators of its nutrient limitation status.

Materials and methods

Experimental site

The experiment was conducted at Shihezi University forage experiment station in Shihezi City, Xinjiang, which is located on the northern slope of Tianshan Mountains and belongs to a temperate continental arid climate. The summer is characterized by abundant Sunlight and significant temperature fluctuations between day and night. The annual average temperature is 7.5℃, the annual average precipitation is 225 mm, and the annual average evaporation is 1250 mm [5]. The experimental site soil is a gray desert soil, with the upper tilled layer characterized with low organic matter (OM) 21.56 $g \cdot kg^{-1}$, total phosphorus 0.53 $g \cdot kg^{-1}$, available P 19.30 $mg \cdot kg^{-1}$, total nitrogen 1.18 g·kg – 1, available N 145.47 $mg \cdot kg^{-1}$, and pH of 7.59.

Experimental design and crop management

This replicated (n = 3) plot (4 m × 6 m = 24 m^2) experiment used a two-factor randomized block design with 16 nutrient treatments; four N application rates: 0 (N_0), 60 (N_1), 120 (N_2), and 180 kg $N \cdot ha^{-1}$ (N_3), and four P application rates: 0 (P_0), 50 (P_1), 100 (P_2), and 150 kg $P_2O_5 \cdot ha^{-1}$ (P_3), (Fig. 1). Urea ($N \geq 46\%$) and monoammonium P ($P_2O_5 \geq 52\%$, $N \geq 12.2\%$) were used as the N and P fertilizer sources, respectively. As monoammonium P contained a small amount of N, additional N was added to maintain consistent N levels between different P treatments when applying the same N level. Specifically, urea

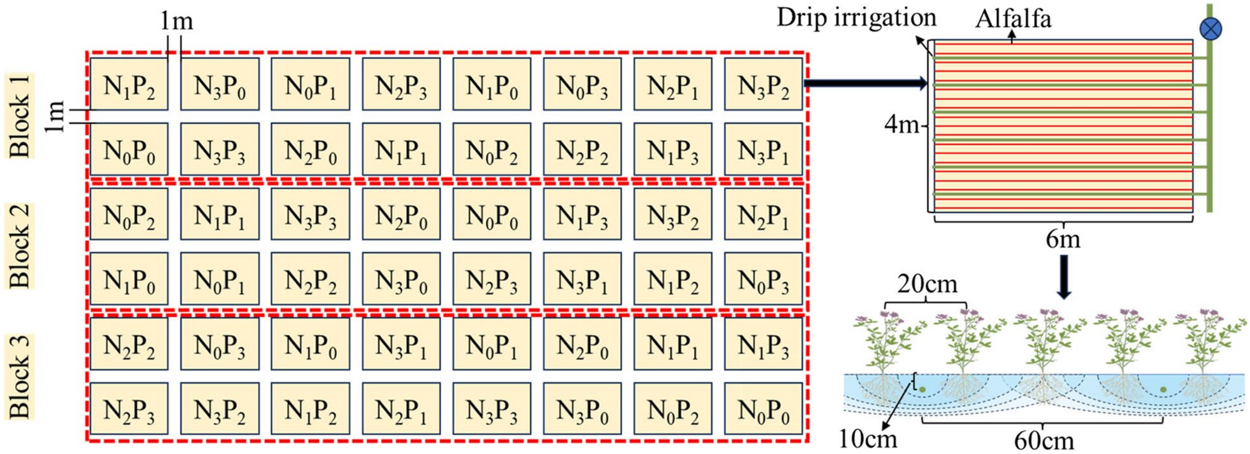


Fig. 1 Experimental plot layout diagram and schematic of fertigation field fertigation system used

addition amounts for P_0, P_1, P_2, and P_3 treatments were 76.5, 51.0, 25.5, and 0 kg·ha^{-1}, respectively. Each of the plots was separated, with a 1 m border to prevent nutrient subsurface movement between plots.

Alfalfa WL366HQ seeds (provided by Beijing Rytway Seed Co., Ltd., Beijing, China), were sown in 2019, at a rate of 18 kg·ha^{-1}, at 2 cm depth, with a row spacing of 20 cm. Alfalfa plots were fertigated 3–5 day after cutting, using a drip-irrigation system, buried at 10 cm below the soil surface and spaced at 60 cm intervals (Fig. 1). Alfalfa crop was harvested on May 25th, July 4th, August 14th, and October 4th, 2020.

To ensure consistent irrigation volume across all plots, a water meter was used to precisely control the water

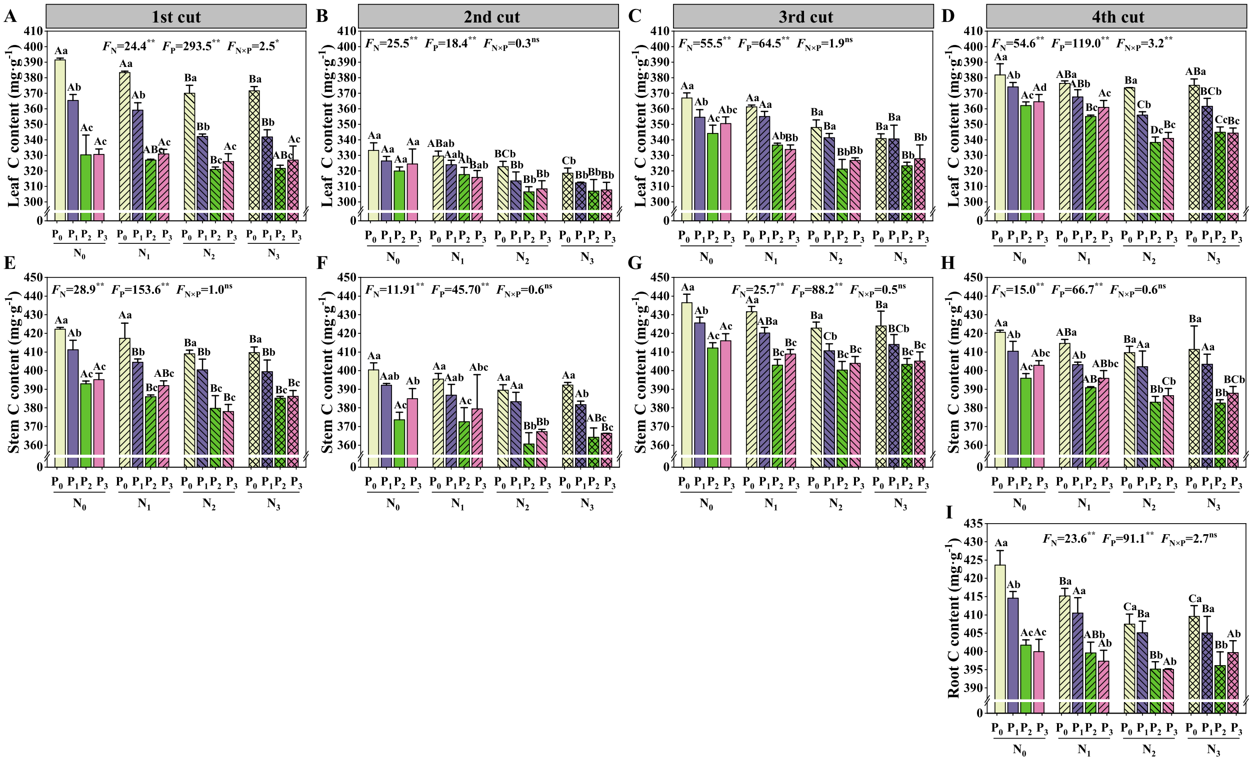


Fig. 2 Carbon (C) content in leaves (**A**, **B**, **C**, and **D**), stems (**E**, **F**, **G**, and **H**), and roots (**I**) of alfalfa (*Medicago sativa* L.) under nitrogen (N) and phosphorus (P) addition prior to each cutting. P_0, P_1, P_2, and P_3 represent application of 0, 50, 100, and 150 kg P_2O_5·ha^{-1}, respectively, and N_0, N_1, N_2, and N_3 represent application of 0, 60, 120, and 180 kg N·ha.$^{-1}$, respectively. Different capital letters indicate significant differences ($P < 0.05$) between the same P level and different N fertilizer levels, while different small letters indicate significant differences ($P < 0.05$) between the same N level and different P fertilizer levels. F_N, F_P, and $F_{N \times P}$ represent the *F* values for the N and P levels, and N-P interaction level, respectively. ns indicates no significant difference ($P > 0.05$), * indicates a significant difference ($P < 0.05$), and ** indicates extremely significant difference ($P < 0.01$)

supply for each irrigation event. Irrigation was scheduled during the green-up stage, 8–10 days before each cutting, and 3–5 days after cutting. The irrigation water was sourced from wells at the experimental site, with pH 7.69 and EC 0.275 mS/cm. The irrigation criterion required contact between wetting fronts of adjacent drip irrigation tapes. The total irrigation volume in 2020 was approximately 6750 $m^3 \cdot ha^{-1}$, with specific irrigation dates as follows: April 19, May 12, May 25, June 21, July 4, August 1, August 14, and September 23, 2020. Fertilization at the plot level was conducted using a 50 L fertilizer tank. To ensure uniform fertilizer application, the fertilizer was fully dissolved in water before application. A portion of the tank's water content was pre-discharged via a water pump to prevent overflow of the fertilizer mixture. The total annual fertilizer amount was equally divided into four applications, which were top-dressed with irrigation water during the green-up stage and during the first irrigation following each cutting. The specific fertilization dates were: April 19, May 25, July 4, and August 14, 2020. All other field management/agronomy was carried out in accordance with local best management practices for uniform high-yield alfalfa fields.

Sampling and measurements

Alfalfa samples (mature leaves and senescent leaves; whole plant leaves, stems and taproots) were collected, Given the progressive accumulation of biomass and nutrients in alfalfa taproots, taproots samples were collected exclusively at the final cut in this study. All samples were immediately brought back to the laboratory, deoxidized at 105°C for 15 min to reduce respiration and decomposition, and then dried at 65°C until it reaches constant mass. Briefly, organic C was determined by $K_2Cr_2O_7$ oxidation method and external heating [21], while total N content was determined by Kjeldahl N determination method after H_2SO_4 and catalyst digestion [21], and the total P content was determined by molybdenum blue colorimetry after the ashing method [21].

Nutrient resorption efficiency [41]was calculated by the following formula:

$$\mathrm{NuRE} = (\mathrm{Nu}_{\mathrm{mature}} - \mathrm{Nu}_{\mathrm{senesced}})/\mathrm{Nu}_{\mathrm{mature}} \times 100\%$$

In the formula, $\mathrm{Nu}_{\mathrm{mature}}$ represents the nutrient concentration of mature alfalfa leaves, and $\mathrm{Nu}_{\mathrm{senesced}}$ represents the nutrient concentration of senescent alfalfa leaves,

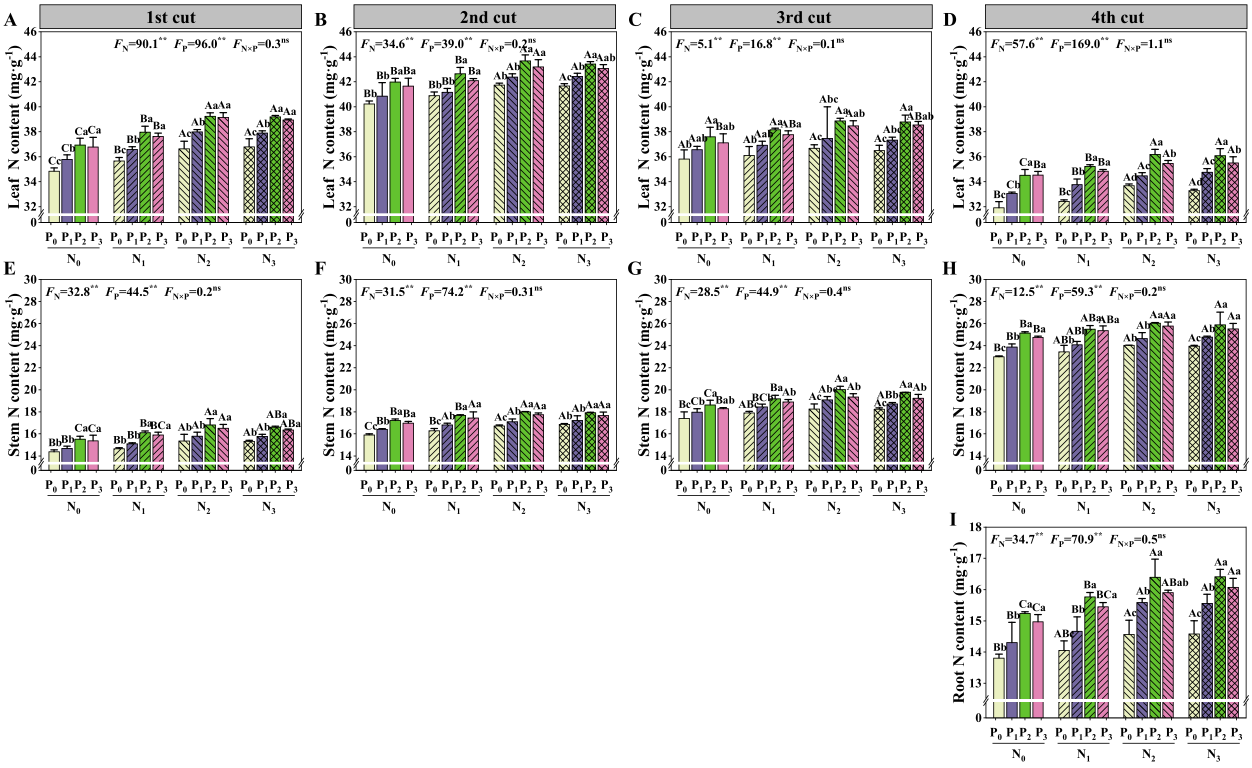


Fig. 3 Nitrogen (N) content in leaves (**A**, **B**, **C** and **D**), stems (**E**, **F**, **G** and **H**) and roots (**I**) of alfalfa (*Medicago sativa* L.) under N and phosphorus (P) addition prior to each cutting. P_0, P_1, P_2, and P_3 represents an addition of 0, 50, 100, and 150 kg $P_2O_5 \cdot ha^{-1}$, respectively, and N_0, N_1, N_2, and N_3 represent an addition of 0, 60, 120, and 180 kg $N \cdot ha.^{-1}$, respectively. Different capital letters indicate significant differences ($P < 0.05$) between the same P level and different N fertilizer levels, while different small letters indicate significant differences ($P < 0.05$) between the same N level and different P fertilizer levels. F_N, F_P, and $F_{N\times P}$ represent the *F* values for the N level, P level, and N-P interaction level, respectively. ns indicates no significant difference ($P > 0.05$), * indicates a significant difference ($P < 0.05$), and ** indicates extremely significant difference ($P < 0.01$)

in which the C, N, and P nutrient reabsorption (RE) of leaves was expressed by CRE, NRE and PRE, respectively.

Soil samples from each experimental plot were randomly collected by 'S' type distribution, and ~ 300 g of 0–30 cm soil was collected by quartering method (NYT1121.1–2006). Collected soil samples from each plot were air dried, sieved (2 mm mesh) to remove stick, roots, stones, and loose organic debris. Samples were then quartered (as described previously) and ground to pass 0.25 mm screen. Soil total N was determined by Kjeldahl N determination method and total P was determined by molybdenum-antimony spectrophotometry after the samples were digested with H_2SO_4 and catalyst, as described previously [21].

Data analysis

Data were sorted using Excel 2021 software (Microsoft, Redmond, WA, USA), and a two-factor analysis of variance (ANOVA) was conducted using SPSS 22 (SPSS Inc., Chicago, IL, USA) to analyze the effects of different fertilization treatments on stoichiometric characteristics. Duncan's test was used for multiple comparisons. The normality (Shapiro–Wilk test) and homogeneity (Bartlett's test) were tested before ANOVA. Regression fitting analysis was conducted using Origin 2024 (Origin Lab, Northampton, MA, USA) for DM yield and different organ stoichiometric ratios. We used partial least squares structural equation modeling (PLS-SEM) to analyze the direct and indirect effects of soil nutrients on leaf nutrient reabsorption, different organ C, N, and P concentrations, and their stoichiometric ratios [22]. Goodness-of-Fit (GoF) was utilized to judge the model.

Results

Carbon allocation in alfalfa leaves, stems and roots

Carbon content of alfalfa leaves, stems, and roots ranged from 306.46–391.52 mg·g^{-1}, 360.66–436.47 mg·g^{-1}, and 395.05–423.67 mg·g^{-1}, respectively (Fig. 2). As the N and P application rates increased, C content of different alfalfa organs initially decreased and subsequently stabilized. The C content among different alfalfa organs showed high synergy. In that, N and P level had a significant effect on the alfalfa C content ($P < 0.05$), while the interaction between N and P had a significant ($P < 0.05$)

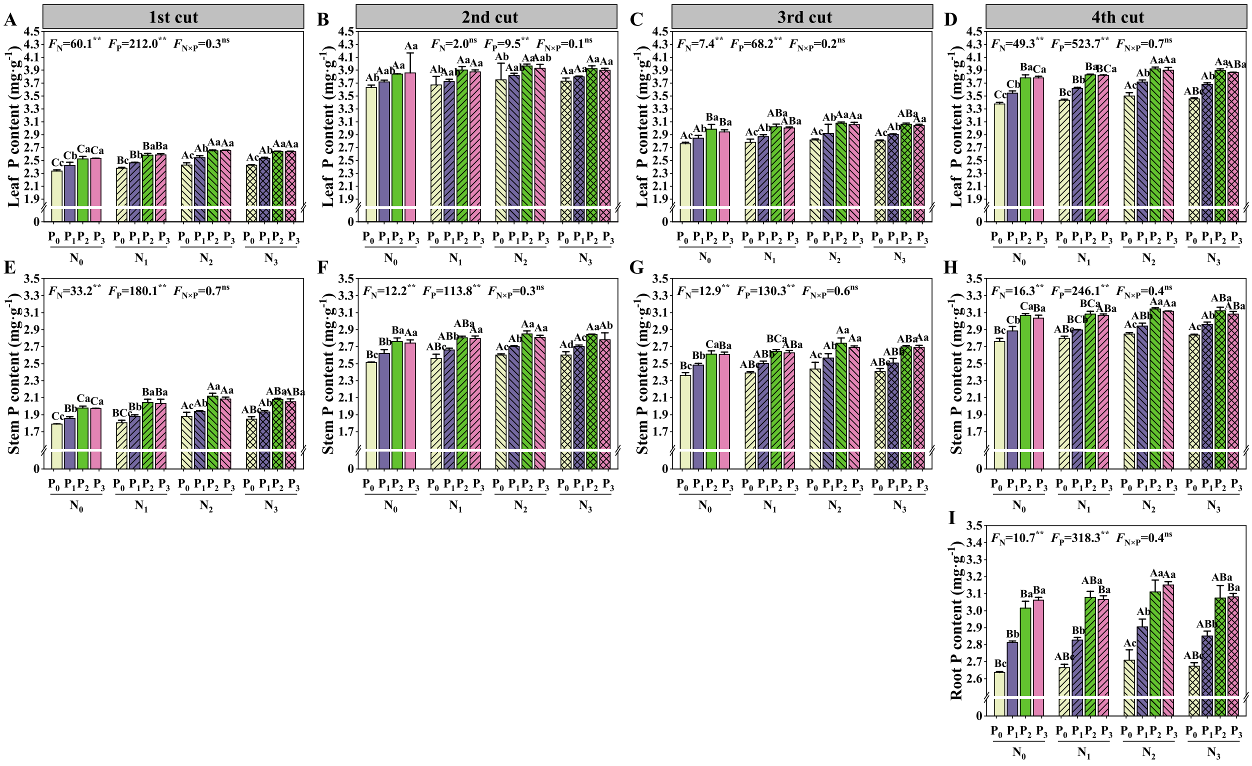


Fig. 4 Phosphorus (P) content in leaves (**A**, **B**, **C** and **D**), stems (**E**, **F**, **G** and **H**), and roots (**I**) of alfalfa (*Medicago sativa* L.) under nitrogen (N) and P addition prior to each cutting. P_0, P_1, P_2, and P_3 represent adding 0, 50, 100, and 150 kg P_2O_5·ha^{-1}, respectively, and N_0, N_1, N_2, and N_3 represent adding 0, 60, 120, and 180 kg N·ha.$^{-1}$, respectively. Different capital letters indicate significant differences ($P < 0.05$) between the same P level and different N fertilizer levels, while different small letters indicate significant differences ($P < 0.05$) between the same N level and different P fertilizer levels. F_N, F_P, and $F_{N×P}$ represent the *F* values for the N and P levels, and N-P interaction level, respectively. ns indicates no significant difference ($P > 0.05$), * indicates a significant difference ($P < 0.05$), and ** indicates extremely significant difference ($P < 0.01$)

effect on C content of alfalfa leaves at the first and fourth cutting, but showed no significant effects ($P > 0.05$) at other cutting times and organs.

Nitrogen allocation in alfalfa leaves, stems, and roots

The N contents of the leaves, stems, and roots of alfalfa ranged from 31.91–43.67 mg·g^{-1}, 14.38–26.04 mg·g^{-1}, and 13.80–16.41 mg·g^{-1}, respectively (Fig. 3). With increased N application, the N content of the different organs showed a significant increasing trend ($P < 0.05$). With increased P application, N content of different organs showed an initial increase and then decreased trend, reaching a maximum value in P_2 treatment. There was no definite regularity of N content of different organs after each cut, but there was a high degree of synergy in N content between different organs under fertilization treatment. The two-factor ANOVA showed that P and N level had a significant effect on the C content in alfalfa ($P < 0.05$), while the interaction level between N and P had no significant effect ($P > 0.05$).

Phosphorus allocation in alfalfa leaves, stems and roots

The P contents of leaves, stems, and roots of alfalfa ranged from 2.33–3.96 mg·g^{-1}, 1.79–3.14 mg·g^{-1}, and 2.64–3.15 mg·g^{-1}, respectively (Fig. 4). Compared with the no-N treatment, in general most high-N treatments significantly increased phosphorus content in different alfalfa organs ($P < 0.05$). With increasing P application, P content in different organs showed a trend of first increasing and then stabilizing. There was a high degree of coordination in the P content of different organs following cutting. Analysis of variance showed that N level had a significant effect on P content of different organs of alfalfa except in the leaves, after the second cutting ($P < 0.05$). Phosphorus level had a significant effect on P content after all cuts ($P < 0.05$). The interaction level between N and P had no significant effect ($P > 0.05$).

The C:N ratios of alfalfa leaves, stems, and roots

The C:N ratios of leaves, stems, and roots of alfalfa under different fertilization conditions ranged from 7.02–11.96, 14.71–29.37, and 24.12–30.70, respectively (Table S1).

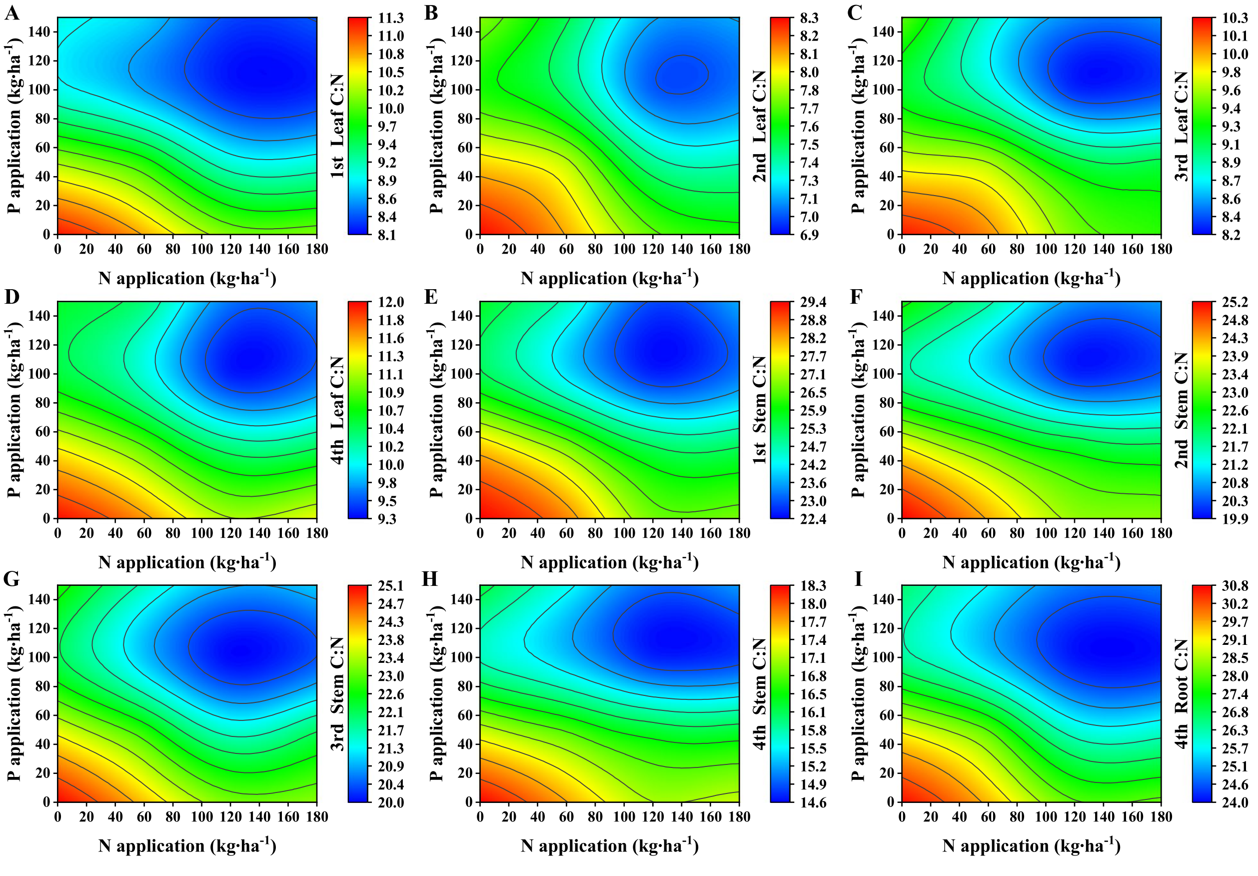


Fig. 5 The Carbon:Nitrogen (C:N) ratios of alfalfa (*Medicago sativa* L.) leaves (**A**-**D**), stems (**E**–**H**), and roots (**I**) under N and P addition prior to each cutting. The x-axis and y-axis represent the application rates of N fertilizer and P fertilizer in the field, respectively, while different colors indicate the levels of the stoichiometric ratio

With increased N application, the C:N ratios of different organs showed a significant decreasing trend ($P < 0.05$). With increased P application, the C:N ratios of different organs initially decreased and then increased slowly (Fig. 5, Table S1). Among them, N and P application levels had a significant effect on organ C:N ratios ($P < 0.05$), while the N-P interaction level, except for the first cut leaves, had no significant effect on the C:N ratios of the different alfalfa organs ($P > 0.05$, Table 1).

The C:P ratios of alfalfa leaves, stems, and roots

The C:P ratios in the leaves, stems, and roots of alfalfa under different fertilization conditions ranged from 77.30–167.70, 121.89–235.91, and 125.39–160.72, respectively (Table S2). With increased N application, the C:P ratios in all organs showed a significant decreased trend ($P < 0.05$). With the increased P application, the C:P ratios in all organs showed a decrease first and then increased trend (Fig. 6, Table S2). Among them, N and P application levels had a significant effect on the C:P ratio ($P < 0.05$), while the interaction between N and P levels, except for the first cut, had no significant effect on the C:P ratios of the alfalfa organs ($P > 0.05$, Table 1).

The N:P ratios of alfalfa leaves, stems, and roots

The N:P ratios in the leaves, stems, and roots of alfalfa under different fertilization conditions ranged from 9.10–15.16, 6.19–8.45, and 4.89–5.46, respectively (Table S3). With the increase of N application, the N:P ratios in all organs showed an increasing trend. With the increase of P application, the N:P ratios in all organs showed a decreasing trend (Fig. 7, Table S3). Except for the third cutting of stems and the fourth cutting of roots, N level had no significant effect on the N:P ratios of alfalfa organs ($P > 0.05$). Except for the second cutting of leaves and stems, and for the fourth cutting of stems, P level had a significant effect on the N:P ratios of alfalfa organs ($P < 0.05$). However, the N-P interaction level had no significant effect on the N:P ratios of alfalfa organs ($P > 0.05$, Table 1).

Relationships between stoichiometry ratios and yield

We fitted the stoichiometric ratios of different organs and their yields (Fig. 8). There was a significant negative correlation between the C:N ratios of leaves, stems, and roots and their yields ($P < 0.05$), with R^2 values of 0.7063, 0.7263, and 0.6559, respectively (Fig. 8A-C). There is a significant negative correlation trend between the C:P ratios of leaves, stems, and roots and their yields ($P < 0.05$), with R^2 values of 0.6449, 0.6263, and 0.5060, respectively (Fig. 8D-F). The N:P ratios of leaves, stems, and roots exhibit a horizontal trend with yield, and the fitting results were not significant (Fig. 8G-I, $P > 0.05$).

Table 1 Variance analysis of stoichiometry ratios of alfalfa (*Medicago sativa* L.) leaves, stems, and roots under nitrogen (N) and phosphorus (P) addition prior to each cutting

Cuts	Treatments	C:N ratio						C:P ratio						N:P ratio					
		Leaf		Stem		Root		Leaf		Stem		Root		Leaf		Stem		Root	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
First cut	N	107.0	< 0.001	59.3	< 0.001	–	–	71.4	< 0.001	58.3	< 0.001	–	–	2.9	0.054	2.5	0.076	–	–
	P	463.6	< 0.001	135.8	< 0.001	–	–	514.7	< 0.001	321.7	< 0.001	–	–	7.7	< 0.001	5.1	0.006	–	–
	N×P	2.6	0.025	0.4	0.907	–	–	2.5	0.027	0.6	0.804	–	–	0.1	0.999	0.1	0.999	–	–
Second cut	N	58.5	< 0.001	40.1	< 0.001	–	–	14.5	< 0.001	23.2	< 0.001	–	–	0.4	0.740	2.5	0.078	–	–
	P	51.7	< 0.001	118.0	< 0.001	–	–	26.0	< 0.001	142.9	< 0.001	–	–	0.7	0.541	2.7	0.062	–	–
	N×P	0.3	0.978	0.3	0.970	–	–	0.1	0.999	0.1	0.998	–	–	0.1	0.999	0.2	0.997	–	–
Third cut	N	40.9	< 0.001	49.2	< 0.001	–	–	66.1	< 0.001	32.6	< 0.001	–	–	0.1	0.979	3.7	0.023	–	–
	P	71.5	< 0.001	104.4	< 0.001	–	–	190.5	< 0.001	228.4	< 0.001	–	–	5.9	0.003	11.3	< 0.001	–	–
	N×P	0.8	0.617	0.2	0.989	–	–	1.4	0.233	0.3	0.973	–	–	0.1	0.999	0.1	0.998	–	–
Fourth cut	N	176.1	< 0.001	35.7	< 0.001	43.4	< 0.001	79.0	< 0.001	32.3	< 0.001	22.3	< 0.001	2.2	0.112	0.9	0.474	15.1	< 0.001
	P	455.1	< 0.001	164.4	< 0.001	105.9	< 0.001	409.9	< 0.001	283.6	< 0.001	362.1	< 0.001	23.9	< 0.001	1.8	0.168	13.6	< 0.001
	N×P	1.2	0.304	0.2	0.231	0.6	0.766	1.4	0.241	0.3	0.965	1.0	0.477	0.5	0.828	0.1	0.999	0.4	0.935

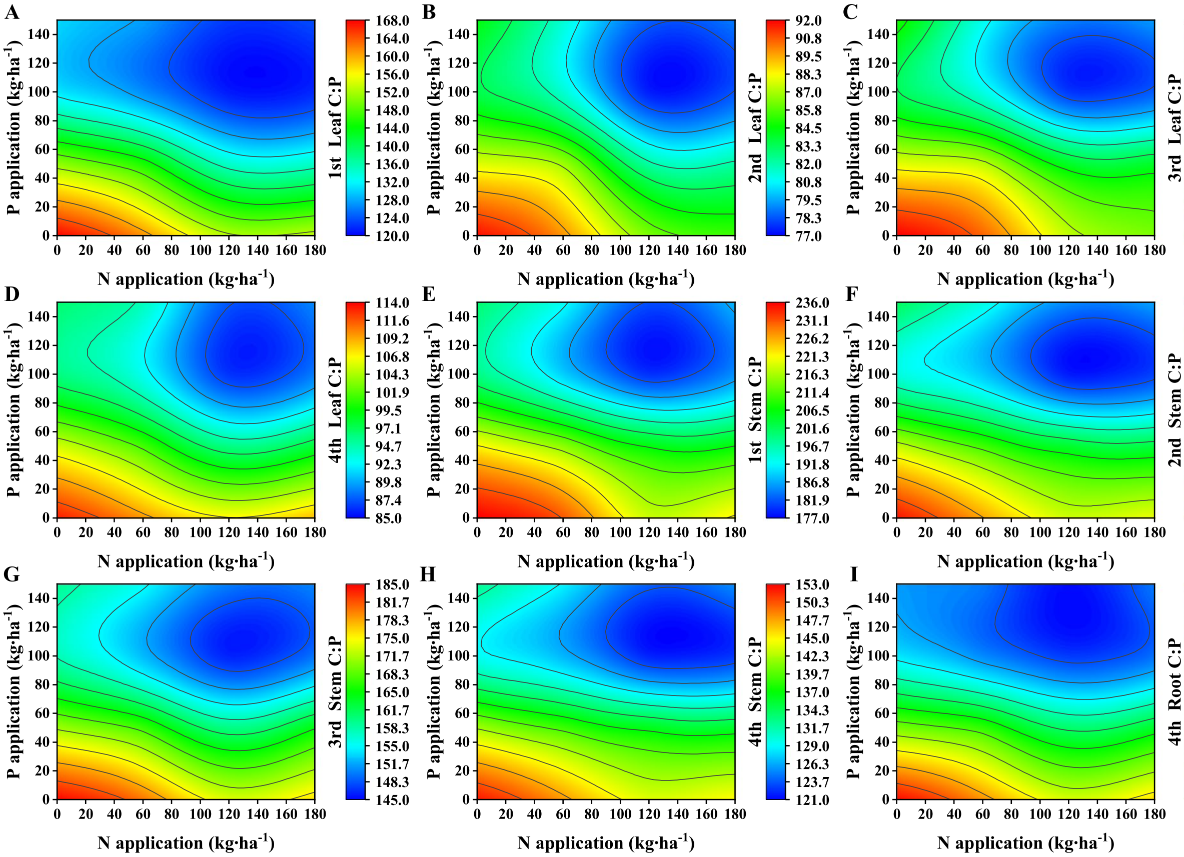


Fig. 6 The Carbon:Phosphorus (C:P) ratios of alfalfa (*Medicago sativa* L.) leaves (**A**-**D**), stems (**E**-**H**), and roots (**I**) under nitrogen (N) and P addition prior to each cutting. The x-axis and y-axis represent the application rates of N fertilizer and P fertilizer in the field, respectively, while different colors indicate the levels of the stoichiometric ratio

Nutrient reabsorption efficiency

With the increase of N and P application, the CRE of four cuts of alfalfa leaves showed a significant increasing trend ($P < 0.05$, Fig. 9A-D). With the increase of N application, NRE showed a significant decreasing trend ($P < 0.05$), while with increased phosphorus application, NRE showed an initial increase and then decreasing trend (Fig. 9E-H). With increased of N application, PRE showed an initial increase and then decreasing trend. With the increase of P application, PRE showed a significant decreasing trend ($P < 0.05$, Fig. 9I-L). The N, P, and N-P interaction levels all had a significant effect on alfalfa leaf CRE, NRE, and PRE ($P < 0.05$, Fig. 9).

Soil nutrient contents and stoichiometric characteristics

With increased N application, there was a significant increase in soil N content (Total N: TN) and the soil N:P ratio ($P < 0.05$, Fig. 10A-C), however, soil P content (Total P: TP) decreased. With increased P application, there was a significant increase in soil N and P ($P < 0.05$), but a significant decrease in the soil N:P ratio ($P < 0.05$). Nitrogen application level, P application level, and N-P interaction level all had a significant effect on soil N and P, and on the soil N:P ratio in alfalfa plots ($P < 0.05$).

Pearson correlation analysis

A Pearson correlation analyses of soil nutrients, leaf nutrient reabsorption, nutrient contents and stoichiometry ratios in different organs (Fig. 11) showed soil total N was significantly positively correlated with CRE and leaf N ($P < 0.05$), and significantly negatively correlated with NRE ($P < 0.05$). Soil total P was significantly positively correlated with N content and P content of different organs of alfalfa ($P < 0.05$), and was significantly negatively correlated with PRE, C content and stoichiometry ratios of different organs ($P < 0.05$), but had no significant correlation with CRE and NRE ($P > 0.05$). The soil N:P ratio was significantly positively correlated with PRE, but negatively correlated with N:P ratios of different organs of alfalfa and root P of alfalfa ($P < 0.05$).

Structural equation modeling

We used PLS-SEM to determine the relationships between soil nutrient content, leaf nutrient reabsorption,

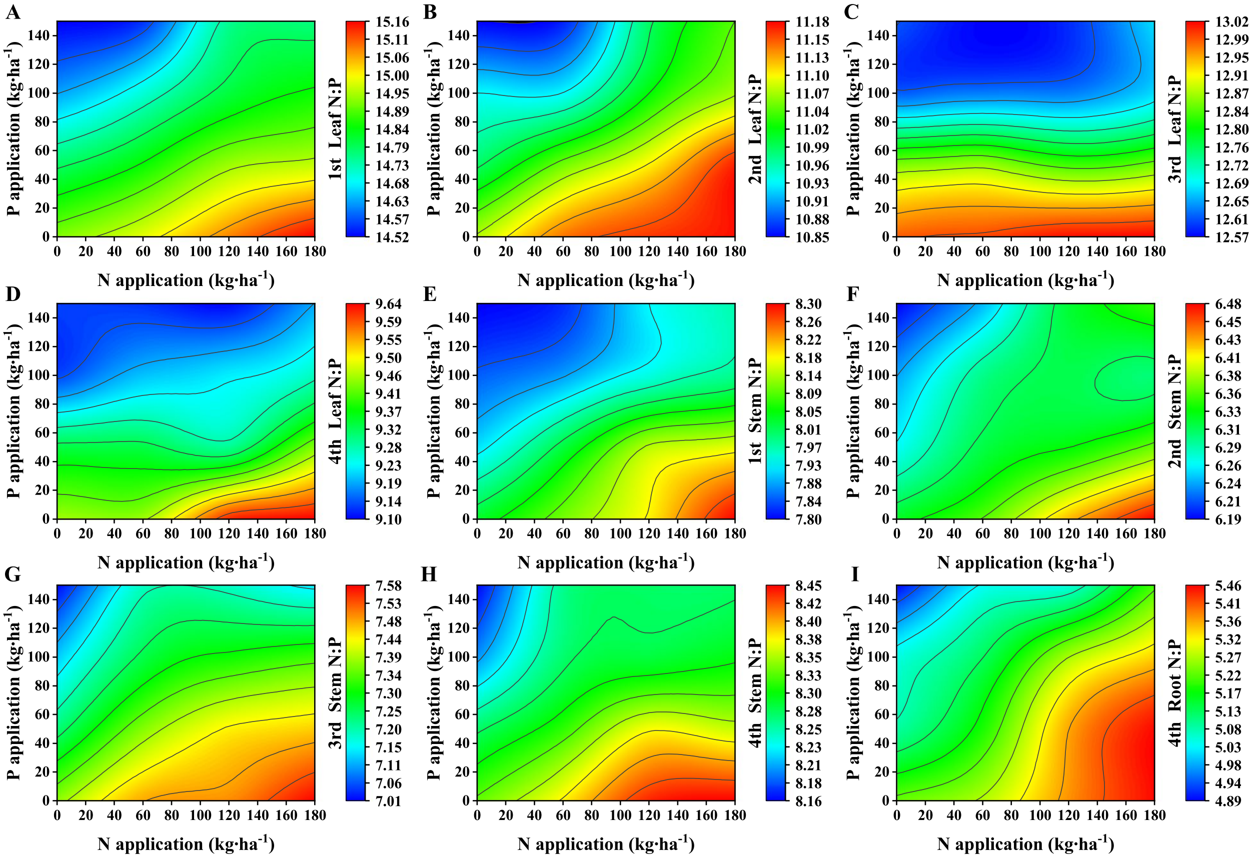


Fig. 7 The Nitrogen:Phosphorus (N:P) ratios of alfalfa (*Medicago sativa* L.) leaves (**A**-**D**), stems (**E**-**H**), and roots (**I**) under N and P addition prior to each cutting. The x-axis and y-axis represent the application rates of N fertilizer and P fertilizer in the field, respectively, while different colors indicate the levels of the stoichiometric ratio

leaf nutrient and stoichiometric ratios, stem nutrient and stoichiometric ratios, and root nutrient and stoichiometric ratios (Fig. 12). This model's Goodness of Fit was 0.8456, indicating that this PLS-SEM can effectively explain the relationships between the above indicators. The results show that soil nutrients have a significant positive effect on leaf (direct regression weight coefficient, rd = 0.80), stem (rd = 0.81), and root (rd = 0.81) nutrient content, explaining 65%, 65%, and 66% of the difference, respectively. Soil nutrients exert direct (rd = 0.79) and indirect (indirect regression weight coefficient, ri = 0.16) effects on the nutrient and stoichiometric ratios of alfalfa organs, explaining 98% of the variation in NuRE.

Discussion

Effects of nitrogen and phosphorus application on nutrient concentrations in different organs of alfalfa and soil

The allocation of C, N, and P nutrients within plants and their interrelationships represent a comprehensive manifestation of plant nutrient allocation strategies and adaptation to external environments. The correlation between the same element in different physiological organs within a plant shows the synergy of the same nutrient absorption and allocation-utilization by different organs, while the correlation between different nutrients within the same organ is a comprehensive reflection of the plant's absorption and utilization of them [23]. In this study, as N and P application rates increased, the N and P contents in the different organs of the plant increased to varying degrees. Probably this is a consequence of the fertilization that leads to a significant increase in available nutrients in the soil, and that the supply of soil nutrients is sufficiently abundant for plant growth (low nutrient stress). In this case, alfalfa may adopt a luxury absorption/consumption strategy, where more nutrients are taken up immediately needed for growth and survival (greater adsorption than maximum crop demand) [42]. In this study, the coefficient of variation of P content in plants under increased N fertilization was larger than the coefficient of variation of N content under increased P fertilization. This phenomenon is primarily attributed to the "P-induced N enhancement" effect unique to leguminous plants and the fact that alfalfa growth is predominantly limited by P availability [24].

In this study, as the fertilization amount increased, nutrient allocation among different organs maintained

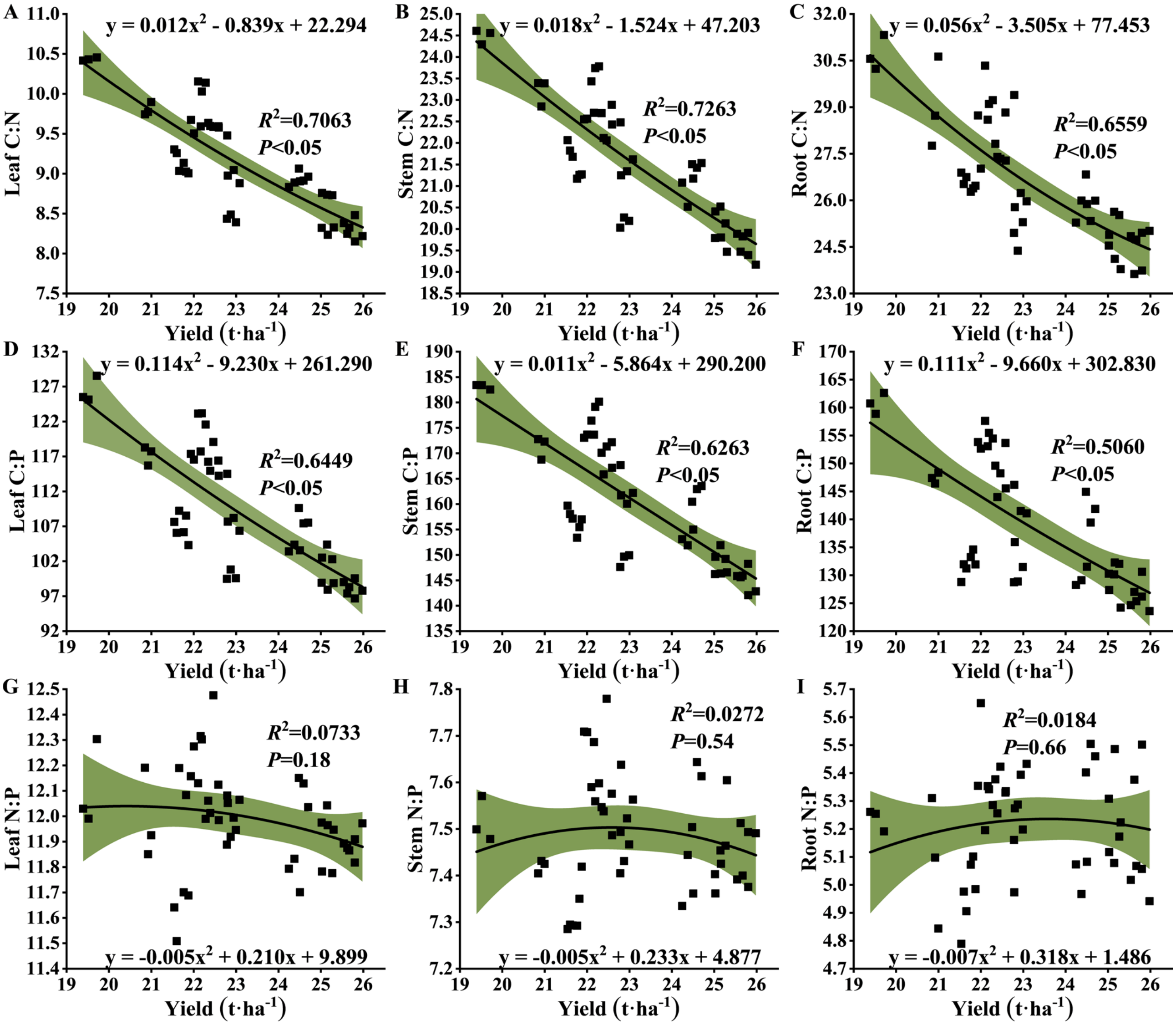


Fig. 8 The relationship between the stoichiometry ratios of alfalfa (*Medicago sativa* L.) leaves (**A**, **D**, and **G**), stems (**B**, **E**, and **H**), and roots (**C**, **F**, and **I**) and dry matter yield. The black curve represents the fitting curve between yield and stoichiometric ratio, while the green shaded area indicates the 95% confidence range

a consistent pattern. However, in the final harvest, increased fertilization led to greater nutrient allocation to root systems. We speculate this may be because root sampling occurred before overwintering when roots need to store more nutrients for winter survival, and high-fertilization treatments provided the conditions necessary to supply more nutrients to the roots. It should be noted that all plants were stimulated to store nutrients due to changes in environmental conditions during winter. However, the results showing more nutrients stored in roots suggest fertilization may enhance nutrient uptake and storage capacity. Additionally, plants receiving higher nitrogen and phosphorus fertilization grew larger (with bigger leaves and root systems), demonstrating greater capacity to capture soil nutrients and sunlight. Concurrently, their larger size creates greater nutrient demands. These factors may be the primary reasons why fertilization led to greater nutrient allocation to roots [25].

Fertilization strategies enhance nutrient content and physicochemical properties of the soil, and improve nutrient content of various plant organs and enhance productivity and yield. Soil N and P constitute essential elements for plant growth and development, and serve as significant indicators for evaluating soil fertility [26]. In this study, increased of N application rate, resulted in a significant increase in soil N content and soil N:P ratio. However, the soil P content decreased, reflects the increased alfalfa growth and yield in response to the greater soil N availability, which increased the plant

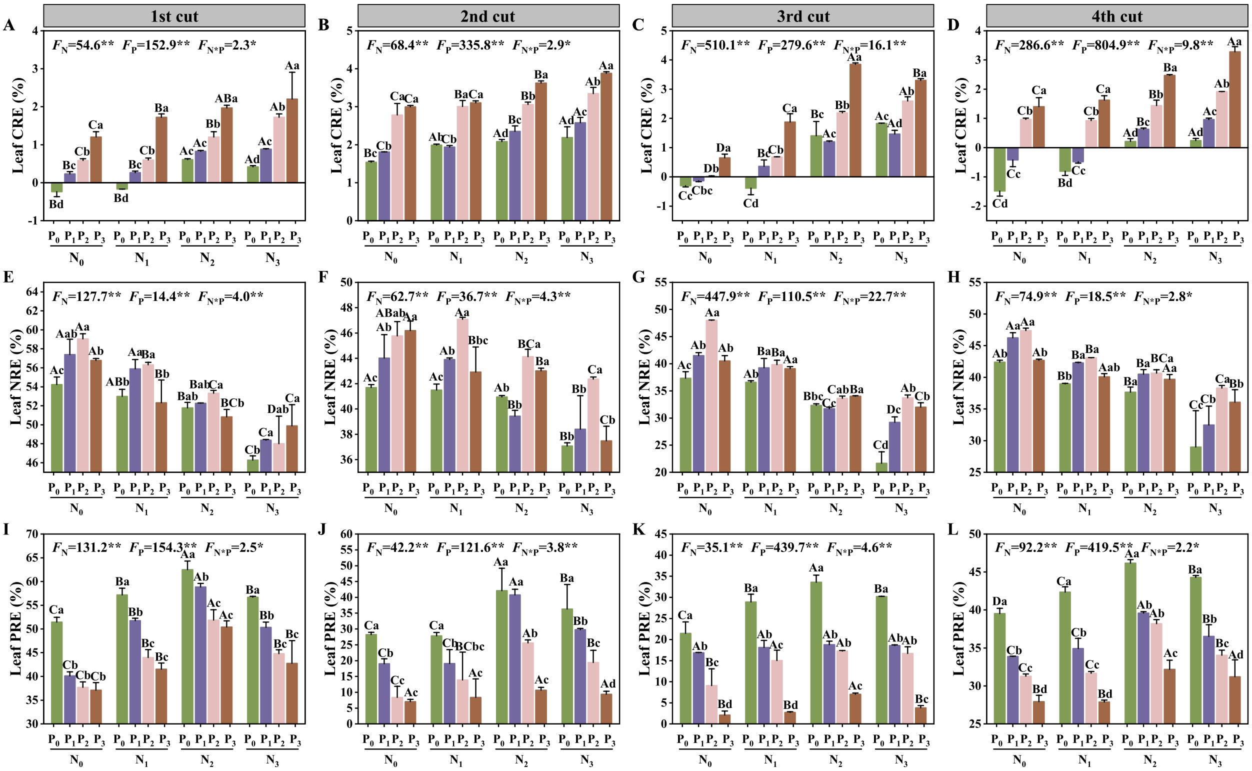


Fig. 9 Nutrient reabsorption in alfalfa (*Medicago sativa* L.) leaves under nitrogen (N) and phosphorus (P) additions prior to each cutting. P_0, P_1, P_2, and P_3 represent adding 0, 50, 100, and 150 kg $P_2O_5·ha^{-1}$, respectively, and N_0, N_1, N_2, and N_3 represent adding 0, 60, 120, and 180 kg N·ha.$^{-1}$, respectively. Different capital letters indicate significant differences ($P < 0.05$) between the same P level and different N fertilizer levels, while different small letters indicate significant differences ($P < 0.05$) between the same N level and different P fertilizer levels. F_N, F_P, and $F_{N×P}$ represent the *F* values for the N and P level, and N-P interaction level, respectively. ns indicates no significant difference ($P > 0.05$), * indicates a significant difference ($P < 0.05$), and ** indicates extremely significant difference ($P < 0.01$)

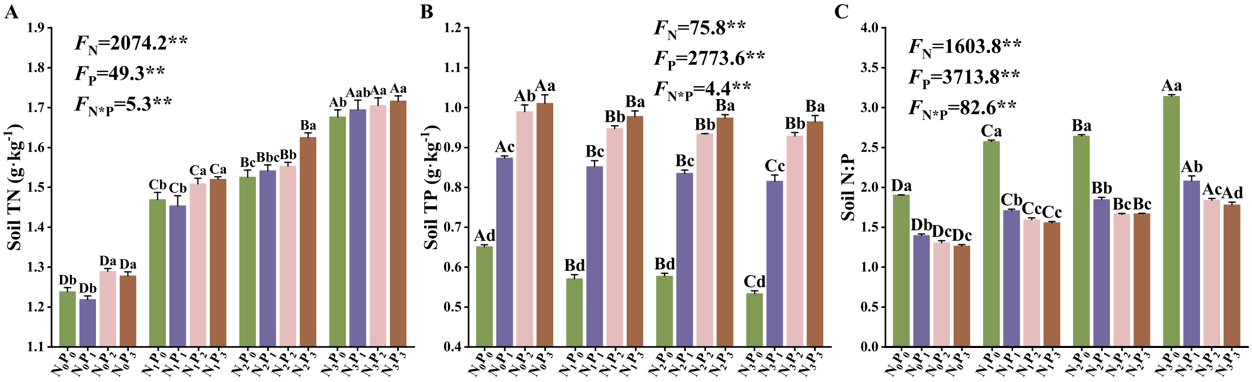


Fig. 10 Soil total nitrogen (TN: graph "A") and total phosphorus (TP: graph "B") contents and their stoichiometry ratio (Soil N:P: graph "C") under N and P additions in the last cut. P_0, P_1, P_2, and P_3 represent addition of 0, 50, 100, and 150 kg $P_2O_5·ha^{-1}$, respectively, and N_0, N_1, N_2, and N_3 represent addition of 0, 60, 120, and 180 kg N·ha.$^{-1}$, respectively. Different capital letters indicate significant differences ($P < 0.05$) between the same P level and different N fertilizer levels, while different small letters indicate significant differences ($P < 0.05$) between the same N level and different P fertilizer levels. F_N, F_P, and $F_{N×P}$ represent the *F* values for the N and P levels, and N:P interaction level, respectively. ns indicates no significant difference ($P > 0.05$), * indicates a significant difference ($P < 0.05$), and ** indicates extremely significant difference ($P < 0.01$)

demand and uptake of P from the soil. Furthermore, soil N and soil P increased significantly with increased P fertilizer level. Adequate P fertilizer application not only directly increases alfalfa growth, but also promotes photosynthetic rates, thereby meeting the energy requirements associated with symbiotic N fixation, including nodule formation, and activation of N-fixing enzymes. In short, P application can increase the symbiotic N fixation

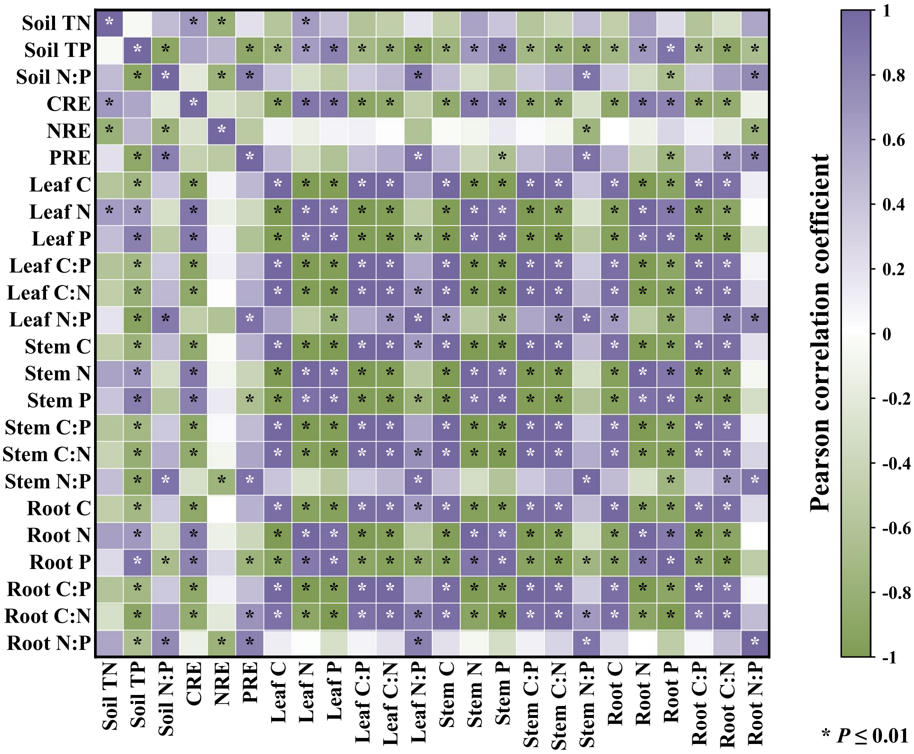


Fig. 11 Correlation analysis between soil nutrients, leaf nutrient reabsorption, and stoichiometry characteristics in different alfalfa (*Medicago sativa* L.) organs. The color gradient represents the magnitude of the Pearson correlation coefficient, with asterisks (*) indicating statistical significance at $P \leq 0.01$

of root nodules and increase N uptake and crop content [43].

Effect of nitrogen and phosphorus fertilization on the stoichiometric ratios of different organs of alfalfa

Carbon, N, and P are crucial elements for plant growth and development, and their equilibrium influences the metabolic activities and energy conversion efficiency of plants [12, 13]. The high plasticity of plant stoichiometry ratios enables plants to optimize their respiration, photosynthesis, and nutrient uptake and utilization under diverse environmental conditions, thereby enhancing nutrient utilization efficiency [27]. In this study, increased fertilizer application, the C:N and C:P of different plant organs showed a decreased trend. We suggest that this is primarily because fertilization changed the uptake and assimilation of P by alfalfa, in that, while fertilization can increase photosynthesis and C fixation, it is lower than the increase in N and P nutrients within the plant.

The N:P threshold hypothesis suggests that a specific N:P ratio can be used to determine the nutrient limitation of plants. Koerselman and Meuleman's [28] comprehensive analysis of 40 fertilization studies found that a N:P ratio greater than 16 indicates P limitation, while a ratio less than 14 indicates N limitation. When the N:P ratio is between 14 and 16, plant growth is limited by both N and P [28]. There have been increasing studies in chemometrics using this hypothesis to evaluate nutrient limitation status of alfalfa [29, 30]. However, our research found that only the first cut leaf N:P ratio was greater than 14, while the other cuts and organs had ratios of less than 14. This inconsistency with other studies suggest that alfalfa is primarily limited by P, and the threshold range in our study is in fact much larger. Güsewell [17] suggested that N:P ratio of less than 10 and a ratio of more than 20 are more suitable for determining N and P nutrient limitations. However, this evaluation standard does not conform to our research results for the N:P ratio of alfalfa (Figure S1). We suggest that the two critical value standards for N and P do not apply to the nutrient limitation evaluation of alfalfa. This may be because reported N:P threshold hypothesis has a large risk of distortion when determining the nutrient limitation of a single plant species and is not applicable to all species [31, 32]. The pattern of plant nutrient absorption and utilization are largely the result of the interaction between the supply of exogenous nutrients, environmental conditions, production practice, and plant physiological and genetic characteristics, which reflect the breeding development and ecotype of the plant material. Although our results are not consistent with the previous N and P critical values, we suggest that alfalfa N and P thresholds are not just dependent on N and P soil levels, they are also related to other biotic and abiotic endogenous

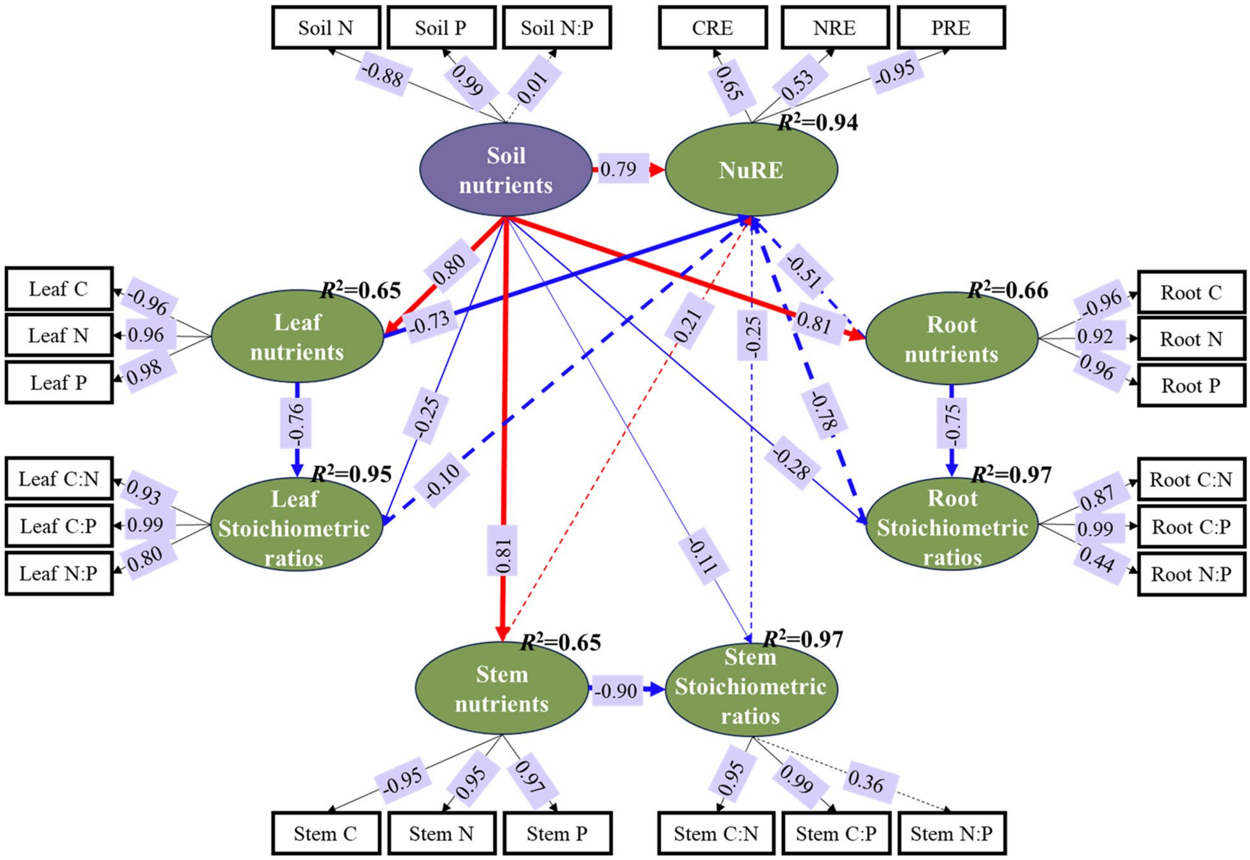


Fig. 12 Partial least squares structural equation model for soil total nitrogen (TN), total phosphorus (TP), leaf nutrient reabsorption, and stoichiometry characteristics of alfalfa (*Medicago sativa* L.) organs. The arrows point to causal relationships, and the red solid lines indicate a significant positive correlation between the two, while the red dashed lines indicate a positive but not significant correlation. The blue solid lines indicate a significant negative correlation, while the blue dashed lines indicate a negative but not significant correlation. The width of the arrows is proportional to the size of the path coefficient, and the numbers on the lines represent the path coefficient. Goodness of Fit was 0.8456

and exogenous factors. In our analysis of the relationship between the N:P ratio and alfalfa yield, we observed a weak correlation. As noted previously, both nitrogen (N) and phosphorus (P) are essential nutrients for alfalfa growth. When soil N and P supply is sufficient, high yields are achieved; conversely, concurrent deficiency in both nutrients severely limits productivity. Thus, alfalfa yield is primarily governed by the absolute supply of N and P, not their relative proportions.

Effects of nitrogen and phosphorus fertilizer on nutrient reabsorption

Nutrient reabsorption refers to the process in which senescent plant organs transfer nutrients to other tissues or are utilized by other organs before they fall off [33]. It not only affects the nutrient acquisition of plants, but also source sink relationships associated with plant growth, nutrient competition and cycling, reflecting to a great extent the adaptive response of plants to environmental and cultural stresses. Under N and P limitations in soil, plants adapt to stress by extending the growth period of senescing tissues. Aside from nutrient stress which may be associated with litter turnover, a variety of factors, including nutrient content of plant biomass, spatial distribution, climate, and soil conditions may also governs nutrient release [34]. Studies have shown that applying fertilizer can reduce the reabsorption of nutrients [35], however, other studies have shown that fertilization can promote nutrient reabsorption from plant senescing tissues, which mainly occurs in areas where nutrients were severely limited [36]. This is mainly because fertilizers increase the nutrient content of mature leaves, while the nutrient content of senescing leaves remains unchanged or only increase marginaly. In that, plants adopt a strategy of fully reabsorbing or partially reabsorbing nutrients supplied from external sources [37]. In this study, with the increase of N application rate, leaf NRE showed a significant decreased trend ($P < 0.05$), but the PRE showed an initial increase first which was then followed by a decreasing trend. Similarly, with increased P, the leaf PRE showed a significant decreased trend, while the leaf NRE increased initially and then decreased. This suggests that increased fertilizer application reduced the reabsorption of the corresponding nutrient elements in the senescent leaves of the plant. While, the nutrients also promote the increase in biomass of alfalfa, which means an increased demand for non-corresponding nutrients, thereby promoting the rise of NuRE. However, this trend stops when alfalfa reaches its peak yield. It must be noted that this study only focuses on short-term nutrient changes, while long-term continuous fertilization may lead to issues such as soil acidification, micronutrient imbalances, and changes in microbial communities, which could affect the sustainable supply of nutrients and soil quality. Therefore, future research should conduct multi-year, continuous field trials to more comprehensively assess the impact of different fertilization strategies on soil quality and alfalfa nutrient absorption.

Conclusions

By analyzing the characteristics of stoichiometry and nutrient reabsorption traits between different organs of alfalfa, we clarified the effects of fertilization factors on the inter-organ stoichiometry and nutrient reabsorption. P fertilization increased the soil TN and TP content, whereas N fertilization increased TN but slightly decreased TP. The increase in soil nutrients enhanced the nitrogen and phosphorus nutritional concentrations of the plant organs, with nutrient concentrations across organs showing high coordination. With increasing single-nutrient fertilizer application, the C:N and C:P ratios in alfalfa organs decreased, while the N:P ratio stabilized under conditions of sufficient or co-limiting soil N and P. Alfalfa N:P ratios under different fertilization treatments were 4.89–5.46 in roots, 6.19–8.45 in stems, and 9.10–15.16 in leaves. It is important to note: although our study showed a certain regularity in the N:P ratio with soil nutrient changes (Fig. 7), the N:P ratio is more influenced by organ type and harvesting (Fig. S1). If specific threshold standards (such as N:P ratio thresholds of 14/16 or 10/20) are directly used to determine nutrient limitation, it may lead to misjudgment of the N and P limitation status in alfalfa. Only when other growth conditions, such as cutting frequency and organ type, are consistent and stable, will the N:P ratio threshold provide a "real" diagnostic value for nutrient limitation in alfalfa. Due to environmental variability, robust N:P thresholds require large-scale dataset analysis. Future research should include more experimental points for further study. In conclusion, stoichiometric methods can be used to identify nutrient limitation in alfalfa and guide field fertilization, but when predicting its nutrient limitation status, the consistency of environmental factors must be ensured.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-07360-6.

Supplementary Material 1.

Authors' contributions

Y.S.: Methodology, Investigation, Software, Formal analysis, Writing-original draft preparation, Visualization. J.H.: Writing-original draft preparation. K.Y.: Writing-original draft preparation. K.W.: Writing-original draft preparation. X.W.: Conceptualization, Writing-review & editing. A.C.: Writing-review & editing, Supervision. I.L.: Writing-review & editing, Supervision. Y.Q.: Conceptualization, Writing-review & editing. C.M.: Conceptualization, Writing-review & editing. Q.Z.: Conceptualization, Funding acquisition, Writing-review & editing, Supervision.All authors reviewed the manuscript.

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Data availability

All data generated or analyzed during the current study are included in this article and its Supplementary Information.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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