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Nutrient Uptake Potential of Nonleguminous Species and Its Interaction with Soil Characteristics and Enzyme Activities in the Agro-ecosystem

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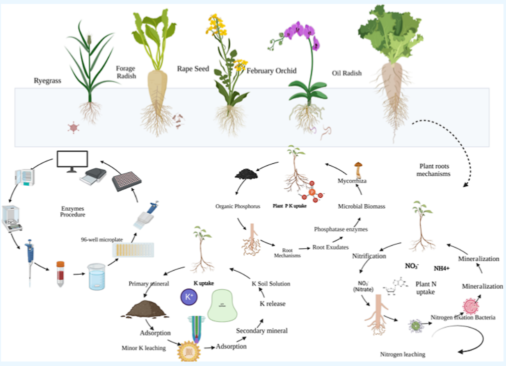
ABSTRACT: The potential nutrient uptake abilities of a plant are essential for improving the yield and quality. Green manures can take up a huge amount of macronutrients from the soil. The mechanisms underlying the differences in nutrient uptake capacity among different nonlegume species remain unclear. The plot experiments were conducted to investigate the performance of nonlegume species including forage radish (*Raphanus raphanistrum* subsp. *sativus*), oil radish (*Raphanus sativus* var. *Longipinnatus*), February orchid (*Orychophragmus violaceus* L), and rapeseed (*Baricca napus*), while a ryegrass (*Lolium perenne* L.) species was used as a control. The study results showed that forage radish had the highest nutrient uptake (N and P), i.e., 322 and 101% in Hunan and 277 and 469% in the Sichuan site, respectively, compared with the control. While the greatest K uptake was found in forage radish, i.e., 123%, and February orchid, 243%, in the Hunan and Sichuan sites. Forage radish also presented higher phosphorus use efficiency in both experimental areas: Hunan by 301% and Sichuan by 633% compared to the control. Significant modifications were found in nutrient availability and enzyme activities after the cultivation of various species. The oil radish enhanced the β-glucosidase (BG) and leucine-aminopeptidase enzyme activities by 324 and 367%, respectively, while forage radish developed the highest phosphatase (Phase) and *N*-acetyl-glucosaminidase (NAG) activities compared to the ryegrass in Hunan. In the Sichuan site, the oil radish promotes enzyme activities such as Phase (126%), BG (19%), and NAG (17%), compared to the control. It is concluded that forage radish, oil radish, and February orchid can easily improve soil nutrient quality in green manuring practices and provide valuable nutrient management systems.



1. INTRODUCTION

Plants require essential macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) in a great amount for their healthy growth.[1−3] N is important for the early development of plants and is involved in the synthesis of amino acids, which is necessary for protein synthesis.[4] P and K are involved in different functional processes, for example, energy transfer, stimulating enzymes, and producing molecules.[5−7] Deficiencies in macronutrients are a major problem for crop production and soil fertility status. An earlier study[8] demonstrated that excessive use of mineral fertilizers not only leads to soil pollution but also can affect the environment. Modern agricultural systems widely adopt sustainable practices that promote soil health and increase crop yield. Green manure crops are well-known as nutrient-efficient plants that have the ability to increase soil nutrient availability in farming systems.[9] Cultivation of green manure is essential for providing food security, improving the sustainable environment, conserving energy, and reducing fertilizer consumption in agriculture.[10] Furthermore, they have a favorable effect on the physicochemical characteristics of soil, increased organic matter, maintaining soil pH, and the ability to control cycles of nutrients.[11,12] In general, these crops are able to reduce soil erosion and greenhouse gas emissions while enhancing nutrient retention, soil fertility, soil productivity, and acting as sources of fertilizer for agricultural crops, thereby increasing overall agricultural sustainability.[13]

The green manures are famous for their unique root mechanisms that are related to various processes, such as the root secretion of organic acid into the soil to improve the nutrient availability for plant uptake. Furthermore, nonleguminous species (green manure) involve the phosphatase

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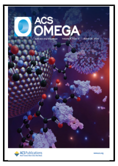

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(Phase) enzymes being released into the soil, which can break down P-containing organic material.[14] Legume species are not only the ones that play a key role in soil nutrient cycling processes; nonlegume species can also improve soil fertility and reduce the demand for synthetic fertilizers. Because some nonlegume species have deep root systems, they are able to minimize soil erosion and compaction, which can enhance soil structure. Their extensive root systems prevent nutrient leaching and maintain the soil fertility.[15] The plant's highest nutrient accumulation abilities mostly depend on its uptake potential, which ultimately improves yield at a low nutrient condition.[16]

A previous study[17] describes that P use efficiency (PUE), the yield of crop per unit of mineral supplied, was determined by how effectively nutrients were used. Whereas, P uptake efficiency (PUpE) refers to the ability of plants to take up P from the soil. The P utilization efficiency (PUtE) defines the biomass yield produced per unit of nutrient uptake by the plant shoots.[18,19] However, a higher nutrient use efficiency (NUE) of a plant could reduce chemical fertilizer consumption, decrease the amount of nutrients lost, and improve crop production.

The root mechanisms of a plant have an influence on their capacity to take up and use nutrients under different conditions. Nonleguminous plant root characteristics have effects on nutrient uptake; the changes vary between species and their groups. For example, deeper root systems of a plant easily take up nutrients from the soil surface and scavenge nutrients from deeper soil layers.[3] Some green manure plants that belong to the *Brassicaceae* family, such as radish, rapeseed, and February orchid plants, effectively accumulate N from the deep soil layers and prevent N leaching loss during the winter and spring periods.[20]

Radish (*Raphanus sativus* L.) is an important root vegetable famous for its rapid growth and different beneficial properties. Mostly, it is cultivated in several countries worldwide overall due to its high nutritional content.[21,22] Rapeseed (*Brassica napus* L.) is a deep-rooted plant that is also known as a high-quality forage crop due to its cold resistance, and its cultivation mostly provides advantages within the rotation of cereals. Rapeseed crops also have the ability to improve nutrients in the soil.[23] February orchid is a nonlegume plant without N fixation ability, but it could absorb residual nitrate (NO_3^-) and reduce N leaching losses. Their extensive rooting system relates to soil N depletion.[24,25] However, microbes and plant roots could secrete enzymes into the soil that support the breakdown of complicated molecules into simpler ones and the intensification of mineralized nutrients to improve the quantity of available nutrient in the soil.[26] Soil enzyme activity is essential for determining soil fertility and plant yield and is an integral part of biological modification.[27] Specific enzymes catalyze particular substrates, and each enzyme does not depend on the entire nutrient cycle.[28] However, phosphatase enzymes are usually related to the conversion of soil P, even though they also catalyze the hydrolysis of ester-phosphate bonds, which releases phosphate and allows plants to survive under P stress conditions.[29] Different activities of enzymes such as β-glucosidase (BG), L-aminopeptidase (LAP), and *N*-acetyl-glucosaminidase (NAG) are correlated to C and N nutrient cycling and critical for soil quality.[30] An effective and conventional approach for the management of agro-ecosystems involves the utilization of green manure, particularly in paddy systems, by alternating the cultivation of rice and green manure. The growing of green manure crops for improving soil N, which is widely used in southern China, has previously attracted a lot of attention.

Numerous studies have emphasized the importance of species identity and an associated high selection effect in cover crop mixtures and their incorporation.[31,32] A previous study showed incorporation of green manure increased soil enzyme activities, which promote plant growth and soil nutrients.[33] We hypothesize that the different nonleguminous species enhance the soil fertility status during their growth period without any fertilizer application. In this regard, the present study investigates the responses of nonleguminous (green manure) species throughout their growth period to soil enzyme activities that are related to N, P, and C cycling and how they interact with nutrient availability. The objectives of this study are to understand how variations in soil enzyme activities influence soil nutrient availability and how greatly these variations depend on the type of green manure (nonleguminous species). Further, the present study also focuses on evaluating the nutrient uptake ability and the PUE between nonlegume species which is beneficial for green manuring the field.

2. METHODOLOGY

2.1. Experimental Location. Two sites were selected for plot experiments in southern China: Qiyang city (Hunan province) and Chengdu city (Sichuan province) for cultivation of various nonleguminous species. Both study areas were selected in subtropical climatic zones with average annual temperatures of 25 and 22 °C and average annual precipitation of 895.6 and 1700 mm in Sichuan and Hunan provinces, respectively. Table 1 provides the fundamental soil characteristics of both of the locations.

Table 1. Basic Soil Characteristics of Sichuan and Hunan Provinces

basic soil properties	Hunan	Sichuan
geographical coordinate	26°34′48″N 111° 50′26″E	30°39′36″N 104°03′48″E
total N (g kg^{-1})	0.73	1.05
SOM (%)	1.0	0.95
NH_4^+ (mg/kg^1)	2.9	4.1
NO_3^- (mg/kg^1)	1.7	2.2
availableP (mg/kg^1)	11.2	18.6
available K (mg/kg^1)	122.0	120.5
pH (1:2.5)	6.3	7.8
Phase (nmol h^{-1} g^{-1})	128	34.2
BG (nmol h^{-1} g^{-1})	38.2	40.8
NAG (nmol h^{-1} g^{-1})	15.2	9.5
LAP (nmol h^{-1} g^{-1})	75.3	57.8

2.2. Experimental Plan. A completely randomized block design was selected for the experiment with four repeats. Each treatment (variety) contained four replicates, with a 4 m^2 area used for each plot and a 144 m^2 whole area used in the Sichuan experimental site, and a 3 m^2 area cultivated for each plot with a 108 m^2 overall area used in the Hunan experimental site. Four nonleguminous species, including forage radish (*Raphanus raphanistrum* subsp. *sativus*), oil radish (*R. sativus* var. *Longipinnatus*), February orchid (*Orychophragmus violaceus* L), and rapeseed (*Barissa napus*) were planted in both study areas, while for the control treatment, a ryegrass (*Lolium perenne* L.)

species was planted. Different species were evaluated without any fertilizer treatment. Nonlegumes species were sown on October 10, 2022, in Hunan and on November 7, 2022, in the Sichuan province.

2.3. Soil and Plant Sampling and Determination. After harvesting of different species, soil samples were taken from a depth of 0−20 cm from each plot and separated into 3 main parts. One portion was quickly kept at −80 °C and subsequently carried to the laboratory for analysis of enzyme activity. Another part was kept at −4 °C for the determination of inorganic N (NH_4^+ and NO_3^-) in the soil and soil moisture content. In order to determine the soil pH range and available P and K, the third portion of the air-dried samples was passed through a 2 mm sieve. For the determination of soil organic matter (SOM) contents and total soil N, a 0.25 mm sieve was used for further processing. However, 10 g of fresh soil was extracted with a 2 M solution of potassium chloride (KCl) (soil: solution 1:10 w/v) and shaken for 60 min to determine inorganic N in the soil using a continuous flow analyzer (Seal AA3, Norderstedt, Germany).[34] The Kjeldahl digestion method was used for the determination of soil total N (TN), as described in the literature.[35] The soil TN analysis involved three main steps. The processes of digesting, distillation, and titration. The process involved digesting 0.5 g of fine dry soil with concentrated H_2SO_4, in addition to an agent mixture to increase the boiling temperature. The solution was heated until it became clear. A steam-distillation setup with an excess amount of sodium hydroxide (NaOH) was used to raise the pH level. The distillate was accumulated in a solution of saturated H_3BO_3, and subsequently subjected to titration using dilute H_2SO_4. The end point of the titration was determined by observing a change in the color of the solution. The SOM content was determined by the Walkley-Black method, as described in the literature.[36] First, 1 g of dry soil was put into a 500 mL conical flask. Ten milliliters of potassium dichromate ($K_2Cr_2O_7$) was added using a pipette and 20 mL of H_2SO_4 was added with a dispenser. The container was shaken in order to mix and kept aside for 30 min. Then, the container was placed on a magnetic stirrer with 10−15 drops of diphenylamine indicator and a Teflon-coated magnetic stirring bar. Titration was done with 0.5 M ferrous ammonium sulfate until violet blue turns green. A pH meter was used (Mettler Toledo 320-S, Shanghai Bante Instrument Co., Ltd., Shanghai, China) for soil pH determination at 1:2.5 soil/water ratios. However, for the determination of the available soil P (Olsen P), 5 g of soil was extracted with 0.5 M $NaHCO_3$ and shaken for 30 min. For analyzing a visible blue light, spectrophotometry (UV−vis spectrophotometer, Model UV-2100, Shimadzu, Tokyo, Japan) was used.[37] The amount of available K in the soil was measured after extracting5 g of dry soil with 1 M ammonium acetate and shaking for 30 min, and a flame photometer was used for analysis by the previously described method.[38]

At the anthesis stage, all nonlegume species were harvested on April 12, 2023, in the Hunan province and April 01, 2023, in the Sichuan province; the plant shoot samples were weighed, dried at 65 °C for 48 h in an oven, and then crushed and stored in order to analyze nutrients. The N, P, and K concentrations in plant shoot samples were analyzed with a mixture of sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) dilution at the high temperatures fallowed by burning procedures, and for the determination of nutrients, an Auto Analyzer (Seal AA3, Norderstedt, Germany) system was used. The N content was examined using the Kjeldahl digestion method described by Nelson,[34] and the molybdovanadate method was used for P determination,[39] while the flame photometry was used for K analysis in plants.[38] The PUtE is measured by the rate at which a plant absorbs a nutrient and the quantity of nutrients available. The PUtE is calculated as plant dry matter per unit of the nutrient taken up by plants. The PUE was estimated as the sum of nutrients available for the plant utilization. The following calculations were used for PUE:[17,40] Nutrient uptake (g/m^2) =

$$[\text{plant uptake contents (\%) in dry matter} \times \text{dry biomass yield (g/m}^2\text{)}]/100 \tag{1}$$

$$\text{P uptake efficiency (PUpE) \%} = (\text{Nu})/(\text{Ps}) \times 100 \tag{2}$$

where Nu is the amount of P accumulated in the shoot dry matter of a plant at maturity, and Ps is the available P amount present in soil.

$$\text{P utilization efficiency (PUtE) \%} = \text{Nu/shoot dry biomass} \times 100 \tag{3}$$

where Nu nutrients accumulate in the dry matter of a plant at maturity and shoot dry biomass weight at maturity.

$$\text{P use efficiency (PUE) \%} = (\text{PUpE}) \times (\text{PUtE}) \tag{4}$$

2.4. Enzyme Activity Determination. According to an earlier described 96-well microplate method by Deforest,[41] the activities of enzymes, i.e., BG, NAG, Phase, and LAP, were determined. For analysis, 1 g of fresh 24 °C overnight incubated soil was put in a 100 mL strainer tube and treated for one min after adding 50 mol of sodium acetate buffer per liter. After that, a 500 mL beaker was filled with the sample suspension. The centrifuge tube was washed with 50 mL of acetate buffer as well, and the mixture was then placed in the same beaker. The suspension solution was mixed with a magnetic stirrer. To maintain the capacity and standardization, 10 M references of buffer solution and 200 M substrates (Table 2) were dispersed in a black 96-well microplate (Scientific Fluoroskan Ascent FL, Thermo) with emission 450 nm and excitation 365 nm filters. The enzyme calculated units are shown in (nmol h^{-1} g^{-1}).

Table 2. Activities of Four Enzymes That Were Found in Different Nonlegumes (Green Manures), along with Their Commission Numbers (EC) and Common Substrates (L-DOPA = L-3,4-Dihydroxyphenylalanine and 4-MUB = 4-Methylumbelliferyl)

enzymes	substrates	EC
BG	4-MUB-β-D-glucoside	3.2.1.21
NAG	4-MUB-*N*-acetyl-*b*-*d*-glucosaminide	3.2.1.30
Phase	4-MUB-phosphatase3	3.1.3.1
LAP	L-leucine-7-amino-4-methylcoumarin	3.4.11

2.5. Statistical Analyses. One-way ANOVA IBM SPSS Statistics version 20.0 (Corp., Armonk, NY, USA) was used to determine the significant variance between different nonlegume species. Duncan's tests at a significant level of $P < 0.05$ were used to evaluate the changes between treatments. Based on Pearson's correlation coefficients, the relationship between P and K uptake and the soil characteristics determined is

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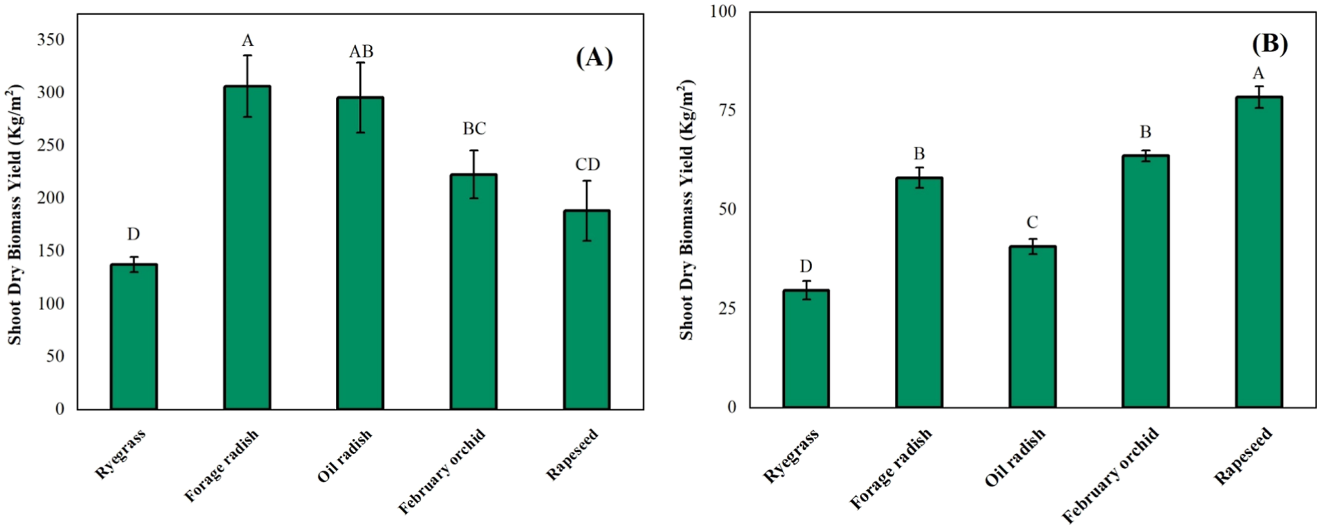

Figure 1. Shoot dry biomass production of different nonlegume species (A) in Hunan and (B) Sichuan regions. The capital letters indicate significant influences at $p < 0.05$, replicates $n = 4$, based on Duncan's tests. Nutrient uptake abilities of different species.

Table 3. Shoot N, P, and K Uptake (g/m^2) of Different Species in Both Experimental Sites[a]

sites	Hunan region			Sichuan region		
species	N uptake (g/m^2)	P uptake (g/m^2)	K uptake (g/m^2)	N uptake (g/m^2)	P uptake (g/m^2)	K uptake (g/m^2)
ryegrass	1.188 ± 0.075B	0.418 ± 0.037C	4.153 ± 0.141C	0.525 ± 0.053C	0.047 ± 0.006D	0.465 ± 0.050C
forage radish	5.005 ± 0.493A	1.468 ± 0.166A	9.260 ± 0.899A	1.981 ± 0.085A	0.268 ± 0.019A	1.509 ± 0.157A
oil radish	4.723 ± 0.498A	1.118 ± 0.131AB	7.365 ± 0.949AB	0.706 ± 0.005C	0.095 ± 0.013C	0.865 ± 0.027B
February orchid	4.745 ± 0.742A	0.733 ± 0.180BA	7.213 ± 0.782AB	1.536 ± 0.158B	0.202 ± 0.015B	1.562 ± 0.167A
rapeseed	2.458 ± 0.491B	0.840 ± 0.155B	6.290 ± 0.989BC	1.368 ± 0.127B	0.220 ± 0.007B	1.293 ± 0.961A

[a] Average ± standard error, replicates $n = 4$, capital letters show significant difference at $P < 0.05$, based on Duncan's tests.

Table 4. PUtE, PUpE, and PUE% of Different Nonlegume Species, Replicates $n = 4$, Capital Letters Show Significant Difference at $P < 0.05$, Based on Duncan's Tests

sites	Hunan region			Sichuan region		
species	PUtE (%)	PUpE (%)	PUE (%)	PUtE (%)	PUpE (%)	PUE (%)
ryegrass	0.303D	3.724D	1.130D	0.314C	0.524C	0.172C
forage radish	0.480A	13.62A	6.041A	0.633A	1.987B	1.257A
oil radish	0.378BC	9.970AB	3.766B	0.265D	0.583C	0.155C
February orchid	0.329CD	6.530BC	2.144BC	0.490BC	1.685 B	0.825B
rapeseed	0.446AB	7.499B	3.342B	0.519B	2.203A	1.144A

indicated by the * and ** to specify the significant values. Origin Pro 9.0 (Northampton, Massachusetts, USA) was used to create the enzyme analysis plots. The CONOCO (Canoco for Windows 4.5, Microcomputer Powering, Willis, TX, USA) was used to identify relationships among different treatments, soil characteristics, and soil enzymes by principle component analysis (PCA) at a significance value of $p < 0.05$.

3. RESULTS

3.1. Shoot dry Biomass Production. Nonlegume species indicated various changes in shoot dry biomass yield in both Hunan and Sichuan sites, as presented in Figure 1. However, in the Hunan region, different species produce a higher biomass yield compared to that in the Sichuan province. Forage radish had the highest shoot dry biomass production (123%), higher than that of ryegrass. Rapeseed had the lowest dry biomass yield (37%), when compared with the control in Hunan. In contrast, the highest dry biomass yield was observed in rapeseed by 165% and the minimum dry biomass production was seen in forage radish by 37% compared to that of the ryegrass at the Sichuan site.

3.2. Nutrient Uptake Capacities. Significant variations ($p < 0.05$) were found in the nutrient uptake capacities of nonlegume species, as shown in Table 3. The highest N uptake abilities were shown in forage radish, i.e., +322, and 277% in the Hunan and Sichuan sites, respectively, compared to the control. The minimum N uptake increased in rapeseed by 107% compared to that in the control in Hunan. While oil radish species showed the lowest shoot N uptake by 35% compared to the control at the Sichuan site. However, forage radish produces the maximum P uptake amount in both experimental sites, i.e., 101 and 469% in Hunan and Sichuan, respectively, compared to the control. Minimum P absorption abilities were noted in February orchid by 76% in Hunan and oil radish by 102% in the Sichuan site compared to the control. Compared to the control, the greatest K uptake was noted in forage radish, i.e., 123%, and February orchid, 243%, in the Hunan and Sichuan sites.

Table 5. Soil Available P and K Determination after Plantation of Nonlegume Species[a]

sites	Hunan region		Sichuan region	
parameters	P (mg/kg)	K (mg/kg)	P (mg/kg)	K (mg/kg)
ryegrass	13.85 ± 0.296B	110.30 ± 0.718C	14.43 ± 0.206B	125.17 ± 1.970B
forage radish	15.12 ± 0.980B	120.90 ± 7.042BC	12.40 ± 0.389B	115.97 ± 0.576C
oil radish	15.70 ± 1.167A	124.11 ± 2.620BC	25.23 ± 2.356A	118.14 ± 1.795C
February orchid	23.87 ± 1.478A	147.20 ± 2.763A	21.35 ± 2.588A	132.00 ± 2.145A
rapeseed	22.67 ± 0.312A	133.2 ± 8.304AB	14.60 ± 0.122B	112.97 ± 2.070C

[a] Average ± standard error, replicates $n = 4$, capital letters show significant difference at $P < 0.05$, based on Duncan's tests.

Table 6. Effects of Various Species on Soil NH_4^+ and NO_3^- Content[a]

sites	Hunan region		Sichuan region	
parameters	NH_4^+ (mg/kg)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	NO_3^- (mg/kg)
ryegrass	0.72 ± 0.52C	0.61 ± 0.12B	2.47 ± 0.29B	0.69 ± 0.17C
forage radish	1.51 ± 0.30AB	1.79 ± 0.30A	4.17 ± 0.43A	2.25 ± 0.33B
oil radish	3.46 ± 0.79A	2.68 ± 1.41A	2.89 ± 0.42B	2.47 ± 0.59B
February orchid	2.07 ± 0.31B	1.90 ± 0.99A	4.23 ± 0.26A	6.33 ± 0.32A
rapeseed	2.30 ± 0.82B	2.02 ± 0.89A	2.46 ± 0.18B	1.86 ± 0.51AB

[a] Average ± standard error, replicates $n = 4$, capital letters show significant difference at $P < 0.05$, based on Duncan's tests.

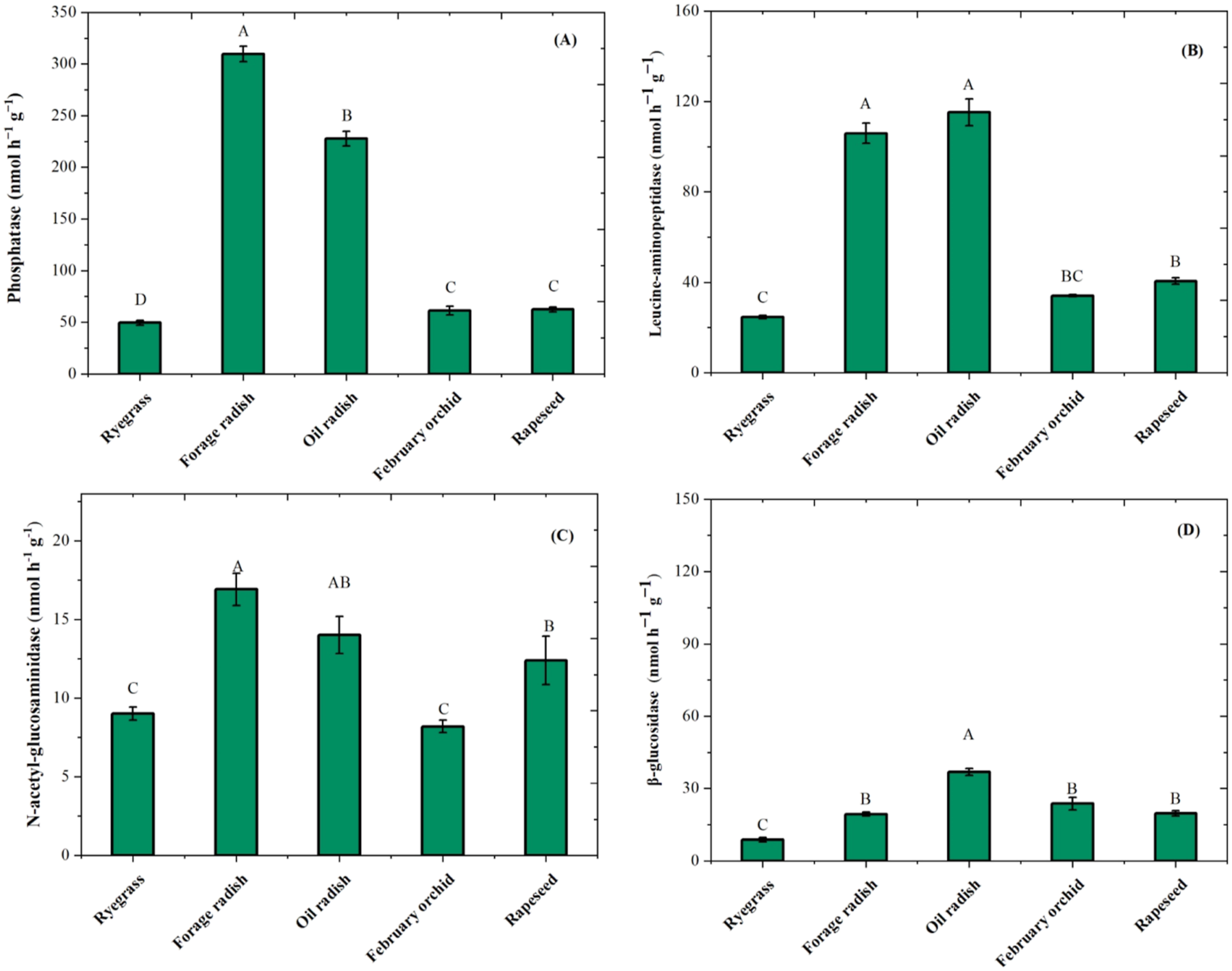

Figure 2. Influence of nonleguminous species on the enzymatic activities of soil in the Hunan site. (A) Phase, (B) LAP, (C) NAG, and (D) BG enzyme activities. The capital letters over the bars indicate significant influences at $p < 0.05$, replicates $n = 4$, based on Duncan's tests.

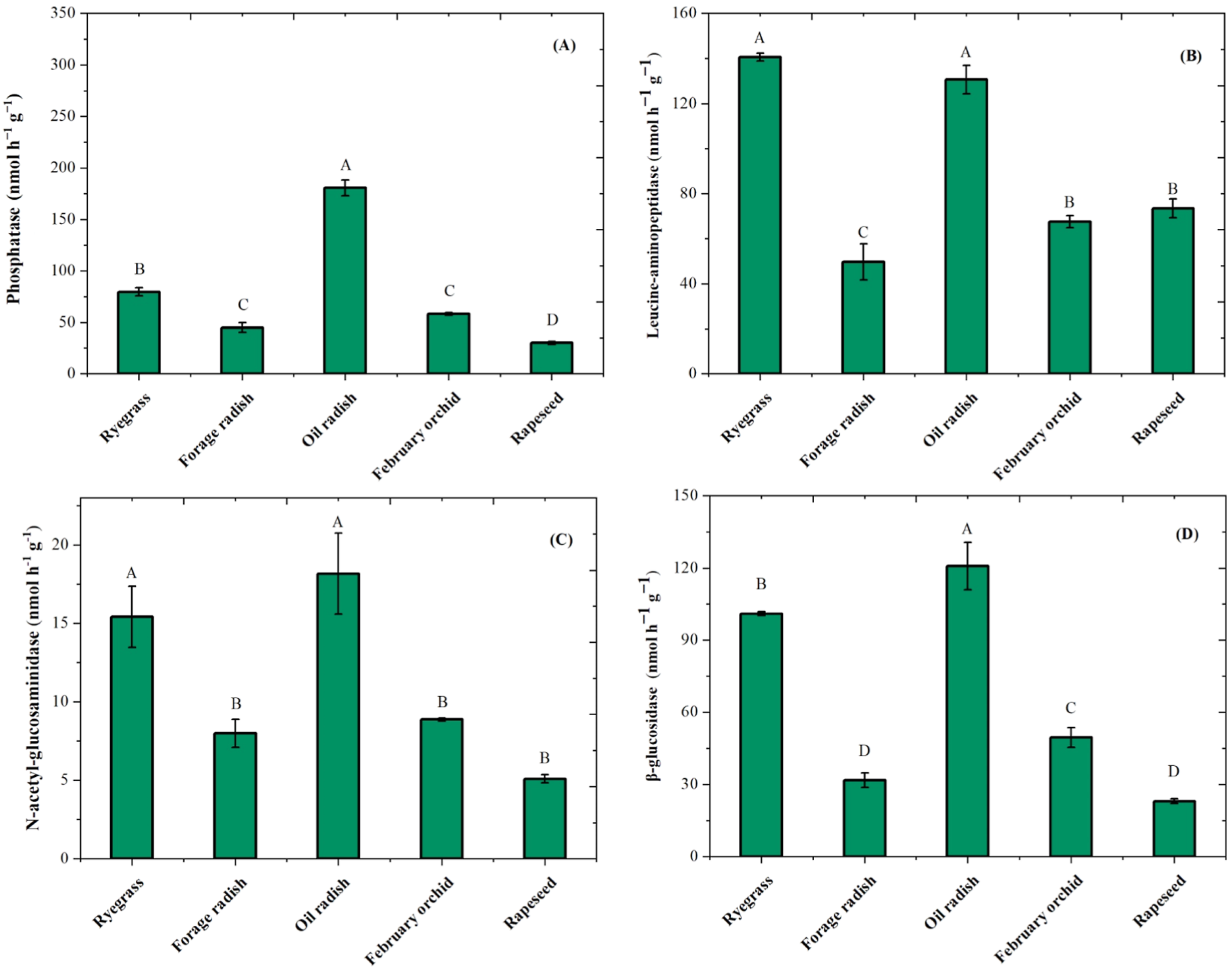

Figure 3. Influence of nonlegume cultivars on soil enzymatic activities in the Sichuan province. (A) Phase, (B) LAP, (C) NAG, and (D) BG enzyme activities. The capital letters over the bars show significant influences at $p < 0.05$, replicates $n = 4$, based on Duncan's tests.

3.3. P use Efficiencies. PUtE, PUpE, and PUE vary among all species in both study sites Table 4. In the Hunan region, the maximum percentages of PUtE, PUpE, and PUE were noted in forage radish, i.e., 56, +252 and +443%. The minimum PUE was observed in February orchid by 85% which is greater than ryegrass in the Hunan region. The forage radish also showed higher PUtE, PUpE, and PUE by 101, 279, and 630% compared to the ryegrass in the Sichuan site. While oil radish and February orchid decreased the PUE compared to the control in the Sichuan site.

3.4. Soil Available P and K Contents. After harvesting various nonleguminous species, the available P and K contents in the soil showed significant changes ($P < 0.05$) in both study regions (Table 5). However, among all species, February orchid performed well for soil available P and K contents. In Hunan, the greatest soil P was found in February orchid, which was 72%, while the lowest soil P was observed in oil radish, by 13% compared to the control. In the Sichuan site, oil radish increased the maximum soil P content by 75% compared with the control. Forage radish decreased soil P by 13% compared to the control. Nonleguminous species effects on soil K content: February orchid increased the highest K content of soil in both study sites; Hunan was 33% and Sichuan was 5%, respectively, higher than the control. The lowest soil K content was shown in forage radish by 9% compared with the control at the Hunan site. In the case of Sichuan, both radish and rapeseed had reduced soil K uptake compared to that of the control.

3.5. Soil Mineral NN. Modification were noted in soil NH_4^+ and NO_3^- N among all experimental species in both study area (Table 6). The maximum NH_4^+ N was increased by oil radish was +383% greater than ryegrass in Hunan site. The minimum NH_4^+ value was recorded in forage radish by 111% compared with the control. While NO_3^- N was increased in the following directions +347, 236, 216 and 197% by oil radish, rapeseed, February orchid, and forage radish, respectively, compared to the ryegrass in Hunan. In the case of the Sichuan region, February orchid showed the highest NH_4^+ N, i.e., 77% compared to the control. Rapeseed decreased the NH_4^+ N as compared to the ryegrass. The highest NO_3^- N was observed in February orchid by +538% and the lowest NO_3^- N was noted in rapeseed compared to the ryegrass in the Sichuan site.

3.6. Influences of Different Species on Enzymatic Activity in Hunan. The enzyme activities showed a significant difference after harvesting the nonlegume species in Hunan (Figure 2). Oil radish promoted the highest BG and

Table 7. Pearson Correlation Analysis Showing Relationship between Shoot N, P, and K Uptake of Nonlegume Species and Soil Properties in the Hunan Province[a]

legumes	N uptake	P uptake	K uptake	SOM	TN	NH_4^+	NO_3^-	AP	AK	pH
N uptake	1	0.672**	0.743**	0.337	0.805**	0.362	0.479*	0.355	0.465*	−0.318
P uptake		1	0.836**	0.136	0.712**	0.284	0.322	−0.135	0.066	−0.251
K uptake			1	0.201	0.779**	0.212	0.378	−0.095	0.247	−0.180
SOM				0.086	0.041	0.598*	−0.389	−0.263	0.410	−0.004
TN				1	1	0.119	0.444*	0.444	0.268	0.446*
NH_4^+						1	0.657**	0.657	0.162	−0.076
NO_3^-							1	0.494	0.162	0.032
available P								1	0.106	−0.159
available K									1	−0.074
soil pH										1

[a]Note: * showed a significant change at $P < 0.05$, and ** showed a significant change at $P < 0.01$. NH_4^+, NO_3^-, SOM, AP (available P), AK (available K), TN, and pH (soil pH).

Table 8. Pearson Correlation Analysis Showing Relationship between Shoot N, P, and K Uptake of Nonlegume Species and Soil Properties in the Sichuan Province[a]

parameters	N uptake	P uptake	K uptake	SOM	TN	NH_4^+	NO_3^-	AP	AK	pH
N uptake	1	0.853**	0.874**	0.021	0.201	0.092	−0.191	0.116	0.486*	0.183
P uptake		1	0.927	0.008	0.164	0.033	−0.005	0.093	−0.499*	0.185
K uptake			1	0.044	0.056	0.044	−0.097	0.076	−0.544*	0.148
SOM				1	−0.235	0.484	0.164	0.457*	0.402	−0.420
TN					1	−0.370	−0.131	−0.292	−0.305	−0.393
NH_4^+						1	0.557*	0.496*	0.503	−0.452*
NO_3^-							1	0.324	0.490	−0.127
available P								1	0.205	0.487*
available K									1	
soil pH										1

[a]Note: * showed a significant change at $P < 0.05$, and ** showed a significant change at $P < 0.01$. NH_4^+, NO_3^-, SOM, AP, AK , TN, and pH (soil pH).

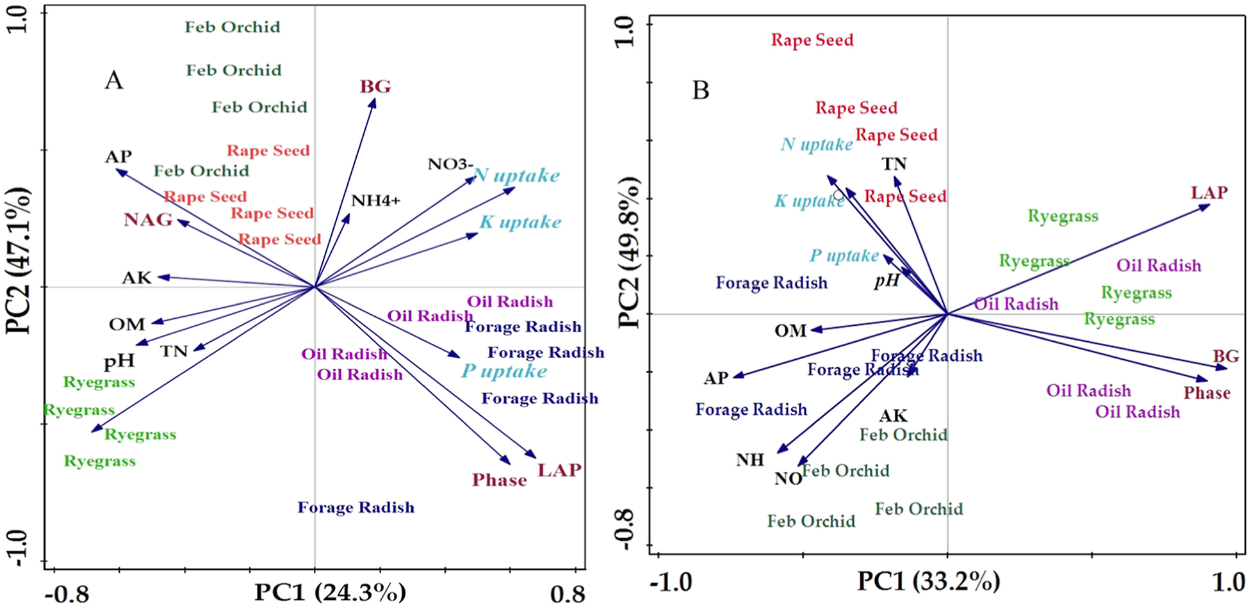

Figure 4. PCA indicated the interaction between various nonlegume species, soil enzymes, and soil properties in two different study areas (A): Hunan and (B) Sichuan. The position variables showed relationships to each other. The crowd of species has a strong relationship with soil properties and enzymes. Note: Soil properties: AP, AK, NH_4^+ , NO_3^-, TN, SOM, and pH (soil pH) are correlated with soil enzymes BG, NAG, LAP, and Phase.

LAP enzyme activities by 324 and 367%, respectively, compared to ryegrass. The lowest BG enzyme activity was shown in forage radish by 125%, and the minimum LAP enzyme activity was noted in February orchid compared to the control. The forage radish increased Phase and NAG enzyme activities, i.e., 526 and 88%, respectively, compared to the control. February orchid produced the lowest Phase activity at 55%; in contrast, February orchid had a decrease, i.e., 2%, in NAG enzyme activity compared to the control.

3.7. Impact of Various Species on Soil Enzymatic Activity in Sichuan. Soil enzymatic activities demonstrate the significant ($P < 0.05$) changes after harvesting the non-leguminous species (Figure 3). Oil radish produced the highest Phase (126%), BG (19%), and NAG (17%) enzyme activity in comparison to the control. In contrast, oil radish reduces LAP enzyme activities by 7% compared to the control. However, other nonlegume species reduces the enzymatic activities. While rapeseed highly decreased the activity of enzymes as follows, i.e., 77, 66, and 62% for BG, NAG, and Phase, respectively, compared to the control. Forage radish reduced the LAP enzyme activity to 52% compared to the control.

3.8. Correlation between Plant Nutrient Uptake and Soil Properties. The Pearson correlation (r) analysis shows that nonleguminous N, P, and K uptake is greatly correlated with soil properties, as shown in Table 7. Shoot N uptake indicated a significant positive relationship with soil TN, NO_3^-, and K, i.e., r = 0.805**, 0.479*, and −0.465*, respectively, in Hunan. While Shoot P and K uptake presented a significant relationship with only TN by r = 0.712** and 0.779**, respectively (Table 8).

In the Sichuan site, shoot N uptake showed a significant positive relationship with soil available K content (r = 0.486*). In contrast, shoot P and K uptake presented a substantial negative correlation with only soil K (r = −0.499* and −0.544*, respectively). Shoot nutrient uptake did not show any significant interaction with other soil properties.

3.9. Relationship between Different Varieties, Soil Properties, and Enzymes Activities. The main differences between nonlegume plants, soil properties, and enzyme activities at both study sites were shown by PCA (Figure 4A,B). For example, the PCA graph showed that both axes explained 24.3 and 47.1% of the differences in between nonlegumes species, enzyme activities, and soil characteristics in the Hunan region. Soil enzyme activities were associated with the right lower corner of the first axis. All species spread through the four corners of the graph. An important negative association between the activity of soil enzymes and SOM (F = 4.2, $p < 0.026$) and P content ($p < 0.04$, F = 3.6) has been found. There were weak relationships between soil enzymes and other soil characteristics. In the Sichuan site, the first and second axes covered 33.2 and 49.8% of the total difference, respectively. Following two species, namely, February orchid and forage radish, were seen on the left side of the primary axis of the PCA graph, which significantly relates to the soil OM, NH_4^+, NO_3^-, P, and K content. While oil radish was nearly seen with enzymatic activities.

4. DISCUSSION

The present research investigates the nonlegume species shoot dry biomass yield of nutrients such as N, P, and K uptake and PUE at two study sites Hunan and Sichuan. According to the current results, in Hunan regions, nonlegume species perform better compared to the Sichuan site. The mean annual temperature in the Sichuan region might be less favorable for some species because it is slightly cooler than in Hunan. The other reason might be due to soil pH values; the study found that the pH levels of the soil were slightly alkaline in Sichuan and slightly acidic in Hunan. Most nutrients are available in slightly acidic and slightly alkaline pH ranges.[42] The maximum dry matter yield of different species at the Hunan site is related to the maximum nutrient uptake of a plant. Forage radish significantly increased the dry matter yield with higher N, P, and K uptakes in the Hunan site. While rapeseed showed maximum biomass yield with greater K uptake, February orchid demonstrated higher N and P uptake in the Sichuan site. A previous greenhouse study showed that annual green manure crops grew faster and quickly reached the flowering stage than the perennial species.[43] The forage radish has higher biomass yields due to its higher nutrient accumulation capacity and NUEs because of its extremely specific root system and rapid growth.[44,45] Our results highlight that rapeseed has the highest biomass and P uptake in the Sichuan site. Previous study focused on developing dry biomass to use all indexes to calculate PUtEs.[46] Increased grain or biomass yield with and without differences in P concentrations in tissues can result in unplanned improvements in PUE.[18] However, biomass and grain yield are not enough to understand the method that evaluates the NUE or the ability of a plant to grow effectively in a low nutrient environment. Biomass reflects the response of the plant species to nutrient availability. Plants are divided into two groups: efficient and inefficient, based on their capacity to transfer nutrients into dry biomass.[47,48] These associations between root traits and nutrient uptake are also dependent on the groups and families of the species.[3,25] Plant's long root hairs frequently improve P acquisition when AM fungi are present in the roots. The root is considered to be the main factor in the growth of aboveground plants, and nutrients are obtained from the subsurface soil.[49] Overall, the nutrient uptake abilities of a plant to acquire nutrients from the soil mainly depend on its root traits because some taproot plants with a higher morphological index may be able to take up nutrients from the soil and store them in the roots. Later, the roots supply nutrients to other parts of the plant.[50]

We hypothesize that without fertilization treatment, green manure species throughout their growth period could potentially take up nutrients and have effects on soil nutrient status. However, significant modifications were recorded in soil nutrient availability among all species. Current results clearly highlight oil radish and February orchid increased soil nutrient availability in both experimental areas (Tables 5 and 6). Similarly, according to a previous study's findings, oil radish crops have longer roots and more mass roots in the topsoil than other species like rye and crimson clover, and the taproot architecture, root strength, and root growth proportion of oil radish increase the nutrient content in deep soil layers.[45,51] Furthermore, this investigation confirms our findings, which used four taproot species that were grown for a P release experiment, including radish crops. Radish crop root length, surface area of the root, and increased volume of roots improved the overall P content of the soil.[50] An earlier study found that February orchid incorporation could decrease mineral fertilizer consumption and increase the TN content in the soil, because nonlegume crops are mostly cultivated to prevent soil erosion loss, reduce N leaching, and improve mineral N.[52] Whereas another study demonstrated that forage radishes also have a positive influence on P status.[53] Some studies emphasize that some green manure, for example, alfalfa, improved available P in the soil.[54,55] It is considered that the high content of soil nutrients after cultivation of green manure is due to their unique root mechanism, in which plant root secrets organic acid compounds into the soil, which in turn can increase the effectiveness of the plant's uptake of soil nutrients.[56] A previous study showed that rye and mustard grew very fine roots in compacted soil compared to other green manure species (such as phacelia, oat, Buckwheat, rye, vetches, radish, and mustard) as rye and mustard have better access to nutrients and water than the other species.[57] Moreover, radish and mustard have the ability to enhance the P in soil due to their shorter and more specific root lengths and high root biomass values.[58]

An earlier research[59] also supported the current study, where green manure species improved soil K content. This might occur when the roots of a particular species of green manure interact with soil microorganisms that have the potential to exude acidic compounds into the rhizosphere of the soil, thereby increasing the availability of P and K.[60,61] It has been proposed that the size of the root system, the physiology of uptake, and plants' capacity to improve K solubility in the rhizosphere as important factors for absorption effectiveness. Significant changes were observed in various enzymatic activities under cultivation of nonlegume species (Figures 2 and 3). Oil radish enhanced enzyme activities at both experimental sites. Similarities were found in a field study, where higher Phase activities were stimulated by different vetch species and fava beans,[62] while red clover boosted more BG activity.[63,64] The present results are in agreement with earlier studies that radish cultivars had increased acid Phase.[65] It is considered that legume roots induced Phase activity to proliferate in the soil, greatly increasing the amount of P that plants could use.[66] Green manures include radishes belonging to the *Brassicaceae* family. These species are popular due to their many unique traits such as root exudation. In this process, roots release compounds such as oxalate, citrate, and malate, and sugar amino acids are the main source of carbon that promote the microbial activity and increase enzymatic activities. Oil radish has a high root density, and symbiotic relationships with mycorrhiza fungi could enhance the Phase activity.[67,68] These species boosted soil microorganism activities not only at the time of incorporation but also during the growing phase. Green manure crops showed a significant influence on the properties of soil and soil enzymes (Figure 4). The RDA analysis indicates a positive interaction among soil enzymes, SOM, and P content. Soil enzyme activity can serve as an indicator of associated SOM, where soil enzymes interact with microbial communities, which may break down SOM and lead to increased nutrient availability in the soil.[69−71] Phase activities play a significant role in P cycling, which supports plant growth and plays a role in soil P conversion.[72] Enzymes are active reservoirs of soil state N modification, and degrade N compounds. In other words, enzymes can significantly affect the availability of soil N.[73] However, various species of green manure play an important role to maintain soil fertility for the next crop.[74] Due to morpho-physiological root traits, these species interact with soil microbes in the soil and obtain high nutrient amounts, thus influencing the soil's fertility.[75] Plant shoot N uptake strategies for efficiency and acquisition will depend not only on external inorganic N concentrations but also the microorganism population, which is present around the root that can deliver *N*-compounds to the plants.

5. CONCLUSIONS

The present study highlights that nonleguminous green manure species can have effects on soil nutrient contents and potentially take up nutrients from the soil throughout their growth period. The study showed that forage radish has great potential to take up nutrients in both study areas, and oil radish increased enzymatic activities among all experimental species. While oil radish and February orchid increase soil available P and K contents. The current data provide information regarding various species such as forage radish, oil radish, and February orchid in green manuring fields that is essential to improving the nutrient management practices in a rice field in southwest China. These species are a better choice to improve the soil nutrient availability for the next crop. Further study is needed to explain the nutrient acquisition traits of roots and the response of soil microbial communities during the different growth stages of green manure crops.

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Notes

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