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Improved Accumulation Capabilities of Phosphorus and Potassium in Green Manures and Its Relationship with Soil Properties and Enzymatic Activities

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Abstract: Cultivation of green manure crops is important strategy for improving soil fertility in South China. Therefore, it is important to obtain plant varieties that can better accumulate nutrients during the green manuring phase. The present study evaluated the phosphorus (P) and potassium (K) uptake efficiencies of various winter leguminous species at two different sites. Varieties tested included six Chinese milk vetch cultivars (i.e., Minzi No.6, Ningbodaqiao, Wanzi No.1, Xiangzi No.1, Yijiangzi, and Yujiangdaye), as well as hairy vetch and common vetch, while ryegrass was planted as a control. All leguminous species showed higher ability for P and K absorption in the two sites compared to the ryegrass. Hairy vetch and common vetch performed better than all six Chinese milk vetch cultivars, and the highest biomass and P and K uptake capacities were observed in hairy vetch. Green manuring had different effects on soil enzymes. Phosphatase (87.0%) and leucine-aminopeptidase (163.8%) were increased by hairy vetch. β -glucosidase (143.4%) and N-acetyl-glucosaminidase (283.3%) were increased by Yijiangzi and Yujiangdaye, respectively, in Guangxi compared to the control. Xiangzi No.1 increased N-acetyl-glucosaminidase (352.6%), leucine-aminopeptidase (477.5%), phosphatase (591%), and β -glucosidase (786.0%) in Hunan compared to the ryegrass. Enzyme activities increased nutrient availability at both experimental sites. P and K uptake efficiencies significantly related with soil enzymes in Guangxi. It is suggested that hairy vetch has the largest capability for P and K uptake and might be a potential choice for improving P and K management in sustainable agriculture.

Keywords: leguminous species; soil enzymes; nutrient availability; P and K uptake

1. Introduction

Phosphorus (P) and potassium (K) are the most significant macronutrients in the soil ecosystem after nitrogen (N) [1,2], which involves various processes, i.e., energy formation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, enzymes activation and redox reactions [3,4]. Nutrient management is important for sustainable crop production. P- and K-efficient cultivars are important options in an agro-ecosystem [5]. Uptake efficiencies of nutrients such as P and K are related to the abilities of plants to produce high yields in low-nutrient conditions [6]. Deficiency in P is mainly attributed to its immobilization in soil [7]. Low K content is highly dependent on soil composition, especially on its fixation to clay particles, leading to deficiency in plant uptake [8]. Immobilized P can be re-mobilized, and non-exchangeable K can also become available in soil solution under biochemical processes [6]. However, they could be strongly dependent on soil chemistry [9] Exploitation of legumes is an essential approach for sustainable agriculture as it can increase nutrient cycling and promote



soil fertility [10] This approach can also potentially improve P and K availability in the soil, which is beneficial for plant uptake and soil microorganisms [11,12]. Root growth and root morphological mechanisms of leguminous plants are the principal factors that usually require greater P and K levels in the soil, which is also related to plant P and K uptake [13,14]. Several green manure crops such as white lupin (*Lupines albas* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L), and faba bean (*Vicia faba*), are able to mobilize soil P through a variety of root mechanisms [15,16].

Recent studies have shown that legumes can regulate soil enzymatic activities more than mineral fertilizers [17]. According to other researchers, legumes such as chickpeas and cowpeas increase phosphatase enzyme activities more than non-legumes [18,19]. Soil enzymes play important roles in catalyzing reactions and are associated, directly or indirectly, with nutrient availability and organic matter decomposition [20]. The specific activity of an enzyme is not considered useful in the complete nutrient cycle of soil because it has a particular substrate [21]. Some hydrolytic enzymes (e.g., β -glucosidase, N-acetyl-glucosaminidase, Leucine-aminopeptidase, and phosphatase), which are related to C, N, and P cycles, are more responsible for the soil properties (pH and soil organic matter) than other variables. Such enzymes could be valuable as early indicators of biological soil modification [22].

Leguminous plants, especially broad bean (*V. faba*), Chinese milk vetch (*Astragalus sinicus* L.), clover (*Trifolium repens* L.), and hairy vetch (*V. villosa* L.), have been used in crop rotation systems worldwide [23–25]. Although, P and K uptake efficiencies have been reported to be different in various legumes crops [26,27], and the relative knowledge and profound information about leguminous green manures in South China are still unclear. Moreover, less information exists in the literature about the changes in enzymatic activities in response to cultivation of different legume species in the field [28,29]. Green manuring has become a popular practice in South China to improve soil fertility and rice yields at present. The most popular winter green manure is milk vetch, which has traditionally been used for several thousands of years, and most attention has focused on N utilization.

The main objectives of this study were to (1) evaluate P and K uptake capabilities in various winter leguminous green manure species in South China and (2) investigate their ability to affect soil enzyme activity by interacting with soil properties.

2. Materials and Methods

2.1. Experimental Design

Plot experiments were conducted at two sites in southern China: Nanning City, (Guangxi province), and Qiyang County, (Hunan province), both study sites were selected in the same climatic zones (Sub-tropical) for the cultivation of different winter leguminous green manure species. The meteorological data of the two experimental sites are given in Table 1. Further, the basic soil properties of the two experimental sites are presented in Table 2.

| Particular | Guangxi Site | Hunan Site |
|-------------------------|--------------------------------|--------------------------------|
| Geographical coordinate | 20° 54′ N, 104° 29′ E | 30° 08′ N, 108° 47′ E |
| Altitude (m) | 99 | 100 |
| MAT (°C) | 27.7 | 20.2 |
| MAP (mm) | 1750 | 1700 |
| Climatic zone | Subtropical | Subtropical |
| Cropping system | Double paddy rice—green manure | Double paddy rice—green manure |
| | | |

Table 1. The principal meteorological data of the two experimental sites.

Note: MAT means annual temperature. MAP means annual precipitation (Year, 2017).

Six Chinese milk vetch (*Astragalus sinicus* L.) cultivars (i.e., Minzi No.6, Ningbodaqiao, Wanzi No.1, Xiangzi No.1, Yijiangzi, and Yujiangdaye), and hairy vetch (*Vicia villosa* L.) and common vetch (*Vicia Sativa* L.), were used for evaluation. A non-legume plant, ryegrass (*Lolium perenne* L.), was planted

as a control. Four replicates were arranged in a randomized complete block design with 1.44 m² per plot and 51.84 m² total area in Guangxi site, while 3 m² per plot with 108 m² total area was used in Hunan, respectively. No extra fertilizer was added during the experimental period. Chinese milk vetches, hairy vetch, common vetch, and ryegrass cultivars were seeded on 24 October 2017 in Guangxi, and on 20 October 2017 in Hunan, respectively.

| Basic Soil Properties | Guangxi Site | Hunan Site |
|--|--------------|------------|
| SOM (%) | 1.2 | 1.0 |
| $TN (g kg^{-1})$ | 0.84 | 0.73 |
| AP (mg kg ⁻¹) | 45.5 | 11.2 |
| AK (mg kg ⁻¹) | 134.9 | 122.0 |
| pH (1:2.5) | 7.5 | 6.3 |
| CEC (cmol kg ^{-1}) | 12.21 | 10.32 |
| Soil Texture | Silty clay | Clay loam |
| Phosphatase (nmol $h^{-1} g^{-1}$) | 73.2 | 128 |
| β -glucosidase (nmol h ⁻¹ g ⁻¹) | 35.0 | 38.2 |
| N-acetyl-glucosaminidase (nmol $h^{-1} g^{-1}$) | 12.1 | 15.2 |
| Leucine-aminopeptidase nmol $h^{-1} g^{-1}$) | 42.6 | 75.3 |

Table 2. The basic soil properties of the two experimental sites.

Note: SOM (organic matter), TN (total nitrogen), AP (available phosphorus), AK (available potassium), pH (soil pH) and CEC (cation exchange capacity).

2.2. Sampling

Soil and plants were sampled on 20 March 2018 in Guangxi and on 12 April 2018 in Hunan, respectively. The soil samples were collected at a depth of 0–20 cm from each plot and divided into three portions. One portion was immediately stored at –80 °C after being carried to the laboratory to analyze the extracellular enzyme activities. The second portion was stored at –4 °C for analysis of mineral nitrogen (NH₄⁺ and NO₃⁻) and soil moisture. The third portion was air-dried and passed through a 0.25 mm sieve for other analyses.

All green manures were harvested at the flowering stage of the milk vetches. The above-ground plant parts were weighted and later were dried in an oven at 65 °C for 72 hours, crushed, and stored for nutrient analyses.

2.3. Determination of Soil and Plant

The contents of NH_4^+ and NO_3^- in fresh soil sub-samples were extracted with 2 M KCl (soil: solution 1:10 *w:v*) and were shaken for 60 minutes to analyze the mineral nitrogen using a continuous flow analyzer (Seal AA3, Norderstedt, Germany) [30]. Soil moisture contents were determined by oven drying at 105 °C for 48 hours. Total N contents were analyzed by the Kjeldahl method [31], and soil organic matter by the Walkley Black method [32]. Soil pH was determined at a 1:2.5 soil/water ratio using a pH meter (Mettler Toledo 320-S, Shanghai Bante Instrument Co., Ltd., Shanghai, China). Soil available P (Olsen) was extracted with 0.5 M NaHCO₃ and analyzed by using visible light spectroscopy in blue light (UV–VIS spectrophotometer, Model UV-2100, Shimadzu, Kyoto, Japan), following the method in ref. [33]. The exchangeable potassium was analyzed by flame photometry using 5 g soil and 1 M ammonium acetate (NH4OAc) [34].

The plant's N, P, and K contents were determined by the Kjeldahl digestion method [35], the molybdovanadate method [36], and flame photometry [37], respectively.

2.4. Soil Enzymatic Activities Measurements

The activities of four enzymes (i.e., Phosphatase (Phos), β -glucosidase (BG), N-acetyl-glucosaminidase (NAG), and Leucine-aminopeptidase (LAP)) were measured using a microplate method, as previously described [38,39]. For determination, 1 g of fresh soil was added into a 100 mL centrifuge tube, and it

was treated with 50 mL of 50 mM sodium acetate buffer ($C_2H_3NaO_2$) using a polytron homogenizer, then the mixture was poured into a 500 mL beaker. Additionally, 50 mL of acetate buffer was used to wash the centrifuge tube and was transferred into the same 500 mL beaker. The mixture was stirred by using a magnetic stirrer to generate a uniform suspension. The buffer solution, 10 μ M references, sample suspension, and 200 μ M substrates (Table 3) were disseminated in a black 96-well microplate to keep the volume and order fixed, following the methods described in ref. [39]. The microplates were covered and incubated in the dark at 25 °C for 4 h, and fluorescence was quantified using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo) with 365 nm excitation and 450 nm emission filters [38]. The activities were expressed in units of nmol h⁻¹ g⁻¹.

Table 3. Hydrolytic enzymes evaluated in green manure, their enzyme commission number (EC), and corresponding substrate ($_L$ -DOPA = $_L$ -3, 4-dihydrox-yphenylalanine, 4-MUB = 4-methylumbelliferyl).

| Enzymes | Substrate | EC |
|--------------------------|------------------------------------|----------|
| Phosphatase | 4-MUB-phosphatase | 3.1.3.1 |
| β-Glucosidase | 4-MUB- β -D-glucosidee | 3.2.1.21 |
| N-acetyl-glucosaminidase | 4-MUB-N-acetyl-b-D-glucosaminide | 3.2.1.30 |
| Leucine-aminopeptidase | L-Leucine-7-amino-4-methylcoumarin | 3.4.11.1 |

2.5. Statistical Analyses

Analysis of variance (ANOVA) using IBM SPSS Statistics Version 20.0 (Corp, Armonk, NY, USA) was carried out to determine the significant difference between the various types of winter legume varieties and their effects on soil properties. Tukey's multiple range tests at (p < 0.05) were used to assess the treatment differences. A correlation matrix of the study was based on Pearson's correlation coefficients using * and ** to indicate the p < 0.05 and p < 0.01 probability levels, respectively. The enzyme analysis figures were made by using Origin Pro. 9.0 (Northampton, MA, USA). Principal component analysis (PCA) and redundancy analysis (RDA) were carried out by using CONOCO (version 4.5) at a significance level p < 0.05 to determine the overall relation between different varieties, soil properties, and enzymatic activities.

3. Results

3.1. Plant Biomass and P and K Uptake

Generally, "above-ground plant biomass" P and K uptakes in leguminous cultivars were higher than ryegrass in both experimental sites, and significant differences were observed for different legumes (Table 4). Moreover, hairy vetch produced the highest above-ground biomass in both sites (i.e., 192.3% and 155.0% higher in Guangxi and Hunan, respectively) compared to the control. The percentages of above-ground biomass increased in Guangxi in the following order: Minzi No.6 85.1%, common vetch 73.9%, and Wanzi No.1 59.5%, respectively, compared to the control. Chinese milk vetch cultivars performed variously, while minimum biomass productions of Hunan cultivars were reported in Ningbodaqiao and ryegrass treatments.

P uptake was also high in hairy vetch (i.e., 260.0% and 294.0% higher in Guangxi and Hunan, respectively) compared to the control. The lowest P uptakes were observed in Xiangzi No.1, only 72.0% higher than ryegrass in Guangxi, and in Minzi No.6, 9.9% higher than the control in Hunan. A similar trend was observed in K uptake, the highest K uptake was found in hairy vetch with increases of 261.8% and 131.0% in Guangxi and Hunan compared to ryegrass treatment. Among the leguminous cultivars, the lowest K uptakes were found in Ningbodaqiao and Wanzi No.1 at 70.9% higher than ryegrass in Guangxi. However, ryegrass treatment showed the lowest K uptake among green manure species in Hunan. Moreover, common vetch had a relatively high ability to uptake P and K.

| Species | | Guangxi Site | | | Hunan Site | |
|--------------|----------------------------------|-------------------------------|-------------------------------|----------------------------------|-------------------------------|-------------------------------|
| -r | Dry Biomass (g m ⁻²) | P Uptake (g m ^{−2}) | K Uptake (g m ⁻²) | Dry Biomass (g m ⁻²) | P Uptake (g m ^{−2}) | K Uptake (g m ⁻²) |
| Ryegrass | 497.5 ± 45.2 c | 1.55 ± 0.09 c | 12.5 ± 0.3 c | $150.0 \pm 10.0 \text{ c}$ | $0.46 \pm 0.04 \text{ c}$ | $4.56 \pm 0.41 \text{ b}$ |
| Minzi No. 6 | 921.04 ± 31.3 b | $3.39 \pm 0.18 \text{ b}$ | 29.9 ± 0.29 b | 194.7 ± 22.2 c | $0.50 \pm 0.07 \text{ c}$ | 7.65 ± 2.62 ab |
| Ningbodaqiao | 636.4 ± 51.4 bc | 2.84 ± 0.38 bc | 21.5 ± 2.23 bc | 152.0 ± 15.0 c | $0.53 \pm 0.06 \text{ c}$ | 7.96 ± 2.06 ab |
| Wanzi No. 1 | 793.5 ± 32.9 bc | 2.84 ± 0.12 bc | 29.6 ± 1.85 b | 206.0 ± 13.2 c | 0.71 ± 0.03 c | 5.99 ± 0.54 ab |
| Xiangzi No.1 | 636.2 ± 27.0 bc | 2.66 ± 0.19 bc | 22.8 ± 1.62 bc | 208.0 ± 13.1 c | $0.70 \pm 0.06 \text{ c}$ | 7.07 ± 0.78 ab |
| Yijiangzi | 652.5 ± 88.6 bc | 2.76 ± 0.22 bc | $24.3 \pm 2.99 \text{ bc}$ | 192 ± 4.62 c | $0.62 \pm 0.08 \text{ c}$ | 6.20 ± 0.33 ab |
| Yujiangdaye | 920.5 ± 94.6 b | $3.14 \pm 0.39 \text{ b}$ | 33.8 ± 3.66 ab | 172 ± 8.33 c | $0.72 \pm 0.05 \text{ c}$ | $5.14 \pm 0.40 \text{ ab}$ |
| Hairy vetch | 1454.2 ± 165.8 a | 5.56 ± 0.60 a | 45.5 ± 5.22 a | 382.5 ± 27.8 a | 1.79 ± 0.13 a | 10.5 ± 0.65 a |
| Common vetch | 865.1 ± 37.2 b | $3.05 \pm 0.31 \text{ b}$ | 30.8 ± 1.70 b | 295.0 ± 15.5 b | $1.16 \pm 0.17 \text{ b}$ | 9.97 ± 0.62 ab |

Table 4. Plant above-ground biomass (g m⁻²) and P and K uptake (g m⁻²) in different varieties of leguminous green manures (average ± standard error).

Ryegrass planted as a control, six Chinese milk vetch varieties, and two other legume species (hairy vetch and common vetch) are reported. The small letter indicates significant differences at (p < 0.05) using Tukey's multiple range tests.

3.2. Effects of Winter Legume Species on Soil P and K

Changes were recorded in available P and K in soil among the studied leguminous varieties in both experimental sites (Table 5). Soil P contents in different treatments were higher than ryegrass and increased in the following order: Wanzi No.1 by 41.2%, Yijiangzi by 29.3%, and common vetch by 25.5%, respectively, higher than ryegrass. The lowest soil P was observed in Xiangzi No.1 among the tested leguminous varieties. Soil K increased in Yujiangdaye, common vetch, and Wanzi No.1 by 59.2%, 58.6%, and 46.7%, respectively, higher than the control. In Hunan, soil P and K contents were significantly increased in Ningbodaqiao by 30.6% and Yujiangdaye by 50.7% compared to the ryegrass, while P and K content decreased in Minzi No.1 by 53.5% and hairy vetch by 5.8% in the Hunan site.

| Species | Guang | gxi Site | Hunan Site | | |
|---------------|----------------------------|--------------------------|----------------------------|----------------------------|--|
| - r | P (mg kg ⁻¹) | K (mg kg ⁻¹) | P (mg kg ⁻¹) | K (mg kg ⁻¹) | |
| Ryegrass | 48.3 ± 1.79 c | 103.4 ± 3.02 d | $13.6 \pm 0.50 \text{ bc}$ | 110.3 ± 0.72 b | |
| Minzi No. 6 | 50.5 ± 3.09 c | 124.6 ± 3.09 cd | 8.84 ± 1.47 d | $114.1 \pm 4.20 \text{ b}$ | |
| Ningbodaqiao | 55.0 ± 1.91 bc | 113.0 ± 1.91 de | 17.7 ± 0.96 a | 110.4 ± 7.89 b | |
| Wanzi No. 1 | 68.3 ± 1.13 a | 151.7 ± 1.13 ab | 13.7 ± 0.43 abc | 127.9 ± 7.93 b | |
| Xiangzi No. 1 | 50.3 ± 2.13 c | 130.7 ± 2.13 bcd | $10.4 \pm 1.31 \text{ cd}$ | 127.5 ± 13.1 b | |
| Yijiangzi | 62.5 ± 2.13 ab | 138.5 ± 2.81 abc | $14.6 \pm 0.70 \text{ ab}$ | 121.9 ± 5.07 b | |
| Yujiangdaye | $56.1 \pm 0.34 \text{bc}$ | 164.7 ± 0.34 a | $14.6 \pm 0.58 \text{ b}$ | 166.2 ± 7.97 a | |
| Hairy vetch | $55.7 \pm 0.82 \text{bc}$ | 136.7 ± 0.82 abc | 13.7 ± 0.61 b | $103.8 \pm 1.00 \text{ b}$ | |
| Common vetch | $60.7 \pm 3.07 \text{bc}$ | 164.0 ± 3.07 a | $15.0 \pm 0.08 \text{ ab}$ | 117.2 ± 5.74 b | |

Table 5. Influence of green manure on available phosphorus and potassium in soil (average ± standard error).

Ryegrass (control), six Chinese milk vetch varieties, and two other legume species (hairy vetch and common vetch) are reported, and different small letters indicate significant differences at (p < 0.05) using Tukey's multiple range tests.

3.3. Soil Enzymatic Activities

"Various species of green manure" showed significant effects (p < 0.05) on phosphatase (Phos), β -glucosidase (BG), N-acetylglucosaminidase (NAG), and leucine-aminopeptidase (LAP) activities in the Guangxi site (Figure 1). They highly depended on different species at both experimental sites. The highest phosphatase and LAP activities were found in hairy vetch (i.e. 87.0% and 163.8% higher than the ryegrass treatment, respectively). The lowest phosphatase activity was in Xiangzi No.1 treatment, only 23.3% higher than the ryegrass, while the lowest LAP activity was reported in the control treatment (Figure 1A,D). The highest β -glucosidase activity was demonstrated in Yijiangzi (143.4% higher), while the lowest activity was in Minzi No.6 (12.2%) compared to the control. The NAG activity was high in Yujiangdaye (283.3% higher), whereas the lowest NAG activity was in Yijiangzi at only 4.3% compared to the control.

More obvious modifications were recorded for the enzymatic activities in the Hunan site (Figure 2). Xiangzi No.1 stimulated the activities of BG (786.0% higher), phosphatase (591.0% higher), LAP (477.5% higher), and NAG (352.6% higher), respectively, compared to the control. Conversely, Ningbodaqiao had smaller effects on the activities of BG (106.3% higher) and NAG (32.6% higher), respectively, when compared to the control. Minor changes in phosphatase activity were also seen in Ningbodaqiao (only 5.3% higher compared to the control). Common vetch showed a 9.6% higher LAP activity compared to the control.





Figure 1. Effects of green manure species on enzyme activities: phosphatase (**A**), β -glucosidase (**B**), N-acetylglucosaminidase (**C**), and leucine-aminopeptidase (**D**) in Guangxi, China. Different letters show significant influences (p < 0.05) using Tukey's multiple range tests.



Figure 2. Effects of green manure species on enzyme activities: phosphatase (**A**), β -glucosidase (**B**), N-acetylglucosaminidase (**C**), and leucine-aminopeptidase (**D**) in the Hunan site. Different letters show significant influences (p < 0.05) using Tukey's multiple range tests.

Based on Pearson's correlation (*r*) analysis, P and K uptake greatly correlated with phosphatase, N-acetylglucosaminidase, and leucine-aminopeptidase activities (r = 0.357 *, 0.333 * and 0.631 **, respectively) and was not significantly related with β -glucosidase enzyme activity in Guangxi (Table 6). Non-significant relationships were observed in Hunan among P and K uptake and soil enzymatic activities.

3.5. Correlation among Soil pH and Soil P, K

However, Pearson's correlation (r) analysis showed non-significant relationship among soil pH and available P in both experimental sites (Table 7), whereas a negative, significant correlation was noted between soil pH (r = -0.369 *) and K contents in the Hunan site.

3.6. Correlation between Soil Enzymes and Soil Properties

The redundancy analysis (RDA) showed that the first and second axes explained 23.1% and 13.5% of the variation for enzyme activities and soil properties in Guangxi (Figure 3A), while both canonical axes described 84.7% and 77.9% of the total modification between enzymatic activities and soil properties. The enzyme activities were significantly correlated with SOM (F = 5.8, p < 0.002), TN (F = 5.7, p < 0.002), NO₃⁻ (F = 8.3, p < 0.002), and AK (F = 4.1, p < 0.006). The right lower corner of the first axis was related to the N-cycling enzymes. The NAG activity was strongly correlated with exchangeable K and negatively correlated with SOM. The upper right corner showed that the C-cycling enzyme BG was associated with soil TN, NO₃⁻, and AP.



Figure 3. Redundancy analysis (RDA) indicates the correlation between soil enzyme activities and soil properties, (**A**) Guangxi, (**B**) Hunan site. Note: Phos (phosphatase), BG (β -glucosidase), NAG (N-acetylglucosaminidase) and LAP (leucine-aminopeptidase) are significantly correlated with soil properties: SOM (organic matter), TN (total nitrogen), NH₄⁺ and NO₃⁻ (mineral nitrogen), AP (available phosphorus) and AK (available potassium).

According to the RDA analysis, soil enzymes showed significant correlations with three soil properties in Hunan site (Figure 3B), and the first (RD1) and second axes (RD2) explained 53.7% and 4.01% of the total variation. Soil enzymes showed a significant relationship with available P (F = 10.9, p < 0.002), TN (F = 16.2, p < 0.002), and available K (F = 5.6, p < 0.024), respectively, whereas, available soil P showed a significant, negative relationship with phosphatase enzymes.

| Parameters | | | Guangxi Site | | | | | Hunan Site | | |
|--------------|----------|---------|--------------|----------|----------|----------|-------|------------|----------|----------|
| i uluineteis | K uptake | Phos | BG | NAG | LAP | K uptake | Phos | BG | NAG | LAP |
| P uptake | 0.909 ** | 0.357 * | 0.184 | 0.333 * | 0.631 ** | 0.605 ** | 0.225 | -0.081 | -0.214 | -0.316 |
| K uptake | 1 | 0.321 | 0.245 | 0.479 ** | 0.641 ** | 1 | 0.183 | -0.002 | -0.156 | -0.204 |
| Phos | | 1 | 0.044 | 0.069 | 0.344 ** | | 1 | 0.754 ** | 0.839 * | 0.832 ** |
| BG | | | 1 | 0.088 | -0.078 | | | 1 | 0.785 ** | 0.802 ** |
| NAG | | | | 1 | 0.133 | | | | 1 | 0.891 ** |
| LAP | | | | | 1 | | | | | 1 |

Table 6. Pearson's correlation (*r*) between P, K uptake, and soil enzymatic activities.

Note: Phos (phosphatase), BG (β-glocosidase), NAG (N-acetylglucosaminidase), and LAP (leucine-aminopeptidase). *, ** significant at *p* < 0.05, *p* < 0.01, respectively.

| Table 7. Pearson's correlation coefficient (r) b | between soil pH and | d available phos | phorus and | potassium. |
|---|---------------------|------------------|------------|------------|
|---|---------------------|------------------|------------|------------|

| Parameters | | Guangxi Site | | | Hunan Site | |
|---------------------------------------|----|---------------------------|---------------------------|----|---------------------------|---------------------|
| - | pH | AP (mg kg ⁻¹) | AK (mg kg ⁻¹) | pН | AP (mg kg ⁻¹) | AK (mg kg $^{-1}$) |
| pН | 1 | 0.02 | -0.323 | 1 | -0.072 | -0.369 * |
| $AP (mg kg^{-1})$ | | 1 | 0.359 * | | 1 | 0.054 |
| AK mg kg ^{-1}) | | | 1 | | | 1 |

AP (available phosphorus), AK (available potassium),* significant at p < 0.05.

3.7. Correlation between Legumes and Soil Properties

The Principal Component Analysis (PCA) showed a clear difference between various legumes and soil properties (Figure 4). The contents of TN, NO_3^- , soil available P and available K were the most different among the different legumes, followed by hairy vetch, Yijiangzi, Wanzi, and Ningbodaqiao. Maximum SOM was related to Wanzi No.1 and Xiangzi No.1 in Guangxi. In Hunan, the first axis contributed to 87.3% of the variation in different varieties and soil properties (Figure 4B). The highest available P, TN, and AK in soil were shown in Ningbodaqiao, Wanzi No.1, and Yujiangdaye treatments.



Figure 4. Principal component analysis (PCA) showed relationship between legumes and soil properties: **(A)** Guangxi, **(B)** Hunan. Note: Phos (phosphatase), BG (β -glucosidase), NAG (N-acetylglucosaminidase) and LAP (leucine-aminopeptidase) are significantly correlated with soil properties, SOM (organic matter), TN (total nitrogen), NH₄⁺ and NO₃⁻ (mineral nitrogen), AP (available phosphorus) and AK (available potassium).

4. Discussion

4.1. Plant. P and K Uptake and Soil Nutrient Availability

The present study compared the P and K uptake efficiencies in different legume species. The dry biomass accumulation and the capability to obtain P and K from the soil varied significantly among species. Maximum P and K uptake abilities were observed in hairy vetch at both sites (Table 4). Our results agreed with previous work, where the morphological traits and a greater biomass relates to a high nutrient uptake in hairy vetch [40]. Above-ground biomass and P and K uptake by mass flow is important, which occurs in the roots and helps in the transportation of P and K to the lateral parts [41,42].

According to the current hypothesis, changes can be observed in available P and K in soil after cultivation of different legume species. The results revealed that the P content significantly increased in Guangxi (Table 5), while both varieties (Ningbodaqiao and common vetch) greatly improved available soil P in Hunan. This study highlighted that legumes like *M. sativa, T. pratense,* and *Lupinus* were also found to have positive influences on P status [43,44]. Legumes can mobilize P, relative to their growth period, because P-solubilizing compounds are secreted in the rhizosphere [45]. Leguminous crops during their growth period showed a key impact on soil K availability in both sites (Table 5). Similarities were found in another study on green manure crops, where K availability was increased, which could be associated with the extensive root systems of leguminous crops [46]. The extensive root system of legumes is beneficial to improve the physical condition of the soil because they have the ability to release organic acids from their roots, which may increase K availability in soil [47].

4.2. Effect of Legume Species on Enzymatic Activities

Legume plants can stimulate microbial proliferation, which could be the cause of the increase in soil enzyme activity. The data show significant changes in the activities of enzymes under different kinds of winter legume varieties. These crops can enhance the catalysts to support the microbial population and promote their growth [48]. The present study showed that phosphatase activities were enhanced in both experimental sites (Figures 1 and 2). Similar results were found, where higher phosphatase levels were observed in broad bean (V. faba) and vetches (Vicieae) in a separate field study [49]. However, phosphatase activity at the Hunan site was higher than that at the Guangxi site. A higher phosphatase activity in Hunan could be due to the soil pH range. A previous study reported that greater phosphate activities were found in acidic to neutral soil [50]. Each species in this study had a unique specialization in morphological traits. However, higher phosphate activity may depend on different factors, for example, high density of root hairs of legumes facilitated the phosphatase activity due to root hairs assisting in the release of phosphate [51]. In addition to the release of organic acids, cluster roots are also efficient at releasing phosphatase [52]. Legume roots release phosphatase to intensify the solubilization and remobilization of phosphate, thus influencing the ability of the plant to survive in phosphorus-stressed conditions [53]. Yijiangzi increased the activity of the BG enzyme in Guangxi (Figure 1), and Xiangzi No.1 promoted it in Hunan. These findings are also supported by other studies, showing that T. pratense L and B. napus leguminous species produced more β -glucosidase activity [54,55]. Green manure add substrate to the soil microorganisms not only at the phase of their incorporation, but also throughout their growth period as well, e.g. through root exudation, root turnover and symbiosis with mycorrhiza [56]. This could be associated with the behaviour of the carbon cycle that catalyzes the hydrolysis of sucrose into glucose and fructose, which activates the microbial population and increases β -glucosidase activity [57,58]. The results also report that there were significant alterations in NAG and LAP activities. The rate of increasing N in the soil through leguminous plants not only involves the N-cycle but also increases enzyme activities [59]. The activity of soil enzymes is the main factor in N transformation in soil, and changes in the activities of N-degrading enzymes will affect the availability of N in soil [60].

4.3. Relationship among P and K Uptake, Soil Properties, and Enzymatic Activities

According to Pearson's correlation coefficient, P and K uptake was strongly related to hydrolytic enzymes in Guangxi (Table 6). Phosphatase, β -glucosidase, N-acetylglucosaminidase, and Leucine-aminopeptidase activities are responsible for the degradation of phosphoric acid into phosphate, carbohydrates into starch, glycogen chitin into cellulose, and proteins into amino acids [61]. Studies concluded that P and K uptake was associated with legume species and phosphatase enzymes [62]. This could be the reason that phosphatase activity is associated with mineralization of P in soils and plays an important role in P cycling that improves plant growth [63]. Our study reported that phosphatase activity was significantly positively related to SOM, and the NAG enzyme was negatively correlated with SOM in Guangxi. The soil's enzymatic activities can act as indicators to identify soil organic matter. Whereas in the some cases, enzymatic activities that interact with the microbial community can be used as indicators of potential SOM decomposition, which leads to an increase in potential nutrient availability [64]. The current study did not find any significant interaction among soil pH with soil P in both experimental sites (Table 7). In this study, soil pH ranges were noted to be slightly acidic in (Hunan) and slightly alkaline in (Guangxi). Most of the nutrients are available in slightly acidic to slightly alkaline soil (pH 6.5 to 7.5) [65]. It could be that the P solubilization mechanism requires a different amount of time, while the pH changes immediately in soil, which may not affect the P solubility [66]. While soil pH was significantly, negatively related with soil K content in the Hunan site (Table 7), this negative correlation might be due to the acidic soil of the Hunan site. Similarities were noted in another study, which also showed a significantly negative interaction between soil pH and K content [67]. Due to K being a basic cation, the K content changed to available

K with the increase in soil pH from slightly acidic to strongly acidic in the chemical reaction. Mostly, cations were leached in acidic conditions [65,68].

The present study measured soil enzyme activities, which provided integrated effects for the changes in soil properties under different legume treatments. The redundancy analysis (RDA) indicated a significant, positive correlation among soil enzymatic activities and soil properties in both experimental sites (Figure 3). However, C- and N-cycling enzymes, BG and NAG, and LAP activities were correlated with NH₄⁺ and NO₃⁻. Some researchers addressed how C-cycle enzymes contribute to alterations in the substrate availability of essential nutrients, and the activities of enzymes are closely related to the changes in chemical properties [69,70]. The N-related enzymes might be the sources to break down the organic N compounds through their action, which decreases N limitations [71]. The principle component analysis (PCA) showed that Legumes were related to the soil properties in the current study, whereas another study showed green manure played a vital role in improving the soil quality [14]. The soil properties were affected by leguminous species because of their shoot and root characteristics, which were related with the high nutrient concentration under low-fertility conditions [72].

The present study indicated that the phosphate activity had no obvious association with available P in soil in Guangxi (Figure 3). Usually, the correlation between phosphatase activity and available phosphorus in the soil environment is complicated. When no relationship is found, there could be other factors that influence the activity of enzymes [73]. In contrast, the P-cycling phosphate enzyme was significantly, negatively related to available P in soil in Hunan (Figure 3). This negative correlation between available P and phosphatase enzyme activity was also documented in a previous study [73]. A higher phosphatase activity could be associated with the basic transformation of P, mainly originating from the hydrolysis of esters and anhydrides of phosphoric acid (H₃PO₄). The microbial biomass of soil would be the most important factor for measuring enzymatic activities in an eco-system, which might be associated with more enhanced enzyme metabolic activities [63,74].

5. Conclusions

Our results emphasize that hairy vetch has the highest P and K uptake capacities, and this was related to the highest biomass and most prominent root morphological mechanisms in both experimental sites. This study revealed that winter green manure that contains hairy vetch might be a potential choice to improve management of P and K in paddy soil in South China, which is conducive to ongoing crop cultivation and agricultural practices. Planting of green manure can also reduce the fertilizer needed for upcoming crop production. Further research needs to clarify the contribution of green manure crops in the soil microbial community structure, and studies into soil enzyme activities to find time scale changes during the cultivation period at different zones and stages, are required to explain the mechanisms behind their correlations.

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