BETTER MANAGEMENT OF PHOSPHORUS FERTILIZER IN INTENSIVE CROPPING SYSTEMS: AN APPROACH BASING ON INTEGRATED AGRONOMIC, ECOLOGICAL AND ENVIRONMENTAL COMPROMISES

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Editorial: Better management of phosphorus fertilizer in intensive cropping systems: An approach based on integrated agronomic, ecological and environmental compromises

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Editorial on the Research Topic
Better management of phosphorus fertilizer in intensive cropping systems: An approach based on integrated agronomic, ecological and environmental compromises

For the last 10,000 years, humans have been growing crops for consumption and trade, but only in the last 200 years have we used industrialized fertilizer production to sustain this. In just two centuries, we have all but used up many of our readily-available mineral resources. We are now reliant on high-yield, yet resource-inefficient, production systems which are wasteful, prone to economic volatility, and environmentally damaging. We need to improve the way in which crop nutrition is managed, by finding ways by which we can use inorganic fertilizer more effectively.

Phosphorus (P), is an essential element for plants, but is easily fixed by mineral surfaces in soils and therefore has a low P use efficiency. Less than 25% of applied P is absorbed by crops in the growing season after application in most soils globally. This lack of efficiency has led to large amounts of P accumulation in soils, known as "legacy P" (Gatiboni et al., 2020). Sattari et al. (2012) suggest that this build-up of legacy P is ca. 550 kg P ha^{-1} between 1965 and 2007 worldwide. This inefficiency enhances the depletion and profligate use of global rock P reserves. This has the potential to deplete the easily extractable and economically viable sources of rock P within the next 100 years if the current consumption rate is not reduced (Cordell et al., 2009). At the same time, some arable lands still need to build up soil P fertility through P fertilizer application, such as in China, where over 50% of arable lands are suffering P deficiency stress (Li et al., 2015) and even more soils in sub-Saharan Africa are in this position. Moreover, recent economic shocks in the fertilizer market due to increasing energy prices and conflict in Ukraine have led to P fertilizer prices reaching peaks not seen since 2008.

Developing better management strategies for the utilization of fertilizer P and soil legacy P are needed and should integrate innovation in agronomy, crop genotype selection, optimization of rhizosphere management for biological mining of sparingly soluble phosphates, improved P uptake efficiency, and implementation of P recycling in soil-cropping systems. In this Research Topic, we have collected together 10 contributions highlighting interactions between soil-plant-microorganisms which determine P use of crops, rhizosphere processes facilitating P uptake of crops, the modification of soil P availability through the addition of organic materials, and the demands of P fertilizer application in different cropping systems.

Soil microorganisms have a critical involvement in the soil P cycle through P mobilization and assimilation, and can also facilitate or reduce the P uptake of plants (Zhang et al., 2018). The paper by Liu et al. demonstrates that combining microbial fertilizer with a reduced conventional fertilizer application increases soil P availability and acid phosphatase activity in soil compared to standard practice, in trials conducted in Jilin province in China. Moreover, the addition of microbial fertilizer increased the relative abundance of beneficial *versus* pathogenic microbes and created a favorable microbial community for maize. In the Inner Mongolia Autonomous Region of China, Qu et al. demonstrate that soil microbial activity was greater with intercropping of legumes and brassica when compared to monocropping. They go on to show that *Proteobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, and *Rokubacteria*, dominant bacterial phyla in soil, are increased in intercropping systems. This modification of soil microorganisms is accompanied by increases in soil organic matter and P availability.

Different plant species show variable adaption strategies to improve P uptake, including morphological and physiological responses. By testing 235 genotypes of Peanut (*Arachis hypogaea* L.) in hydroponic experiments, Zhu et al. find that P deficiency not only improves the P absorption efficiency of the roots but also induces root growth, which means the roots contribute more to the P uptake capacity. Furthermore, Wang et al. show that the over-application of P fertilizer causes O_2-deficient stress of amaranth (*A. mangostanus*). They demonstrate that when rhizosphere O_2 concentration was 250.6 μmol L^{-1}, it stimulated root growth in a pot experiment. Oxygenation also increased Olsen-P in the rhizosphere by promoting organic P mineralization and was related to improved yield and amaranth quality.

Organic material addition can improve soil P bioavailability by desorbing P fixed by soil minerals and P released from organic material. Wang et al. modify the Langmuir equation to describe P adsorption properties in organic material-incubated soils. They find organic material addition decreased the amount of P adsorption by soil. This suggests that organic material addition could be used to improve P availability, but should be controlled in high P soil to avoid increases in the risk of P loss to the environment. Importantly, the addition of organic material to soil was shown to be an efficient approach to building up soil P fertility and promoting the P uptake of maize, as shown by Khan et al. based on a pot experiment.

Sub-optimal P fertilizer application still occurs in some areas of China. The dynamic P pool simulator model was used to predict P pools from 1985 to 2100 in pomelo orchards of Pinghe County in China. Yan et al. find that the labile P pool would increase by more than twofold if the current P application rate was maintained, resulting in serious P resources waste and risk of P loss to the environment. Scenario analyses showed the P application rate can be reduced from 413.63 kg ha^{-1} to 31 kg P ha^{-1} without any yield loss. Shi et al. assess the yield gap of spring maize in the Jilin province of China and found that farmers only achieved 52% of the model yield potential. Suboptimal soil Olsen P levels are one of the major contributors to this yield gap. Therefore, efficient soil P management is still needed in the region. In contrast, in the Yangtze River delta of China, Xiao et al. recommend an optimal P application rate for the rice-wheat rotation system of 72–75 kg P_2O_5 ha^{-1}. Wei et al. assess the P uptake requirements of rice (*Oryza sativa* L.) grown in saline-sodic soils of Northeast China and show that the P requirement ranged from 4.21 kg P Mg^{-1} to 4.61 kg P Mg^{-1} in this region.

Overall, this Research Topic of papers demonstrates that there are a number of ways in which the P use efficiency, and therefore the sustainability, of cropping systems can be improved. Taken together, the papers demonstrate that there are a number of interventions that can be considered including the optimization of fertilizer applications, optimized plant-microbe interactions, consideration of rhizosphere processes, and the judicious use of organic material additions. It is clear that an integrated approach is needed to help secure the sustainability of the use of P resources in the future.

In the end, we would like to thank all the reviewers for their valuable comments and suggestions, which helped to improve the quality of the papers. We hope this Research Topic will stimulate further research into better management of P fertilizer in intensive cropping systems, which needs a multidisciplinary approach to generate novel management strategies.

Author contributions

HL, GF, and TG contributed equally to defining the scope for this Research Topic. They edited the manuscripts submitted to this Research Topic.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Spatio-Temporal Variation of Soil Phosphorus and Its Implications for Future Pomelo Orcharding System Management: A Model Prediction From Southeast China From 1985–2100

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Phosphorus (P) is a non-renewable source, requires in large amount for maintaining better crop growth and development. The excessive P fertilizer contributes to the accumulation of P in the soil and results in increased soil total P and Olsen P. However, the spatio-temporal variation of soil P remains unclear in pomelo orchard systems. Therefore, this study aimed to assess the temporal and spatial variation of soil P in pomelo orchards and future to predict P pool from 1985 to 2100, based on the dynamic P pool simulator (DPPS) model. We found that an average of 282.23 kg P ha^{-1} yr^{-1} accumulated in pomelo orchard soil, resulting in increased concentration of Olsen P (i.e., 5–212 mg kg^{-1}) and total P (i.e., 80–1883 mg kg^{-1}) in the topsoil. It showed that Olsen P and total P pools increased in topsoil about 42 and 25 folds, respectively from 1985 to 2015. Soil P accumulation occurred not only in topsoil but also found in deeper soil horizon of pomelo orchard. Compared with the natural forest, the concentration of Olsen P and fractions (Al-P and Fe-P) in 20-year-old pomelo orchard increased significantly in soil depth of 0–120 cm, while Sol-P increased significantly in 0–60 cm soil depth. Scenario analyses from 1985 to 2100 indicated that the P application rate at 31 kg P ha^{-1} could maintain pomelo yield at its optimum level. These findings could provide the synthesized novel insight for understanding the soil P status and its sustainable management in the pomelo orchard systems.

Keywords: pomelo orchard, P budget, temporal and spatial variation, soil P management, DPPS

INTRODUCTION

Phosphorus (P) is one of the essential nutritional elements for plant growth and development (Smith et al., 2011). It plays an important role in maintaining soils fertility and sustainable agricultural production. However, it could be a future constraint for the agricultural system owing to the depletion of P resources (Van Vuuren et al., 2010; Reijnders, 2014; Yan et al., 2020). The modern agricultural system is mainly dependent on P derived from phosphate rock, a non-renewable resource, and current P global reserves could be depleted in the next 50–100 years (Cordell et al., 2009). Therefore, future sustainable agriculture development is needed to exploit P legacy reserves in soil for improving P use efficiency in various cropping systems.

The excess use of P fertilizer results in a large amount of P accumulation (Sharma et al., 2017). Zhang et al. (2017) investigated the global croplands and found the P pool increased rapidly from 1900 to 2010 in soils of Europe (+31%), South America (+2%), North America (+15%), Asia (+17%), and Oceania (+17%). In China, the consumption of chemical P fertilizers has been increased from 1.23 million metric tonnes (Mt) (1980) to 5.54 Mt (2007), and only 15–20% of the P applied was taken up by plants in the growing season (Zhang et al., 2008). An average of 8.96 kg P ha^{-1} yr^{-1}accumulated in soil for field crop production during 1980–2007, whereas soil Olsen P increased from 7.4 to 24.7 mg kg^{-1} (Li et al., 2011). Besides, Chen et al. (2017) found that the P budget per cropland area ranged from 35 to 66 kg ha^{-1} yr^{-1} in the Yong'an watershed, eastern China, and total P in surface soil (0–20 cm) significantly increased by 107–640 mg kg^{-1} from 1984 to 2009. To date, it is well established that the level of P pools in field cropland soils increases with the increase of cropping history, but the status of P pools in orchards of acidic soil regions, i.e., characterized by high nutrient input, is far from being understood (Zhang et al., 2011).

So far, P over-fertilization builds up P reserves in soils that may remain accessible to plants (Roberts and Johnston, 2015). Dynamic P pool simulator (DPPS) is a simple two P pool model used to simulate the P transformations in soil and buildup of the residual soil P pools (Sattari et al., 2012). As a widely used model, DPPS has reported that the soil has a high P supply potential from residual soil P pool due to the cumulative input of P grossly exceeding the cumulative P uptake by crops in Europe (Sattari et al., 2012). It is also suggested that 20% of P fertilizer could be saved until 2050 by exploiting the residual P in China (Sattari et al., 2014). Residual soil P pool could also supply for decades of cultivation in Brazilian agriculture, which also can buffer the impact of a sharp increase in the price of P fertilizer (Withers et al., 2018). Nevertheless, the prediction of P pools by the DPPS model should be implemented in the orchard systems (Lu et al., 2012), so that dynamic changes of P pool could be predicted and sensible P management strategies could be adopted for agriculture sustainability.

Likewise, the deep soil P reserves may not be neglected for P sustainable management in orchards system. The previous report has found that high P reserves availability in deep soil layers results in a higher P leaching rate (Khan et al., 2019). The variation of soil P pools along the soil horizons is preferentially influenced by agronomic management practices, e.g., P fertilizer application rate (Damian et al., 2020). However, the majority of studies on soil P pools have mainly focused exclusively on the topsoil layer (0–20 cm) rather than deep soils (García-Oliva et al., 2018; Jiménez et al., 2019). Furthermore, unveiling the availability of P reservoirs for plants requires detailed chemical speciation of abundant soil P components by sequential extraction schemes (Maranguit et al., 2017). Two broad categories of soil P pools are inorganic P (Pi) and organic P (Org–P). Inorganic P (Pi) is considered a major source of available P in soils (Waqas et al., 2019). Most of the Pi is accumulated in association with aluminum and iron compounds in the acidic soils, whereas in calcareous soils, calcium phosphate is the main form (Pizzeghello et al., 2011; Turner and Blackwell, 2013). Long-term excessive P application significantly impacted the soil P fractions in pomelo orchards (Chen et al., 2022). However, most of the previous studies have focused on the soil P pools in topsoil, and only limited studies have explicitly focused on the P pools and their components in whole soil profiles under long-term cultivation.

Citrus is the leading fruit crop globally. Over 140 countries have been producing citrus fruits, and China is one of the leading producers, with an annual citrus production of 5,121 $\times 10^4$ tons in 2020 (NBS, 2021). Pomelo (*Citrus grandis*) is the third major type of citrus after *Citrus reticulata* and *Citrus sinensis*. The pomelo production in Pinghe County have increased rapidly over the past three decades, and it is the most famous and largest pomelo production county estimated the output accounts for more than 80% of the domestic production (Wei et al., 2020). Although pomelo industry has become important source of finance, but it also faced a huge challenge in P fertilizer management, e.g. most farmers are still applying much more P fertilizer than recommended in the past decades (Li et al., 2019; Chen et al., 2020). Therefore, this study was designed to answer the following research questions: 1) what is the difference of the P pools in surface soil (0–20 cm) during 1985, 2005, 2010, and 2015 (temporal variation of P pools) in Pinghe County? 2) What is the impact of the long-term P fertilizer on P pools in the 20-year-old pomelo orchard compared to the natural forest (spatial variation of P pools) in soil profiles from 0 to 200 cm? 3) How could the P application influence the P reservoirs? The key objectives of this study were to 1) estimate the temporal and spatial variation of P pools in pomelo production in Pinghe County 2) predict the P dynamics during 2016–2100. We hypothesize that P fertilizer input in pomelo production may increase the P accumulation from 1985 to 2015 and could affect the P distribution in the whole soil profile.

MATERIALS AND METHODS

Study Area

The study area is in Pinghe County (24°02′–24°35′ N, 116°54′–117°31′ E, and 10–1,545 m above sea level), Zhangzhou City, Fujian Province, Southeast China (**Figure 1**). It is characterized by a subtropical oceanic monsoon climate with an annual average temperature of 17.5–21.3°C, annual precipitation 1,600–2,000 mm, and the soil type is ferrallisols, which is classified as the red soil in the Chinese soil classification (Smith, 2014). Low-elevation mountains and hills constitute the main types of landforms in Pinghe County. These landforms are mostly distributed in the valleys and intermountain regions of the Huashanxi basin, accounting for 91.5% of the total area.

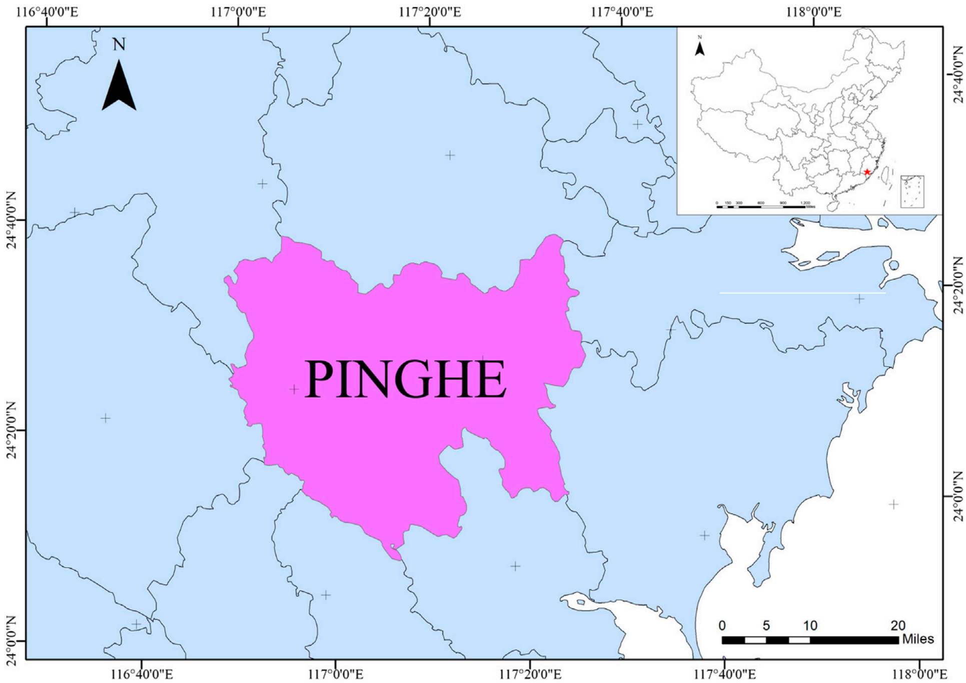

FIGURE 1 | Geographical distribution of the Pinghe County in China.

Soil Analysis and Data Collection

Data on P fertilizer input and pomelo yield from 1985 to 2015 were collected by interviewing the local farmer and Pinghe Statistical Yearbook 1985–2015. The concentration of P in the pomelo orchard system was collected from the published literature (Xu, 2019). The value of P uptake was calculated by multiplying the pomelo yield by its P concentration.

Soil samples were collected randomly from surface soil (0–20 cm) in 4 years (i.e., 1985, 2005, 2010, and 2015). Each soil sample was a composite of four sub-samples taken from a single orchard. Finally, 3,040 composite samples (1985, 12; 2005, 2,386; 2010, 297; and 2015, 345) were collected for this study. Besides, in early November 2019, soil samples from natural forest and 20-year-old pomelo orchard were also collected. Three sampling sites were selected for each treatment. Each soil sample was a composite of five sub-samples, and soil samples were collected from 10 soil layers (i.e., 0–200 cm, each at 20 cm intervals). All soil sampling was performed away from the trunk at the drip line of the plant, using a soil core sampler (3 cm diameter) after removing plant biomass and litter. To avoid the fertilization and edge effects, soil samples were collected from the center of each orchard after harvesting.

All soil samples were air-dried, visible root segments and organic debris were removed by shaking and with forcep. Air-dried soil samples were sieved through a 2 mm sieve to determine soil available phosphorus (Olsen P), representative sub-samples were passed through a 0.25 mm sieve to determine soil organic matter (SOM), total phosphorus, and phosphorus fraction (P fraction). P fraction was determined only in soils collected from natural forest and 20-year-old pomelo orchard. Briefly, Olsen P was extracted using 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 (Li Y. et al., 2015), total P was extracted using aqua regia (HCl/HNO_3 mix) (Aainaa et al., 2018). Both these P forms were analyzed using the molybdate blue procedure (Murphy and Riley, 1962). SOM was determined using K_2CrO_7 oxidation with the heated oil bath method (Li Y. et al., 2015). P fraction was measured according to the method described by (Yan et al., 2020). Specifically, the soil Pi was divided into easily soluble P (Sol-P; extracted with ammonium chloride at 1 mol L^{-1} NH4Cl), aluminum P (Al–P; extracted with ammonium fluoride at 0.5 mol L^{-1} NH_4F, pH = 8.2), iron P (Fe–P; extracted with sodium hydroxide at 0.1 mol L^{-1} NaOH), occluded P (O-P; extracted with sodium citrate and dithionite at 0.3 mol L^{-1} $Na_3C_6H_5O_7.2H_2O$ and 1 g $Na_2S_2O_4$) and calcium P (Ca-P; extracted with sulfuric acid at 0.25 mol L^{-1} H_2SO_4), and Org-P was estimated by pyrolyzing in a 550°C muffle furnace under ignition (Chen et al., 2022).

Model Descriptions and Scenarios Analysis

The dynamic P pool simulator (DPPS) model, including two dynamic P pools, was used to simulate the long-term historical annual P uptake by plants for a time series of P inputs and to estimate the future P inputs for a specific future target P uptake (Wolf et al., 1987; Sattari et al., 2012). In general, the major inputs of P for this model mainly included P fertilizer, weathering, and atmospheric deposition, and output sources mainly included P uptake and P loss. The P budget (the differences between inputs and uptake) is allocated to the labile (80%) and stable (20%) pools. These two P pools are mutually transformed every year. A detailed description and application guide of this model can be found in related references (Wolf et al., 1987; Sattari et al., 2012).

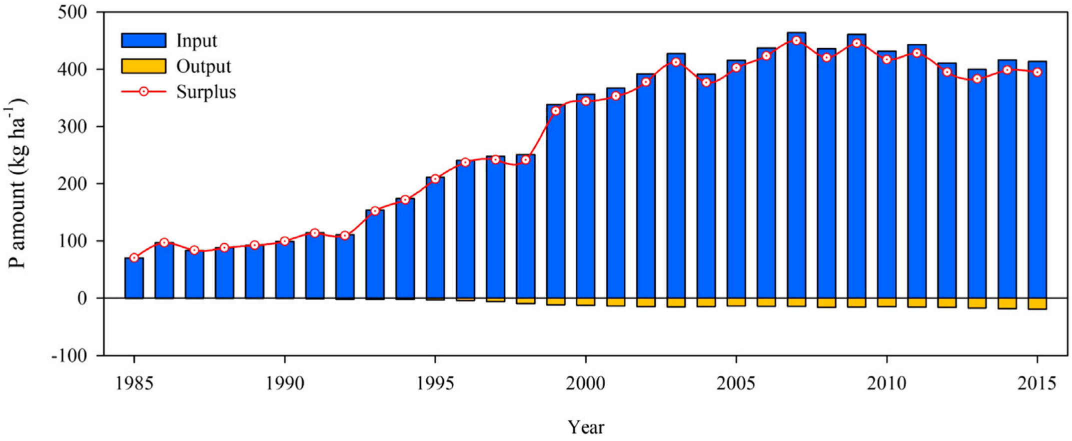

FIGURE 2 | Historical P input, output, and surplus in pomelo production for the period 1985–2015.

Three different scenarios for the 2100 years were built in this study to simulate P legacy changes in the DPPS model; 1) P application rate would be constant at 413.63 kg ha^{-1} [S1], 2) P application rate would maintain pomelo yield at 2015 level (28.14 t ha^{-1}) [S2], 3) P application rate would be constant at 0 kg ha^{-1} [S3].

Formula and Statistical Analysis

We calculated the P pool (Olsen P pool and total P pool) based on **Eq. 1**

$$\text{P pool }(\text{kg ha}^{-1}) = \frac{BD \times PC \times 20 \times 10^4}{10^5} \quad (1)$$

Where BD (g cm^{-3}) is the soil bulk density, which was calculated with Adams equation (Adams, 1973) (**Eq. 2**). SOM (g kg^{-1}) is the soil organic matter. PC (mg kg^{-1}) represents the concentration of P (Olsen P, total P) in soil. 20 cm was the surface soil depth. 10^4 square meters are equal to 1 ha.

$$BD\ (\text{g cm}^{-3}) = \frac{100}{\frac{SOM}{0.244} + \frac{(100-SOM)}{1.64}} \quad (2)$$

Data processing was performed using Microsoft Office Excel 2016, and all statistical analyses were conducted using SPSS 21.0. One-way analysis of variance (ANOVA) and Least Significant Difference (LSD) tests were used to check the differences in P concentrations (Olsen P, total P) among each measurement year and soil layers.

RESULT

Status of Excessive P Fertilization in the Pomelo Orchard

The average amount of annual P input in Pinghe pomelo production was 70.34 kg ha^{-1} in 1985, peaked at 463.86 kg ha^{-1} in 2007, and then slightly changed around 413.63–460.76 kg ha^{-1} from 2008 to 2015. The amount of P output was much lower than those of P application. Average annual P output increased from

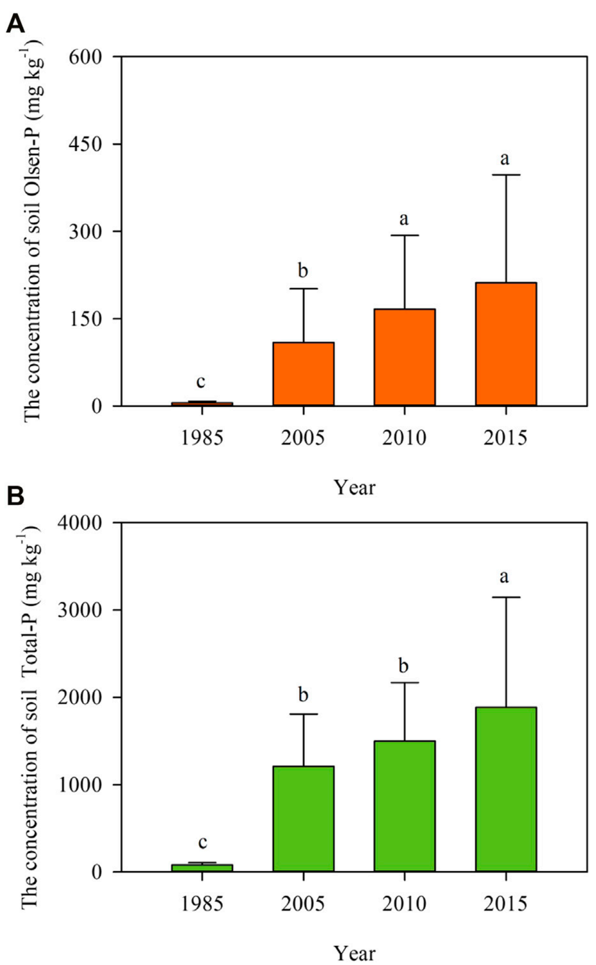

FIGURE 3 | Temporal changes in the concentration of Olsen-P **(A)** and Total-P **(B)** in Pinghe County over the 1985–2015 study period. Bars indicate mean ± SD. Different letters on the bars indicate a significant difference between different years.

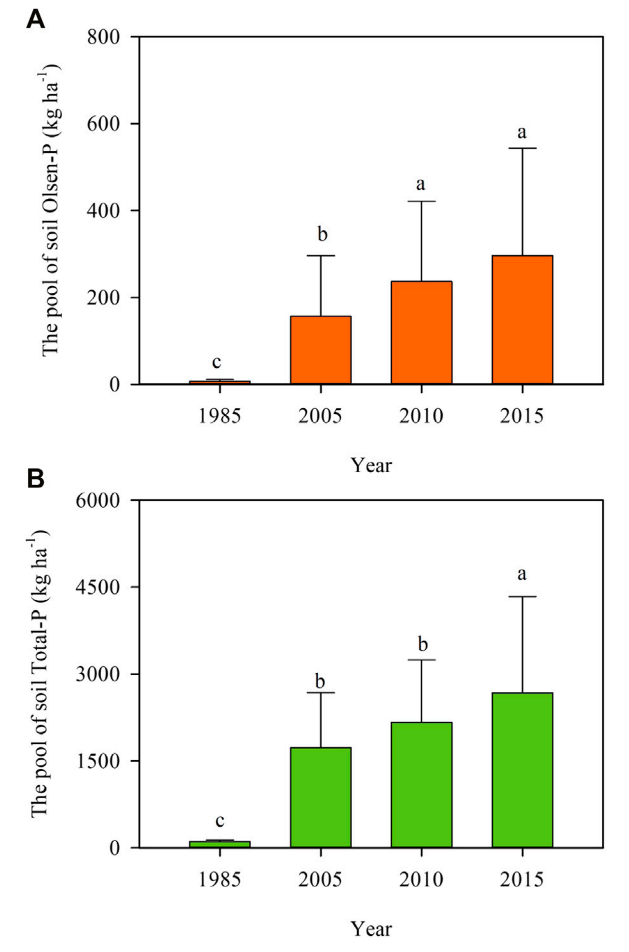

FIGURE 4 | Temporal changes in the Olsen-P pool **(A)** and Total-P pool **(B)** in Pinghe County over the 1985–2015 study period. Bars indicate mean ± SD. Different letters on the bars indicate a significant difference between different years.

0.15 kg ha^{-1} in 1985 to 19.13 kg ha^{-1} in 2015. Therefore, annual P surplus ranged from 70.19 to 449.12 kg ha^{-1} with an average of 282.23 kg ha^{-1} (**Figure 2**).

Temporal Characteristic of Soil Phosphorus

Generally, the average soil P concentration and P pool showed an increasing trend during the 30 years (**Figures 3**, **4**). The results of the analysis of variance showed that the concentration of soil P was significantly affected by years. Compared with 1985, Olsen P concentration in 2015 increased by 41-fold, while total P concentration increased by 24-fold (**Figure 3**). The corresponding Olsen P pool and total P pool increased by 42 and 25-fold, which increased from 7 to 297 kg ha^{-1} and 105–2,675 kg ha^{-1}, respectively (**Figure 4**).

Spatial Characteristic of Soil Phosphorus

Overall, the concentration of soil P and P pools in the natural forest did not change significantly with different soil layers. Comparing the 20-year-old pomelo orchard with the natural forest, the concentration of soil P and P pools significantly increased in 0–120 cm soil layers, but the increase rate decreased gradually with the increase in soil depth and then remained almost stable below 120 cm soil layers (**Figures 5**, **6**).

The average of soil Olsen P concentration and pool in 0–200 cm soil profile was 2.16 mg kg^{-1} (ranged from 0.29 to 5.80 mg kg^{-1}) and 5.76 kg ha^{-1} (ranged from 0.66 to 15.66 kg ha^{-1}) for the natural forest (**Figure 5**). By comparison, the soil Olsen P concentration in 20-year-old pomelo orchard under 0–20, 20–40, 40–60, 60–80, 80–100 and 100–120 cm was 338.66, 257.74, 212.37, 60.62, 63.06 and 30.39 mg kg^{-1}, respectively, and the soil Olsen P concentration ranged from 1.85 to 6.41 mg kg^{-1} under the soil depth of 120–200 cm (**Figure 5**). Correspondingly, in the 20-year-old pomelo orchard, the Olsen P pool at 0–120 cm depth decreased from 666.40 to 90.43 kg ha^{-1} with increase in soil depth, and then stabilized below 120 cm soil depth (**Figure 5**).

The differences in different P components were very small among different soil layers of the natural forest. The average concentration of Sol-P, Al-P, Fe-P, O-P, Ca-P, and Org-P along with the 0–200 cm soil profile for the natural forest was 0.64 (ranged from 0.56 to 0.88), 0.97 (ranged from 0.45 to 1.47), 8.03 (ranged from 4.65 to 11.64), 12.27 (ranged from 5.01 to 19.15), 8.46 (ranged from 5.50 to 11.94), and 60.64 (ranged from 39.08 to 84.71) mg kg^{-1}, respectively (**Figure 6**). On contrary, a significant increase in the concentration of soil P fractions was found between 0 and 120 cm soil layers of the 20-year-old pomelo orchard (**Figure 6**).

The total P pool in the natural forest was increased first and decreased later from 0 to 200 cm soil layer, but the variation was minor. In a natural forest, Org-P was the dominant P fraction, accounting for 55.2–76.6% of the sum of the P fractions, and others in order as follow: O-P > Ca-P > Fe-P > Al-P > Sol-P (**Figure 7**). For 20-year-old pomelo orchard soil, Al-P and Fe-P were the dominant P fractions with the proportion ranging from 77.2 to 82.0% in the layer of 0–60 cm and 62.4–69.9% in the layer of 60–120 cm, respectively. However, in the soil layer below 120 cm, Org-P and O-P were the dominant P fractions, which was similar to that of natural forest (**Figure 7**).

Scenario Analysis

Labile and stable P pools simulated by DPPS showed a continuous increase from 1985 to 2015 with the annual P application. The labile and stable P pools in 0–20 cm soil layer was increased from 67.49 to 2,235.70 kg ha^{-1} and 155.15–5,532.95 kg ha^{-1} from 1980 to 2015, respectively (**Figures 8**, **9**).

The scenario analysis also showed that the labile P pool would increase from 2,235.70 to 6,892.56 kg ha^{-1} for the S1; In the S2, the amount of P application decreased and remained relatively constant at approximately 31 kg P ha^{-1} after 2015 (**Figure 8**). the labile P pool would remain fairly constant around 2,233.56–2,353.00 kg ha^{-1} for the S2; and the labile P pool would decrease grafually firstly and then to equilibrium in S3. Meanwhile, the average size of a stable P pool would continue to rise from 5,532.95 kg ha^{-1} in 2016–29,592.92, 11,423.81, and 6,104.48 kg ha^{-1} in 2100 for the S1, S2, and S3 scenarios, respectively (**Figure 9**).

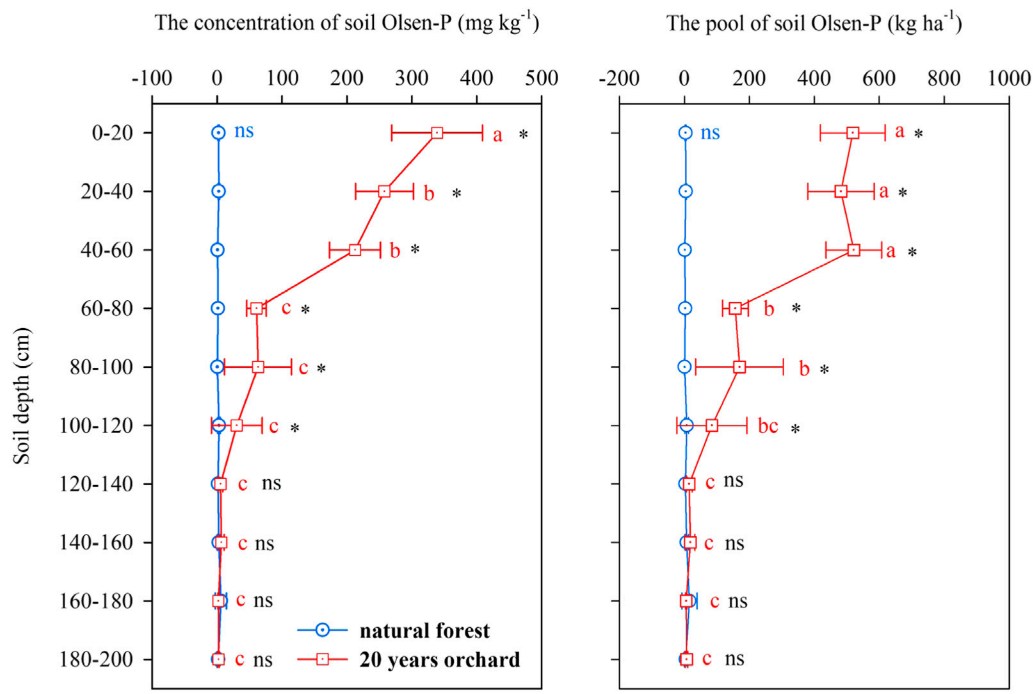

FIGURE 5 | Spatial variations of the concentration and pool of Olsen-P in different land-use treatments. Dots indicate mean ± SD ($n = 3$). Different letters on the dots for the same land use indicate a significant difference between different soil layers. ns indicates no significant difference, *indicates significant difference at $p < 0.05$ between natural forest and 20 years orchard.

DISCUSSION

Temporal and Spatial Variations of Soil Phosphorus Pools

P application was much higher than the uptake by pomelo orchards (**Figure 2**), thereby, the P surplus in this study (282.23 kg P ha^{-1}) was much higher than the annual average P surplus for field crops production (8.96 kg P ha^{-1}) from 1980 to 2007 in China (Li et al., 2011). Due to study limited resources, it was not possible to quantify the P stored in above ground biomass of the tree, hence, soil P surplus was over-estimated. However, it could not affect the final results because we measured the P accumulation in the soil, and it is generally accepted that soil P accumulation is much higher than tree accumulation (Li G. et al., 2015; Chen et al., 2022). A previous study reported that when the soil P surplus exceeds 4,128 kg P ha^{-1}, there would be a serious risk of P leaching (Chen et al., 2022). In the past 30 years, the soil P surplus exceeds 8,467 kg P ha^{-1}, which was much larger than the safe value. With the P accumulation in the soil, soil Olsen P and total P concentration of surface soil in 2015 were 42 and 24-folds higher, respectively, in comparison with the year 1985 (**Figures 3**, **4**). The increase rate of soil P in the current study was much higher than previous findings (Khan et al., 2018). This difference was mainly attributed to the higher P budget value. There was no big difference in P uptake between pomelo production and field crops production (Macdonald et al., 2011; Sattari et al., 2012; Chen and Graedel, 2016). However, the average amount of P application in pomelo production was about 15-fold higher than field crop production (Macdonald et al., 2011; Sattari et al., 2012; Chen and Graedel, 2016). In the present study, the increased rate of Olsen P and total P was different, which was in agreement with the observations of other authors Bai et al. (2013), who also found that the relationship between total P and Olsen P can be split into two straight lines with two different slopes by a change point, when above the change point, Olsen P exhibited a quicker increase with soil total P.

Typically, the red soils are ancient and highly weathered, and as a result, they are abundant in duricrusts with low P content (Dent et al., 2005). The primary source of soil P in mineral weathering and atmospheric deposition to the natural forest is minimal (Yu et al., 2018). Hence, a small range of variation in Olsen P and P fractions among different soil layers was detected in the natural forest (**Figure 5**). In contrast, the concentration of Olsen P and P fractions of 20-year-old pomelo orchard increased quickly among 0–120 cm soil layers (**Figures 5–7**). The difference in soil P status between natural forest and agricultural lands has been verified in many studies, which was strongly influenced by high P fertilizer application (Korai et al., 2018; Xu et al., 2019). Also, a higher level of P input in long-term tea cultivation increased the concentration of soil P significantly at 0–90 cm soil depth (Yan et al., 2018). Overall, the Org-P and O-P were the dominant fractions in natural forest soil. However, the dominant P fractions in the soil layer of 0–120 cm in 20-year-old pomelo orchard were converted to Al-P and Fe-P fraction. The proportion of P fraction changed was closely associated with the size of total P pool, the proportion of Al-P and Fe-P increased with total P increased and became the predominant fractions. The previous study also demonstrated that

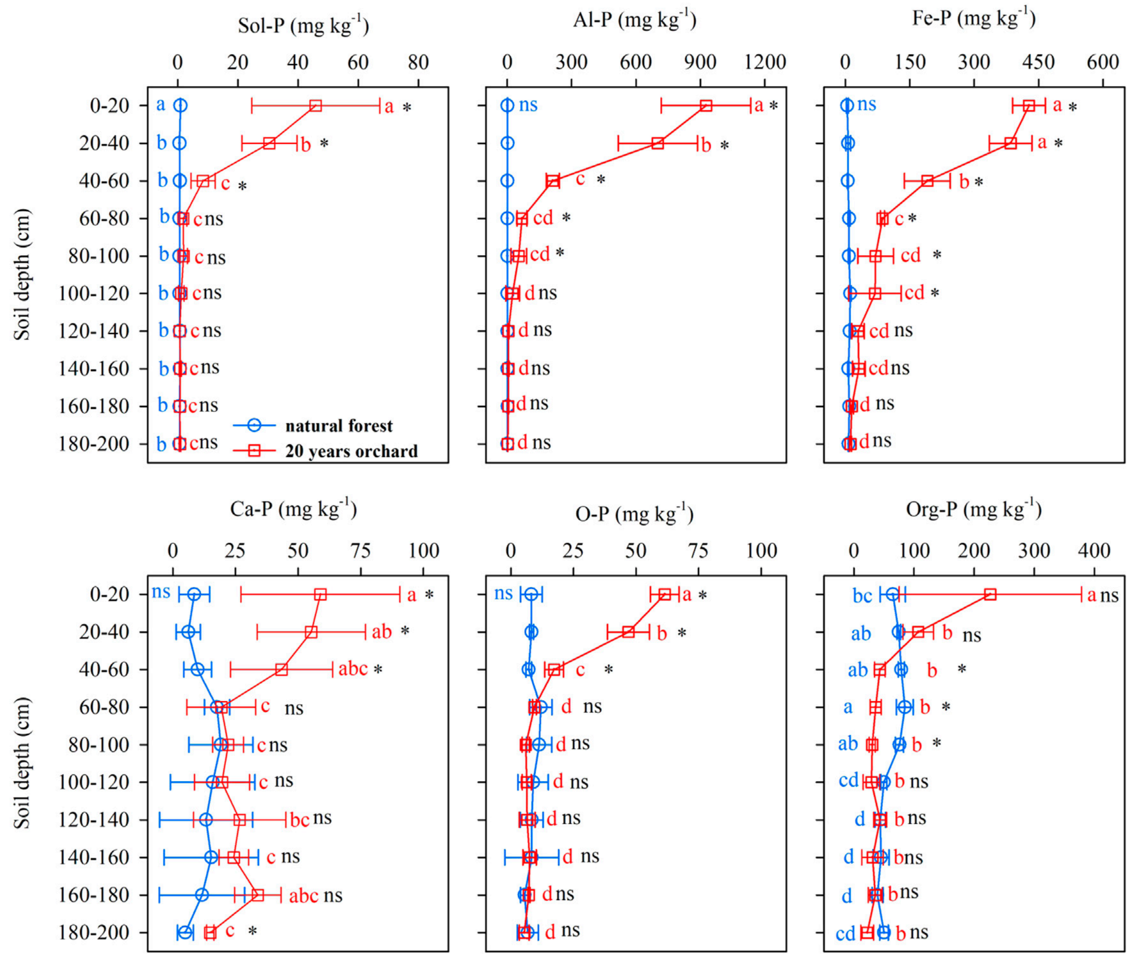

FIGURE 6 | Spatial variations of the concentration of P fraction in different land-use treatments. Dots indicate mean ± SD ($n = 3$). Different letters on the dots for the same land use indicate a significant difference between different soil layers. ns indicates no significant difference, *indicates significant difference at $p < 0.05$ between natural forest and 20 years orchard.

Al-P presented the greatest liability as plant absorption occurred (Wang et al., 2014). Therefore, the bioavailability of soil P was increased with the net P accumulation in soil. On the other side, the results in the study also implied that there was an increase environmental risk of P leaching in soil layer 0–120 cm in the current pomelo production system. Bai et al. (2013) reported that the P losses via leaching and overland flow greatly increases once the critical Olsen-P values has surpassed 90 mg kg–^{1} in red soil. Therefore, maximizing the P utilization and mitigation of its environment by P leaching in fruit orchards should be investigated on a priority basis. Hence, an integrated optimal P management for the intensive pomelo production with the high level of P fertilizer application in Pinghe County is urgently needed.

Scenarios Simulations and Phosphorus Management in Pomelo Orchard Systems

P pool plays a crucial role in sustaining the P uptake, and it has been confirmed by the conceptual model (Sattari et al., 2014). The annual increase rate of soil labile and stable P pools in pomelo orchards from 1985–2015 was almost 16-fold and 8-fold (**Figure 9**) higher than those of the field crop production system in China (4.4 and 21.7 kg ha^{-1} yr^{-1} for labile and stable P pools, respectively) (Yu et al., 2021), and around 43-fold and 20-fold higher than those of field crop production system in the United States (1.6 and 8.7 kg ha^{-1} yr^{-1} for labile and stable P pools, respectively) (Zhang et al., 2017), respectively. So, a significant legacy P pool has accumulated in soils of the pomelo orchards where it may be transformed to becoming a vital P source for future agriculture development.

The effects of the different P application rates on the sizes of soil labile and stable P pools during 2015–2100 were simulated by the DPPS model in the study. P pools displayed a sharp rise in S1 compared to past 30-year (**Figure 9**), consequently, the P management in S1 would have a more significant risk impact on the environment in the future. A recent study reported that the increasing trend of P concentration in the river water would continue in the foreseeable future (Chen et al., 2018). Such

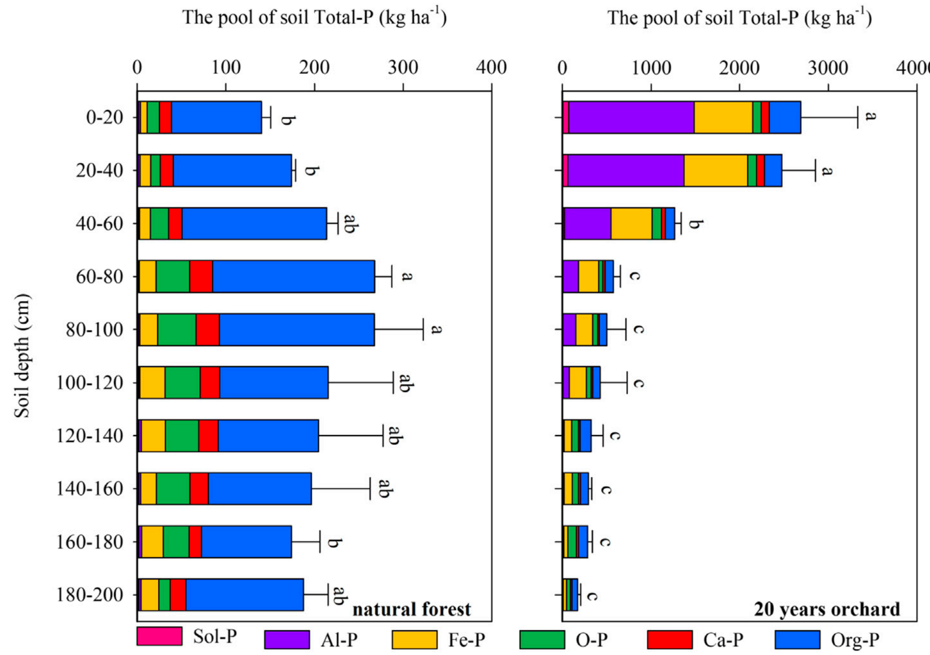

FIGURE 7 | Spatial variations of total-P pool in different land-use treatments. Dots indicate mean ± SD (*n* = 3). Different letters on the dots for the same land use indicate a significant difference between different soil layers.

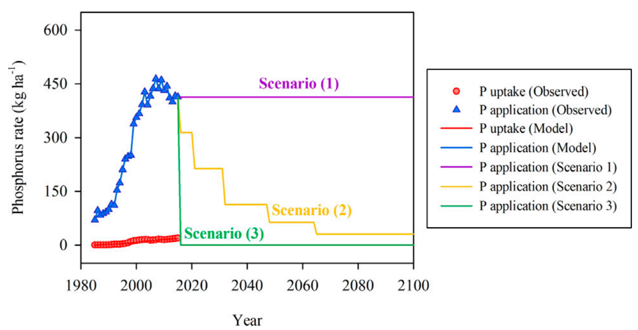

FIGURE 8 | Historical P and modeled P application/uptake (observed and simulated by DPPS model) in pomelo production for the period 1985–2015 and the trends of annual P application in pomelo production for the period 2016–2100 according to different scenarios. Scenarios 1 to 3 means the P application rate would be constant at 413.63 kg ha^{-1}, the P application rate would maintain pomelo production at 2015 level (28.14 t ha^{-1}), and the P application rate would be constant at 0 kg ha^{-1}, respectively.

increasing trends have also been observed in the United States (Stackpoole et al., 2019). Hence, improved incentives and optimal management strategies are necessary to reduce soil P leaching losses and alleviate environmental water pollution. In the S2, P application would decrease and remain relatively constant at approximately 31 kg ha^{-1} after 2015 (**Figure 8**). It was very close to 30–40 kg P ha^{-1} in United States (Obreza and Morgan, 2008) and 43 kg P ha^{-1} per year at the yield of 28 t ha^{-1} for China's citrus orchard (Li et al., 2019). Even in the S3, the labile P pool firstly decreased gradually in the first 10 years, and then decrease very slowly to the same level as in 2003 until 2100 (**Figure 9**). Correspondingly, the simulated pomelo yield would be stable in the first 6 years and would decrease gradually to 80% of the yield in 2015 until 2032, then the yield simulated in 2100 would finally drop slowly to approximately 75% of the yield in 2015 (**Supplementary Figure S1**). However, the situation is not always so straightforward. On the one hand, the agronomically-optimum critical level of Olsen P concentration ranges from 15–80 mg kg^{-1} for pomelo grown (Liu et al., 2021), but in the current soil Olsen-P is about 212 mg kg–^{1} (**Figure 3**). And continued agricultural innovations such as uncover mechanisms by which soil legacy P is mobilized through root physiological and morphological processes and through arbuscular mycorrhizal fungi and P-solubilizing bacteria in rhizosphere and hyphosphere are likely to improve the P use

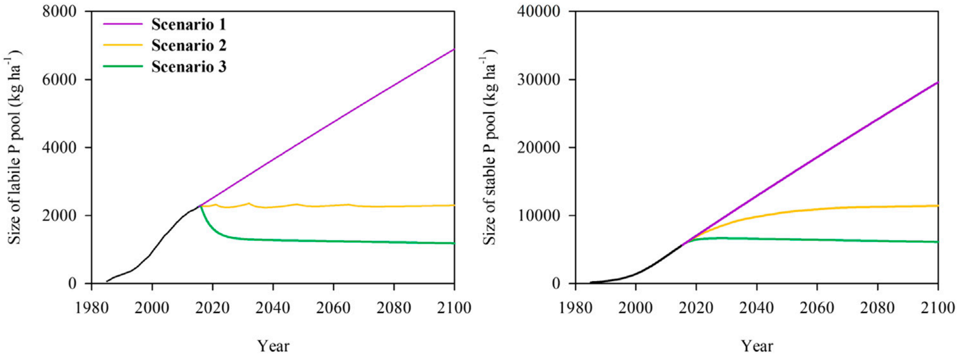

FIGURE 9 | The size of labile and stable P pools in 0–20 cm from 1985–2100 according to different scenarios. Scenarios 1 to 3 means the P application rate would be constant at 413.63 kg ha^{-1}, the P application rate would maintain pomelo production at 2015 level (28.14 t ha^{-1}), and the P application rate would be constant at 0 kg ha^{-1}, respectively.

efficiency further, but these potentials were not taken into account in our analysis (Feng et al., 2019). On the other hand, root of the tree much larger than 20 cm, but the current model only considered the topsoil, so 0–20 cm soil layer may provide incomplete information about soil P pool. So, without P application until 2100, this pomelo yield will be no less than 75% of 2015. Therefore, sustainable P management decisions and environmental policymaking strategies must be based on the status quo of the P pool, and also establish the recommended standards for P application in pomelo orchards.

CONCLUSIONS

Temporal and spatial variations of P concentrations and P pools were recorded for the pomelo orchard system in China. For temporal analysis, P concentration and P pool increased significantly from 1985 to 2015 in the topsoil of the pomelo orchard system. For spatial analysis, pomelo planting affected the Olsen P concentration and P pool in the deep soil at 0–60 cm. In addition, the scenario analyses indicated that P Pools from 2016 to 2100 could be affected by different P management strategies and could provide a vital resource for future P management in the pomelo orchard system in China. Overall, these findings suggest that the accumulation of P in soil is an important source for future agriculture development and it is very crucial for optimal P management in the pomelo orchard system to prevent the excessive P accumulation for sustainable and green agriculture development, and P resource protection.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

XY analyzed the data and wrote the manuscript. GL provided the DPPS model and revised the manuscript. WZ analyzed the data and wrote the manuscript. MAM revised the manuscript. WY analyzed the data. CM analyzed the data. LW designed the study and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.858816/full#supplementary-material

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Study on the Relationship of Root Morphology and Phosphorus Absorption Efficiency With Phosphorus Uptake Capacity in 235 Peanut (*Arachis hypogaea L.*) Germplasms

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Peanut (*Arachis hypogaea L.*) is a significant oil and protein crop. Its yields greatly depend on the availability of phosphorus (P). Root morphology and P absorption efficiency are important factors affecting the P uptake capacity, but their relationships in peanuts are rarely reported. Here, we report the effect of root morphology and P absorption efficiency on the P uptake capacity in peanuts using 235 germplasms. In this work, we use the P uptake rate per plant to reflect the P uptake capacity. The P uptake capacity was significantly increased after low-P treatment and showed great differences among the germplasms. The germplasms with higher P absorption efficiency and a well-developed root system have higher P uptake capacity. Under both P conditions, the P absorption efficiency plays more important roles than root morphology in P uptake capacity, and the P uptake rate per unit root dry weight and the P uptake rate per unit root surface area contributed the most. Root morphology contributes more to the P uptake capacity under low-P treatment than under sufficient-P conditions, and root surface area contributed the most. Forty-eight germplasms with higher P uptake capacity were screened, and they had three different uptake strategies under low-P treatment. These findings indicated that low-P stress induces root growth and improves the P absorption efficiency of peanuts to ensure the plant gets enough P; provides new insights into the relationship between the P uptake capacity, P absorption efficiency, and root morphology; and furnishes important evaluation indexes for high P-efficient germplasm selection.

Keywords: peanut germplasms, phosphorus uptake rate, phosphorus uptake capacity, root morphology, phosphorus absorption efficiency

INTRODUCTION

Phosphorus (P) is an essential macroelement for crop growth and development, which participates in multiple important biological processes (Lorenzo-Orts et al., 2020). Although P is abundant in soil, little available P can be directly absorbed and utilized by plants due to fixation of the soil (Li et al., 2011; Manschadi et al., 2014). Under low-P conditions, plants improve the P uptake and utilization efficiency by a series of morphological, physiological, and biochemical adaptations; however, these functions were significantly different among crops or genotypes (Yuan et al., 2017; Kale et al., 2020; Soumya et al., 2021). Previous research found that P uptake is more important than P utilization when P supply is limited, while P utilization has a greater impact on P efficiency for a sufficient P supply (Manske et al., 2001; Wang et al., 2010a). In coffee, the contribution of P uptake efficiency to yield formation increased significantly under low-P (LP) conditions (Neto et al., 2016). Therefore, studying the P-efficient absorption characteristics is of great significance to enhance the adaptability of crops in the LP environment.

Plants have evolved a variety of beneficial root traits and physiological traits to obtain more amounts of P under the LP environment (López-Arredondo et al., 2014; Nestler et al., 2016; Liu, 2021; Zhang et al., 2021). On the one hand, the increase in the P absorption area is conducive to higher P uptake capacity. Under P deficiency, the plants distribute more photosynthetic products to the root to improve their development and growth. Excellent root morphology and root configuration were promoted to upgrade the P absorption area (Wang et al., 2010b; Falk et al., 2020; Bello, 2021). On the other hand, when available P diffuses to the root surface, a higher P absorption efficiency per unit area is important for P acquisition. The root acquires P mainly in the form of inorganic phosphate (Pi, orthophosphate) by Pi transporters in plasma membranes, and the plasma membrane-localized Pi transporter activity affects P acquisition and allocation (Liao et al., 2019). Under LP conditions, cells express predominantly high-affinity Pi transporters, which could enhance P uptake ability by promoting the Pi transport from the root surface to the cytoplasm (Lapis-Gaza et al., 2014; Sudhakar et al., 2018; Wang et al., 2018).

Plant P acquisition is generally driven by cooperations of morphological and physiological responses (Lynch and Ho, 2005; Honvault et al., 2020). Because these traits require carbon costs, plants tend to rely primarily on a few of these favorable traits (Pearse et al., 2006; Raven et al., 2018; Wen et al., 2019). In maize and wheat, the root morphology plays more important roles than the physiological responses under LP conditions (Lyu et al., 2016; Wen et al., 2017), whereas root exudates increased more significantly in chickpeas (Lyu et al., 2016). Trade-offs and coordination between morphological and physiological traits resulted in multiple P uptake strategies, resulting in similar total P uptake levels (Lyu et al., 2016; Honvault et al., 2020). Due to the diversity and complexity of the P uptake strategies among germplasms, it is unilateral to evaluate P uptake efficiency by a few germplasms. Therefore, it is necessary to evaluate and identify P uptake efficiency, as well as its influencing factors, in a wide range of populations.

The peanut (*Arachis hypogaea L.*) is an important oil and protein crop. P deficiency is one of the factors limiting its production (Singh et al., 2015; Naga Madhuri et al., 2018). The P uptake capacity reflects the adaptability to certain P conditions. The P uptake rate per plant is defined as the P absorption per plant per unit time and can be used to reflect the P uptake capacity. At present, few studies are available regarding P uptake capacity in peanuts, and related studies were only based on recombinant inbred line (RIL) populations or dozens of germplasms to evaluate the changes of few root morphology traits (Krishna, 1997; Jadhav and Gowda, 2012; Kumar et al., 2015). For example, Jadhav and Gowda (2012) studied the root morphology in a recombinant inbred line (RIL) population and found significant differences in root traits between germplasms under different P treatments. Krishna (1997) and Kumar et al. (2015) found that increased root biomass, root length, and P accumulation per unit root length are beneficial to P absorption, but different varieties rely on different uptake strategies. The P uptake capacity under P deficiency was affected by the expression of root traits associated with soil foraging and P transporter activity associated with P absorption efficiency. But limited information is available on the contribution of the root morphology and P absorption efficiency. Moreover, the selection and evaluation of P-efficient uptake peanut cultivars and germplasms were rarely reported, which restricted the extension of P-efficient cultivars and the development and use of P-efficient germplasms. In our research, the total root length, root surface area, root volume, average root diameter, root tip number, specific root length, and root dry weight per plant were measured to reflect the root morphology; the P uptake rate per unit root dry weight/root length/root surface area/root volume was calculated to represent the P absorption efficiency. A diverse panel including 235 peanut germplasms with extensive phenotypic variations in P efficiency was grown in LP and sufficient-P (SP) conditions. The objectives of this study were to (i) evaluate the effects of LP stress on the P uptake capacity, P absorption efficiency, and root morphology; (ii) evaluate the contribution of the P absorption efficiency and root morphology to P uptake capacity under LP and SP conditions; and (iii) screen P-efficient germplasms and explore P-efficient uptake strategies. These analyses clarify the key factors affecting the P uptake capacity and the P uptake strategies of different P-efficient germplasms, providing the experimental method and theoretical basis for the investigation of the P efficiency in peanuts.

MATERIALS AND METHODS

Plant Materials

A diverse panel consisting of 235 peanut germplasms was used in this study (**Supplementary Table S1**). These lines covered 87 (37.02%) lines of ssp. *fastigiate* (62 var. *vulgaris*, 22 var. *fastigiata*, and 3 var. *aequatoriana*), 85 (36.17%) lines of ssp. *hypogaea* (65

TABLE 1 | Summary of traits and their calculation methods in hydroponic culture.

Trait	Abbreviation	Units	Calculation methods
Total root length	TRL	Cm	
Root surface area	RSA	Cm^2	
Root volume	RV	Cm^3	WinRHizo LA-S image analysis system
Average root diameter	ARD	Mm	
Root tip number	RTN	-	
Root dry weight per plant	RDW	G $plant^{-1}$	Weight using 1/100 balances
Specific root length	SRL	M g^{-1}	(TRL/1000)/RDW
P uptake rate per unit root dry weight	PUR_RDW	μmol h^{-1} g^{-1}	PUR_RDW
P uptake rate per unit root length	PUR_RL	nmol $h^{-1}cm^{-1}$	(PUR/TRL) × 1000
P uptake rate per unit root surface area	PUR_RSA	nmol $h^{-1}cm^{-2}$	(PUR/RSA) × 1000
P uptake rate per unit root volume	PUR_RV	μmol $h^{-1}cm^{-3}$	PUR_RV

var. *hypogaea* and 20 var. *hirsuta*), and 63 (26.81%) lines of irregular types (Zhang et al., 2017). These germplasms included main cultivars, native varieties, a peanut core collection, important breeding parents, and advanced breeding lines. The lines also showed a broad geographic distribution: 169 (71.91%) lines were from different areas of China, 55 (23.40%) were from 17 countries, and 11 (4.68%) were unknown (**Supplementary Table S1**). There was a significant phenotypic variation within the population, and key agronomic traits showed normal distributions (Zhang et al., 2017). All the germplasms were stored by our group.

Experiments Design

Hydroponic experiments were carried out in a greenhouse. Intact plump seeds with similar sizes were surface-sterilized with 0.1% H_2O_2 for 6 h and thoroughly rinsed by sterilizing deionized water. The seeds were then placed on wet degreasing cotton in germination disks and grown in the dark for about 5 days at 26°C. The seeds should stay moist during germination. After unfolding, 10 seedlings similar in size were transplanted to a hydroponic box with deionized water for 3 days and then cultured in 1/5 Hoagland's nutrient solution (1.4 L) with sufficient (0.6 mmol/L) and low (0.01 mmol/L) KH_2PO_4. Sixteen days after P treatment, plants were cultured in deionized water for 24 h, and then transferred to 1/5 Hoagland's nutrient (1.4 L) with 0.2 mmol/L KH_2PO_4 for 8 h after LP treatment (then transferred to deionized water for 16 h) and 24 h after the SP condition (**Supplementary Figure S1**). Three biological repeats were performed for each treatment, and 10 seedlings were tested in each replication. The composition of Hoagland's solution was as follows: 1 mmol/L KNO_3, 1 mmol/L Ca $(NO_3)_2{\cdot}4H_2O$, 0.4 mmol/L $MgSO_4{\cdot}7H_2O$, 1 mmol/L KH_2PO_4, 0.01 mmol/L EDTA·Fe, 0.1 × 10^{-3} μmol/L H_3BO_3, 0.15 × 10^{-3} μmol/L $MnSO_4$, 0.03 × 10^{-3} μmol/L, $ZnSO_4{\cdot}7H2O$, 1.0 × 10^{-6} μmol/L $Na_2MoO_4{\cdot}2H_2O$, 0.16 × 10^{-6} μmol/L $CuSO_4{\cdot}5H_2O$, and 0.21 × 10^{-6} μmol/L $CoCl_2{\cdot}6H_2O$ (Hoagland and Arnon, 1950). The nutrient solution was changed every 4 days, and pH was maintained at 6.0 using HCl or NaOH. The LP nutrient solution was supplemented with KCl to ensure that the K concentration was consistent with that of the SP nutrient solution. The seedlings were grown in a greenhouse under 16-h light/8-h dark photoperiod (11.4K LuX light intensity) at 24°C.

P Concentration and Root Morphology Measurement

The P uptake rate was measured by using the depletion method after 16 days of P treatment (Claassen and Barber, 1974; Bhadoria et al., 2004). To maximize the P uptake rate, plants were cultured in deionized water for 24 h and then transferred to 1/5 Hoagland's nutrient (1.4 L) with 0.2 mmol/L KH_2PO_4. To minimize P consumption in the solution under the condition of different P uptake rates after high- and low-P treatments, the plants were treated for 8 h after LP treatment (then transferred to deionized water for 16 h) and 24 h after the SP condition. Then the nutrient solution was replenished to 1.4 L, and 3 ml nutrient solution was taken from each hydroponic box after 8-h LP treatment and 24-h SP condition. The P concentration in nutrient solution was determined by using the vanadium molybdate blue colorimetric method (Murphy and Riley, 1962). The P uptake rate per plant (PUR, μmol h^{-1} $plant^{-1}$) is the P absorption per plant per unit time and reflects the P uptake capacity of the peanut. Considering the diversity of root morphology of different germplasms, the P uptake rate per unit root dry weight (PUR_RDW), P uptake rate per unit root length (PUR_RL), P uptake rate per unit root surface area (PUR_RSA), and P uptake rate per unit root volume (PUR_RV) were calculated to reflect the P absorption efficiency.

$$PUR = \frac{C_1 V_1 - C_2 V_2}{h \times N},$$

where *C1* is the P concentration of nutrient solution before absorption, *V1* is the volume of nutrient solution before absorption, *C2* is the P concentration of nutrient solution after absorption, *V2* is the volume of nutrient solution after absorption, *h* is the absorption time, and *N* is the number of plants in each box.

At harvest (18 days after P treatment), the roots from 6 plants per treatment were harvested and rinsed with deionized water. The total root length (TRL), root surface area (RSA), root volume (RV), average root diameter (ARD), and root tip number (RTN) were measured using the WinRHIZO LA-S image analysis system (WinRHizo LA-S, Re´gent Instr. Inc., Quebec, Canada). Then the root samples were dried in an oven at 80°C until the dry weight was constant, and then root dry weight (RDW) was measured.

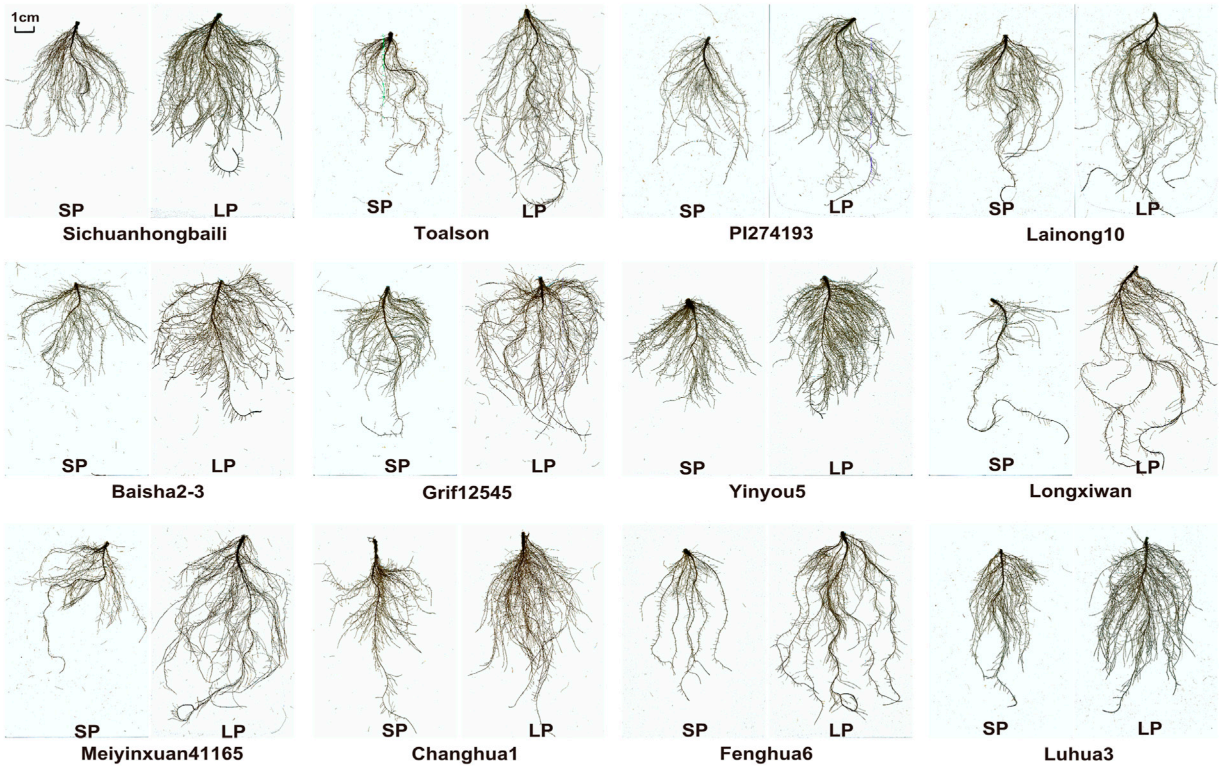

FIGURE 1 | Root morphological of 12 germplasms under low-P (LP) and sufficient-P (SP) conditions.

The specific root length (SRL) (m g^{-1}) was determined by dividing the RDW by TRL (**Table 1**). These seven indices were used to reflect the root morphology. We also measured the shoot dry weight (SDW) and calculated the total dry weight (TDW) and root/shoot ratio (RSR). Three biological repeats were carried out for each treatment.

To better understand the responses of LP treatment, the relative values of all traits were calculated by dividing the values of LP treatment by the values of the SP condition (Yuan et al., 2017).

Data Analysis

Descriptive statistics, analyses of variance (ANOVA), and correlation were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, United States), and then we calculated the broad-sense heritability (hB^2) by $hB^2 = \sigma g^2/(\sigma g^2+\sigma e^2)$, where σg^2 means genetic variance and σe^2 means environmental variance (Knapp et al., 1985). Clustering based on Ward's method was executed using a squared Euclidean distance matrix of the PUR after LP treatment, germplasms were divided into low P uptake capacity, medium P uptake capacity, and high P uptake capacity. Principal component analysis (PCA) was carried out using the "FactomineR" package in R version 4.1.0 (R Foundation for Statistical Computing, Vienna, 2005). Hierarchical classification on principal components (HCPC) was then performed using the first two principal components (80% of the total variability) to define groups with similar patterns of P absorption efficiency and root morphology. To underline the relative ability of the P absorption efficiency and root morphology for P uptake capacity, partial square path modeling (PLS PM) was performed using the "pls pm" package in R version 3.6.0 (Sanchez, 2013). Two clusters of variables, or "latent variables," were defined, respectively: the "root morphology" variables encompassing TRL, RSA, RV, ARD, RTN, SRL, and RDW, and the "P absorption efficiency" variables encompassing PUR_RDW, PUR_RL, PUR_RSA, and PUR_RV. Overall model quality was evaluated with the goodness of fit (GoF) index, and significance was determined with 95% confidence. Graphs were plotted using the "ggplot2" package in R version 4.1.0.

RESULTS

Effects of LP Stress on the P Uptake Capacity, P Absorption Efficiency, and Root Morphology

P deficiency enhanced root production and induced root morphological remodeling in peanuts. Some germplasms produced more and longer lateral roots and increased the root volume and root surface area for P exploration (Jadhav and Gowda, 2012; Kumar et al., 2015). To investigate the root morphology differences among peanut germplasms under LP treatment, we performed scanning and analysis of roots using 235

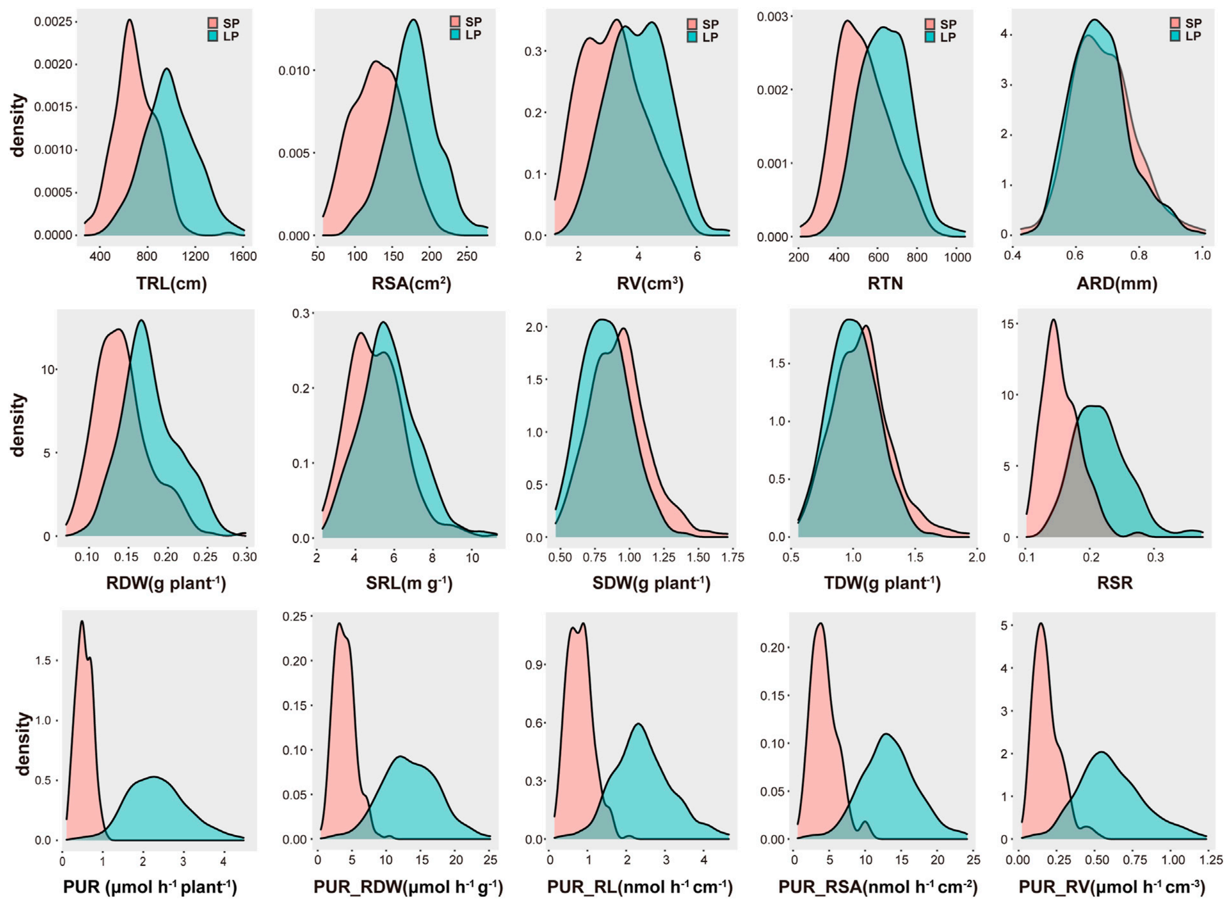

FIGURE 2 | Phenotypic distribution of 12 traits under low-P (LP) and sufficient-P (SP) conditions.

germplasms. Scanning of root systems revealed variable changes among germplasms when exposed to LP (**Figure 1**). After quantifying the TRL, RSA, RV, ARD, and RTN of all germplasms, we found that these indexes display normal distribution through the population (**Figure 2**).

To clarify the effects of LP treatment on the P uptake capacity and root morphology, we performed comprehensive analysis of 12 traits (**Table 2**). Significant ($p < 0.001$) differences were detected among the populations under SP and LP conditions (**Table 2**; **Figure 2**). Compared to the SP condition, a significant increase (335%) was noted in PUR after LP treatment in all germplasms (**Table 2**). However, the range of relative value showed that the root morphology varied differently among germplasms after LP treatment. For example, fenghua6, Sichuanhongbaili, PI274193, and Toalson significantly increased (**Figure 1**, **Supplementary Figure S3**); Baisha1016, 03F032, and 07H226 decreased; and 98D080-2, Silicai (1), and Ji0608-228 significantly decreased (**Supplementary Figure S2**). Similar results were also found in the P uptake capacities among germplasms (**Table 2**). The mean value of RDW, TRL, RV, RSA, RTN, and SRL of the population all increased after LP treatment in comparison to SP condition, their relative values ranged from 1.17 to 1.46, and the increasing range was 28.57, 41.30, 27.44, 36.39, 21.88, and 13.19%, respectively. The ARD showed a relatively small change and decreased by 1.45%. The PUR_RDW, PUR_RL, PUR_RSA, and PUR_RV of the populations all increased under LP treatment. Their relative values were all greater than 3.4, and they increased significantly by 243, 201, 206, and 221%, respectively. The coefficients of variation (CVs) of all traits, except ARD, were close to or above 20% (**Table 2**). The hB^2 of all traits ranged from 57.42 to 78.14%.

These findings indicated that LP stress induces the root growth and improves the P absorption efficiency of peanuts to ensure the plant gets enough P for the shoot growth (**Figure 2**). This regular pattern is universally valid throughout the peanut population.

Comparison of P Uptake Capacity Among Germplasms

Based on the average PUR of 235 germplasms after LP treatment, these germplasms were divided into three clusters: high P uptake capacity, medium P uptake capacity, and low P uptake capacity (**Supplementary Figure S4**, **Figure 3A**), which make up 20.4% (48), 46.4% (109), and 33.2% (78) of the population, respectively. Under SP conditions, the P uptake capacity of the high, medium,

TABLE 2 | Phenotypic variation and analysis of variance (ANOVA) of investigated traits among peanut germplasms under low-P (LP) and sufficient-P (SP) conditions.

Trait	Range (min-max)		Mean ± SD		CV (%)		Relative value		F-value			hB^2
	LP0	SP	LP	SP	LP	SP	Range	Mean	G	T	G*T	(%)
PUR (μmol h^{-1} plant $^{-1}$)	0.37 ~ 4.47	0.11 ~ 1.10	2.35 ± 0.73	0.54 ± 0.20	31.04	36.52	1.03 ~ 14.48	4.80	8.17***	7495.888***	10.6***	65.64
TRL (cm)	453.20 ~ 1610.51	273.52 ~ 1479.94	986.88 ± 214.44	698.444 ± 172.63	21.73	24.72	0.78 ~ 2.87	1.46	16.29***	2732.9***	5.59***	66.4
RSA (cm^2)	95.35 ~ 277.67	57.40 ~ 219.86	177.91±33.13	130.44±33.15	18.62	25.41	0.84 ~ 2.98	1.43	11.73***	2007.86***	4.18***	59.98
RV (cm^3)	1.69 ~ 7.09	1.22 ~ 5.57	4.04 ± 0.98	3.17 ± 1.02	24.34	32.12	0.66 ~ 3.21	1.35	11.98***	661.48***	2.65***	57.42
ARD (mm)	0.47 ~ 0.98	0.43 ~ 1.01	0.68 ± 0.09	0.69 ± 0.10	13.42	14.19	0.72 ~ 1.43	0.99	30.4***	22.5***	3.67***	75.16
RTN	338 ~ 1044	211 ~ 886	639.85 ± 121.73	524.97 ± 130.45	19.02	24.85	0.61 ~ 2.69	1.27	14.98***	958.07***	4.16***	62.00
SRL (m g^{-1})	2.66 ~ 11.29	2.30 ~ 11.28	5.75 ± 1.50	5.08 ± 1.45	26.14	28.53	0.64 ~ 2.55	1.17	17.4***	293.112***	17.4***	78.14
RDW (g $plant^{-1}$)	0.09 ~ 0.28	0.07 ~ 0.30	0.18 ± 0.03	0.14 ± 0.04	19.78	24.58	0.78 ~ 2.02	1.27	14.24***	909.94***	2.56***	72.30
PUR_RDW (μmol h^{-1} g^{-1})	2.22 ~ 25.17	0.47 ~ 10.47	13.52 + 4.09	3.94 + 1.59	30.22	40.4	1.02~12.86	3.79	8.63***	5157.56***	10.04***	72.22
PUR_RL (nmol h^{-1} cm^{-1})	0.39 ~ 4.65	0.15 ~ 2.08	2.44 ± 0.76	0.81 ± 0.33	31.10	40.75	0.65 ~ 12.4	3.41	5.81***	3215.38***	8.35***	59.15
PUR_RSA (nmol h^1 cm^{-2})	1.99 ~ 24.15	0.64 ~ 10.7	13.36 ± 3.83	4.36 ± 1.88	28.69	43.12	0.70~15.52	3.50	5.4***	3471.02***	7.5***	59.23
PUR_RV(μmol h^{-1} cm^{-3})	0.09 ~ 1.23	0.02 ~ 0.53	0.61 ± 0.21	0.19 ± 0.09	34.74	48.98	0.78 ~ 22.1	3.77	6.45***	3086.19***	6.42***	64.09

*SD, standard deviation; CV, coefficient of variation; G, genotypes; T, treatments; G*T, genotypes*treatments; hB^2, broad-sense heritability; PUR, P uptake rate per plant; RDW, root dry weight per plant; TRL, total root length; RSA, root surface area; RV, root volume; ARD, average root diameter; RTN, root tip number; SRL, specific root length; PUR_RDW, P uptake rate per unit root dry weight; PUR_RL, P uptake rate per unit root length; PUR_RSA, P uptake rate per unit root surface area; PUR_RV, P update rate per unit root volume.*** indicates significance at p<0.001.*

and low clusters was 0.63, 0.57, and 0.44 μmol h^{-1} plant $^{-1}$, while under LP treatment, it was 3.42, 2.43, and 1.58 μmol h^{-1} plant $^{-1}$, respectively.

The root morphology of germplasms belonging to each cluster was analyzed, respectively, to explore their relationship with P uptake capacity. As shown in **Figure 3**, under SP conditions, none of the 12 indexes showed significant differences among the three clusters. Under LP treatment, RDW, TRL, RSA, PUR_RDW, PUR_RL, PUR_RSA, and PUR_RV represented the extremely significant difference between the three clusters (**Figures 3B–D,I–L**), while the difference was not detected in RV and RTN of moderate and low clusters (**Figures 3E,F**). The ARD and SRL showed little variation between the three clusters (**Figures 3G,H**). These findings indicated that germplasms whose RDW, TRL, and RSA respond strongly to LP stress usually showed higher P uptake capacity.

Contributions of P Absorption Efficiency and Root Morphology to the P Uptake Capacity

To investigate the relationships between the root morphology and P uptake capacity, correlation analysis was conducted. As shown in **Table 3**, the PUR was significantly and positively correlated with the PUR_RDW, PUR_RL, PUR_RSA, and PUR_RV, with correlation coefficients greater than 0.600 under two P conditions. Significantly positive correlations were also found between the PUR and the RDW, TRL, RV, RSA, and RTN with correlation coefficients between 0.143 and 0.430 after LP treatment. In comparison with LP treatment, the PUR showed a weaker positive correlation with RDW, TRL, RV, and RSA, with correlation coefficients between 0.163 and 0.240 under SP conditions. These results show that the P absorption efficiency and root morphology were significantly correlated with P uptake capacity, and the correlation of P absorption efficiency was greater than that of root morphology. The PCA also underlined the importance of P absorption efficiency and root morphology under LP treatment, and the contribution of P absorption efficiency on the P uptake capacity was larger than that of root morphology (**Figure 4**).

To further explore the effects of P absorption efficiency and root morphology on the P uptake capacity, the PLS PM was also used. As shown in **Figure 5**, PLS PM (R^2 = 0.960 under LP treatment, R^2 = 0.875 under SP condition) showed that the effects of P absorption efficiency (coef = 0.868 under LP treatment, coef = 0.911 under SP condition) on P uptake capacity were larger than that on root morphology (coef = 0.520 under LP treatment and coef = 0.385 under SP condition) under the two P conditions. The effects of root morphology on P uptake capacity after LP treatment were larger than that under the SP condition, while the opposite result occurred in P absorption efficiency. The most impactful contributor to the root morphology was RSA > TRL > RDW under LP treatment and RDW > RSA under the SP condition. Under the LP treatment, PUR_RDW and PUR_RSA were the better single predictor in the P absorption efficiency component, followed by PUR_RL and PUR_RV. Under SP conditions, PUR_RDW and PUR_RL were both the best single predictor in the P absorption efficiency component, followed by

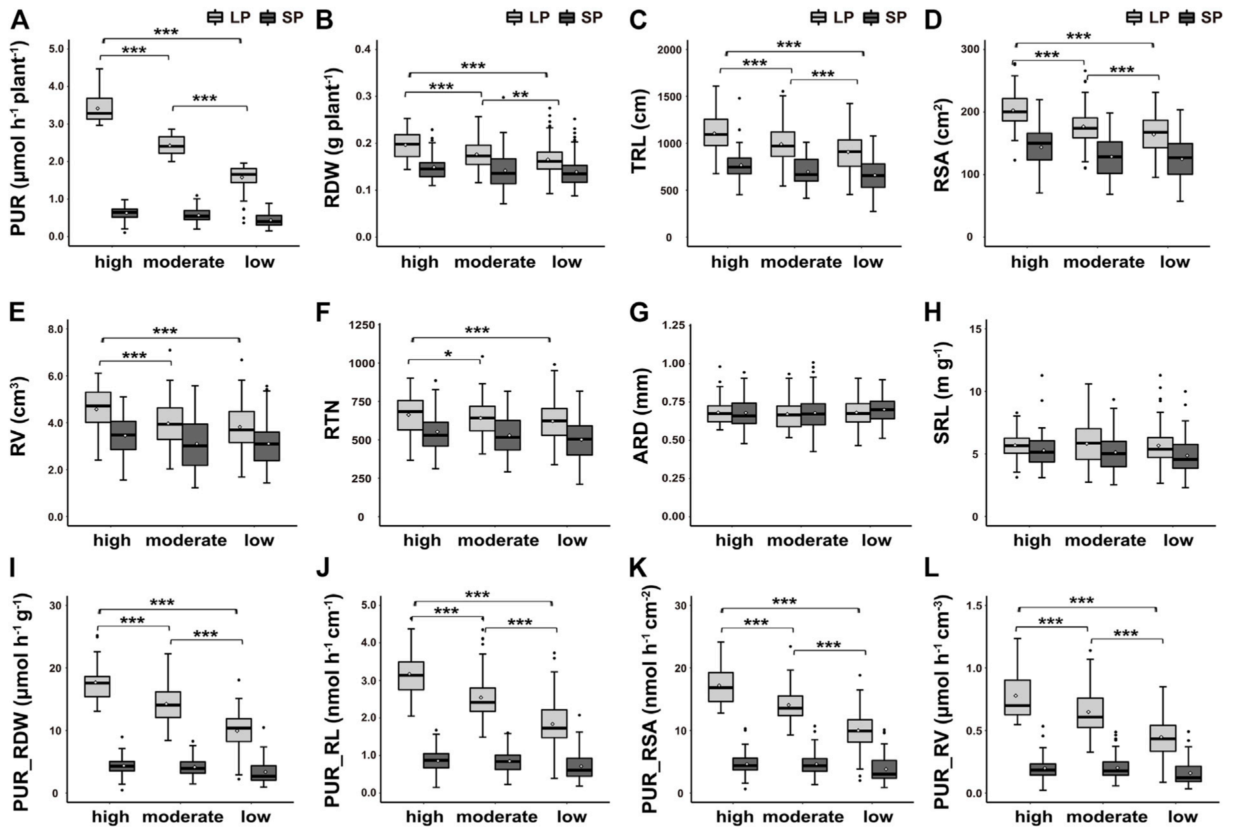

FIGURE 3 | Box and whisker plot showing differences in 12 traits among different P uptake capacity of peanut germplasms under low-P (LP) and sufficient-P (SP) treatments. **(A)** P uptake rate per plant (PUR), **(B)** root dry weight per plant (RDW), **(C)** total toot length (TRL), **(D)** root surface area (RSA), **(E)** root volume (RV), **(F)** root tip number (RTN), **(G)** average root diameter (ARD), **(H)** specific root length (SRL), **(I)** P uptake rate per unit root dry weight (PUR_RDW), **(J)** P uptake rate per unit root length (PUR_RL), **(K)** P uptake rate per unit root surface area (PUR_RSA), and **(L)** P uptake rate per unit root volume (PUR_RV). High, moderate, and low represent high, moderate, and low P uptake rates per plant, respectively. Horizontal lines inside boxes, median; white dot, mean; box hinges, first and third quartiles; whiskers, full range of the data; black dots represent outliers. *, **, and *** indicate significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

TABLE 3 | Relationships between PUR and root morphology/P absorption efficiency traits under low-P (LP) and sufficient-P (SP) conditions.

	PUR	PUR_DW	PUR_TRL	PUR_RSA	PUR_RV	TRL	RSA	RV	ARD	RT	SRL	RDW
PUR		0.778**	0.698**	0.770**	0.661**	0.380**	0.430**	0.254**	-0.050ns	0.143*	0.045ns	0.326**
PUR_DW	0.767**		0.601**	0.814**	0.798**	0.222**	0.035ns	-0.175**	-0.299**	0.040ns	0.417**	-0.310**
PUR_TRL	0.767**	0.729**		0.855**	0.546**	-0.360**	-0.138*	0.094ns	0.379**	-0.307**	-0.429**	0.155*
PUR_RSA	0.721**	0.861**	0.912**		0.888**	-0.106ns	-0.224**	-0.260**	-0.130*	-0.127ns	-0.064ns	-0.055ns
PUR_RV	0.632**	0.871**	0.764**	0.953**		0.135*	-0.229**	-0.517**	-0.515**	0.032ns	0.253**	-0.198**
TRL	0.190**	-0.038ns	-0.430**	-0.351**	-0.250**		0.758**	0.215**	-0.550**	0.565**	0.644**	0.230**
RSA	0.210**	-0.246**	-0.329**	-0.470**	-0.503**	0.817**		0.770**	0.094ns	0.409**	0.163*	0.596**
RV	0.163*	-0.343**	-0.189**	-0.444**	-0.581**	0.501**	0.889**		0.647**	0.111ns	-0.332**	0.674**
ARD	-0.003ns	-0.374**	0.141*	-0.242**	-0.472**	-0.277**	0.294**	0.635**		-0.391**	-0.746**	0.391**
RT	0.064ns	-0.099ns	-0.431**	-0.341**	-0.241**	0.818**	0.635**	0.360**	-0.310**		0.327**	0.155*
SRL	-0.020ns	0.302**	-0.362**	-0.097ns	0.104ns	0.614**	0.149*	-0.211**	-0.716**	0.532**		-0.563**
RDW	0.243**	-0.375**	-0.014ns	-0.242**	-0.371**	0.328**	0.691**	0.803**	0.569**	0.211**	-.0507**	

RDW, root dry weight per plant; TRL, total length; RSA, root surface area; RV, root volume; ARD, average root diameter; RTN, roots tip number; SRL, specific root length; PUR, P uptake rate per plant; PUR_RDW, P uptake rate per unit root dry weight; PUR_RL, P uptake rate per unit root length; PUR_RSA, P uptake rate per unit root surface area; PUR_RV, P uptake rate per unit root volume. The upper-right diagonal and lower-left diagonal number indicated correlations obtained under LP condition and SP condition, respectively. "," "**," and "ns" indicate significance at p<0.05, p<0.01, and no significant correlation, respectively.*

PUR_RSA. These findings indicate that the P absorption efficiency, especially PUR_RDW and PUR_RSA, has a larger contribution to the P uptake capacity than root morphology under two P conditions; and the contribution of root morphology (mainly RSA) to P uptake capacity increased under LP treatment compared to the SP condition.

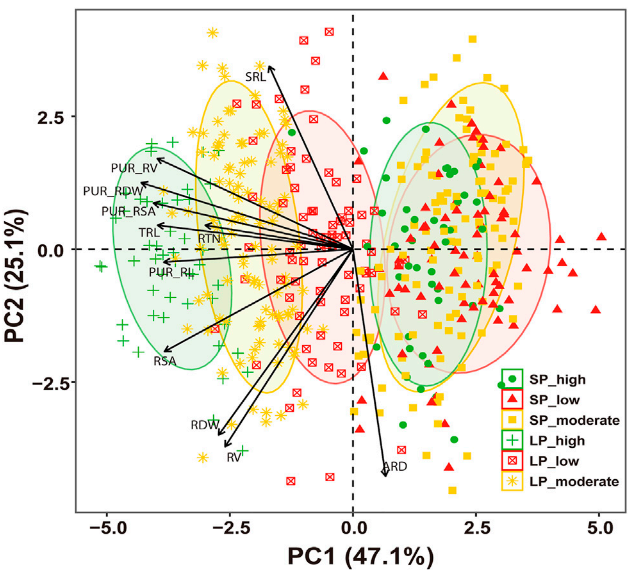

FIGURE 4 | Principal component analysis (PCA) of genotype and genotype × trait interaction. SP_high, high P uptake rate per plant under sufficient-P treatment; SP_low, low P uptake rate per plant under sufficient-P treatment; SP_moderate, moderate P uptake rate per plant under sufficient-P treatment; LP_high, high P uptake rate per plant under low-P treatment; LP_low, low P uptake rate per plant under low-P treatment; LP_moderate, moderate P uptake rate per plant under low-P treatment. RDW, root dry weight per plant; TRL, total toot length; RSA, root surface area; RV, root volume; ARD, average root diameter; RTN, root tip number; SRL, specific root length; PUR_RDW, P uptake rate per unit root dry weight; PUR_RL, P uptake rate per unit root length; PUR_RSA, P uptake rate per unit root surface area; PUR_RV, P uptake rate per unit root volume.

Screening and Evaluation of Germplasms With High P Uptake Capacity

To compare the P uptake differences among germplasms, PCA and HCPC were performed on the P absorption efficiency and root morphology of 48 germplasms with high P uptake capacity after LP treatment. As shown in **Figure 6A**, the first two PCA components represented 74.4% of the total variability. Based on the PCA scores, the germplasms with high P uptake capacity were divided into four groups via HCPC (**Figures 6B**, **7**). Group 1 (G1), encompassing 11 germplasms (such as EC7746 and Yangfanhuasheng), showed the higher PUR_RDW, PUR_RL, PUR_RSA, and PUR_RV after LP treatment but poor root morphology compared with other high P uptake capacity germplasms; group 2 (G2), encompassing 18 germplasms (such as Putiangoubi and Miandianhuasheng), showed medium to strong P absorption efficiency and well-developed root system after LP treatment; group 3 (G3), encompassing 7 germplasms (such as Huayu22 and Haiyangdalidun), presented the greatest PUR_RL, RV, ARD, and RDW, while the rest showed average behavior; and group 4 (G4), encompassing 12 germplasms (such as Fenghua4 and Sichuanhongbaili), showed higher RDW, TRL, RV, RSA, RTN, and SRL after LP treatment, but P absorption efficiency was lower than that in other high P uptake capacity germplasms. Under SP conditions, similar behaviors were found in the root morphology between four groups with LP treatment, but there was no significant difference in P absorption efficiency. These results suggest that the trade-off and coordination between P absorption efficiency and root morphology could result in similar levels of total P uptake under LP stress.

DISCUSSION

P Uptake Strategies of Peanut Germplasm Under LP Stress

With the decrease in available P in soil, it is more and more important to select varieties with high P uptake efficiency and P utilization efficiency. Identification and evaluation of the P-efficient germplasms have been studied in many crops, including wheat (Soumya et al., 2021), rice (Kale et al., 2020), and soybean (Wang et al., 2010b). P-efficient germplasms are suitable for growth under LP conditions, which is conducive to decreasing the P fertilizer utilization and protecting the environment. In our study, 48 germplasms with higher P uptake capacity, possessing the stronger P absorption efficiency and well-developed root system under LP stress (**Figure 3**), were selected. These excellent varieties include both main cultivars (Zhonghua4 and Fenghua5), which are dominant in certain areas of cultivation, and some selected germplasms (02P175, etc.). This provides a basis for the selection and utilization of P-efficient germplasms in peanuts.

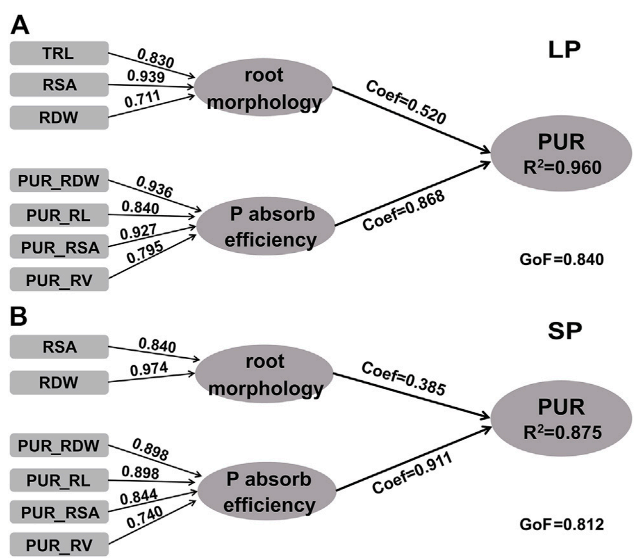

FIGURE 5 | Partial least square-path models predicting PUR. **(A)** Low-P (LP) condition and **(B)** sufficient-P (SP) condition. Coef is the path coefficient. GoF, goodness of fit of the model; R^2, coefficients of determination of the endogenous latent variables; RDW, root dry weight per plant; TRL, total toot length; RSA, root surface area; RV, root volume; ARD, average root diameter; RTN, root tip number; SRL, specific root length; PUR, P uptake rate per plant; PUR_RDW, P uptake rate per unit root dry weight; PUR_RL, P uptake rate per unit root length; PUR_RSA, P uptake rate per unit root surface area; PUR_RV, P uptake rate per unit root volume. Coef are significant at 95% confidence intervals (p value < 0.05).

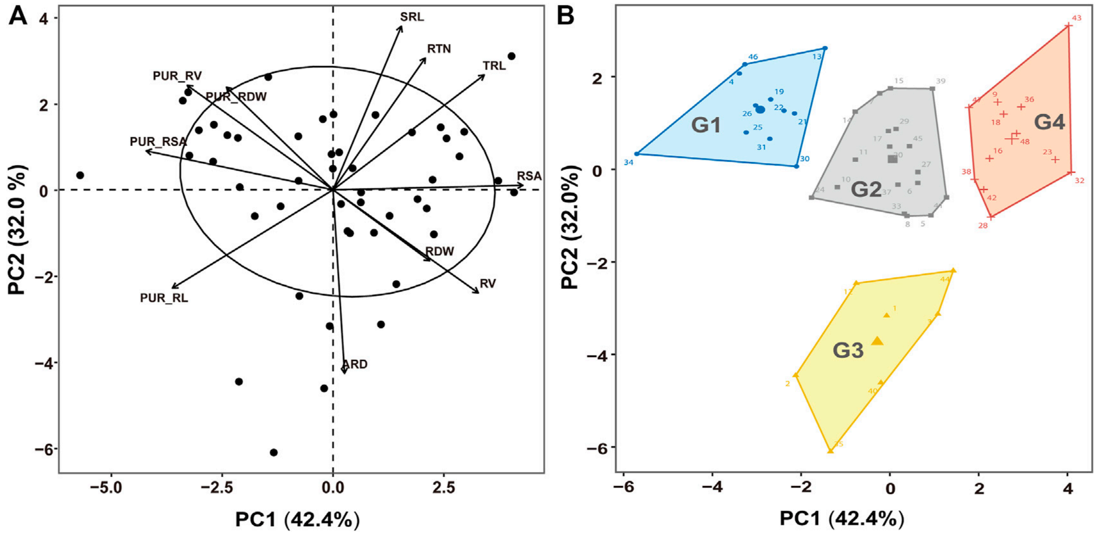

FIGURE 6 | Principal component analysis (PCA) of P absorption efficiency traits and root traits under low-P (LP) treatment. **(A)** Variable covariance along with the first two components and **(B)** clusters formed with hierarchical classification on principal components (HCPC). RDW, root dry weight per plant; TRL, total toot length; RSA, root surface area; RV, root volume; ARD, average root diameter; RTN, root tip number; SRL, specific root length; PUR_RDW, P uptake rate per unit root dry weight; PUR_RL, P uptake rate per unit root length; PUR_RSA, P uptake rate per unit root surface area; PUR_RV, P uptake rate per unit root volume.

Although both P absorption efficiency and root morphology are essential for P acquisition, their costs and benefits in a specific context resulted in multiple coordination and trade-offs among traits (Lynch and Ho, 2005; Raven et al., 2018). Different P-uptake strategies were formed among germplasms (Lyu et al., 2016; Wen et al., 2019; Honvault et al., 2020). In this study, three P uptake strategies were identified among germplasms with high P uptake capacity according to their P absorption efficiency and root morphology (**Figure 7**). The first strategy presented a well-developed root system (with the largest TRL and RSA) but lower P absorption efficiency, encompassing Sichuanhongbaili, 51015-2, etc. This strategy was probably oriented toward available P uptake area, a well-developed root system could increase soil foraging, and lager TRL and RSA denote an important exploration of a given soil volume (Liu, 2021). The other strategy presented stronger P absorption efficiency, possessing the highest PUR_RDW, PUR_RSA, and PUR_RV, but poor root morphology compared with other high-P uptake capacity germplasms, encompassing Funongbaipi and Yinyou5. The high P absorption efficiency of these germplasms is probably due to the higher activity of P transporters in the root (Sun et al., 2012; Liu C. et al., 2018). The third strategy focused on the interaction of the root morphology and P absorption efficiency, including two situations: one is that all traits showed good performance; for example, Zhonghua4 had both stronger P absorption efficiency and a well-developed root system; the other presented high expressions of partly traits; for example, Huayu22 showed outstanding performance in PUR_RL, RV, and ARD, with other traits showing poor performance. This phenomenon also indicates that the regulation and interaction between traits are very complex (McCormack et al., 2015; Honvault et al., 2020). In practical application, the selection of germplasms with optimal P uptake strategies according to the change of environment not only improves the application value of germplasms but also the environmental P utilization efficiency.

The Variation of Root Morphology and P Absorption Efficiency After LP Treatment and Their Effects on P Uptake Capacity

The P uptake efficiency varied widely among genotypes and environments (Wissuwa and Ae, 2001; Patricio, 2016). The improvement of P uptake capacity is the key to coping with P-deficiency stress. Our results also demonstrated this view (**Table 2**, **Figure 1**). The higher P uptake efficiency is generally achieved by modifying root growth, such as superficial root architecture, exudation of organic acid anions (Wang et al., 2015; Falk et al., 2020; Bello, 2021; Liu, 2021), or enhancing the ability to transport P from the root surface into the plant body (such as the expression of high-affinity Pi transporters) (Sun et al., 2012; Lapis-Gaza et al., 2014). In this study, the P absorption efficiency and root morphology were significantly correlated with the P uptake capacity after LP treatment (**Table 3**), which showed that a higher P uptake capacity may be related to a stronger P absorption efficiency and well-developed root system.

The influx of P in soil is mainly by diffusion, but poor diffusion rates and mobility result in insufficient effectiveness of soil rhizosphere P, so modifying root growth plays an important role in P uptake (Havlin, 1999; Liu, 2021). It has been reported in rice (Vejchasarn et al., 2016), wheat (Deng et al., 2018), maize (Liu Z. et al., 2018), and cotton (Mai et al., 2018) that root trait response to P nutrition is complex and shows different variations among genotypes. For peanuts, the study of the changes in the root morphology under LP conditions is limited; only root length,

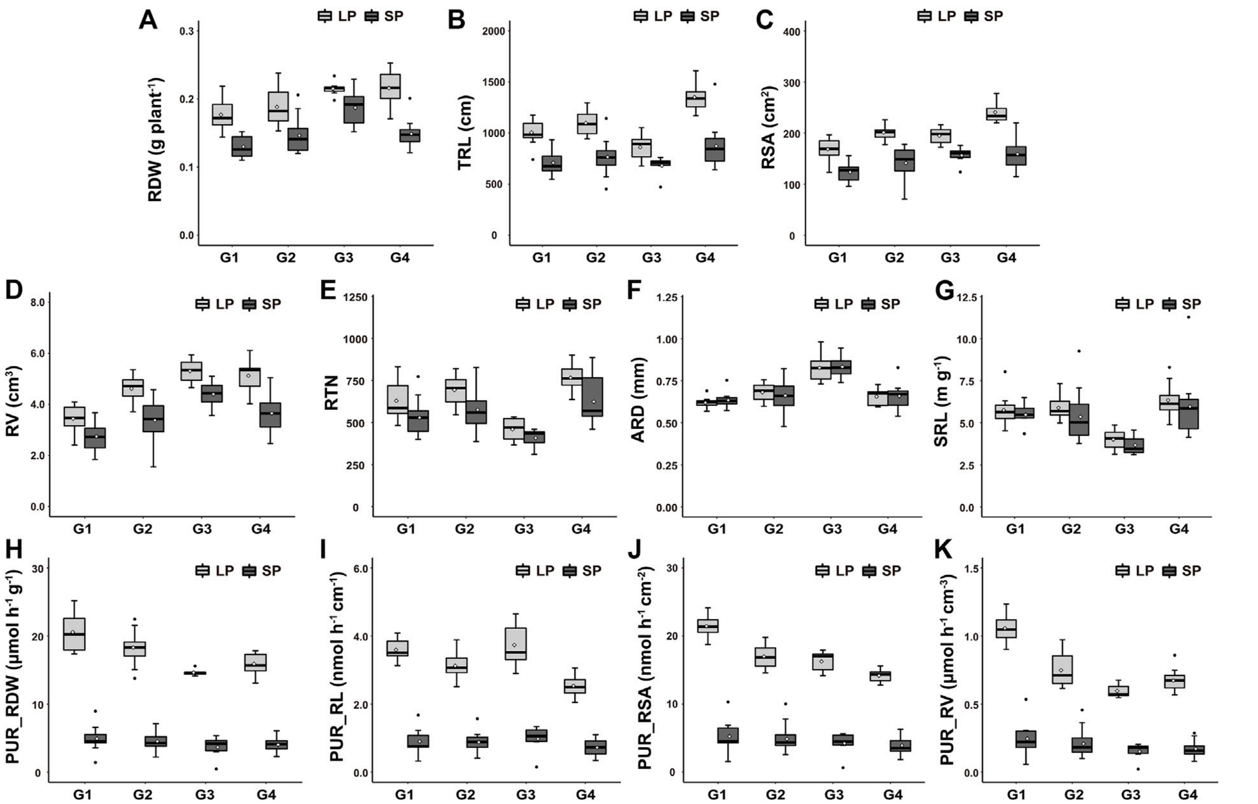

FIGURE 7 | Box and whisker plot showing differences in 11 traits among high-P uptake capacity peanut germplasms under low-P (LP) and sufficient-P (SP) treatments. **(A)** Root dry weight per plant (RDW), **(B)** total toot length (TRL), **(C)** root surface area (RSA), **(D)** root volume (RV), **(E)** root tip number (RTN), **(F)** average root diameter (ARD), **(G)** specific root length (SRL), **(H)** P uptake rate per unit root dry weight (PUR_RDW), **(I)** P uptake rate per unit root length (PUR_RL), **(J)** P uptake rate per unit root surface area (PUR_RSA), and **(K)** P uptake rate per unit root volume (PUR_RV). High, moderate, and low represent high, moderate, and low P uptake rates per plant, respectively. Horizontal lines inside boxes, median; white dot, mean; box hinges, first and third quartiles; whiskers, full range of the data; black dots represent outliers.

root dry weight, secondary root number, and root volume have been studied (Krishna, 1997; Jadhav and Gowda, 2012; Kumar et al., 2015); and only dozens of peanut varieties were used in some studies (Krishna, 1997; Jadhav and Gowda, 2012). In this study, we evaluated 7 root morphology traits of 235 peanut germplasms with a broad geographic distribution and significant phenotypic variation in P efficiency. Extensive and significant differences in roots were found among germplasms after LP treatment (**Table 2**; **Figure 1**). For example, PI274193 significantly increased root dry matter accumulation, Baisha2-3 and Sichuanhongbaili display longer main roots and more lateral roots, Meiyinxuan41165 and Lainong10 exhibit longer lateral roots, Grif 12545 and Toalson showed more and longer lateral roots (**Figure 1**, **Supplementary Figure S3**), while Silicai (1), Ji0608-228, and 98D080-2 showed shorter root length and less lateral roots, and Baisha1016 and 03F032 were less affected (**Supplementary Figure S2**). This comprehensively reflected the diversity of root changes among peanut germplasms under LP conditions. Previous studies have found that the differences in root morphology traits such as root configuration and root length resulted in the change in P uptake efficiency among crops and varieties (Bello, 2021; Liu, 2021). In this study, significantly positive correlations were found between the PUR and the RDW, TRL RV, RSA, and RT after LP treatment (**Table 3**). Similar results were also found in maize (Gu et al., 2016) and wheat (Soumya et al., 2021). On this basis, we further underlined the RSA was the most impactful contributor to the P uptake capacity, followed by the TRL and RDW under LP treatment through PLS PM (**Figure 5**). This is mainly because lager RSA and TRL lead to a lager P uptake area, which is conducive to higher P uptake capacity (Liu, 2021). The aforementioned results were rarely reported in peanuts. These findings revealed the changes of root morphology traits among peanut germplasms under LP stress and proposed that root traits mainly influence P uptake capacity.

The plasma membrane-localized Pi transporter in the root regulates the P uptake (Sudhakar et al., 2018; Wang et al., 2018). The high-affinity transporter activity was enhanced after LP treatment, and a low concentration of extracellular P could be transported across the membrane into the cell, which was the main way to absorb P under LP conditions (Sudhakar et al., 2018; Wang et al., 2018; Liao et al., 2019). The transmembrane transfer activity can be improved by increasing the amount of transporter proteins while the unit transporter activity is unchanged. For example, the overexpression of *ZmPT7* in maize (Wang et al., 2020), *OsPHT1;3* and *OsPht1;8* in rice (Jia et al., 2011; Chang et al., 2019), and *TaPht1;4* in wheat (Liu et al., 2013) significantly enhanced the P uptake capacity, while the knockout lines represented reduced P uptake capacity. These may be the underlying mechanisms of high P absorption efficiency in some germplasms. Interestingly, we found that P absorption efficiency was positively correlated with P uptake capacity (**Table 3**; **Figure 5**). The contributor for the P absorption efficiency component was PUR_RDW > PUR_RSA > PUR_RL > PUR_RV, and the path coefficient of PUR_RDW and PUR_RSA was greater than 0.9. An increase in transporting activity of each transporter protein also increased P uptake capacity. In this study, the P absorption efficiency showed extensive variations among germplasms after LP treatment, but most germplasms significantly increased and showed different increasing ranges (**Table 2**; **Figure 2**). We hypothesized that the expression level of Pi transporter-encoding genes or transporting activity of Pi transporter was induced under LP stress in these germplasms. This will be helpful in the identification of high-affinity Pi transporters, selection, and breeding of high P-efficient cultivars.

While both higher P absorption efficiency and root morphology should benefit the P uptake, their contribution varied in different crops, varieties, and P conditions (Lyu et al., 2016; Wen et al., 2019; Honvault et al., 2020). Identification of P efficiency in the field requires heavy workload and a long working cycle and is susceptible to environmental factors. In this study, we found that P absorption efficiency was the critical factor affecting P uptake capacity for peanuts. After LP treatment, the correlation between P uptake capacity and P absorption efficiency (correlation coefficient >0.6) was larger than that between P uptake capacity and root morphology (correlation coefficient <0.5) (**Table 3**). PLS-PM also proved that the effect of P absorption efficiency on P uptake capacity (coef = 0.868) was greater than that of root morphology (coef = 0.520) (**Figure 5**). These findings provide new insights into the relationship between P uptake capacity and root morphology/P absorption efficiency and furnish important evaluation indexes for high P-efficient uptake germplasm selection. These results provide a reference for functional gene screening, mechanism studies, and further identification in the field.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Conceptualization: YW, KZ and FL; methodology: SZ; software: SZ and LL; validation: SZ, LL and XZ; investigation: SZ, MZ, XW, JZ, XL, QW and LL; resources: YW and KZ; writing—original draft preparation: SZ; writing—review and editing: LL, YW and SZ; visualization: SZ and LL; project administration: YW, KZ and FL; and funding acquisition: KZ and FL. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.855815/full#supplementary-material

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Link Between Aeration in the Rhizosphere and P-Acquisition Strategies: Constructing Efficient Vegetable Root Morphology

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Excessive application of phosphate fertilizer is common in vegetable fields and causes deterioration of the rhizosphere environment, that is, the soil oxygen (O_2) environment, which further constrains root morphology construction and limits vegetable yield. Nevertheless, the interaction between root morphology and the response of the rhizosphere O_2 environment to vegetable P utilization has rarely been reported. Therefore, we carried out an experiment applying different concentrations of O_2 generator, 10%, 30%, 50%, and 80% urea hydrogen peroxide (as pure nitrogen) instead of urea as a top dressing in the rhizosphere, to study the effect on root morphology and P adsorption, and its mechanism. We found that there were O_2-deficient and P-deficient zones in the rhizosphere, and oxygenation could alleviate the rhizosphere O_2 and P consumption in roots. The rhizosphere O_2 concentration was maintained at approximately 250.6 μmol L^{-1}, which significantly promoted total root length, root volume, average diameter, and root activity by 29.0%, 30.9%, 3.9%, and 111.2%, respectively. Oxygenation promoted organic P mineralization and increased the Olsen-P content in the rhizosphere. The characteristics of root morphology and increased available P in the rhizosphere jointly contributed to high P absorption and utilization, and the P use efficiency was improved by 9.3% and the shoot P accumulation by 10.9% in the 30% urea hydrogen peroxide treatment compared with CK. Moreover, this treatment also improved yield and quality, including vitamin C and the soluble sugar content. However, at a still higher O_2 concentration (260.8 μmol L^{-1}), vegetable growth exhibited O_2 damage, resulting in reduced yield and quality. Our study provided new insights into constructing efficient root morphology by regulating the rhizosphere O_2 environment to improve vegetable yield and quality, as well as to increase P use efficiency in vegetable fields.

Keywords: aeration, vegetable, phosphorus, root morphology, urea hydrogen peroxide

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INTRODUCTION

Driven by economic growth, China has undergone a rapid increase in vegetable production: the area devoted to vegetables has been increased from 3.3 million hm^2 in 1976 to 21.5 million hm^2 in 2020, which now accounts for 12.8% of the farmland area in China (NBSC 2020). In intensive vegetable production systems, farmers often apply large amounts of fertilizer, especially phosphate fertilizers,

TABLE 1 | Fertilizer application amounts and times.

Treatment	Base fertilizer (mg kg^{-1})			Top dressing at 13 days (mg kg^{-1})		Top dressing at 23 days (mg kg^{-1})	
	Urea-N	P	K	Urea-N	UHP-N	Urea-N	UHP-N
CK	80	80	120	40	0	40	0
UHP1 (10%)	80	80	120	36	4	36	4
UHP2 (30%)	80	80	120	28	12	28	12
UHP3 (50%)	80	80	120	20	20	20	20
UHP4 (80%)	80	80	120	8	32	8	32

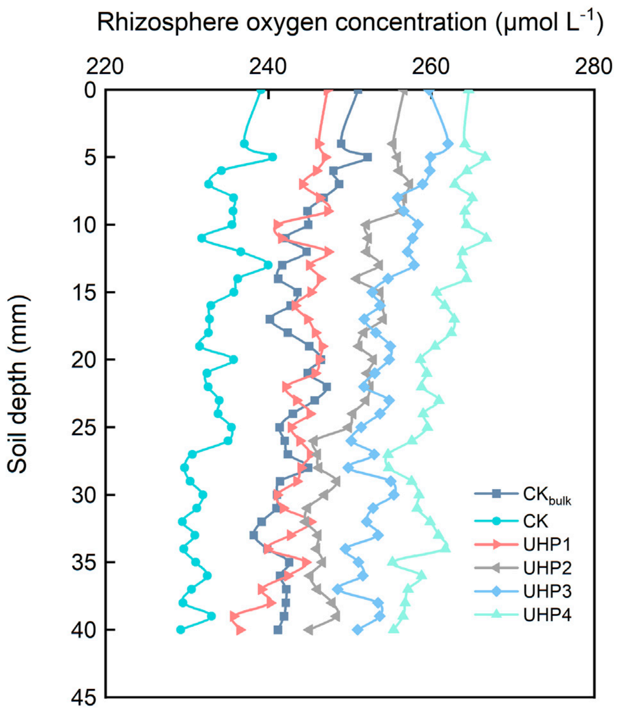

FIGURE 1 | Representative graph showing the O_2 profiles of the rhizosphere soil (4 cm away from the root surface) harvested at 25°C. CK_{bulk} represents no UHP application and no amaranth growth; CK represents no UHP application. 3 mm from the soil surface was defined as "0" point. The O_2 profiles obtained in each replication of the same treatment were generally similar, and results of triple averaging are shown.

to obtain high yields (Liang et al., 2013). The nitrogen (N) fertilization applied to vegetables was 1.26 times that of upland crops per season in China, while the phosphorus (P) fertilization of vegetable fields was 1.85 times that of upland crops per season in China (Wang et al., 2019). Based on the published field studies in China, the P fertilizer applied in greenhouse (571 kg P hm^{-2}) and open-field (117 kg P hm^{-2}) vegetable systems exceeded the P removal in harvested vegetables (44 and 25 kg P hm^{-2} in greenhouse and open-field vegetable systems, respectively) per season, and organic P application accounted for half of the total P fertilization (Yan et al., 2014). However, most P can be fixed by iron and manganese oxides or locked up in other insoluble forms (Hao et al., 2002; McBeath et al., 2005). The root system, as the main interface between the plant and soil, is important for nutrient and water uptake, and storage (Tian et al., 2014). Vegetable roots are characterized by comparatively low root length densities and a shallow distribution, thus requiring high soil P concentrations in the root zone for optimal production (Yan et al., 2014). However, the diffusion of P to the vegetable root zone is the major way for P absorption; since P uptake occurs at a much higher rate than P diffusion within the root-zone soil, a P depletion zone is quickly established (Morgan and Connolly 2013). Therefore, there is a spatial dislocation between P uptake of vegetable root and P supply, which cannot be completely solved by a large amount of P fertilizer applied.

Moreover, massive and repeated P fertilization could lead to soil compaction because the phosphate ions combine with cations such as Al^{3+}, Fe^{3+}, Ca^{2+}, and Mg^{2+} in the soil to form insoluble phosphates, and the decreased cation content in the soil solution decreases the soil structure stability. Long-term intensive tillage, such as high-density planting and a high cropping index, also causes soil compaction. Soil compaction reduces the soil air capacity (Schnurr-Putz et al., 2006). The degradation of organic matter accompanied by high consumption of O_2 further decreases the soil O_2 content since massive amounts of organic fertilizer are customarily applied in vegetable fields (Ratering and Schnell 2001; Li et al., 2014). Indeed, the phenomenon of O_2 limitation occurs even within seemingly aerobic, well-drained soils. The lack of soil O_2 directly affects root growth because root growth is sensitive to O_2 deficiency, which reduces root elongation (Aguilar et al., 2003). The root growth of wheat is suppressed, and yield is substantially decreased when soil O_2 levels are below the critical level (Meyer et al., 1985). The poor mobility of P in the soil, accompanied by the decreased P uptake ability resulting from the poor growth of the root system caused by hypoxia, further reduces P use efficiency (PUE) and yield. Therefore, the rhizosphere O_2 environment is crucial to efficient P uptake, and vegetables might not reach their maximum biological potential in poor rhizosphere environments, that is, at low O_2 concentrations.

Soil aeration is considered the third most important factor, after water and nutrient availability, in affecting soil fertility (Ben-Noah et al., 2021) and accelerating the yield of crops and vegetables (Zhu et al., 2015; Li et al., 2016a; Li et al., 2020). Several techniques for aeration of the root zone have been developed, including physical and chemical oxygenation. Physical oxygenation usually includes pumping pressurized air or sucking air (bubbles) into the irrigation water (Goorahoo et al., 2002; Bhattarai et al., 2004; Bhattarai et al., 2006; Abuarab et al., 2013). Chemical oxygenation is achieved by adding various peroxides (usually considered a kind of O_2

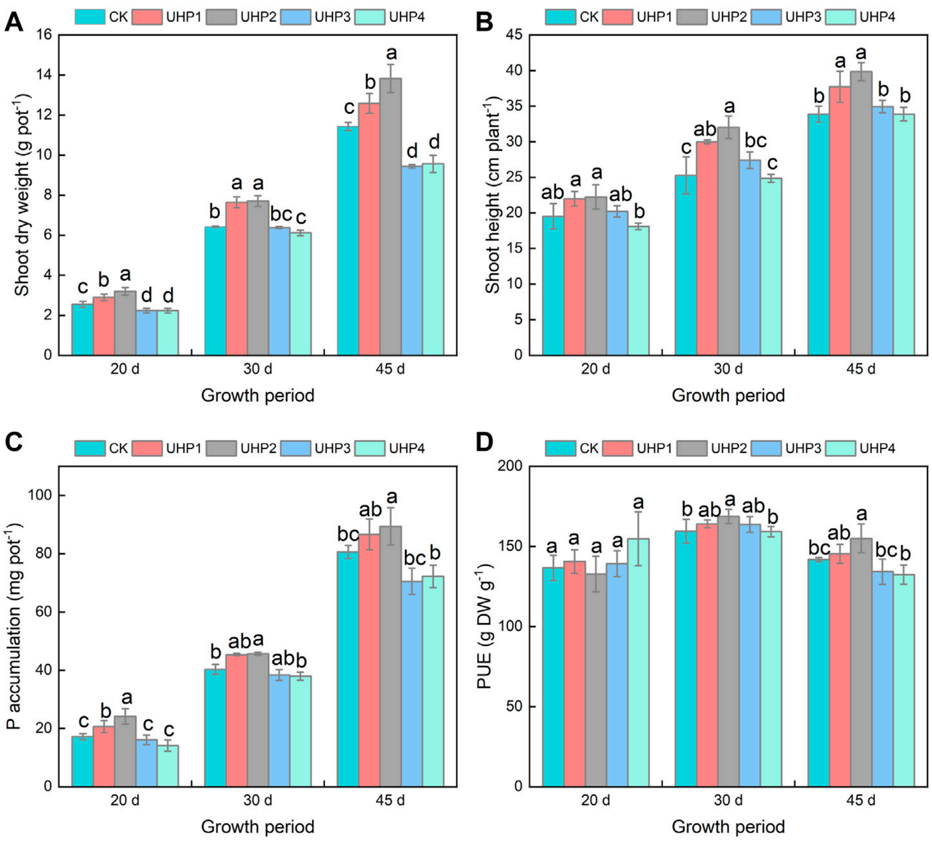

FIGURE 2 | Effects of aeration on the growth, absorption, and utilization of P in amaranth at different growth stages. **(A)** Shoot dry weight, **(B)** shoot height, **(C)** P accumulation, and **(D)** phosphorus use efficiency (PUE). Bars indicate mean ± SD. Different letters on the bars indicate a significant difference ($p < 0.05$) between different treatments in the same growth period.

TABLE 2 | Effects of aeration on root architecture of amaranth at different growth stages.

Growth stage (d)	Treatment	Total root length (cm)	Total root surface area (cm^2)	Root average diameter (mm)	Total root volume (cm^3)	Specific root length (mg^{-1})
20	CK	598 ± 9.93c	119 ± 1.86c	0.97 ± 0.14b	1.78 ± 0.07b	39.1 ± 2.41b
	UHP1	730 ± 4.61b	148 ± 0.93b	0.98 ± 0.12b	1.92 ± 0.08b	45.7 ± 2.59a
	UHP2	829 ± 16.9a	190 ± 3.00a	1.21 ± 0.07a	2.17 ± 0.13a	46.1 ± 2.41a
	UHP3	527 ± 25.9d	90.6 ± 2.96d	0.68 ± 0.05c	1.53 ± 0.11c	39.6 ± 2.46b
	UHP4	487 ± 21.2d	89.5 ± 2.83d	0.68 ± 0.04c	1.48 ± 0.02c	37.7 ± 4.53b
30	CK	1,656 ± 4.64c	375 ± 4.27b	1.33 ± 0.02b	5.00 ± 0.06b	23.0 ± 0.48c
	UHP1	2,173 ± 53.7b	388 ± 7.12a	1.33 ± 0.03b	5.76 ± 0.38a	28.7 ± 0.37b
	UHP2	2,553 ± 29.3a	392 ± 4.78a	1.49 ± 0.07a	5.98 ± 0.67a	33.8 ± 0.52a
	UHP3	1,609 ± 21.5c	317 ± 3.93c	1.25 ± 0.02c	4.32 ± 0.05c	24.0 ± 0.17cd
	UHP4	1,448 ± 15.1d	312 ± 1.74c	1.22 ± 0.01c	3.97 ± 0.09c	22.6 ± 1.15d
45	CK	2,627 ± 13.2c	386 ± 2.98b	1.79 ± 0.03b	6.84 ± 0.05c	22.5 ± 0.53b
	UHP1	3,090 ± 25.8b	400 ± 4.26a	1.85 ± 0.01a	8.47 ± 0.08b	24.6 ± 0.51a
	UHP2	3,389 ± 104a	402 ± 4.99a	1.86 ± 0.01a	8.96 ± 0.04a	25.6 ± 0.63a
	UHP3	2,116 ± 87.9d	354 ± 3.75c	1.72 ± 0.07c	5.80 ± 0.17d	21.3 ± 0.70c
	UHP4	1,601 ± 66.3e	311 ± 2.36d	1.60 ± 0.04d	5.67 ± 0.12d	16.2 ± 1.11d

fertilizer), such as H_2O_2, urea hydrogen peroxide (UHP), and potassium peroxide, into the soil or irrigation water (Bryce et al., 1982; Bhattarai et al., 2004; Urrestarazu and Mazuela, 2005). Most studies have concentrated on physical oxygenation in vegetable production (Goorahoo et al., 2002; Li et al., 2016b). However, the larger scale use of physical oxygenation has been limited by oxygenation equipment, non-uniform aeration in the field, and the limited time supersaturated O_2 remains dissolved in irrigation water (Lei et al., 2016). Oxygen fertilizer is convenient and targeted, providing O_2 in the root zone by direct application

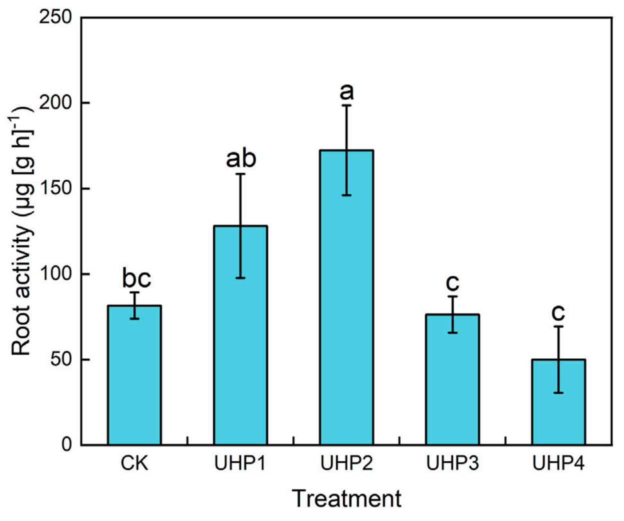

FIGURE 3 | Effects of aeration on the root activity of amaranth at harvest. Bars indicate the mean ± SD. Different letters on the bars indicate significant differences (p < 0.05).

to the rhizosphere. Numerous studies have concentrated on the use of O_2 fertilizer in flooded conditions, especially the use of UHP in paddy fields. UHP is an environmentally friendly hydrogen peroxide that provides higher O_2 concentrations than physical oxygenation by irrigation water venting (Frankenberger, 1997). It has been proven that UHP can increase rice yields by 3.1%–11.5% compared with the control (Zhao et al., 2010). However, there have been few studies on the effect of UHP on the P uptake of vegetables. Thus, the experiments in this study were carried out to study the effect and mechanism of UHP applied to the rhizosphere as an oxygen generator on vegetable yield and P adsorption to realize efficient utilization of vegetable phosphate fertilizer.

MATERIALS AND METHODS

Soil Collection

Soil samples were collected from open vegetable fields where Chinese cabbage had been cultivated for 7 years in Wuxi, Jiangsu Province (China) (31°23′ N, 119°58′ E). The region has a subtropical monsoon climate with a mean air temperature of 15.6 C and an annual precipitation of 1,100 mm. The soil properties were as follows: 15.6 g kg^{-1} soil organic matter (SOM), 0.89 g kg^{-1} total nitrogen (TN), 0.82 g kg^{-1} total phosphorus (TP), 119.2 mg kg^{-1} Olsen-P, and 6.35 pH (with a soil: water ratio of 1:2.5). The soil samples were air-dried, ground, sieved through a 0.85-mm mesh, and stored for further incubation experiments.

Experimental Design and Sample Collection

The experiment was carried out to study the effect of optimizing urea hydrogen peroxide (UHP) input on amaranth

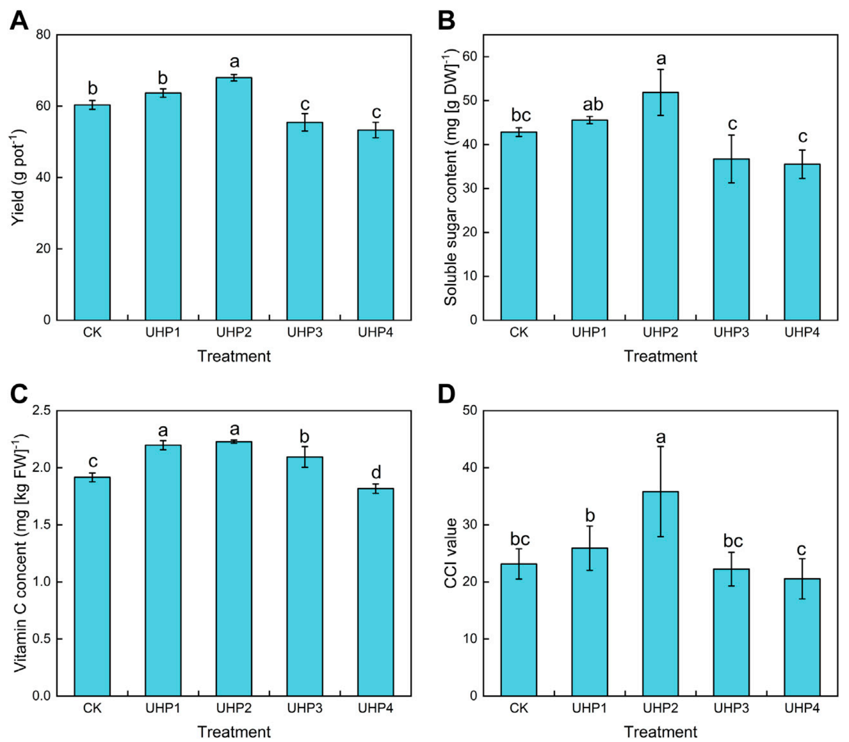

FIGURE 4 | Effects of aeration on amaranth yield and quality at harvest. **(A)** Yield, **(B)** soluble sugar content, **(C)** ascorbic acid (vitamin C) content, and **(D)** chlorophyll content index (CCI). Bars indicate mean ± SD. Different letters on the bars indicate a significant difference between different treatments.

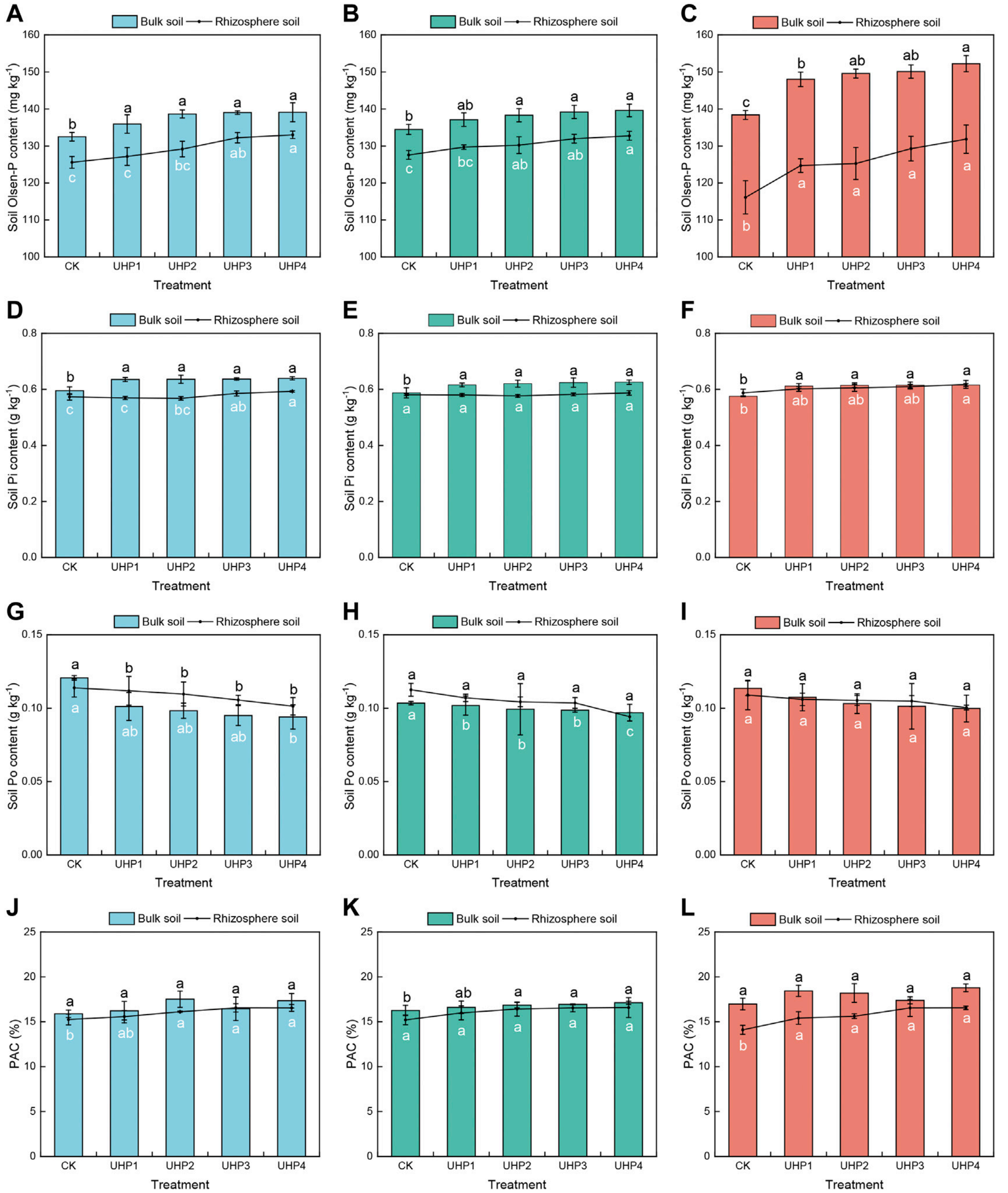

FIGURE 5 | Effects of aeration on the P turnover of amaranth rhizosphere soil and bulk soil at different growth stages. **(A)** Soil Olsen-P content at 20 days, **(B)** soil Olsen-P content at 30 days, **(C)** soil Olsen-P content at 45 days, **(D)** soil inorganic P (Pi) content at 20 days, **(E)** soil Pi content at 30 days, **(F)** soil Pi at 45 days, **(G)** soil organic P (Po) content at 20 days, **(H)** soil Po content at 30 days, **(I)** soil Po at 45 days, **(J)** P activation coefficient (PAC) at 20 days, **(K)** PAC content at 30 days, and **(L)** PAC at 45 days. Black letters represent significant differences in bulk soil, while white letters represent rhizosphere soil. Bars indicate mean ± SD. Different letters on the bars indicate a significant difference between different treatments.

(*A. mangostanus*) growth. Based on our pre-experiments, the UHP concentration gradient included five treatments: no UHP(CK), 10% UHP (UHP1), 30% UHP (UHP2), 50% UHP (UHP3), and 80% UHP (UHP4), as a replacement for urea, according to the principle of equal N fertilization in top dressing. A 2.5-kg sample of soil mixed thoroughly with urea (80 mg N kg^{-1}, 50% of the total N fertilization), $CaP_2H_4O_8$ (80 mg P_2O_5 kg^{-1}), and K_2SO_4 (120 mg K_2O kg^{-1}) as a base fertilizer, according to the conventional amounts used by the local farmers, was used to fill each pot. All pots were moistened with tap water and equilibrated for 24 h before sowing amaranth by the direct-seeding method. The remaining N fertilizer (50% N) was divided equally and applied twice as top dressing at 13 days and 23 days after sowing. The detailed fertilization strategy is shown in **Table 1**. UHP was applied in aqueous solution injected by a syringe into the rhizosphere (around the root base) at a depth of 4 cm.

Amaranth seeds were immersed in NaClO (1%) for 20 min and then washed thoroughly with tap water. The seeds were then immersed in water for 3 h before being placed on a moist filter paper to germinate at 25 C overnight in darkness. Fifteen seeds were sown in each pot, and 2-week-old seedlings were thinned to eight with similar growth. To exclude the influence of amaranth roots, one more treatment without amaranth planting was defined as bulk soil. CK_{bulk} represents the treatment without amaranth growth and UHP addition, which reflects the background soil O_2 concentration. The moisture content in the soil was maintained at 60% of the field water-holding capacity using daily distilled water replenishment. Sampling was performed at 20 days (seedling stage), 30 days (growth period, 7 days after the first UHP application), and 45 days (harvest, 7 days after the second UHP application) of amaranth growth. The fresh weights of amaranth shoots and roots were determined, and the materials were dried at 105 C for 30 min and then to constant weight at 75 C for 48 h to determine the dry weight. The rhizosphere soil of the amaranth root was gathered by removing the loose soil and collecting the remaining soil that was tightly adhered to the roots, which was then air-dried for chemical analysis.

Soil Property Analysis

Soil pH was measured using a pH meter (Beckman, Inc. in California) with a soil: water ratio of 1:2.5, inorganic P (Pi) was determined by H_2SO_4 extraction and followed by the Mo–Sb anti-spectrophotometric method, and organic P (P_O) was measured by the ignition method (Bao, 2000); Olsen-P was extracted with 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) for 30 min on an orbital shaker (180 rpm, 25 C) at a soil solution ratio of 1:20 (Olsen et al., 1954); total P (TP) was measured using the $HClO_4$–H_2SO_4 digestion and Mo–Sb anti-spectrophotometric methods (Liu, 1996). The P activation coefficient (PAC) was the ratio of Olsen-P to TP, which reflected the soil P availability and was an important indicator of soil fertility (Wu et al., 2017).

Root Morphology and Aboveground Growth Parameters

Root morphological parameters, including total root length, root surface area, root volume, and average diameter, were analyzed using root analysis instrument WinRhizo-LA1600 (Regent Instruments Inc., Quebec, QC, Canada). The aboveground growth was monitored by shoot height, dry weight, and leaf fresh weight (yield). The specific root length (SRL) was calculated as the fresh length of the root sample divided by its dry mass (m g^{-1}). The root activity was monitored using the triphenyltetrazolium chloride (TTC) method (Lindström and Nyström, 1987). TTC is reduced by dehydrogenases, and the dehydrogenase activity is regarded as an indicator of the root activity (Li et al., 2011). The P concentration in shoots was determined after digestion with a mixture of 5 ml of concentrated sulfuric acid and 8 ml of 30% v/v H_2O_2, followed by spectrophotometric analysis using the molybdovanado phosphate method (UVmini-1240; Shimadzu, Kyoto, Japan) (Johnson and Ulrich, 1959). P accumulation (mg pot^{-1}) and P use efficiency (PUE) (g DW g^{-1}) were calculated by **Eq. 1** and **Eq. 2**. The concentration of leaf soluble sugars was determined using the anthrone method for spectrophotometric determination (Fales, 1951). Ascorbic acid (vitamin C) was measured using the titration method with 2,6-dichloroindophenol (Commission, 2016). The chlorophyll content index (CCI) values of leaves were quantified using a handheld CCM-200 chlorophyll meter (Opti-Sciences, USA), which is a reliable method for estimating chlorophyll contents (Silla et al., 2010).

$$\textbf{P accumulation} = \textbf{Dry weight} * \textbf{P concetration} \quad (1)$$

$$\textbf{PUE} = \frac{\textbf{Dry weight}}{\textbf{P accumulation}} \quad (2)$$

In Situ Measurements of Soil O_2 Concentration

The O_2 microelectrode, a miniaturized Clark-type O_2 electrode with a guard cathode (OX 50, ø = 40–60 μm, Unisense, Aarhus, Denmark) (Revsbech, 1989), was used to measure soil O_2 concentration *in situ* at harvest (45 days of amaranth growth). The sampling plant for each replication pot was chosen randomly, and the soil O_2 profile measurements were performed in the center of the range at a horizontal distance of 1 cm from the plant root base with a depth of 4 cm for all treatments. A micromanipulator (MM33-2, Unisense) with a motor controller (MC-232, Unisense) was used and fixed in a lab stand (LS18, Unisense) to avoid shaking during the microelectrode movement, and treatment was performed with a 1 mm depth interval measurement, and the periods for "wait before measure" and "measure" were both set to 5 s.

Data Analysis

Data were analyzed statistically by statistical software program SPSS version 20 (SPSS Inc., Chicago, IL, USA). Means were compared using one-way analysis of variance (ANOVA) with Duncan's multiple-range test. Significant differences ($p < 0.05$) between treatments are indicated by different letters in the figure and table legends. Graphs were produced using Origin Pro 2021.

RESULTS

Distribution of the Soil O_2 Concentration

The distribution of the soil O_2 concentration in the rhizosphere soil was used to clarify the aeration effect of UHP application. Although certain values at certain depths showed abnormal peaks, the O_2 concentration decreased slowly with increasing soil depth and increased with increasing UHP concentration (**Figure 1**). The average values were approximately 233.4, 242.9, 250.6, 254.3, and 260.8 μmol L^{-1} in the CK, UHP1, UHP2, UHP3, and UHP4 treatments, respectively (**Figure 1**). The soil O_2 concentration under the CK_{bulk} treatment (no UHP applied and without amaranth growth) was significantly higher than that of the CK treatment (**Figure 1**). Consumption of O_2 by the amaranth root resulted in an "O_2-deficient zone" in the rhizosphere.

Effects of Aeration on the Growth, P Accumulation, and PUE of Amaranth

The shoot dry weight of the UHP2 treatment was significantly higher than that of CK, especially at 20 days, when it was 25.1% higher than that of CK; 30 days, when it was 20.1% higher; and 45 days, when it was 21.0% higher. However, the shoot dry weight showed significant decrease in the UHP3 and UHP4 treatments compared with CK (**Figure 2A**). The shoot height of all the plants under the UHP1, UHP2, and UHP3 treatments was higher than that under the CK treatment, and the tallest plants were grown under the UHP2 treatment, with an increase of 17.7% compared with CK at harvest (**Figure 2B**), while there were no significant differences among the UHP3, UHP4, and CK treatments (**Figure 2B**). The P accumulation in the shoots of the UHP2 treatments was significantly higher than that in CK, with increases of 40.7% at 20 days, 13.4% at 30 days, and 10.9% at 45 days (**Figure 2C**). The differences in shoot PUE among different treatments at 20 days were not prominent (**Figure 2D**); PUE in the shoots of UHP2 was significantly increased by 5.8% at 30 days and 9.3% at 45 days compared with that in CK ($p < 0.05$) (**Figure 2D**).

Effects of Aeration on the Root Architecture and Activity of Amaranth at Different Growth Stages

The UHP2 treatment resulted in larger root systems than the other treatments, especially total root length and surface area. The total root length under the UHP2 treatment was significantly increased by 38.5% at 20 days, 56.1% at 30 days, and 29.0% at 45 days compared with the CK ($p < 0.05$) (**Table 2**). UHP2 treatment also significantly promoted the root surface area by 60.1% at 20 days, 4.5% at 30 days, and 4.1% at 45 days compared with CK ($p < 0.05$) (**Table 2**). In contrast, all root indices under the UHP3 and UHP4 treatments were significantly lower than those under CK ($p < 0.05$), especially root length, which was 19.4% and 39.0% lower than that under CK at 45 days, respectively (**Table 2**). The specific root length (SRL) in the UHP2 treatment was significantly enhanced by 17.9% at 20 days, 48.9% at 30 days, and 13.8% at 45 days compared with CK (**Table 2**). We further measured the root activity of different treatments at harvest, indicating that UHP2 treatment had the highest promotional effect, and the root activity was significantly enhanced by 111.2% compared with the CK treatment, while UHP4 treatment decreased root activity by 38.7% compared to CK (**Figure 3**).

Effects of Aeration on Amaranth Yield and Quality

The highest yield and the best quality were both observed in the UHP2 treatment compared with the CK treatment ($p < 0.05$). The yield of the UHP2 treatment was 12.6% higher than that of CK (**Figure 4A**). However, the UHP3 and UHP4 treatments exhibited significant inhibitory effects on amaranth yield, which was 8.1% and 11.7% lower than that of CK, respectively (**Figure 4A**). The soluble sugar contents of the UHP2 treatment were significantly higher than those of CK by 21.1%, and there was no significant difference between other UHP treatments and CK (**Figure 4B**). The ascorbic acid content of the UHP1, UHP2, and UHP3 treatments increased by 14.6%, 16.1%, and 8.9%, respectively, compared with CK, while it decreased by 5.2% in the UHP4 treatment (**Figure 4C**). The CCI value of the UHP2 treatment was significantly greater than that of CK by 54.8% (**Figure 4D**).

Effects of Aeration on Rhizosphere P Turnover of Amaranth at Different Growth Stages

To investigate the effect of UHP addition on soil P turnover, several main nutritional parameters of soil, including rhizosphere and bulk soil (without vegetable planting), were measured, and the results are shown in **Figure 5**. The nutrient contents in the bulk soil, including Olsen-P and Pi, were much higher than those in the rhizosphere soil, and P turnover showed an obvious "rhizosphere effect" (**Figure 5**). Olsen-P, Pi, and PAC showed upward trends as the UHP concentration increased. The Olsen-P contents of all aeration treatments were higher than those of CK, with an increase of 7.4%–13.5% in rhizosphere soil and 6.9%–10.0% in bulk soil (**Figure 5C**). The Pi contents in the bulk soil were not significantly different among the aeration treatments but were significantly higher than those in CK (**Figures 5D–F**). In the rhizosphere soil, the Pi content was significantly increased with the addition of UHP at 20 days and 45 days, and the highest content was observed under the UHP4 treatment, with a gap of 3.5% and 5.1% at 20 and 45 days, respectively, compared with CK (**Figures 5D,F**). Po decreased with the application of UHP, which was opposite to the change in the Pi content. No significant differences in the Po content in the bulk soil were observed between aeration treatments and CK at 30 days and 40 days (**Figures 5H,I**), but the UHP2, UHP3, and UHP4 treatments had significantly lower Po content than CK at 20 days by 18.3%, 20.8%, and 21.7%, respectively (**Figure 5G**). Oxygenation had a greater effect on PAC in the rhizosphere than in the bulk soil, especially at harvest, which increased by 7.1%–21.4% compared with that of CK (**Figure 5L**).

DISCUSSION

Alleviation of the Rhizosphere O_2-Deficient Zone Coupled With the P-Deficient Zone Under Oxygenation

The soil oxygenation status results from the equilibrium between the physical processes of gas transport between the atmosphere and the soil pores, and the biological processes of O_2 consumption and CO_2 accumulation (Brzezińska et al., 2001). O_2 consumption is caused by root and soil organism respiration; it consumes several tons of O_2 per hectare and per year under field conditions; and the presence of plants may elevate O_2

consumption more than twofold (Stępniewski, 2011). The dissolved O_2 concentration in the soil was approximately 243.6 μmol L^{-1} without amaranth plants, which was higher than the corresponding control (233.4 μmol L^{-1} with amaranth) in this study (**Figure 1**). This confirmed that there was an O_2-deficient zone in the rhizosphere soil. There was also a significant change in the rhizosphere ventilation environment with UHP addition, and the dissolved O_2 concentration increased with increasing UHP concentration. Soil is heterogeneous with vertical differentiation of the oxygenation status, and the O_2 concentration gradually decreases with soil depth (Stepniewski and Stepniewski, 2009), which was also confirmed by this research (**Figure 1**).

Similar to the rhizosphere anoxic zone, there was also a P-deficient zone in the rhizosphere. Olsen-P in the rhizosphere was lower than that in the corresponding bulk soil, and the phenomenon of P deficiency became increasingly serious with vegetable growth (**Figures 5A–C**). Indeed, there still existed a relatively P-deficient zone in the rhizosphere, although the bulk soil P maintained a high concentration due to the strong absorption of P by vegetable roots. Oxygenation could alleviate P deficiency in the rhizosphere, promote P turnover, and increase available P in the rhizosphere (**Figure 5**). Aerobic microorganisms are more active in well-aerated soils, and more nutrients can be released for plants to absorb (Bhattarai, Su et al., 2005). Research has proven that aeration promotes the abundance of microorganisms related to phosphate solubilization, that is, *Pseudomonas* and *Aspergillus* (Zhao et al., 2019), which can solubilize insoluble Pi and mineralize Po to available P (Zhang et al., 2018). The reduction in Po compared to Pi (**Figures 5D–I**) suggested the conversion of Po to Pi, and Pi was the main form for root acquisition by the Pi transporter at plasma membranes (Liao et al., 2019). Increasing the soil air content could effectively increase soil enzyme activity, such as phosphatases (Li et al., 2016b). Soil phosphatase activity directly affects the transformation and utilization of Po (Dodor and Tabatabai, 2003; Wang et al., 2013). The ratio of Olsen-P to TP is defined as the PAC, which was an important indicator of soil fertility, and higher PAC in soils could stimulate plant growth (Xiao et al., 2012; Wu et al., 2017). In this study, the PACs were greater than 2.0% (**Figure 5J-L**), which was usually defined as the threshold for P effectiveness and bioavailability (Xiao et al., 2012), indicating that soil P was sufficient for plant growth. Furthermore, PAC was higher with UHP added than that in CK treatment (**Figure 5J-L**), indicating that aeration could accelerate the P transformation process and provide more available P for vegetable growth. Further studies would be conducted for the effect of oxygenation on microbial, as well as enzymatic activity and thus on P turnover processes.

Root Morphology Construction and P-Acquisition Strategies Under Oxygenation

Soil aeration is essential to the normal growth and development of crops. Vegetable roots are the first plant tissue to perceive soil O_2 changes and then grow toward the "air-rich" zone (Bhattarai et al., 2005), while there exists reduced root elongation in O_2-deficient environment (Aguilar et al., 2003). Compared with the CK treatment, the UHP2 treatment significantly increased the total root length, root surface area, root diameter, and root volume (**Table 2**), especially root activity at harvest (**Figure 3**). In other words, increasing O_2 supply in the rhizosphere could promote earlier root development. The significant effect of UHP2 treatment on root growth and root activity might be one of the reasons for promoting the formation of amaranth biomass. The yield of the UHP2 treatment increased 12.6% compared with CK (**Figure 4A**). H_2O_2 was injected through subsurface drip irrigation at the rate of 5 L hm^{-2} into a zucchini crop (*Cucurbita pepo*), and the fruit yield, fruit number, and shoot weight increased by 25%, 29%, and 24%, respectively, compared to the control (Bhattarai et al., 2004). However, it was not the case where higher O_2 concentrations led to better plant growth. The total root length, surface area, volume, and average diameter were significantly reduced under the UHP3 and UHP4 treatments compared with CK ($p < 0.05$) (**Table 2**). Dunand et al. (2007) found that a high concentration of H_2O_2 considerably reduced *Arabidopsis* root length, and the root hardly developed when supplied with 1 mmol L^{-1} H_2O_2. A high concentration of UHP damaged root growth due to its strong oxidizing properties, resulting in growth inhibition. Treatment with UHP3 and UHP4 inhibited amaranth growth, and the yield decreased by 8.1% and 11.7%, respectively, compared with those in CK (**Figure 4A**). The increased O_2 concentration could promote the aerobic respiration of vegetables, but under a continuous high O_2 environment, amaranth showed different degrees of O_2 damage, which affected growth and metabolism. However, a high concentration of UHP damaged root growth (**Table 2**) due to its strong oxidizing properties, resulting in growth inhibition. Therefore, changes in root morphological characteristics in response to the rhizosphere O_2 environment might be one of the main reasons for the difference in amaranth biomass.

Moderately enhanced rhizosphere O_2 levels not only were conducive to the development of a more extensive root system but also influenced the P acquisition ability of roots. The P accumulation of amaranth increased significantly under the UHP2 treatment compared with the CK treatment (**Figure 2C**), and PUE was also significantly higher than that in CK at 30 days and 45 days (**Figure 2D**). The significant gains in P accumulation in the UHP2 treatment might be attributed to root morphological strategies to increase P acquisition and increase the vigor of root systems. Thicker roots could secrete greater amounts of P-mobilizing exudates to improve soil P availability, like high carboxylate exudation (Wen, Li et al., 2019), and reduced the roots turnover rate to help root effectively maintain P nutrition (De la Fuente Cantó et al., 2020). A larger root length and surface area were observed in the UHP2 treatment (**Table 2**), which could increase the larger P foraging area and lead to a higher P uptake capacity (Liu, 2021). The root system responds to the changes of O_2 environment in the rhizosphere mainly by adjusting root length, therefore leading to changes in SRL. High SRL facilitates faster growth through more rapid acquisition of soil resources (Kramer-Walter et al., 2016). Therefore, moderately enhanced rhizosphere O_2 levels (250.6 μmol L^{-1}) could enhance the P bioavailability by increasing the P-mobilizing exudates of thicker roots, expand root foraging for P, and improve the root metabolism, thereby realizing the efficient absorption of P. Improving P acquisition and utilization could enhance P efficiency (Wang et al., 2010), which might be the root P acquisition strategy of PUE increased in the UHP2 treatment (**Figure 2D**). Therefore, building a well-developed root system is crucial for increasing PUE in vegetable fields.

Improving Vegetable Quality by Regulating the Rhizosphere O_2 Environment

Vegetables are rich in essential nutrients such as vitamins and amino acids, and people are increasingly concerned about vegetable quality and safety as living standards improve. Aeration in the rhizosphere could improve the rhizosphere soil O_2 environment and physiological metabolism, and has a certain promoting effect on vegetable quality. Vitamin C and soluble sugars, two beneficial substances for the human body, were significantly enhanced under the UHP2 treatment (**Figures 4B,C**). Root hypoxia led to decreased contents of ascorbic acid (vitamin C), and the low levels of ascorbate might result from a modification of the leaf hormone status caused by disturbed root function (Horchani et al., 2008). The ascorbic acid concentration is reported to be the highest in mature leaves with highest chlorophyll (Gill and Tuteja, 2010), and hypoxia caused leaf epinasty and reduced photosynthetic activity (Pezeshki, 2001). Therefore, the VC content first increased and then decreased with increasing UHP concentration in this study, which was consistent with the change in the CCI value. The CCI value indicated a close positive correlation with the chlorophyll content in leaves (Richardson et al., 2002) and was used to characterize the relative chlorophyll content. Chlorophyll is the main substance that absorbs light energy in plant photosynthesis, and its content was closely related to the photosynthetic rate of plants. A reduction in the chlorophyll content would affect photosynthetic performance and dry matter accumulation, decreasing yield reduction (**Figures 4A,D**). Soluble sugar content reflects dry matter accumulation, the soluble sugar content of UHP2 treatment was significantly higher than that of CK (**Figure 4B**), and the dry matter accumulation in leaf tissue by promoting photosynthesis with elevated O_2 was another important factor in the increased yield.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RW conducted the experiments, analyzed the data, and wrote the manuscript. YL designed the study and revised the manuscript. WS supervised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Using a Modified Langmuir Equation to Estimate the Influence of Organic Materials on Phosphorus Adsorption in a Mollisol From Northeast, China

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The use of organic materials has been increasing due to improving soil fertility by affecting phosphorus (P) adsorption and desorption behavior of soils. However, previous studies ignored the influences of increased P concentrations in equilibrium solutions caused by P released from applied organic materials in bath P adsorption experiments. To eliminate these influences, a modified Langmuir equation was applied to describe P adsorption properties in dairy manure composts and biosolids-incubated soils. The adsorption and desorption trends shifted around the initial P concentration of 70 mg/L. The fitted parameter of correlation coefficients showed that the modified Langmuir model fitted well for the adsorption data covering only the lower initial P concentrations (0–70 mg P/L) and covering the whole P concentrations applied in the present study (0–400 mg/L). For the fitted results covering the whole P concentrations, adding organic materials generally decreased both the $\Gamma_{\max}$ (maximum adsorption capacity for P) and the *MABC* (maximum adsorption buffering capacity), except for the 20-g/kg biosolids incubation, indicating that the number of adsorption sites for P decreased as the organic materials applied, and the binding energy for the P adsorption to the soil also decreased to some extent with some fluctuation as reflected by *K* values which represent adsorption strength. Higher DOC content, lower molecular weight, and higher humic acid might result in the higher $\Gamma_{\max}$ of 20-g/kg biosolids incubated soil. The linear equations are described well for P desorption. The constant *a* value of slope in a linear equation reflects the P desorption capacity of soils increased due to the application of organic materials, especially in high P concentrations with *a* value of 0.45 for the control soil increased to 1.02 for 10-g/kg biosolids treated soil. Thus, it would be important to control the P application amounts when the application of P fertilizers to the soils with organic materials applied, as the effects of organic materials on P adsorption and desorption characteristics were more efficient at high P concentrations.

Keywords: black soil, organic materials, modified Langmuir equation, phosphorus adsorption, phosphorus desorption, UV spectroscopy

INTRODUCTION

Phosphorus (P) is the second most important macronutrient for plant growth and nutrition as a nonrenewable resource. Application of P fertilizers is vital for food production, especially for enhancing agricultural production in soils with low P availability, as all plants need an ample supply of P. When phosphate fertilizers are applied to the soils, their utilization efficiency for crops is only 10–25% and up to 75–90% of P would be accumulated in soils (Lu et al., 1995; Wang et al., 2011). Excessive phosphate fertilizers are annually applied to the soils and P input is often greater than its demand, and it will thus result in contamination of aquatic systems and underground water if P applied into soils is leached into surface water and groundwater (Heredia and Cirelli, 2007; Holman et al., 2010).

Adsorption and desorption of P in soils combined with mineralization and immobilization form the dynamic soil P cycling, and adsorption-desorption is an important internal cycle for determining the environmental fate of P and for understanding the soil fertility (Barros et al., 2005). Adsorption and desorption processes depend on many factors and soil conditions, such as soil types, soil texture and pH, contents of Al, Fe, soil organic matter, and clay minerals (Antelo et al., 2007; Weng et al., 2011; Wang and Liang, 2014; Fink et al., 2016; Kurnain, 2016). Soil organic matter plays an important role in P adsorption and desorption, as it could involve the competition between organic matter and P for mineral adsorption sites, removing adsorption sites by complexation of surface metals and release of these metals into solution, repulsion of phosphate anions by sorption of organic matter to positive sites, and formation of cation bridge leading to increase in P sorption sites (Hunt et al., 2007; Debicka et al., 2016).

The application of organic materials can change the content and constitute of soil organic matter, and thus will affect the adsorption and desorption characteristics of P in soil. Organic materials applied to acid sandy soils would commonly decrease P adsorption and increase P availability (Borggaard et al., 1990; Nziguheba et al., 1998; Hafiz et al., 2016). The application of manure in calcareous cinnamon soil also decreased the P adsorption and buffering capacity (Zhang et al., 2008). However, the exceptional results were also determined. Ma and Xu (2010) found that the application of rice straw decreased P adsorption to red soil and paddy soil, but there was nearly no effect on latosol soil, although these three soils all belong to the acid soils with low soil organic matter. Humic acids incubated in black soil enhanced the maximum adsorption capacity of P, soil available P, and P activation coefficient (Yang et al., 2019).

Furthermore, when calculating P sorption from solution to soils applied with organic materials, the release/desorbing of P from the organic materials have not been accounted for in these studies. Indeed, there is considerable evidence that increased P in soil solutions arises from the applied organic materials (Guppy et al., 2005). P equilibrium concentrations in batch adsorption experiments would be greater than expected due to the release of P, and finally, P adsorbed to soils would be underestimated. Therefore, the incubation of organic materials in soils has commonly been reported as reducing P sorption in soils. Whether the reported decreases in P sorption (as measured by the differences between P in the solutions before and after adsorption) are related to competition with organic matter (such as humic acid) decomposition products of organic materials breakdown or the results of P release from organic materials, need to be further determined.

For avoiding underestimating the effects of P release from organic materials applied into soils on P adsorption, the soil organic matter removal combated with batch adsorption experiments were applied to investigate the influence of organic matter on P adsorption and desorption. Hiradate and Uchida (2004) found that removing organic matter from the Andisol soil increased the P adsorbed amount, indicating that soils organic matter occupied sites that could adsorb P, and soil organic matter could inhibit P adsorption through competitive adsorption. But Debicka et al. (2016) found that the adsorption capacity for P in most of the topsoils that were tested decreased, and the desorption of P increased when organic matter was removed, indicating that soils organic matter was potentially P binding soil constituents and could adsorb P. These contradictory results may have been caused by the P adsorption capacity of soil depending on the type of organic matter present and the soil type. In addition, P adsorption experiments on matrix minerals (such as goethite, ferrihydrite, gibbsite, and kaolin) covered with organic matter (such as humic acid) were carried out to determine the effect of organic matter on P adsorption (Antelo et al., 2007; Hunt et al., 2007; Kurnain, 2016; Yan et al., 2016), and inhibition of P sorption to matrix minerals was commonly observed. Also, correlation analyses and path analyses were applied to find out the influences of organic matter on the adsorption and availability of P in soils (Kang et al., 2009; Yadav et al., 2017). But in these studies, the soil organic matter was not manipulated or controlled. To both control the soil organic matter (increasing soil organic matter only without changing the other soil components) and avoid the underestimating the effects of P release from organic materials applied to soils, Yang et al. (2019) applied humic acid-containing negligible P as the exogenous organic materials to identify the mechanism through which organic matter affects the adsorption of P in black soil, and found that P adsorption and availability were enhanced by increasing soil organic matter. Although humic acid was one of the most important decomposition products of organic materials breakdown applied to soils, it could not represent the organic materials applied to soils. So, the effects of organic materials applied to soils on the adsorption and availability of P in soils need to be further investigated by considering both the release of P from organic materials and the actual conditions of organic materials applied to soils.

Of all the obvious studies, the Langmuir equation has been used to describe P sorption reactions to soils. To account for the contributions of P releasing from organic materials-incubated soils, various Langmuir equation modifications have been developed (Villapando and Graetz, 2001; Brock et al., 2007; Zhang et al., 2010; Wang et al., 2014; Wang et al., 2016). The Langmuir equation is typically expressed as follows:

$$\Gamma_T = \frac{\Gamma_{max} KC}{1 + KC}$$

where Γ_T (mg/kg) is the total amount of P adsorbed by the soil, which includes P that was already adsorbed to the soil before adsorption experiments (Γ_0) and P adsorbed/desorbed from/into the equilibrating solution (Γ_s), C (mg/L) is the P concentration in the equilibrium solution, Γ_{max} (mg/kg) is P adsorption maximum, and K (L/mg) represents P adsorption strength. When there is net P desorption into the equilibrating solutions, Γ_0 represents the P that could be desorbed if the solution P concentration is maintained at or near zero (Barrow, 2008). For low-P soils, the amount of desorption/releasing is too small to be measured by standard techniques, and Γ_0 is often ignored (Barrow, 2008; Zhang et al., 2010). But for P-enriched soils, Γ_0 should be included in the Langmuir equation to describe P adsorption data because the amount of P releasing/desorbing should not be ignored. Based on comparisons of estimated adsorption parameters or goodness of fit for different Langmuir equation modifications, Wang et al. found that the estimate of Γ_0 by fitting a linear equation to P adsorption data at low-P equilibrium concentration might be preferred (Wang et al., 2014). The Γ_0 estimated in this way could cover the effect of P releasing from P-enriched soil and the effect of P releasing has been adjusted. The final fitting result is the result, that is, expected and P adsorbed to soil would not be underestimated or overestimated. So, in the present, a modified Langmuir equation was chosen and used to study the effects of organic materials applied in soils on the adsorption and availability of P in a Mollisol soil of northeast China (Wang et al., 2014; Wang et al., 2016). Dairy manure composts (MC) and biosolids (Bios) were chosen as the organic materials applied to the soil.

Furthermore, the previous research covers a different range of P concentrations applied to sorption experiments, and generally, it can be divided into two groups according to the upper initial P concentration limit, i.e., 10–60 mg P/L (McDowell and Condron, 2001; Guppy et al., 2005; Heredia and Cirelli, 2007; Wang et al., 2014; Debicka et al., 2016; Wang et al., 2016; Yang et al., 2019) with the Γ_{max} of hundreds of in order of magnitude and 170–500 mg P/L (Bhatti et al., 1998; Hiradate and Uchida, 2004; Brock et al., 2007; Xue et al., 2014) with the Γ_{max} of thousands of in order of magnitude. These also would affect the results of P sorption. To compare with the above results at a similar range of P concentrations, this study applied more P concentrations (up to 25 different concentrations of P) within a range of 0–400 mg P/L, with a smaller interval at lower initial P concentrations and with a larger interval at higher initial P concentrations. Then, the modified Langmuir equation has respectively been used to investigate P sorption behavior in the organic materials-amended soils at low P concentrations and high P concentrations.

MATERIALS AND METHODS

Soil Samples Collection and Organic Materials Preparation

A typical black soil (BS, a mollisol) was collected from an agricultural experimental base of Jilin University in Changchun City (latitude 43°56.603′-43°57.274′ N, 125°14.231′-125°14.914′ E). Surface soil samples (0–20 cm) were collected and then were air-dried, ground, homogenized, and sieved to pass through a < 2-mm sieve after removing stones and residual roots. Pre-decomposed dairy manure composts (MC) and biosolids (Bios) were chosen as exogenous organic materials. Organic materials were air-dried, ground to pass a < 2-mm sieve, and stored in a brown wide-mouth bottle prior to applying to the soils.

The soil of 3.0 kg was amended with 5, 10, and 20 g OM (organic matter)/kg soil from each of the organic material sources to increase the organic matter content and incubated for 30 days at 60% of the maximum field moisture capacity with adding deionized water (Hafiz et al., 2016; Yang et al., 2019). The soil without organic materials was also incubated for 30 days under the same condition and considered as the control. Three replicates have been applied for each treatment. Then the soils were air-dried, sieved to pass through a < 2-mm sieve, and stored in a brown bottle prior to use for adsorption and desorption experiments. Properties of soils and organic materials before and after incubation are listed in **Table 1**.

Solid samples were mixed with deionized water at the ratio of 1:1, shaken for 20 min, and then stood for 10 min to determine the pH and EC (Accumet AB 200, Fisher Scientific). The total organic carbon (TOC) of solids was measured by a Vario TOC cube (Elementar, German). The total P (TP) and available P contents were determined by an AutoAnalyzer 3 (Bran Luebbe Gmbh Company, German) after performing sulfuric acid—perchloric acid digestion and sodium bicarbonate extraction.

After incubation for 30 days, the release of P from soils by using 0.01 mol/L KCl at pH 7.0 was carried out (Guppy et al., 2005). Each soil sample (2.5 g) was placed into a 50-ml centrifuge tube and then was equilibrated with 25 ml of KCl (0.01 mol/L) at pH 7.0 for 24 h. After reaching equilibrium, the mixed suspensions were centrifuged at 4,000 r/min for 30 min followed by being filtered through 0.45-μM filters (Millipore, Billerica, MA, United States). P in supernatants was determined by an AutoAnalyzer 3 (Bran Luebbe Gmbh Company, German) and the release amounts of P were obtained (**Table 1**). Meanwhile, dissolved organic carbon (DOC) contents were also measured by a Vario TOC cube (Elementar, German), and the structural characteristics of DOC were determined by UV-Visible absorption spectroscopy (Kalbitz et al., 2000; Xie et al., 2018). UV/Vis spectra were recorded with a UV-2550 spectrophotometer (Shimadzu, Japan) in the wavelength range 200 ± 500 nm using 1 cm cuvettes. All spectra were normalized by the measured DOC contents and standardized at a concentration of 10 mg C/L by dilution.

Adsorption and Desorption Experiments

Batch adsorption and desorption experiments were performed in this study. Each soil sample (1.0 g) was equilibrated with 10 ml of KCl (0.01 mol/L) at pH 7.0 supplied with P at different concentrations (0–400 mg/L) using KH_2PO_4. The mixed suspensions were added with three drops of chloroform to avoid microbial growth and horizontally shaken for 24 h, and then centrifuged at 4,000 r/min for 30 min followed by being filtered through 0.45-μM filters (Millipore, Billerica, MA, United States). P in the initial or equilibrium solutions were measured by an AutoAnalyzer 3 (Bran Luebbe Gmbh Company, German).

The solid samples were washed with deionized water for 30 min, centrifuged at 4,000 r/min for 30 min and the supernatants were discarded. Then the solid samples were shaken and re-equilibrated with 10 ml of KCl (0.01 mol/L) for 24 h. The suspensions were centrifuged at 4,000 r/min for 30 min followed by being filtered through 0.45-μM filters, and P in supernatants were determined by an AutoAnalyzer 3 (Bran Luebbe Gmbh Company, German) and the amounts of desorbed P were obtained.

Data Analysis

A modified Langmuir model was used to analyze adsorption data in this study (Wang et al., 2014; Wang et al., 2016), and a sorption curve could be described as follows:

$$\Gamma = \frac{\Gamma_{\max} \times K \times C}{1 + K \times C} - \Gamma_0 \tag{1}$$

where C (mg/L) is the equilibrium P concentration of the solution, Γ (mg/kg) is the amount of P sorbed/desorbed from/into the solution during shaking, Γ_0 (mg/kg) is the initial sorbed P that was already sorbed to the soil before analysis, $\Gamma_{\max}$ (mg/kg) is the maximum amount of P, K (L/mg) is a constant related to the binding strength of P at the adsorption sites, and $K \times \Gamma_{\max}$ is the maximum adsorption buffering capacity (*MABC*, L/kg). The releasing experiment results indicated that P desorption reactions indeed occurred in the present study, especially for the soils incubated with more organic materials (**Table 1**).

If $\Gamma = 0$, C can be designated as C_e, which indicates the solution P concentration after shaking at zero netted sorption, and the following equation is obtained:

$$\Gamma_0 = \frac{\Gamma_{\max} \times K \times C_e}{1 + K \times C_e} \tag{2}$$

Combining **Eq. 1**, **2**, the Langmuir equation could be modified as follows:

$$\Gamma = \frac{\Gamma_{\max} \times K \times C}{1 + K \times C} - \frac{\Gamma_{\max} \times K \times C_e}{1 + K \times C_e} \tag{3}$$

At low solution P concentrations, the relationship between Γ and C could be assumed to be linear (Wang et al., 2014; Wang et al., 2016):

$$\Gamma = K_1 C - \Gamma_0 \tag{4}$$

where K_1 (L/kg) is the partition coefficient. Accordingly, from **Eq. 4**, C_e can be calculated by the following equation:

$$C_e = \frac{\Gamma_0}{K_1} \tag{5}$$

In our study, the relatively lower initial P solutions (i.e., 0, 1, 3, 5 mg P/L) were used to determine the fitted values of the parameters of **Eq. 4**.

P desorption data were described by linear model $D = a \times \Gamma + b$, where D (mg/kg) is the amount of P desorbed from the soil after the adsorption experiment, Γ (mg/kg) is the amount of P adsorbed to the soil in the former P adsorption, slope a is the constant related to desorbing capacity.

RESULTS AND DISCUSSION

Soil Properties

The soil used in the present study is mollisol, a typical black soil with a TOC of 24.28 g/kg. The characteristics of organic materials used in this study were different from each other with a higher pH for MC and the lower pH for Bios (**Table 1**). On the contrary, the higher EC was found in Bios and the lower EC was present in MC.

TABLE 1 | Selected properties of soils and organic materials used in the study (*n = 3*).

Treatments	pH	EC (μS/cm)	TOC (g/kg)	TP (g/kg)	Available P (mg/kg)	PAC%	P releasing (mg/kg)	DOC (g/kg)
Black soil (BS)	6.06 ± 0.03	96.57 ± 2.15	24.28 ± 0.44	0.48 ± 0.08	42.35 ± 0.63	8.82	1.32 ± 0.08	0.69 ± 0.08
Dairy manure compost (MC)	7.82 ± 0.05	58.55 ± 1.76	176.86 ± 6.91	0.86 ± 0.12	464.66 ± 3.34	54.03	--	--
Biosolids (Bios)	6.07 ± 0.04	580.60 ± 5.85	188.92 ± 2.07	5.27 ± 0.34	462.27 ± 4.12	8.77	--	--
BS with 5-g/kg OM from MC	6.76 ± 0.08	195.15 ± 3.18	28.33 ± 2.59	0.47 ± 0.03	48.92 ± 0.48	10.41	8.45 ± 0.12	1.25 ± 0.09
BS with 10-g/kg OM from MC	6.66 ± 0.09	186.00 ± 3.25	33.79 ± 0.87	0.49 ± 0.05	52.64 ± 0.42	10.74	10.18 ± 0.41	1.33 ± 0.10
BS with 20-g/kg OM from MC	6.08 ± 0.07	228.30 ± 1.27	43.49 ± 7.80	0.52 ± 0.04	66.67 ± 0.63	12.82	13.16 ± 0.13	1.61 ± 0.08
BS with 5-g/kg OM from Bios	6.21 ± 0.05	317.40 ± 25.73	28.70 ± 4.79	0.87 ± 0.07	77.33 ± 0.66	8.89	3.77 ± 0.04	1.24 ± 0.07
BS with 10-g/kg OM from Bios	5.99 ± 0.07	1,267.50 ± 26.16	34.37 ± 1.19	1.01 ± 0.07	133.16 ± 0.78	13.18	10.51 ± 0.08	1.36 ± 0.11
BS with 20-g/kg OM from Bios	6.09 ± 0.07	1,690.95 ± 74.24	43.34 ± 1.40	1.49 ± 0.08	188.90 ± 0.97	12.68	26.95 ± 0.18	1.77 ± 0.09

TOC, total organic carbon; TP, total phosphorus; PAC, P activation coefficient obtained by the ratio of available P to total; DOC, dissolved organic carbon.

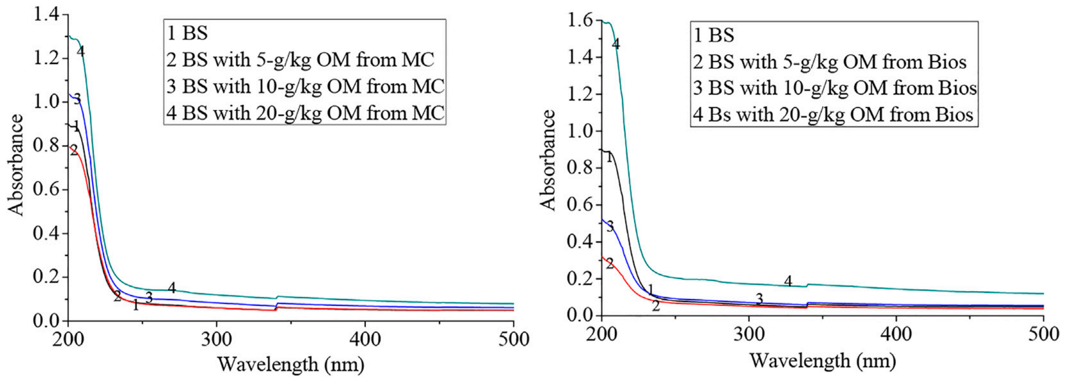

FIGURE 1 | UV spectra of dissolved organic matter extracted from incubated soils (absorbance was standardized to 10 mg C/L).

The organic matter contents of these two organic materials were similar to each other as described by TOC. Although the total P content of Bios was significantly higher than that of MC, the available P content of MC was close to that of Bios.

After adding the organic materials, the EC, TOC, TP, and available P of soil were all increased to some degree. The total P contents of the soils provided little information about the transformation of P in the soil and the availability of P to crops. The total P contents of the soils varied significantly with different organic materials being added, especially with the addition of bios and it increased the total P contents in the soils by 0.8–2.1 times. Also, available P contents were obviously increased due to the addition of Bios. The influences of MC on total P and available P in the soils were lower. The available P to total P ratio is called the P activation coefficient (PAC), which represents the degree of difficulty with which transformations between total P and available P. A higher coefficient indicates more P will be available to promote crops growth (Wu et al., 2017). The PAC increased after both MC and Bios were added to the soil.

Like the available P, organic materials applied in soils also enhanced the releasing/desorbing of P from the incubated soils (**Table 1**). The more organic materials added, the higher P released from the soils, and the highest P release was found from the soil incubated with 20-g/kg organic matter of Bios and almost 20 times more than that released from the control soil. So, it is important to account for the effects of P releasing/desorbing on the P adsorption experiments, and the modified Langmuir model rather than the original Langmuir model should be used in the present study.

Also dissolved organic carbon (DOC) released from the incubated soils increased with the application of the organic materials (**Table 1**). UV/Vis spectra of DOC extracted from the soils incubated with 20-g/kg OM from Bios show a higher absorbance over a wide range of wavelengths, with little change for 5- and 10 –g/kg OM incubated soils (**Figure 1**). A similar result was also obtained for the soils incubated with MC, but the absorbance change in the soil added with 20-g/kg OM from MC was smaller than that for the Bios added to the soil. MC and Bios application could increase UV spectra absorbance of DOM at 254, 260, and 280 nm, indicating that the fragrance, hydrophobicity, and molecular weight of DOM might increase to some extent (Xie et al., 2018) and the highest change was found in the 20-g/kg Bios-incubation soil. The UV spectra ratio of A250/A365 decreased for MC incubated soils as compared to control soil; it further proved that MC addition increased the molecular weight of organic matter in the soil. The ratio of A250/A365 also decreased for the soils incubated with 5- and 10 -g/kg OM from Bios (from 1.43 to 1.27 and 1.40), but increased to 1.48 for the soil incubated with 20-g/kg OM from Bios indicating that 20-g/kg Bios incubation decreased the molecular weight of soil organic matter. The ratio of A300/A400 were all below 3.5, which indicated that the soils with or without OM incubation were rich in humic acid, but humic acid in Bios was higher than that in MC (Xie et al., 2018).

Phosphorus Adsorption Characteristics

In the current study, we applied up to 25 initial P concentrations of adsorption experiments to cover both the lower and higher P concentrations. The more P added, the more P adsorbed to the soils. Supporting the general adsorption characteristics, the P adsorption increased rapidly as the initial P concentrations increased at lower P concentrations with the equilibrium P concentration increased to 40 mg/L, and then increased slowly to a lesser extent of higher P concentrations. When the equilibrium P concentration was lower than 40 mg/L, especially lower than 20 mg/L, the P adsorption amounts of the soils with MC and Bios were similar to the control soil which was also incubated under the same incubation condition but without organic materials added (**Figure 2**). The P adsorption by soils is regarded as being a multi-stage kinetic process (Wang and Liang, 2014), the present study results indicated that P adsorbed by soils went through an initial fast adsorption stage and then a slower adsorption stage. Previous studies have observed H-curve type isotherms (Azeez and Van Averbeke, 2011; Yu et al., 2013) or S-curve type isotherms (Hafiz et al., 2016) in P adsorption on soils incubated with organic materials. No matter H-curve type isotherms or S-curve type isotherms, the P adsorption trends both shifted at some specific P concentration. Yu et al. (2013) found that P adsorbed to soil

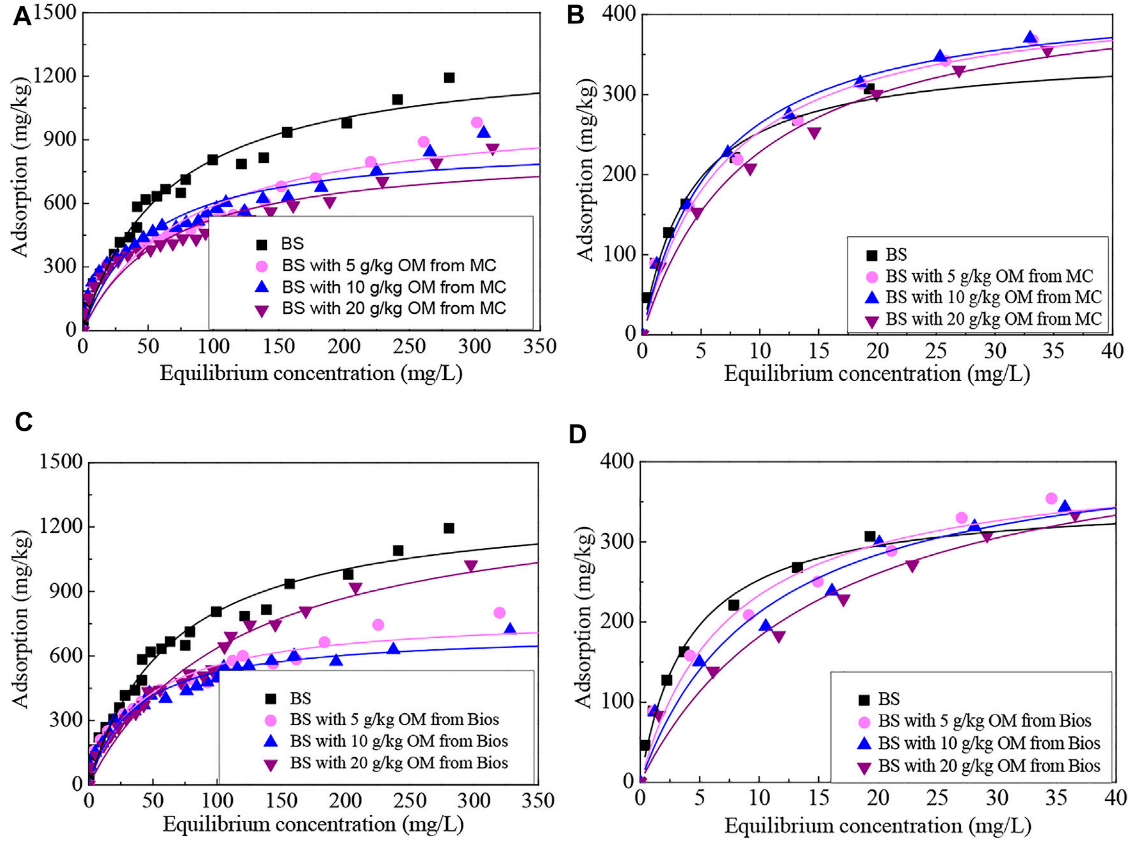

FIGURE 2 | Langmuir isotherms of P adsorbed onto soils incubated with different contents of organic matter derived from **(A) (B)** manure compost and **(C) (D)** biosolids.

parent materials with poultry manure compost and organic fertilizer application showed an H-shaped increasing trend, with P adsorption reaching a plateau when the initial P concentration was about 40–100 mg/L, but increased further beyond 100 mg/L. P adsorption to the dairy, poultry, and goat manure-incubated soils conformed to the S-curvature, with the lower P adsorption at low equilibrium concentration and become easier as the equilibrium concentration was rising to 50–100 mg/L (Hafiz et al., 2016). A careful observation of the adsorption isotherms in the present study indicated that the isotherms have more in common with the H-curve type isotherms, with P adsorption reaching a plateau when the equilibrium P concentration was 50–120 mg/L, and then increased further beyond 120 mg/L. High affinity with P for soils caused a high initial slope of the isotherms and likely appeared as the H-shaped isotherms. On the contrary, the low initial slopes of the isotherm curves were caused by the low affinity with P for soils and appeared as S-shaped curves (Hafiz et al., 2016). P adsorption isotherms forms depended on adsorption mechanisms and the P adsorption process could be divided into chemical and physical adsorption processes. When the initial P concentrations were relatively low, the chemical process could dominate the adsorption processes and would be completed rapidly, and ligand exchange and ion exchange were likely to be the dominant mechanisms contributing to the high adsorption rates (Lai and Lam, 2009). Correspondingly, the chemical adsorption process would slow down rapidly at high P concentrations due to the quickly being saturated of the available adsorption sites, and the P adsorption changed to the physicochemical and physical adsorption processes and presented slower rates (Lai and Lam, 2009; Wang and Liang, 2014).

Many models have been developed to describe adsorption isotherms and the Langmuir equation is one of the most used models to quantitatively fit P adsorption isotherms (Hussain et al., 2003; Lair et al., 2009; Rossi et al., 2012; Yang et al., 2019). The modified Langmuir model which is suitable for the P-enriched soils was applied to fit the adsorption data into this study (Wang et al., 2014; Wang et al., 2016). The adsorption data covering only the lower initial P concentrations (i.e., 0-70 mg P/L, **Figures 2B,D**) and the data covering the whole P concentrations applied in the present study (i.e., 0-400 mg/L, **Figures 2A,C**) were respectively fitted by the modified Langmuir model. Simulated Langmuir curves for the soils with or without organic materials incubation are illustrated in **Figure 2** and the resulting fitted parameters are presented in **Table 2**. Results revealed that the modified Langmuir equation properly described the P adsorption properties of the soils, and

TABLE 2 | Parameters of the modified Langmuir model for P adsorbed in soils.

	Treatments	Γ_{max} (mg/kg)	*K* (L/mg)	*MABC* (L/kg)	*r*
Coverage of Sorption data (0–400 mg/L)	BS	1,326.53 ± 61.91	0.016 ± 0.0018	21.22	0.9864**
	BS with 5-g/kg OM from CM	1,058.84 ± 93.16	0.013 ± 0.0027	13.76	0.9541**
	BS with 10-g/kg OM from CM	893.24 ± 55.13	0.021 ± 0.0038	18.76	0.9637**
	BS with 20-g/kg OM from CM	857.94 ± 69.91	0.016 ± 0.0035	13.73	0.9494**
	BS with 5-g/kg OM from Bios	795.49 ± 41.00	0.023 ± 0.0037	18.30	0.9717**
	BS with 10-g/kg OM from Bios	711.89 ± 26.25	0.028 ± 0.0034	19.93	0.9858**
	BS with 20-g/kg OM from Bios	1,376.25 ± 90.99	0.0086 ± 0.0011	11.84	0.9904**
Coverage of Sorption data (0–70 mg/L)	BS	355.32 ± 18.67	0.25 ± 0.037	88.83	0.9913**
	BS with 5-g/kg OM from CM	431.92 ± 25.56	0.14 ± 0.027	60.47	0.9856**
	BS with 10-g/kg OM from CM	429.17 ± 14.16	0.16 ± 0.017	68.67	0.9948**
	BS with 20-g/kg OM from CM	440.41 ± 25.93	0.11 ± 0.018	48.44	0.9891**
	BS with 5-g/kg OM from Bios	407.37 ± 34.71	0.13 ± 0.037	52.96	0.9700**
	BS with 10-g/kg OM from Bios	431.01 ± 49.82	0.097 ± 0.031	41.81	0.9630**
	BS with 20-g/kg OM from Bios	461.47 ± 38.40	0.065 ± 0.019	29.99	0.9721**

Γ_{max}, the maximum adsorption amount of P; K, the binding strength of P at the adsorption sites; MABC, the maximum adsorption buffering capacity obtained by K × Γ_{max}.

the relationships indicated by the correlation coefficients were statistically significant.

The maximum adsorption capacity for P (Γ_{max}) reflects the number of P adsorption sites per unit weight of the soil and is generally used to evaluate the adsorption capacity of soil for P (Hiradate and Uchida. 2004; Yan et al., 2016; Fink et al., 2016; Yang et al., 2019). Γ_{max} obtained from the fitted curves covering only the lower initial P concentrations between 0–70 mg P/L were similar to each other for different organic materials-incubated soils at different levels, and all higher than that of the control soil with the increased rate of 14.6–29.9%, and the highest value was obtained in the soil with 20-g/kg bios incubation. Γ_{max} changed slightly with MC added rates increased, but with Bios added rates increased, Γ_{max} also increased significantly (**Table 2**). However, when we used the modified Langmuir model to fit the whole initial P concentrations, the higher Γ_{max} values have been obtained, whether for the soils incubated with organic materials or not. Γ_{max} for the control soil was 1,326.53 mg/kg and organic materials application clearly influenced the Γ_{max}. The Γ_{max} decreased as the spiked MC increased, indicating that the P storage capacity of the soil decreased as the MC incubation amounts increased. The Γ_{max} also reduced along with the Bios addition when the Bios application was at lower rates of 5 g/kg and 10 g/kg, but when the application rate was up to 20 g/kg, the Γ_{max} instead increased. Hafiz et al. (2016) found that the Γ_{max} values of a silt loam agricultural soil also decreased after incubation with dairy, goat, and poultry manures for 1 month. Yu et al. (2013) found that poultry manure incubation reduced the Γ_{max} of the soils derived from basalt, granite, and river alluvial deposits, no matter whether incubated for 30 days or 60 days; but organic fertilizer incubation increased the Γ_{max} of the soils derived from granite and river alluvial deposits, although it also reduced the Γ_{max} of the soils derived from basalt. The reduction in the Γ_{max} due to the addition of MC and Bios in lower rates in the current study might be due to the affinity of organic ligands for sorption sites. Most ligands are capable of displacing phosphate ions in site-specific organic ligands such as oxalate, malate, citrate, tartrate, malonate, humic acid, and fulvic acid (Violante and Gianfreda, 1993; Bhatti et al., 1998; Guppy et al., 2005). Sharma et al. (2006) found that the possible blockade of P-fixing sites in the soil by soluble humic substances present in manure might reduce the adsorption capacity of the soil. The reduction in P adsorption capacity and consequently increase in P availability to plants or increase in P loss to water has commonly ascribed to competition between the decomposition products of organic matter and P for soil sorption sites, and the evidence for competition inhibition of P sorption by dissolved organic carbon compounds, derived from the breakdown of organic matter, includes the competition between P and low molecular weight organic acids, humic and fulvic acids, and organic matter leachates in soils with a high P sorption capacity (Guppy et al., 2005; Bayon et al., 2006; Zamuner et al., 2008). If the organic ligands decomposed from the incubated organic materials were previously adsorbed, the surface charge of the solids would be altered, and then might cause P to be electrostatically repelled and decrease P adsorption (Antelo et al., 2007). Meanwhile, Guppy et al. (2005) reported that decreases in P sorption as measured by P in the soil solution might also be the result of P release from the organic materials that were not considered when calculating the reduction in P sorption. In the present study, when calculating the P adsorption amounts, the P release from organic materials was accounted for by using the modified Langmuir model and MC incubation still reduced the P adsorption capacity of the soil. So, it could be assumed that the competition mechanism played a leading role in P adsorption processes in the current MC applied soils. The pH value changes in the incubated soils might also reflect the shifts in P adsorption capacity. Zhao et al. (2006) found that pH 6.0 was a turning point and increasing the organic matter content increased the P adsorption by soil at pH > 6.0, but decreased the amount of P adsorbed by soil at pH < 6.0. However, Guppy et al. (2005) considered that the magnitude of the inhibition of P sorption by the decomposition products of organic materials leachate is negligible at rates equivalent to those of organic materials applied field. Organic materials might also increase the P adsorption capacity through metal-chelate linkages provided by these organic compounds. The suddenly significant increase in the Γ_{max} of the soil with 20 g/kg of Bios incubation resulted in part from the above reason. Besides, Bios was commonly characterized by containing more humic acid-like and fulvic acid-like compounds (Torrecillas et al., 2013; Campuzano and González-Martínez, 2016). Humic acid and fulvic acid do not simply compete with P for sorption sites in soils, but in many instances, act as P adsorbing surfaces also (Appelt et al., 1975; Perrott, 1978). In the present study, the DOM content and structure of the soil incubated with 20 g/kg of Bios were different from the other incubated soils, with higher DOC content, lower molecular weight, and higher humic acid (**Figure 1**), and this might result in higher $\Gamma_{\max}$. In some allophonic soils, reactions to metals with humic acid and fulvic acid greatly increase P sorption and are critical to P cycling processes (Borie and Zunino, 1983). In highly weathered soils, sorption of P to humic acid and fulvic acid might help alleviate problems associated with high P fixation through the slow mineralization of these complexes (Pushparajah et al., 1998). Yang et al. (2019) reported that the addition of humic acid to increase the soil organic matter content could efficiently increase the P adsorption capacity $\Gamma_{\max}$. This is possibly another reason to explain the increase of $\Gamma_{\max}$ for the soil incubated with 20 g/kg of Bios.

The P adsorption energy (*K*) is another one of the most important parameters describing the P adsorption affinity with soils and related to binding energy to sorption sites. The higher value of *K* means a stronger trend of P adsorption, and the degree of spontaneous reaction is stronger, with weaker P supplying intensity (Wang and Liang, 2014; Hafiz et al., 2016). *K* obtained from the fitted curves of the lower initial P concentrations of 0–70 mg P/L were all significantly higher than those obtained from the fitted curves of the whole initial P concentrations. At lower initial P concentrations, organic materials incubation reduced *K* values as compared with control soil. But the *K* values changed significantly, and different shifting trends were found for the soils with different organic materials applied at whole initial P concentrations. With the OM incubation rates increasing, MC treatment first decreased *K* and then increased *K*, but Bios applied first enhanced *K* and finally reduced *K*, as compared with the control soil. These two organic materials had one thing in common was that incubation at the rate of 10-g/kg all increased *K* up to the highest. Without considering control soil, the *K* values approximately followed parabolic trends, which were consistent with the results previously reported by Xia and Wang (2009), first increasing, and then decreasing when the OM contents of the lime concretion black soil and the calcareous yellow fluvo-aquic soil increased. Hafiz et al. (2016) reported that manure treatments decreased *K* for dairy and goat manure incubated soil and increased for poultry manure incubated soil. *K* decreased by the lower MC incubation indicating that organic matter decomposed from MC competed with P for adsorption sites and decreased the amount of P adsorbed from the soil solution. When the soil was applied with higher OM, higher *K* values were obtained and this might be due to the high reactivity of the organic matter with phosphate anions (Yang et al., 2019). Then *K* decreased with OM further increasing might be possible as a result of P adsorption with

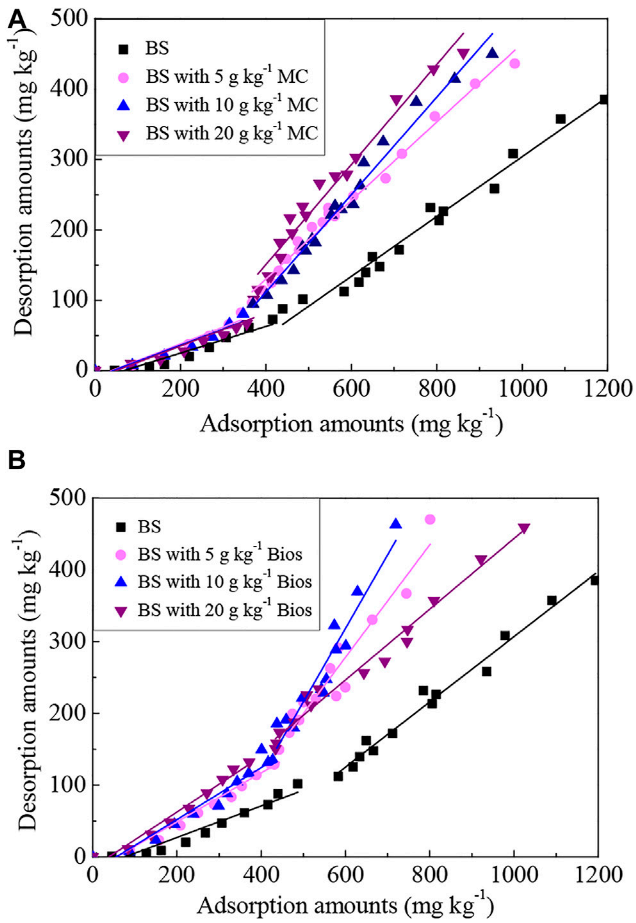

FIGURE 3 | P desorption from soils incubated with different contents of organic matter derived from **(A)** manure compost and **(B)** biosolids.

little energy, such as cation bridges provided by organic materials (Kreller et al., 2003).

MABC is an integrated parameter of the Langmuir model $\Gamma_{\max}$ and *K*. This is a useful index if evaluating the P supply and immobilization capacity and rates by soils (Holford and Patrick, 1979 ; Hafiz et al., 2016). The higher *MABC* means a higher P adsorption amount caused by changed unit equilibrium concentration when equilibrium liquid concentration tends to be very low (close to zero) (Wang and Liang, 2014). Organic materials treatments reduced the *MABC* of the soils for P. The similar results were also obtained by Hafiz et al. (2016), and this suggested that smaller P application rates would be better for maintaining a desired P concentration in the soil solution. Without considering control soil, the changing trend of *MABC* values was similar to that of *K* values and also likely followed parabolic trends, just like the results on the lime concretion black soil and the calcareous yellow fluvoaquic soil previously obtained by Xia and Wang (2009).

The $\Gamma_{\max}$ and *MABC* values of the black soil obtained at the whole initial P concentrations decreased with the application of the organic materials, except the $\Gamma_{\max}$ of the soil incubated with 20-g/kg OM from Bios, indicating that the number of adsorption

TABLE 3 | Parameters of the linear model for P desorbed from soils with and without organic matter incubation.

	The first segment		The second segment	
	Linear equation	*r*	Linear equation	*r*
BS	$D = -16.87 + 0.22 \times \Gamma$	0.9657**	$D = -147.48 + 0.45 \times \Gamma$	0.9907**
BS with 5-g/kg CM	$D = -9.42 + 0.24 \times \Gamma$	0.9650**	$D = -93.01 + 0.56 \times \Gamma$	0.9930**
BS with 10-g/kg CM	$D = -9.53 + 0.23 \times \Gamma$	0.9564**	$D = -165.65 + 0.69 \times \Gamma$	0.9894**
BS with 20-g/kg CM	$D = -11.42 + 0.23 \times \Gamma$	0.9202**	$D = -133.69 + 0.71 \times \Gamma$	0.9834**
BS with 5-g/kg Bios	$D = -19.50 + 0.34 \times \Gamma$	0.9782**	$D = -192.34 + 0.78 \times \Gamma$	0.9567**
BS with 10-g/kg Bios	$D = -20.39 + 0.36 \times \Gamma$	0.9704**	$D = -296.39 + 1.02 \times \Gamma$	0.9497**
BS with 20-g/kg Bios	$D = -14.96 + 0.39 \times \Gamma$	0.9845**	$D = -49.07 + 0.49 \times \Gamma$	0.9895**

sites for P decreased as the organic materials applied, and the binding energy for the P adsorption to the soil also decreased to some extent with some fluctuation as reflected by *K* values.

Phosphorus Desorption Characteristics

P desorption from soils is considered the reverse process of the adsorption process. It is more important than adsorption for reusing immobilized P in soils and possibly inducing the environmental problems caused by the release of P from soils (Wang and Liang, 2014). The amounts of P desorbed from the soils were less than the amounts of P adsorbed previously, indicating that the P adsorbed in soils was not fully reversible, although P that adsorbed to the soils could desorb to some extent and re-release to the soil solution (**Figure 3**). P desorption amounts from the soils tended to increase with increasing P adsorption amounts, and the desorption rates also approximately tended to increase and the obvious turning point appeared at the adsorption amounts of 350–450 mg/kg. When the adsorption amounts were lower than 400 mg/kg, the desorption rates were relatively low, and then increased higher. Considering the shift of P desorption rates, the desorption data before and after the turning points were described separately by the linear equation as shown in **Figure 3**, and the parameters for the linear equation were listed in **Table 3**.

The constant *a* of slope in the linear equation reflects the P desorption capacity from soils, and the higher *a* value, the stronger the P desorption ability. For the control soil, the *a* value is 0.22 when the adsorption amounts were low (corresponding to the low P concentration in the solution in the previous adsorption experiment), and increased to 0.45 with the P adsorption amounts increasing. P desorbed from the soils incubated with organic materials also presented similar trends with lower *a* values (0.23—0.39) in low P concentrations and higher *a* values (0.49–1.02) in high P concentrations. This indicated that P adsorption sites in soils were abundant when the P concentrations were relatively low and that the P-soil chemical adsorption binding capacity was high, resulting in a high degree of adsorption and a low degree of desorption. With the P concentrations of the solution increasing, the adsorption sites in soils gradually became saturated and finally decreased the P-soil adsorption binding capacity. The physical adsorption mechanism might be responsible for this adsorption stage and thus P bound by physical adsorption could readily be desorbed and re-released (Wang and Liang, 2014; Yang et al., 2019). So, it is important to control the amounts of P applied to soil, as a less level of P application will supply a less P concentration in the soil solution and more P will be absorbed by soils, and thus P fertilizer will not play its due role for plants; a higher P application will increase the amounts of P desorbed, then P concentration of the soil solution will increase and P fertilizer utilization will be improved. However, from the perspective of environmental risk, higher P concentration in the soil solution caused by higher P application to the soil will enhance the risk of P releasing and losing from soil to the surrounding environment, such as surface waters or underground waters.

The *a* values of the soils with organic materials incubation were generally greater than that of the control soil both in the low P concentrations and in the high P concentrations. In low P concentrations, *a* value increased with the Bios incubation rate increasing, and in high P concentrations, *a* value increased with MC added rate increasing. Also, it could be found that organic materials incubation promoted the desorption turning point ahead. The results suggested that incubation of organic materials can enhance the P desorption of the chemical adsorption phase in low P concentration to a lesser extent, and significantly promote the P desorption of the slow physical adsorption phase in high P concentration. This process could affect the activity and bioavailability of soil P, and then increase the amounts of bioavailable P; also, the risk of P loss may increase, especially in high P concentrations.

CONCLUSION

The effects of dairy manure compost and biosolids on adsorption and desorption of P were studied in a typical black soil (a mollisol) from Northeast China by using the batch equilibrium experiments. After being incubated by organic materials, P releasing/desorbing amounts of the soil significantly increased and should be accounted for when estimating P adsorption amounts. The modified Langmuir equation which has been proved to effectively account for the released P effect for P-enriched soil was chosen to fit P adsorption and the fitted results proved this modified model sufficiently to describe P adsorption. Except for the 20-g/kg biosolids incubation, adding organic materials generally decreased both the maximum adsorption capacity for P and the maximum adsorption buffering capacity at the whole P concentrations, indicating that the number of adsorption sites for P decreased as the organic materials were applied, and the binding energy for the P adsorption to the soil also decreased to some extent with some fluctuation as reflected by *K* values. The higher adsorption of 20-g/kg biosolids incubated soil might be due to the higher DOC content, lower molecular weight, and higher humic acid of 20-g/kg biosolids spiked soil. The linear equations described well for P desorption and organic materials incubation enhanced P desorbed from the soils. The constant *a* value of slope in a linear equation reflects the P desorption capacity from soils increased due to the application of organic materials, especially in high P concentrations with *a* value of 0.45 for the control soil increased to 1.02 for 10-g/kg biosolids treated soil.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

ZW conceived and designed the experiments, analyzed the data, wrote the original draft, and discussed the results. LH further analyzed the data, verified the analytical methods, and edited the manuscript. ZL reviewed and edited the manuscript. NC supervised the work. XW supervised the work and reviewed and edited the manuscript.

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Response of Soil Microbial Community Structure to Phosphate Fertilizer Reduction and Combinations of Microbial Fertilizer

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The excessive application of phosphorus (P) fertilizer is becoming a major agricultural problem, which reduces the utilization rate of the P fertilizer and degrades soil quality. The following five P fertilizer treatments were investigated to know how they affect soil properties, enzyme activity, bacterial and fungal community structure. 1) no P fertilizer (P0); 2) farmers' traditional P fertilization scheme (FP); 3) 30% reduction in P fertilizer application (P1, microbial blended fertilizer as base fertilizer); 4) 30% reduction in P fertilizer application (P2, diammonium phosphate as starting fertilizer); 5) 30% reduction in P fertilizer application (P3, microbial inoculum seed dressing). The P fertilizer reduction combined with microbial fertilizer significantly increased soil organic matter (SOM), total phosphorus (TP), available phosphorus (AP) available potassium (AK) contents, and acid phosphatase activity (ACP), however, soil urease activity was significantly reduced. Moreover, the P fertilizer reduction combined with microbial fertilizer significantly increased the relative abundance of a potential beneficial genus (i.e., *Bacillus*, *Pseudomonas*, *Penicillium*, and *Acremonium*) and potentially pathogenic genus (i.e., *Fusarium*, *Gibberella*, and *Drechslera*). The structural equation model (SEM) revealed that different P fertilizer reduction systems had significant indirect effects on bacterial and fungal community structures. The results suggested that the P fertilizer reduction combined with microbial fertilizer systems regulated the pathogenic and beneficial genus which created a microbial community that is favorable for maize growth. Moreover, the findings highlighted the importance of soil properties in determining the soil bacterial and fungal community structure.

Keywords: phosphate fertilizer reduction, microbial fertilizer, bacteria, fungi, high-throughput sequencing

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INTRODUCTION

Phosphorus (P) is the most abundant essential nutrient in plants after nitrogen and potassium. According to reports, about 40% of the world's arable land is limited by P deficiency, which affects crop yield. As such the application of P fertilizer has become one of the important agronomic measures to improve crop yield and soil quality (Elser, 2012). However, P fertilizers are easily adsorbed and fixed after being applied to the soil, and their utilization rate in the planting season is only 10%–25%, and the remaining 85% accumulates in the soil in an ineffective state (Zhu et al., 2018). This does not only reduce the application efficiency of P fertilizers but also accelerates the depletion of non-renewable P resources, increasing the risk of environmental pollution (Meinikmann et al., 2015). Therefore, finding ways to improve the utilization efficiency of P fertilizers and slow down the depletion of P rock resources is of great significance for the realization of sustainable agricultural development. This will optimize the application of P fertilizers in order to fully benefit the biological potential of crops and soil microorganisms.

Microorganisms are the key drivers in the cyclic transformation of fertilizer nutrients into the soil and are directly related to nutrient use efficiency and its agronomic and environmental effects (Wang et al., 2022). Soil microorganisms play a very important role in the transformation of soil P and about 40% of soil microorganisms can activate insoluble P and convert it into microbial P to form a soil microbial biomass P pool (Jorquera et al., 2008). Application of P fertilizer can increase the content of soil available P, promote the release of P in soil microorganisms, and provide available P for crop absorption and utilization. Studies have shown that different fertilization methods can directly or indirectly affect the structure and composition of soil microbial communities and their functions by changing the soil microenvironment. This process is driven by factors such as soil pH, texture, moisture, organic carbon content, and nutrient availability (Zhang et al., 2017; Ding et al., 2018; Lin et al., 2019). However, long-term excessive application of P fertilizers not only leads to the decline of soil organic matter content and quality (Zhang and Zhang, 2010), but also leads to the depletion of soil resources and energy, water, air pollution, and other environmental problems (Liu et al., 2007). Therefore, the partial replacement of chemical fertilizers with microbial fertilizers has gradually become an important way to improve soil fertility and realize the reduction and efficiency of chemical fertilizers in this era of intensified agricultural activities. Microbiome studies have proved that the microbial flora in the ecological environment is complex and diverse, and it is difficult to achieve artificial directional control. The most effective solution to promote the growth and development of plants is to inoculate crops with beneficial microbial fertilizers. This will promote the formation of new microbial flora at the root of crops and improve the soil environment (Pineda et al., 2017). Microbial fertilizers mainly include microbial inoculants, compound microbial fertilizers, and bio-organic fertilizers. Studies have reported that microbial fertilizers can promote corn growth and increase yield and can replace 10%–30% of compound chemical fertilizers. At present, there is a lack of targeted research in the case of reducing P fertilizers and replacing them with microbial fertilizers (Atieno et al., 2020). Although there have been much research on the effects of different fertilization measures on soil nutrient transformation and the changes in microbial community structure etc, however, the microbiological mechanism and regulation principle of the changes in soil nutrients and enzyme activities in the case of reducing P fertilizers and replacing it with microbial fertilizers remains unknown. Therefore, this study used Illumina Miseq sequencing technology to analyze the effect of different P reduction systems on soil bacterial and fungal community structure, to elucidate the changes in soil microbial community composition, diversity, and functional bacteria caused by P reduction. The results can provide a theoretical reference to understand the microbiology related to P fertilizer reduction and synergy in the black soil region of Northeast China.

MATERIALS AND METHODS

Experimental Design

This experiment was set up in an experimental field in the southern area of Jilin Agricultural University. The average annual temperature in the area is 4.9°C and the average annual precipitation is 593.8 mm. The soil tested was typical black soil and the experimental site was a large-scale test site with a test area of 312 m^2. The study included five treatments: 1) no P fertilizer (P0); 2) farmers' traditional P fertilization scheme (FP, 120 kg/hm^2 P_2O_5 of diammonium phosphate as base fertilizer); 3) 30% reduction in P fertilizer application (P1, 84.0 kg P_2O_5/hm^2 and microbial blended fertilizer as base fertilizer); 4) 30% reduction in P fertilizer application (P2, 67.2 kg P_2O_5/hm^2 and microbial blend fertilizer as base fertilizer +16.8 kg P_2O_5/hm^2 and diammonium phosphate as starting fertilizer); 5) 30% reduction in P fertilizer application (P3, microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 and diammonium phosphate as base fertilizer). The fertilizer application rate of each treatment is shown in **Supplementary Table S1**, and the field management followed the conventional management system. The planting density was 65,000 plants/hm^2 and the tested corn variety was Liangyu 99. The microbial blended fertilizer and microbial inoculum were purchased from Jilin Province Jiabo Biotechnology Co., Ltd. (the number of viable bacteria ≥200 million/g).

During the maize tasseling period, soil samples of each treatment were taken from the 0–20 cm layer, using a random sampling method with three replicates. After collection, the samples were placed in an icebox and brought back to the laboratory. Part of each sample was stored in the refrigerator at −80°C for total soil DNA extraction, and the other part was naturally air-dried in a cool place from which a small sample was taken, ground, and sieved according to the quartering method, for the determination of soil physicochemical properties.

Soil Physicochemical Properties and Enzyme Activity Determination

Soil pH was measured by pH meter (the water-soil ratio was 2.5:1), soil organic matter (SOM) was measured by potassium dichromate oxidation-external heating method, and soil total nitrogen (TN) was measured by Kjeldahl method. The alkaline hydrolysis diffusion method was used to determine the available nitrogen (AN) content of the soil. The total phosphorus (TP) content of the soil was digested with H_2SO_4-$HClO_4$ and antimony resistance was measured by the colorimetric method. Soil total potassium (TK) and soil available potassium (AK) were measured by a flame spectrophotometer. The phenol-sodium hypochlorite colorimetric method was used for the determination of soil urease by using urea as the matrix and the soil phosphatase was determined by the phenyl disodium phosphate colorimetric method.

Soil DNA Extraction

DNA was extracted using the FastDNA® SPIN Kit for Soil (MP Biomedicals, United States), and the extracted DNA was detected and analyzed using gel electrophoresis.

Illumina MiSeq Sequencing

The corresponding primers for the bacterial 16S rRNA V3-V4 region were: 338F (5′-GTG CCA GCM GCC GCG G-3′) and 806R (5′-CCG TCA ATT CMT TTR AGT TT-3′) (Xu et al., 2016). The fungal ITS (ITS1-ITS2) primers used were ITS1F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) and ITS2 (5′-GCT GCG TTC ATC GAT GC-3′) (Adams et al., 2013). 16S rRNA and ITS gene were performed using a 25 µl reaction system: forward Primer (5 µM) 1 µl, reverse Primer (5 µM) 1 µl, BSA (2 ng/µl) 3 µl, template DNA 30 ng, 2xTaq plus master mix 12.5 µl, ddH_2O 7.5 µl. 16S rRNA gene PCR amplification procedure was 94°C 5 min, 94°C 30 s, 55°C 30 s, 72°C 60 s, 72°C 7 min, for 28cycles. ITS gene PCR amplification procedure was as follows: 94°C 5 min, 94°C 30 s, 55°C 30 s, 72°C 60 s, 72°C 7 min, for 34 cycles. All samples were replicated three times, and PCR products from the same sample were pooled. The axyprep DNA recovery kit was used to recover the resulting product. Samples were eluted with Tris-HCl and detected by electrophoresis.

Illumina Miseq Analytical Methods for High Throughput Data

The off-machine data was split by the QIIME software (V1.8.0) according to the barcode sequence, and the Pear software (V0.9.6) was used to filter and splice the data. The minimum overlap during splicing was set to 10 bp, and the mismatch rate was set to 0.1. Short and chimeric sequences were removed using the Vsearch software (V2.7.1) and denovo method. The high-quality sequences obtained were clustered by OTU (Operational Taxonomic Units) using the Vsearch software (V2.7.1) and UPARSE algorithm (version7.1 http://drive5.com/uparse/), and the similarity threshold was set to 97% (Edgar, 2013). The OTUs obtained from the 16S rRNA gene were aligned on the Silva database (Release128 http://www.arb-silva.de) and the minimum similarity was 0.8. The OTUs obtained from the ITS gene were aligned on the UNITE database (version7. 2 https://unite.ut.ee/) and the lowest similarity was set to 0.8 (Koljalg et al., 2014). In order to analyze the microbial communities at the same sequencing depth, the lowest sequencing number was randomly selected per sample of which 24,477 sequences were selected for bacterial 16S rRNA gene and 27,192 sequences for fungal ITS gene. The raw sequences were deposited into NCBI under the accession number SRP365800.

Statistical Analysis

The significant difference between treatments was analyzed by the F test in one-way ANOVA and multiple comparisons of means ($p < 0.05$) were conducted with a Fisher's protected least significant difference (LSD) using SPSS 20. Pearson analysis was used to analyze the correlation between soil enzyme activity and soil physicochemical properties. Heatmaps were drawn using the "pheatmap" package in R (V3.4.2) (R-project, 2010). Partial least squares discriminative analysis (PLS-DA) was completed in the muma package of R software (version 3.5.0). Bacterial function prediction analysis was done by using FAPROTAX (http://www.zoology.ubc.ca/louca/FAPROTAX). The fungal function was predicted by FUNGuild (http://www.stbates.org/guilds/app.php) (Nguyen et al., 2016). Canonical correspondence analysis (CCA) was done with Canoco software (version 5.0) and the structural equation model (SEM) was established using AMOS 24 (Malaeb et al., 2000). This was used to establish the direct and indirect effects of different P reduction systems on soil bacterial and fungal community diversity and composition. A priori model of predictors were constructed and the soil bacterial and fungal community structures were represented by PC1. Model accuracy was tested with χ^2 ($p > 0.05$), goodness-of-fit index (GFI>0.9), and Akaike information criterion (AIC) (Markland, 2007).

RESULTS

Soil Physicochemical Properties

The application of P significantly increased soil nutrient content compared with P0. Compared with P0, treatments FP, P1, P2, and P3 significantly increased the content of SOM, TP, AN, and AK, while P2 and P3 significantly increased the content of TN (**Table 1**). Moreover, P1, P2, and P3 significantly increased the content of AP. Compared with high P application (FP), the treatments with phosphorus reduction systems (P1, P2, and P3) significantly improved the contents of SOM, TN, TP, AN, AP, and AK (**Table 1**). Compared with FP, the SOM and TP contents of P1, P2, and P3 were significantly increased by 10.81%, 16.93%, 13.67% and 13.73%, 22.61%, 23.69%, respectively. In addition, the TN and AN contents of P2 significantly increased by 7.50% and 8.20%, respectively. The AP contents of P2 and P3 were significantly increased by 24.50% and 36.51%, respectively.

Soil Enzymatic Activity

Compared with P0, the application of P fertilizer significantly reduced soil urease activity. Compared with FP, P2 and P3 significantly reduced soil urease activity (**Figure 1**). Also, P2 and P3 significantly increased acid phosphatase (ACP) activity compared with P0 and FP. There was no

TABLE 1 | Soil physicochemical properties under different P fertilizer application systems.

Treatment	pH	SOM (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)	An (mg/kg)	AP (mg/kg)	AK (mg/kg)
P0	6.09 ± 0.06b	17.01 ± 0.34d	1.07 ± 0.02cd	0.40 ± 0.02d	21.82 ± 0.44a	118.13 ± 4.19c	11.33.±0.63cd	129.85 ± 0.79c
FP	6.21 ± 0.04a	18.29 ± 0.63c	1.13 ± 0.02bc	0.45 ± 0.02c	21.75 ± 0.34a	134.96 ± 6.06b	14.04 ± 1.84bc	134.77 ± 2.28b
P1	6.23 ± 0.03a	20.27 ± 0.45b	1.13 ± 0.09bc	0.51 ± 0.02b	21.66 ± 0.41a	133.42 ± 3.75b	14.34 ± 1.18b	135.33 ± 2.25b
P2	6.27 ± 0.04a	21.39 ± 0.52a	1.22 ± 0.03a	0.55 ± 0.02a	21.84 ± 0.26a	146.04 ± 5.04a	17.48 ± 0.95a	140.36 ± 0.77a
P3	6.27 ± 0.06a	20.79 ± 0.49 ab	1.17 ± 0.04 ab	0.55 ± 0.01a	21.86 ± 0.37a	139.79 ± 3.74 ab	19.17 ± 3.22a	141.89 ± 3.42a

Values followed by different letters within the same column are significantly different among treatments tested by one-way ANOVA., P0 and FP, represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate). SOM, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium.

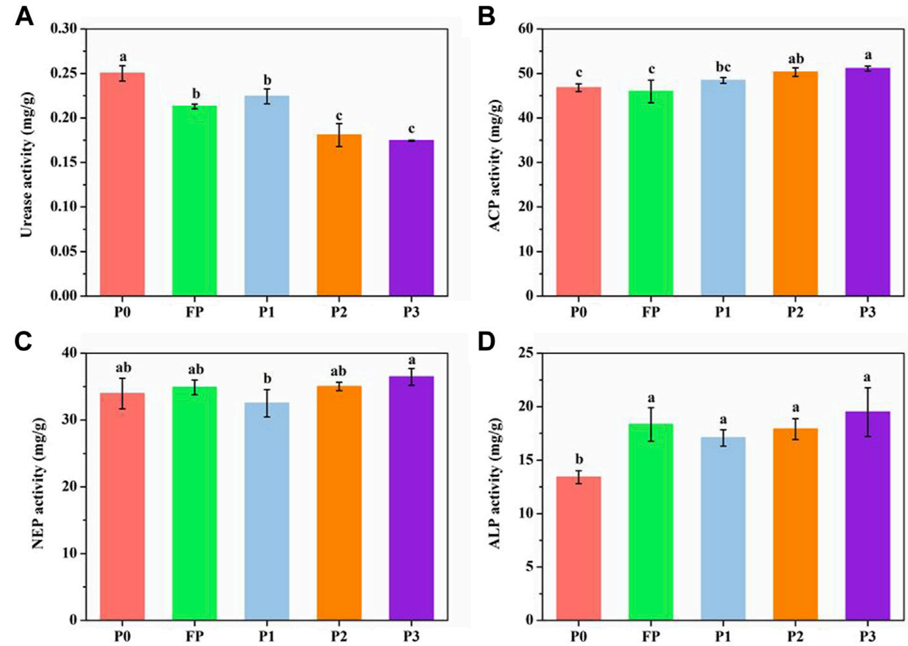

FIGURE 1 | Soil enzymatic activity under different P fertilizer application systems. Urease **(A)**; ACP, acid phosphatase **(B)**; NEP, neutral phosphatase **(C)**; ALP, alkaline phosphatase **(D)**. P0 and FP represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate).

significant difference in neutral phosphatase (NEP) activity among the different P application systems. Compared with P0, the application of P fertilizer significantly increased alkaline phosphatase (ALP) activity. Urease activity was significantly negatively correlated with TP and AP, while ACP activity and ALP activity were significantly positively correlated with TP and AP (**Supplementary Figure S1**). Neutral phosphatase was significantly positively correlated with AP.

Soil Bacterial and Fungal Community Composition

In the Illumina-miseq sequencing of the 16S rRNA gene in this experiment, 596,004 optimized sequences were obtained from all samples, with 31,258–48,530 optimized sequences per sample (an average of 39,734 optimized sequences) (**Supplementary Table S2**). In the Illumina-miseq sequencing of ITS genes, 660,412 optimized sequences were obtained from all samples, with 35,632–49,544 optimized sequences per sample (44,027 optimized sequences on average) (**Supplementary Table S3**). The dominant bacterial phyla under different phosphorus application systems were Proteobacteria (29.5%–40.3%), Actinobacteria (19.2%–22.8%), Acidobacteria (12.0%–16.0%), Bacteroidetes (5.84%–9.77%), and Saccharibacteria (3.95%–9.53%) (**Supplementary Figure S2A**). Moreover, P2 and P3 significantly increased the relative abundance of Saccharibacteria compared with P0 and FP. The relative abundance of Ascomycota (57.3%–85.8%) was the highest under the different P application systems,

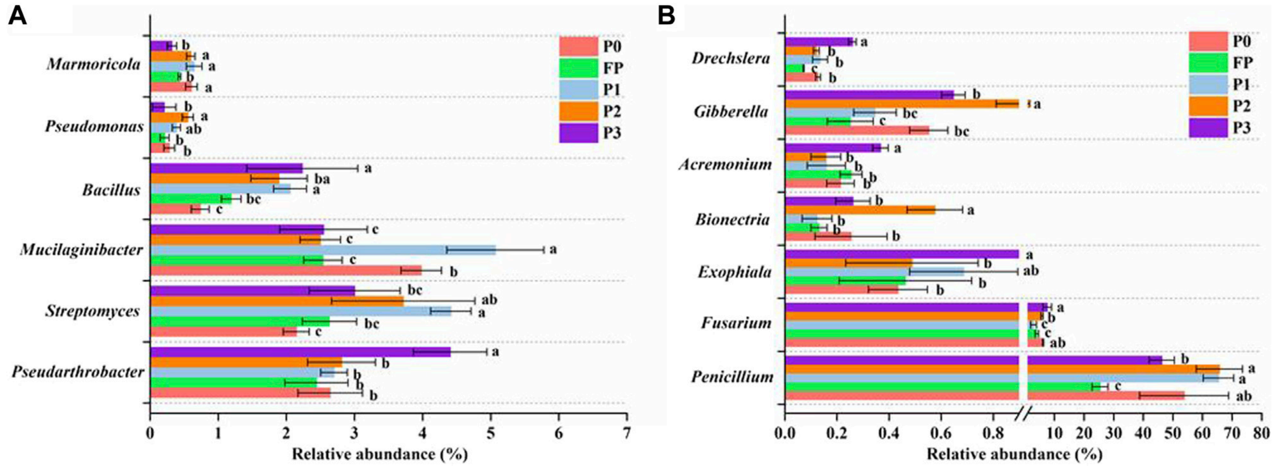

FIGURE 2 | Relative abundance of the dominant functional bacterial genera **(A)** and functional fungal genera **(B)** under different P fertilizer application systems. P0 and FP represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate).

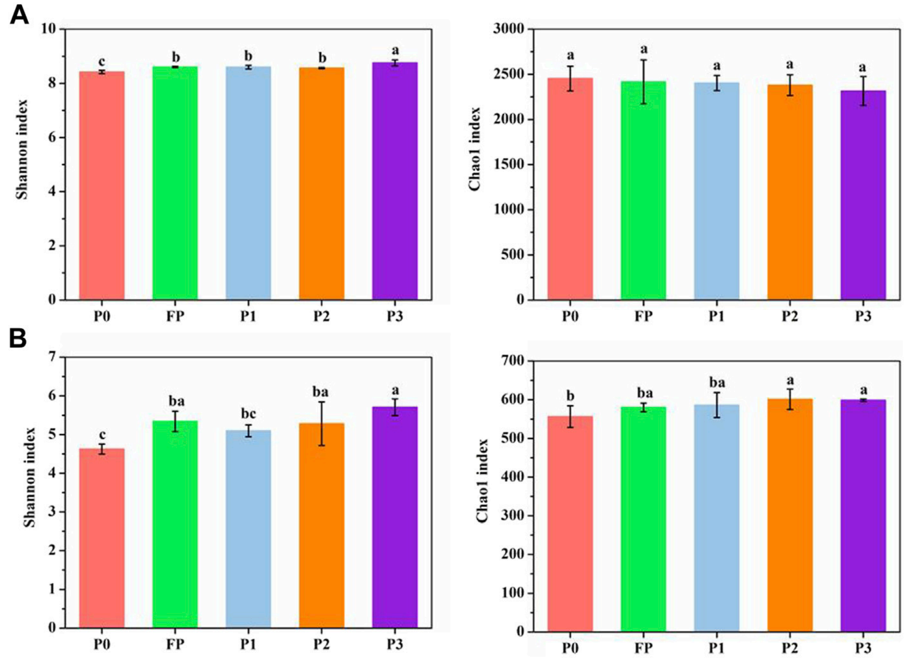

FIGURE 3 | Soil bacterial **(A)** and fungal **(B)** community Shannon and Chao1 index under different P fertilizer application systems. P0 and FP represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate).

followed by Basidiomycota and Mortierellomycota (**Supplementary Figure S2B**). Compared with FP, the combination of P reduction and microbial fertilizer significantly increased the relative abundance of Ascomycota.

The relative abundance of some functional genera changed after P reduction and microbial fertilizer application. P3 significantly increased the relative abundance of *Pseudarthrobacter* (**Figure 2A**), P1 significantly increased the relative abundance of *Mucilaginibacter* and *Streptomyces*, and P1

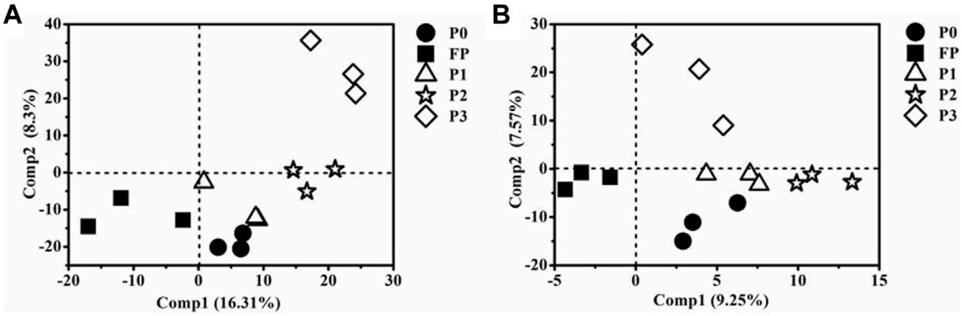

FIGURE 4 | Partial least squares discriminant analysis (PLS-DA) of soil bacterial **(A)** and fungal **(B)** community under different P fertilizer application systems. P0 and FP represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate).

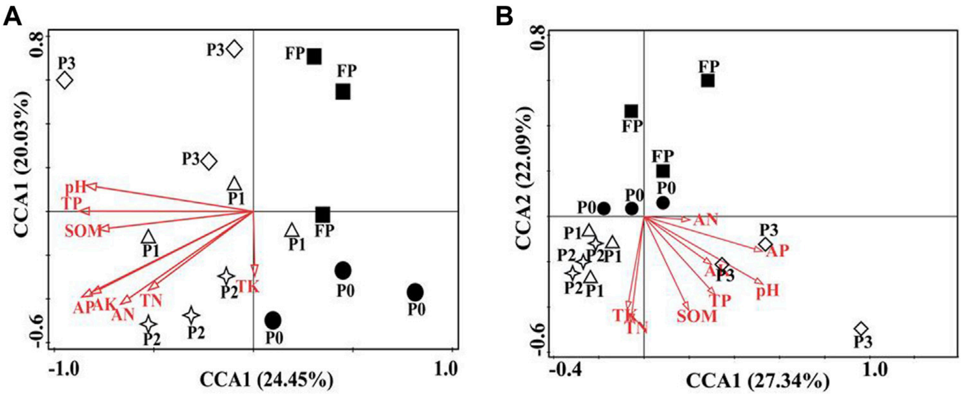

FIGURE 5 | Canonical correspondence analysis (CCA) of soil bacterial **(A)** and fungal **(B)** community structure and soil properties under different P fertilizer application systems. P0 and FP represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate).

and P3 significantly increased the relative abundance of *Bacillus*. The relative abundance of *Pseudomonas* and *Marmoricola* significantly increased in P2. The dominant fungal genus under the different P application systems was *Penicillium* (25.4%–65.7%) (**Figure 2B**). The relative abundance of *Penicillium* increased significantly after P reduction and microbial fertilizer application. P2 and P3 significantly increased the abundance of *Fusarium* and *Gibberella*. Also, P3 significantly increased the relative abundance of *Exophiala*, *Acremonium*, *Drechslera*, and P2 significantly increased the relative abundance of *Bionectria*.

Soil Bacterial and Fungal Community Diversity

Compared with FP, P3 significantly increased the Shannon index of bacteria (**Figure 3A**), as well as the Shannon index and Chao1 index of fungi (**Figure 3B**). PLS-DA analysis showed that the soil bacterial and fungal communities on PC1 both formed their cluster distributions between high and low P, and the bacterial and fungal communities of P3 were significantly different on PC2 (**Figure 4**).

Correlation Analysis Between Microbial Community and Environmental Factors

The CCA analysis showed that AP was the main driver of changes in bacterial and fungal community structure ($p < 0.05$) (**Figure 5**, **Supplementary Tables S4, S5**). SEM was used to predict the direct and indirect effects of different P reduction systems on soil microbial diversity and composition. The final fitted model complies with the established criteria of this experiment and gives a better model effect GFI = 0.997, AIC = 54.161, df = 1, χ^2 = 0.161 (**Figure 6B**, **Figure 6**). Different P reduction systems indirectly affected the bacterial community through their effects on soil pH, SOM, AP, AN, TP, ACP, NEP, and urease. Again, the P reduction systems indirectly affected the fungal community structure through their effects on soil pH, SOM, AN, TN, ACP, and urease (**Figures 6A, B**).

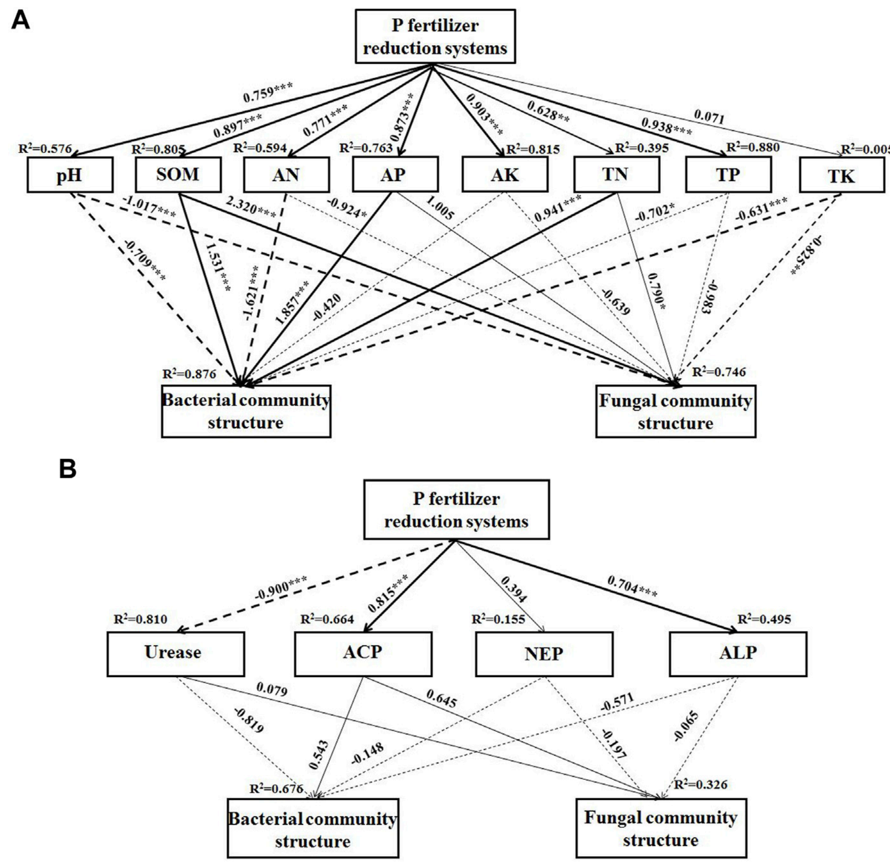

FIGURE 6 | The structural equation model (SEM) for the relationship between P fertilizer reduction systems and soil properties **(A)**, soil enzymatic activities and their relationship with soil bacterial and fungal community structure **(B)**. The thickness of the arrow was proportional to the strength of the path coefficient. A solid arrow represents a positive path coefficient, a dotted arrow represents a negative path coefficient. *, **, *** respectively indicated $p < 0.05$, $p < 0.01$, $p < 0.001$. SOM, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium. ACP, acid phosphatase; NEP, neutral phosphatase; ALP, alkaline phosphatase. P0 and FP represent 0 and 120 kg P_2O_5 ha^{-1} of P fertilizer application rates, respectively. P1, 30% reduction in P fertilizer application (84.0 kg P_2O_5/hm^2 of microbial blended fertilizer); P2, 30% reduction in P fertilizer application (67.2 kg P_2O_5/hm^2 of microbial blend fertilizer +16.8 kg P_2O_5/hm^2 of diammonium phosphate); P3, 30% reduction in P fertilizer application (microbial inoculum seed dressing +84.0 kg P_2O_5/hm^2 of diammonium phosphate).

DISCUSSION

Shifts in Soil Physicochemical Properties and Enzymatic Activity in Response to Different P Fertilizer Reduction Systems

The results of this study showed that compared with FP, treatments P1, P2, and P3 significantly increased the contents of SOM and TP, indicating that soil organic matter conditions were improved after P reduction and microbial fertilizer application, and P fixation was reduced, thereby improving soil P availability. This may be due to the fact that the microbial fertilizer contains a variety of microorganisms, which can increase the content of SOM, thereby improving soil fertility (Juwarkar and Jambhulkar, 2008; Tosic et al., 2016). P2 and P3 significantly increased ACP activity compared with FP, as similarly indicated by Clarholm (1993) that chronic P application decreased ACP activity. This indicated that there was a positive interaction between microbial fertilizers with reduced P fertilizers and host microorganisms in the soil. This may be because the rich active substances in microbial fertilizer can have an impact on the activity of soil ACP to promote the absorption and utilization of P nutrients by crops and also enhance the availability of soil available P, which leads to an increased ACP activity (Prasanna et al., 2012).

Effects of Different P Fertilizer Reduction Systems on Soil Microbial Community Composition

In this study, Proteobacteria, Actinobacteria, and Acidobacteria were the most abundant bacterial phyla at the phylum level (**Supplementary Figure S2A**), which is consistent with previous studies which have shown that Proteobacteria, Actinobacteria, and Acidobacteria are the most common bacterial phyla in different agricultural systems (Shen et al., 2014). Similarly as has been indicated in previous studies almost all fungi isolated from farmland are Ascomycota (Viaud et al., 2000), in this study the dominant fungal phylum with the highest relative abundance was Ascomycota (**Supplementary Figure S2B**). This study showed the relative abundance of most potential beneficial genera (i.e., *Pseudarthrobacter*, *Mucilaginibacter*, *Streptomyces*, *Bacillus*, *Pseudomonas*, *Penicillium*, and *Acremonium*) and pathogenic genera (i.e., *Fusarium*, *Gibberella*, *Exophiala*, and *Drechslera*) increased in P reduction and microbial fertilizer application. Some studies have reported that the application of microbial bacterial fertilizer can significantly increase the diversity of microbial communities and promote the proliferation of beneficial bacteria (Araujo et al., 2020; Wang et al., 2022). In Chen et al. (2021) *Pseudarthrobacter defluvii E5* was isolated from agricultural soils and showed efficient phthalic acid esters degradation and mineralization abilities. *Mucilaginibacter* is known for the degradation of cellulose and hemicellulose (López-mondéjar et al., 2016) while *Streptomyces* from a disease-suppressive soil produced volatile organic compounds (Cordovez et al., 2015). *Bacillus* with P solubilizing function may be a potential inoculant to regulate the biotic process of P transformation (Zhang, et al., 2021). *Pseudomonas* resistance against pathogen growth improves P cycling and also serves as inorganic P solubilizing bacteria that can be cultured (Jones and Oburger, 2011; Panpatte et al., 2016). *Penicillium* is an antagonistic phytopathogenic fungus and plays a critical role in the control of plant diseases. *Penicillium oxalicum* controls tomato fusarium wilt (Sabuquillo et al., 2006) while *Penicillium bilaii* has been shown to increase P solubility (Karamanos et al., 2010). Large and complex fungal genera containing many plant pathogens such as *F. oxysporum* and *F. equiseti* are important pathogenic bacteria that cause root rot (Zhou et al., 2018). Additionally, *Gibberella zeae* causes head blight in wheat, and can produce estrogenic toxins such as zearalenone, trichothecene, and deoxynivalenol (Khan et al., 2001). *Exophiala* are common saprobes in soil, opportunistic pathogens of animals, or endophytes in plant roots (Cheikh-Ali et al., 2015). *Acremonium* is an antagonistic plant pathogenic fungus and plays a certain role in the control of plant diseases. *Acremonium obclavatum* controls peanut rust (Sathiyabama and Balasubramanian, 2018). *Drechslera tritici-repentis* was noted in a previous study as a potentially pathogenic bacteria genus that caused wheat tan spots (Carmona et al., 2006). After the P reduction and microbial fertilizer application, crops can stimulate microorganisms to increase the number of beneficial microorganisms. This is done by increasing the number of certain secretions and crop root exudates that can act as signal molecules to regulate microbial populations and increase the number of antagonistic pathogenic bacteria in the soil. This promotes the healthy development of the soil, which is ultimately beneficial to the growth of crops (Henkes et al., 2011). Microbial function is closely related to soil function. Functional microorganisms can fix nitrogen and dissolve phosphorus, and also produce polysaccharides and other substances through reproduction and metabolism. This improves soil physical and chemical properties, alleviate soil hardening, improve soil fertilizer and water retention, enhance root absorption capacity and improve nutrient utilization. Functional microorganisms donot only ensure proper soil function, but also serve as the basis for restoring soil function (Yang et al., 2020).

Effects of Different P Fertilizer Reduction Systems on Soil Microbial Diversity

In the present study, P3 significantly increased bacterial diversity (**Figure 3A**), fungal diversity, and richness (**Figure 3B**) compared with FP. This may be due to the increase in soil nutrient content, especially the increase in organic matter content, which is the main reason for the increase in microbial diversity. Yue et al. (2022) also found that the combined application of microbial fertilizers and chemical fertilizers significantly increased the Shannon index of soil bacteria. The results of Chhabra et al. (2013) also showed that the addition of P reduced the microbial Shannon diversity index in the soil. Li et al. (2021) found that adding bio-organic fertilizer increased bacterial diversity but decreased fungal diversity. The results of Liu et al. (2018) showed that different P application systems had no significant effect on bacterial diversity, while fungal richness and diversity indices decreased significantly with the increase of P application rates. The reason for the different responses maybe that fungi are more sensitive to soil fertility and P addition than bacteria. Typically, fungal hyphae can extend into the soil, increasing the surface area in the soil for water and nutrient uptake. This gives fungi easy access to AP in soil culminating in its increased sensitivity to P.

Driving Factors Affecting Changes in Soil Microbial Community Structure

The results of this study showed that AP was the main driver of bacterial and fungal community structural changes ($p < 0.05$) (**Figure 5**, **Supplementary Tables S4, 5**). Liu et al. (2018) also found that AP was the dominant factor affecting fungal community changes in different P application systems. Similarly, Siciliano et al. (2014) showed that soil P content is an important driver of soil bacterial and fungal diversity. The effect of P availability on microbial diversity may depend on increasing the relative abundance of some genera and altering competition patterns between different populations (He et al., 2016). These results suggest that P input is a determinant of changes in soil microbial community structure in agricultural systems. Increased AP content may affect microbial community structure, possibly by exerting selective pressure on microbial communities and altering competitive interactions (Crowther et al., 2011).

CONCLUSION

Reduced application of P fertilizer combined with the application of microbial fertilizer significantly increased the content of SOM, TP, AP, AK, and ACP activity and also improved bacterial and fungal diversity. Moreover, the relative abundance of potentially beneficial genera (i.e., *Bacillus*, *Pseudarthrobacter*, *Penicillium*, and *Acremonium*) and potentially pathogenic genera (i.e., *Fusarium*, *Gibberella*, and D*rechsle*ra) increased significantly. Different environmental factors also have different degrees of influence on the changes in soil bacterial and fungal communities, among which AP had the greatest impact. The results suggested that the P fertilizer reduction combined with microbial fertilizer systems regulated the pathogenic and beneficial fungi populations which created a microbial community that is favorable for maize growth. Therefore we recommend the reduced application of P fertilizer combined with the application of microbial fertilizer as the most effective agronomic practice for improving soil fertility and crop yields. This will help to rationalize the application of P fertilizer and also ensure proper nutrient use efficiency and sustainable agriculture.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI (accession: SRP365800).

AUTHOR CONTRIBUTIONS

CL and QG designed the study; HL performed the work; SL, RQ, and EL analysed the data; and ZZ revised the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.899727/full#supplementary-material

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Improvement of P Use Efficiency and P Balance of Rice–Wheat Rotation System According to the Long-Term Field Experiments in the Taihu Lake Basin

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Phosphorus (P) accumulation in rice–wheat rotation fields around the Yangtze River delta have been enriched during the last decades. To protect the environment and save P resources, we conducted field experiments to optimize the P application scheme. First, one field experiment was designed as a series of P fertilizer application doses of 0–100 kg P_2O_5 hm^{-2}. Grain yield and P uptake by crops were analyzed to calculate P surplus and P use efficiency. Soil P fractions were extracted and tested. According to the P balance, we optimized fertilization by reducing the chemical P amount, which was used by local farmers; furthermore, we substituted chemical P with organic fertilizer. To verify these management strategies, another field experiment was conducted with five treatments: no N, P, or K fertilizer (CK); only no P fertilizer (NK); farmers' fertilization of P (90 kg P_2O_5 hm^{-2}) (FFP); reducing 20% P (FFP-20%P); and reducing 20% P and replacing 20% P by manure (FFPM-36%P). The grain yield was enhanced by increased P fertilizer and reached a constant level after 75 kg P_2O_5 hm^{-2}. Moreover, the annual P surplus was balanced around the input of 150 kg P_2O_5 hm^{-2}. Accordingly, by optimizing fertilization (FFP-20%P) and further replacing manure (FFPM-36%P), we also achieved crop yield equivalent to that of FFP treatment (90 kg P_2O_5 hm^{-2}). Thus, the 72–75 kg P_2O_5 hm^{-2} application rate is a threshold for the production of rice and wheat and P balance. Total P content in soil was enhanced by increased input of P fertilizer and mainly divided into labile Pi and middle stable Pi fractions. Soil Olsen-P content increased by P fertilization accordingly, while the content of organic P and stable P content was relatively constant. Reducing P fertilizer by 20% had similar results for soil P fractions when compared with farmers' P fertilization treatment. Therefore, reducing at least 20% current input of P by farmers (annual 180 kg P_2O_5 hm^{-2}) according to the balance of P surplus in rice and wheat rotation systems is an imperative measure to guarantee crop production with enhanced P use efficiency, and meanwhile, it can alleviate environmental risk.

Keywords: rice–wheat rotation, P fertilizer, P surplus, P use efficiency, soil P fractions

INTRODUCTION

Food security is always an important issue in Asia, where there is a large population (Thangavel and Sridevi, 2017). In China, the rice–wheat rotation system is one of the traditional agricultural practices along the Yangtze River Basin, with a planting area of 4.8 million hectares (Zhang et al., 2017), which provides important staple foods for people. In 2018, China's total rice and wheat production accounted for 27% and 18% of the global production, respectively (Muthayya et al., 2015). These huge crop productions in China were strongly dependent on the large inputs of chemical fertilizers, especially N and P (Qiu, 2009; Qiu, 2010; Cui et al., 2018). According to the data from the National Bureau of Statistics (https://www.ceicdata.com/zh-hans/china/consumption-of-chemical-fertilizer-phosphate), P fertilizer applied in 2019 amounts to 6.815 million tons. The annual application amount of P fertilizer from 1979 to 2019 is an average of 6.890 million tons. The value peaked in 2014, at about 8.453 million tons from the lowest value in 1979, about 2.235 million tons. The heavy fertilizer use has made China one of the biggest fertilizer consumers in the world. Therefore, such an agricultural mode had adverse negative effects on the environment, such as the increase in greenhouse gas emissions (Chen et al., 2014; Cui et al., 2018), soil acidification (Guo et al., 2010), eutrophication (Le et al., 2010), and biodiversity loss in soil (Clark and Tilman, 2008). The Yangtze River Basin is an important agricultural area in China due to enough water and temperature resources for paddy rice cultivation. However, the Yangtze River Basin has consistently accumulated phosphorus since 1980 due to intensive agriculture with high input of P (Powers et al., 2016).

Thus, it is urgent to re-evaluate the input of fertilizers, seasonal crop production, accumulation, and balance of nutrient elements in the local agricultural system. Phosphorus (P) is one of the important fertilizers for crop growth and yield. However, P is easily absorbed by oxides of Al or Fe or precipitated by Ca in the soil, which leads to low P use efficiency (Holford, 1997). To achieve high crop production, farmers apply the P fertilizer every season in high amounts, due to the lack of information regarding the present values of available P in soil. In the Yangtze River delta near the Taihu Lake, average yields of rice and wheat in the area were reported to be around 7,500 and 4,500 kg hm^{-2} during the wet and dry rotation, respectively (Zhao et al., 2009). However, due to the high rate of chemical P fertilizer application, the phosphorus content in farmland soil in Taihu Lake Basin has been increasing over the last 40 years. The Olsen-P concentration in 65% of soil samples in this region was over 20 mg kg^{-1} soil (Wang S. Q. et al., 2012; Wang et al., 2022); in contrast, it was only several mg·kg^{-1} in the 1980s. Since the environmental threshold of soil Olsen-P in the local fields is about 30 mg kg^{-1}, the overapplication of chemical P fertilizer or

TABLE 1 | Fertilizers for wheat and rice cultivation (kg·hm^{-2}).

Treatment	Base fertilizer			Tillering fertilizer	Panicle fertilizer	
	N	P_2O_5	K_2O (wheat/rice)	N	N	K_2O (wheat/rice)
P_0	120	0	45/90	90	90	45/0
P_{25}	120	25	45/90	90	90	45/0
P_{50}	120	50	45/90	90	90	45/0
P_{75}	120	75	45/90	90	90	45/0
P_{100}	120	100	45/90	90	90	45/0
CK	0	0	0	0	0	0
NK	120	0	45/90	90	90	45/0
FFP	120	90	45/90	90	90	45/0
FFP-20%P	120	72	45/90	90	90	45/0
FFPM-36%P	120	57.6	45/90	90	90	45/0

TABLE 2 | Effect of P fertilization rates on rice yield.

Treatment	Straw yield (kg·hm^{-2})	Grain yield (kg·hm^{-2})	Increased (%)	Harvest index(%)
2016 rice				
P_0	13208c	7519d	-	56.9b
P_{25}	14905ab	8585c	14	57.5a
P_{50}	15690ab	9089a	20	57.9a
P_{75}	16080a	9325a	24	57.9a
P_{100}	15958b	9145b	21	57.3a
2017 rice				
P_0	13593c	8208b	-	60.3a
P_{25}	15424ab	8806b	7	57.1b
P_{50}	15455ab	9210a	12	59.6a
P_{75}	15799a	9390a	15	59.4a
P_{100}	15544b	9180b	12	59.1a

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

TABLE 3 | Effect of P fertilization rates on wheat yield.

Treatment	Straw yield (kg·hm^{-2})	Grain yield (kg·hm^{-2})	Increased (%)	Harvest index (%)
2016–2017 wheat				
P_0	9311c	3299c	-	35.4c
P_{25}	10935b	4075b	13	37.2b
P_{50}	12520ab	4708a	29	36.7b
P_{75}	12709a	4898a	44	39.2a
P_{100}	12911a	4947a	38	38.1a
2017–2018 wheat				
P_0	9487c	3345d	-	35.2b
P_{25}	10753b	4277c	27	37.0ab
P_{50}	11986a	4598b	37	38.2ab
P_{75}	12825a	5047a	50	39.3a
P_{100}	12589a	4854ab	45	38.0a

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

compound fertilizer brought a great risk to the environment (Powers et al., 2016), and further decreased P use efficiency and wasted P resources (Alewell et al., 2020).

In the present study, we conducted a field experiment with a series of P fertilization rates to check the effect of P input on the crop yield and P surplus in the current rice and wheat rotation system. Furthermore, we reduced the current farmers' application dose of P fertilizer and combined it with organic manure to get an optimized regime of P fertilization in the Taihu Lake delta. Our aim is to find a practical and feasible fertilization strategy to guarantee crop production and reduce risk to the environment for sustainable agriculture.

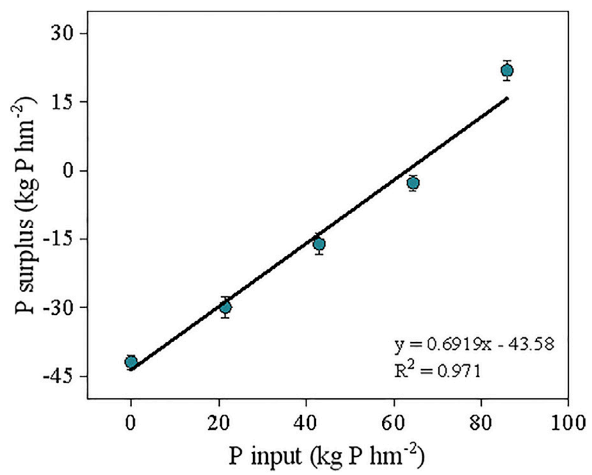

FIGURE 1 | The relationship between the annual P input and the P surplus in rice and wheat rotation according to two years under a series of P fertilization amount treatment.

MATERIALS AND METHODS

Field Experiments Description

Field trials were conducted on a farm in Yixing City, in the Yangtze River delta in the northeast of China (31°41′N, 120°40′E). This region has a subtropical semi-humid monsoon climate, with an average annual temperature of 15.2°C and annual rainfall of 1,286 mm. About 50% of the precipitation is distributed from April to June. The soil was paddy soil, consisting of 12% sand, 45% silt, and 43% clay. The initial soil pH was 6.21 (1:1, water/soil, w/w). In the surface soil (0–20 cm), the total nitrogen is 13.6 g kg^{-1}, the organic carbon is 12.8 g kg^{-1}, the available P is 22.8 mg kg^{-1}, and the available K is 64.9 mg kg^{-1}.

The field trials were divided into two parts. First, the different P fertilization amounts were applied beginning in 2016. There are five treatments: P_0, P_{25}, P_{50}, P_{75}, and P_{100} (representing 0–100 kg P_2O_5 hm^{-2} of P fertilizer, respectively). The plot size was 7 × 5 = 35 m^2 with a 50-cm wide walkway between the plots. Another field experiment had five treatments, including control (CK), no phosphate fertilizer (NK), conventional fertilization by farmers (FFP), FFP reduced P by 20% (FFP-20%P), and organic substitution plus or minus P by 36% (FFPM-36%P). The plot size was 8 × 10 = 80 m^2 with a 50-cm wide walkway between the plots. All treatments had four repetitions, and plots are randomly arranged in the fields.

The local cultivar of rice and wheat were Nangeng 46 and Yangmai 5, respectively. In the rice season, rice seedlings were prepared and transplanted at 30,000 holes per hectare, while wheat was directly planted at 50,000 plants per hectare. Urea was used as N fertilizer, and superphosphate and potassium chloride were used as P and K fertilizers. The organic fertilizer was manufactured by a local factory with organic matter of 40% and total N, P, and K of 6%. Urea was applied as basal fertilizer and two top dressings with a ratio of 4:3:3 (**Table 1**). P, K, and organic fertilizers were applied as basal fertilizers.

Crop Yield Estimation

Crops were manually harvested with all the above-ground parts from each plot. The grain and straw were separated by a threshing machine. Yield components and P concentration of grain and straw were analyzed.

Soil P Analysis

Soil samples (0–20 cm depth) from each plot were collected after crop harvest, air-dried, ground, sieved (< 2 mm sieve), and stored

TABLE 4 | Effect of P fertilization rates on P uptake and P surplus.

Treatment	Wheat		Rice		Anniversary	
	P uptake (kg P·hm^{-2})	P surplus (kg·hm^{-2})	P uptake (kg P·hm^{-2})	P surplus (kg P·hm^{-2})	P uptake (kg P·hm^{-2})	P surplus (kg P·hm^{-2}kg·hm^{-2}
Year 2016–2017						
P_0	16.9c	−16.9	26.9d	−26.9	43.8c	−43.8
P_{25}	18.4bc	−7.5	31.3c	−20.4	49.8c	−28.0
P_{50}	22.5ab	−0.7	36.2b	−14.3	58.7b	−15.0
P_{75}	26.6a	6.1	40.8a	−8.0	67.4a	−1.9
P_{100}	23.6ab	20.0	43.6a	0.1	67.2a	19.9
Year 2017–2018						
P_0	16.2d	−16.2	26.1d	−26.1	42.4c	−42.4
P_{25}	19.5c	−8.6	30.1c	−19.1	49.6bc	−27.8
P_{50}	22.3b	−0.4	35.0b	−13.2	57.3b	−13.6
P_{75}	25.1a	7.6	41.2a	−8.4	66.3a	−0.8
P_{100}	24.4a	19.2	42.3a	1.3	66.7a	20.6

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

TABLE 5 | Effect of optimized fertilization on rice yield.

Treatments	Straw yield (kg·hm^{-2})	Grain yield (kg·hm^{-2})	Increased (%)	Harvest index (%)
2018 rice				
CK	11550c	6480c	-	56b
NK	13692b	8391b	29.4	57a
FFP	15647a	8983a	38.6	57a
FFP-20%P	15790a	9190a	41.8	58a
FFPM-36%P	15960a	9219a	42.2	57a
2019 rice				
CK	11932c	6172c	-	51b
NK	13769b	8410b	36.2	61a
FFP	15772a	9012a	46.0	57a
FFP-20%P	16187a	9135a	48.0	56a
FFPM-36%P	16356a	9245a	49.8	56a

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

TABLE 6 | Effect of optimized fertilization on wheat yield.

Treatments	Straw yield (kg·hm^{-2})	Grain yield (kg·hm^{-2})	Increased (%)	Harvest index(%)
2018–2019 wheat				
CK	8045c	2915c	-	36b
NK	9368b	3669b	25.84	39a
FFP	12885a	4939a	69.43	38ab
FFP-20%P	13087a	4881a	67.44	37ab
FFPM-36%P	13290a	4994a	71.3	37ab
2019–2020 wheat				
CK	7682c	2674d	-	34b
NK	8679b	3782c	41.45	43a
FFP	12702a	4872b	82.23	38ab
FFP-20%P	12914a	4992a	86.72	38ab
FFPM-36%P	13309a	5022a	87.83	37ab

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

TABLE 7 | Effect of optimized fertilization on P uptake and P surplus.

Treatment	Wheat		Rice		Anniversary	
	P uptake (kg P·hm^{-2})	P surplus (kg·hm^{-2})	P uptake (kg P·hm^{-2})	P surplus (kg P·hm^{-2})	P uptake (kg P·hm^{-2})	P surplus (kg P·hm^{-2})
2018–2019 year						
CK	10.5c	–10.5	18.8b	–18.8	29.4c	–29.4
NK	16.0b	–16.0	25.6ab	–25.6	41.6b	–41.6
FFP	23.9a	15.3	34.4a	4.9	58.3a	20.3
FFP-20%P	26.5a	4.96	36.5a	–5.1	63.0a	–0.1
FFPM-36%P	27.1a	4.36	34.3a	–2.9	61.4a	1.4
2019–2020 year						
CK	10.1c	–10.1	18.0b	–18.0	28.1c	–28.1
NK	15.6b	–15.6	24.3ab	–24.3	40.0b	–40.0
FFP	24.7a	14.5	35.9a	3.3	60.7a	17.9
FFP-20%P	26.4a	5.0	38.9a	–7.4	65.3a	–2.3
FFPM-36%P	27.5a	3.9	36.0a	–4.5	63.6a	–0.6

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

TABLE 8 | Effect of P fertilization rates on P use efficiency.

Treatment	Rice		Wheat	
	AE_P (kg·kg^{-1})	RE_P (%)	AE_P (kg·kg^{-1})	RE_P (%)
2016–2017 year				
P_{25}	42.6a	17.8a	31.0a	6.1b
P_{50}	31.4b	18.6a	28.2a	11.3a
P_{75}	24.1b	18.6a	21.3b	12.9a
P_{100}	16.3 c	16.7a	16.5c	6.7b
2017–2018 year				
P_{25}	46.5 a	15.8a	37.3a	13.2a
P_{50}	30.1b	17.7a	25.1b	12.2a
P_{75}	22.7c	20.1a	22.7b	11.9a
P_{100}	17.7d	16.1a	15.1c	8.2b

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

TABLE 9 | Effect of optimized fertilization on P use efficiency.

Treatment	Rice		Wheat	
	AE_P (kg·kg^{-1})	RE_P (%)	AE_P (kg·kg^{-1})	RE_P (%)
2018–2019 year				
FFP	27.8c	17.3b	22.2c	14.9c
FFP-20%P	37.6b	24.6a	30.9b	22.1b
FFPM-36%P	47.6a	26.9a	40.5a	28.7a
2019–2020 year				
FFP	31.6c	19.9b	24.4c	16.3c
FFP-20%P	41.2b	29.0a	32.2b	22.7b
FFPM-36%P	53.4a	31.3a	40.8a	30.3a

Note: Different lowercases indicate significant differences between the treatments of the same year at 5% level by Duncan test.

at room temperature for chemical analysis. Available P in soil was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and determined using the molybdenum blue method (Olsen et al., 1954).

Various P fractions were sequentially extracted from soils by deionized H_2O and anion exchange resin, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1M HCl according to Tiessen and Moir (1993). 0.5 g air-dried soil samples were dispersed in the aforementioned 20 ml solution step by step and shaken for 16 h on a rotary shaker. The soil and supernatant were then separated by centrifugation for 10 min at 10,000 × g at 4°C. The P concentration in the supernatant was determined by a spectrometer at 700 nm (UV 2500, Japan;) using the ascorbic acid molybdenum blue method (Murphy and Riley, 1962). After all the extraction steps, the soil residual was digested with H_2SO_4 and H_2O_2 at 360°C for P analysis. Moreover, $NaHCO_3$ and NaOH extracts were halved to measure the total P and inorganic P. The amount of organic P in the extract was calculated based on the difference between total P in digestion and inorganic P in the extracts.

P Use Efficiency Calculation

Agronomic efficiency of P: AE_P (kg·kg^{-1}) = (yield under P fertilization − yield of blank)/P application dose; recovery efficiency of P: REP (%) = (P uptake by crop under P fertilization − P uptake by crop of blank)/P fertilization dose ×100%;

P uptake (kg·hm^{-2}) = biomass per unit area × P content;
P surplus (kg·hm^{-2}) = (P application dose − P accumulation) per unit area.

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted to explore the effects of different treatments on each variable (SPSS Statistics 19.0). Differences between treatments were tested using Duncan's test with the significance level set as alpha = 0.05. The structural equation modeling (SEM) was used to study the interaction and transformation of different P components (Hou et al., 2016) using IBM SPSS AMOS 22.0. Root mean square error of approximation (RMSEA) (<0.08), chi-

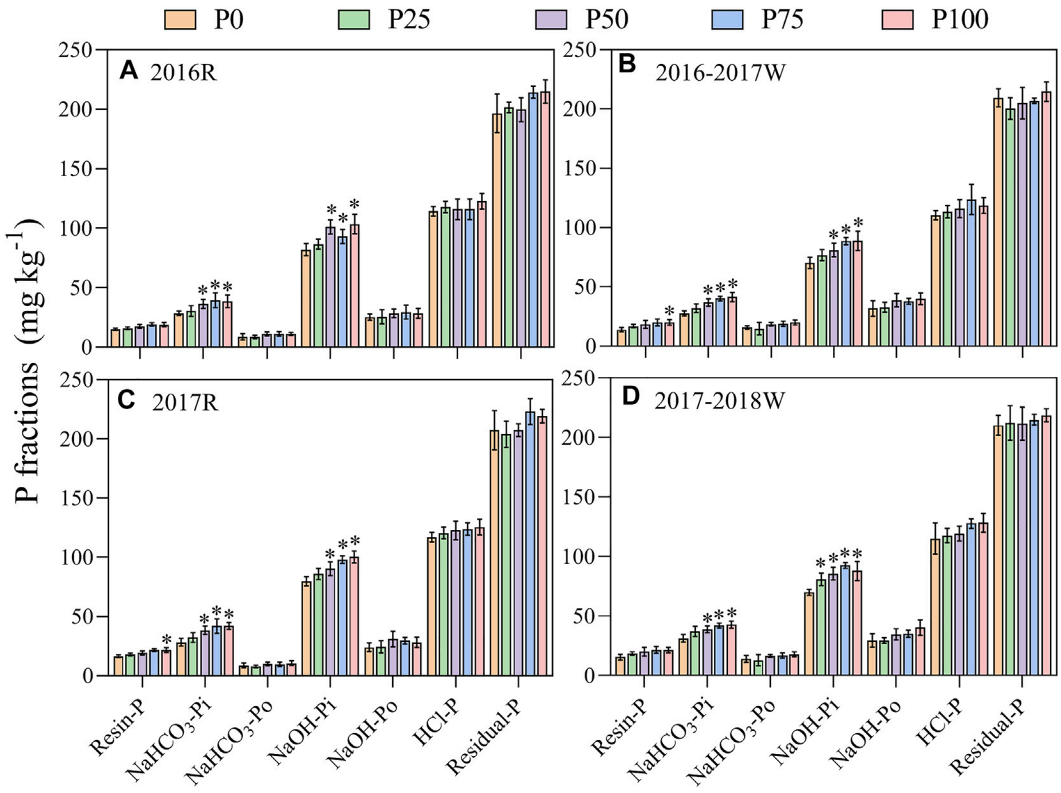

FIGURE 2 | Variations in sequentially extracted P fractions under P fertilization rates on two-year rice-wheat rotation paddy field from 2016 to 2018.

square (χ^2) (χ^2/df < 2), and the p value of χ^2 ($p > 0.05$) were used to evaluate the model fitting.

RESULTS

The Yield of Rice and Wheat Under Different P Fertilization Rates

Rice yield was enhanced by P fertilizer application rates from 0~100 P_2O_5 hm^{-2} (**Table 2**), including both grain and straw yield. However, the harvest index was not variable and ranged from 56~59%. The yield components of rice showed that the P fertilizer application rates significantly affected the panicle number and grain number per panicle, but not the thousand-grain weight and filled-grain percent (**Supplementary Table S1**). Combining the data from the two rice seasons, it was found the P_{75} treatment had the highest grain yield (**Supplementary Figure S1A**) with the highest panicle number and grain number per panicle (**Supplementary Table S1**) among all the treatments. Nevertheless, the yield of rice did not further increase with the treatment of P_{100}.

The wheat yield was also influenced by the application rates of P fertilizer (**Table 3**), with increased panicle number by elevated P input, while the grain number per panicle or thousand-grain weight was not changed (**Supplementary Table S2**). The grain yield of two wheat seasons under the P_{100} treatment was similar to that under P_{75} (**Figure 1A**).

P Uptake by Crops and P Surplus in Rice–Wheat Rotation System Under Different P Fertilization Rates

Since the total yield of aboveground biomass (straw and grain) of rice or wheat was related to the P application rates (**Tables 2**, **3**), the total uptake of P, which accumulated in aboveground biomass of rice and wheat, also seemed dependent on the P application rates (**Table 4**). 75 kg P_2O_5 hm^{-2} input caused the highest P uptake by rice and wheat; however, additional input of P did not enhance P uptake as under P_{100} treatments. P surplus showed negative values before P input around 75 kg P_2O_5 hm^{-2} and became positive after P_{75} treatment.

Taken together, we found the annual P surplus equilibrium is around 150 kg P_2O_5 hm^{-2} application rate (=65.5 P hm^{-2}, **Figure 1**). Therefore, we decided to cut down the currently annual P fertilizer amount (180 kg P_2O_5 hm^2) of farmers (FFP) by 20% (FFP-20%P) and further substitute partial P with organic manure (FFPM-36%P).

The Yield of Rice and Wheat Under Optimized P Fertilization

In the subsequent field trial from 2018 to 2019, we found that the application of P had a significant effect on rice yield compared to the no P application (NK) or no fertilization of N, P, K (CK) (**Table 5**). Reducing 20% P did not decrease the panicle number,

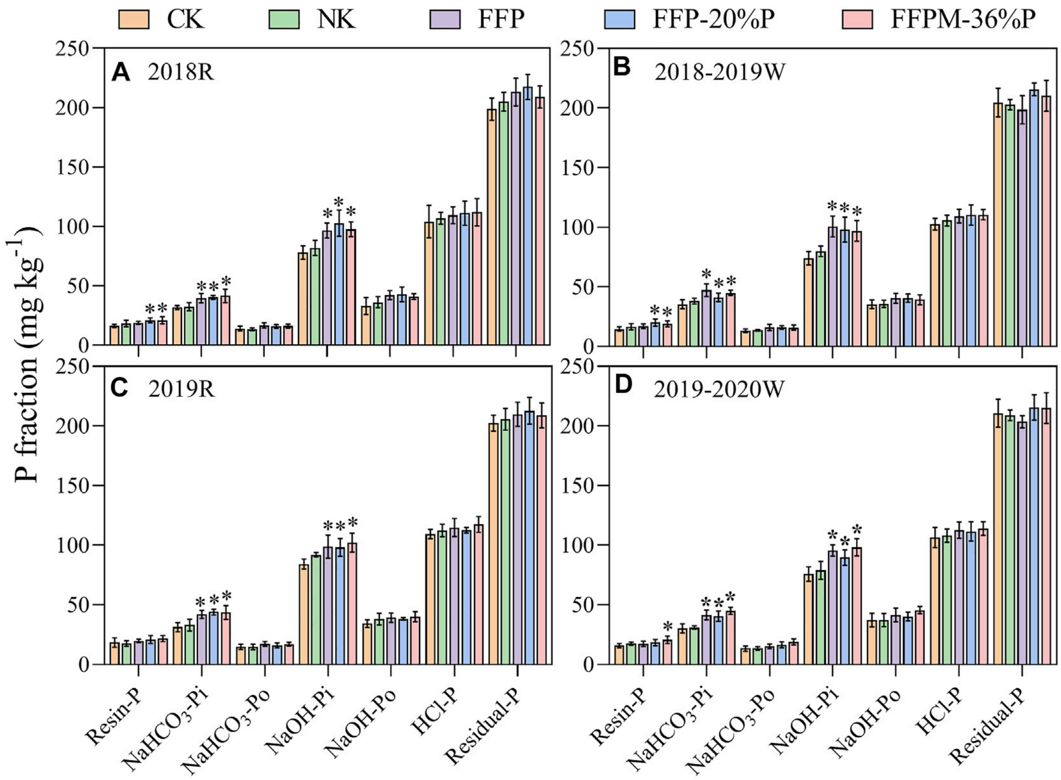

FIGURE 3 | Variations in sequentially extracted P fractions under optimized fertilization on two-year rice-wheat rotation paddy field from 2018 to 2020.

grain number per panicle, thousand-grain weight, and seed setting rate, in comparison with the farmers' P fertilization rate (**Supplementary Table S3**). Combining data from the seasons revealed that the rice yield can be guaranteed by reducing at least 20% of the current P fertilization (**Supplementary Figure S1B**). Similar results were also found in wheat seasons. Application of P fertilizer strongly improved wheat yield (**Table 6**), with significantly increased panicle number and grain number per panicle (**Supplementary Table S4**). Compared to FFP, the yield of wheat in two seasons under FFP-20%P and FFPM-36%P treatments was similar and without the differences.

P Uptake by Crops and P Surplus in the Rotation System Under Optimized P Fertilization

Optimized P fertilization in rice and wheat rotation showed reduced 20% P input did not cause a decrease in P uptake but significantly depressed the P surplus in comparison with the farmer's P fertilization dose (**Table 7**). It seemed that the uptake of P showed no difference between farmer's treatment (FFP) and reduced 20% P fertilizer treatments (FFP-20%P and FFPM-36% P). Furthermore, rice took up more P than wheat and caused a net deficit of P surplus. But taken together, the annual P surplus was near equilibrium under treatments of reduced 20% P fertilizer (FFP-20%P and FFPM-36%P).

P Use Efficiency in Rice and Wheat Season

The effect of different P fertilizer application rates on the P use efficiency in rice and wheat rotation was calculated (**Table 8**). With elevated application rates of P fertilizer, the agronomic efficiency of P (AE_P) continuously decreased, while the recovery efficiency of P (RE_P) increased and reached a maximum under P_{75} treatment, and then decreased under P_{100} treatment for both rice and wheat season (**Table 8**).

The optimized P fertilization with 20% reduced P significantly enhanced the P use efficiency of AE_p and RE_P both in the rice and wheat season (**Table 9**). FFPM-36%P treatment had even higher AE_P and RE_p than FFP-20%P treatment, indicating that organic fertilizer could improve the P availability in soil compared with chemical fertilizer.

Changes of P Fractions in Soil

Different P fractions were extracted from the soil after every season. P extracted with anion-exchange resin and $NaHCO_3$ is considered labile P, and that extracted with NaOH is moderately labile P. P extracted with HCl and residual P is considered stable P (Hedley et al., 1982; Tiessen and Moir 1993).

After treatment of P fertilizer application rates, labile P and moderately labile P in soils were consequently elevated by the increase of P input, either in rice season (**Figures 2A,C**) or wheat season (**Figures 2B,D**). This is also true after the optimized fertilization treatments (**Figure 3**) that labile P and moderately labile P significantly increased, compared with no P treatments

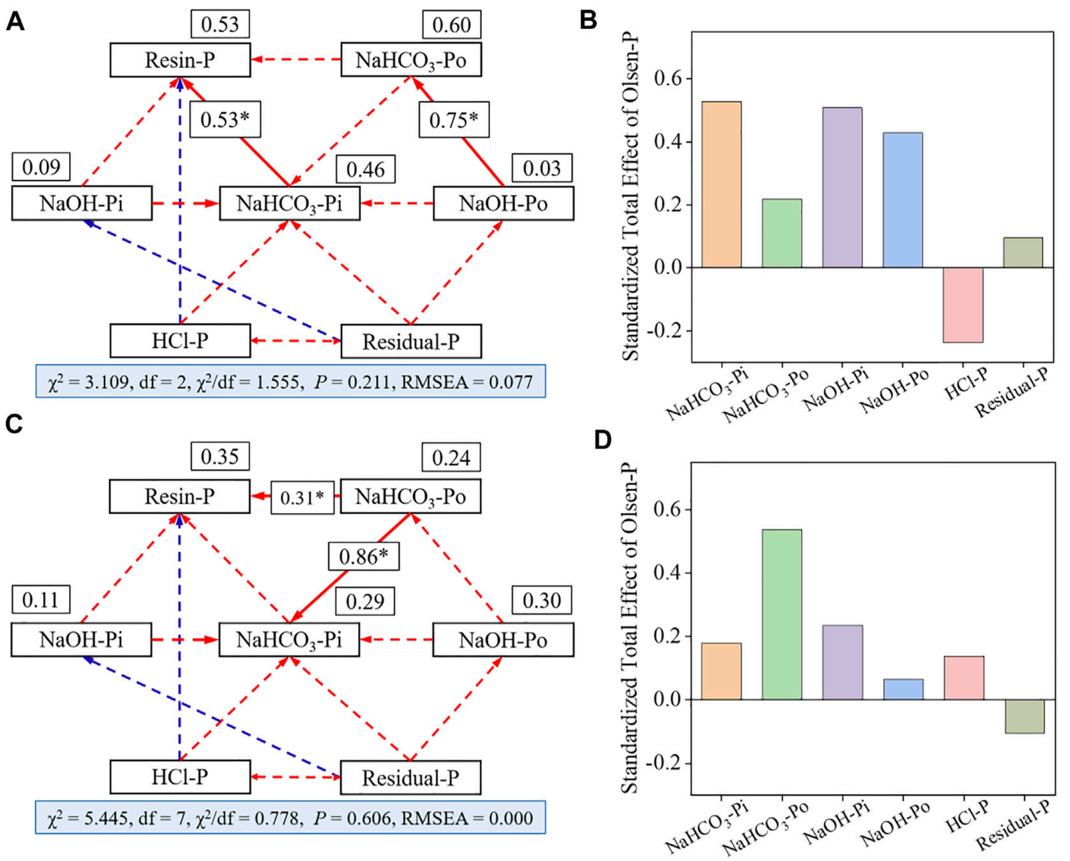

FIGURE 4 | Structural equation modeling (SEM) analysis of different P fractions transformation under P fertilizer input and optimization on four-year rice-wheat rotation paddy field from 2016 to 2020.

(CK and NK). While organic P content and stable P content were not influenced by the input of P fertilizer in the field experiments.

Further SEM was used to explore how different P fertilizer input and optimization affected soil P fraction transformation. The results showed that $NaHCO_3$-Pi had a direct and positive effect on resin-P (path coefficient = 0.53); NaOH-Po has a direct and positive effect on $NaHCO_3$-Po (path coefficient = 0.75) (**Figure 4A**); among them, $NaHCO_3$-Pi (standard coefficient = 0.53) and NaOH-Pi (standard coefficient = 0.51) have the most significant influence on the total standard of resin-P (**Figure 4B**). In the experiment of optimization of P fertilization, the results showed that $NaHCO_3$-Po had a direct and positive effect on $NaHCO_3$-Pi (path coefficient = 0.86) and resin-P (path coefficient = 0.31) (**Figure 4C**). Among them, $NaHCO_3$-Po (standard coefficient = 0.55) has the most significant influence on the total standard of resin-P (**Figure 4D**).

Taken together, the total P in the soil is strongly elevated by the input dose of P fertilizer (**Figures 5A,B**). Accordingly, soil Olsen-P content was significantly elevated with increasing P fertilizer application rates (**Figures 6A,B**). A positive correlation existed between the soil Olsen P content and the P input dose in both the rice and wheat seasons (**Supplementary Figure S2**).

DISCUSSION

Effects of P Fertilization Regimes on Crop Yield

Our field experiments showed that the application amount of P fertilizer had a major effect on the yields of rice and wheat (**Tables 1, 2, Supplementary Figure S1**), which was related to the formation of the panicle number of these crops (**Supplementary Tables S1, S2**). The results indicated that the response of the panicle number rather than grain weight to fertilization regimes is the crucial factor determining crop yield. This is consistent with various previous studies (Peng et al., 2007; Gao et al., 2009; Ding et al., 2013; Song et al., 2013).

However, crop yield could not cross the threshold of a P fertilizer amount. In our experiment, 75 kg P_2O_5 hm^{-2} input caused the maximum rice or wheat yield. Excessive application of P fertilizer could not further promote the crop yield in this area. (**Supplementary Figure S1**). Similar results were also found by other researchers (Gong et al., 2011; Li et al., 2015). It was explained that if the amount of P fertilizer exceeded the requirement of crop growth, excessive P would not further promote crop yield.

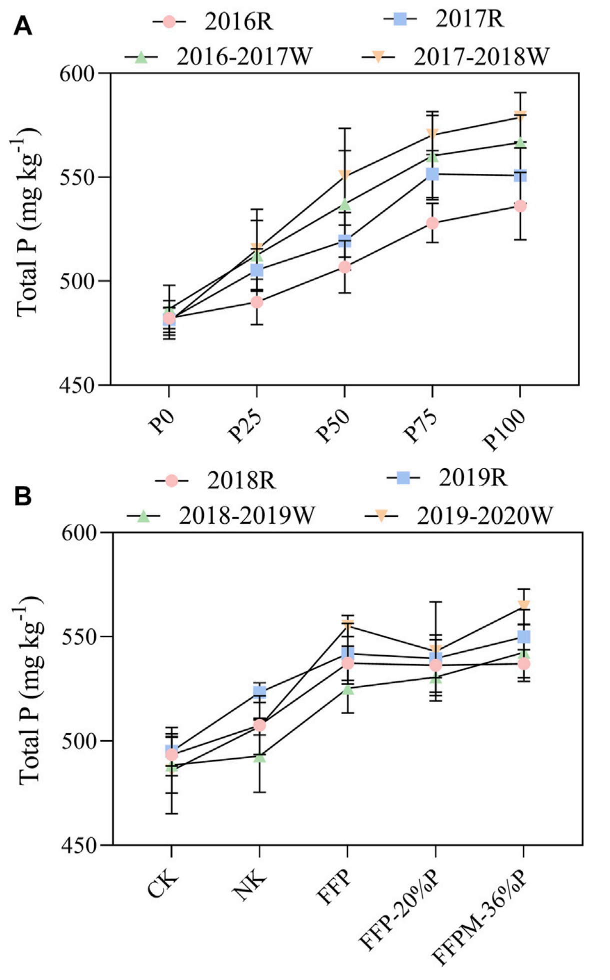

FIGURE 5 | Total P (TP) concentration under P fertilizer input and optimization on four-year rice-wheat rotation paddy field from 2016 to 2020.

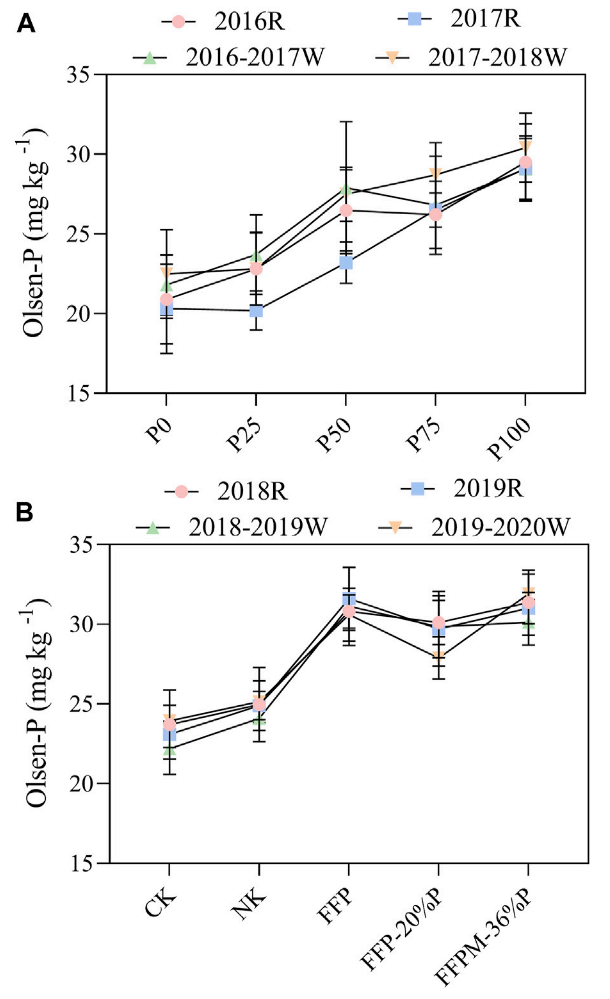

FIGURE 6 | Olsen-P concentration under P fertilizer input and optimization on four-year rice-wheat rotation paddy field from 2016 to 2020.

In addition, we found that organic fertilizer replacing partial chemical fertilizer can ensure the panicle number and grain number of rice or wheat (**Supplementary Tables S1, S2**) necessary to achieve a similar or even higher yield like that under farmers' fertilization (**Tables 2**, **3**), albeit not statistically significant (**Supplementary Figure S1**). The results here indicated that organic manure could replace some chemical P fertilizer to further cut down the input of P. The involved mechanism may be due to the activation of soil microorganisms to mobilize the otherwise unavailable P in soil (Kaur et al., 2008). Organic manures increased organic carbon and nitrogen contents in soil (Luo et al., 2019), which in turn could stimulate the abundance of microorganisms to promote the release of ALP and accelerate the transformation of P in soil (Xavier et al., 2009; Chen et al., 2022). In addition, organic matters could help form soil aggregates and improve the physical properties of soil (Ye et al., 2019). Thus, crop production could be improved by the addition of organic manures. It is also true that 28 years of organic-inorganic fertilization field trials showed that the rice yield was 19.9% higher than that under chemical fertilization treatment (Huang et al., 2013).

Effects of Different Fertilization Regimes on P Use Efficiency

Different inputs of P fertilizer had a positive effect on P uptake by crops (**Table 4**), which in turn increased the accumulation of P in crop biomass (Li et al., 2015). In this experiment, it was also found that applying too much P fertilizer did not further increase P accumulation in crops when the P input was over 75 kg P_2O_5 hm^{-2} (**Table 4**). Meanwhile, crop yield did not continue to increase when P input exceeded 75 kg P_2O_5 hm^{-2} (**Supplementary Figure S1**); this might have been caused by the limitation of other nutrients in the soil. Accordingly, the agronomy efficiency of P (AE_p) decreased when the P input increased, but the recovery efficiency of P (RE_p) reached maximum value under the treatment of 75 kg P_2O_5 hm^{-2} (**Table 8**). Our results indicated that 75 kg P_2O_5 hm^{-2} was currently the optimum to meet the requirement of rice or wheat yield in Taihu Lake Basin with the highest P fertilizer use efficiency. In addition, the annual P surplus reached a level of equilibrium under the treatment of 75 kg P_2O_5 hm^{-2} for the rice season or the wheat season (**Table 4** and **Figure 1**).

Therefore, to optimize P fertilization for local farmers in Taihu Lake Basin, we cut down 20% input of chemical P fertilizer used by farmers' (90 kg P_2O_5 hm^{-2}) and tested it in field trials. The P uptake by crops was not disturbed by the 20% decrease in P fertilizer amount used by local farmers (**Table 7**); meanwhile, the P use efficiency was enhanced to different degrees by the FFP-20%P and FFPM-36%P treatments, compared to the conventional FFP treatment. This also suggests that organic fertilizer might improve P fertilizer use efficiency (Ayaga et al., 2006).

Effects of Different Fertilization Regimes on Soil P Pool

Fertilization is one of the major factors that can affect the various P fractions in the soil (Hong et al., 2015). Understanding the composition and transformation characteristics of soil P is of great significance for improving the recycling of P in soil (Wang et al., 2009). In this study, we analyzed P fractions extracted from the soil after harvest. The application of P fertilizer increased the total P content of the soil (**Figures 5A,B**) and mainly in inorganic P fractions of $NaHCO_3$-Pi, and NaOH-Pi (**Figures 2**, **3**). According to the structural equation modeling analysis, all the P application treatments caused the transformation of moderately labile P (NaOH-P) to labile P (Resin-P and $NaHCO_3$-P) (**Figure 4**), which indicated that P application was beneficial to the mobilization of P (Chen et al., 2021; Chen et al., 2022). In this way, the Olsen-P content was significantly elevated by P fertilization (**Figure 6**). Our results showed that changes in soil P within a certain range were positively correlated with the amount of P fertilizer input. The content of Olsen-P in soil increased with the increase of the P application rates (**Supplementary Figure S2**). Our results were in line with other findings (Wang S. X. et al., 2012). In addition, some studies reported that P fertilization could also enhance the organic P in the soil due to the stimulation of microorganisms (Feng et al., 2010). However, our study did not detect significantly increased organic P content after P fertilization, which may be limited by C sources in the soil (Zhang et al., 2018).

Nevertheless, it could not be forgotten that crops also need to consume inorganic P from soil (Penuelas et al., 2013), whose root systems in soil could transfer moderately labile P from solid phase to liquid phase through abiotic or biological processes (Frossard et al., 2000), such as secretion of root exudates (Menezes-Blackburn et al., 2016; Zou et al., 2018; Chai and Schachtman, 2022) or microbial-related enzymes (Sinsabaugh et al., 2009; Nannipieri et al., 2011). Soil resin-P is a dynamic P pool and is easily absorbed by plants and utilized by soil microorganisms, which may also explain the reason why Resin-P is relatively less than other P fractions and kept relatively constant under all treatments.

CONCLUSION

In this study, P fertilization rates caused P surplus variability, providing solid evidence to guide the nutrition management for input and balance of P in the rice and wheat rotation system. Results of our field experiments showed that applying appropriate P fertilizer is not only important for the improvement of crop yields but also to keep a relatively higher PUE, which in turn can cut down the P accumulation in soil and alleviate the risk of P pollution in the ecosystem. Nevertheless, our research also proved that combining organic fertilizer with reducing P fertilizer can ensure crop yield and enhance the PUE of rice and wheat. Thus, our findings provided a basis for formulating efficient and reasonable P fertilizer optimization methods for the rice–wheat rotation system.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; and further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

LX: Conceptualization, data curation, investigation, methodology, and writing—original draft. GC: Conceptualization, data curation, formal analysis, investigation, and writing. HW: Investigation, methodology, formal analysis, and writing—editing. YL conducted the field experiment. CL conducted the field experiment. LC: Software. YZ: Project leader and writing—review and editing. XX: Resources and writing—review and editing. WW: Review and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.932833/full#supplementary-material

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Change in phosphorus requirement with increasing grain yield for rice under saline-sodic stress in Northeast China

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It is possible to simultaneously reduce both food security and environmental impact by understanding the relation between rice (*Oryza sativa* L.) grain yield and phosphorus (P) uptake requirements. The goal of this study was to determine P uptake requirements and relationship of P accumulation with yield formation at different rice grain yield levels under saline-sodic stress. A database comprising measurements in 28 plots in four on-farm research station located in saline-sodic soil area during the period 2018–2019 in Jilin province of Northeast China was used for the analyses. The grain yields of rice averaged 9.0 Mg ha^{-1} and varied from 5.11 to 13.41 Mg ha^{-1}. The P uptake at late growth stages (heading and maturity) of rice gradually increased with increasing grain yield levels. The P requirement for producing 1 Mg grain ($P_{req.}$) were 4.61, 4.60, and 4.21 kg Mg^{-1} for grain yields ranging from <7.0, 8.0–9.5, and >10.0 Mg ha^{-1}, respectively. The decrease in $P_{req.}$ values with increasing grain yield was mainly attributable to the increase in the harvest index from 0.25 to 0.33. A larger proportion of the P was accumulated from heading to maturity stage when grain yields were higher than 8.0 Mg ha^{-1}. The P uptake in leaves, stems and panicles at the maturity stage gradually increased with increasing grain yield levels. The results give a contribution to rice production in saline-sodic soils, and greatly optimize P management especially in high-yielding rice systems, furtherly improving food security in the Jilin province of China.

KEYWORDS

phosphorus uptake requirements, yield level, rice, saline-sodic stress, yield formation

Introduction

Food production needs to increase 100% by 2050 to meet projected food demands (Pastor et al., 2019). Marginal lands have received an increased attention for world food crisis, especially in China, which has a large population and relatively limited cultivated land. Saline-sodic soils are the typical marginal land, which have a serious adverse impacts on agricultural productivity and sustainability in arid and semi-arid climates (Qadir et al., 2007; Chi et al., 2012; Huang et al., 2017). Rice-planting could potentially reduce the adverse effects of these soil degradation, and has been recommended as the preferred method of biological improvement for saline-sodic soils (Chi et al., 2012; Huang et al., 2016; Huang et al., 2017). So far, the area of saline-sodic paddy fields in the Western Songnen Plain of China had reached 1.1 million ha, accounting for 3.67% of China's total rice area (Huang et al., 2016). However, the yield of rice under saline-sodic stress responses to nutrient supply are not well understood. Especially about phosphorus (P), which as an essential macronutrient plays an important role for plant growth (Wu et al., 2015; Dai et al., 2022; Javed et al., 2022). Under saline-sodic conditions, the soil organic matter is low (Huang et al., 2016), and soils contain extremely high ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}, and Cl^-/NO_3^- (Huang et al., 2017), the Olsen-P is easily immobilized by Ca^{2+}, and competes with Na^+, Cl^-, CO_3^{2-}, and other salt ions (Hu and Schmidhalter, 2010; Tian et al., 2016). The high pH conditions of saline-sodic soils generally result in the reduction of phosphorus absorption (Tian et al., 2016). The availability of P to the plant is limited by insoluble state of tricalcium phosphate in saline-sodic soils (Saito et al., 2019; Guo et al., 2020), so the P use efficiency (PUE) in saline-sodic soils are very low. P has become the main limiting factor affecting the growth and yield of rice under saline-sodic stress (Guo et al., 2020).

To improve the crop yields and maintain soil fertility, phosphate fertilizers are regularly recommended based on target yields and soil test values (Zhang et al., 2008; Zhang et al., 2015). The typical P fertilization rate applied for rice paddy in Northeastern China is about 40–70 kg P_2O_5 ha^{-1} under a agronomist's recommendation (Zhang et al., 2010). But, most farmers and even agricultural extension staff still blindly apply P fertilizer. Applying excess P fertilizer beyond crop needs increases P accumulation in soil and the risk of P loss, which leads to serious environmental problems, like eutrophication of water bodies (Conley et al., 2009; Bai et al., 2013). The consequences of these soil and water problems are meaningful on a global scale. Therefore, understanding the yield formation of rice under saline-sodic stress from the aspect of P uptake requirements, and improving PUE while increasing crop productivity and reducing environmental risk are very important for efficient use of marginal land and food security (Li et al., 2011; Zhang et al., 2012; Tian et al., 2017).

Grain yield of rice are associated with P concentrations in grains. Previous studies have reported differences in the rice grain yield -P uptake relationships over regions (environments), genotypes, irrigation, and nutrient (nitrogen and phosphorus) management strategies (Dobermann et al., 1996; Vandamme et al., 2016; Wang et al., 2017; Wei et al., 2017; Song et al., 2019; Guo et al., 2020). For example, Dobermann et al. (1996) reported that uptake of 1.8–4.2 kg P was required to produce one ton of grain yield by using the data from long-term experiments at 11 sites in five countries of Asia. Wei et al. (2017) found that the higher yield produced by japonica/indica hybrid of "super rice" was accompanied by a higher total P accumulation, and the grain yield was positively correlated with P accumulation from stem elongation to heading stage of rice. Wang et al. (2017) reported low straw P concentrations improved grain yield by enhancing P translocation into grain. In addition, the yield of rice in saline-alkaline soils was positively correlated with P accumulation and rates of nutrient mobilization to the leaves, stem-sheaths, and panicles at maturity (Guo et al., 2020).

Many studies have provided information about P requirements and the relationships with grain yield of rice, but quantitative physiological P requirements in terms of yield relationships across a wide range of farming environments under saline-sodic stress are very few. In this study, we collected data in 28 plots of saline-sodic paddy field from 2018 to 2019 in Jilin province of Northeastern China. The objectives of this study were to investigate P uptake during main growth periods and relationship of P accumulation with grain yield, quantify the P requirement per ton grain produced for different yield levels, explore the key factors affecting the formation of rice yield under saline-sodic stress.

Materials and methods

Data collection

The data were collected from on-farm research station experiments conducted in four locations in the saline-sodic soil area during 2018–2019 in Jilin province (41°–46°N, 121°–130°E), Northeastern China. Measurements were conducted in 28 plots of saline-sodic paddy field in total. Specific detailed information of these on-farm studies are shown in Table 1. Rice was transplanted in early May and harvested in early October. Annual precipitation in this area is 500–800 mm, with 60–70% of the rainfall occurring during the rice growth periods. Fertilizer were recommended by agronomists and were based on targeted yield and soil fertility. All experimental fields received phosphorus applications that ranged from 50 to 130 kg P_2O_5 ha^{-1}, nitrogen application rates range from 97 to 278 kg N ha^{-1} and potassium application rates range from 52 to 129 kg K_2O ha^{-1}. Plant densities ranged from 200,000 to 300,000 plants ha^{-1}.

TABLE 1 Specific detailed information of four on-farm research station.

Station	Year	No. of plot	pH	EC (μS cm^{-1})	Treatment type	Variety	Plant density (plant ha^{-1})	Fertilizer application rate (kg ha^{-1})	Yield range (Mg ha^{-1})
S1	2019	9	7.58 ± 0.19	1007.1 ± 46.2	Nitrogen fertilizer application of direct-seeded rice	Longdao 5	300,000	N:120–180 P_2O_5:50 K_2O:70	5.1–7.0
S2	2018 2019	9	7.72 ± 0.04	1082.3 ± 54.1	Screening of rice varieties, Nitrogen fertilizer application	Jigeng 302 Jigeng 809 Tonghe 899	300,000	N:190–225 P_2O_5:80 K_2O:100	8.4–10.8
S3	2019	4	8.14 ± 0.10	1136.2 ± 50.8	Farmers' high yield key fields	Jigeng 809	200,000 300,000	N:97–160 P_2O_5:72–130 K_2O:75–129	12.7–13.4
S4	2019	6	8.10 ± 0.22	2123.6 ± 22.0	Screening of rice varieties	Jigeng 302 Jigeng 809 Baidao 8	300,000	N:278 P_2O_5:96 K_2O:52	8.0–10.7

Commercial rice varieties with high yield potential that were considered suitable at each site were used. All experiments were managed according to the individual farmers' practices, and no manure was applied. Insects were intensively controlled using chemicals to avoid biomass and yield losses.

Sampling and measurements

At the rice tillering, stem elongation, heading and maturity stages, ten consecutive plants of each plot were sampled to determine the aboveground total dry matter (DM) and phosphorus (P) content. These plants were separated into leaves, stems (culm and sheath) at four stages, and panicles at the heading and maturity stages. All plant samples were oven-dried at 105°C for 0.5 h and then dried at 80°C until a constant weight was reached for biomass measurements. After weighing, the samples were ground to fine powder (0.5 mm sieve), and the P concentrations (mg g^{-1}) of each sample were determined by the molybdate-blue colorimetric method (Murphy and Riley, 1962). The P accumulation (kg ha^{-1}) were calculated by multiplying the P concentration by the aboveground total dry weight.

At the maturity stage, plants covering an area of 1 m^2 (excluding the border rows) in each plot were collected to determine the grain yield components, includes number of panicles per hectare, the number of spikelets per panicle, filled-grain percentage (%) and 10^3-grain weight (g). The percentage of filled grains was calculated in the ratio of filled grains (specific gravity ≥1.06 g cm^{-3}) to the total number of spikelets (Wei et al., 2017). All plants in each plot were harvested and grain yield was reported with 14% moisture content.

The $P_{req.}$ (kg Mg^{-1} grain) was defined as the amount of aboveground P needed to produce 1 Mg grain. The harvest index (HI) was determined by dividing the grain yield by the total aboveground plant dry matter. The partial factor productivity (PFP_P, kg kg^{-1}) was calculated as the grain yield divided by the amount of P fertilizer applied. The P harvest index (PHI) was calculated as the ratio of the grain P accumulation to the total P accumulation of the aboveground parts at the maturity stage (Tang et al., 2011; Wu et al., 2015; Wang et al., 2017).

Data analysis

According to the rice yield of Jilin province of China in recent 5 years, the data of grain yields were split into three groups: <7.0, 8.0–9.5, and >10.0 Mg ha^{-1}. Experiments with P accumulation data collected at four stages, and grain yield components at the maturity stage were also grouped on the basis of grain yield (<7.0, 8.0–9.5, and >10.0 Mg ha^{-1}). A one-way ANOVA was used to compare the means of P accumulation, grain yield components and yield levels based on the least significant difference at a 0.05 level of probability with SPSS 18.0. And the relationship between yields and P accumulation were calculated using Pearson correlation coefficient.

Results

Grain yield components with different yield levels of rice under saline-sodic stress

Considering all 28 observations, the average rice grain yields of <7.0, 8.0–9.5, and >10.0 Mg ha^{-1} were 6.3 ± 0.5, 8.7 ± 0.4 and

TABLE 2 Grain yield components with different yield levels.

Yield level ($Mg\ ha^{-1}$)	Yield ($Mg\ ha^{-1}$)	Panicles ($10^4\ ha^{-1}$)	Spikelets per panicle	Filled-grain percentage (%)	10^3-grain weight (g)
<7.0 ($n^a = 9$)	6.3c ± 0.5[b]	429.9b ± 20.9	93.5b ± 10.3	96.2a ± 1.5	27.1a ± 0.9
8.0–9.5 ($n = 8$)	8.7b ± 0.4	440.7a ± 25.8	125.3a ± 9.7	95.1a ± 1.4	23.3b ± 1.0
>10.0 ($n = 11$)	11.4a ± 1.4	456.6a ± 26.1	133.3a ± 7.2	96.2a ± 1.2	23.9b ± 0.9
Pearson correlation					
Correlation coefficient	0.49*	0.88**	0.14	−0.67**	

[a]n = number of plots.
[b]Mean ± standard deviation.
Different letters in the same column indicate significant different ($p < 0.05$). * and ** indicate significance at $p < 0.05$ and $p < 0.01$, respectively.

TABLE 3 The total dry matter (DM) at main growth stages of rice with different yield levels.

Yield level ($Mg\ ha^{-1}$)	Total DM ($Mg\ ha^{-1}$)			
	Tillering	Elongation	Heading	Maturity
<7.0 ($n^a = 9$)	1.0a ± 0.1[b]	7.5a ± 0.7	11.5b ± 0.7	25.5c ± 1.7
8.0–9.5 ($n = 8$)	1.1a ± 0.2	6.1b ± 1.0	11.8b ± 2.2	29.3b ± 1.6
>10.0 ($n = 11$)	1.1a ± 0.2	6.7b ± 0.7	16.5a ± 2.2	32.0a ± 1.1

[a]n = number of plots.
[b]Mean ± standard deviation.
Different letters in the same column indicate significant different ($p < 0.05$).

11.4 ± 1.4 (Table 2). The number of panicles per hectare and the number of spikelets per panicle gradually increased with increasing grain yield levels, and the yields of 8.0–9.5, and >10.0 $Mg\ ha^{-1}$ were significantly higher than the yield of <7.0 $Mg\ ha^{-1}$. The filled-grain percentages remained stable at ~96.0% among the three yield levels. However, the 10^3-grain weight decreased with increasing grain yield levels. Overall, there was a significant positive correlation between rice yield and the number of spikelets per panicle (Table 2).

P requirements with different yield levels of rice under saline-sodic stress

For total DM accumulation, the greatest difference among the three yield ranges was observed at maturity stage (Table 3). The total DM gradually increased with increasing grain yield levels at the heading and maturity stages, which caused P uptake to increase with an increase in grain yield (Figure 1). The yield level of >10.0 $Mg\ ha^{-1}$ showed higher P uptake at heading stage (39.5 $kg\ ha^{-1}$) and maturity stage (64.5 $kg\ ha^{-1}$) compared with the yields of <7.0, and 8.0–9.5 $Mg\ ha^{-1}$. The P uptake at late growth stages of rice gradually increased with increasing grain yield levels (Figure 1).

The $P_{req.}$ were decreased with increase in grain yield, average $P_{req.}$ values were 4.61, 4.60, and 4.21 $kg\ Mg^{-1}$ for grain yields ranging from <7.0, 8.0–9.5, and >10 $Mg\ ha^{-1}$, respectively (Table 4). The increase in HI also contributed to the decrease in $P_{req.}$ with increasing yield levels. Average PHI around 0.75 for all of the yield levels. The PFP_P were 125.4 $kg\ kg^{-1}$, 109.5 $kg\ kg^{-1}$ and 126.5 $kg\ kg^{-1}$ as yield increased from <7.0 $Mg\ ha^{-1}$ to 8.0–9.5 $Mg\ ha^{-1}$ to >10.0 $Mg\ ha^{-1}$, respectively (Table 4).

P accumulation during main growth periods with different yield levels under saline-sodic stress

To better understand the P accumulation and partitioning pattern of rice under saline-sodic stress, P accumulation during main growth periods (Table 5) and P uptake in each organ at the maturity stage (Figure 2) were calculated. From tillering to stem elongation stage, the P accumulation rate in the yield of <7.0 $Mg\ ha^{-1}$was highest (58.1%), followed by the yield of >10.0, and 8.0–9.5 $Mg\ ha^{-1}$. From stem elongation to heading stage, the P accumulation rate increased from 9.2% to 19.7% to 21.3% as yield increased from <7.0 $Mg\ ha^{-1}$ to 8.0–9.5 $Mg\ ha^{-1}$ to >10.0 $Mg\ ha^{-1}$, respectively, while the same trend was observed from heading stage to maturity stage. The yield level of >10.0, and 8.0–9.5 $Mg\ ha^{-1}$ had the highest P accumulation rate from heading stage to maturity stage, which were 40.3 and 54.5%, respectively (Table 5).

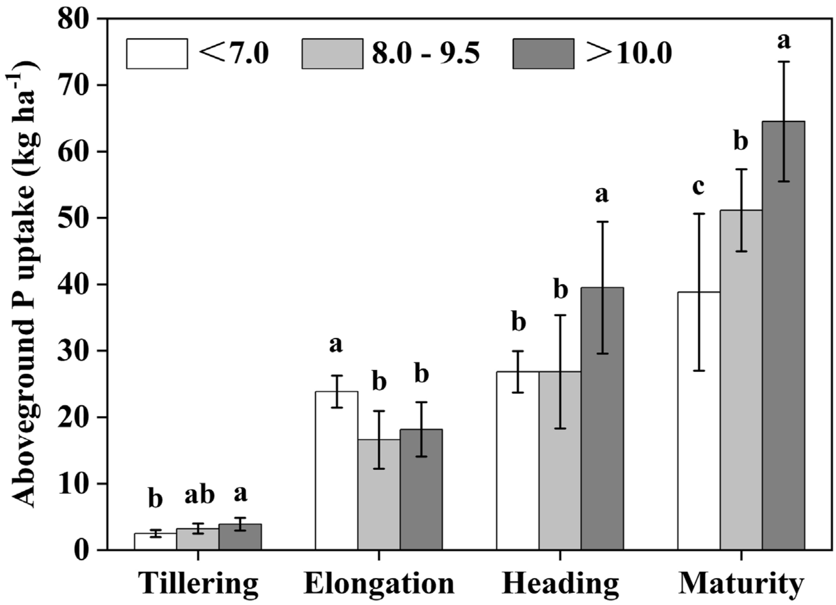

FIGURE 1
Aboveground P uptake at main growth stages of rice with different yield levels. Different letters above the bars are significantly different for different yield levels ($p < 0.05$). Yield level: <7.0, 8.0–9.5, and >10.0 Mg ha^{-1}.

TABLE 4 The P requirement (Preq.), harvest index (HI), P harvest index (PHI), and partial factor productivity (PFPp) of rice with different yield levels.

Yield level (Mg ha^{-1})	$P_{req.}$ (kg Mg^{-1} grain)	HI	PHI	PFP_p(kg kg^{-1})
<7.0 (n[a] = 9)	4.61a ± 1.82[b]	0.25b ± 0.02	0.74a ± 0.11	125.4a ± 10.9
8.0–9.5 (n = 8)	4.60a ± 0.79	0.30a ± 0.01	0.78a ± 0.05	109.5b ± 3.7
>10.0 (n = 11)	4.21a ± 1.04	0.33a ± 0.05	0.72a ± 0.05	126.5a ± 26.1

[a]n = number of plots.
[b]Mean ± standard deviation.
Different letters in the same column indicate significant different ($p < 0.05$).

TABLE 5 P accumulation and rate during main growth periods for different yield levels.

Yield level (Mg ha^{-1})	Tillering to elongation		Elongation to heading		Heading to maturity	
	P Accumulation (kg ha^{-1})	Rate (%)	P Accumulation (kg ha^{-1})	Rate (%)	P Accumulation (kg ha^{-1})	Rate (%)
<7.0 (n[a] = 9)	21.4a	58.1	3.4c	9.2	14.3b	32.6
8.0–9.5 (n = 8)	13.4b	25.8	10.2b	19.7	28.3a	54.5
>10.0 (n = 11)	15.7b	25.3	21.3a	34.4	25.0a	40.3

[a]n = number of plots.
Different letters in the same column indicate significant different ($p < 0.05$).

At the maturity stage, leaves dry matter in the yield of <7.0 Mg ha^{-1}was highest, followed by the yield of 8.0–9.5, and >10.0 Mg ha^{-1} (Table 6). Dry matter of stems and panicles gradually increased with increasing grain yield levels. For the P concentration, grain yield levels did not show the difference on leaf, stem, and panicle P concentration at the heading stage. At

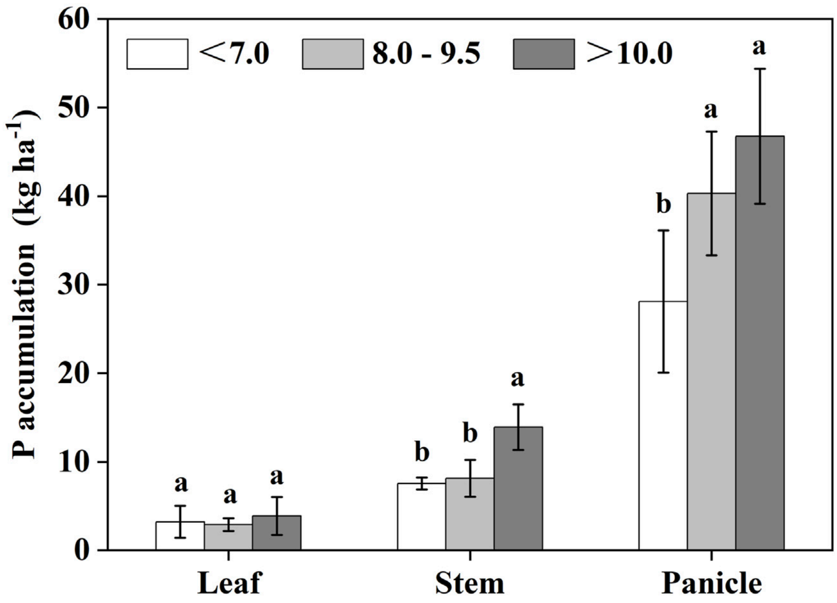

FIGURE 2
P accumulation in each organ at the maturity stages of rice with different yield levels. Different letters above the bars are significantly different for different yield levels ($p < 0.05$). Yield level: <7.0, 8.0–9.5, and >10.0 Mg ha^{-1}.

TABLE 6 The total dry matter (DM) and P concentration in each organ at the maturity stages of rice with different yield levels.

Yield level (Mg ha^{-1})	Total DM (Mg ha^{-1})			P Concentration (g kg^{-1})					
	Maturity stage			Heading stage			Maturity stage		
	Leaf	Stem	Panicle	Leaf	Stem	Panicle	Leaf	Stem	Panicle
<7.0 (n^a = 9)	4.4a	7.2b	13.8b	2.1a	2.8a	1.7b	0.7b	1.3ab	2.1a
8.0–9.5 (n = 8)	3.3b	7.4b	19.0a	1.6b	2.9a	2.2a	1.0ab	1.2b	2.2a
>10.0 (n = 11)	3.0b	9.6a	19.2a	1.8ab	3.0a	1.8ab	1.1a	1.4a	2.5a

[a]n = number of plots.
Different letters in the same column indicate significant different ($p < 0.05$).

the maturity stage, the P concentration in leaves, stems, and panicles also increased with increasing grain yield levels, but there was no significantly different among the three yield levels (Table 6). A considerable amount of stem and leaf P was translocated to the grain in the yield of >10.0 Mg ha^{-1} (Table 6). For the P uptake in each organ at the maturity stage, the P uptake in leaves, stems and panicles gradually increased with increasing grain yield levels. There was no significant difference among the three yield levels in leaves, while was significantly different between the yield of >10.0 Mg ha^{-1} and <7.0 Mg ha^{-1} in stems and panicles (Figure 2).

Overall, the relationship between aboveground P accumulation and grain yield of rice under saline-sodic stress were shown in Figure 3. The grain yield was significantly and positively affected by the P accumulation from stem elongation to heading stage, and P uptake in panicle at the maturity stage ($P < 0.01$).

Discussion

Among yield components, panicle and spikelet number was the basis of stable high yield of rice (Sheehy et al., 2001; Yan et al., 2010; Guo et al., 2020; Li et al., 2021). Our results showed that the number of panicles per hectare and spikelets per panicle were significantly increased with increasing grain yield levels (Table 2). Generally, the number of spikelets per panicle was negatively

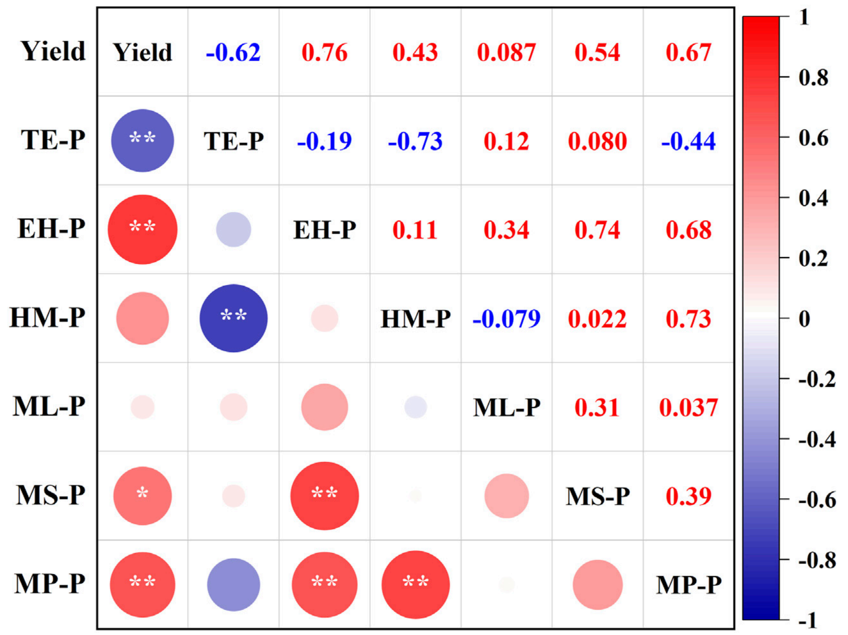

FIGURE 3
Pearson correlation between aboveground P accumulation and grain yield of rice (n = 28). * and ** indicate significance at $P < 0.05$ and $P < 0.01$, respectively. TE-P, P accumulation from tillering to stem elongation stage; EH-P, P accumulation from stem elongation to heading stage; HM-P, P accumulation from heading to maturity stage; ML-P, P uptake in leaf at maturity stage; MS-P, P uptake in stem at maturity stage; MP-P, P uptake in panicle at maturity stage.

correlated with the filled-grain percentage generally (Mohapatra and Sahu, 1991). But in the present study, there was no significant difference in filled-grain percentage among the three yield levels (Table 2), which probably means that the high-yielding rice in our study can achieve the synchronous improvement of spikelet number and filled-grain percentage.

Soil salinity and sodicity can affect the absorption of essential nutrients, and cause nutritional disorders and reduction of grain yield. Especially about P, which is easily immobilized by Ca^{2+}, and competes with salt ions (Huang et al., 2017). In addition, the organic matter (OM) in soils are able to rapidly sorb applied P fertilizer and improved P availability of plants (Guppy et al., 2005). However, OM in saline-sodic soils (6–8 g kg^{-1}) were lower than that of conventional paddy field (>20 g kg^{-1}) (Huang et al., 2016; Wei et al., 2017; Song et al., 2019). In practice, to maximize grain yield of rice in saline-sodic soils, the application of P fertilizer was higher than that of conventional paddy field, so the PUE in saline-sodic soils are very low. In the present study, the yield level of >10.0 Mg ha^{-1} showed higher total DM and P uptake at late growth stages of rice compared with other two yield levels (Table 3 and Figure 1). A similar observation of "super rice" was also reported by Wei et al. (2016). Our results showed that $P_{req.}$ of rice decreased as grain yield improved (Table 4), and the $P_{req.}$ value in this study (4.2–4.6 kg Mg^{-1}) was higher than the 1.8–4.2 kg Mg^{-1} reported by Dobermann et al. (1996), based on the data from long-term experiments of rice in Asia. Moreover, some similar trend about $P_{req.}$ of maize (*Zea mays* L.) was reported by Wu et al. (2015), and decreased $P_{req.}$ as grain yield improved means that improvements in grain yield do not require proportional increases in the use of chemical P fertilizers. Therefore, the $P_{req.}$ of rice as affected by grain yield level should be of consideration in appropriate P fertilizer recommendation for simultaneously reducing cost and P losses of rice in saline-sodic soils.

Rice HI have been documented in several previous studies, which range of 0.17–0.56 (Bueno and Lafarge, 2009; Ju et al., 2009; Yang and Zhang, 2010). In the present study, HI (0.25–0.33) increased when the yield range increased (Table 4). An improvement of HI means an increase in the economic portion of the plant, and rice HI is the result of various integrated processes, including the number of panicles per unit area, the number of spikelets per panicle, filled-grain percentage, and 10^3-grain weight (Li et al., 2012). The PHI (0.72–0.78) in this study was consistent with the results of 0.73–0.83 (indica rice) reported by Song et al. (2019), and higher than the 0.50–0.68 of two indica rice reported by Wang et al. (2017). Our results showed that the HI increased as yield increased, but PHI has no significant difference (Table 4), which probably related with the greater increasing in the allocation of carbohydrates (Wu et al., 2015).

P accumulation during main growth periods and the relationship of P accumulation with grain yield have been documented in several previous studies (Rose et al., 2010; Bi et al., 2013; Wang et al., 2017; Wei et al., 2017; Guo et al., 2020). 60–90% of P taken up by the rice is typically accumulated in the grains at maturity (Rose et al., 2010; Bi et al., 2013). Wei et al. (2017) found that the grain yield of "super rice" was positively correlated with P accumulation from stem elongation to heading stage of rice. Wang et al. (2017) reported that low straw P concentrations improved grain yield by enhancing P translocation into grain. In saline-alkaline soils, there was a positively correlation between grain yield with P accumulation and rates of nutrient mobilization to the leaves, stem-sheaths, and panicles at maturity were reported by Guo et al. (2020). In this study, a larger proportion of the P (40.3%, 54.5%) was accumulated from heading to maturity stage when grain yields were higher than 8.0 Mg ha^{-1} (Table 5). The P uptake in leaves, stems and panicles at the maturity stage gradually increased with increasing grain yield levels (Figure 2). A considerable amount of stem and leaf P was translocated to the grain in the yield of >10.0 Mg ha^{-1} (Table 6). Moreover, We found that the grain yield was positively affected by the P accumulation from stem elongation to heading stage, and P uptake in panicle at the maturity stage (Figure 3), which was consistent with previous studies (Wang et al., 2017; Wei et al., 2017; Guo et al., 2020). This results highlights the importance of maintaining P accumulation at late growth stages in high-yielding rice system under saline-sodic stress.

Conclusion

In summary, from the data analysis, the P uptake at late growth stages of rice were gradually increased with increasing grain yield levels. The $P_{req.}$ were decreased with increase in grain yield. The grain yield was significantly and positively affected by the P accumulation from stem elongation to heading stage, and P uptake in panicle at the maturity stage. The knowledge with relationship between P requirement and grain yield can play a vital role in increasing rice yield in saline-sodic soils and in optimizing P management especially in high-yielding rice system.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

NC and ZW designed the research and supervised the project. YZ, ZL, TW, and MP were key players for the field trials and collected data. ZL and TW analyzed the data and verified the analytical methods. YZ, ZW, and NC wrote the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Closing county-level yield gaps through better phosphorus fertilizer management in Northeast China

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The limited available information on variations in yield gaps (differences between actual yields and the theoretically attainable yields) restricts the development of rational strategies to optimize yields and reduce environmental costs. Quantifying the yield potential and the variations in yield gaps will help identify factors that limit yields and will enable a narrowing of the current yield gap. Here, we applied an analytical framework to yield data to identify options for closing the yield gap at the county level. We used a database containing yields for 40 counties and data from 87 representative on-farm experiments in Jilin Province, China, from 2006 to 2008. The yield potential was simulated for each region-year using a Hybrid-Maize model (http://www.hybridmaize.unl.edu/) and weather data. We then conducted a systematic and spatial analysis of actual yields to identify yield gaps at the county level. The simulated average potential yield at 27 representative sites was 15.2 Mg ha^{-1} (range 8.1–17.6 Mg ha^{-1}) in Jilin Province. The on-farm experiments suggested an attainable potential yield ranging from 8.7 to 16.7 Mg ha^{-1} across Jilin Province. During this period, the actual maize yield varied between 4.1 and 11.9 Mg ha^{-1}, according to the county-level data. Farmers' fields, therefore, achieved 52% of the model yield potential and 77% of the attainable potential yield. Widely different amounts of P fertilizer input among farmers contributed significantly to regional variations in YG_E. Soil Olsen-P and rainfall were also major factors. The results indicate that there is great potential to substantially increase the maize yield in non-optimal P management regions, such as in the western Jilin Province. Hence, improvements in regional P management strategies, such as at the county level, need to be assessed separately to provide a basis for increasing the actual maize yield.

KEYWORDS

Northeast China, spring maize, yield gap, phosphorus fertilizer management, yield potential

1 Introduction

Global agriculture is facing a great challenge to ensure food security with an urgent need to increase crop production by around 60% before 2050 (David Tilman et al., 2011). In 2014, China produced 20.7% of the global maize on 19.6% of global the agricultural land and consumed 21.5% of the global maize production (FAO, 2015). As a consequence of expected economic growth and changing diet, the demand for maize in China is anticipated to increase by 47% by 2030 compared to the present time (Cui et al., 2014). However, the rates of gain in maize yields have slowed markedly in the past 10–20 years, even though agricultural inputs such as phosphorus (P) have continued to increase (Chen et al., 2014). As a result, the net P surplus (P input minus output) was 23.6 kg ha^{-1} $year^{-1}$ in the current maize system in China (Zhang et al., 2015). P surplus on land with already adequate P availability accelerates the rate of phosphate reserve depletion and increases the risk of water problems (Townsend and Porder, 2012). The consequences of these problems are meaningful on a global scale.

Northeast China is one of the major agricultural production areas in China. Maize production in this area accounts for approximately 53% of total production in China and for approximately 52% of the land devoted to this crop (2001–2015). A recent study reported the modeled potential yield of maize in this region varies from 8.3 t ha^{-1} (Lv et al., 2015) to 17.7 t ha^{-1} (Meng et al., 2013). Yield gaps for maize production (defined as the difference between yield potential and the yield actually achieved) are large and vary among different regions (Meng et al., 2013; Tao et al., 2015). In practice, most farmers and even extension staff still believe that high P fertilization rates and high grain yields are always directly related (Li et al., 2015). However, our previous studies showed that phosphorus fertilizer use efficiency (PUE) conflicts with increased yield under the current maize system in Northeast China (Zhang et al., 2015). This variation in yield gap and PUE underlines the importance of accurate estimates across meaningful land scales (Meng et al., 2013). Understanding the variation of yield gaps and their responses to P fertilization is necessary to devise improved broad-scale P management strategies and minimizing negative environmental impacts.

A previous study reported that potential yield and actual yield did not have good relevancy by region (Ciampitti and Vyn, 2014). Observations on maize yields in different regions indicate that different factors contribute to the sizes of their yield gaps and their responses to P management (Lv et al., 2015; Tao et al., 2015). However, there is still a considerable knowledge gap that makes it difficult for decision makers to develop appropriate P management strategies to increase maize production and P use efficiency.

In China, the county is the lowest administrative tier for executive policy implementation; most agricultural policies and recommended practices are conducted at a county scale. Therefore, understanding the variation of yield gaps and assessing the related impacts of P management strategies at the county level will aid policymakers to optimize future management strategies. The objectives of this study were (i) to estimate the model-based yield gap (YG_M, model yield potential–actual farm yield) and the attainable yield gap (YG_A, attainable maximum yield–actual farm yield) at the county level in Jilin Province; and (ii) to quantify yield gaps and their responses to P fertilization.

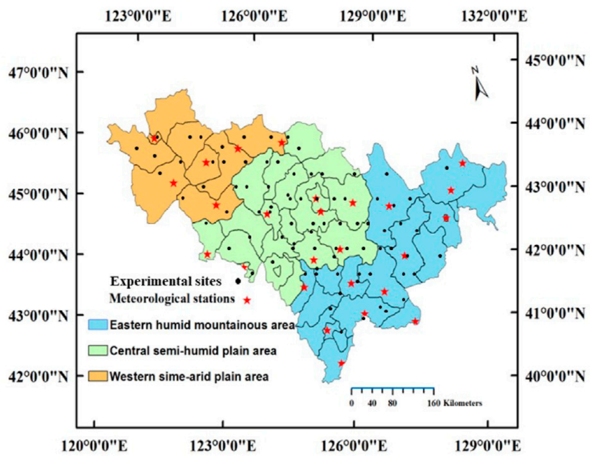

FIGURE 1
Locations of the eastern humid mountainous area (EHMA), central semi-humid plain area (CSPA), western semi-arid plain area (WSPA), and 27 meteorological stations in Jilin province.

2 Materials and methods

2.1 Study site

The study region in Jilin Province (41°–46°N, 121°–130°E) was divided into three areas based on their ecological function: eastern humid mountainous area (EHMA), central semi-humid plain area (CSPA), and western semi-arid plain area (WSPA) (2013) (Figure 1). The most important crop in this region is spring maize, which is sown at the end of April and matures in early October. This region of Jilin Province has a warm-temperate and sub-humid climate, with cold winters and hot summers (Cui et al., 2013). Soil types include ablic luvisol, calcaric arenosol, calcaric fluvisol, haplic chernozem, haplic kastanozem, haplic luvisol, and luvic phaeozem (Gong et al., 1999; FAO, 2015). Long-term (1960–2012) average temperature in the study region during the growing season was 16.2–20.9°C, increasing from east to west (Figure 2A); average annual precipitation was 258.3–756.3 mm, increasing from northwest to southeast (Figure 2B). More than 70% of the annual precipitation (600–800 mm in EHMA, 450–500 mm in CSPA, and 250–350 mm in WSPA) falls during the May–September

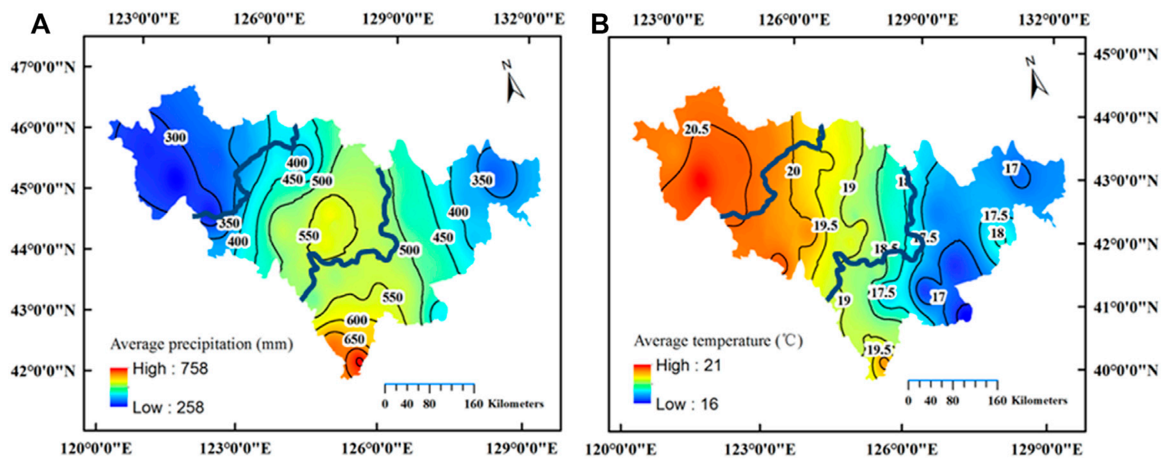

FIGURE 2
Spatial distribution of average precipitation **(A)** and temperature **(B)** during the growing season (May to September) from 1960 to 2012. Black lines are the boundaries of counties, and dark-blue lines are the boundaries of the eastern humid mountainous area (EHMA), central semi-humid plain area (CSPA), and western semi-arid plain area (WSPA).

growing season of the rain-fed maize. Data were collected from farm land, and no specific permissions were required for these locations. The field studies did not involve endangered or protected species.

2.2 Database description

The county-level modeled potential yield was simulated by Hybrid-Maize model, which has been validated and widely used in China, and has been shown to be reasonably accurate at estimating maize potential yield (Chen et al., 2011; Meng et al., 2013). In this study, water-limited yield potential (Yw) was estimated as 72% as the farmland grows maize under rain-fed conditions (2007–2009). The daily weather data, including maximum and minimum temperatures, relative humidity, precipitation, and wind speed used for the model were based on the 1960–2012 averages and were obtained from 27 meteorological stations in Jilin Province (Figure 1), operated by the National Meteorological Networks of China Meteorological Administration. The sowing and harvest dates, plant populations, and hybrid maturity dates for simulations at each site were similar to those for the actual crops grown by the local farmers. The spatial distribution of Yw was estimated by interpolation based on the simulated results from all 27 weather stations and a map using the ordinary Kriging method in ArcGIS (http://www.esri.com/software/arcgis). We used the Lambert-projected coordinate system with a spatial resolution of 200 m per pixel, and the latitude and longitude of each sampling point were provided by Google Earth (http://www.google.com/earth/).

The attainable potential yield (Y_A) was obtained from a total of 139 on-farm experiments, which were conducted from 2006 to 2008 on 40 farms in 40 counties of Jilin Province (41°–46°N, 121°–130°E); these experimental farms covered the humid and sub-humid areas, and included seven soil types (Figure 1). The experiments were conducted in farmers' fields based on the recommendations of local agronomists as to the selection of cultivars, plant density, and fertilizer rates.

Our experimental design consisted of a single replication of three P treatments, no P as a control (P_0), the recommended P rate (RPR), and 150% of the recommended P rate (150% RPR). The RPR was determined by an agronomist's recommendation based on soil test results and a target yield (1.1 times the average yield of the past 5 years) (Zhang et al., 2010), which received 60–130 kg P_2O_5 ha^{-1} as triple superphosphate before planting. All plots received 75–220 kg N ha^{-1} (one-third was applied before sowing, and the remaining two-thirds, during sowing) and 60–120 kg K_2O ha^{-1} before planting, based on soil N and K test results. The highest yield recorded from each experiment represents the Y_A for the farmer at the selected location.

A questionnaire survey on crop yield and nutrient inputs was conducted from 2006 to 2009 by the Soil and Fertilizer Station of Jilin Province (Li et al., 2011). We used data of maize yield and P input from 261 farmers located in the same villages as the experimental sites. Average farmers' yields (Y_F) at the county level were calculated as average yields for 2006–2008 to increase accuracy and were mapped to the county level.

2.3 Yield gap calculation

Y_W and Y_A at the county level were obtained by converting grid map data into county map data using the command tools of

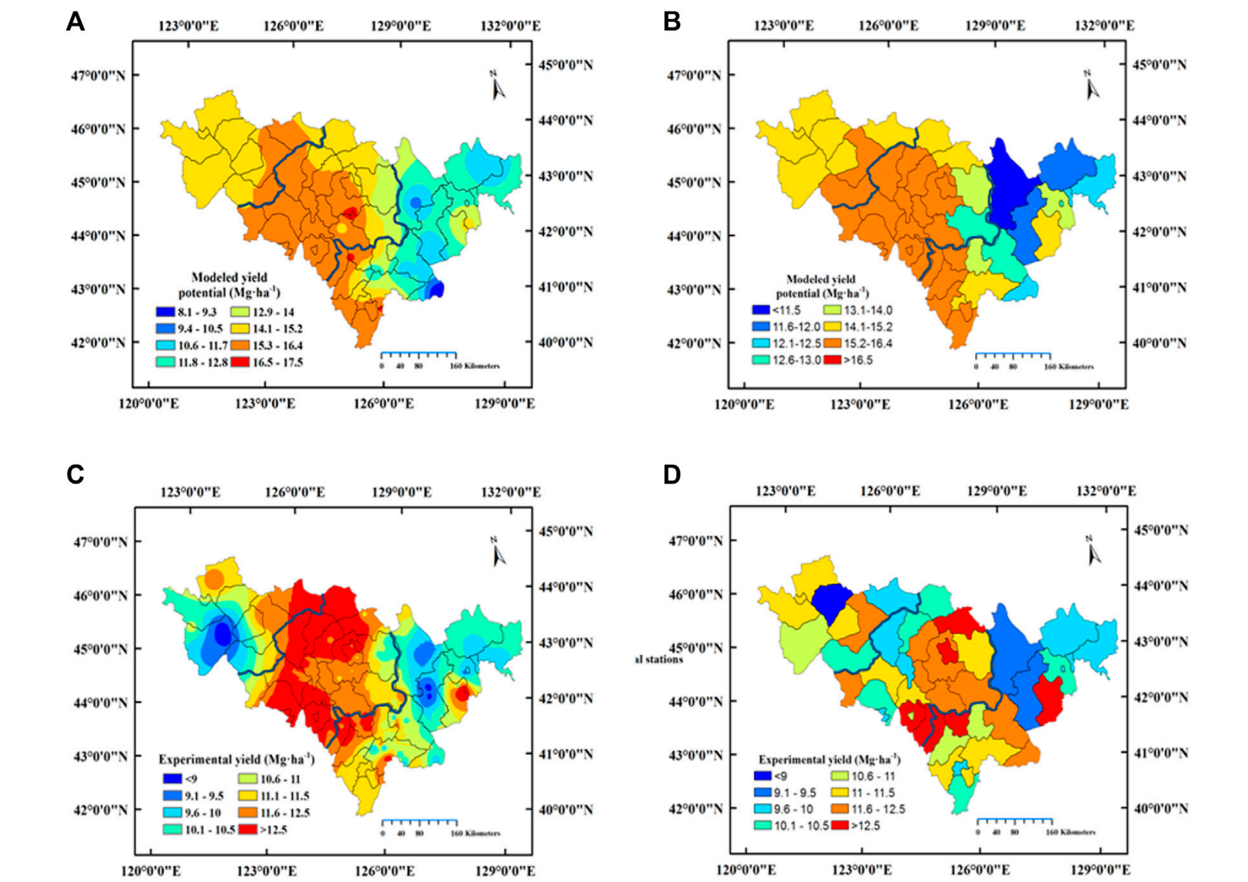

FIGURE 3
Spatial distribution of the modeled yield potential and attainable yield potential for Jilin province. **(A)** Modeled yield potential using the ordinary Kriging method in ArcGIS based on the simulated results of all 27 weather stations; **(B)** modeled yield potential at the county level using the tool command of "zonal statistics as table" according to the maize sowing area for 2006–2008. **(C)** Attainable yield potential was estimated by interpolation and a map using ArcGIS based on the simulated results of all 87 sites; **(D)** attainable yield potential at the county level using the tool command of "zonal statistics as table" according to the maize sowing area for 2006–2008. Black lines are the boundaries of counties, and dark-blue lines are the boundaries of the eastern humid mountainous area (EHMA), central semi-humid plain area (CSPA), and western semi-arid plain area (WSPA).

"zonal statistics as table" according to the maize sowing area for 2006–2008. In combination with the county map, we used the Y_W and Y_A at the county level based on weighted averages according to the maize sowing area in 2006–2008. We estimated two different yield gaps using different measures of yield potential or attainable yields: (i) model-based yield gap (YG_M), which was estimated as the difference between Yw and Y_F in each county; and (ii) the experiment-based yield gap (YG_E), which was estimated as the difference between Y_A and Y_F in each county.

A mixed-model ANOVA was used to assess the variability of modeled yield potential, experimental yield potential, the average yields YG_M and YG_E among counties, [degrees of freedom (df) = 39], P input (df = 260), soil types (df = 6), and rainfall levels (df = 26) for all the experimental site-years.

3 Results

Modeled yield potential (Yw) was estimated by interpolation (Figure 3A) and by "zonal statistics as table" (Figure 3B). Average potential yield at the county level showed no significant differences among counties using these two methods of estimation. This means that it is reasonable to convert yield potential from a grid map into a county map. Yw varied across Jilin Province from 8.1 to 17.6 t ha^{-1}, with a weighted average of 15.2 t ha^{-1} for the entire province based on the average maize sowing area during 2006–2008. Average potential yield increased with decreasing longitude for most of the Jilin Province except for the arid western part. In the EHMA, potential yield varied from 11.4 to 15.7 t ha^{-1}, with a weak trend to decrease with increasing longitude. In the CSPA, potential yields ranged from 12.9 to 16.3 t ha^{-1}, with a trend to increase with decreasing longitude.

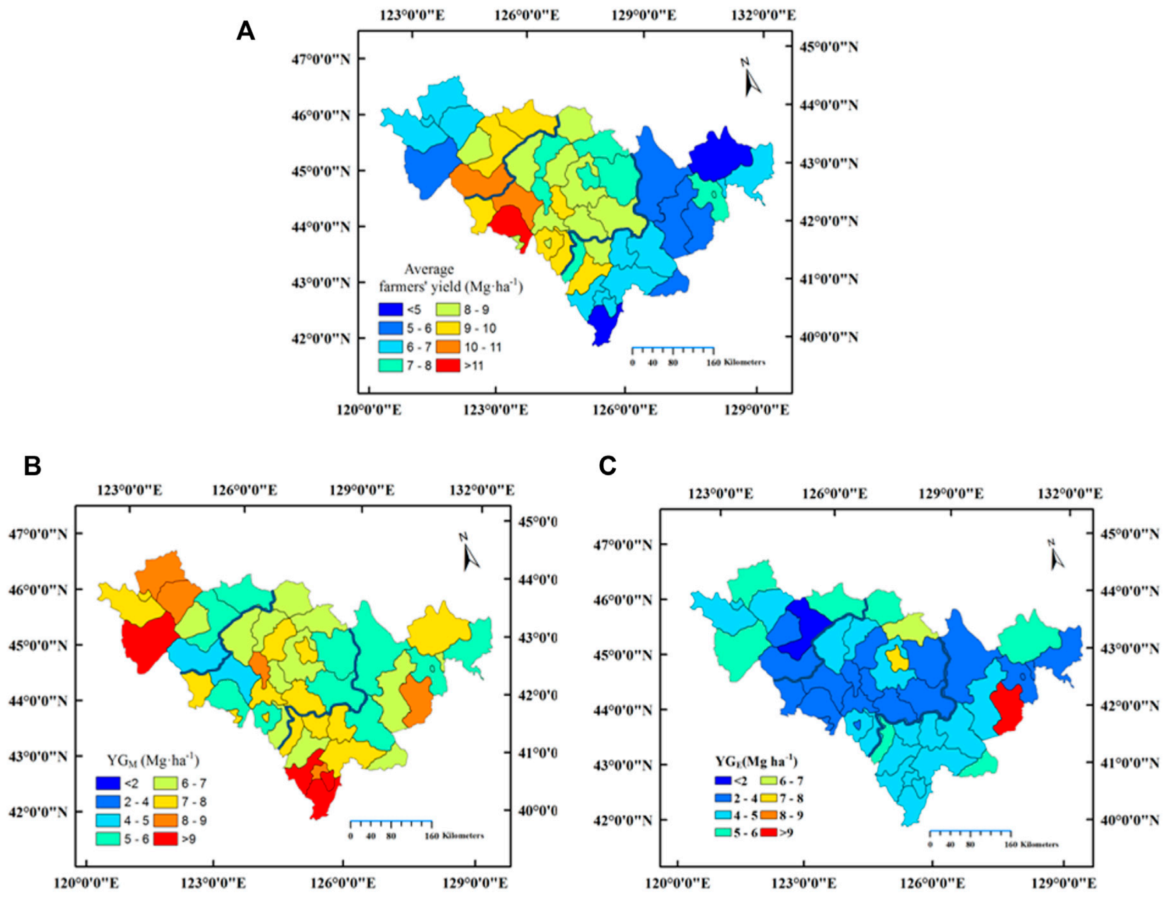

FIGURE 4
Spatial distribution of actual farms' yield **(A)**, YG_M **(B)**, and YG_E **(C)** for Jilin province. YG_M was estimated as the difference between the modeled yield potential and average farmers' yield in each county; and YG_E was estimated as the difference between the attainable yield potential and average farmers' yield in each county. Black lines are the boundaries of counties, and dark-blue lines are the boundaries of eastern humid mountainous area (EHMA), central semi-humid plain area (CSPA), and western semi-arid plain area (WSPA).

Attainable rain-fed maize yield in Jilin Province, which is the average experimental yield potential, was 11.6 Mg ha^{-1} (range, 8.7–16.7 Mg ha^{-1}) across all 87 experimental sites (Figures 3C,D). In the WSPA, the experimental yield ranged from 8.5 to 12.0 Mg ha^{-1}. The lowest experimental yield was found in Tongyu Prefecture that suffers from the effects of soil salinization. The EHMA showed a large variation in experimental yields with a range from 8.7 to 15.6 t ha^{-1}. The areas included Dunhua Prefecture and a narrow zone in the northeastern part that contained both plains and hilly areas where maize yield are low (less than 9.0 t ha^{-1}). In the CSPA, traditionally, the main maize production area due to low temperature stress and high soil quality, the experimental yield ranged from 10.0 to 16.7 t ha^{-1}.

The weighted average value of farmers' yields in Jilin Province during 2006–2008 was 8.7 Mg ha^{-1} based on the harvest area (range, 4.1–11.9 Mg ha^{-1}; Figure 4A). In 80% of the maize area, yields were above 7.5 Mg ha^{-1}; in 43% of the area, mainly in the central part of Jilin Province, the yield exceeded 9.0 Mg ha^{-1}. In 8% of the maize area, yields were between 4.1 and 6.5 Mg ha^{-1}, and this area comprised mainly the eastern mountainous and semi-mountainous areas. Generally, the productivity of spring maize was much higher in the center than in the eastern and western parts of Jilin Province (Figure 4A). The yield gap between the average farmers' yield and the modeled yield potential (YG_M) at the county level was between 4.9 and 11.0 Mg ha^{-1} (Figure 4B). YG_E totaled 3.6 Mg ha^{-1} (range, 2.3–10.8 Mg ha^{-1}; Figure 4C).

In our study area, averaged soil Olsen-P was 23.7 mg kg^{-1} and ranged from 8.3 to 48.9 mg kg^{-1}. Mean soil Olsen-P values was 13.9 mg kg^{-1} for low soil P fertility (Olsen-P <20 mg kg^{-1}) counties (n = 16), 23.4 mg kg^{-1} for medium soil P fertility (20 mg kg^{-1}<Olsen-P <30 mg kg^{-1}) counties (n = 18), and 31.9 mg kg^{-1}for high soil P fertility (Olsen-P>30 mg kg^{-1}) counties (n = 16). The areas of high soil P fertility and low soil P fertility were mainly located in EHMA and WSPA,

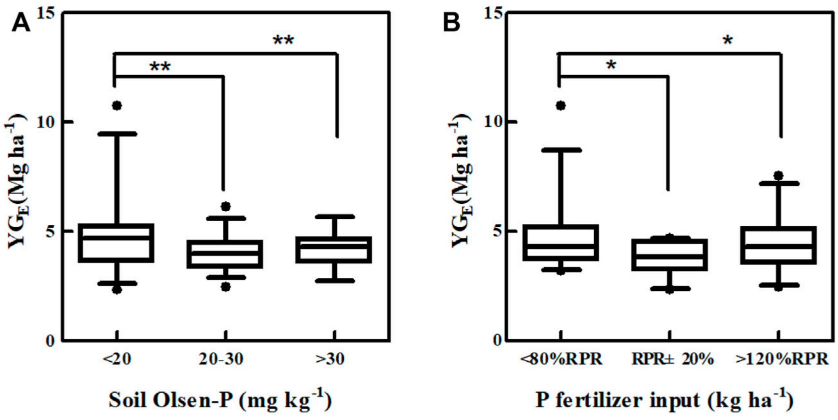

FIGURE 5
YG_E with different soil Olsen-P ranges **(A)** and P fertilizer input **(B)** among farmer's field (*n* = 261) in Jilin province.

TABLE 1 Variability of the modeled yield potential, experimental yield potential, the average farmers' yields, YG_M, and YGE among counties and factors contributing to variation for YG_E.

	Model yield potential	Experimental yield potential	Average farmers' yield	YG_M	YG_E
	———————————	———————————	Mg ha^{-1}———————	———————	———————
25% percentile	13.7	11.0	6.6	5.9	3.5
Median	15.2	11.7	7.8	6.9	4.3
75% percentile	15.7	13.3	8.9	7.8	4.9
Mean	14.6 ± 1.4	12.0 ± 1.8	7.6 ± 1.6	7.0 ± 1.3	4.4 ± 1.4
Source of variation for YG_E					
Source					YG_E
Counties (46)					**
Soil Olsen-P (260)					**
P input (260)					*
Soil type (6)					ns
Precipitation (26)					ns

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† ns, non-significant at 0.05 probability levels.

respectively (Zhang et al., 2015). Under these conditions, YG_E averaged 5.0, 4.1, and 4.2 Mg ha^{-1} (ranged from 4.5 to 10.7 Mg ha^{-1}, 3.1–6.6 Mg ha^{-1} and 3.3–5.6 Mg ha^{-1}) for the counties with low, medium, and high soil P fertility, respectively (Figure 5A). The maximum yield classification distributions of P0 and maximum YG_E were both located in Jiutai and Helong, as was the minimum yield distribution, indicating a close relationship between the attainable yield gap and P0 yield. Based on established regression models, the yield gap increased with an increase in yield of the P0 treatment ($YG_E = 0.79\ Y_{P0} + 4.6$; $R^2 = 0.80$). Across all 261 farmers' site-years, YG_E with suitable P input (RPR±20%) averaged 3.8 Mg ha^{-1} and varied from 2.3 to 4.7 Mg ha^{-1} (Figure 5B). Comparing suitable P input to overuse of P (>120% RPR), adding more P did not close yield gaps, but reduced PFP_P. A relative decrease in P (<80% RPR) led to a 16% yield gap expansion, confirming that the RPR±20% was within a reasonable range for farmers' practice. Non-optimal P management significantly increases yield gap in the field with low soil P fertility.

The YG_E averaged 4.4 Mg ha^{-1} and ranged from 2.3 Mg ha^{-1} to 10.7 Mg ha^{-1}, varying significantly between counties ($p < 0.01$; Table 1). Little change occurred in mean YG_E among soil types, which ranged from 3.8 Mg ha^{-1} to 5.6 Mg ha^{-1}. In contrast, the mean YG_E among different P input ranged from 2.3 Mg ha^{-1} to 10.7 Mg ha^{-1} (Table 1, $p < 0.01$), indicating that P input significantly affected variation in YG_E. Precipitation and soil Olsen-P among different areas also led to significant variation in YG_E ($p < 0.05$). The YG_E increased with an increase in seasonal total rainfall, but decreased with an increase in soil Olsen-P. These data indicate that farmers' P fertilizer practices and environmental conditions contribute significantly to regional variation in yield gap.

4 Discussion

Sustainably increasing global food output to feed the world's growing population is a crucial challenge, and closing the yield gap is one of the strategies to achieve this goal (Zhang et al., 2015).

On-farm experiments provide data that demonstrate the variations in yield gap among different regions. In this study, the average model yield potential was 15.2 t ha^{-1} in Jilin Province, and this varied between different areas from 8.1 to 17.6 t ha^{-1}. This yield potential is similar to those reported previously for this province (Lv et al., 2015; Liu et al., 2016). For Jilin Province as a whole, farmers achieved approximately 57% of the predicted yield potential; this rate is similar to that achieved in global maize production (Licker et al., 2010) but is considerably lower than that in the western United States where farmers achieve more than 80% of the yield potential (Grassini et al., 2011). The large YG_M in Jilin Province indicates a great potential to substantially increase yield.

Evidence suggests that the large YG_M for maize production in Northeast China is related to climatic factors (Liu et al., 2012; Tao et al., 2015). In this study, a positive correlation was found between the long-term average annual temperature and YG_M. One of the ways to close the existing YG_M is redesigning the crop system in different regions to improve light and heat resource use efficiency, for example, by increasing maize plant density in the central regions, adjusting the sowing dates, using long-duration varieties in the western regions, and using cold-resistant varieties in the eastern regions. A second possible approach will be to persuade farmers to adopt water-saving techniques such as residue mulching and no-tillage systems in Jilin Province, particularly in the arid western areas.

We found that farmers' fields achieved 77% of the associated experimental yield potential, and YG_E averaged 3.6 Mg ha^{-1}. This yield gap is similar to those reported for other major maize-producing areas in Northeast China (Meng et al., 2013). Evidence shows that inefficient crop management practices constitute a principal factor for a large YG_E in maize production (Chen et al., 2011; Li et al., 2015; Zhang et al., 2015). In the present study, farmers' P fertilizer practices and environmental conditions contributed significantly to regional variations in the gap between actual farmers' yields and attainable potential yields. Non-optimal P management by farmers is a major factor in YG_E in the counties with low soil P fertility. For example, in Yongji Prefecture, a high YG_E (exceeding 9.0 Mg ha^{-1}) was mainly due to the low rate of P application (less than 60 kg ha^{-1}). Yongji Prefecture is traditionally a rice-growing area and much less attention has been paid to the P fertilizer requirements for maize production. There is great potential to substantially increase maize yield by improvement of non-optimal P management in areas with low soil P fertility, such as in western Jilin Province.

The use of variable levels of P application among farmers is an important factor in the wide variation in YG_E. In the short term, closing YG_E seems to be a more effective approach to increase grain yield due to the similarities between experimental and farmers' field conditions. In this study, the YG_E increased with an increase in yield of the P0 treatment. However, in those areas with P0 yield over 8.9 Mg ha^{-1}, the YG_E increased, but the increased yield due to phosphorus fertilization decreased (data not shown). A potential explanation is that excessive P is no longer necessary to closing YG_E though another yield-limiting factor may be present (e.g., hybrids, plant density, N supply, pest/disease management). In Northeast China, improving P management strategies other than applying more P fertilizer are crucial to closing yield gap and achieving high PUE, due to low soil temperature during spring that inhibits development of maize even in areas with high soil P fertility. Rhizosphere-based P managements, such as banding starter P fertilizer (Shen, et al., 2018) or increasing the colonization of roots by AM fungi (Hou, et al., 2021), provide effective approaches to improving PUE and closing the yield gap by exploiting the biological potential for efficient P acquisition by maize and achieve higher crop yields.

For the regional level, uniform P management strategies in hot spots would be a valuable first step to narrowing the variation in YG_E. First, improvements in agro-technical service provision and agricultural machinery support could be offered to help farmers improve sowing quality, balance P concentrations, and to adopt proper plant-protection controls. Second, encouraging land transfer to merge small farms into larger land holdings should improve P use efficiency and yield.

The Chinese government has more than tripled its investment in agricultural research since 2000 to realize its goal of a 30–50% increase in crop yields. It has allocated 3 billion RMB annually since 2008 to a national network of organizations for developing modern agricultural technology (Zhang et al., 2013). Identifying the regional attribution benefits could help show the priorities for future adaptation practices aimed at resuming yield growth. This information may help policymakers identify areas of high potential for increased maize yield and enable the development of targeted innovative approaches.

5 Conclusion

In this study, we explored factors associated with yield potential and yield gaps in spring maize production. Our analyses provided insights into the causes of regional variation among counties in Northeast China and highlighted the constraints imposed by current P management practice and also the potential for closing the yield gap. Our results showed that farmers' fields achieved 52% of the model yield potential and 77% of the attainable potential yield. Widely different amounts of P fertilizer input among farmers contributed significantly to regional variations in the YG_E. Soil Olsen-P and rainfall were also major factors. Our results indicate that there is great potential to substantially increase maize yield in non-optimal P management regions, such as western Jilin Province. Hence, improvements in regional P management strategies, such as at the county level, need to be assessed separately to provide a basis for increasing actual maize yield. This study has demonstrated how to identify areas that would benefit most from improved cultivation practices and provides useful information for proper management strategies to close the yield gap.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

NC and JC designed the research and supervised the project. WS, YZ, MP, YS, WL, PL, ZL, and LS were key players for the field trials and collected data. MP and YS analyzed the data and verified the analytical methods. WS, YZ, NC, and ZC wrote the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effects of rape/common vetch intercropping on biomass, soil characteristics, and microbial community diversity

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Legume–brassica intercropping is widely used to increase productivity in modern, sustainable agricultural systems. However, few studies have assessed the linkages between soil properties and soil microorganisms. Soil microorganisms play a key role in soil nutrient turnover and plant community composition. To elucidate the responses of soil microbial community diversity and structure to intercropping, we conducted a 2-year experiment based on common vetch (CV) monoculture, rape (R) monoculture, and common vetch–rape intercropping (IRCV) with phosphorus (P) addition in alkaline soil. The microbial communities of bacteria and fungi in the rhizosphere soil were examined based on high-throughput sequencing targeting the 16S rRNA and ITS genes, respectively. In addition, we analyzed changes in soil properties and enzyme activities. Intercropping significantly increased dry matter (up to 98.86% and 81.48%, respectively dry matter is the aboveground biomass.) compared with common vetch monoculture. Intercropping decreased soil bulk density and pH and enhanced soil available phosphorus (AP) by 14.54–34.38%, 7.25–22.67%, soil organic matter (SOM) by 15.57–22.85, 6.82–15.57%, soil sucrase (Suc.) by 13.69–16.10%, 35.57–40.24% compared to monoculture common vetch and rape, respectively. However, bacterial alpha diversity was higher under rape monoculture than IRCV. In addition, the dominant soil bacterial phyla Proteobacteria (1.25–3.60%), Gemmatimonadetes (7.88–15.16%), Bacteroidetes (9.39–11.76%), and Rokubacteria (0.49–5.69%) were present at greater abundance with IRCV relative to those with CV and R, but phyla Chloroflexi was significantly decreased by 11.56–12.94% with IRCV compared with the other two treatments. The redundant analysis showed that SOM and AP were positively correlated with the dominant bacterial and fungal flora. Common vetch–rape intercropping resulted in increased biomass and altered soil microbial community composition as well as soil properties. Our results showed that intercropping systems positively improve soil microbial activity; this strategy could help in the cultivation of multiple crops and improve soil properties through sustainable production.

KEYWORDS

common vetch, intercropping, rape, soil microbial community diversity, soil characteristics

Introduction

In terms of area, China is the third-largest country in the world; with a population of 1.41 billion, it accounted for 18% of the global population in 2020 (Wang et al., 2021). This poses a challenging task in providing food security, including the demand for dairy products, beef, and mutton, which is gradually increasing due to the increasing population. High-quality pastureland plays an indispensable role in producing high-quality and safe dairy products, beef, and mutton. Since 2008, China's imports of both alfalfa hay and oat grass have shown an exponentially increasing trend (Wang et al., 2015). Inner Mongolia's farming–pastoral ecotone comprises most of the farming–pastoral ecotone in north China, which is suitable for pasture cultivation (Wei et al., 2020). Common vetch (*Vicia sativa* L.) and rape (*Brassica napus* L.) are high-quality forage crops known for their drought and cold resistance (Yuan et al., 2021; Ping et al., 2022). The growing of common vetch and rape in Inner Mongolia is of great importance for the development of animal husbandry.

Intercropping is the practice of growing two or more crops on the same land at the same time, which is a typical pattern of aboveground crop diversity (Gong et al., 2019). Intercropping has been widely used all over the world because of its advantages in increasing yield, preventing diseases, and utilizing resources (He et al., 2010; Yin et al., 2017). Following intercropping, the quantity and composition of organic matter in the input soil increases, and the physical and chemical properties of the soil also change (Nyawade et al., 2020). One study showed that, compared with monoculture, the soil water content following millet and mung bean intercropping increased by 12.1% (Wang et al., 2021). After continuous intercropping of wheat and corn for many years, the soil organic carbon (SOC) content increased by an average of 3% compared with the SOC following monoculture (Cong et al., 2014). Additionally, intercropping has a positive effect on the number of soil microorganisms and enzyme activity. Compared with monoculture, intercropping of maize and peanuts improved the functional diversity of soil microbial communities and soil enzyme activity (Zhang et al., 2012). Changes following intercropping can directly or indirectly affect the microbial activity and functional diversity of microorganisms (Zhao et al., 2017; Nyawade et al., 2019). A comparison of microbial diversity could be useful in understanding how rhizosphere communities mediate the impacts of various agricultural practices on crop yields (Zhang et al., 2010). However, few studies have investigated common vetch and rape intercropping, and the mechanism underlying the changes observed following their intercropping remains unclear.

Microbial characteristics are one of the most critical indicators of soil health because soil microorganisms play a critical role in soil nutrient transformation and cycling (Franchini et al., 2007; Yang et al., 2021). Soil management and planting systems can increase soil microbial biomass and the activity and quantity of beneficial microorganisms (Balota et al., 2003). The change in soil available phosphorous (AP) concentration is a key parameter that previous studies have shown can shift the diversity and composition of the soil microbial community (He et al., 2016; Ling et al., 2017). Soil enzymes are a type of bioactive substance and mainly come from the decomposition of plant roots, soil microorganisms, and animal and plant residues (Meng and Wu, 2004). They participate in soil biochemical reactions, promote the process of soil physiological and biochemical reactions, and are an indispensable part of soil ecology (Xue et al., 2003); therefore, the study of soil microorganisms and enzymes is of great importance for the evaluation of soil quality and fertility. Some chemicals secreted by microorganisms living in soil can have an important effect on the formation and activity of enzymes. Therefore, it is worth investigating how the changes in soil physical and chemical properties caused by intercropping affect the metabolic activity and functional diversity of underground soil microorganisms.

To explore the responses in soil properties and soil microbial community diversity and structure to common vetch–rape intercropping compared with monocropping, we conducted a 2-year experiment, with additional application of P. Our objectives were to 1) investigate the responses in the soil microbial community composition to the intercropping system and 2) identify the main properties of soil that drive changes in soil microbial community composition in three crop intercropping systems.

Materials and methods

Experimental site

The field experiment was conducted at an agricultural experimental station (40°56′ N, 110°48′ E), Inner Mongolia Autonomous Region, North China, in 2019 and 2020. The altitude is 1,052 m, and the climate is a typical semi-arid and temperate continental monsoon climate, with mean annual precipitation and temperature 400 mm and 7.2°C, respectively, and with average annual evaporation of 1851.7 mm and accumulation temperature of 2,800°C. The initial soil chemical properties (depth 0–20 cm) in 2019 were as follows: pH 8.45 (soil: water = 1:2.5), soil organic matter content (SOM) 16.56 g kg^{-1}, total nitrogen (TN) 0.53 g kg^{-1}, total phosphorus (TP) 0.54 g kg^{-1}, total potassium (TK) 16.64 g kg^{-1}, available nitrogen (AN) 90.4 mg kg^{-1}, available phosphorus (AP) 15.51 mg kg^{-1}, and available potassium (AK) 140.20 mg kg^{-1}.

Experimental design

The random block design was adopted in this experiment, and the plots were 5 × 6 m in size. As shown in Figure 1, three treatments were set up, with five replicates of each: 1) monoculture of common vetch (CV), 2) monoculture of rape (R), and 3) intercropping of rape/

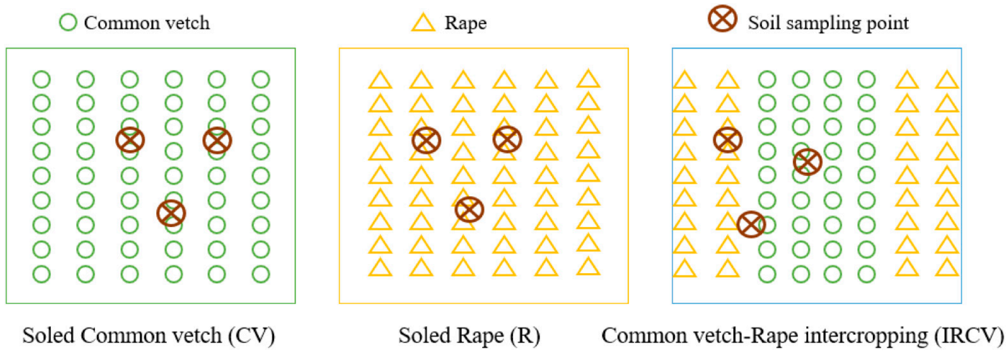

FIGURE 1
Schematic diagram of the planting pattern and soil sampling. CV, R, and IRCV represent common vetch monoculture, rape monoculture, and two rows of rape alternated with four rows of common vetch, respectively.

common vetch (IRCV). In the intercropping system, four rows of common vetch and two rows of rape were planted. The inter-row distance between rows of rape and adjacent rows of common vetch was 25 cm, the same as on the monoculture plots (Figure 1). In all treatments, rape and common vetch were sown at a seeding rate of 15 kg hm^{-2} and 75 kg hm^{-2}, respectively. Basal nutrients supplied were 90 kg P hm^{-2} (as calcium superphosphate) and 120 kg N hm^{-2} (as urea), with no further fertilizers applied during the following growth period. All fertilizers were uniformly broadcast and incorporated into the upper 30 cm of the soil prior to sowing. Two applications of irrigation of 180 mm (1800 m^3 hm^{-2}) each were made on 1 to 10 June and 1 to 5 July in both years. No herbicides were used during the growth period, but insect control followed standard farming practices (Li et al., 2018) and was carried out as needed. Common vetch, as well as rape, was sown on 30 April 2019 and 4 May 2020 and harvested on 25 July 2019 and 3 August 2020.

Soil sampling

After harvesting the common vetch and rape in July 2019 and August 2020, soil augers were use to collect soil samples (0–30 cm deep) from three points on each plot (Figure 1). The soil from each plot was mixed thoroughly and sieved (2 mm). Each soil sample was divided into three portions. One part was stored in a −80°C freezer and subsequently used for the analysis of soil microbial community structure and diversity detection; the second portion was stored in a 4°C refrigerator prior to being used detect the soil microbial biomass; and the third portion of each soil sample was dried at room temperature and ground to a powder for the detection of soil nutrients, pH, and soil enzymes. The ring knife method was used to determine soil bulk density (SBD), with the ring soil samples scraped out and any roots manually removed. The soils were dried at 105°C to constant weight.

Analysis of soil nutrients, pH, and SBD

Soil organic matter (SOM) content was determined by the potassium dichromate volumetric method (Finzi et al., 2015). TN content was measured using the method described by Finzi et al. (2015). TP content and AP were determined using the NaOH molybdenum stibium anti-color method (Ren et al., 2016), and soil pH was determined by the potentiometric method, where the soil to water ratio was 1:2.5 (Bao, 2005). Soil bulk density (SBD) was calculated by the gravimetric weight (Vos et al., 2005).

Analysis of soil microbial biomass and soil enzymatic activity

Soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) were measured using the chloroform fumigation-extraction technique, modified from the methods of Brookes et al. (1982), Brookes et al. (1984), and Brookes et al. (1985). Soil urease activity was determined by the indophenol blue colorimetric method (Wang et al., 2009), soil sucrase activity was determined by the 3,5-dinitrosalicylic acid method (Mi et al., 2018), and soil acid phosphatase activity was determined by the disodium phenyl phosphate method (Guan et al., 1986).

Microbial DNA extraction, PCR amplification, and Illumina NovaSeq sequencing

A Power Soil ® DNA Isolation Kit (MO BIO Laboratories) was used to extract soil DNA. The 16S rRNA gene of bacteria (in the V3–V4 region) was amplified using the primers 338 F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-

GGACTACHVGGGTWTCTAAT-3′) (Mori et al., 2014). We amplified the rRNA gene of fungi (ITS1 region) using ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2-R (5′-GCTGCGTTCTTCATC GATGC-3′) primers (White et al., 1990; Gardes and Bruns, 1993).

The paired-end reads of the fungal ITS genes and bacterial 16S rRNA genes were processed, and the ITS regions and 16S sequences were screened for quality control according to the methods of Guo et al., 2019. We clustered all tags with >97% identity into operational taxonomic units (OTUs). The tags were then classified into different taxonomies according to the Silva and UNITE databases for soil bacterial and fungal communities, respectively. There were 44,529 OTUs for soil bacteria and 3,237 OTUs for soil fungi after removing those OTUs that did not belong to either the soil bacterial or fungal communities.

Statistical analysis

Microsoft Excel 2019 software and SPSS 23.0 software were used for the statistical analysis of data. Duncan's multiple range test was used for the analysis of variance ($p < 0.05$). The relationships among soil properties and forage aboveground biomass were examined by performing Pearson's correlation analysis. The Canoco 5 (Microcomputer Power, Ithaca, NY, United States) was used for principal coordinates analysis (PCoA) and redundancy discriminatory analysis (RDA). Origin software (Version 8.5; Northampton, MA, United States) was used to draw figures.

Results

Aboveground biomass

The yield from rape/common vetch intercropping was 31.44 t hm^{-2} in 2019 and 24.99 t hm^{-2} in 2020. In 2019, the yield of common vetch monoculture was 15.81 t hm^{-2}, and the yield of rape monoculture was 31.08 t hm^{-2}. In 2020, the yield of common vetch monoculture was 13.77 t hm^{-2}, while the yield of rape monoculture was 27.60 t hm^{-2}. Intercropping significantly increased dry matter (refers to aboveground biomass, up to 98.86% and 81.48%, respectively) compared with that obtained by common vetch monoculture.

Soil properties

As shown in Table 1, the intercropping patterns affected soil nutrients and pH. Specifically, regarding the soil physical properties, pH was lower with intercropping compared with the cropping systems of common vetch or rape monoculture, which were decreased by 0.42 and 0.43, respectively. Compared with the common vetch or rape monoculture cropping systems, SBD under intercropping significantly decreased by 1.85% and 2.75% in 2019 and 4.92% and 1.69%, in 2020, respectively. For soil chemical properties, however, intercropping significantly increased SOM, TN, and AP levels ($p < 0.05$), ranging from 19.26 to 23.66 g kg^{-1} for OM, 0.52 to 0.62 g kg^{-1} for TN, 0.88 to 0.94 for g kg^{-1} TP, and 22.19 to 29.82 mg kg^{-1} for AP (in 2020). Similarly, compared with monoculture, the SOM, TN, and AP content was higher under intercropping treatment (in 2019, $p > 0.05$).

Both monoculture and intercropping systems had significant effects on the soil MBC, MBN, and MBP (Figure 2). Compared with common vetch monoculture, the content of MBC, MBN, and MBP in the rhizosphere soil of the intercropping system was significantly higher ($p < 0.05$), by 29.13%, 39.44%, and 35.46% in 2019 and by 33.70%, 31.92%, and 32.70% in 2020, respectively.

Soil sucrase and urease activities in the intercropping soil were significantly higher by 40.24% and 16.47% in 2019 and 35.57% and 16.84% in 2020, respectively, than with rape monoculture (Figures 3A,B, $p < 0.05$). Intercropping increased sucrase activity by 16.10% and 13.69% compared with common vetch monoculture in both years (Figure 3A). However, the soil alkaline phosphatase activity in the monoculture soil (except for common vetch monoculture in 2019) was higher than that in the intercropping soil, but this difference was not significant (Figure 3C).

Correlation between aboveground biomass and soil properties

Pearson correlation coefficients were calculated between the aboveground biomass and soil properties (Table 2). The aboveground biomass of common vetch and rape was significantly positively correlated with SOM and AP. In addition, aboveground biomass was significantly negatively correlated with SBD and ALP.

Soil bacterial and fungal community diversity

The soil bacterial abundance index values (observed OTUs and Chao1 index) were significantly lower in the intercropped soil than in the monoculture soil. Bacterial diversity index values based on the Shannon and Simpson indices showed no significant difference between monoculture and intercropping. The Shannon index was the highest with the R treatment, followed by the IRCV and CV treatments (Table 3). The soil fungi abundance index values (observed OTUs and Chao1 index) were highest with the CV treatment, followed by the IRCV and R treatments (Table 4). However, the Shannon and Simpson indices were lowest with the CV treatment (Table 4).

TABLE 1 Soil physicochemical properties.

Year	Soil physicochemical properties	CV	R	IRCV	*F*	*P*
2019	pH	8.53 ± 0.08a	8.56 ± 0.06a	8.55 ± 0.10a	0.185	0.833
	SBD ($g\cdot cm^3$)	1.08 ± 0.01 ab	1.09 ± 0.02a	1.06 ± 0.01b	4.377	0.037
	OM ($g\cdot kg^{-1}$)	20.36 ± 3.14a	20.36 ± 1.25a	23.53 ± 3.49a	2.125	0.162
	TN ($g\cdot kg^{-1}$)	0.61 ± 0.03a	0.58 ± 0.02a	0.61 ± 0.03a	1.389	0.287
	TP ($g\cdot kg^{-1}$)	0.91 ± 0.05a	0.92 ± 0.02a	0.90 ± 0.02a	0.041	0.960
	AP ($mg\cdot kg^{-1}$)	21.05 ± 1.05b	22.48 ± 2.45 ab	24.11 ± 1.76a	3.432	0.066
2020	pH	8.58 ± 0.08a	8.59 ± 0.05a	8.16 ± 0.24b	14.004	0.001
	SBD ($g\cdot cm^3$)	1.22 ± 0.02a	1.18 ± 0.02b	1.16 ± 0.02b	12.205	0.001
	OM ($g\cdot kg^{-1}$)	19.26 ± 3.33b	22.15 ± 2.25 ab	23.66 ± 2.36a	3.446	0.066
	TN ($g\cdot kg^{-1}$)	0.59 ± 0.04 ab	0.52 ± 0.03b	0.62 ± 0.09a	3.007	0.087
	TP ($g\cdot kg^{-1}$)	0.89 ± 0.04b	0.94 ± 0.02a	0.88 ± 0.01b	6.848	0.010
	AP ($mg\cdot kg^{-1}$)	22.19 ± 0.60c	24.31 ± 0.98b	29.82 ± 0.62a	135.852	<0.001

The data represent the mean ± SD; $n = 5$. Different letters within a column indicate significant differences among treatments at the $p < 0.05$ level. CV, R, and IRCV represent common vetch monoculture, rape monoculture, and R and CV intercropping, respectively.

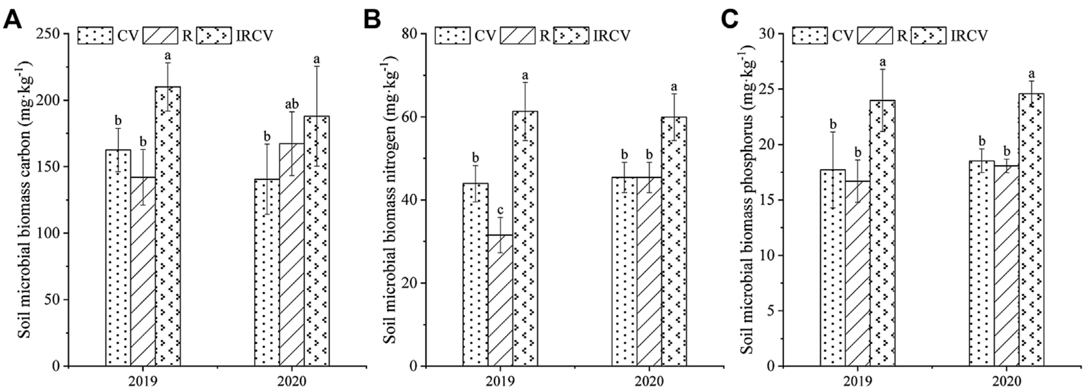

FIGURE 2
Changes in soil MBC **(A)**, MBN **(B)**, and MBP **(C)** under three cropping systems in 2019–2020. Different lowercase letters represent significant differences among treatments ($p < 0.05$). CV, R, and IRCV represent the common vetch monoculture, rape monoculture, and R and CV intercropping, respectively.

PCoA showed that the fungal communities seen between rape and common vetch were clearly separated (Figure 4B), which indicates that the crop species influenced the soil microbial community. However, the difference in bacterial communities between treatments was not significant (Figure 4A).

Shifts of soil bacterial and fungal community composition and responses to soil properties and enzyme activities

None of the bacterial phyla showed a significant difference between treatments (one-way ANOVA $p > 0.05$). Proteobacteria (31.92–33.07%) were the most abundant, followed by Acidobacteria and Actinobacteria, and Gemmatimonadetes (Figure 5A). The relative abundance of Proteobacteria increased by 3.60% under IRCV treatment compared with CV treatment. The CV treatment had the highest relative abundance of Acidobacteria (21.35%), which was higher compared with their relative abundance under R treatment (7.94%). The IRCV treatment increased the relative abundance of Gemmatimonadetes, Bacteroidetes, and Rokubacteria by 15.12%, 9.55%, and 5.81%, respectively, compared with the R treatment. The relative abundance of Chloroflexi was significantly ($p < 0.05$) decreased under IRCV (7.08%)

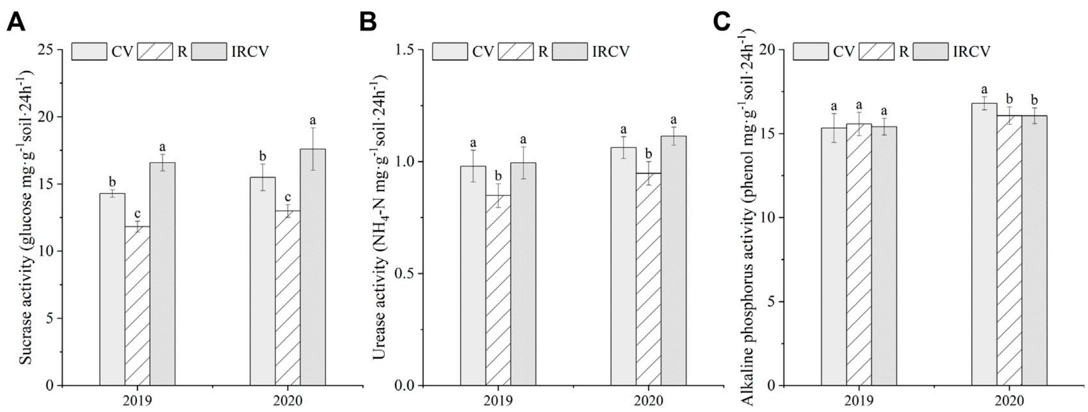

FIGURE 3
Changes in soil enzyme activities under the three cropping systems in 2019–2020. Different lowercase letters represent significant differences among treatments (p < 0.05). Soil sucrase **(A)**, urease **(B)**, and soil alkaline phosphatase **(C)**. CV, R, and IRCV represent common vetch monoculture, rape monoculture, and R and CV intercropping, respectively.

TABLE 2 Correlation coefficients between aboveground biomass and soil properties for all treatments.

	AGB	pH	SBD	OM	TN	TP	AP	MBC	MBN	MBP	SUC	Ure	ALP
AGB	1	−0.31 ns	−0.72[b]	0.56[a]	−20 ns	0.42 ns	0.57[a]	0.49 ns	0.51 ns	0.27 ns	−0.17 ns	−0.28	−0.70[b]
pH		1	0.53[a]	−0.47 ns	−0.49 ns	0.35 ns	−0.80[b]	−0.40 ns	−0.56[a]	−0.81[b]	−0.82[b]	−0.59[a]	0.33 ns
SBD			1	−0.80[b]	−0.41 ns	−0.14 ns	−0.73[b]	−0.59[a]	−0.59[a]	−0.59[a]	−0.22 ns	−0.17 ns	0.63[a]
OM				1	0.33 ns	0.14 ns	0.52[a]	0.31 ns	0.54[a]	0.34 ns	0.18 ns	0.15 ns	−0.34 ns
TN					1	−0.41 ns	0.28 ns	0.46 ns	0.01 ns	0.49 ns	0.60[a]	0.32 ns	0.15 ns
TP						1	−0.34 ns	0.13 ns	−0.26 ns	−0.47 ns	−0.68[b]	−0.43 ns	−0.01 ns
AP							1	0.50 ns	0.83**	0.88[b]	0.59[a]	0.44 ns	−0.58[a]
MBC								1	0.01 ns	0.50 ns	0.26 ns	0.01 ns	−0.39 ns
MBN									1	0.69[b]	0.33 ns	0.37 ns	−0.33 ns
MBP										1	0.73[b]	0.69[b]	−0.32 ns
SUC											1	0.70[b]	−0.02 ns
URE												1	−0.01 ns
ALP													1

[a]Correlation is significant at the 0.05 level.
[b]Correlation is significant at the 0.01 level, and ns means no significant difference. AGB, aboveground biomass, Suc., soil sucrase; Ure., soil urease, and ALP, soil alkaline phosphatase.

TABLE 3 Richness, diversity of the bacterial communities of the rhizosphere soil under different treatments.

Cropping pattern	Richness		Diversity	
	Observed OTUs	Chao 1	Shannon	Simpson
R	6,812.04 ± 147.76a	8,685.29 ± 341.08a	11.55 ± 0.04a	0.99925 ± 0.00003a
CV	6,411.92 ± 357.90b	8,103.21 ± 620.75 ab	11.46 ± 0.09a	0.99921 ± 0.00004a
IRCV	6,151.46 ± 148.07b	7,555.34 ± 291.48b	11.48 ± 0.04a	0.99924 ± 0.00005a

Changes in microbial (bacterial) alpha diversity under different cropping patterns. CV, R, and IRCV represent the common vetch monoculture, rape monoculture, and intercropping of R and CV, respectively.

TABLE 4 Richness, diversity of the fungal communities of the rhizosphere soil under different treatments.

Cropping patterns	Richness		Diversity	
	Observed OTUs	Chao 1	Shannon	Simpson
R	512.06 ± 71.46a	518.02 ± 70.36a	5.61 ± 0.92a	0.93 ± 0.05a
CV	493.30 ± 64.23a	496.85 ± 65.26a	6.28 ± 0.36a	0.97 ± 0.01a
IRCV	517.84 ± 67.07a	521.22 ± 67.43a	5.98 ± 0.54a	0.95 ± 0.02a

Changes in microbial (fungal) alpha diversity under different cropping patterns. CV, R, and IRCV represent common vetch monoculture, rape monoculture, and R and CV intercropping, respectively.

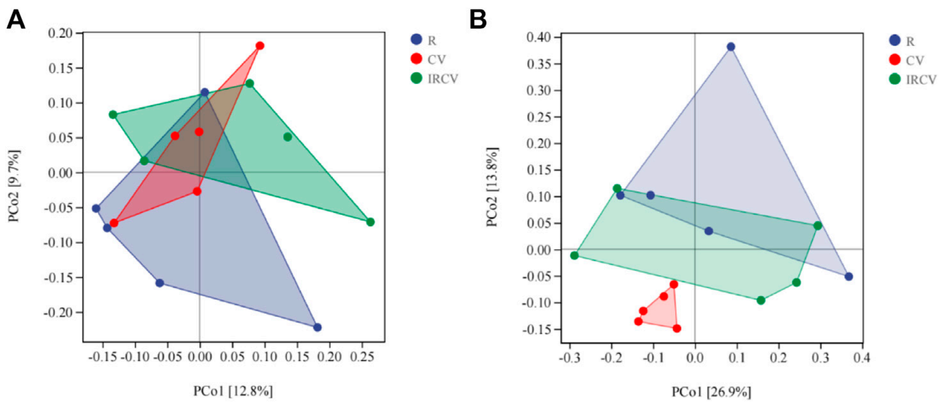

FIGURE 4
Principal coordinates analysis (PCoA) representation of soil bacterial **(A)** and fungal communities **(B)** in the three cropping sequences (OTUs with relative abundance of more than 0.01%). CV, R, and IRCV represent common vetch monoculture, rape monoculture, and R and CV intercropping, respectively.

treatment compared with CV (8.14%) and R (8.01%) treatments. The relative abundances of the bacterial genera are shown in Figure 5C. The predominant phyla of the bacterial community were Subgroup_6, MND1, RB41, Sphingomonas, and KD4-96. The IRCV treatment increased the relative abundance of Subgroup_6, RB41, Rokubacteriales, 67_14, Subgroup_7, and Haliangium by 5.12%, 6.22%, 5.69%, 41.82%, 1.60%, and 4.10%, respectively, compared with the R treatment. Moreover, compared with R and CV, the relative abundance of Sphingomonas was decreased with the IRCV treatment by 12.90% and 20.37%, respectively.

Soil fungal communities were dominated by Ascomycota (68.92%–75.84%, average 72.25%), Mortierellomycota (6.73%–8.86%, average 7.84%), Olpidiomycota (0.21%–6.83%, average 3.68%), and Basidiomycota (3.19%–4.94%, average 4.09%), which made up more than 87.86% of the total sequences. Blastocladiomycota, Chytridiomycota, Zoopagomycota, Glomeromycota, Mucoromycota, and Rozellomycota were detected at relatively low abundances (relative abundance <1%). Compared with the R treatment, IRCV increased the relative abundance of Mortierellomycota and Basidiomycota by 17.68% and 29.47%, respectively (Figure 5B). The fungal genera with a high relative abundance (>1%) are shown in Figure 5D. The community following CV treatment had a significantly lower relative abundance of Olpidium, which decreased by 96.93% and 94.75%, respectively, compared with R and IRCV. The abundance of Mortierella, *Alternaria*, *Fusarium*, Botryotrichum, Dokmaia, Solicoccozyma, Didymella, and Tausonia were elevated under CV treatment compared with R and IRCV treatments.

Redundancy analysis (RDA) was conducted to determine the influence of variations in soil properties on microbial community composition at the phylum level. For the bacterial community, axis one explained 71.85% and axis two explained 8.93% of the total variations. The variation in the bacterial community was mainly influenced by the soil ALP content ($F = 7.1$, $p = 0.02$) and soil TP content ($F = 4.9$, $p = 0.048$) (Figure 6A). Soil sucrase ($F = 4.0$, $p = 0.04$) was found to be the most important parameter influencing the community composition of soil fungi (Figure 6B).

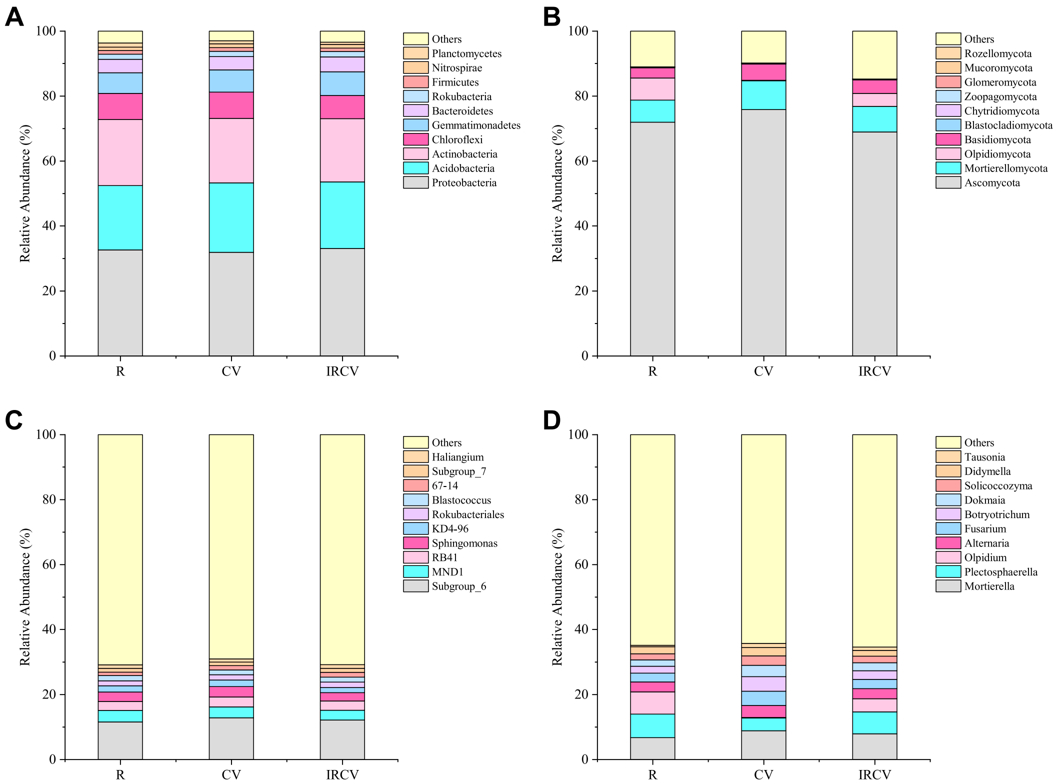

FIGURE 5
Relative abundances of the dominant bacteria [**(A)**, at phylum level; **(C)**, at genus level] and fungi [**(B)**, at phylum level; **(D)**, at genus level] in the three cropping patterns. The top ten dominant phyla and genera of bacteria and fungi, respectively, were used in the analysis. The relative abundance of each phylum or genus was calculated by the average relative abundance of this phylum or genus across all soils divided by the average total relative abundance of all phyla in each cropping sequence. CV, R, and IRCV represent common vetch monoculture, rape monoculture, and R and CV intercropping, respectively.

Discussion

Biomass and soil properties

Intercropping increased dry matter (Figure 7), but this was not our primary interest as these effects have already been shown, with higher uptake of nitrogen (Blessing et al., 2022) and phosphate (Li et al., 2007) through intercropping maize and beans. In 2019, there was no significant difference between the biomass obtained with intercropping treatment and that obtained with rape monoculture. In 2020, the yield with the intercropping treatment was significantly lower than that with the R treatment. This was due to the fact that intercropping treatment involved two rows of rape and four rows of common vetch. The biomass per plant of common vetch was lower than that of rape, leading to the above results.

The physical and chemical properties of soil are generally regarded to be indicators of soil quality (Schoenholtz et al., 2000). Gong et al. (2019) noted that proso millet/mung bean intercropping significantly increased soil nutrient retention, including N, P, K, and soil temperature, findings that were reflected in the current study. We found that SBD was significantly lower with the IRCV treatment than with the monocultures (Table 1) and corresponded with the opposite trends with higher SOC, TN, and available P content (Table 1) compared with the monoculture cropping systems. This indicated that intercropping can effectively maintain soil moisture and soil porosity. Soil pH showed a decreased trend with IRCV treatment relative to that seen with the R and CV treatments, although the differences among these three treatments were not significant in 2019, which contrasts with previous results reported by Betencourt et al. for chickpeas (2012). This result may be due to the fact that the distribution and infiltration of roots in the soil may help to improve the physical properties and structure of soil and further increase the nutrient supply to rhizosphere soil (Li et al., 2016). The root nodules of common vetch can increase soil N retention and reduce SOM loss, which helps to maintains a healthy ecosystem (Amusan et al., 2011; Jayaraman et al., 2020; Wang et al., 2022). Similar results have been reported by Soltangheisi et al. (2018) as

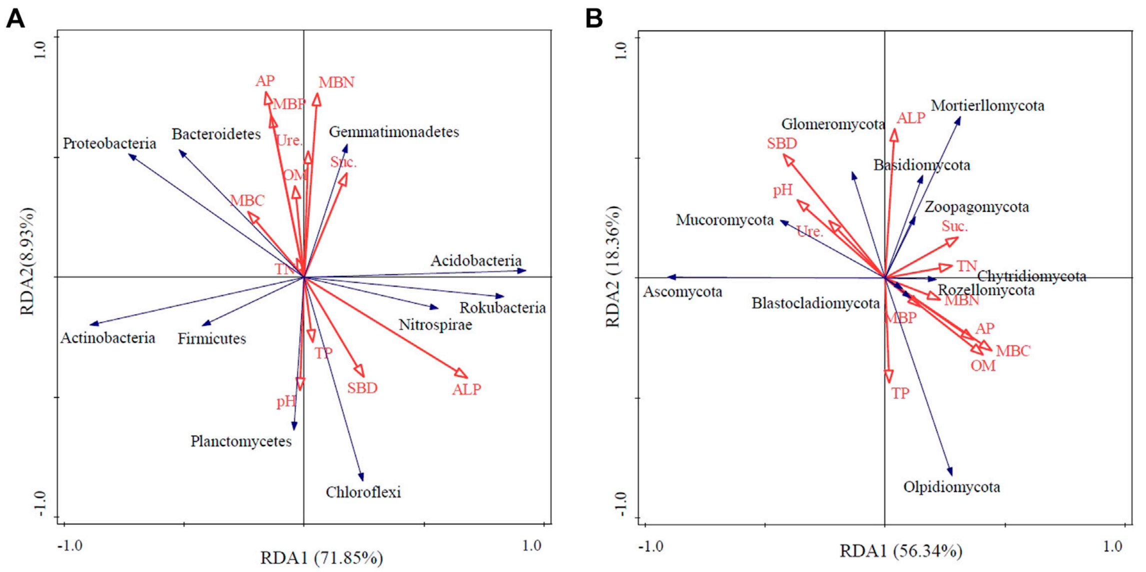

FIGURE 6
Redundancy analysis (RDA) of bacterial **(A)** and fungal **(B)** communities at the phylum level with soil variables. SBD, soil bulk density; SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus, AP, available phosphorus, MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBP, microbial biomass phosphorus, Suc., soil sucrase; Ure., soil urease, and ALP, soil alkaline phosphatase.

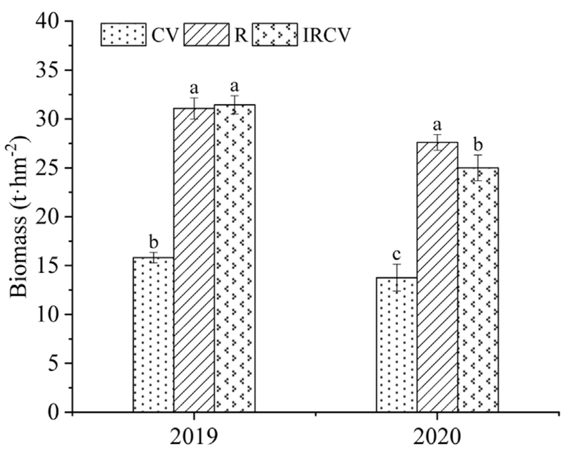

FIGURE 7
Biomass in the three cropping systems in 2019–2020. Different lowercase letters represent significant differences among treatments ($p < 0.05$).

the AP content increased in IRCV, as rape and common vetch can be both a valuable source of phosphorus for mobilizing more P. However, Zhang and Li (2003) found soil TN and AP content decreased, as intercropping used soil nutrients more efficiently than monoculture cropping. Our correlation analysis also showed that SOM and AP were positively correlated with aboveground biomass (Table 2), indicating that higher levels of soil nutrients increased the biomass of common vetch and rape.

As a result of management practices, microbial biomass and soil enzyme activity are also essential for soil metabolic capacity and nutrient cycling (Saha et al., 2008; Spohn and Kuzyakov, 2013). MBC, MBN, and MBP did indeed significantly increase with the intercropping treatment (Figure 2), which is direct evidence of the well-documented stimulation of microbial communities by intercropping. Besides microbial biomass, we also reported that soil sucrase, soil urease, and soil alkaline phosphatase (Figure 3) were enhanced in the rhizosphere, which are additional indications of the advantages of intercropping. These findings are in accordance with those of a previous study conducted by Ahmed et al. (2019). Liu et al. (2021) reported that *F. multifora-A. paniculate* intercropping system significantly increased microbial biomass and soil enzyme activity, which promoted the release of soil nutrients. These results could be due to variations in the origin, form, or persistence of different enzymatic groups under different crop patterns (Trasar-Cepeda et al., 2008). Pearson's correlation analysis showed that MBP was significantly positively correlated with soil urease (Table 2), probably because the numbers of microorganisms present in soil are believed to be the drivers of enhanced microbial activities due to the presence of phosphatases (Turner and Haygarth, 2005). The findings of Dakora and Phillips (2002) are also in agreement with the factthat fungi is responsible for ALP. If this is the case, the observed changes in soil properties when common vetch was intercropped with rape could have contributed to mediating aboveground–belowground cycling in this intercropping system (Tang et al., 2016).

Microbial diversity and microbial composition

Differences in soil characteristics can explain differences between soil bacterial and fungal communities, because microbes provide nutrients for plant growth (Tian et al., 2019). Our results confirmed that bacterial alpha diversity was higher under monocropping, especially the R treatment (Table 3), but it was difficult to predict fungal alpha diversity for different intercropping systems (Table 4), indicating the higher sensitivity of the bacterial community than that of the fungal community to the common vetch–rape intercropping system. This result is supported by the work of Gong et al. (2019), which suggested that intercropping was beneficial for the control of pests and diseases (Jarvis et al., 2015). Additionally, the diversity of bacterial communities associated with intercropping of sugarcane with soybean was significantly increased compared with monoculture and played an indispensable role in shifting the root environment to help healthy plant growth (Malviya et al., 2021).

Soil nutrient content and soil structural characteristics are crucial factors affecting bacterial and fungal community composition (Mouhamadou et al., 2013). In this study, with respect to bacterial composition, we found that the phyla Proteobacteria, Gemmatimonadetes, Bacteroidetes, and Rokubacteria were significantly increased under intercropping treatments. This result is consistent with the findings of a recent study that investigated peanut–cassava intercropping, which reported that the percentage of Gemmatimonadetes in the rhizospheric soils of intercropping systems was higher than in monoculture soils (Tang et al., 2020). In contrast, Chloroflexi significantly decreased, which is similar to Li et al.'s (2021) results showing the effect on microbial community structure of intercropping *Ophiopogon japonicus* in a tea garden. Chloroflexi grows slowly and tends to congregate in low-nutrient environments (NüBel et al., 2001). Our correlation analysis between soil environmental variables and the dominant bacteria further confirmed this result, and there was a negative correlation between Chloroflexi and TN, TP, OM, and AP (Figure 6A). Regarding the fungal community, intercropping increased the relative abundance of Mortierellomycota and Basidiomycota, a finding that was in line with those described for arid farmlands on a global scale (Li et al., 2018).

At the genus level, our study showed that the relative abundance of Sphingomonas decreased under IRCV treatment. Sphingomonas has been shown to exhibit N cycling and has been associated with the antagonistic activity of soil nutrients (Videira et al., 2009; Prakash et al., 2012; Xu et al., 2018). The fungal genus with a low relative abundance under IRCV treatment compared with CV treatment was *Fusarium*. *Fusarium* can cause legume stem disease (Cannon et al., 2012). The change in soil microbial community composition among different cropping patterns might be due to the soil temperature and moisture and indicated that common vetch–rape intercropping can maintain a healthy soil ecosystem.

Conclusion

In conclusion, IRCV significantly increased the biomass of common vetch. As a result of the intercropping of common vetch with rape, soil quality was improved by increasing the microbial biomass, the activity of some soil enzymes, bacterial diversity, and the relative abundances of potentially beneficial microorganisms. Our results also indicated that intercropping led to more bacterial diversity than that seen for fungi. The common vetch–rape intercropping system is a good example of a new strategy for ecological planting of pasture, which will be of great importance for sustainable development and animal husbandry. Moreover, intercropping significantly increased the soil AP content, which is a useful finding for improving the activation of insoluble P in soil.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

JQ and LL conceived and designed the experiments; YW and JY performed the experiments; XZ performed the statistical analysis; JQ and LL wrote the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Co-application of organic amendments and inorganic P increase maize growth and soil carbon, phosphorus availability in calcareous soil

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Phosphorus (P) constraint can be alleviated by increasing C inputs, which can help to improve crop production and P fertilizer use efficiency. However, the effects of different manures on soil microbial biomass P (MBP) and P fractions as well as C fractions in calcareous soils remain poorly understood. Soil MBP pool involves the P mineralization and immobilization processes, potentially changing P fractions and P availability. Therefore, the effects of different manures on soil microbial biomass (MBP, MBC) pool, P, and C fractions and crop P utilization were evaluated in greenhouse experiments with maize plantation. Treatments included no manure (control), poultry manure (PM), cow manure (CM), goat manure (GM), mixed manure (MM), and three inorganic P (Pi) rates; P0: 0 mg kg-1, P50: 50 mg kg-1, and P100: P100 mg kg-1 (P2O5). For plant growth comparison, crop physiological growth indices, shoot P contents and total P uptake were increased by PM and P100 as compared to other treatments. The PM with P100 significantly increased the plant growth by inducing P uptake of ~18% compared with control. The results exhibited that Pi (P100) combined with manure (PM) significantly ($p < 0.05$) increased the soil physicochemical properties, that is, 683.76 mg kg-1 total P, 21.5 mg kg-1 Olsen P, 4.26 g kg-1 SOC, 2.41 g kg-1 POC, as well as microbial biomass C and P increased by 152.84 mg kg-1 and 36.83 mg kg-1, respectively. Consequently, we concluded that PM with Pi (P100) application builds up soil microbial biomass, which is more beneficial for promoting P utilization for maize.

KEYWORDS

carbon addition, microbial biomass, physical fractions, P bioavailability, sequential fractionation

Introduction

Phosphorus deficiency is the world's second most serious soil fertility issue and a major constraint to agricultural productivity (Gichangi et al., 2011). In Pakistan, soils have low organic matter levels, ranging from 2.6 to 6.4 mg C $g^{-1,}$ and extracted phosphate ($NaHCO_3$), about 6 μg P g^{-1} (Khan and Joergensen 2009). Low P availability in soil (pH 8.2–9.3) is mainly due to the rapid formation of non-labile P forms after inorganic P (Pi) addition (Khan and Joergensen 2012). P precipitated and adsorbed with native soil P and calcium, iron, and aluminum ions which become unavailable to plants. According to Huang et al. (2017), the Olsen P contents in soil ranging from 5 to 10 mg kg^{-1} would be optimum for crop production, while lower contents (<5 mg kg^{-1}) affect plant growth.

Many agricultural soils require a substantial amount of costly fertilizer to achieve adequate crop production (Muhammad et al., 2007). This causes serious environmental challenges such as P leaching, soil acidity, and soil hardness, which reduce soil fertility (Ahmed et al., 2019). To address these issues, Khan et al. (2019) found the impact of the combined application of organic addition with various inorganic inputs on soil fertility and rice production. Their findings show that the cumulative use of organic and inorganic fertilizer increased P availability and crop yield. The inorganic fertilizer's use efficiency could be decreased by improving soil fertility with organic manure (Ayaga et al., 2006). The combination of inorganic and organic amendments such as farmyard manure and crop residues is recommended to overcome soil fertility challenges (Biswas et al., 2017).

Organic amendments, including farmyard, poultry, and goat manure, are commonly used to mitigate soil alkalinity by improving the nutrient supply and water retention capacity of soils (Ojo et al., 2016). The P availability for plants was increased by adding organic amendments to calcareous soils (Huang et al., 2017). This was attributed to the release of organic acids contending with phosphates for adsorption sites on the soil colloid's surface or P mineralization during decomposition. Many cumulative mechanisms might be involved, such as soil pH improvement and inorganic P release from decomposing residues (Chen et al., 2016). There is considerable information that applying organic manure to soil can increase P solubility, decrease P fixation, and improve P availability to plants (Bader et al., 2020).

Soil microbial biomass is a living component of soil organic matter and an active pool for plant-available N and P. Several scientists have previously documented increases in microbial biomass carbon after adding organic amendments to the soil, such as compost, poultry manure, and compost (Marschner, 2008). Phosphorus (P) incorporation into the microbial biomass and its associated pool are reported mechanisms that substantially increase P availability in soil-plant systems (Muhammad et al., 2007). The use of organic manure and inorganic P together promotes soil microbial biomass growth and labile microbial P enriched metabolites, which serve as a labile P pool in soil (Brookes, 2001). During the microbial biomass turnover process, this phosphorus could be released slowly and more efficiently taken up by the plants (Kouno et al., 2002). Several studies indicated that when organic manure and P fertilizer were applied together, the microbial biomass phosphorus (MBP) pool was larger than when P fertilizer or organic manure were applied separately (Reddy et al., 2005; Ayaga et al., 2006; Khan and Joergensen 2009; Li and Marschner 2019; Peng et al., 2021). According to most studies, calcareous soils have a negative impact on plant development by restricting root carbon release into the soil (Zhang et al., 2016). As a result, soil alkalinity causes a decrease in soil microbial biomass (Halvorson et al., 1990).

Soil organic carbon (SOC) is the largest C pool in the terrestrial ecosystem, which directly or indirectly affects the soil's physicochemical and biological properties (Kan et al., 2020; Zhao et al., 2020). Particulate organic matter (POC) is a labile fraction of SOC that could reflect initial changes in the soil immediately as compared to other fractions. Furthermore, mineral-associated organic carbon (MAOC) is a relatively non-labile fraction associated with silt and clay-sized particles, and it is primarily composed of low molecular weight compounds derived from microbial activities (Kleber et al., 2015). Soil fertility is influenced due to variations in SOC under organic and inorganic amendments (Lal 2013).

Soil phosphorus forms and their distributions in the soil vary under different sources of manure application, including compost and animal manure (Schachtman et al., 1998). The soil's chemical characteristics are potentially affected by the application of different types of manure sources, which change the quantity and distribution of distinct soil P forms. Calcium-bounded phosphorus is the dominant fraction and is frequently present in calcareous soils (Khan and Joergensen, 2009). Calcium phosphate minerals were divided into three groups: 1) Ca_2-P (brushite and monetite) is water-soluble P, which is readily available to plants; and 2) Ca_8-P (b-tricalcium and P octacalcium P) is a moderately labile P fraction that can be used by selective plants (Jiang and Gu 1989); and 3) Ca_{10}-P (hydroxyapatite), is a stable form that is unavailable to plants. Al-P and Fe-P are less available to plants, and occluded P (goethite) is not available to plants (Adhikari and Pandey 2019). In maize soils, organic and Pi addition can influence the total P storage and create changes in soil P contents, which could provide useful

TABLE 1 Selected properties of the soil and different manure used in the present study.

Properties	Soil	Manures		
		PM	CM	GM
Total N%	0.056	2.04	1.34	0.21
Total P%	0.041	2.06	0.78	0.66
Total K%	0.0086	1.86	0.15	0.71
pH	7.93	#FF0000; 6.67	#FF0000; 7.23	#FF0000; 7.87
Soil texture	Sandy clay loam	—	—	—
EC (dSm^{-1})	1.89	—	—	—
Organic C ($mg\ kg^{-1}$)	3.34	#FF0000; 23.90	#FF0000; 19.64	#FF0000; 18.45
Olsen P ($mg\ kg^{-1}$)	2.37	—	—	—
MBC ($mg\ kg^{-1}$)	36.75	—	—	—
MBP ($mg\ kg^{-1}$)	3.37	—	—	—
#FF0000; $NaHCO_3$-Pi ($g\ kg^{-1}$)	—	#FF0000; 1.73	#FF0000; 0.96	#FF0000; 0.76
#FF0000; NaOH-Pi ($g\ kg^{-1}$)	—	#FF0000; 0.34	#FF0000; 0.20	#FF0000; 0.16
#FF0000; HCl-Pi ($g\ kg^{-1}$)	—	#FF0000; 3.28	#FF0000; 2.01	#FF0000; 1.26

information on the effects of P inputs and P transformations (Linu et al., 2019). In P-limited soil conditions, the bioavailability of both the soil organic and inorganic P chemical fractions can be different (Bol et al., 2016). Consequently, exploring the behaviors of soil P at the expense of organic and inorganic inputs is critical to clarifying the P fraction distribution and maize P uptake mechanism in calcareous soil.

Recent studies have resulted that P-enriched organic treatments could improve P availability and plant growth more than the sole addition of Pi fertilizers (Aziz et al., 2010). This might be mainly because of microorganisms that act as a sink for inorganic P during the decomposition of soil organic matter or organic amendments (Rawat et al., 2020). According to Muhammad et al. (2007), the addition of P-enriched compost significantly increased P availability and maize yield, possibly due to higher microbial growth and turnover rate. Some studies have suggested that soil microbial activities are quite linked with SOC, P fractions, and utilization in different soil systems.

However, the relationship of soil microbial pools with SOC and P factions has not been fully understood. Therefore, this study was conducted to evaluate the response of SOC, P fraction, and microbial pool as well as plant growth. We hypothesized that organic amendments could induce microbial activity by providing soil C as an energy source, and in return, microbes could acquire more P from the soil Pi pool, thereby increasing plant growth. The basic objectives of this study are 1) to evaluate variation in MBP and P fractions in maize soil under inorganic and organic applications to better understand soil P behavior; and 2) to assess the relationship between SOC and P fractions and the plant's P uptake.

Materials and methods

Collection and pre-analysis sample

Organic manures used in this study were collected from a livestock farm located at the University of Agriculture in Faisalabad, Pakistan, and were sieved (2 mm) before the start of the experiment in order to ensure a homogeneous product. A subsample of manure is sieved and dried at 65°C for elemental analysis by following standard procedures (Peters et al., 2003). Soil samples were collected from the university ISES research farm (Table 1).

Growth conditions and plant material

A pot trial was set up in the greenhouse of the Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. The study treatments were organized in a completely randomized design (CRD) with four replicates using organic C sources (goat, cow, poultry, and mixed manures) and inorganic P (P_2O_5 form) applied at three different application rates, that is, P0 = 0, P50 = 50, and P100 = 100 $mg\ kg^{-1}$ by using diammonium phosphate (DAP) fertilizer source. The treatments consisted of: no manure (control treatment with P0, P50, and P100 mg P_2O_5 kg^{-1}; first, goat manure (GM) with P0, P50, and P100 $mg\ kg^{-1}$ P_2O_5; second cow manure (CM) with P0, P50, and P100 $mg\ kg^{-1}$ P_2O_5; third, poultry manure (PM) with P0, P50, and P100 $mg\ kg^{-1}$ P_2O_5 and finally, mixed manure (MM) fertilization with P0, P50, and P100 $mg\ kg^{-1}$ P_2O_5. The mixed manure (MM) was developed by the combination of all of the three aforementioned manures in an equal ratio (1:1:1). Considering a manure input rate of 5% (w/w) (oven-dried weight) was taken. The soil was thoroughly mixed and three maize seeds (cv Nilam) were initially sown in each plastic pot (32 cm in diameter, 24 cm in depth). Water holding capacity was maintained at 50%. In addition, the recommended dose of nitrogen and potassium were applied to the pots at the rate of 120:40 kg ha^{-1} in split doses for normal plant growth, and development and to avoid nutrient deficiency (Reddy 2012). After 7 days of emergence, the plants were thinned to one plant per pot. Eight weeks of plants were harvested to measure the plant growth indexes. Plant height was measured by using a measuring tape. Leaf chlorophyll contents (SPAD) were recorded with a SPAD-502 m, while leaf area (LA) was recorded by using a measurement tape. Plant roots were removed gently from each pot and washed to remove soil particles and allowed to be stored in paper bags. Furthermore, an electric balance was used to measure the fresh root and shoot weights.

Determination of plant nutrient uptake

Shoots and roots were carefully washed using water (distilled) and were dried in an oven (105°C) for 1 h and then at 70°C to ensure final weight. Dry shoot and root samples of 200 mg were processed with sulfuric acid 8 ml in a sealed chamber (Li and Marschner 2019). Total P concentration in dried samples was measured with the vanadate molybdate method by visible spectrophotometer/UV as recommended by El-Gizawy et al. (1992). Total P uptake was calculated from the formula given below;

$$\text{Total plant P} - \text{uptake} = \text{P} - \text{uptake (shoot)} + \text{P} - \text{uptake (root)}.$$

After the final harvest (56 days), the soil sample (50 g moist bases) was taken and analyzed for different P fractions, microbial biomass C, and microbial biomass P contents. Additionally, samples collected (air-dried) were analyzed for soil available P.

Determination of microbial biomass C, P, and olsen P

Microbial biomass P was estimated by the chloroform fumigation extraction method (Brookes et al., 1982) in the 50 g sample taken from each treated pot. Three parts of soil weighing 5 g (oven-dried for 24 h at 105°C), were separated into a 50 ml flask. One part was untreated, the second part was treated with P KH_2PO_4, and the third part was fumigated (24h, 25°C) with ethanol-free chloroform. These three soil parts were extracted with 0.5 M $NaHCO_3$ by shaking at 150 rev min^{-1} for 30 min and filtered by using a 0.45 μm filter paper. MBP was calculated by using a 0.4 conversion digit. The MBC was analyzed by the chloroform fumigation-extraction method and calculated as follows: $C = E_C/K_{EC}$, where E_C is the difference between organic carbon extracted from fumigated and non-fumigated samples, and K_{EC} is 0.4 (Wu et al., 1990). The Olsen-P concentration was analyzed by extracting 0.5 M $NaHCO_3$ (8.5 pH) following Olsen (1954).

Determination of soil C fractions

For soil organic C analysis, the soil samples (oven-dried) were ground, sieved (0.25-mm), and analyzed as suggested by Walkley and Black, (1934). Particulate organic matter (POM) and mineral-associated organic carbon (MAOC) were analyzed by the method of Cambardella and Elliott, (1992). POM was determined by shaking 10 g of oven-dried soil for 18 h in 30 ml of sodium hexametaphosphate (5 g/L). The supernatant was passed through a sieve (53-μm). The retained material passed on the screen was oven-dried at 50°C for 24 h and used to measure C according to the aforementioned method. POC was determined as the carbon (C) content in the screen retained POM. Meanwhile, the screen-passed material was also measured for C and identified as mineral-associated organic carbon (MAOC).

Determination of soil pi fractions

According to Jiang and Gu (1989), soil inorganic P (Pi) was divided into distinct inorganic P fractions; this extraction method has been used for P fractionation of alkaline soils (Wang et al., 2010b). Different inorganic P fractions such as Ca_2-P, Ca_8-P, Ca_{10}-P, Al-P, Fe-P, and O-P were extracted by following the sequential extraction method (Figure 1).

Briefly, the soil sample of about 0.5 g (oven-dried bases) was added into centrifuge tubes of 50 ml. By following the sequence shown in Figure 1, the selected extractants of 25 ml (with a defined pH) were taken into tubes and shaken by using an automated reciprocating shaker machine for the required time. After shaking, the tubes were allowed to centrifuge (10 min, 4500 rpm). After, completing the centrifugation process, the supernatants were transferred carefully into a new tube to avoid any contamination. Finally, the extracted supernatants were evaluated with a UV/visible spectrophotometer at 880 nm following the molybdate blue method of Murphy and Riley (1962) to analyze the inorganic phosphorus (Pi) contents in the soil fractions.

Statistical analysis

SPSS version 25.0 was used for the statistical analysis. Two-way ANOVA (analysis of variance) was used to analyze the influence of organic manure and inorganic P (Pi) on recorded data. To compare the cumulative effects of organic C and Pi

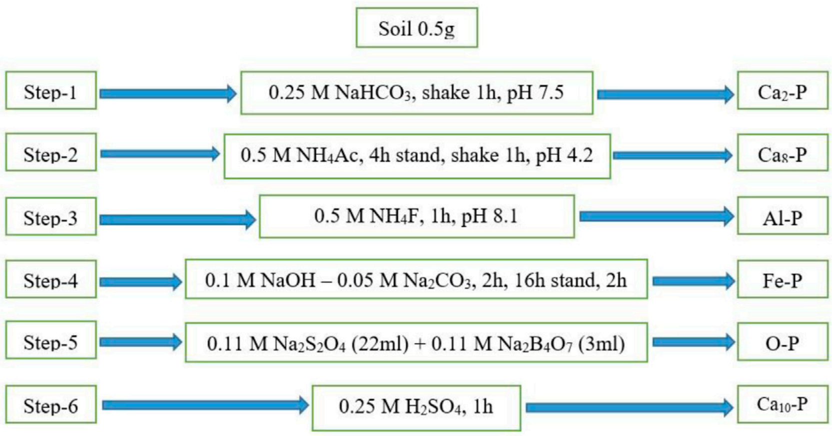

FIGURE 1
Sequential extraction steps for the inorganic P fractions by Jiang and Gu 1989.

amendments, the significance of the difference between treatments was evaluated by the HSD test (honestly significant difference test).

Results

Plant growth and P uptake

Inorganic and organic fertilization alone or in combination affect the growth indexes of maize. In this study, the combined application of PM and P100 significantly increased the maize growth attributes (Table 2). The average increase in plant height (117 cm), shoot fresh weight (101 g), shoot dry weight (13 g), root fresh weight (33.34 g), root dry weight (4.8 g), and leaf area (422 cm^2) was observed under the combined application of PM and P100 to the maize plant. The chlorophyll content of maize leaf increased under the cumulative effect of manures and Pi than that of control (Table 2). The addition of PM and P100 together increased the 180 mg P uptake by the plant compared to the control. Compared with the control, the P contents were significantly increased in the root (0.21%) and shoot (0.4%) of maize (Table 2). The P concentration in different plant parts of maize (shoot and root) were increased in the order of PM > CM > MM > GM > control, respectively (Table 2).

Variation in soil pi fractions

In the current study, the size of the individual P pool was significantly ($p < 0.05$) affected by organic and inorganic amendments (Table 3). The ANOVA demonstrated a significant effect of the treatments with inorganic P (Pi) addition rates of P0, P50, and P100 on the relative proportion of extracted P in bulk soil fractions. The overall organic treatment effect indicated the increasing trends, that is, PM > CM > GM > MM > control. The PM showed a significant increase in the Pi fractions $NaHCO_3$-P, NH_4Ac-P, NH_4F-P, and NaOH-P occluded P (O-P), and Ca_{10}-P in calcareous soils.

In general, under no manure treatment (control) the average size of Pi fractions increased in the order of Ca_8-P > Ca_2-P (labile pool), Al-P > Fe-P (moderately labile pool), and Ca_{10}-P > O-P (non-labile pool), respectively (Table 3). In the control treatment, the Ca_2-P and C_8-P were 1.89 and 9.16% of the total P in the soil (Figure 2). The P100 showed the highest increase in Ca_2-P that was about 40.3%, whereas, the C_8-P was increased by 14.8% (was more or less constant) as compared to P0. Moreover, Fe-P and Al-P constituted 2.31 and 4.51% of the total P in the soil in control, respectively (Figure 2). Compared with the control, the average increase of Fe-P was about 32.6% of the added P50 rate and 35.22% of the added P100 rate. The P50 and P100 exhibited the highest increase in Al-P about 27.3 and 30.75% with respect to control, respectively. In addition, P100 showed the maximum concentration of occluded P approximately, 46.96%. Similarly, P100 depicted the highest Ca_{10}-P about 7.4% compared to P0. The occluded P and Ca_{10}-P were 0.68 and 81.34% of the total P in the soil in control (Figure 2).

Data in Table 3 showed that the PM ($p < 0.05$) increased the concentration of Pi fractions in the following sequence: C_8-P > Ca_2-P, Al-P > Fe-P and Ca_{10}-P > O-P. By applying PM, the Ca_2-P and C_8-P fractions were significantly increased, with a mean value of 32.30 and 76.56 mg kg^{-1}, respectively. These fractions had a higher concentration of about 5.09% (Ca_2-P) and 12.06% (C_8-P) of the total soil P under PM (Figure 2). The increase in Ca_2-P was observed in other organic manure that is 28.91 (CM), 19.33 (GM), 23.34 (MM), and 8.3 mg kg^{-1} (control). Whereas,

TABLE 2 Maize plant growth characteristics and P uptake under different manure types and inorganic P rates.

Treatment	Plant height (cm)	Leaf area (cm^2)	Chlorophyll content	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	Shoot P concentration (%)	Root P concentration (%)	Plant P uptake (mg/plant)
#FF0000; Control	75.25c	230d	30.09d	57.00d	7.70d	24d	3.42e	0.47e	0.18e	57.00e
GM	86.57b	328.59c	33.68c	72.98c	8.10d	25.50cd	3.66d	0.59d	0.24d	72.98c
CM	105.92a	403.66a	40.54a	92.23 ab	10.66b	30.52 ab	4.26b	0.78b	0.35b	92.23 ab
PM	112.93a	415a	42.18a	98.00a	12.22a	32.08a	4.60a	0.86a	0.39a	98.00a
MM	95.02b	362.83b	36.13b	84.05b	9.72c	27.89bc	3.87c	0.67c	0.28c	84.05b
P0	90.08b	336.18b	34.87c	73.33a	8.36c	26.42b	3.74c	0.62c	0.26c	73.33b
P50	95.10 ab	346.13 ab	36.59b	82.64a	9.96b	27.95 ab	3.97b	0.67b	0.29b	82.64a
P100	100.23a	361.73a	38.07a	86.57a	10.72a	29.62a	4.18a	0.72a	0.31a	86.57a
P0-Control	72.34	215.16	27.90	46.50	6.40	22.22	3.23	0.43	0.15	46.50
P0-GM	80.83	313.61	32.79	64.73	7	23.91	3.54	0.55	0.22	64.73
P0-CM	100.04	398.81	39.19	84.47	9.47	28.86	3.96	0.72	0.31	84.47
P0-PM	108.38	407.94	40.45	93.00	10.34	30.17	4.45	0.83	0.37	93.00
P0-MM	88.82	345.37	34.01	77.99	8.59	26.97	3.60	0.59	0.24	77.99
P50-Control	75.47	228.04	30.23	61	7.70	23.92	3.48	0.47	0.18	61.00
P50-GM	86.34	321.09	33.04	74.20	8.37	25.21	3.64	0.59	0.24	74.20
P50-CM	105.73	402.61	41.35	93.86	10.85	30.06	4.30	0.78	0.35	93.86
P50-PM	113.12	414.90	42.02	100	13.01	32.74	4.54	0.86	0.36	100.00
P50-MM	94.81	364.04	36.31	84.19	9.87	27.84	3.90	0.66	0.28	84.19
P100-Control	77.94	246.80	31.91	63.50	9.00	25.85	3.56	0.50	0.20	63.50
P100-GM	92.54	351.07	35.20	80.01	8.94	27.39	3.81	0.63	0.25	80.01
P100-CM	111.97	409.55	41.08	98.39	11.67	32.63	4.54	0.83	0.38	98.39
P100-PM	117.29	422.16	44.09	101	13.30	33.34	4.88	0.89	0.41	101.00
P100-MM	101.43	379.07	38.08	90	10.70	28.88	4.11cd	0.74	0.33	90.00
p-Value (OT)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
p-Value (P)	<0.01	<0.01	<0.01	<0.01	<0.01	0.0130	<0.01	<0.01	<0.01	<0.01
p-Value (OT*P)	0.9959	0.9389	0.7664	0.9386	0.3816	0.9989	0.3582	0.8278	0.5663	0.0049

the Ca_8-P average increase by using other manure sources was about 70.61 (CM), 50.52 (GM), 57.08 (MM), and 40.57 mg kg^{-1} (control). The Ca_2-P was increased by 17.89, 24.32, and 25.11 mg kg^{-1}, while the Ca_8-P was increased by 53.77, 61.67, and 61.76 mg kg^{-1} in P0, P50, and P100, respectively. The current study results showed that the Ca_8-P was larger than the Ca_2-P with a mean difference value of 44.29 mg kg^{-1} under the P100 and PM (Table 3). The significantly increased in Al-P with PM, the mean value was 49.27 mg kg^{-1}, while other manures had mean values of 41.24 (CM), 29.95 (GM), 37.40 (MM), and 20 mg kg^{-1} (control) (Table 3). In addition, Al-P had almost 7.8% contribution to total P (Figure 2). Compared with P0, the maximum increase of Al-P was about 31% by P50, and similar results were observed with P100. When the PM and P100 were applied together, the average increase in Al-P contents was about 53.07 mg kg^{-1}. The PM also significantly increase the Fe-P by about 36.92 mg kg^{-1} compared to the control. This fraction made up 6.28% of total P in soil (Figure 2). By using other manure, the mean value of Fe-P was approximately 47.51 (CM), 31.10 (GM), 33.03 (MM) and 10.57 mg kg^{-1} (control), respectively. The P0, P50, and P100 significantly increased the Fe-P concentration by approximately 26.42, 35.08, and 35.73 mg kg^{-1}, respectively. In this study, the combined application of P100 and PM significantly increased the Fe-P fraction by almost 43.29 mg kg^{-1}. In comparison to the labile and moderately labile pools, the occluded P (O-P) was slightly affected by PM addition (Table 3). The PM increased the O-P contents by 13.89 mg kg^{-1}, which accounted for 2.24% of total P in soil (Figure 2). The O-P fraction was significantly affected with other manure types approximately of 12.49 (CM), 7.89 (GM), 5.84 (MM), and 2.53 mg kg^{-1} (control). The PM addition and P100 together increased the O-P concentration by about

TABLE 3 Different phosphorus and carbon fractions distribution under different manure types and inorganic P rates.

Treatment	Olsen P (mg kg^{-1})	Ca_2P (mg kg^{-1})	Ca_8P (mg kg^{-1})	Al-P (mg kg^{-1})	Fe-P (mg kg^{-1})	Occ-P (mg kg^{-1})	Ca_{10}-P (mg kg^{-1})	MBP (mg kg^{-1})	MBC (mg kg^{-1})	SOC (g kg^{-1})	POC (g kg^{-1})	MAOC (g kg^{-1})
#FF0000; Control	6.05e	8.34e	40.57e	20.0e	10.58d	2.53e	358.8d	7.82e	70.60d	2.90c	1.20c	1.70a
GM	11.03d	19.33d	50.52d	29.96d	31.11c	7.89c	381.19cd	14.27d	90.25c	3.98b	1.97b	2.01a
CM	15.79b	28.91b	70.61b	41.24b	33.04c	12.49b	410.29 ab	25.87b	110.21b	4.30 ab	2.50a	1.80a
PM	18.87a	32.30a	76.56a	49.56a	39.81b	14.24a	421.05a	32.87a	140.09a	4.50a	2.71a	1.79a
MM	12.52c	23.31c	57.07c	37.40c	33.04c	5.84d	391.53bc	17.12c	105.35b	4.10b	2.10b	1.99a
P0	8.44c	17.89b	53.78b	29.85b	26.43b	6.68b	375.23b	15.63c	76.90b	4.61a	2.76a	1.84a
P50	14.49b	24.32a	61.67a	38.0a	35.05a	9.37a	399.17a	19.87b	115.06a	3.69b	1.77b	1.91a
P100	8.44c	25.10a	61.76a	39.03a	35.74a	9.73a	403.31a	23.28a	117.93a	3.57b	1.74b	1.82a
P0-Control	2.54	5.60	35.11	13.94	6.60	1.30	348.91	3.395	37.93	3.63	2.11	1.51
P0-GM	5.76	14.04	45.02	22.64	23.47	5.99	366.70	11.00	60.57	4.96	2.60	2.36
P0-CM	11.82	24.90	64.41	36.98	40.15	10.16	389.05	22.15	90.10	4.84	3.08	1.76
P0-PM	14.48	27.56	71.02	44.15	34.21	12.32	399.94	28.65	115.32	4.94	3.32	1.62
P0-MM	7.62	17.32	53.34	31.53	27.74	3.66	371.53	12.96	80.61	4.68	2.71	1.97
P50-Control	7.65	9.35	43.06	22.44	12.39	2.97	361.60	9.11	84.66	2.60	0.74	1.85
P50-GM	13.32	21.85	53.02	33.02	34.74	8.59	383.94	14.36	102.72	3.54	1.68	1.85
P50-CM	16.69	30.76	73.01	43.05	50.70	13.45	419.26	25.46	119.06	4.18	2.21	1.97
P50-PM	20.65	34.04	79.057	51.44	41.92	15.10	430.70	33.12	152.09	4.30	2.42	1.88
P50-MM	14.14	25.60	59.70	40.07	35.49	6.75	400.36	17.29	116.77	3.80	1.82	1.97
P100-Control	7.96	10.06	43.55	23.62	12.76	3.30	365.88	10.97	89.20	2.49	0.73	1.75
P100-GM	14.02	22.09	53.51	34.20	35.12	9.08	392.91	17.46	107.46	3.43	1.62	1.81
P100-CM	18.85	31.07	73.92	43.66	51.68	13.84	422.57	10.97	121.49	3.86	2.20	1.66
P100-PM	15.80	35.29	79.61	51.44	43.29	15.32	432.50	36.83	152.84	4.26	2.41	1.85
P100-MM	21.49	27.01	58.19	53.08	35.87	7.10	402.70	21.12	118.67	3.80	1.75	2.04
p-Value (OT)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.197
p-Value (P)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.767
p-Value (OT*P)	<0.01	0.085	0.773	0.327	0.096	0.160	0.996	0.152	0.490	0.284	0.342	0.348

14.02 mg kg^{-1}, as compared to the control. The O-P increased about 45.75% under P100 (same for P50) compared to P0. Moreover, the Ca_{10}-P was 66.46% of the total soil P (Figure 2). By PM, the highest Ca_{10}-P contents (17.59%) were observed with respect to control. The PM and P100 together increased the Ca_{10}-P concentration by about 432.50 mg kg^{-1}. Whereas, the Ca_{10}-P concentration increased about 410.26 (CM), 381.50 (GM), 391.53 (MM) and 358.8 mg kg^{-1} (control). In P0, P50, and P100, the Ca_{10}-P fraction was increased by almost 375.23, 399.17, and 403.31 mg kg^{-1}, respectively.

Variation in soil C fractions, olsen P and microbial biomass indices

The SOC, POC, and MAOC concentrations varied between the different organic amendments. The SOC fraction increased in the following order: PM > CM > MM > GT > control (Table 3). The maximum SOC was 4.2 g kg^{-1} under the combined addition of P100 and PM. Compared to the control, the average increase in SOC was about, 1.6 (PM), 1.4 (CM), 1.08 (GM), and 1.2 (MM) g kg^{-1}. The Pi had increased the SOC, approximately 4.61, 3.69, and 3.57 g kg^{-1} under P0, P50, and P100, respectively. The different organic manure additions significantly increased POC that ranging between 1.2 and 2.7 g kg^{-1} (Table 3). The Pi significantly ($p < 0.0\ 5$) increased the POC that was 1.77 g kg^{-1} under P50 and 1.74 g kg^{-1} under P100. The higher POC was 2.4 g kg^{-1}observed in the combined addition of P100 and PM. The effect of treatments on MAOC fraction increased in the following order: GT > MM > CM > PM > control (Table 3). Overall, the maximum C fractions size increased in PM by the following order: SOC > POC > MAOC.

The different organic manure amendments with Pi caused variations in Olsen P, MBC, and MBP (Table 3). The maximum Olsen P 21.5 mg kg^{-1} was observed by the combined addition of

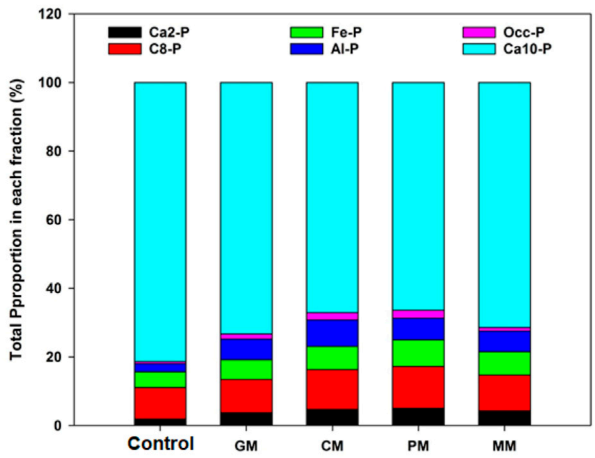

FIGURE 2
Total Pi proportion in each fraction under organic manure addition.

PM and P100. The Olsen P was increased by about 72 and 85% under P50 and P100. The average increase of Olsen P was 18.88 (PM), 15.79 (CM), 11.04 (GM), and 12.51 (MM) mg kg^{-1} than that of control (Table 3). Collectively, the MBC and MBP pools were higher at almost 69.4.5, 39.9, 20.25, 34.75 mg kg^{-1} and 25.07, 18.07, 6.47, 9.32 mg kg_{-1} in PM, CM, GM, MM in comparison to control, respectively (Table 3).

Discussion

Changes in soil properties (SOC, POC, and olsen P)

In our study, organic and inorganic fertilization significantly ($p < 0.05$) affected the soil chemical properties, microbial biomass indices, and inorganic P (Pi) fractions (Table 3), that could be due to the manure types and P application rates. Many previous studies have presented that the Pi forms are mainly influenced by microbial activities and soil chemical properties (Spohn and Widdig, 2017). Furthermore, the soil physicochemical properties significantly affected the microbial biomass and Pi fraction in calcareous soil. Among different organic amendments, PM had the greater effect on soil chemical and biological properties, resulting in native soil P lability. This was mainly due to organic amendments chemical composition, where PM had higher organic-P contents as compared to other amendments (CM, GM). Moreover, higher microbial activities (MBC, MBP) under PM might increase the soil available and total P by inducing SOC and POC concentration in the soil (Table 3). In the present research, total soil P (683.76 mg kg^{-1}) and Olsen-P (21.5 mg kg^{-1}) contents were highest in the PM with P100 (Figure 2; Table 3). According to Hassan et al. (2012), the critical Olsen-P level for wheat and maize growth is 14.6 and 13.2 mg kg-1, respectively. This study achieves quite a similar Olsen-P threshold hold levels with PM and P100 applications. The significant increase in the Olsen-P showed a positive correlation with Pi fractions pools, showing that the combined organic and inorganic fertilizer addition had positive effects on Olsen-P contents. This might be due to the organic manures being sufficient in P supply and organic manures with inorganic P are prevented from the adsorption and insoluble complexes in the soils (Chen et al., 2006). Moreover, organic inputs mobilized native soil P by accelerating microbial activities (Kouno et al., 2002).

Overall, organic amendments significantly stimulated the soil microbial pool by increasing the availability of C sources (POC). Among other amendments, PM had a higher moderately labile (Ca_8-P) fraction, possibly due to higher nutrient contents, POC availability, and water holding capacity (Hoover et al., 2019). The MAOC had no relationship with microbial activities because that was known to be a recalcitrant C and a non-usable pool (Virk et al., 2021). This indicates that the only labile pool of C in the organic manures could increase P availability by inducing microbial activity. Natural manure fertilization is the source of essential nutrients for plants. Plants require P in the vegetative, and during reproductive development (Richardson et al., 2009). The data in Table 2 shows that plant biomass is considerably affected by both manures and Pi. The higher amount of approximately 236 mg of P uptake by the maize plant was estimated as compared to CM (197.74 mg) and GM (113.54 mg) under the dose rate of P100 (Table 2). This might be because PM contains about three to four times higher P concentration than manure from other livestock, except for pigs (Kleinman et al., 2005), which was also seen in the current study. The nutrient concentration for all types of manure varied when compared to other published studies, which was probably attributed to dietary habits, animal age, and the season (Penhallegon 2003; Brown 2013). In addition, this higher plant P uptake might be due to PM-treated soil having the potential to increase the labile SOC pool mainly POC, resulting in higher microbial activities and higher P availability (Ca_8-P) inducing plant growth. Similarly, our results are also consistent with the previous research, which reported that applied Pi is mostly transformed into Ca_2-P (more available for plant growth) instead of Ca_8-P in calcareous soil (Marriott and Wander 2006; Liebisch et al., 2014). Furthermore, Hassan et al. (2012), found that organic input with Pi leads to a synergistic effect on plant growth compared to the application of the sole fertilizer in calcareous soils. The labile P and moderately labile P are always known as bioavailable P forms for plant uptake. Consequently, it improves the physicochemical and biological soil conditions for plant growth and development.

Changes in microbial biomass indices

Soil microbial dynamics are affected by various organic amendments. In C and P deficient soils, the microbial community acts more effectively when energy source C is added. A significantly higher MBC of 152.8 mg kg^{-1} was observed under the cumulative effect of organic and inorganic amendments (Table 3). In addition, a significant positive relationship between MBC with Pi fractions and a negative correlation with SOC and POC was observed, suggesting that an increase in MBC at the expense of SOC and POC resulted in P release from Pi fractions, in parallel to these results. Wu et al. (1990) reported that MBC pool mainly depends on the organic C availability. Moreover, the native microbial population is stimulated in response to the Pi addition (Ayaga et al., 2006). However, the relationship of MBC to organic C is inconsistent, showing either a positive or no correlation due to the microbial community structure and organic C quality (Brookes 2001).

The SOC and POC had a positive correlation with MBP, the increase in microbial biomass indicating the strong potential to accumulate radially available P into their bodies (Spohn and Widdig, 2017). The maximum increase in MBP of 36.8 mg kg^{-1} due to the cumulative effect of organic manure and Pi amendments was observed (Table 3). As suggested before, organic amendments induce MBC (in response to POC and SOC increment), but Pi with organic amendments could stimulate MBP and Olsen-P availability (Tang et al., 2014). An important finding was that the manure-treated soil (PM) had a larger size of microbial biomass C and microbial biomass P than that of other organic manures. This might be due to the difference in microbial P acquisition ability in different manure sources, or might be due to the difference in availability of C and P in manure sources (Kouno et al., 2002). In the current study, microbial biomass dynamics showed variations in different organic amendments, indicating that microbial activity depends on organic C availability (Nyawade et al., 2019).

Changes in soil P fractionation

The application of Pi and different manure together ($p <$ 0.05) enhanced the P concentration in soil, this result is consistent with Zhang et al. (2021). P input increased the size of all Pi fractions such as the labile, moderately-labile, and non-labile (Table 3). Our study describes that the manure applied to soil has a direct influence on P availability. This was possibly due to different Pi amendment rates and manure having various impacts on P immobilization and its mobilization in calcareous soils (Khan and Joergensen 2009).

The Ca-P fraction was identified as the dominant P fraction in calcareous soil. Soil Pi forms Ca_2-P considered as labile P or bio-available P form for plant uptake (Jiang and Gu 1989). In the current study, Ca_2-P accounted for 5.09%, of total P as shown in

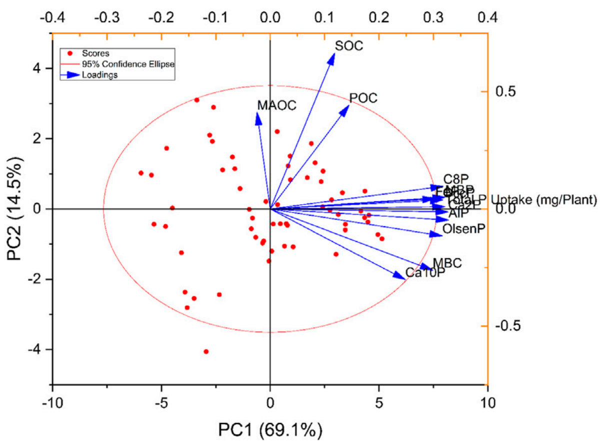

FIGURE 3
PCA analysis of phosphorus and SOC fractions.

Figure 2 by the combined addition of PM and P100. This indicated that the available P supply ability was well maintained by the Pi input combined with manure over inorganic input only (control with P100). According to Delgado et al. (2002), P fixation with Ca-P reduces the P accessibility for plant growth that may be available after the manure input into the soil, which has been proven in our PCA analysis (Figure 3). These study results are in accordance with Ahmed et al. (2019), which found that the Pi and pig manure addition increased the P labile forms by inducing microbial activities in response to C addition in soil. The average labile Pi content increases in the PM, possibly due to the higher SOC (4.5 g kg^{-1}) and POC (2.7 g kg^{-1}) concentration in the soil (Virk et al., 2021), that might move the P adsorption sites by exchanging the clay mineral surface charges, decreasing the sorption sites and improving the soil P availability. Noticeably, the association of Pi fractions and soil carbon (SOC, POC) also indicated that the input of organic manure reduced the Pi fixation and increase Ca_2-P availability (Delgado et al., 2002).

The inorganic fractions (Ca_8-P, Fe-P, and Al-P) were known as the moderately labile P pool. Ca_8-P, Fe-P, and Al-P fractions accounted for 79.61, 43.29, and 50.03 mg kg^{-1}, respectively, with the PM and Pi. During the manure decomposition process, organic acids are released, which lead to chemical bonding of P with minerals ions (Fe and Al), decreasing the soil P availability. As the labile P, the moderately labile P also improved by the PM, compared to the other manure source and control (Table 3). That is possibly due to the high nutrient content in PM mainly POC and SOC as compared to other manures (GM, CM), which have higher nutrients and water holding capacity. Several studies also resulted that the various source of organic amendments increased the moderately labile Pi pool as compared to inorganic fertilizer (Li et al., 2012). Malik et al. (2012) identified that manure sources containing P content can have synergetic effects on increasing organic P content and retention in soils that could be due to the P adsorption in organic matter that is transformed into inorganic P by plant roots. In calcareous soil, the Ca_8-P fraction is mostly greater than Ca_2-P this may explain that Ca_8-P is more stable in P-limited soil and it needs higher Pi in the soil for the solution to transform into Ca_2-P, which may not be possible under low Pi addition (Lindsay et al., 1962; Ayaga et al., 2006). However, the increase in the moderately labile P pool in the combined use of PM and Pi may explain the slow P release from an organic source which can contribute to the P cycling process in soil.

The non-labile P fractions which are stable and insoluble P form, such as occluded P (O-P) and apatite P (Ca_{10}-P), are known as the unavailable P pools for plant uptake (Cao et al., 2020). The Ca_{10}-P fraction is dominated by all the P fractions in the soils. Noticeably, it had a significant correlation with microbial index, this might be due to higher organic source amendments (Yong-Fu et al., 2008). The Ca_{10}-P is the recalcitrant pool due to geochemical properties on P cycling and higher $CaCO_3$ contents in the soils, as suggested by Mehmood et al. (2015). Our results support the study of Zhang et al. (2021), who suggested that adding P fertilizer had a slight influence on the residual P forms but substantially enhanced the soluble Pi. While the stable P was supposed to be less available P pool, the variation in this P pool shows P cycling on a long-term basis in the soil system (Huang et al., 2017). It was concluded by Meason et al. (2009) that applying P adsorbed on the surface of the mineral can contribute to increasing the stable pool. Our study resulted that non-labile P concentrations were higher in Pi with manure addition, that resulted in the P sorption under excessive Pi input, that may be transformed to the moderately or labile P pools over time.

Conclusion

The Poultry manure addition increased the plant growth, P uptake, and Ca_8-P pool (after Ca_2-P is depleted), by improving the total SOC contents and labile organic C (POC) pool in P-limited soil. Present research demonstrated that SOC and microbial biomass (MBP, MBC) are the key drivers to improve the soil P availability and the cumulative effect of PM and Pi is not only a better approach for P acquisition for the maize plant but also has a positive effect on the chemical properties (SOC, POC and Olsen P) of the soil. Indeed, the increase in the soil labile and moderately labile P fractions are likely due to MBP turnover processes mediated by PM. Therefore, we concluded PM with Pi promotes soil MBP pool, which in turn improves soil P availability and utilization efficiency in maize crops. This study shed light on the Pi addition rate with PM should be further evaluated for P balance and crop yield in field conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

Conceptualization, KK, and GF; methodology, KK, MN, and MR; software, KK; validation, MA and MN; data curation, MN; writing—original draft preparation, KK; writing—review and editing, KK, MA, MN, MS, NA, HA, and RG; supervision, MN and GF.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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