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RESEARCH ARTICLE

Liming reduces soil phosphorus availability but promotes yield and P uptake in a double rice cropping system

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Abstract

Liming is often applied to alleviate soil acidification and increase crop yield on acidic soils, but its effect on soil phosphorus (P) availability is unclear, particularly in rice paddies. The objective of this study was to examine the effect of liming on rice production, yield and P uptake in a three-year field experiment in a double rice cropping system in subtropical China. We also conducted an incubation experiment to investigate the direct effect of liming on soil available P and phosphatase activities on paddy soils in the absence of plants. In the incubation experiment, liming reduced soil P availability (measured as Olsen-extractable P) by 14–17% and inhibited the activity of soil acid phosphatase. Nonetheless, lime application increased grain yield, biomass, and P uptake in the field. Liming increased grain yield and P uptake more strongly for late rice (26 and 21%, respectively) than for early rice (15 and 8%, respectively). Liming reduced the concentration of soil available P in the field as well, reflecting the increase in rice P uptake and the direct negative effect of liming on soil P availability. Taken together, these results suggest that by stimulating rice growth, liming can overcome direct negative effects on soil P availability and increase plant P uptake in this acidic paddy soil where P is not the limiting factor.

Keywords: crop yield, subtropical, phosphatase activity, phosphorus availability, soil acidification

1. Introduction

China is the largest rice (*Oryza sativa* L.) producer in the world (Alexandratos and Bruinsma 2012). The double rice cropping system (i.e., rice is cropped twice annually), mostly located in the subtropical region of China, accounts for approximately 40% of China's total rice planting area and thus plays a critical role in its rice production (MARA 2019).

Chinese rice yield per hectare has increased by a factor of three over the past several decades (1961–2016), largely

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due to fertilizer applications of particularly nitrogen (N) (Zhang *et al.* 2012; MARA 2019). However, soil acidity, resulting from a combination of inherent low soil pH values and excess use of N fertilizers, now constrains rice yield improvement in large areas of subtropical China (Guo *et al.* 2010). Liming is therefore commonly used to ameliorate soil acidity and improve rice yield (Liao *et al.* 2018, 2020; Holland *et al.* 2019).

In addition to N, phosphorus (P) is a limiting nutrient in many rice production areas, particularly in highly weathered soils in low-latitude regions (Nishigaki *et al.* 2019). It is still unclear how liming affects P availability to rice in these areas. On one hand, liming-induced increases in soil pH help to promote the release of P anions from Al- and Fe-(hydr) oxide surfaces (Barrow 2017; Penn and Camberato 2019). Because liming generally increases microbial activity, it often stimulates the mineralization of crop residues and soil organic matter (e.g., Aye *et al.* 2016; Liao *et al.* 2018, 2020), which in turn can enhance soil available P (Simonsson *et al.* 2018). On the other hand, liming may reduce soil P availability due to increased P precipitation as Ca-phosphate at higher pH (Barrow 2017; Penn and Camberato 2019). Liming can also alleviate deficiencies of other nutrients besides P (e.g., N) (Liao *et al.* 2018, 2020), which allows for more P uptake by crops and thus results in reduced amounts of readily available P in soil. Thus, to fully understand liming effects on soil P dynamics, plant responses and direct liming effects on soil chemistry need to be studied simultaneously.

To date, most studies on liming and soil P availability have focused on upland soils but the effect of liming on soil available P and P uptake in rice paddies is still unclear (Holland *et al.* 2019). Therefore, we conducted a 3-year field experiment to investigate the effect of liming on soil P availability, plant P uptake and yield in a double rice cropping system on an acidic soil in subtropical China. To examine the effect of liming on soil P availability and enzyme activities that are not mediated through plants, we also conducted an incubation experiment, using soils from the same experimental site.

2. Materials and methods

2.1. Experimental site

From 2015 to 2017, we conducted an experiment in a rice paddy field in Zengjia Village, Shanggao County, Jiangxi Province, China (115°09´E, 28°31´N). Early rice was grown from April to July and late rice was grown from July to November, followed by a winter fallow period (November until April of the following year). The site is characterized by a subtropical climate with a mean annual precipitation of 1 650 mm and mean annual temperature of 17.5°C. The paddy soil is classified as a Typic Stagnic Anthrosol (IUSS Working Group and WRB 2006). The surface soil properties (0–15 cm) before the experiment were: pH (soil:H_2O=1:2.5, w/w), 5.2; organic matter, 18.1 g kg^{-1}; total N, 1.1 g kg^{-1}; total P, 0.4 g kg^{-1}; total K, 3.9 g kg^{-1}; alkaline hydrolyzable-N, 115.0 mg kg^{-1}; Olsen-P, 15.9 mg kg^{-1}; available K, 64.0 mg kg^{-1}; exchangeable Al^{3+}, 2.3 cmol kg^{-1}; exchangeable Ca^{2+}, 7.6 cmol kg^{-1}; exchangeable Mg^{2+}, 0.7 cmol kg^{-1} and clay content (<0.002 mm), 17.0% (Liao *et al.* 2018, 2020).

2.2. Experimental design

Details of the experimental treatments are reported by Liao *et al.* (2018, 2020) and Jiang *et al.* (2018). Briefly, in 2015 we established a factorial field experiment that included two factors (liming and rice straw retention) in a completely randomized block design with three replicates. Each plot was 25 m^2 in size (5 m×5 m); plots were separated by 20-cm thick levees with plastic covering. For this study, we focused on plots with straw incorporation, comparing two treatments, i.e., liming (+L) and no liming (–L). The +L plots received lime in the form of $Ca(OH)_2$ powder with particle size<0.16 mm. Lime was broadcast only once (2.1 t ha^{-1}) before soil plowing in the early rice season of 2015. All the treatments received the same rates of inorganic N, P_2O_5, and K_2O fertilizers as 120, 75, and 75 kg ha^{-1} for the early rice and 150, 75, and 75 kg ha^{-1} for the late rice, respectively. These application rates are representative of fertilizer practices of local farmers.

The inbred rice variety Zhongjiazao 17 was planted as the early rice cultivar, and the hybrid rice variety Wuyou 308 as the late rice cultivar. Pre-germinated seeds were sown in a seedbed. Further details on crop management operations are presented in Table 1. After transplanting, we maintained a standing water depth of about 3 cm in each plot. We initiated a one-week midseason drainage about 28 and 18 days after transplanting the early and late rice, respectively. Following midseason drainage, we applied intermittent irrigation (i.e., alternate wetting and drying) until one week before harvest. After the harvest of early rice, aboveground rice straw was chopped into 10-cm long pieces with10-cm residual stubbles and then was incorporated into soil by plowing. After the harvest of late rice, the straw was left on the soil surface until the following year, when it was incorporated into the soil by plowing before transplanting early rice (Liao *et al.* 2018, 2020).

2.3. Soil incubation

For the incubation experiment, we collected soil samples (0–15 cm) from unlimed plots after the harvest of late rice in

Table 1 Overview of crop management operations throughout our field experiment

Year	Crop	Planting density (cm×cm)	Seedling number (no. per hill)	Date (d/mon)			Growth period (d)
				Sowing	Transplanting	Harvest	
2015	Early rice	13.2×23.1	4	22/03	24/04	10/07	110
	Late rice	13.2×26.4	2	24/06	22/07	28/10	126
2016	Early rice	13.2×23.1	4	20/03	21/04	8/07	110
	Late rice	13.2×26.4	2	22/06	20/07	30/10	130
2017	Early rice	13.2×23.1	4	23/03	22/04	9/07	107
	Late rice	13.2×26.4	2	27/06	26/07	2/11	128

2017. The experiment included three treatments: no liming (control) and liming at a rate of 0.99 g $Ca(OH)_2$ kg^{-1} soil (L) and 1.98 g $Ca(OH)_2$ kg^{-1} soil (2L), equivalent to a rate of 2.0 and 4.0 t $Ca(OH)_2$ ha^{-1} in the field, respectively. Each treatment had three replicates, i.e., one replicate for each corresponding unlimed field plot. For each replicate, 50 g of fresh soil (2-mm sieved) was put into a 200-mL plastic bottle. For the L and 2L treatments, lime was added and thoroughly mixed with the soil. Soils were then incubated at 25°C in the dark for one month. Deionized water was added every two days to maintain a 1-cm water layer in the bottles during the incubation period. We indicated target water levels by drawing a line on each plastic bottle. Water was then added until the water level reached this target.

2.4. Sampling and measurements

Grain yield, aboveground biomass, P concentration, and P uptake At plant maturity, we measured grain yield and aboveground biomass (i.e., stems, leaf blades, and panicles) for early and late rice in all three years, following the method of Liao *et al.* (2018, 2020). Plant samples were ground to pass a 0.25-mm sieve and then digested with H_2SO_4-H_2O_2. Their P concentration was determined by the molybdenum-blue colorimetric method at 880 nm (Pansu and Gautheyrou 2007). Plant P uptake was calculated by multiplying P concentration by biomass.

Soil pH and soil available P We collected five soil cores (3 cm in diameter) to a depth of 15 cm in each plot after the harvest of late rice from 2015 to 2017. Soils were pooled as a composite sample for each plot, air-dried, and then passed through a 2-mm sieve for analyses. We measured soil pH using a pH meter in a 1:2.5 (w/w) soil:water mixture. Soil available (measured as Olsen-extractable P) P pools were determined following Pansu and Gautheyrou (2007).

Soil acid and alkaline phosphatase At the end of the soil incubation, we determined soil pH and available P as described above. Soil acid and alkaline phosphatase activities were determined according to Guan (1986) and Su *et al.* (2015). Briefly, soil phosphatase activities were measured using *p*-nitrophenyl phosphate disodium as a substrate, incubated at 37°C for 24 h, after which the liberated phenol was determined by a colorimetric method. Phosphatase activities were expressed as μmol phenol g^{-1} d^{-1}. Acid phosphatase and alkaline phosphatase activities were determined using a modified universal buffer pH of 5.0 and 9.4, respectively.

2.5. Statistical analyses

We used model simplification on linear mixed-effects models (R package nlme, Pinheiro *et al.* 2016) to examine the effects of liming, rice crop (early or late rice), year, and their interactions on plant P uptake and concentration, rice yield and biomass, and soil properties in the field experiment. The fixed effects were liming, crop, and year, while blocks were included as a random effect. For model simplification we iteratively removed higher order interactions and assessed model performance before and after each removal (Crawley *et al.* 2013). One-way analysis of variance (ANOVA) was performed to determine the difference in soil pH, soil available P, and enzyme activities among treatments in the incubation experiment. Following the ANOVA we used Tukey's honest significant difference (HSD) test to identify significant differences among means in the incubation experiment. We performed all analyses in R (v3.5.1; R Core Team 2018). To ease interpretation, we used tables to report main effects and figures to highlight interactions of various factors (i.e., liming, crop, and year).

3. Results

3.1. Yield and biomass

Liming significantly increased grain yield and total aboveground biomass in the double rice cropping system (Table 2). We found a significant liming×crop interaction for yield and biomass (Fig. 1-A and B), whereby liming enhanced grain yield and biomass more strongly in the late rice season than in the early rice season. The effect of liming on total biomass varied with year, with year 2 showing the largest effect of liming on total biomass (+23.6%; Appendix A).

Table 2 Yield, total aboveground biomass, and P uptake at maturity as affected by different factors

	Yield (t ha^{-1})	Total aboveground biomass (t ha^{-1})	Total P uptake (kg ha^{-1})
Liming (L)[1)]			
+L	8.22***	14.78***	37.65***
–L	6.80	12.79	32.67
Crop (C)			
Early rice	6.71	13.01	31.53
Late rice	8.31***	14.56***	38.79***
Year (Y)			
2015	7.54	15.18	33.77
2016	7.63	13.75	42.35
2017	7.35	12.42***	29.36***
F-value			
L×C	13.16**	9.34**	18.93***
L×Y	1.46	4.56*	1.19
C×Y	3.48*	8.95**	105.24***

[1)] +L and –L represent with and without lime addition, respectively. *F*-values are provided for interactions. There were no significant three-way interactions. Significant differences between averages and significance of *F*-values are indicated by * (0.01<*P*≤0.05), ** (0.001<*P*≤0.01) and *** (*P*≤0.001).

3.2. Plant P uptake and concentration

Liming significantly increased total P uptake in aboveground biomass (Table 2). We found a significant liming×crop interaction for P uptake, with a greater increase in P uptake under liming in the late rice season (21.4%) than in the early rice season (8.0%) (Fig. 1-C). Liming did not affect P concentrations in any of the plant organs (Appendix B).

3.3. Soil properties

Liming significantly increased soil pH, while reducing soil available P (Table 3). The liming effect on pH diminished over time (Appendix C). Liming consistently reduced soil available P during the three years, with the strongest reduction in the second year of 2016 (Appendix C). The average total soil P content at the end of the experiment was 0.38 g kg^{-1}. Total soil P was not affected by liming (data not shown).

In the incubation experiment, liming increased the initial soil pH from 5.3 to 5.7 and 6.2 by applying low and high rates of lime, respectively (Table 4). Similar to the field experiment, liming significantly reduced soil available P at the end of the incubation. Liming also significantly reduced the activity of soil acid phosphatases, but it increased alkaline phosphatase activity (Table 4).

3.4. Relationship between soil pH, soil available P, and P uptake

A three dimensional figure shows the relationships between total P uptake by rice, soil pH, and soil available P (Fig. 2-A). Soil pH was positively correlated with soil available P in the limed plots, while no significant relationship was found in

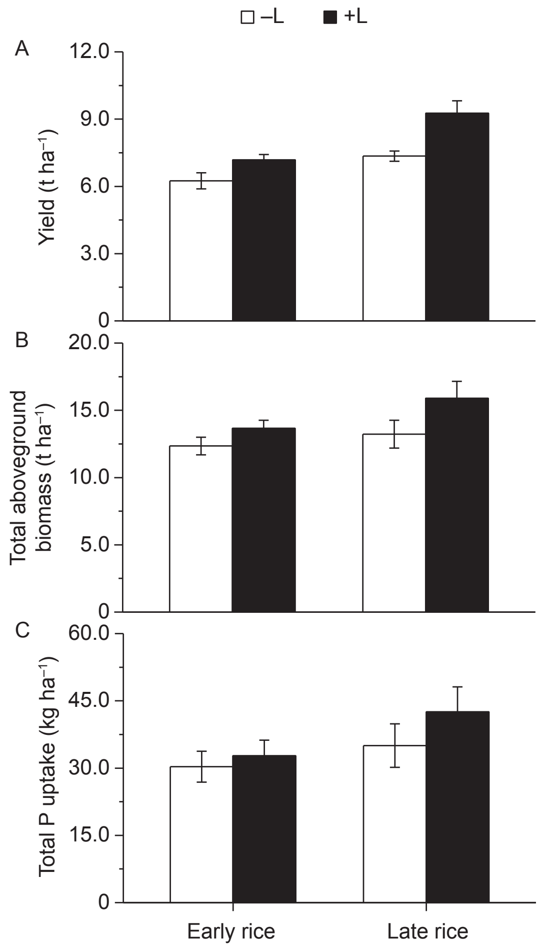

Fig. 1 Yield, total aboveground biomass, and total P uptake as affected by liming and crop (early rice and late rice). +L and –L represent with and without lime addition, respectively. Error bars represent the 95% confidence interval of the mean (*n*=3).

Table 3 Mean soil properties after the harvest of the late rice as affected by different factors

	pH	Soil available P (mg kg^{-1})[2)]
Liming (L)[1)]		
+L	5.95***	17.96***
–L	5.43	21.69
Year (Y)		
2015	5.77	18.20
2016	5.82	26.26
2017	5.48**	15.02***
F-value		
L×Y	6.79*	6.87*

[1)] +L and –L represent with and without lime addition, respectively.
[2)] Measured as Olsen-P (Pansu and Gautheyrou 2007).
F-values are provided for interactions. Significant differences between averages and significance of *F*-values are indicated by * (0.01<*P*≤0.05), ** (*P*≤0.01) and *** (*P*≤0.001).

Table 4 Soil properties as affected by liming in the incubation experiment

Treatment[1)]	pH	Soil available P (mg kg⁻¹)[2)]	Soil acid phosphatase (μmol g⁻¹ d⁻¹)	Soil alkaline phosphatase (μmol g⁻¹ d⁻¹)
Control	5.3 c	19.6 a	14.6 a	1.1 b
L	5.7 b	16.2 b	13.4 b	1.5 a
2L	6.2 a	16.8 b	10.4 c	1.4 a

[1)] Control, no liming; L, liming at a rate of 0.99 g $Ca(OH)_2$ kg^{-1} soil; 2L, liming at a rate of 1.98 g $Ca(OH)_2$ kg^{-1} soil.
[2)] Measured as Olsen-P (Pansu and Gautheyrou 2007).
Different lowercase letters in the same column indicate statistically significant differences at $P \leq 0.05$.

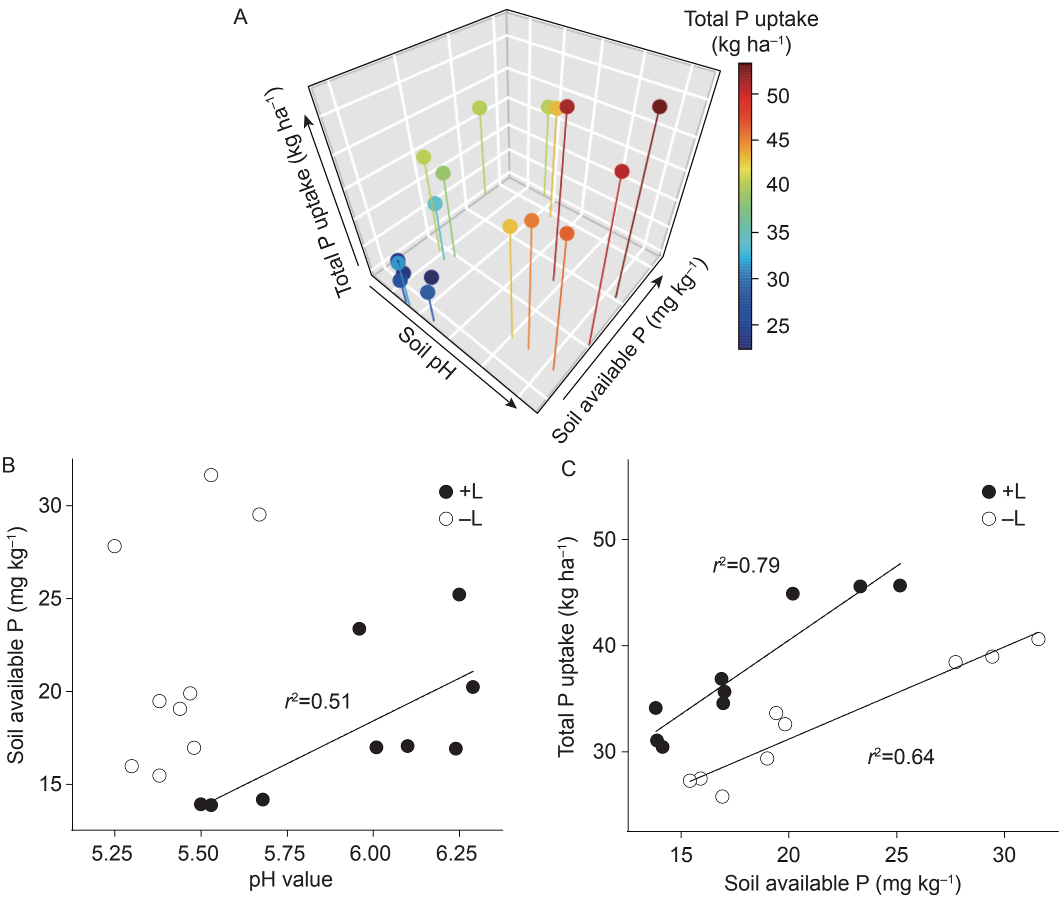

Fig. 2 Relationships between total P uptake by rice, soil pH and soil available P. +L and –L represent with and without lime addition, respectively. The lines represent significant relationships based on mixed-effects model results.

the unlimed plots (Fig. 2-B). Soil available P was positively correlated with total P uptake in both the limed and unlimed treatments (Fig. 2-C).

4. Discussion

Our incubation experiment clearly indicates a direct negative effect of liming on soil P availability, but our field data show that liming promoted P uptake in an acidic paddy soil. Previous studies have showed that liming-induced precipitation of Ca-P minerals effectively decreases the amount of P available to plants (Barrow 2017). Our results show that although liming reduced soil available P (i.e., a lower intercept in limed soils than in unlimed soils), soil available P positively correlated with soil pH (Fig. 2-B). Thus, the decrease in soil available P under lime application is likely driven by fixation of P by Ca and adsorption of P to Fe and Al oxide surfaces, and not driven through changes in soil pH (Penn and Camberato 2019).

Liming may also affect P availability by altering the activity of soil phosphatases (Ma *et al.* 2018). Indeed, our results indicate that whereas lime addition promoted soil alkaline phosphatase activities, it significantly reduced the activity of acid phosphatase. The change in soil phosphatase activities was probably due to liming-induced shifts in soil P forms (Dinesh *et al.* 1998; Holland *et al.* 2018). For instance, Li *et al.* (2008) reported that acid phosphatase activities were negatively correlated to the concentration of $NaHCO_3$-extracted organic P in rice rhizosphere. However, as the activity of soil acid phosphatase was one order of magnitude greater than that of alkaline phosphatase (Table 4), liming-induced decreases in soil acid phosphatase activities may reduce P mobilization, thereby lowering soil P availability (Su *et al.* 2015).

The pool of readily available soil P in the field is determined by various processes, including direct liming effects and plant P uptake. Higher P uptake by rice plants in limed plots can reduce amounts of soil available P relative to unlimed plots (Shen *et al.* 2011). However, our results indicate that P uptake was positively correlated with soil available P, suggesting that higher P uptake did not result in lower soil available P (Fig. 2-C). On the other hand, liming-induced reductions in soil P availability in the field and in the incubation experiment were quantitatively similar (Tables 3 and 4), suggesting that field observations may be explained by direct liming effects rather than higher P uptake. To increase mechanistic understanding of liming effects on soil P dynamics, we suggest that future research should focus on separating direct from indirect (i.e., affected by plant uptake) effects of liming. For instance, measurements of Ca-P minerals in the field could help to quantify direct negative effects of liming on P availability. Regardless of the mechanism underlying the reduction of P availability in the field, our results show that direct effects of liming on soil P availability did not limit plant growth in the current study.

Why does liming promote rice P uptake even when it reduces soil available P in the absence of plants? We suggest that the amounts of available P in the limed plots were not low enough to limit rice growth (Li *et al.* 2011). Indeed, our results showed that there were no significant relationships between rice yield and soil available P (Appendix D). Furthermore, a comprehensive study suggests that an Olsen-P value of 10 mg kg^{-1} is sufficient to support normal rice growth in China (Shen 1998). Similarly, Bai *et al.* (2013) also found a critical Olsen-P value of 10.9 mg kg^{-1} for rice, beyond which there was no response to fertilization of additional P. Thus, the average soil P availability of 17.96 mg kg^{-1} in the limed plots was likely ample to meet the P requirement of rice (Table 3). The relatively high P availability in our soils is representative for the middle-lower Yangtze Plain (e.g., Li *et al.* 2011; Ma *et al.* 2016), a result of decades of P application rates that exceed crop P removal rates. Total P input including fertilizer and straw (40.4 kg ha^{-1}) also surpassed the total P uptake in the +L plots (37.7 kg ha^{-1}) at our site (Table 2), which is another indication that P availability was not limiting growth.

Two other processes likely helped to prevent reductions in soil available P with liming. First, lime application significantly enhanced soil dissolved organic carbon (Appendix E), probably because liming stimulated rice growth and thus straw inputs (Table 2). By providing additional substrate, higher straw incorporation with liming may stimulate soil microbial biomass and activity, thereby promoting enzyme activity (e.g., soil phosphatase activities) and soil P availability (Akhtar *et al.* 2018; Han *et al.* 2018; Liao *et al.* 2018, 2019). This explanation is consistent with Wei *et al.* (2016), who reported that straw incorporation increased both organic C content and organic P content of soil particulate fractions. Second, the improved P uptake with liming also increased P inputs through straw retention in the following season, thereby supplementing the available P pool.

Taken together, our data suggest that although liming has direct negative effects on soil P availability in the absence of plants, it does not limit rice growth as the soil P status is sufficient to sustain optimal growth. Therefore, given the high levels of soil acidification and available P in the middle-lower Yangtze Plain area (Guo *et al.* 2010; Ma *et al.* 2016), we suggest that lime should be applied to increase both rice yield and soil pH.

Our results indicated that liming did not affect P concentrations in any of the plant organs even though lime application significantly enhanced rice biomass. Thus, we conclude that the increase in P uptake with liming is driven by the improvement in rice growth. It is well known that liming can alleviate soil acidity and promote crop growth on acidic soils by increasing nutrient availability (e.g., N, Ca, and Mg) and by reducing Al^{3+} and Mn^{2+} toxicity (e.g., Holland *et al.* 2018). Indeed, previous studies at our experimental site indicate that liming increases soil N availability and rice N uptake (Liao *et al.* 2018, 2020), and that liming reduces soil exchangeable Al^{3+} (Jiang *et al.* 2018).

Liming increased total P uptake more strongly in the late rice season than in the early rice season, which is mainly due to the greater increase in rice biomass. Our previous study demonstrated that a stronger N uptake response to liming for late rice than early rice contributed to the greater increase in biomass (Liao *et al.* 2018, 2020). As liming promotes the decomposition of organic matter, it alleviates N immobilization in the late rice season caused by straw incorporation following early-rice harvest (Aye *et al.* 2016; Jiang *et al.* 2018). In addition, the hybrid variety used for late rice has a higher yield potential than the inbred variety for early rice, and thus may show a greater increase in biomass and nutrient uptake in response to liming-induced reductions in soil acidification and improved growth conditions (Huang *et al.* 2017; Liao *et al.* 2018, 2020).

Our results indicated that liming can be used to increase rice yield and P uptake in the double rice cropping system, even though it reduces soil available P. However, we note that these results are from a system in which soil P availability was relatively high. Therefore, we suggest that future research should quantify the effects of liming in soils with a range in pH and available P levels (Barrow 2017). In addition, the effect of liming on soil P fractionation should be examined to clarify the mechanism underlying the decrease in soil P availability under lime application (Eslamian *et al.* 2020).

5. Conclusion

In a three-year experiment on an acidic paddy soil, we found that liming increased rice yield, biomass, and P uptake, but reduced soil P availability and the activity of acid phosphatase. Lime application did not significantly affect rice P concentrations. Therefore, we conclude that in the short term, liming promotes rice production and P uptake in acidic paddy soils as soil P availability is not the growth-limiting factor. However, further research is needed to investigate the long-term effect of liming on the use efficiency of inorganic P fertilizer and soil P pool dynamics.

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Appendices associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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