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Full Length Research Paper

The mechanism on rhizosphere phosphorus activation of two wheat genotypes with different phosphorus efficiency

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A short-term greenhouse experiment was carried out with two phosphorus (P) levels of purple soil to investigate P availability and associated processes in the rhizosphere of two different P-efficiency wheat genotypes using a thin slicing technique. Two genotypes with different P efficiencies were grown in a root-compartment experiment under low P (P 10: 10 P mg/kg) and high P (P 100: 100 P mg/kg) treatments. Results show that readily extracted forms of soil inorganic P were depleted by the two wheat genotypes, depletion zones extended further adjacent to 4 to 6 mm. Enhanced depletion of sodium hydroxide extractable organic P apparent in the rhizosphere of high P-efficient wheat cultivar 10098 compared with low P inefficient wheat cultivar 10026 was related to the presence of greater concentrations of microbial biomass and higher soil acid phosphatase and phosphodiesterase enzyme activities. These results confirm that microorganisms and soil enzymic activities played important roles in the mineralization of soil organic P, particularly under high P-efficient wheat cultivar 10098. These results suggest that improving P efficiency based on the character of P efficiency acquisition in P-efficient genotype would be a potential approach for maintaining wheat yield potential in soils with low P bioavailability.

Key words: Wheat, P efficiency, rhizosphere properties, P fractions, phosphates activity.

INTRODUCTION

Wheat is one of the most important crops in the world. However, its production is largely limited by the phosphorus (P) deficiency in many soils (Zhu et al., 2002). This fact particularly applies to soils with a high iron or aluminum oxide content, where P is strongly bound and largely unavailable for crop uptake (Ae et al., 1990). P fixation is a serious problem in many parts of the world as only a small fraction of the applied fertilizer P is usually taken by the crop; the rest is being fixed by the soil (Thung, 1990). Development of crop varieties that can efficiently utilize these fixed forms of soil P (mostly iron- and aluminum bound-P) would result in lower input, eco-friendly sustainable production systems for countries (Subbarao et al., 1997). Previous studies have shown that the phosphorus absorption, transport and utilization efficiency of different P-efficient wheat genotypes varied significantly (Ma et al., 1998; Wang et al., 2000). Therefore, screening and developing phosphorus-efficient wheat genotype can be a favorable way to ease China's lack of phosphate rock resources and increase phosphate utilization. It is necessary to clarify the different phosphorus efficiency mechanism of different wheat genotypes before the foresaid screening and development.

Previous studies have also shown that under phosphorus stress conditions, the P efficient genotype can take advantage of more insoluble soil phosphorus than the

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P inefficient genotype and its mechanism has been researched in-depth. Root-associated factors such as root morphology, architecture, root hair density, nutrient absorption rate, ability to modify the rhizosphere and mycorrhizal fungi symbiosis, root exudations by plant roots as organic acids and acid phosphatase can strongly influence inorganic phosphate (Pi) acquisition (Li and Zhang, 2001; Fohse et al., 1991; Schweiger et al., 2007; Ma et al., 2001; Shane and Lambers, 2005; Asmar et al., 1995). It has generally been accepted that P absorption by plants is influenced by the chemical and biochemical changes in the rhizosphere (Trolove et al., 2003). Plant derived organic constituents enhance microbial activity and thus influence nutrient transformations (especially phosphorus) in the rhizosphere (Rovira, 1991). However, genotypic differences in rhizosphere soil biological and chemical properties as a result of root activities remain poorly understood. Little information is available about the changes in chemical and biochemical properties in the rhizosphere of different wheat genotypes and their relationships with P depletion in soils.

Slicing technology using frozen samples, which scarcely alters the soil biological and chemical properties, has been used to study the variety of nutrients in soil slices taken certain distances from the root surface. Application of Hedley (Hedley et al., 1982a) fractionation in research of rhizosphere processes has given a further understanding of P form transformation resulting from root uptake. Previous studies showed that depletion of different forms of phosphorus varied greatly in different plant species rhizosphere, even in different genotypes of the same plant (Zhang et al., 2009; Zoysa et al., 1999; Chen et al., 2002). Base on the previous screening test, this present study selected two phosphorus efficiency wheat geno-types. By studying the dynamics of rhizosphere pH, root surface phosphatase and different forms of phosphorus (varied in the distance from the root surface), this research explored the relationship between wheat rhizo-sphere chemical and biology characteristics and the P use of two wheat genotypes, and provided scientific basis to further clarify the mechanism of wheat to adapt to phosphorus stress.

MATERIALS AND METHODS

Experimental soil and plant

The experiment was carried out in the glasshouse of Southwest University, Chongqing Province, China. The tested soil was collected from the topsoil base of purple fertility and fertilizer monitoring plots of China (0~20 cm). Soil organic matter content was 23.9 g/kg, total nitrogen content of 1.23 g/kg, total phosphate content of 0.24 g/kg and available phosphate content of 1.87 mg/kg (extracted by NH_4F-HCl). The soil had a pH in water of 6.1 (1:2.5; w/w soil to water ration). Air-dried soil samples were ground and passed through a 2 mm sieve then steam-sterilized for 3 h to minimize the effects of micro-organisms.

The test materials were relatively high phosphorus efficient wheat cultivar 10098 and phosphorus inefficient wheat cultivar 10026 screened from 150 cultivars. Field test results showed that the P accumulation and biomass of cultivar 10098 were both significantly higher than cultivar 10026. For the convenience of writing, high P-efficient wheat cultivar 10098 was expressed as HG, while low P inefficient wheat cultivar 10026 was expressed LG.

Experimental design and treatment

A rhizo-box (17 × 12 ×17 cm in length × width × depth) with three compartments was used to study the rhizosphere process. The rhizo-box consisted of three compartments, one root and two soil compartments. The soil compartments were separated from the root compartment by a 30-μM pore diameter polyester mesh. The middle compartment was 3 cm in width, while the two side compartment were 6 cm in width. The three compartments were filled with tested soil, and the soil bulk density was around 1.20 g/cm^3. The interface was sealed with glass sealant. Wheat seeds (cv. 10098 and 10026) were sterilized with 5% (w/v) NaOCl solution for 5 min and then thoroughly washed with distilled water. Nine seeds were sown in the middle compartment of the rhizo-box. With the growth of wheat root, root-soil interface zone was formed between the inside and outside chamber. By the method of water weighing, the soil remained at about 60% of field capacity. After 70 days, the box was opened and the wheat was harvested. Wheat root and over ground part were separated, dried, weighed, and then made into dry ground sample.

At harvest, the rhizo-box systems were dismantled, and the rhizosphere soil samples were obtained according to the method of George et al. (2002). In this method, 1-mm thick stainless steel pane was pushed into one side of the soil compartment, flush to the compartment edge and 1-mm thick soil slices were protruded successively from the other side of the soil compartment. By the aforementioned method, six layers of soil samples were collected respectively from 0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5 and 5 to 6 mm distance from the nylon mesh in the same soil layer of the both side of the box, and the same soil layer of both sides were consolidated to test soil phosphorus levels, soil acid phosphatase (AcPME) and phosphodiesterase (PDE) activity, pH, microbial biomass and other indicators. The test was set in two treatments, low amount of applied phosphorus 10 mg P/kg (P10) and high levels of applied phosphorus 100 mg P /kg (P100), with KH_2PO_4 as source of phosphorus. 150 mg N/kg and 150 mg K/kg were added as basal K fertilizer before planting, while adding a solution containing other nutrients, 25 mg/kg $MgSO_4 \cdot 7H_2O$; 2.86 mg/kg H_3BO_3; 1.81 mg/kg $MnCl_2 \cdot 4H_2O$; 0.22 mg/kg $ZnSO_4 \cdot 7H_2O$; 0.08 mg/kg $CuSO_4 \cdot 5H_2O$ and 0.22 mg/kg $(NH_4)_6MO_7O_2 \cdot 4H_2O$. Rhizobox systems without plants were also incubated in the experiment and used as controls, and repeated three times.

Soil and plant analyses

Soil pH was determined using a soil: water ratio 1:2.5. For all harvests, roots were excised from shoots, then roots and shoots were weighed, and after drying at 65°C for 36 h, dry matter was determined. In order to measure the P concentration herein, plant samples were wet digested in a mixture of HNO_3, $HClO_4$ and H_2SO_4 in the ratio of 7:2:1 and phosphorus was determined with the molybdate-vanadium method of Kitson and Mellon (1944).

Soil P fractionation by sequential extraction

The soil samples taken from the rhizo-boxes were used for P fractionation after plants were harvested. The soil P fractions of 0.5 g air-dried soil (sieved through a 2 mm mesh) were determined sequentially according to the procedure of Tiessen and Moir (1993)

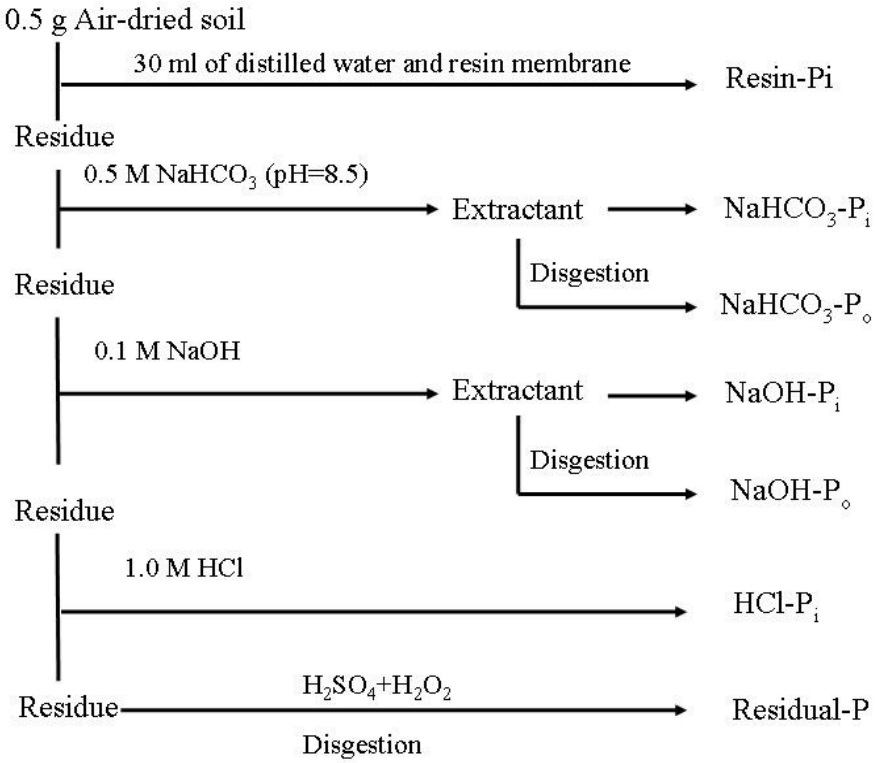


Figure 1. Soil P fractions sequentially.

based on the method of Hedley et al. (1982b), which uses progressively more aggressive extractants to remove P. Specific classification is shown in Figure 1. The content (mg/kg) of different P forms of the soil background before planting wheat were Resin-P_i 1.4, $NaHCO_3$-P_i 7.8, NaOH-P_i 35.1, HCl-P_i 12.5, $NaHCO_3$-P_o 5.1, NaOH-P_o 25.1, Residual-P 81.

Determination of acid phosphatase activity, microbial biomass and phosphodiesterase activity

Soil acid phosphatase activity was determined following the method of Shen et al. (2005) as modified by Hedley et al. (1982b). Samples of approximately 0.3 g soil (< 2 mm) were used with 4 ml extraction buffer composed of 40 mM NaOAc-HAc (pH 6.5), and 2 ml of 15 mM *p*-nitrophenol phosphate as substrate. After incubation at 37°C for 1 h, the reaction was stopped with 1 ml of 1 M NaOH and absorbance was measured spectrophotometrically at 400 nm. One unit of acid phosphatase activity was defined as the activity per gram soil that produced 1µmol *p*-nitrophenol/h.

Microbial biomass C (MBC) was measured using the fumigation-extraction method (Vance et al., 1987), except that 0.5 g soil (oven-dry equivalent) and 10 ml 0.5 M K_2SO_4 extractant were used. Acid phosphatase (AcPME) activities were measured using the method described by Adams (1992), and phosphodiesterase (PDE) activity was determined according to the method described by Browman and Tabatabai (1978) using 0.25 g soil.

Statistical analysis

All data were tested for normality and homogeneity of error variances prior to comparing means. One-way analysis of variance (ANOVA) was performed to test the effects of soil P fractions, acid phosphatase activity, pH and P uptake data.Tukey's honestly significant difference (HSD) test was used for post hoc multiple comparisons (α=0.05). All statistical analyses were performed using the SPSS statistical software package version 18.0 (SPSS inc., 2002).

RESULTS

Impact on biomass and phosphorus accumulation of different wheat genotypes

The plant uptake of phosphorus was indicated by phosphorus accumulation. The biomass production and P accumulation by the two wheat genotypes under the two P fertilizer treatments is given in Table 1. The rhizo-boxes experiment (Table 1) showed that the phosphorus accumulation of HG was more than LG. On the P10 treatment, both genotypes showed distinct P deficiency symptoms; they were small and stunted, and with darkish green old leaves, the latter was particularly evident in P-inefficient genotype. Moreover, both genotypes grew well under P100 treatment. In the P10 and P100 treatment, the HG P accumulation increased by 18.6, 20.7 and 1.5% compared to LG, in P10 conditions, the phosphorus accumulation differences of the two species reached a significant level. In high level phosphorus applied conditions, this difference narrowed. This showed that the absorption of phosphorus of HG was stronger than LG especially in l ow-phosphorus stress conditions. In low-

Table 1. Biomass production and P accumulation of two wheat genotypes under different P treatments.

Treatment	Genotype	Biomass production (g/pot FW)			Increase (%)	P accumulation (mg/pot)	Increase (%)
		Shoot	Root	Total			
P10	HG	8.77[a]	4.87[b]	13.64[b]	32.6[a]	26.2[b]	20.7[a]
	LG	7.65[b]	2.64[c]	10.29[c]	-	21.7[c]	-
P100	HG	8.83[a]	5.38[a]	14.21[a]	1.3[c]	35.2[a]	1.5[c]
	LG	8.72[a]	5.31[a]	14.03[a]	-	34.7[a]	-

Each value was the average of three replicates. The different letters in the same column indicate the significant difference at the 0.05 levels using Tukey's honestly significant difference (HSD) test.

phosphorus stress, HG can absorb more phosphorus or use phosphorus efficiently to meet photosynthesis and photosynthate transport in terms of phosphorus, resulting in a higher biomass.

Impact on rhizosphere soil pH of different wheat genotypes

pH is an important factor in the transformation and effectiveness of P. The rhizosphere pH changes of the two different phosphorus efficiency wheats are shown in Figure 2. It can be seen that the two wheat soil acidification was more obvious within the chamber. In both high and low phosphorus levels, HG rhizosphere soil pH was significantly lower than LG rhizosphere soil pH, which indicated that HG had higher rhizosphere soil acidification ability. In low-phosphorus conditions, HG and LG rhizosphere soil pH were lower than non-rhizosphere soil by 0.02 to 0.46 units. In high-phosphorus conditions, HG and LG rhizosphere soil pH were lower than non-rhizosphere soil by 0.01 to 0.58 units, the lower pH range of P efficient varieties were wider than phosphorus inefficient varieties, and the HG had stronger rhizosphere soil acidification ability.

Impact on different forms of soil phosphorus levels of different wheats rhizosphere

Resin-Pi and $NaHCO_3$-P_i, two forms of inorganic phosphorus, are effective phosphorus in plant. NaOH-P_i may be buffer library of $NaHCO_3$-P_i, when there are little phosphorus in soil solution, it can be absorbed and used by plants (Zoysa et al., 1999). Two varieties of wheat in low-phosphorus (P10) and high phosphorus (P100) conditions, NaOH-P_i and $NaHCO_3$-P_i suffered depletion in the rhizosphere of wheat. The depletion levels of Resin-P_i, $NaHCO_3$-P_i and NaOH-P_i of HG were higher than LG (Figure 3).

Furthermore, the different uses of NaOH-P_o and $NaHCO_3$-P_o of different wheat varieties reflected that different varieties had different abilities of organic phosphorus mineralization. Under P10 treatment, the lower contents of NaOH-P_o and $NaHCO_3$-P_o were observed in the depletion zones of HG than LG. While in P100 treatment, the lower content of NaOH-P_o and $NaHCO_3$-P_o were observed in the rhizosphere of HG than LG depletion zone of 0 to 1.5 mm area. It can be interpreted that the secretion amount of acid phosphatase in rhizosphere of two varieties were different, the activity of acid phosphatase secretion of HG rhizosphere was significantly higher than that of LG, which resulted in low levels of rhizosphere NaOH-P_o and $NaHCO_3$-P_o (Figure 3).The rhizosphere HCl-P_i and residual P_i content was not significantly different from the control, so the two forms of phosphorus cannot be absorbed by wheat.

Plant root and microbial activity mediated transformation of organic P in the soil (Magid et al., 1996). It is well known that root-derived organic carbon (C) stimulates the growth of microorganisms and increases microbial activity in the rhizosphere (Martin, 1983; Toal et al., 2000). The amount of root-derived C flow through the rhizosphere has a significant impact on transformations of soil organic P (Helal and Sauerbeck, 1989). In the present study, MBC accumulated in the rhizosphere of both HG and LG were significantly greater in the rhizosphere soil compared with bulk soil. MBC accumulation in the rhizosphere of HG was significantly greater in the rhizosphere compared with LG especially in the low level P soil (Figure 4).

Impact on rhizosphere soil acid phosphatase activity of different varieties of wheat

Rhizosphere soil phosphatase is of great significance to biochemical cycle of organic phosphorus. The measurement results of the impact of two varieties of wheat on rhizosphere soil acid phosphatase activity are shown in Figure 5, which showed that the rhizosphere soil acid phosphatase activity of two different wheat were significant. Phosphorus supply had obvious impact on the secretion of acid phosphatase of wheat rhizosphere; the acid phosphatase activities of the two wheat in low P soil were higher than that in high P soil. In the two phosphorus levels, the acid phosphatase activities of HG were

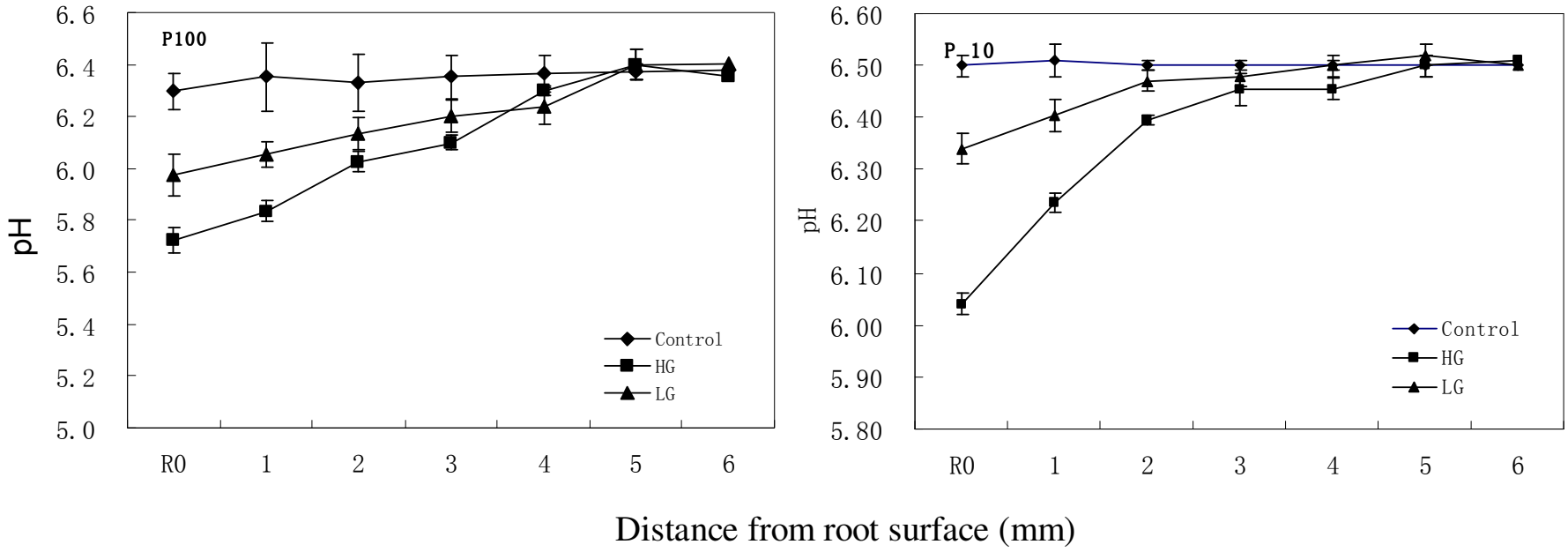


Figure 2. Development of pH in rhizosphere zones of wheat genotypes under P10 and P100 treatments after 70 days growth in a rhizobox experiment.

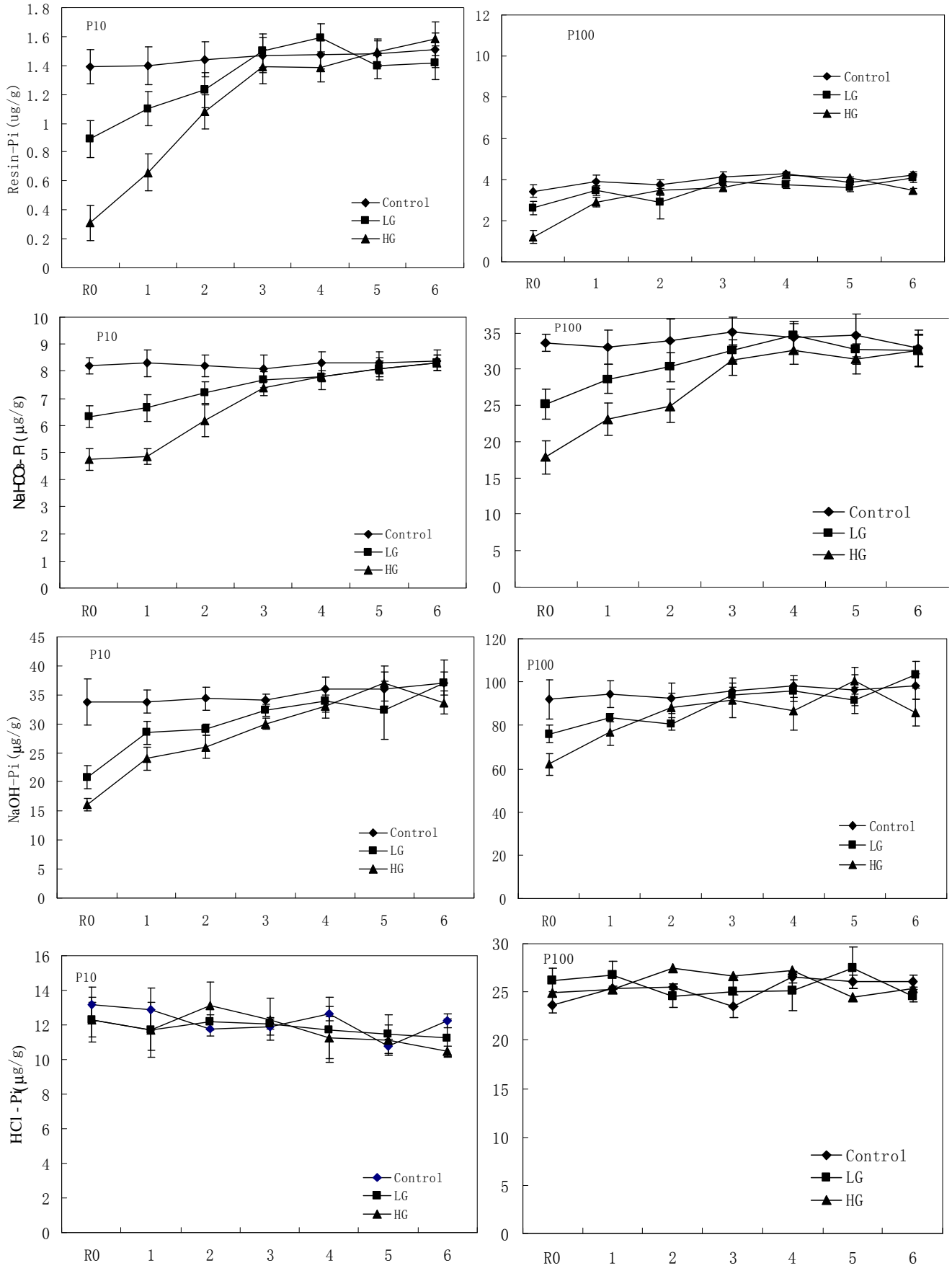
significantly higher than that of LG. In the same distance from the root surface, the acid phosphatase activities of low P soil were significantly higher than P100 treatment.

It has been suggested that both plant roots and microorganisms produce AcPME and PDE (Tarafdar and Claassen, 1988). It was found that AcPME and PDE activities were higher in the rhizosphere of HG and LG compared with bulk soil (Figures 5 and 6), which is consistent with the findings from several other studies (Tarafdar and Jungk, 1987; Asmar et al.,1995). Moreover, AcPME and PDE activities were directly related to concentrations of MBC in soils under HG ($r = 0.8546^* - 0.9863^{**}$, $n = 7$) and LG ($r = 0.8864^* - 0.9023^{**}$, $n = 7$). This confirms that these enzymes were at least partly of microbial origin. Therefore, increased root exudation in the rhizosphere of HG compared with LG may account for the enhanced levels of microbial biomass and enzyme activity and consequently greater depletion of soil P.

The relevance of rhizosphere acid phosphatase, phosphodiesterase activity and the organic phosphorus

It can be seen from Figure 7 in P10 conditions that the rhizosphere acid phosphatase activity of different phosphorus efficiency wheat was significantly negatively correlated with NaOH-P_o content. In other words, the stronger the activity of acid phosphatase was, the lower the organic phosphorus of NaOH-P_o was. However, in high P conditions, only the acid phosphatase activity of P efficient wheat was negatively correlated with rhizosphere NaOH-P_o, and that of the P inefficient was not related with rhizosphere NaOH-P_o (Figure 7). This also showed that in low-phosphorus conditions, acid phosphatase activity played a very important role in the mineralization of rhizosphere organic phosphorus. Similarly, for the two wheat varieties in low-phosphorus conditions, the rhizosphere phosphodiesterase activity were significantly negatively correlated with rhizosphere NaOH-P_o (Figure 8), which also showed that in low-phosphorus conditions, rhizosphere acid phosphatase activity also played a very important role in the mineralization of rhizosphere organic phosphorus.

Moreover, concentration of MBC was also negatively correlated with levels of NaOH-P_o especially in low P level soil under HG and LG (Figure 9). However, in high phosphorus soil conditions,


P10
P100
Control
LG
HG
Resin-Pi(ug/g)
NaHCO3-Pi(μg/g)
NaOH-Pi(μg/g)
HCl-Pi(μg/g)
R0
1
2
3
4
5
6

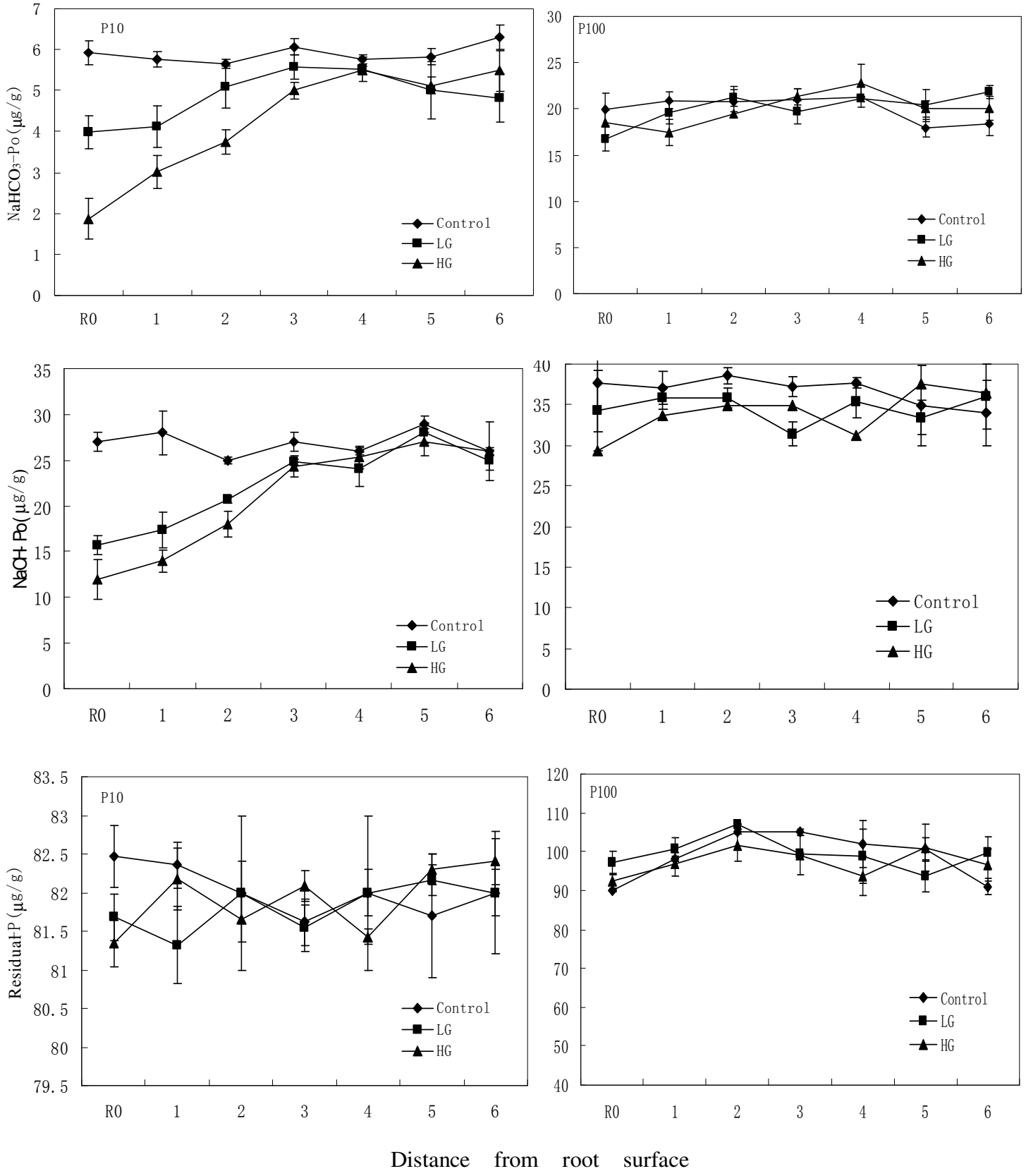


Figure 3. Phosphorus fractions in rhizosphere zones of two wheat genotypes, LG and HG, under P10 and P100 treatments after 70 days growth in a rhizo-box experiment. Control represents no plant.

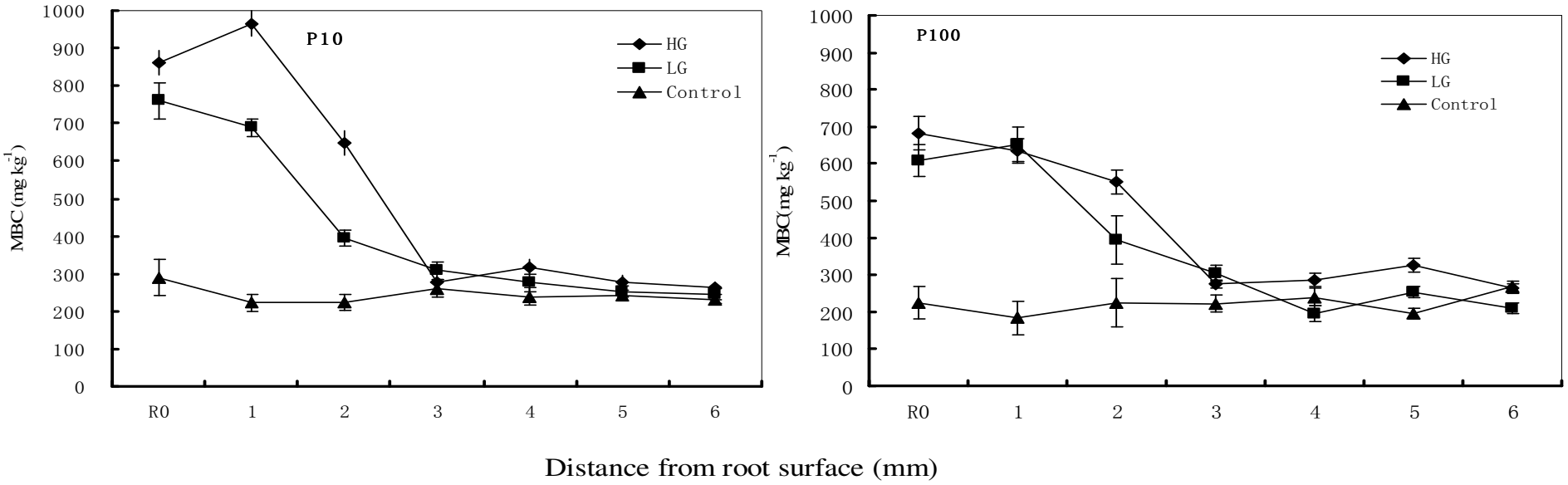


Figure 4. Effect of different P efficient wheat on microbial biomass C (MBC) determined in the rhizosphere of soil after 70 days (n=7).

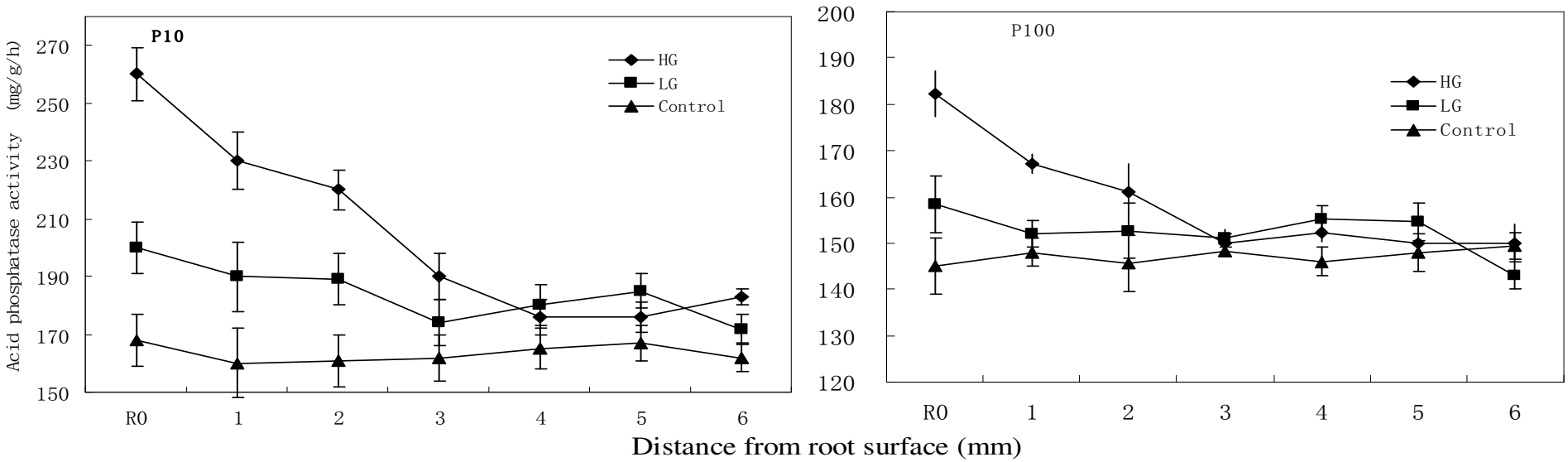


Figure 5. Development of soil Acid phosphatase activities (AcPME) in rhizosphere zones of wheat genotypes under P10 and P100 treatments after 70 days growth in a rhizo-box experiment.

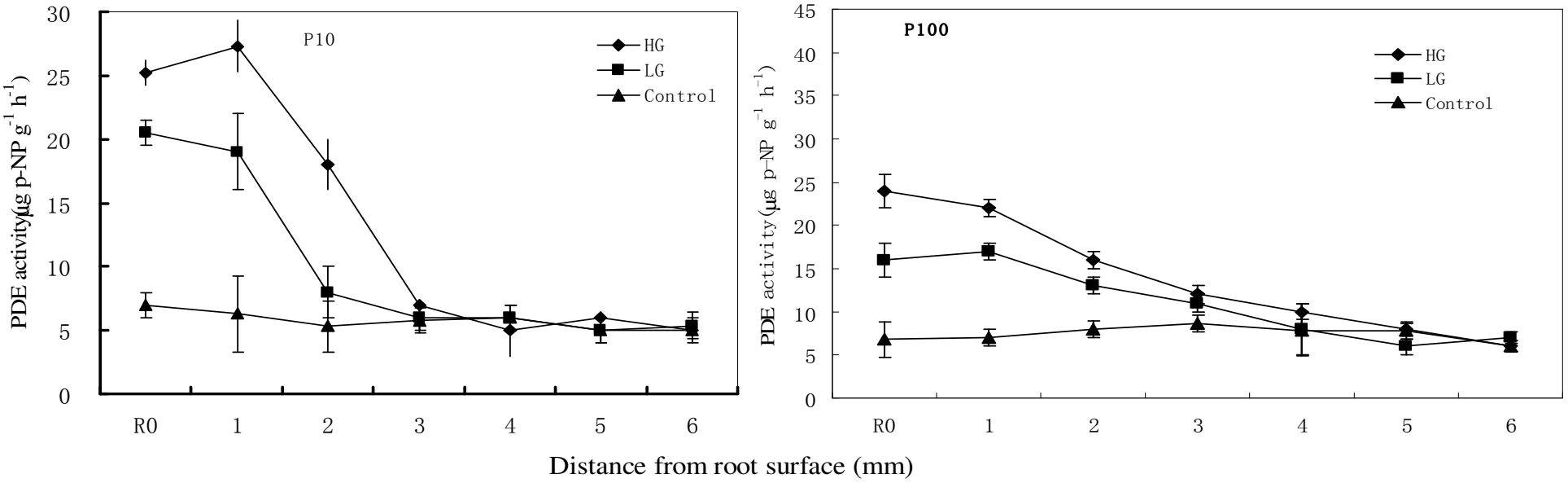


Figure 6. Development of soil phosphodiesterase activities (PDE) in rhizosphere zones of wheat genotypes under P10 and P100 treatments after 70 days growth in a rhizo-box experiment.

NaOH-P_o and rhizosphere MBC is not correlated. This tend to indicate that in addition to enhancing microbial activity in the rhizosphere, root exudation might improve solubility and consequent mineralization of organic P especially under low P level soil (Comerford and Skinner, 1989; Fox and Comerford, 1990).

DISCUSSION

The present study shows that HG had a greater P uptake than LG under low-P treatments and plus-P treatments (Table 1), suggesting that HG had a greater ability to take up P from either soil- or fertilizer-P, especially to low-P soil. More also, pH value is an important factor in the transformation of phosphorus forms (Dakora and Phillips, 2002). Our results show that the wheat rhizosphere pH was significantly lower than the control and regardless of high or low phosphorus conditions, HG rhizo-sphere pH was lower than LG. Hinsinger et al. (2003) studied that P uptake of plant was exponentially related with rhizosphere soil pH. Within a certain range, with the decline of rhizosphere pH, phosphorus absorption was significantly increased. The rhizosphere available phosphorus content were significantly lower in the P efficient genotypes than in the P inefficient genotypes, indicating that phosphorus efficient wheat genotype had a high activation and absorption capacity of the insoluble phosphorus in soil, and also that the decrease of rhizosphere pH was root-induced adaptive mechanisms of the phosphorus efficient wheat genotypes to soil phosphorus deficiency. Previous research showed that in P stress conditions, wheat roots released citric, malic, tartaric, benzoic and other organic acids, and these organic acids amount secreted by the roots were in positive relation with phosphorus concentration of rhizosphere soil solution (Bhattacharyya et al., 2003). Organic acid anions mobilize inorganic P by anion exchange, solubilization of Ca phosphates and chelation of Fe (Gerke, 1993) and hence release P from soil sparingly phosphate. It is therefore possible that the low-weight organic acids released by phosphorus efficient wheat was higher than phosphorus inefficient wheat, large amount secretion of small molecular organic acids can promote rhizosphere soil acidification and increase the amount of water-soluble phosphorus in soil, thereby increasing the P uptake and accumulation of HG, especially in the condition of low P soil, the acidification was important to the P absorption.

Cross's (1995) study showed that in the rhizosphere soil, available P was in a significant

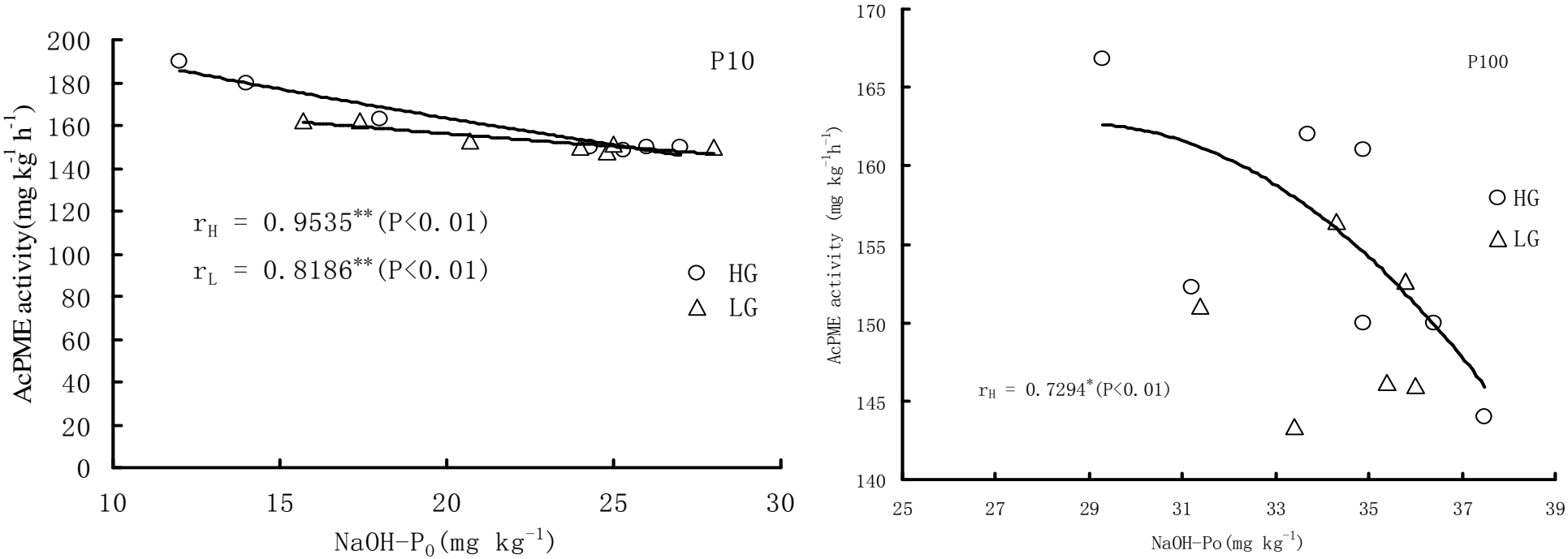


Figure 7. Relationship between phosphatase activity and NaOH-P_o status in the rhizosphere.

positive correlation with Resin-P_i, $NaHCO_3$-P_i and NaOH-P_i, indicating that the rhizosphere micro-domain, Resin-P_i, $NaHCO_3$-P_i and NaOH -P_i played an important role in the effectiveness of soil phosphorus (Cross and Schlesinger 1995). Previous experi-mental results proved this point, many plants such as tea and corn plants were observed that the three forms of P were in a serious deficit pheno-menon (Zoysa, 1999; Xue et al., 2010). The phosphorus deficiency of Resin-P, NaOH-P_i and H_2SO_4-P_i of high P uptake ability of tea variety TRI2023 were greater than S106. Therefore, the depletion of these three P fractions was easily observed in the rhizosphere of many plant species like Brassica, rice and maize. These three P fractions had a significant positive correlation with various growth parameters and P uptake by plants (Li et al., 2008; Zhang et al., 2009; Sharma and Subehia, 2003; Verma et al., 2005). The present results also showed that significant depletions of resin-Pi, $NaHCO_3$-Pi and NaOH-P_i occurred in the rhizosphere of both HG and LG under zero-P and Plus-P treatments. These three P fractions within soil solution may be absorbed by plant roots under P deficient condition, which resulted in a larger degree of depletion near the root zone. Results of soil phosphorus levels showed that in low-P and Plus-P treatments, the most important component of soil phosphorus was NaOH-P_i. More also, the deficit of NaOH-P_i was far greater than the other forms of phosphorus' deficiency (Figure 3). Therefore, it can be concluded that the P uptake of wheat was mainly due to the NaOH-P_i deficit of rhizosphere soil. In this trial, the deficit of NaOH-P_i in HG rhizosphere soil was higher than LG; this may be the main factors of HG's strong uptake capacity.

Rhizosphere HCl-P_i inorganic phosphorus of two varieties of wheat did not appear obvious deficiency phenomenon, but Sood and Bhardwaj (1992) reported that root exudates can activate HCl-P_i. The fact that HCl-P_i was not deficient may be related to different soil properties and wheat secretions. Residual-P mainly consisted of stable organic phosphorus. Compared with other forms

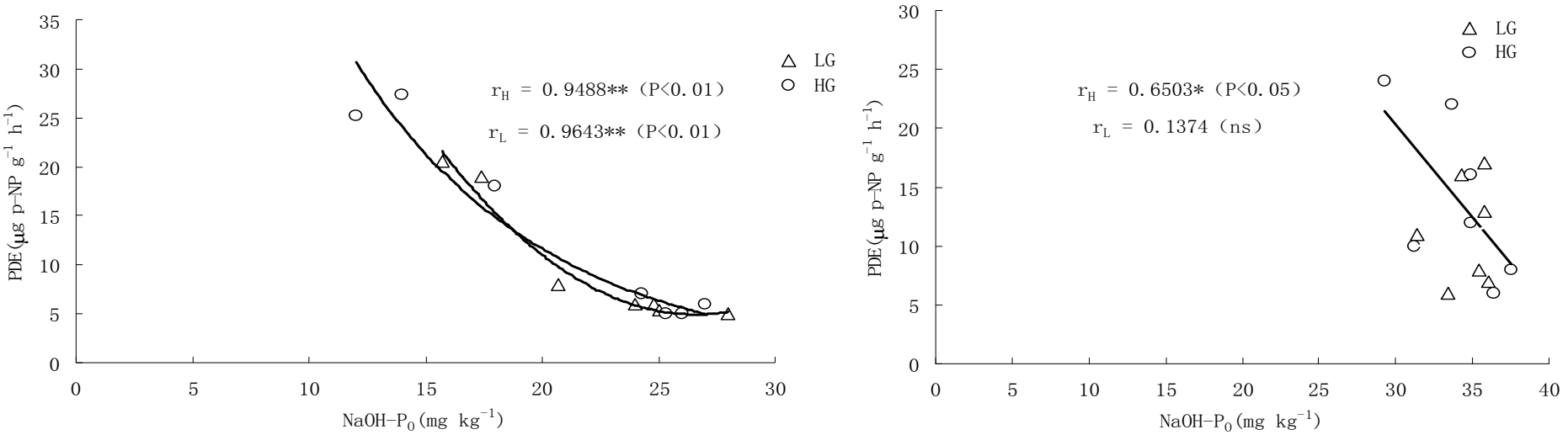


Figure 8. Relationship between PDE activity and NaOH-P_o status in the rhizosphere.

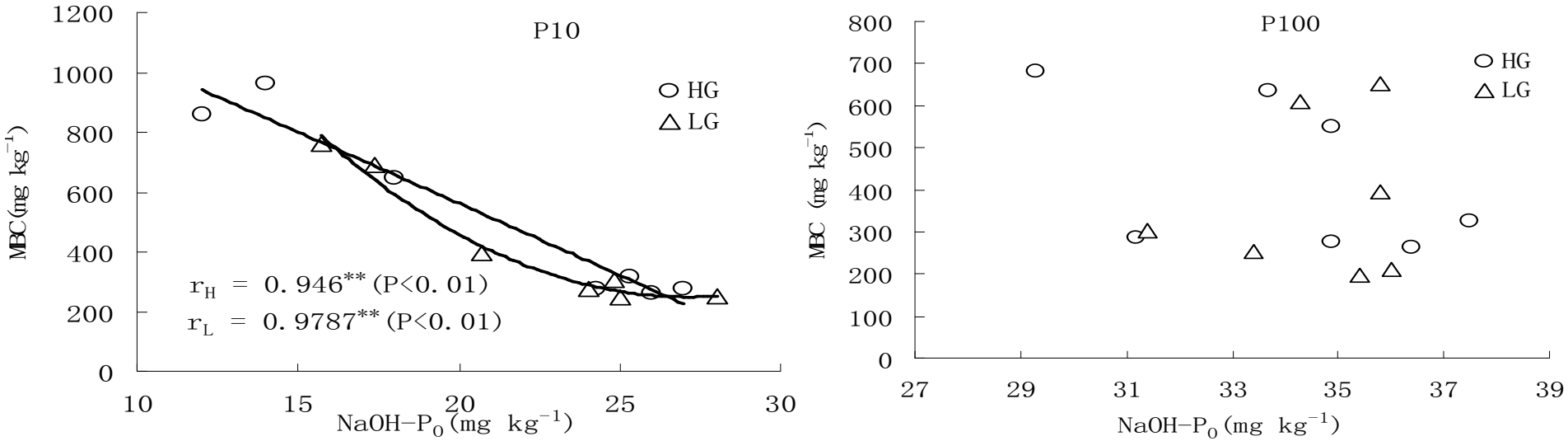


Figure 9. Relationships determined between concentrations of sodium hydroxide extractable organic P (NaOH-P_o) and microbial biomass C (MBC) and in the rhizosphere of HG and LG after 70 days (n=7; r_H and r_L are correlation coefficients for HG and LG, respectively).

of phosphorus, residual-P had minimum effectiveness on the plant (Lin et al., 2006). The test results also showed that regardless of phosphorus or not, residual-P of two wheat genotypes rhizosphere had no significant deficit phenomenon. It is possible that in short-term test period, it was difficult for the wheat to take advantage of this form of phosphorus, or residual-P was partially absorbed by the wheat, but during the experiment other forms of phosphorus quickly turned into residual-P, resulting in the stable content of the residual-P.

Soil organic phosphorus is an important component of soil phosphorus, which can be of 80% of total soil phosphorous. The importance of the mineralization of organic P compounds in regulating the supply of plant-available P has reported by Asmar et al. (2002). Plant root and microbial activity mediate transformation of organic P in the soil (Magid et al., 1996), and root-borne acid phosphatase enzyme together with fungal acid phosphatase are believed to be responsible for hydrolysis of organic P in the rhizosphere. Nuruzzaman et al. (2006) and Tarafdar and Jungk (1987) also reported that significant depletion of $NaHCO_3$-P_o and NaOH-P_o occurred in the rhizosphere, and the depletion depended on acid phosphatase activity. As shown in Figure 7, significantly negative correlation was observed between soil acid phosphatase activity and content of NaOH-P_o under P 10 and P100 by only HG, confirming the above conclusions.

It has been suggested that both plant roots and microorganisms produce AcPME and PDE (Tarafdar and Claassen, 1988). It was found that AcPME and PDE activities were higher in the rhizosphere of HG and LG compared with control soil, which is consistent with the findings from several other studies (Tarafdar and Jungk, 1987; Asmar et al., 1995). Moreover, AcPME and PDE activities were directly related to concentrations of MBC in soils under HG ($r = 0.876^* - 0.986^{**}$) and LG ($r = 0.854^* - 0.975^{**}$). This confirms that these enzymes were at least partly of microbial origin. Therefore, increased root exudation in the rhizosphere of HG compared with LG may account for the enhanced levels of microbial biomass and enzyme activity and consequent greater depletion of soil P. The study of Marschner et al. (2007) have shown that in low-phosphorus conditions, P uptake of wheat was in significant positive correlation with rhizosphere acid phosphatase activity. This test results also show that under the conditions of low phosphorus (P10), both HG and LG rhizosphere had a phenomenon of NaOH-P_o deficit, and the deficit degree of HG was higher than LG (Figure 3), indicating that the potential, transformable organic phosphorus reduced into useful inorganic phosphorus of plant, thus confirming that soil organic P is the important phosphorus for wheat. The AcPME and PDE activities in HG rhizosphere were greater compared with LG in high level and in low level P soils. Depletion of NaOH-P_o was significantly related to increased activities of AcPME and PDE activities except for high level P soil of LG. These findings suggest that phosphatase (particularly AcPME and PDE) activities play an important role in the mineralization of soil organic P in soils. Moreover, concentration of MBC was also negatively correlated with levels of NaOH-P_o in both soils under HG and LG. This tended to indicate that in addition to enhancing microbial activity in the rhizosphere, root exudation might improve solubility and consequent mineralization of organic P under two genotypes especially for HG (Comerford and Skinner, 1989). Previous study has suggested that low-molecular-weight organic acids dissolved Al (Fe)-organic P complexes by chelation, thus releasing organic P (Fox and Comerford, 1990). On the other hand, significant relationships were found between depletion of NaOH-P_o and phosphatase enzyme activities.

Conclusion

Based on the study conducted, the following conclusions were derived;

1. The soil acidification ability of P efficient genotype is stronger compared with P inefficient genotype, resulting in the dissolution of some rhizosphere insoluble phosphate, which is one of the major reasons why the P uptake of HG is significantly higher than that of LG.
2. For the two wheat genotypes, whether in low-P soil or high-P soil, four forms of phosphorus as $NaHCO_3$-Pi, $NaHCO_3$-P_o, NaOH-P_o and NaOH-P_i in soil has a significant deficit phenomenon; resin-P appears enrichment; and HCl-P_i and residual-Pi has no obvious deficit phenomenon. In comparison, $NaHCO_3$-Pi, NaHCO3-P_o, NaOH-P_o and NaOH-P_i of HG rhizosphere has a slightly higher deficit phenomenon than LG, which may be another reason for the higher P uptake ability of HG.
3. Higher concentrations of MBC and AcPME and PDE activities in the rhizosphere of HG are responsible for greater mineralization of soil organic P under HG compared with LG.
4. The short-term study of the rhizosphere can quickly acquire different information of P utilization efficiency of different varieties.

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REFERENCES

Adams MA (1992). Phosphatase activity and phosphorus fractions in Karri (Eucalyptus diversicolor F. Muell.) forest soils. Biol. Fert. Soils.

14(3): 200-204.
Ae N, Arihara J, Okada K, Yoshihara T, Johansen C (1990). Phosorus uptake by pigeonpea and its role in cropping systems of the Indian subcontinent. Science, 248: 477-480.
Asmar F, Gahoonia TS, Nielsen NE (1995). Barley genotypes differ in activity of soluble extracellular phosphatase and depletion of organic phosphorus in the rhizosphere soil. Plant Soil, 172(1): 117-122.
Browman MG, Tabatabai MA (1978). Phosphodiesterase activity of soils. Soil Sci Soc. Am J. 42: 284-290.
Chen CR, Condron LM, Davis MR, Sherlock RR (2002). Phosphorus dynamics in the rhizosphere of perennial ryegrass(Lolium Perenne L.) and radiate pin(Pinus radiate D. Don) . Soil Biol. Biochem. 34: 487-499.
Comerford NB, Skinner MF (1989). Residual phosphorus solubility for an acid, clayey, forested soil in the presence of oxalate and citrate. Can J. Soil Sci. 69: 111-117.
Cross AF, Schlesinger WH (1995). A literature review and evaluation of the Hedley fractionation: applications to the biogechemical cycle of soil phosphorus in natural ecosystems. Geoderma, 64: 197-214.
Dakora FD, Phillips DA (2002). Root exudates as mediators of mineral acquisition in low nutrient environments. Plant Soil, 245: 35-47.
Fohse DN, Claassen, Jungk A (1991). Phosphorus efficiency of plants. Ⅱ. Significance of root radius, root hairs and cation anion balance for phosphorus influx in sever plant species. Plant Soil. 132: 261-272.
Fox TR, Comerford N B (1990). Low-molecular-weight organic acids in selected forset soils of the Southeastern USA. Soil Sci. Soc. Am J. 54: 1139-1144.
George TS, Gregory PJ, Robinson JS, Buresh RJ (2002). Changes in phosphorus concentrations and pH in the rhizosphere of some agroforestry and crop species. Plant Soil. 246: 65-73.
Gerke J (1993). Solubilization of Fe (Ⅲ) from humic-Fe complexes, humic/Fe-oxide mixtures and from poorly ordered Fe-oxide by organic acids-consequences for P adsorption. J. Plant Nutr. Soil Sci. 156: 253-257.
Hedley MJ, Stewart JWB, Chauhan BS (1982a). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46: 970-976.
Hedley MJ, White RE, Nye PH (1982b). Plant induced changes in the rhizosphere of rape (Brassica napus var. Emerald) seedlings. III Changes in L value, soil phosphate fractions and phosphatase activity. New Phytol. 91: 45-56.
Helal HM, Sauerbeck DR (1989). Carbon turnover in the rhizosphere. J. Plant Nutr. Soil Sci. 152: 211-216.
Hinsinger P, Plassard C, Tang CX, Jaillard B (2003). Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil, 248: 43–59.
Kitson RE, Mellon MG (1944). Colorimetric determination of phosphorus as molybovanadophosphoric acid. Ind. Eng. Chem. Anal. Ed. 16: 379-383.
Li H, Zhang XM (2001). Responses of different genotypic wheat seedlings to phosphorus and water deficiency. (In Chinese). Agric. Res. Arid Areas, 1(19):72-78.
Li YF, Luo AC, Wei XH, Yao XG (2008). Changes in phosphorus fraction, pH, and Phosphatase activity in rhizosphere of two rice genotypes. Pedosphere, 18(6): 785-794.
Lin DX, Fan XH, Hu F Yang LZ, Han XZ (2006). Forms of phosphorus in hapli-udic isohumosols soils after long-term fertilization. (In Chinese). Acta Pedologica Sinica,43(4): 605-610.
Ma J, Gahoonia TS, Nielsen NE (1998). Phosphorus uptake from rhizosphere soil by two wheat cultivars. Pedosphere. 8(4): 325-330.
Ma Z, Bielenberg DG, Brown KM, Lynch JP (2001). Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. Plant Cell Environ. 24: 459-467.
Magid J, Tiessen H, Condron LM (1996). Dynamics of organic phosphorus in soil natural and agricultural ecosystem. In: Piccolo A. (Ed.). Humic Substances in Terrestrial Ecosystems. Elsevier. Amsterdam. pp. 429-466.
Marschner P, Solaiman Z, Rengel Z (2007). Brassica genotypes differ in growth, phosphorus uptake and rhizosphere properties under P-limiting conditions. Soil Biol. Biochem. 39: 87-89.
Martin JK (1983). Biology of the rhizosphere. Soils: An Australian Viewpoint. CSIRO/Academic Press. Melbourne. pp. 685-692.
Nuruzzaman M, Lambers H, Bolland MDA, Veneklaas EJ (2006). Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant Soil. 281: 109-120.
Rovira AD (1991). Rhizosphere research-85 years of progress and frustration. In: Keister DL, Cregan PB (Eds.). The rhizosphere and plant growth. Kluwer academic publishers. Dordrecht. pp. 3-13.
Schweiger PF, Robson AD, Brarrow NJ, Abbott LK (2007). Arbuscular mycorrhizal fungi from three genera induce two-phase plant growth responses on a high P-fixing soil. Plant Soil, 292: 181-192.
Shane MW, Lambers H (2005). Cluster roots: a curiosity in context. Plant Soil, 274: 101-125.
Sharma SP, Subehia SK (2003). Effects of twenty-five years of fertilizer use on maize and wheat yields and quality of an acidic soil in the western Himalayas. Exp Agric. 39: 55-64.
Shen JB, Li HG, Neumann G, Zhang FS (2005). Nutrient uptake, cluster root formation and exudation of protons and citrate in Lupinus albus as affected by localized supply of phosphorus in a split-root system. Plant Sci.168: 837-845.
Sood RD, Bhardwaj RK (1992). Effect of liming and phosphorus fertilization on the P fractions in an acid Alfisol. J. Indian Soc. Soil Sci. 40: 299-301.
Subbarao GV, Ae N, Otani T (1997). Genotypic variation in iron-, aluminum- phosphate solubilizing activity of the pigeonopea root exudates under P deficient conditions. Soil Sci. Plant Nutr. 43: 295-305.
Tarafdar JC, Claassen N (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol. Fertil. Soil, 5: 308-312.
Tarafdar JC, Jungk A (1987). Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertil. Soils 3: 199-204.
Thung M (1990). Phosphorus: A limiting nutrient in bean (Phaseolus vulgaris L.) production in Latin America and field screening for efficiency and response. In Genetic Aspects of Plant Mineral Nutrition, Ed. N.EI. Bassam, M. Dambrote and BC Loughman, Kluwer Academic Publisher. The Dordrecht. pp. 501-521.
Tiessen H, Moir (1993). Characterization of available P by sequential extraction. In: Carter MR (ed) Soil sampling and methods of analysis. Canadian Society of Soil Science. CRC. Lewis. Boca Raton. FL. pp. 75-86.
Toal ME, Yeomans C, Killham K, Meharg AA (2000). A review of rhizosphere carbon flow modelling. Plant Soil. 222: 263-281.
Trolove SN, Hedley MJ, Kirk GJD, Bolan NS, Loganathan P (2003). Progress in selected areas of rhizosphere research on P acquisition. Aust. J. Soil Res. 41(3): 471-499.
Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19: 703-707.
Verma S, Subehia SK, Sharma SP (2005). Phosphorus fractions in an acid soil continuously fertilized with mineral and organic fertilizers. Biol. Fertil. Soil, 41:259-300.
Wang QR, Li JS, Li ZS (2000). Adapted responses in the rhizosphere of P-efficient wheat genotype to stress of phosphorus deficiency. (In Chinese). Acta. Bot. Boreal. Occident Sin. 20(1): 1-7
Xue ZY, Zhou ZY, Zhan YY, Ren W (2010). Changing characteristics of phosphorus in the rhizosphere soil of the xeromorphic shrubs in arid deserts. (In Chinese) Acta Ecologica Sinica 30(2): 341-349.
Zhang HW, Huang Y, Ye XS, Shi L, Xu FS (2009). Genotypic differences in phosphorus acquisition and the rhizosphere properties of Brassica napus in response to low phosphorus stress. Plant Soil, 320: 91-102.
Zhu YG, Howes NK, Smith SE (2002). Phosphorus uptake and utilisation efficiencies of different wheat cultivars based on a sand-culture screening system. Pedosphere, 12(4): 329-337.
Zoysa AKN, Loganathan P, Hedley MJ (1999). Phosphorus utilisation efficiency and depletion of phosphate fraction in the rhizosphere of three tea (Camellia sinensis L.) clones. Nutr. Cycl. Agroecosys. 53: 189-201.