

1 **Distribution of inorganic phosphorus in profiles and particle-size fractions across**  
2 **an established riparian buffer and adjacent cropped area at the Dian lake**

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6

7 Abstract

8 Riparian buffer can trap sediment and nutrients sourced from upper cropland and  
9 minimizing eutrophication risk of water quality. This study aimed to investigate the  
10 distributions of soil inorganic phosphorus (Pi) forms among profile and particle-size  
11 fractions in an established riparian buffer and adjacent cropped area at the Dian lake,  
12 Southwestern China. The Ca-bound fraction (62%) was the major proportion of the Pi in  
13 the riparian soils. Buffer rehabilitation from cropped area had a limited impact on total  
14 phosphorus (TP) concentrations after 3 years, but has contributed to a change in Pi forms.  
15 At 0-20 cm soil layer, levels of the Olsen-P, nonoccluded, Ca-bound and total Pi were  
16 lower in the buffer than the cropped area; however, the Pi distribution between the  
17 cropped area and the buffer did not differ significantly as depth increased. The clay  
18 fraction corresponded to 57% of TP and seemed to be both a sink for highly recalcitrant  
19 Pi and a source for labile Pi. The lower concentration of Pi forms in the silt and sand  
20 particle fraction in the surface soil was observed in the buffer area, which indicating that  
21 the Pi distribution in coarse particle fraction has sensitively responded to land-use  
22 changes.

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24 separation; Dian lake

25

## 26 **1 Introduction**

27 Eutrophication of surface water has been linked to runoff of excess nutrients from  
28 agricultural soils in many parts of the world. The improper management or use of  
29 phosphorus (P) fertilizer during cultivation enhances the P transport affecting the water  
30 quality, leading to alterations in water ecosystems (Sharpley *et al.* 2003; Troitiño *et al.*  
31 2008). Riparian buffer is an efficient and economical tool to reduce agricultural non-point  
32 source pollution (Correll, 2000). Because of the filtering function of soil, the effectiveness  
33 of riparian buffers in reducing sediment and nutrient loading in surface and subsurface  
34 flows from cropland has been shown across many geographic regions (Dosskey *et al.*,  
35 2010; Keesstra *et al.* 2012).

36 In the basin of the Dian lake, Southwestern China, agriculture development from  
37 conventional field crop to intensive horticultural crop has occurred for more than 20  
38 years. Due to severe eutrophication of the Dian lake, a conservation program of Dian lake  
39 was established in 2009. Since its inception, thousands of hectare of buffers have been  
40 established by converting strips of cropland around the lake riparian zones to permanent  
41 vegetation. It is critical to know how the change of land-use affects soil P and its various  
42 forms which in turn affect its potential as a pollutant.

43 Much experimental evidence from research on both arable and buffer soils indicates that  
44 P are transported from soil to water with eroded soil (Abrams and Jarrell, 1995)

45 Minimizing the risk of P enrichment of surface water bodies with P transported from  
46 agricultural soils will require specific attention to forms of P in soils. Mooer and Reddy



47 (1994) reported that soil inorganic P may represent a greater risk for eutrophication than  
48 the organic forms of P. P distribution in chemical fractions can vary among land uses.  
49 Chalmers *et al.*(2001) observed only a slight increase in extractable P in buffer area as  
50 compared with crop field after 3-5 years set-aside period. Schroeder and Kovar (2006)  
51 reported that levels of the iron (Fe)-P fractions were higher in the buffer area than crop  
52 field over a 20-year period.

53 The chemical fractions of inorganic P include iron (Fe)-P, aluminum (Al)-P, calcium  
54 (Ca)-P, and occluded P according to Chang and Jackson (1957) fractionation procedure.

55 Kuo (1996) developed a sequential extraction technique for separating soil inorganic P  
56 into various forms, which integrated several fractionation procedures. Application of the  
57 Kuo fractionation to particle-size fractions can achieve better understanding on Pi  
58 transformations because of soil texture is an essential factor dominating soil P dynamics  
59 (Christensen, 1992).

60 In the present study we applied the Kuo fractionation to whole soil samples and particle-  
61 size fractions of different land uses. The objective of this study was to identify the  
62 transformations of the inorganic P forms in the plateau lake riparian anthrosols after land-  
63 use changes from cropped area to vegetation buffer. This will allow us to sustainably  
64 manage these systems within the landscape and minimize non-point source pollution.

## 65 **2 Materials and methods**

### 66 *2.1 Site description*

67 The study area is located on the southeastern basin of the Dian lake in the mid-Yunnan  
68 Plateau (24°42'-46'N, 102°41'-43'E), China, which is the last large agricultural  
69 production area in the basin of the Dian lake. This semi-humid region within the sub-  
70 tropical climatic zone is characterized by a dry winter and a moist summer. Mean annual

71 air temperature in the area is 14.7 °C and mean annual rainfall is 953 mm, of which 80%  
72 falls between June and October. The soil at the site is a paddy soil (Soil Taxonomy:  
73 Anthrosols, according to IUSS Working Group WRB, 2014). The cropped area has been  
74 in vegetable cropping (4-6 harvests per year) in plastic greenhouse for more than 20 years.  
75 After each harvest in a year, the field is rotary plowed to a 0.15-m-depth. A large amount  
76 of chemical fertilizer and animal manure is applied in those production area, resulting in  
77 P application of approximately 136-195 kg/ha in each year. The buffer area, which is  
78 approximately 100-200 m wide, was converted from the cropped area in 2009 and planted  
79 indigenous tree plantation (*Salix babylonica L.* and *Metasequoia glyptostroboides*).

## 80 2.2 Soil sampling

81 All the soils in this study were collected in the Niulian (NL), Haigeng (HG) and Xinglong  
82 (XL) sites which are located at the southern part of the basin of the Dian lake in 2012  
83 (Figure1). In the drought season, the shallow ground water levels were about 0.3 m, 0.7  
84 m and 1.2 m deep in the buffer at NL, HG and XL, whereas the shallow ground water  
85 levels were about 0.1 m, 0.4 m and 1.0 m deep at NL, HG and XL in the rainy season,  
86 respectively. Because of drainage, the shallow ground water levels were below 1.0 m  
87 deep in the cropped area both at NL and HG. Soils were collected during April and May  
88 of 2012 to a depth of 100 cm at 20 cm- interval. At each site five cores were collected  
89 from randomly selected locations across a 50- by 50-m area of both the buffer and the  
90 cropped area with a soil drill. There were 15 cores samples of soil profiles for buffer and  
91 cropped area respectively. All soil samples were air dried, passed through a 2 mm sieve,  
92 and stored at 20°C.

## 93 2.3 Laboratory analyses

94 Soil subsamples were ground with a mortar and pestle prior to total soil nutrient analysis.  
95 Subsamples were analyzed for pH (soil:water = 1:2.5); Soil organic carbon and total  
96 nitrogen were determined by the oil-bath potassium dichromate ( $K_2Cr_2O_7$ ) titration  
97 method and the macro-Kjeldahl method (Shi, 1998). Particle size distribution of soil was  
98 determined by the hydrometer method (Gee and Bauder, 1986). Particle density of soil  
99 ( $Mg/m^3$ ) was measured using the liquid pycnometer method, with desired water as the  
100 displacing liquid (Blake and Hartge, 1986). All the measurements were triplicated.  
101 The particle-size fractionation followed a fractionation procedure of Christensen (1992)  
102 and Neufeldt et al. (2000). 50 g of soil in 100 ml deionised water was intensely shaken  
103 for 3 h to disaggregate the coarse-sand fraction. The coarse sand ( $> 250 \mu m$ ) was obtained  
104 by passing the suspension through a  $250 \mu m$  sieve, and dried at  $40^\circ C$ . The remaining  
105 suspension was exposed to  $1500 J/ml$  ultrasonic energy using a probe-type disintegrator.  
106 The clay fraction ( $< 2 \mu m$ ) was freeze-dried after separating it from the fine sand and the  
107 silt by repeated centrifugation until the supernatant was clear. The fine sand ( $250-50 \mu m$ )  
108 was obtained by passing through a  $50 \mu m$  sieve, and dried at  $40^\circ C$ , whereas the  
109 remaining silt ( $2-50 \mu m$ ) in the remaining suspension was freeze-dried. Due to the low  
110 recovery of sand fractions, it was necessary to combine coarse-sand and fine-sand  
111 fractions to give one sample for chemical analyses.  
112 Total soil P was determined using wet oxidation (Shi, 1998). Olsen P was determined by  
113 adding 20 ml of 0.5 M  $NaHCO_3$ , pH 8.5 extractant to 1.00 g of soil, shaking for 30 min,  
114 and filtering through Whatman 42 filter paper (Kuo, 1996). Inorganic P fractions were  
115 conducted by Kuo fractionation schemes (Zhang and Kovar, 2000). A 0.5 g soil sample  
116 was placed in a 50 ml centrifuge tube and sequentially extracted with 25 ml each of 1.0  
117 M  $NH_4Cl$ , 0.5 M  $NH_4F$  (loosely bound  $P_i$ ), and 0.1 M  $NaOH$  (non-occluded  $P_i$ ) in that

118 order. Each extraction was performed for 0.1, 1, and 17 h using a horizontal shaker  
119 followed by centrifugation at 3300 rpm for 15 min, respectively. The occluded Pi was  
120 subsequently extracted by adding 20 ml of 0.3 M  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ , 2.5 ml of 1 M  $\text{NaHCO}_3$ ,  
121 and 0.5 g  $\text{Na}_2\text{S}_2\text{O}_4$  to the residue in each tube and heating for 15 min at 85°C, followed  
122 by centrifugation at 3300 rpm for 15 min. The calcium-bound Pi was subsequently  
123 extracted by adding 25 ml of 0.25 M  $\text{H}_2\text{SO}_4$  followed by centrifugation at 3300 rpm for  
124 15 min. All the measurements were triplicated.

#### 125 *2.4 Statistical analysis*


126 All data was submitted to analysis of variance (ANOVA) and treatment means were  
127 detected using Fisher's LSD and Duncan's Multiple Range Tests. Statistical analyses  
128 were performed with the PASW Statistics 17.0.2 for Windows (SPSS Inc., 2009).

### 129 **3 Results and discussion**

#### 130 *3.1 Land-use effects on soil physicochemical properties*


131 Summarized results from particle size analysis are presented in Figure 2. The soils in the  
132 Xinglong (XL) and Haigeng (HG) sites have less clay than those at the Niulian (NL) sites.  
133 Soil textures under the buffer and crop fields were determined as a clay in all soil depths  
134 at the NL sites, whereas a clay loam both at the XL and HG sites, according to soil texture  
135 classification of USDA system. The higher percentages of sand in the buffer upper soils  
136 (0-40 cm) at the NL and HG sites indicated that the buffer has acted as a sink for coarse  
137 sediments but not for finer particles.

138 Soil organic carbon (SOC) and nitrogen (N) concentrations at the NL sites were  
139 significantly higher than the HG and XL sites (Figure 3). This was probably related to the  
140 shallower groundwater table and higher clay content of the soils (Parton *et al.*, 1987).  
141 Organic carbon and nitrogen concentrations in the buffer soils (0-100 cm at the NL sites

142 and 0-20 cm at the HG and XL sites) were significantly higher than in the cropped soils  
143 (Figure 3). The greater accumulation of plant debris by the revegetation and the slower  
144 rate of organic matter decomposition by the lack of cultivation might have contributed to  
145 the build up of soil organic carbon (SOC) in the buffer soils (Reddy *et al.*, 2008). There  
146 was a significant relationship between SOC and N at the research area. Several studies   
147 have indicated that the rate of SOC accumulation is controlled by the rate of N  
148 accumulation in the early stages of vegetation regeneration of abandoned agricultural  
149 soils (Knops *et al.*, 2000; Poulton *et al.*, 2003). Differences of SOC and N between the  
150 crop and the buffer soils were less significant as depth increased at the HG and XL sites  
151 but at the NL sites. The organic-rich subsurface soil in the buffer area at the NL sites may  
152 been due to the buried river channel sediment or peat.

153 The higher pH of surface soil (0-20 cm) in the cropped area reflect the regular fertilization  
154 increasing salt concentration in the soil solution (Godsey *et al.*, 2007). In addition, the pH  
155 of the NL soils was 0.2 to 0.5 higher than the pH of the XL and HG soils. Compared as  
156 the cropped soils, the lower pH of subsurface buffer soils in the NL sites may be partly  
157 explained as the higher soil organic matter which can be a potential source of soil acidity  
158 (Coleman and Thomas, 1967). Soil particle density ( $\rho_d$ ) of the NL sites was significantly  
159 lower than that of the XL and HG sites.

### 160 *3.2 Land-use effects on P fractions in whole soil samples*

161 Fertilizer application has a significant effect on increasing the available P forms in the   
162 cropped soils (Figure 4). Comparison of Olsen-P levels in the crop and buffer soils  
163 indicated that higher amount of available P (99.3 mg/kg) was accumulated in topsoil (0-  
164 20 cm) in crop areas. The higher extractable P in the cropped soil implicated that it would  
165 be more easily depleted by movement from the soil surface by erosion and leaching

166 (Sharpley *et al.* 2003). Also, non-occluded, Ca-bound and total inorganic P (Pi) levels at  
167 0-20 cm layer were higher in the cropped soil than in the buffer soil. This may be due to  
168 the regular fertilization in surface soil for crop growth. Sharply and Smith (1985) and  
169 Negassa *et al.* (2009) also reported amounts of soil Pi increased with fertilizer application,  
170 with non-occluded and Ca-bound Pi constituting the major proportion of the change.  
171 Neufeldt *et al.* (2000) reported that fertilizer P has also been sorbed in a highly recalcitrant  
172 form and suggested that all P fractions are linked. No significant difference in loosely-  
173 bound and occluded Pi and total P distribution between these crop and buffer soils was  
174 observed in this study. However, Sharply and Smith (1985) reported that cultivated soils  
175 had lower occluded Pi as compared with their virgin analogues. Cooper and Gilliam  
176 (1987) reported that topsoil total P concentrations has significantly increased in riparian  
177 areas as compared with an adjacent cropland over a 20-year period.  
178 Furthermore, Olsen-P, Pi fractions and TP levels were higher in the 0-20 cm samples  
179 compared to other sampling depths in both areas, suggesting that P was stratified in both  
180 areas. Within each sampling site (crop or buffer soil), TP concentrations decreased as  
181 sampling depth increased. Loosely-bound, non-occluded, occluded and total Pi were  
182 differed significantly in the 0-40 cm depth but not below the 40 cm depth in these soils.  
183 Additionally, the percentage of loosely-bound, non-occluded Pi also decreased as  
184 sampling depth increased except occluded and Ca-bound Pi (Figure 4).  
185 Distribution of total Pi in the soils was, on the average, 5% loosely-bound, 9% non-  
186 occluded, 24% occluded and 62% Ca-bound. High levels of Ca-P in these soils suggested  
187 that at least some of the soil's alluvial parent material probably originated from the  
188 limestone bedrock in the area above the catchment of the Dian lake (Schroeder and Kovar,  
189 2006). Except for the occluded Pi in 0-20cm layer, the percentages of Pi fractions did not



190 differ significantly between the cropped and the buffer soils. Compared with the cropped  
191 area topsoils (0-20 cm), the higher percentages of occluded Pi was found in the buffer  
192 topsoils (Table 1). In general, this observation suggests the increasing possibility of  
193 occlusion of P with Fe/Al hydrous oxides or formation of insoluble Al/Fe phosphates in  
194 buffer soils relative to cropped soils.

195 Although subsoil Ca-P (below 0-20 cm) alone did not differ among those areas, the others  
196 Pi fractions were greater in the XL soils than in the HG and NL soils (Figure 4). The NL  
197 soil had the lowest loosely-bound, non-occluded, occluded and total Pi (Figure 4).  
198 However, total P concentration did not differ significantly among those areas. Soil TP  
199 concentration was positively correlated with clay content ( $r=0.508$ ,  $n=30$ ,  $P<0.01$ ).  
200 However, there was not a significant relationship between Pi fractions and clay content.  
201 Occluded Pi was negatively correlated with soil pH ( $r=-0.621$ ,  $n=30$ ,  $P<0.01$ ).  
202 Furthermore, there were strong correlations among the P fractions.

### 203 *3.3 Land-use effects on inorganic phosphorus fractions in particle-size fractions*

204 Although the error of individual fractions was up to 12%, the sum of all Pi fractions in  
205 the particle-size fractions was similar to Pi in the whole soil (Table 2). In these soils, the  
206 proportion of P froms decreased continuously with increasing particle size of the  
207 fractions. The high proportion of P froms in the clay fraction reflected that the clay  
208 fraction not only seemed to be a sink for highly recalcitrant Pi but also a source for labile  
209 Pi forms (Neufeldt et al., 2000; Suñer and Galantini, 2015). The concentrations of Pi  
210 forms in the sand fraction were both significantly lower than in the clay and silt fraction,  
211 whereas the concentrations of Pi forms did not differ significantly between the clay and  
212 silt fraction. The higher concentration of TP in the clay fraction could be attributed to the  
213 enrichment of organic phosphorus in the finer particle size class. Christensen (2001)

214 reported that in arable soils, clay-sized complexes ( $< 2\mu\text{m}$ ) have the largest concentrations  
215 of OM, silt-sized ( $2\text{--}20\mu\text{m}$ ) particles are less enriched, and size separates  $> 20\mu\text{m}$  usually  
216 contain little OM.

217 Figure 5 shows the complex distribution of surface soil (0-20 cm) Pi fractions in the  
218 particle-size fractions under different land-use types. Buffer rehabilitation had no effect  
219 on the concentration of Pi fractions within clay fraction, but the coarse particle fraction  
220 exhibited an decrease in the concentration of loosely-bound, nonoccluded, occluded (not  
221 in the silt fraction) and Ca-bound Pi. Chen *et al.* (2015) also reported that topsoil nutrients  
222 stored in coarse particle fractions were more sensitive than those stored in the fine fraction  
223 to soil recovery. However, Suñer *et al.* (2014) reported that the coarse fraction of the  
224 cultivated field had low levels of Pi as a consequence of the particulate organic matter  
225 decomposition and coarse mineral particle weathering. The reduction of Pi in coarse  
226 particle fractions in the buffer soil could have a positive influence on the function of the  
227 buffer to act as a sink for P. In the cropped soil, however, the accumulation of labile Pi in  
228 coarse particle fractions was considered to be more susceptible to lose P via leaching.  
229 Zheng *et al.* (2003) reported that soil particles containing high amounts of extractable P  
230 suggested to have a higher P release potential. The higher nonoccluded and occluded Pi  
231 in the sand and silt fractions of the cropped soil indicated that the dissolved fertilizer P  
232 was easy transformed into more stable Pi forms (Neufeldt et al., 2000). The significant  
233 accumulation of Ca-bound Pi in the sand and silt fractions of the cropped soils as  
234 compared to the buffer soils could be explained by the presence of undissolved P fertilizer  
235 granules in these fractions, because of rock phosphate had been often used together with  
236 soluble P fertilizers in these areas. Tiessen *et al.* (1983) also observed a similar  
237 enrichment of recalcitrant P in the coarse silt fraction of a cropped soil. Below 20 cm, the

238 concentrations of Pi fractions in the particle-size fractions did not differ significantly  
239 between the crop and buffer soil.

240 To enable a better understanding of P transformations in those riparian soils, regardless  
241 of land uses, the average proportions of the P fractions in the particle size fractions are  
242 presented in Figure 6. Compared with the sand and silt fractions, the proportions of  
243 occluded Pi was increased in the clay fraction, which should be related to the increasing  
244 adsorption to Fe/Al hydrous oxides with finer particle size classes. Agbenin and Tiessen  
245 (1995) and Neufeldt *et al.* (2000) also reported a similar change of the proportions in  
246 entisols, inceptisols and oxisols from Brazil. The proportions of Ca-bound Pi in the clay  
247 and silt sand fraction were significantly lower than that in the sand fractions, which  
248 suggested that the increase in occluded Pi with finer particle size fractions may be at the  
249 expense of Ca-bound Pi. The occluded Pi, which consisted of insoluble Al/Fe phosphates,  
250 are progressively sequestered in finer particle size fractions and therefore more difficult  
251 to extract.

252 The proportion of nonoccluded Pi was nearly twice as high in the silt and clay fractions  
253 as compared to the sand fractions, which could be attributed to their comparatively higher  
254 amounts of discrete Fe/Al hydrous oxides. However, Neufeldt *et al.* (2000) reported that  
255 the proportions of NaOH-Pi showed no consistent trends between the particle-size  
256 classes. In contrast, the proportions of loosely-bound Pi did not differ significantly  
257 between the particle-size classes, which suggested the potential of labile Pi lose would  
258 not decrease with decreasing the particle-size fractions.

#### 259 **4 Conclusion**

260 Inorganic phosphorus forms and their distribution in particle-size fractions were different  
261 between the conservation buffer and the continuously cropped area. Amounts of Pi

262 increased in the surface soil of crop field associated with fertilizer P application, with  
263 nonoccluded and Ca-bound Pi constituting the major proportion of the change. Lower  
264 concentration of Pi forms in the silt and sand particle fraction was found in the surface  
265 soil of the buffer, suggesting that P in these fractions had a lower release potential. The  
266 enrichment of labile Pi in the coarse particle fraction of cropped soil implicated that it  
267 would be more easily depleted by movement from the soil surface by leaching. More  
268 important, the application of particle-size separation of P forms determination can be  
269 represented to a better understanding of soil P distribution between different land uses.

270

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379 Table 1 Percentage of inorganic phosphorus forms on total Pi at 0-20cm soil layer of  
 380 different sites

	Percentage of Pi forms on total Pi			
	loosely-bound	non-occluded	occluded	Ca-bound
Niulian site				
Buffer area	5 c	5c	26b	64b
Crop area	4 c	4 c	23c	69a
Haigeng site				
Buffer area	7b	11b	31a	51d
Crop area	6 b	12b	26b	56c
Xinglong site				
Buffer area	11 a	18a	28ab	42f
Crop area	11 a	18a	25c	46e

381 Data followed by the same lower case letter at same column indicate that the percentage of Pi forms were not significantly different at  $P < 0.05$   
 382 according to lsd test.

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395 Table 2 Phosphorus forms in particle-size fractions of the riparian soil. Percentage of sum  
 396 ( $\Sigma$ ) in parentheses

P forms	Particle size classes			$\Sigma$ g/kg	Whole soil g/kg
	<2 $\mu$ m g/kg	2-50 $\mu$ m g/kg	50-2000 $\mu$ m g/kg		
loosely-bound	0.05A(49a)	0.05A(38b)	0.04B(13c)	0.05	0.05
non-occluded	0.09A(46a)	0.11A(44a)	0.06B(10b)	0.09	0.09
occluded	0.25A(53a)	0.23B(33b)	0.19C(14c)	0.24	0.21
Ca-bound	0.52A(51a)	0.51A(34b)	0.42B(15c)	0.50	0.48
TPi	0.91A(51a)	0.91A(35b)	0.71B(14c)	0.87	0.82
TP	1.75A(57a)	1.25B(28b)	1.21B(15c)	1.48	1.39

397 For each phosphorus form, data followed by the same capital case letter indicate that the P concentrations between particle size fractions were  
 398 not significantly different at  $P < 0.05$  according to lsd test. Data in parentheses followed by the same lower case letter indicate that the proportion  
 399 of P forms between particle size fractions were not significantly different at  $P < 0.05$  according to lsd test.

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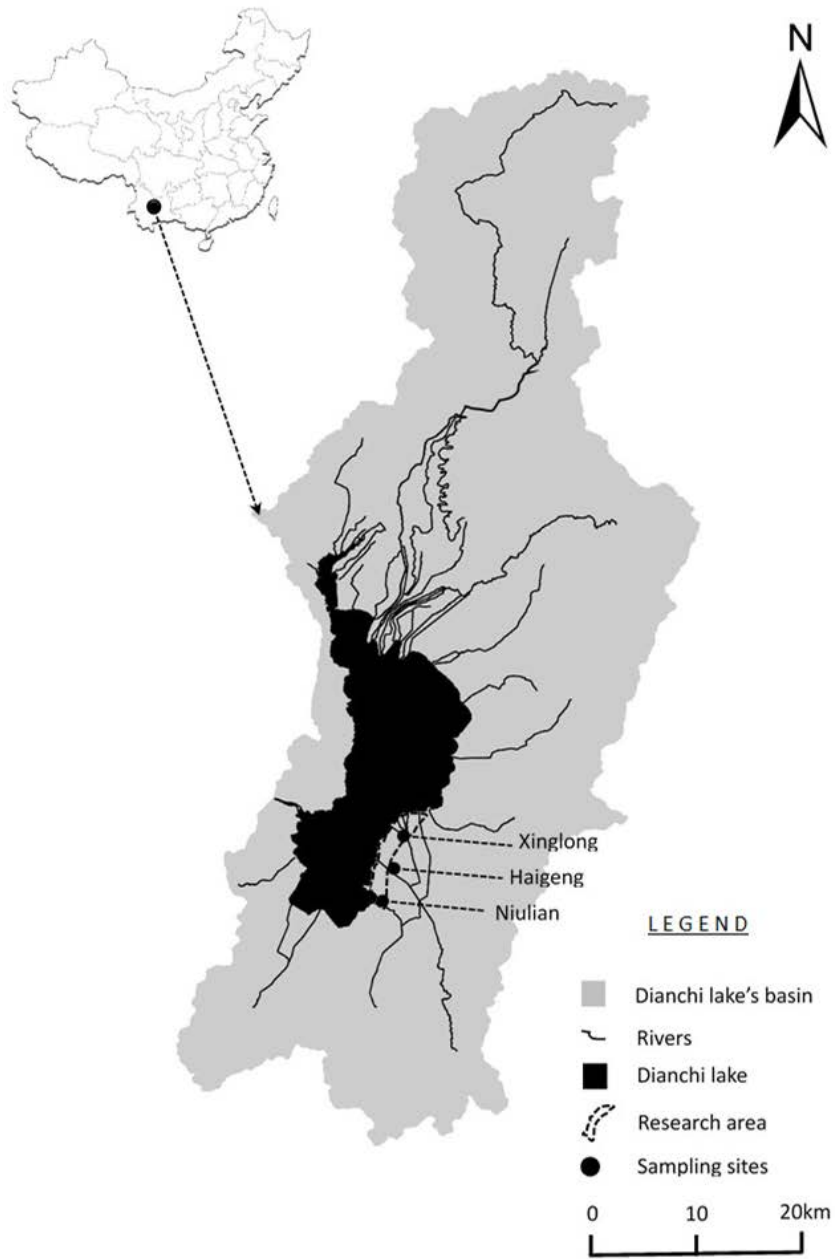
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414 Figure 1. Map of the research area with sampling sites

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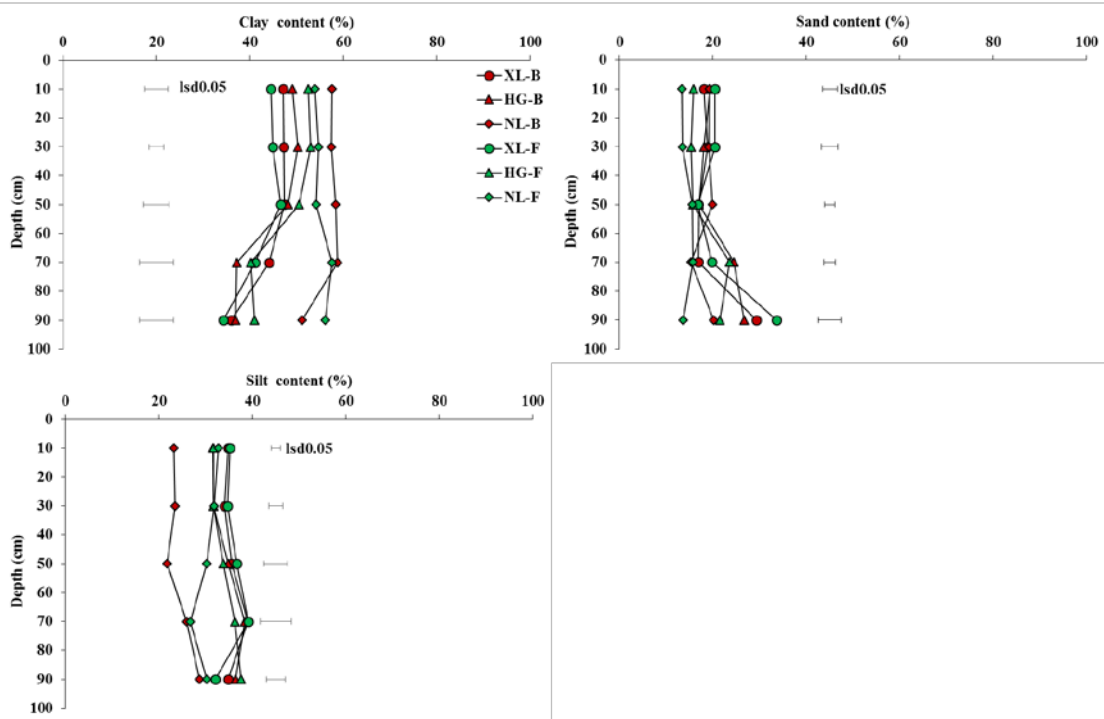
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422 Figure 2. The distribution of soil particle size fractions in profile at different land use  
 423 sites. Buffer of Xinglong site (XL-B); Buffer of Haigeng site (HG-B); Buffer of Niulian  
 424 site (NL-B); Crop field of Xinglong site (XL-F); Crop field of Haigeng site (HG-F); Crop  
 425 field of Niulian site (NL-F).

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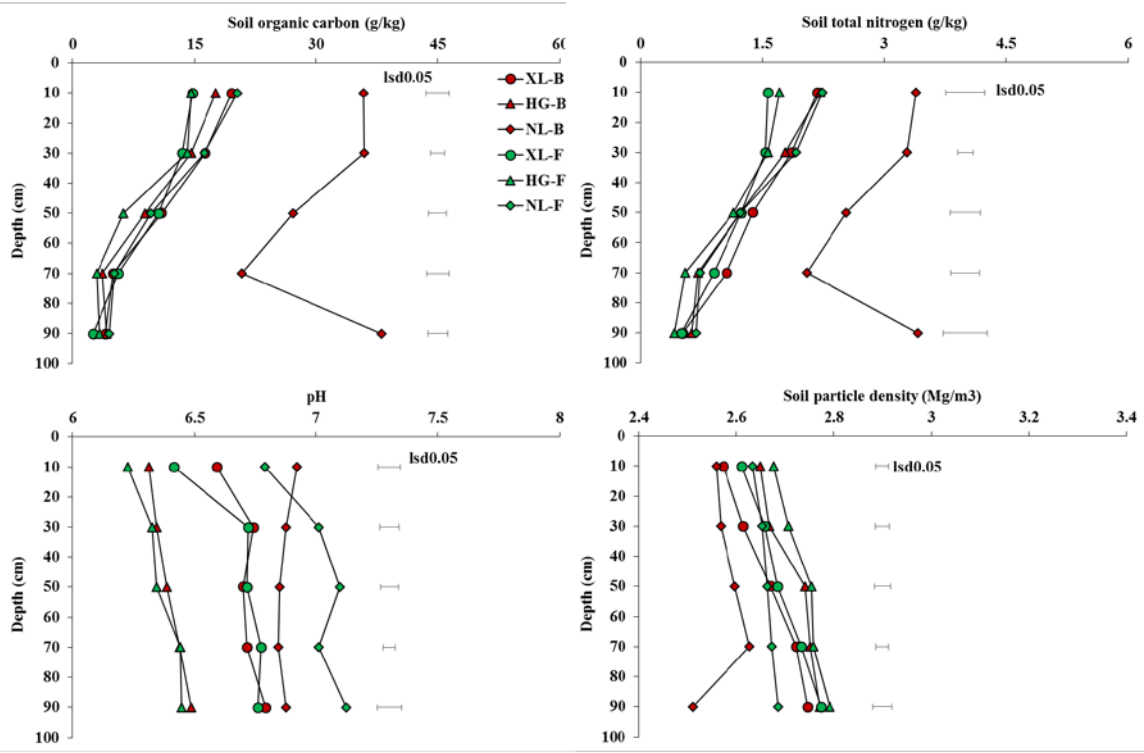
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437 Figure 3. The distribution of soil physicochemical properties in profile at different land  
 438 use sites. For abbreviations, see Figure 2.

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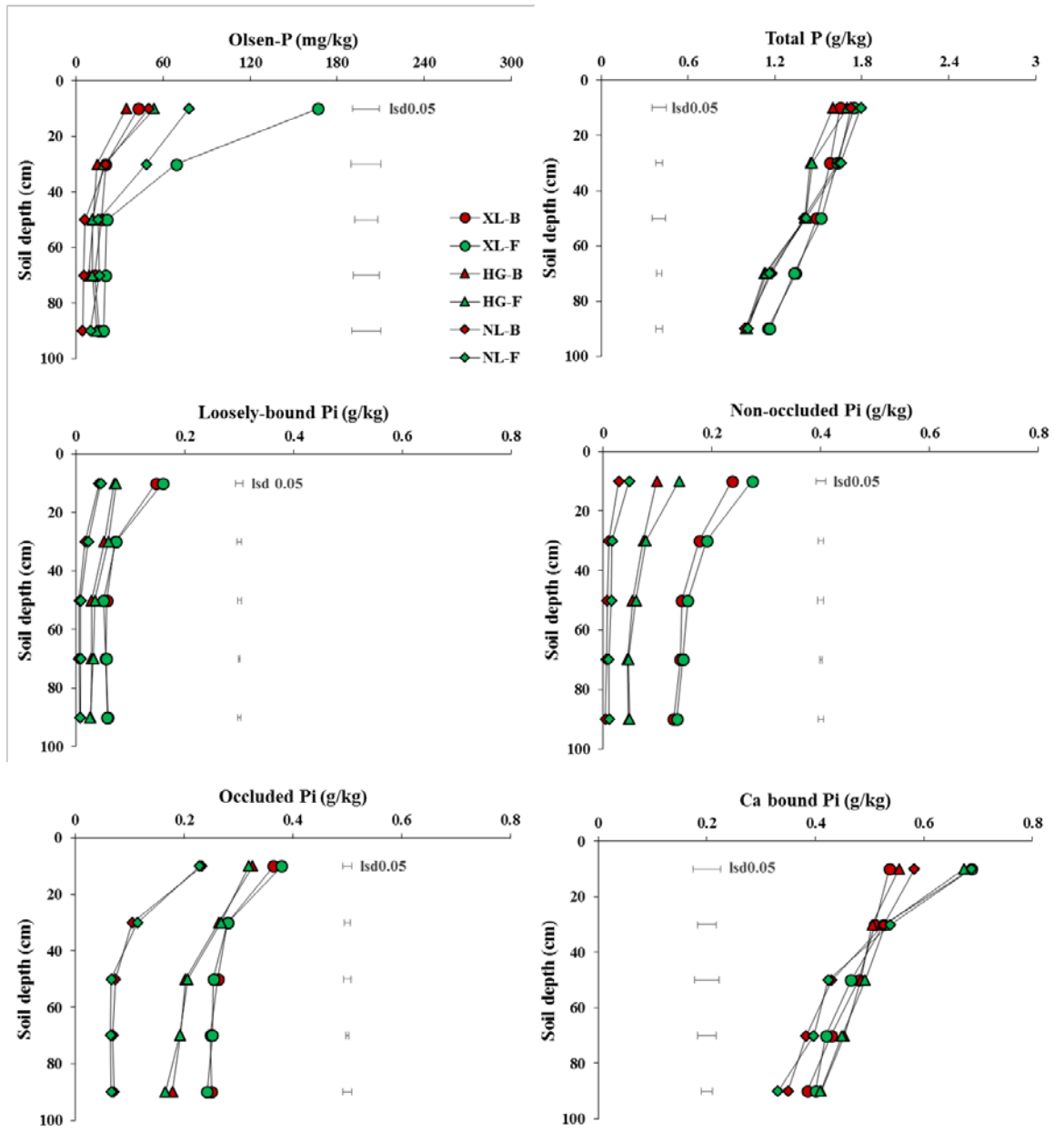
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452 Figure 4. The concentrations of P forms in soil profile at different land use sites. For  
 453 abbreviations, see Figure 2.

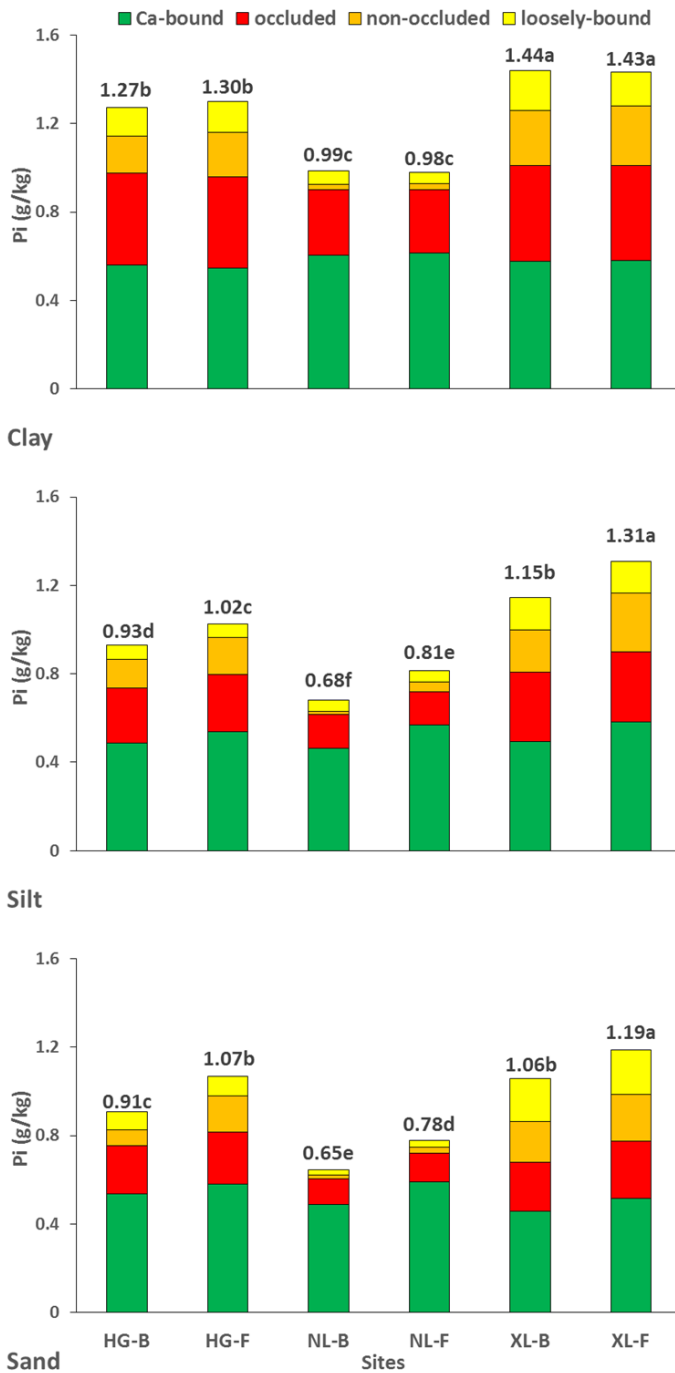
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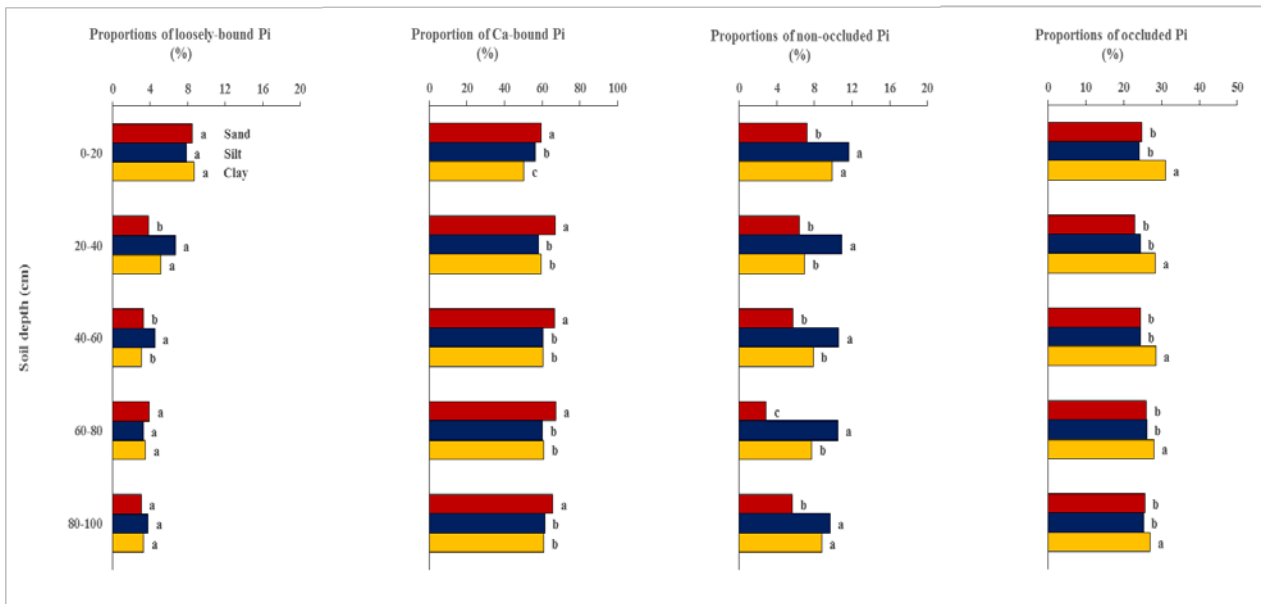
460 Figure 5. The P storage in particle size fractions for Pi fractions of different land use sites.

461 Values on the column are the sum values of Pi fractions at same site. Values followed by

462 a similar letter are not significantly different at  $p < 0.05$  level of significance according to

463 lsd test. For abbreviations, see Figure 2.

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466 Figure 6. The proportion of Pi forms in particle size fractions in soil profile. Values

467 followed by a similar letter in same layer are not significantly different at  $p < 0.05$  level of

468 significance according to lsd test.

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