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### Relationships of phosphorus fractions to organic carbon content in surface soils in mature subtropical forests, Dinghushan, China

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**Abstract.** Exploring the relationship between the accumulation of soil organic carbon (C) and the form and availability of soil phosphorus (P) is important for improved understanding of soil P availability and its regulation of C storage in forest ecosystems. Here, we investigated the relationships among soil organic C, sequentially extracted P fractions and P sorption index in 32 surface soils (0–0.15 m depth) across eight mature subtropical forests (80–400 years) in Dinghushan, China. Results showed that soil organic P (Po) accounted for 40–63% (mean 54%) of soil total P. Soil organic C was significantly positively correlated with both the content and the percentage of soluble inorganic P (Pi), Al-Po and Fe-Po fractions and the content of the Al-Pi fraction. The content of soil total Po increased significantly with soil organic C, whereas the percentage of soil total Po tended to increase with soil organic C only when soil organic C was low (<30 Mg/ha) but was relatively stable when soil organic C was high ( $\geq$ 30 Mg/ha). Moreover, soil organic C was highly correlated with P sorption index. Our results suggest that accumulation of organic C may increase, rather than decrease, the availability of P in surface soil in mature subtropical forests.

Additional keywords: organic carbon, organic phosphorus, phosphorus sorption index, soil phosphorus fractionation, subtropical forest.

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#### Introduction

Phosphorus (P) is one of the important nutrients determining the function and primary productivity of terrestrial ecosystems (Aerts and Chapin 1999; Elser *et al.* 2007). Despite the typically large P stock present in soils (Smil 2000; Imai *et al.* 2010), soil P supply is rarely adequate in meeting the P demands of plants in terrestrial ecosystems (Aerts and Chapin 1999; Elser *et al.* 2007). This is largely due to multiple forms of P existing in the soils, which differ in their availability for plant uptake across time scales (Hedley *et al.* 1982; Chen *et al.* 2003). Soil P fractions determined by sequential fractionation methods can provide useful information on the availability and dynamics of soil P and its responses to various environmental or anthropogenic factors in terrestrial ecosystems, and thus they have been widely used to date (Chang and Jackson 1957; Hedley *et al.* 1982; Yang and Post 2011).

The important role of soil organic matter in maintaining soil availability of nutrients (including P) in terrestrial ecosystems has long been recognised (Tiessen *et al.* 1994; Johnson *et al.* 2003). Soil organic matter provides an important source of P that can be utilised by plants and soil microbes following mineralisation (Tiessen *et al.* 1994; Kirkby *et al.* 2011), as well as energy reserves and carbon (C) substrates used in microbial activities such as the production of enzymes

involved in P mineralisation (Allison and Vitousek 2005; Allison et al. 2007). It can also maintain soil P availability by providing binding sites (Kang et al. 2009). However, some studies have suggested that an accumulation of soil organic C (or organic matter) may decrease soil P availability due to the inclusion of P in organic form (Huang et al. 2012; Schlesinger et al. 1998). For example, Schlesinger et al. (1998) proposed that P was limiting plant growth in soil at an early stage of development (110 years) on Rakata Island, Indonesia, perhaps as a result of the observed rapid accumulation of organic P. Wang et al. (2006) found that soil organic C concentration was positively related to the concentrations of soil soluble organic-P fractions, whereas it was negatively related to the concentrations of soil soluble inorganic-P fractions in depressional and riparian freshwater wetlands in North-east China. Further studies on the relationships between soil organic C and P fractions are needed to clarify the relationship between soil organic C accumulation and soil P availability, which has important implications in understanding the limitation P places on the storage of C in terrestrial ecosystems (Wang et al. 2010; Kirkby et al. 2011).

China has  $0.97 \text{ million } \text{km}^2$  of subtropical and tropical forests, representing 62% of its total forested area and 72% of its total forest net primary productivity (Zhao *et al.* 2004).

A long-term monitoring study reported that soil organic C stock in the top 0.2 m soil layer had accumulated at an average rate of 0.61 Mg C/ha.year in an old-growth (>400 years) subtropical forest in Dinghushan, China, between 1979 and 2003 (Zhou *et al.* 2006). Recently, Huang *et al.* (2012) proposed that the continuous accumulation of soil organic C might have occluded P in its organic form, thereby decreasing soil P availability in forest ecosystems in this area, as the site with a relatively higher soil organic matter pool possessed a relatively lower soil soluble P pool.

To test this hypothesis, we sampled 32 surface mineral soils (0-0.15 m depth) from eight mature subtropical forests in Dinghushan that varied in topographic conditions, plant communities and land use history. Soil organic C content, P fractions extracted by a sequential extraction procedure and P sorption index were determined. Surface soil was investigated, as it is usually the major source of nutrients for plant uptake. It also features the highest quantities of organic C in soil profiles, and consequently, the effects of organic C accumulation on soil P forms and availability was expected to be the most significant. We hypothesised that the amounts of soil organic-P fractions might be positively related to soil organic C content, whereas the amounts of soil inorganic-P fractions might be negatively related to it. General characteristics of soil P fractions from the studied forests were also explored to advance our knowledge of soil P fractions in strongly weathered and acidified subtropical forest soils.

#### Materials and methods

#### Site description

The study was conducted in Dinghushan Biosphere Reserve, in the middle of Guangdong province in China  $(112^{\circ}31'-112^{\circ}34'E, 23^{\circ}09'-23^{\circ}12'N)$ . The Reserve covers an area of 1155 ha, and has a typical subtropical monsoon climate. Mean annual temperature in the Reserve is 21°C, and mean annual precipitation is ~1900 mm. Nearly 80% of the precipitation falls in the wet season (April–September) and 20% in the dry season (October–March). Elevation ranges from 10 to 1000 m above sea level. Soil thickness mostly varies from 0.3 to 0.9 m. Surface soils are Ferralsols (FAO-UNESCO 1974), and have developed on sandstone during the early Holocene (Shen *et al.* 2001). Basic information of the eight selected forest sites is summarised in Table 1. The eight forest sites represent the major vegetation types and topographical conditions in Dinghushan, and also represent five of the major vegetation types as well as the major soil type and geological conditions of natural subtropical forests in south China. All forests are mature-growth ( $\geq$ 80 years) and have been well protected since their establishment, except for the forest at site 1, which had been disturbed by the harvest of understory vegetation and litter by local residents between the 1950s and 1990s (Mo *et al.* 1995).

#### Sampling and sample preparation

Field sampling was conducted in October 2010. At each forest plot, four subplots (20 m by 20 m) were randomly set up with a distance of at least 10 m between them. At each subplot, three sampling points were randomly selected with the constraints that they were 1-2 m away from the nearest tree (diameter at breast height >0.05 m) and at least 5 m from its nearest neighbour. After forest floor materials were removed, a soil profile was excavated in the bare soil area. Mineral soil at the 0-0.15 m depth was sampled by three successive cutting rings (height 0.05 m, volume  $100 \text{ cm}^3$ ) from top to bottom (0.05 m depth per cutting ring). Nine soils (3 cutting rings per area  $\times$  3 areas) of each subplot were bulked together as one composite soil sample. In total, 32 composite soil samples were collected. By using a cutting ring to sample soil, the bulk density at the time of soil sampling was also determined. Soil C and nutrient pools investigated in this way should be more accurate than if separate samplings of soil for bulk density and soil C and nutrient concentration measurements were performed. Large roots and stones were separated from the sample and weighed during sieving (4-mm mesh) in the laboratory. Soil was air-dried for 2 weeks, and then ground and passed through 0.15-mm mesh for soil property determination.

#### Analytical methods

Total C and total N concentrations were determined using an Isoprime isotope ratio mass spectrometer with an elemental analyser (Isoprime-Euro EA 3000; Eurovector, Milan, Italy). Since surface soils are strongly acidic in the study area (pH 3.6–4.2; Liu *et al.* 2010*a*), the measured soil total C should be mainly in the organic form and, thus, is used to represent soil

 Table 1. Characteristics of the eight study forest sites in Dinghushan, China

Site no.	Forest type	Stand age (years)	Aspect	Slope	Altitude (m)	Major tree species
1	Pine forest (litter and understory harvested)	80	SE	25°	50-150	Pinus massoniana, Schima superba, Euodia lepta
2	Pine and broadleaved mixed forest	80	SE	30°	50-200	Pinus massoniana, Schima superba, Castanea henryi
3		80	NE	40°	150-200	Pinus massoniana, Schima superba, Castanea henryi
4		80	NE	25°	300-350	Pinus massoniana, Schima superba, Castanea henryi
5	Ravine evergreen broadleaved forest	300	Е	20–25°	200–250	Aporusa yunnanensis, Cryptocarya concinna, Ormosia fordiana
6		300	W	30–40°	100-150	Gironniera subaequalis, Sterculia lanceolata, Caryota ochlandra
7	Monsoon evergreen broadleaved forest	~400	NE	30°	250-300	Schima superba, Castanea henryi, Cryptocarya concinna
8	Mountainous evergreen broadleaved forest	~100	NE	30°	550-600	Engelhardtia roxburghiana, Rhododendron henryi, Machilus breviflora

organic C throughout the text. Total P concentration was measured using a nitric acid–perchloric acid digestion, followed by a colourimetric analysis (Murphy and Riley 1962) using a UV-Vis spectrophotometer (UV1800; Shimadzu, Kyoto, Japan).

As suggested by Wu *et al.* (2000), NH<sub>4</sub>F is preferable to NaHCO<sub>3</sub> in extracting readily available P fractions in acidic soils. A modified procedure of McDowell and Condron (2000) that included an NH<sub>4</sub>F extraction procedure (3.0 g air-dried soil) was thus adopted in the present study to extract soil P fractions. The protocol was as follows:

- Soluble P [NH<sub>4</sub>Cl]: 30 mL 1.0 M NH<sub>4</sub>Cl, shaken for 30 min, centrifuged (relative centrifugal force, RCF, 10 000, 10 min), supernatant filtered (<0.45 mm);</li>
- (2) Aluminium (Al)-associated P [NH<sub>4</sub>F]: 30 mL 0.1 M NH<sub>4</sub>F, shaken for 16 h, centrifuged (RCF 10 000, 10 min), supernatant filtered (<0.45 mm);</li>
- (3) Wash: 30 mL distilled H<sub>2</sub>O, shaken 30 min, centrifuged (RCF 10 000, 10 min), supernatant discarded;
- Iron (Fe)-associated P [NaOH]: 30 mL 0.1 M NaOH, shaken for 16 h, centrifuged (RCF 10 000, 10 min), supernatant filtered (<0.45 mm);</li>
- (5) Wash: 30 mL distilled H<sub>2</sub>O, shaken 30 min, centrifuged (RCF 10 000, 10 min), supernatant discarded;
- (6) Calcium (Ca)-associated P [H<sub>2</sub>SO<sub>4</sub>]: 30 mL 0.5 M H<sub>2</sub>SO<sub>4</sub> (H<sub>2</sub>SO<sub>4</sub> I), shaken for 16 h, centrifuged (RCF 10 000, 10 min), supernatant filtered (<0.45 mm);</li>
- (7) Wash: 30 mL distilled H<sub>2</sub>O, shaken 30 min, centrifuged (RCF 10 000, 10 min), supernatant discarded;
- (8) Residual P: residue was equally divided into two subsamples, one subsample directly extracted with 15 mL 0.5 M H<sub>2</sub>SO<sub>4</sub> (H<sub>2</sub>SO<sub>4</sub> II), the other subsample extracted with 15 mL 0.5 M H<sub>2</sub>SO<sub>4</sub> after being ashed at 550°C for 2 h (H<sub>2</sub>SO<sub>4</sub> III); the extraction procedure was the same as for step 6.

Inorganic P concentration in the extracts of 0.1 M NH<sub>4</sub>F (Al-Pi), 0.1 M NaOH (Fe-Pi), 0.5 M H<sub>2</sub>SO<sub>4</sub> I (Ca-Pi), and 0.5 M H<sub>2</sub>SO<sub>4</sub> II and III was directly measured using the molybdate blue method (Murphy and Riley 1962). Inorganic P concentration in the 1.0 M NH<sub>4</sub>Cl extract (soluble Pi) was below the detection limit by the molybdate blue method, and therefore reanalysed using the malachite green method (Ohno and Zibilske 1991). Organic P in the 0.1 M NH<sub>4</sub>F (Al-Po) and 0.1 M NaOH (Fe-Po) extracts was calculated as the difference between total P determined after persulfate digestion (Ormaza-González and Statham 1996) and inorganic P. Residual organic P (residual Po) was calculated as the difference of inorganic P between the H<sub>2</sub>SO<sub>4</sub> II and III extracts. Residual inorganic P (residual Pi) was calculated as the difference between total P and the sum of soluble Pi, Al-Pi and -Po, Fe-Pi and -Po, Ca-Pi, and residual Po. Total inorganic P (total Pi) was calculated as the sum of soluble Pi, Al-Pi, Fe-Pi, Ca-Pi and residual Pi, whereas total organic P (total Po) was calculated as the sum of Al-Po, Fe-Po and residual Po. Soil organic C, total N, and total P and P fractions are all presented on an area basis (Mg/ha or kg/ha), and soil P fractions are also presented as a percentage of soil total P content.

Phosphorus sorption index was determined following the procedure of Kovar and Pierzynski (2009). The amount of P

sorbed, x (mg P/kg), from an addition of 1.5 g P/kg soil (as KH<sub>2</sub>PO<sub>4</sub> in 0.01 M CaCl<sub>2</sub>), was determined after shaking for 18 h at a soil weight : solution volume ratio of 1 : 20. The P sorption index (L/kg) was calculated as the quotient  $x/\log C$ , where C is the solution concentration in the filtrate (mg/L).

#### Statistical analyses

The Pearson correlation method (P < 0.05, Bonferronicorrected) was adopted to investigate the relationships among the contents of P fractions and P sorption index in the soils using SPSS 16.0 (SPSS Inc., Chicago). Relationships of organic C content with the contents and percentages of P fractions and P sorption index in the soils were investigated visually by plotting on an x-y scatter by using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA).

#### Results

#### Basic soil properties

Soil bulk density varied between 0.78 and 1.39 (mean 1.00) g/  $cm^3$  across all 32 soils (Table 2). Soil organic C content was 13.6–52.5 (mean 33.9) Mg/ha and soil total N content 0.9–3.4 (mean 2.1) Mg/ha (Table 2). Soil organic C and total N contents were both markedly lower at site 1 than at all other sites (Table 2). Soil total P content ranged between 155 and 492 (mean 271) kg/ha, and was highest at sites 5–7 (321–394 kg/ha), intermediate at site 8 (281 kg/ha), and lowest at sites 1–4 (185–243 kg/ha) (Table 2). Phosphorus sorption index ranged between 276 and 1130 (mean 662) L/kg. Soil C : N, C : P and N : P mass ratios ranged from 12.5 to 20.4 (mean 16.2), from 58.8 to 209.2 (mean 126.9) and from 4.1 to 12.3 (mean 7.7), respectively.

#### Soil P fractions

Similar to soil total P content, the contents of all soil P fractions were generally higher at sites 5-7 than at other sites (Table 3). Soil organic P accounted for 40-64% (mean 54%) of soil total P (Table 4). Of the soil organic P, about half was present in the residues, represented by soil residual Po (mean 24% in soil total P, range 12-36%) (Table 4). Among the extractable organic P fractions, Fe-Po was the dominant fraction, accounting for 13-37% (mean 25%) of soil total P, whereas Al-Po accounted for only 0.8-8.1% (mean 4.8%) of soil total P (Table 4). The averaged percentages of soil soluble Pi, Al-Pi, Ca-Pi and Al-Po fractions were 0.08%, 0.9%, 2.3% and 4.8%, respectively (Table 4). The coefficient of variance of the percentage was smaller than that of the content for all soil P fractions (Tables 3 and 4).

#### Relationships between soil P fractions, P sorption index and soil organic C

The contents of soil Ca-Pi, Fe-Pi and Fe-Po fractions were significantly correlated with each other (r=0.60-0.76, P<0.05; Table 5). Soil residual Po content was significantly correlated with soil Fe-Pi and Ca-Pi fraction contents (r=0.65-0.68, P<0.05; Table 5). The contents of all soil P fractions except for soil soluble Pi, Al-Pi and residual Pi fractions were significantly correlated with soil total Po and

Site no.		Bulk density (g/cm <sup>3</sup> )	Organic C (Mg/ha)	Total N (Mg/ha)	Total P (kg/ha)	C : N ratio	C : P ratio	N : P ratio	P sorption index (L/kg)
1	Mean	1.35	17.4	1.1	212.9	15.1	80.1	5.3	359.9
	s.e.	(0.02)	(2.5)	(0.1)	(12.6)	(0.3)	(9.2)	(0.5)	(46.1)
2	Mean	0.92	34.5	1.8	200.4	19.7	166.8	8.5	634.3
	s.e.	(0.03)	(1)	(0.1)	(11.4)	(0.4)	(7.5)	(0.3)	(54.9)
3	Mean	1.02	31.4	1.8	243.0	17.7	124.0	7.0	618.3
	s.e.	(0.07)	(4.8)	(0.3)	(29.1)	(0.4)	(5.7)	(0.3)	(60.2)
4	Mean	0.98	33.2	2.0	184.9	16.5	177.1	10.7	653.9
	s.e.	(0.04)	(1.7)	(0.1)	(12.2)	(0.8)	(16.8)	(0.7)	(46.6)
5	Mean	1.09	27.6	2.2	394.0	12.8	69.2	5.4	471.4
	s.e.	(0.04)	(2.3)	(0.2)	(35.6)	(0.3)	(3.9)	(0.2)	(25.8)
6	Mean	0.89	42.9	2.8	330.2	15.3	125.9	8.2	970.7
	s.e.	(0.04)	(4.3)	(0.3)	(15.4)	(0.5)	(12.9)	(0.6)	(74.6)
7	Mean	0.84	39.7	2.7	321.0	14.8	119.1	8.0	814.9
	s.e.	(0.04)	(4.3)	(0.3)	(19.3)	(0.3)	(10.9)	(0.6)	(65.4)
8	Mean	0.88	44.1	2.5	280.3	17.4	152.8	8.8	774.2
	s.e.	(0.03)	(3)	(0.2)	(16.4)	(0.2)	(5.8)	(0.4)	(13.2)
All	Mean	1.00	33.9	2.1	270.8	16.2	126.9	7.7	662.2
	s.e.	(0.03)	(1.8)	(0.1)	(13.9)	(0.4)	(7.2)	(0.3)	(36.3)
	CV (%)	17.2	29.8	30.2	29.1	13.3	31.9	24.9	31.0
	Range	0.78-1.39	13.6–52.5	0.9–3.4	154.9-491.8	12.5-20.4	58.8-209.2	4.1-12.3	276.2-1129.5

Table 2. Basic properties of the 0–0.15 m soils in the eight study forests in Dinghushan n=4 for each site and 32 for all sites

Table 3. Contents of phosphorus fractions (kg/ha) in the 0–0.15 m soils in the eight study forests in Dinghushan

Pi, Inorganic P; Po, organic P. n=4 for each site and 32 for all sites

Site no.		Soluble Pi	Al-Pi	Al-Po	Fe-Pi	Fe-Po	Ca-Pi	Residual Pi	Residual Po	Total Pi	Total Po
1	Mean	0.07	2.3	6.1	19.2	32.0	4.6	93.9	54.6	120.1	92.8
	s.e.	(0.03)	(1.2)	(1.6)	(3.4)	(1.7)	(0.5)	(13.3)	(1.3)	(10.3)	(3)
2	Mean	0.26	1.4	13.3	15.8	49.7	4.2	65.6	50.0	87.4	113.0
	s.e.	(0.05)	(0.2)	(1.8)	(1.4)	(2.3)	(0.4)	(3.4)	(4.8)	(4.2)	(7.3)
3	Mean	0.21	1.7	11.9	15.4	51.6	5.4	78.9	78.0	101.6	141.4
	s.e.	(0.08)	(0.8)	(1.5)	(2.5)	(8.5)	(0.7)	(9)	(10.7)	(11.4)	(20.4)
4	Mean	0.14	1.9	9.5	16.6	48.6	3.9	50.3	54.1	72.8	112.1
	s.e.	(0.02)	(0.5)	(1.6)	(1.1)	(2.6)	(0.4)	(9.7)	(4.8)	(9.4)	(6.8)
5	Mean	0.34	3.1	19.2	61.5	98.2	11.3	85.7	114.5	162.0	232.0
	s.e.	(0.04)	(0.4)	(2.1)	(6)	(12.7)	(1.4)	(20.8)	(7.5)	(17.6)	(19.9)
6	Mean	0.32	4.4	18.7	34.2	118.9	8.5	95.3	49.9	142.7	187.5
	s.e.	(0.08)	(0.4)	(3.6)	(3.8)	(7.1)	(0.5)	(4.9)	(4)	(7.4)	(10.5)
7	Mean	0.33	3.6	16.1	29.3	95.1	7.2	108.5	60.8	148.9	172.0
	s.e.	(0.05)	(0.7)	(1.1)	(2.4)	(7.7)	(0.3)	(9)	(4.7)	(11.6)	(8)
8	Mean	0.21	1.9	11.8	23.3	75.7	6.9	96.0	64.5	128.3	152.0
	s.e.	(0.05)	(0.7)	(1.7)	(2.9)	(8.7)	(0.7)	(9)	(1.2)	(11.3)	(10.8)
All	Mean	0.23	2.5	13.3	26.9	71.2	6.5	84.3	65.8	120.5	150.3
	s.e.	(0.02)	(0.3)	(1)	(2.8)	(5.6)	(0.5)	(4.7)	(4.1)	(6.3)	(8.6)
	CV (%)	55.6	62.3	41.7	58.9	44.5	41.2	31.4	34.9	29.4	32.4
	Range	0-0.52	0-5.8	1.4-27.7	8.2–74.5	26.1-136.1	3.2-14.3	32.3-138.2	37.7–129	53.7-211.3	80.8-280.5

total P contents (r=0.50-0.95, P<0.05; Table 5). All of these correlations were positive.

Soil organic C content was significantly positively correlated with both the contents and percentages of soil Al-Po and Fe-Po fractions (r=0.36-0.64, P<0.05; Fig. 1a-d). By contrast, the content of soil residual Po fraction did not correlate with soil organic C content (r=-0.05, P=0.80; Fig. 1e) and the percentage of soil residual Po fraction tended to decrease with an increase in soil organic C content when soil organic

C content was >30 Mg/ha (quadratic curve, r=0.48, P<0.05; Fig. 1*f*). The content of soil total Po fraction significantly increased with soil organic C content (r=0.39, P<0.05; Fig. 1*g*), while the percentage of soil total Po fraction tended to increase with soil organic C content only when soil organic C content was <30 Mg/ha (quadratic curve, r=0.59, P<0.01; Fig. 1*h*). For inorganic P fractions, both the content and the percentage of the soil soluble Pi fraction and the content of soil Al-Pi fraction were significantly and positively correlated with

Site no.		Soluble Pi	Al-Pi	Al-Po	Fe-Pi	Fe-Po	Ca-Pi	Residual Pi	Residual Po	Total Pi	Total Po
1	Mean	0.03	1.0	2.7	9.3	14.8	2.1	44.4	25.5	56.9	43.1
	s.e.	(0.01)	(0.5)	(0.6)	(2.4)	(0.5)	(0.2)	(4.3)	(2.1)	(1.8)	(1.8)
2	Mean	0.12	0.7	6.3	7.6	23.9	2.0	35.4	23.9	45.8	54.2
	s.e.	(0.03)	(0.1)	(0.7)	(0.5)	(0.7)	(0.1)	(1.3)	(1.4)	(0.9)	(0.9)
3	Mean	0.08	0.6	4.7	6.1	20.2	2.2	35.1	31.0	44.0	56.0
	s.e.	(0.03)	(0.3)	(0.2)	(0.4)	(1.4)	(0.1)	(3.1)	(1.6)	(2.8)	(2.8)
4	Mean	0.08	1.0	5.1	8.8	25.7	2.0	29.0	28.3	40.9	59.1
	s.e.	(0.01)	(0.3)	(1)	(0.8)	(1.7)	(0.1)	(3.6)	(1.2)	(2.9)	(2.9)
5	Mean	0.09	0.8	4.8	15.9	24.2	2.8	22.6	28.8	42.2	57.8
	s.e.	(0.01)	(0.1)	(0.5)	(2.7)	(1.1)	(0.3)	(3.4)	(1.7)	(1.6)	(1.6)
6	Mean	0.10	1.3	5.5	10.0	34.8	2.5	31.1	14.6	45.0	55.0
	s.e.	(0.03)	(0.1)	(1)	(1)	(1.1)	(0.1)	(0.7)	(1.1)	(1.2)	(1.2)
7	Mean	0.10	1.1	4.8	8.8	28.4	2.2	36.3	18.4	48.4	51.6
	s.e.	(0.01)	(0.2)	(0.2)	(0.2)	(1.1)	(0.1)	(0.7)	(1.6)	(0.8)	(0.8)
8	Mean	0.07	0.6	4.1	8.0	26.0	2.4	36.3	22.6	47.3	52.7
	s.e.	(0.02)	(0.2)	(0.5)	(0.6)	(2.2)	(0.1)	(2.8)	(1.5)	(2.7)	(2.7)
All	Mean	0.08	0.9	4.8	9.3	24.8	2.3	33.8	24.1	46.3	53.7
	s.e.	(0.01)	(0.1)	(0.3)	(0.7)	(1.1)	(0.1)	(1.4)	(1)	(1)	(1)
	CV (%)	52.0	59.0	32.3	39.8	24.3	16.5	23.2	24.6	12.7	11.0
	Range	0-0.2	0-2.4	0.8-8.1	5.1-22.7	13.3–37.4	1.6-3.6	16-50.1	11.9–35.7	36.7-60.2	39.8-63.4

Table 4. Percentages of phosphorus fractions in total P in the 0-0.15 m soils in the eight study forests in Dinghushan Pi, Inorganic P; Po, organic P. n=4 for each site and 32 for all sites

 Table 5. Coefficients (r value) of correlations between the contents of soil phosphorus fractions and P sorption index in the 0-0.15 m forest soils in Dinghushan

Pi,	Inorganic	P; Po,	, organic P.	Values in	n bold indicate	e significant	correlations	$(P \cdot$	< 0.05)	(Bonferroni	i-corrected	); <i>n</i> = 1	32
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	Soluble Pi	Al-Pi	Al-Po	Fe-Pi	Fe-Po	Ca-Pi	Residual Pi	Residual Po	Total Pi	Total Po	Total P
Al-Pi	0.47										
Al-Po	0.61	0.37									
Fe-Pi	0.40	0.44	0.52								
Fe-Po	0.51	0.53	0.76	0.60							
Ca-Pi	0.45	0.44	0.66	0.76	0.81						
Residual Pi	0.23	0.28	0.23	0.13	0.51	0.49					
Residual Po	0.28	0.15	0.41	0.68	0.30	0.65	0.12				
Total Pi	0.41	0.49	0.48	0.62	0.74	0.80	0.86	0.45			
Total Po	0.54	0.46	0.81	0.77	0.88	0.91	0.42	0.71	0.75		
Total P	0.52	0.50	0.71	0.76	0.88	0.92	0.64	0.64	0.91	0.95	
P sorption index	0.46	0.38	0.46	-0.07	0.56	0.17	0.20	-0.21	0.15	0.32	0.26

soil organic C content (r=0.46-0.57, P<0.05; Fig. 2). Neither the content nor the percentage of other inorganic P fractions was significantly correlated with soil organic C content (data not shown). Moreover, P sorption index was highly correlated with soil organic C content (r=0.83, P<0.001; Fig. 3).

#### Discussion

## General characteristics of soil properties and soil *P* fractions

The markedly lower soil organic C and total N contents at site 1 than at the other sites could be mainly explained by the long-term litter and understory harvest at this site, which had removed a substantial proportion of litterfall from the soil surface (Mo *et al.* 1995). However, the impact of litterfall harvest on soil total P content seems small (Table 2). Although stand age at sites 5-7 was much greater than at the other sites (300–400 v.

80–100 years), soil organic C content did not reveal much difference between them (Table 2). This result seems inconsistent with the study by Zhou *et al.* (2006), which reported that surface-soil organic C stock accumulated rapidly at site 5 between 1979 and 2003. However, it is possible that soil organic C content at all study sites had accumulated at a high rate, possibly due to the continuously high atmospheric N deposition (~40 kg N/ha.year) (Mo *et al.* 2008; Huang *et al.* (2009) reviewed soil C stock changes with stand development in many biomes and found that chronosequence estimates of soil organic C accumulation (–4.5 to 17.6 g C/m<sup>2</sup>.year) were generally much lower than the estimates based on repeated sampling (32–165 g C/m<sup>2</sup>.year).

As expected, soil organic P accounted for more than half of the soil total P in the study forests (Table 4). Half of the organic P existed as soil Al-Po and Fe-Po fractions, both of which are



**Fig. 1.** Relationships of organic carbon content with the contents and percentages in total P of organic P fractions in the 0-0.15 m forest soils in Dinghushan: specifically the contents of soil (*a*) Al-Po, (*c*) Fe-Po, (*e*) residual Po and (*g*) total Po fractions, and the percentages in soil total P of (*b*) soil Al-Po, (*d*) Fe-Po, (*f*) residual Po and (*h*) total Po fractions.

potentially available for plant uptake after mineralisation (Chen et al. 2002; Hamer et al. 2013). Inorganic P fractions associated with Al (Al-Pi) and Fe (Fe-Pi) were only one-tenth to half of the corresponding organic P fractions (Al-Po and Fe-Po), suggesting that mineralisation of soil organic P might be a limiting process of soil P cycling in the study forests (Huang et al. 2013). As expected, the percentage of soil Ca-Pi fraction (1.6-3.6%) was low, because surface soils in the study area have developed over thousands of years (Shen et al. 2001) and are strongly acidified (Liu et al. 2010a). High annual temperature (21°C) and rainfall (1900 mm) in the study area promote soil weathering. It is notable that more than half of the soil total P in the study soils existed within the soil residual P (Pi + Po) fractions, which are bound with Al and Fe oxides and are either very weakly available or unavailable for plant uptake (Chang and Jackson 1957; Chen et al. 2003; Wang et al. 2006). This result suggests that the large proportion of soil P occluded with Al and Fe oxides may be an important factor in the low P availability and P limitation on the growth of plants and microbes in the study area (Hou et al. 2012; Liu et al. 2012), in addition to the high

atmospheric N deposition level and the low-P parent rock (Hou *et al.* 2012).

# Relationship between soil organic C content and soil P fractions

As expected, increases in both the absolute and relative amounts of soil Al-Po and Fe-Po fractions with soil organic C content were found in this study (Fig. 1a-d), which is consistent with previous studies on the relationships between soil organic C and extractable organic P fractions in forest ecosystems (Schlesinger *et al.* 1998; Dieter *et al.* 2010). In contrast to the extractable organic P fractions, the content of soil residual Po fraction did not correlate with soil organic C content (Fig. 1e). The result was consistent with the study by Brandtberg *et al.* (2010), which found that the concentration of soil non-occluded Po fractions (NaHCO<sub>3</sub>-Po and first NaOH-Po) was significantly and positively correlated with soil organic C concentration, whereas the concentration of soil occluded P fraction (second NaOH-Po) was uncorrelated with organic C in surface forest

**Fig. 2.** Relationships between organic carbon content and (*a*) soluble Pi content, (*b*) soluble Pi percentage in total P and (*c*) Al-Pi content in the 0-0.15 m forest soils in Dinghushan.

soils in New Zealand. A negative relationship between soil organic C content and occluded Po percentage (Fig. 1*f*) corresponded to the positive relationships between soil organic C content and the percentages of soil Al-Po and Fe-Po fractions (Fig. 1*b*, *d*).

Soil total Po percentage increased with soil organic C content only when soil organic C content was low (<30 Mg/ha in the 0–0.15 m soil) but was relatively stable when soil organic C content was high ( $\geq$ 30 Mg/ha) (Fig. 1*h*). This result fits well with the notion that the soil organic P pool would increase with stand development or soil organic C accumulation and finally reach a steady-state characterised by an equilibrium between the build-up and decomposition of organic P in forest ecosystems (De Schrijver *et al.* 2012). Increases in the activity of enzymes participating in soil organic P mineralisation in line with



increases in soil organic C content have been reported by many studies (Allison and Vitousek 2005; Sinsabaugh *et al.* 2008). Increases in soil organic C content can provide more energy and C-structure materials for microbes, leading to elevated production of phosphatase enzymes involved in the mineralisation of soil organic P (Allison and Vitousek 2005; Sinsabaugh and Moorhead 1994). Soil organic matter can also provide binding sites for phosphatases to protect them from degradation (Tabatabai *et al.* 2002).

Our test of the relationships between soil organic C content and soil inorganic P fractions revealed that soil soluble and labile Pi contents would increase with soil organic C accumulation (Fig. 2). These relationships may be partly because that labile P is largely derived from the decomposition of organic matter (Tiessen et al. 1994; Johnson et al. 2003). Soil organic matter can also absorb labile Pi via poorly crystalline or non-crystalline Al and Fe (Kang et al. 2009), as supported by the strong correlation between soil organic C content and P sorption index in our study (Fig. 3). Similarly, Johnson et al. (2003) found strong relationships between soil organic C and resin-P contents (r=0.73-0.96, P<0.05) at sites without a history of recent agriculture. Frizano et al. (2002) studied the change in soil organic C and soil P fractions during the early soil succession stage (1–55 years) on landslide scars in the Luquillo mountains, Puerto Rico; they also found a significant relationship between soil organic C and resin-P contents (r=0.82, P<0.01) and a significant relationship between soil organic C and labile P (resin-Pi+HCO<sub>3</sub>-Pi+HCO<sub>3</sub>-Po; defined by Hedley method) contents (r = 0.87, P < 0.01).

Our integrated results of the relationships between soil organic C content and soil P fractions suggest that P availability in surface soils may increase with soil organic C accumulation in mature subtropical forests. The results did not support the hypothesis that continuous accumulation of soil organic C could essentially lead to binding of the P pool, thus reducing soil P availability and leading to P limitation on plant growth (Schlesinger *et al.* 1998; Huang *et al.* 2012). Soil extractable Po (Al-Po and Fe-Po) contents might linearly increase with the accumulation of soil organic C, but the increased soil extractable Po was not transferred from soil





extractable Pi (soluble Pi, Al-Pi and Fe-Pi) or residual Pi or Po fraction in the surface soil. Instead, it might be, at least partly, transferred from subsoil by root-uplifting of P. Plants can assimilate P and other mineral nutrients in both topsoil and subsoil by roots and return C, P and other nutrients onto the topsoil through litterfall (Jobbágy and Jackson 2001). In the long term, P can continuously accumulate in the topsoil with forest development (Jobbágy and Jackson 2001, 2004). The observed trend of increase in soil total P content with stand age (Tables 1 and 2) supported this hypothesis. We did not investigate organic C content and P fractions in the subsoil. However, a previous study on soil total P concentration in soil profiles at sites 1, 2 and 5 in the study area (Liu et al. 2010b) supports this hypothesis. Soil total P concentration tends to increase, or not change, with soil depth at sites 1 and 2 (stand age 80 years), while markedly decreasing with soil depth at site 5 (stand age 400 years). Moreover, the increase in soil organic C content can enhance soil P sorption capacity (Fig. 3); therefore, the retention capacity of P input from litterfall, the atmosphere or rock weathering may be relatively higher in soil with relatively higher organic C content (Frizano et al. 2002).

#### Conclusion

It has been suggested that organic C had continuously accumulated at a high rate in the surface soil in mature subtropical forests in south China during the past three decades (Zhou *et al.* 2006). Our results suggest that this accumulation of soil organic C might have enhanced, rather than reduced, P availability in surface soil in mature subtropical forests in China. Further study is warranted on the whole-ecosystem cycling of P and its relationship with the cycling and storage of C in natural subtropical forests in south China.

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