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ORIGINAL PAPER

Soil potential labile but not occluded phosphorus forms increase with forest succession

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Abstract Quantifying soil P fractions is essential for understanding soil P cycling because these fractions are potential sources of bioavailable P. Here, we investigated whether soil P fractions (i.e., labile inorganic P [P_i], intermediately available P_i, organic P, occluded P, and apatite P) differed among three tropical forests at different stages of succession (early, middle, and late) in the Dinghushan Biosphere Reserve (DBR), Southern China. We also determined which soil P fractions was closely related to soil microbial and chemical properties in these forests. Soil microbial biomass and chemical properties (except pH, exchangeable Ca, and Mn) were higher in the late successional forest than those in the other two forests. We found that soil organic and occluded P were the dominant fraction in all three forests and together accounted for 78.9, 84.2, and 78.6 % of total P in the early, middle, and late successional forests, respectively. Soil P fractions and acid phosphomonoesterase activity differed significantly among forest successional stages; intermediately available P_i and organic P (potential sources of labile P_i) were highest in the late successional forest, occluded P was highest in the middle successional forest, and acid phosphomonoesterase activity significantly increased in middle and late successional forest. Soil labile P_i fraction and its potential sources (i.e., intermediately available P_i and soil organic P) were both positively correlated with soil microbial and chemical properties, suggesting that these properties may play critical roles in maintaining high levels of available or potentially available P fractions during this forest succession. Overall, our study indicates that succession in tropical forest ecosystems may not always lead to lower soil P availability.

Keywords Soil P fractions · Soil organic P · Soil microbial properties · Forest succession · Tropical forest

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Introduction

Phosphorus (P) is often thought to be one of the most limiting nutrients to primary production in tropical forests (Elser et al. 2007; Vitousek et al. 2010; Reed et al. 2011; Sullivan et al. 2014) because of the low availability of soil inorganic P (P_i) (Olander and Vitousek 2000; Wardle et al. 2004; Huang et al. 2013). However, labile P_i provided by highly weathered tropical soils is generally several times higher than annual vegetation demands (Johnson et al. 2003; Yang and Post 2011). One possible explanation for this contradiction is the complex chemical behavior of P in tropical soils. Most P is bound or immobilized in various stable inorganic and organic fractions (Walker and Syers 1976; Cross and Schlesinger 1995; Lawrence and Schlesinger 2001; Yang and Post 2011), which could, over time, serve as sources of labile P to meet the biological demands (Chapin et al. 1978; Tiessen et al. 1984; Chen et al. 2003; Richter et al. 2006; Yang and Post 2011). Thus, an understanding of P availability in tropical forest ecosystems depends on an understanding of the relative composition of different P fractions in soils.

The P fractions in highly weathered tropical soils are complex but mainly consist of occluded P, organic P, and intermediately available P_i associated with aluminum/iron compounds (Cross and Schlesinger 1995; Crews et al. 1995; Reed et al. 2011; Yang and Post 2011). Occluded P which is physically encapsulated or surrounded by secondary minerals is recognized as the most recalcitrant form, i.e., the least available to plants and other organisms (Walker and Syers 1976). Organic P and intermediately available P_i, in contrast, may serve as potential sources of labile P_i at relatively short time scales (days or months) (Reed et al. 2011; Yang and Post 2011). The organic P and intermediately available P_i fractions dynamically interact with soil chemical components (e.g., aluminum/iron minerals) and with soil microorganisms (Frossard et al. 1995; Olander and Vitousek 2004; Richter et al. 2006; Lambers et al. 2008; Vandecar et al. 2009; Richardson and Simpson 2011) and are involved in intricate biogeochemical processes (Olander and Vitousek 2004), which ultimately determine available P content and the relative quantities of various P fractions in soil. Labile P can be continuously supplied to the soil solution, for instance, by organic P mineralization through microbial activity (McGill and Cole 1981; Chen et al. 2004; Jones and Oburger 2011; Nannipieri et al. 2011; Marklein and Houlton 2012; Spohn and Kuzyakov 2013) and by mineral P desorption through chemical reaction (Sanyal and De Datta 1991; Frossard et al. 1995; Sims and Pierzynski 2005). At the same time, P in the soil solution can be quickly absorbed by aluminum/iron minerals and then transformed into secondary mineral P_i (Sanchez 1976; Frossard et al. 1995; Olander and Vitousek 2004; Sims and Pierzynski 2005). Soil solution P also can be immobilized by soil microorganisms and subsequently transformed into organic P (Olander and Vitousek 2004; Oberson and Joner 2005; Liebisch et al. 2014).

In tropical regions, deforestation and other anthropogenic activities have transformed many primary forests in secondary forest successional gradients (FAO 2010). These forests often differ in vegetation composition and soil chemical and biological properties (Brown et al. 1995; Tang et al. 2006) and, thus, in soil P cycling (Fox et al. 2011; Hou et al. 2012; Huang et al. 2013). Since Walker and Syers (1976) proposed the classical conceptual model which predicts that total P will decrease and the P fractions will be transformed into more stable forms, many previous P fraction studies were focused on long-term soil chronosequence and supported the predictable model (Crews et al. 1995; Eger et al. 2011; Izquierdo et al. 2013). However, how soil P fractions change along the secondary forest succession is still unclear. The dynamics of P fractions along such secondary succession will be inconsistent with long-term soil chronosequence studies based on short-term plant succession studies which showed that labile P and organic P increased while occluded P decreased with succession (Frizano et al. 2002; Zhou et al. 2013). A recent study (Huang et al. 2013) conducted at UNESCO/MAB Dinghushan Biosphere Reserve (DBR) in tropical China which contains three typical tropical forests that represent different stages of a secondary successional gradient, indicated that soil P limitation increases with this forest succession and used soil acid phosphomonoesterase activity and N:P ratio as proxies. However, information on soil P fractions along this successional gradient is still lacking, which provides a good opportunity to study how soil P fractions change across secondary forest succession.

The objectives of this study were (1) to determine whether the soil P fractions change with secondary succession in the three forests at the DBR and (2) to examine the relationships between soil P fractions and soil microbial and chemical factors. We hypothesized that soil P fractions would significantly differ among the three successional forests at the DBR because of differences in plant composition and soil properties (Tang et al. 2006; Hou et al. 2012). We also predicted that changes in soil P fractions, especially soil organic P and intermediately available P_i, would be closely related to soil microbial and chemical properties as these two P fractions are likely to be involved in biological and chemical processes at relatively short time scales (Frossard et al. 1995; Olander and Vitousek 2004).

Materials and methods

Study sites

This study was conducted in the Dinghushan Biosphere Reserve (DBR), an UNESCO/MAB site located in the middle of Guangdong Province in subtropical China (23° 09′ 21″–23° 11′ 30″ N, 112° 30′ 39″–112° 33′ 41″ E). The reserve covers an area of 1155 ha and is characterized by a typical subtropical humid monsoon climate. The mean annual temperature is 21 °C, with the lowest monthly mean of 12.6 °C in January and the highest monthly mean of 28.0 °C in July. The average annual precipitation is 1927 mm, with 80 % falling between April and September. The soils are classified in the Ultisol group and Udult subgroup according to the USDA soil classification system and have developed form kaolinite and halloysite. These soils are naturally acidic (pH 4.0–4.9) and low with base saturation (<10 %). The bedrock is typically sandstone and shale belonging to the Devonian Period.

The three forests in our study included a pine forest (PF), a mixed pine and broadleaf forest (MF), and a monsoon evergreen broadleaf forest (BF). These three forests were well accepted as a sequence of secondary forest succession from

early pioneer (PF), middle transition (MF), and late succession stages (BF) (Huang et al. 2013). The PF is located between 200 and 300 m a.s.l, which is about 60 years old and has developed from a Masson pine plantation planted in the 1950s after the original forest was clear cut. *Pinus massoniana* is the predominant species in the PF and accounts for about 90 % of the total standing biomass (Brown et al. 1995). The MF is about 90 years old and has developed from a PF that was planted in the 1930s and that has been naturally invaded by some pioneer broadleaf species. The dominant canopy species in the MF are *Castanopsis chinensis*, *P. massoniana*, and *Schima superba*; these species account for >90 % of total standing biomass in the MF (Huang et al. 2013). The BF has been undisturbed for more than 400 years and is recognized as mature forest in the DBR (Zhou et al. 2006). The dominant canopy species in BF are *Acmena acuminatissima*, *Castanopsis chinensis*, *Gironniera subaequalis*, *Schima superba*, and *Syzygium rehderianum*; these species account for >65 % of the total standing biomass in the BF (Huang et al. 2013). Both the MF and BF are located between 220 and 300 m a.s.l. The three forest types are located in the same climate region and with the same soil pedogenesis, slope, and aspects.

Soil sampling

In July 2013, we established three replicate 0.09-ha (30 m× 30 m) plots in each of the three forests (PF, MF, BF), resulting in nine plots in total. The three sampling plots in each forest were selected with the similar plant composition, microclimate, and soil type and were separated by 300 to 400 m to make sure the separation of sampling points. In 2013, soil was sampled twice (in July and December) in each plot, resulting in six samples per forest type. For each plot at each sampling, we randomly dug eight soil pits to 60-cm depth after removing the thin litter layer and then collected mineral soil at four depth intervals based on soil genetic horizons: 0–10 cm (the surface soil), 11–25 cm, 26–40 cm, and 41–60 cm. Because of the higher spatial heterogeneity of the surface soils, another 10 soil samples were randomly taken with a soil corer (2.5-cm diameter) to 10-cm depth in each plot. The soil samples in each soil layer were combined for each plot. The soil samples were put in sealed plastic bags, stored in an ice chest, and immediately transported to the laboratory at the South China Botanical Garden. After visible roots, stones, debris, and soil macrofauna were removed, each soil sample was sieved (<2 mm plastic sieve) and used for determination of soil acid phosphomonoesterase activity. A subsample of the fresh and sieved (<2 mm) soil was passed through a 1-mm sieve and used for determination of P fractions. Another subsample of the soil that had been passed through the 2-mm sieve was freeze-dried and used for extraction of soil microbial phospholipid fatty acids (PLFAs). The remaining soil samples were air-dried and used for soil chemical analysis.

Soil chemical analysis

Soil pH values were measured on a soil/deionized water suspension (1:2.5) with a pH meter (Mettler Toledo, Shanghai, China). Soil exchangeable cations were measured as described by Hendershot et al. (2007). Briefly, the soils were extracted in 0.1 M $BaCl_2$, and exchangeable Fe, Al, Ca, and Mn in the extracts were measured with an inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer, Waltham, MA, USA.). Soil organic C (SOC) was determined using dichromate redox titration methods as described by Skjemstad and Baldock (2007). Soil total N (TN) was determined by semimicro-Kjeldahl digestion followed by steam distillation and final titration of ammonium (Rutherford et al. 2007).

Soil microbial PLFAs

The composition of soil microbial community was determined by PLFA analysis as described by Bossio and Scow (1998). For each soil sample, lipids were extracted in a single-phase mixture of chloroform/methanol/phosphate buffer (1:2:0.8 by vol.; pH 7.4) and later were analyzed with a gas chromatograph equipped with a flame ionization detector (Agilent 6890, Agilent Technologies, Palo Alto, CA, USA). The abundance of individual PLFAs was expressed as nanomole PLFAs per gram dry soil (nmol g^{-1}), and the sum of all PLFAs was used as a measure of total microbial biomass (TB) (Frostegård and Bååth 1996). Different PLFAs were considered to be the representative of different functional groups of soil microorganisms. The branched and saturated PLFAs i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0 are common in Gram-positive bacteria (G^+), and the mono-unsaturated and cyclopropyl PLFAs 16:1ω7c, 18:1ω7c, cy17:0, and cy19:0 are indicative of Gram-negative bacteria (G^-) (Frostegård and Bååth 1996; Zogg et al. 1997; Bossio and Scow 1998; Zelles 1999; Cusack et al. 2011; Fanin et al. 2013). Saprotrophic fungi (F) were considered to be represented by the PLFA markers 18:1ω9 and 18:2ω6 (Frostegård and Bååth 1996; Cusack et al. 2011; Fanin et al. 2013), whereas the fatty acid 16:1ω5 was used as an indicator of arbuscular mycorrhizal fungi (AM) (Nordby et al. 1981; Olsson 1999; van Diepen et al. 2010; Cusack et al. 2011). The methyl (Me)-branched fatty acids 16:0 10Me and 18:0 10Me are indicative of actinomycetes (A) (van Diepen et al. 2010; Cusack et al. 2011). We characterized the general composition of the soil microbial community with two parameters: G^+:G^- biomass ratio and F:B biomass ratio (B is bacteria and here equals G^+ plus G^-) (Frostegård and Bååth 1996; Cusack et al. 2011; Fanin et al. 2013).

Soil P fractions

The sequential extraction procedure originally developed by Hedley et al. (1982) and modified by Tiessen and Moir (2007) was used to assess soil P fractions. Field moist soil samples were sequentially extracted using the following extraction agents as described by Tiessen and Moir (2007): anion-exchange resin membrane (AERM) strips (9×62 mm, bicarbonate form) in deionized water, 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH, 1 M HCl, and hot concentrated HCl. Extractions were shaken end-over-end in 50-mL centrifuge tubes with 30 mL of reagent for 16 h. AERM strips were extracted with 0.5 M HCl. Other extracts were collected by centrifuging soil suspension at 25,000*g* for 10 min at 0 °C, followed by decanting the supernatant through a 0.45-μm membrane filter into a clean vial for later analysis. The remaining residual soil P was determined by digestion in boiling concentrated H_2SO_4 with repeated additions of a 30 % H_2O_2. The concentration of inorganic P in each extract was determined with an UV-vis spectrophotometer at 712 nm using a molybdate-ascorbic acid method (Murphy and Riley 1962). For 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH, and hot concentrated HCl extracts, total P was determined by persulfate digestion, and organic P was estimated by the difference between total P and inorganic P in each of these extracts (Tiessen and Moir 2007). Therefore, the sequential extraction procedure results in nine specific P fractions: resin-P_i, $NaHCO_3$-P_i, $NaHCO_3$-P_o, NaOH-P_i, NaOH-P_o, HCl-P_i, hot concentrated HCl-P_i, hot concentrated HCl-P_o, and residual P. We combined P fractions to reflect ecological significance based on previous studies (Hedley et al. 1982; Crews et al. 1995; Cross and Schlesinger 2001; Tiessen and Moir 2007; Selmants and Hart 2010; Yang and Post 2011): labile inorganic P (labile P_i) is the sum of resin-P_i and $NaHCO_3$-P_i; intermediately available P_i associated with aluminum/iron minerals is the NaOH-P_i; organic P is the sum of $NaHCO_3$-P_o, NaOH-P_o, and hot concentrated HCl-P_o; apatite P (Ca-associated P, presumably from primary minerals such as apatite) is the HCl-P_i; and occluded P is the sum of hot concentrated HCl-P_i and residual P. Total P content was the sum of all nine P fractions.

Soil acid phosphomonoesterase activity assay

Potential soil acid phosphomonoesterase activity was measured using a colorimetric *p*-nitrophenyl-ester-based method described by Acosta-Martínez and Tabatabai (2011), which was modified from Tabatabai and Bremner (1969). Briefly, field moist soil was well mixed with modified universal buffer (MUB, pH 5.5) and substrate (*p*-nitrophenyl phosphate) solution and then incubated at 37 °C for 1 h. After incubation, the absorbance of the resultant soil filtrates was measured with an UV-vis spectrophotometer at 410 nm. Potential acid phosphomonoesterase activity in soil was calculated on a dry weight basis and expressed as micromole *p*-nitrophenol (*p*-NP) per gram of soil per hour (μmol*p*-NP g^{-1} h^{-1}).

Statistical analysis

Prior to statistical analyses, all data were tested for normality using the Shapiro-Wilk test and for homoscedasticity using the Levene test. The differences in soil P fractions (concentrations and percentage of total P present as labile P_i, intermediately available P_i, organic P, occluded P, and apatite P), acid phosphomonoesterase activity, microbial properties, and chemical properties were compared among the three successional forests using one-way analysis of variance (ANOVA), and multiple comparisons were conducted using a post hoc Tukey HSD test. Differences were considered to be significant at the 0.05 level. The general relationships between soil P fractions and soil chemical and microbial properties were first examined by Spearman rank correlation analysis. Then, redundancy analysis (RDA) was applied to elucidate the relationships among soil P fractions and corresponding soil environmental variables (chemical and microbial properties) among the three forests and four soil layers. SPSS 18.0 (SPSS, Inc, Chicago, IL) was used for all statistical analyses except for RDA, which was performed using CANOCO software for Windows 4.5 (Ithaca, NY, USA).

Results

Soil chemical and microbial properties

SOC, TN, and exchangeable Al and Fe were higher in the BF than those in the PF and MF (Table 1). The mean concentrations of exchangeable Mn were highest in MF, especially at 11–25 cm and 26–40 cm depth (Table 1). The C:N ratio significantly decreased with succession, while pH and exchangeable Ca did not change significantly across the three successional forests. Microbial biomass (TB) and all individual functional groups except for saprophytic fungi significantly increased with forest succession (Table 2). Microbial community composition (F:B biomass ratio and G^+:G^- biomass ratio) did not significantly differ among the three successional forests except that the G^+:G^- biomass ratio was lower in the PF than that in the MF and BF at 26–40 cm depth (Table 2).

Total P and individual soil P fractions

Both the concentrations of total P and occluded P significantly changed along the three successional forests for all soil layers (Fig. 1a, e). At 0–10 cm and 11–25 cm depth, total P concentration was higher in the BF than that in the PF and was intermediate in MF (Fig. 1a). At 26–40 cm and 41–60 cm

Table 1 Soil chemical properties of the three successional forests at four depths in the Dinghushan Biosphere Reserve (DBR), Southern China

Soil depth	Forest	pH	SOC (g kg^{-1})	TN (g kg^{-1})	C:N	Al (μg g^{-1})	Fe (μg g^{-1})	Ca (μg g^{-1})	Mn (μg g^{-1})
0–10 cm	PF	3.69±0.02^{a}	48.06±2.92^{ab}	2.54±0.13^{b}	18.91±0.36^{a}	624.6±12.70^{b}	27.64±5.70^{a}	184.3±41.6^{a}	6.10±1.17^{a}
	MF	3.78±0.03^{a}	37.74±2.25^{b}	2.15±0.11^{b}	17.52±0.41^{b}	643.0±33.03^{b}	34.49±9.65^{a}	150.7±106.6^{a}	10.21±2.54^{a}
	BF	3.73±0.03^{a}	53.31±3.29^{a}	3.34±0.19^{a}	15.97±0.30^{c}	1085.5±58.11^{a}	53.46±5.33^{a}	53.83±9.19^{a}	5.34±0.97^{a}
11–25 cm	PF	4.09±0.02^{a}	10.54±0.44^{c}	0.72±0.03^{c}	14.71±0.40^{a}	340.7±14.25^{c}	3.61±0.71^{b}	118.7±99.77^{a}	2.05±0.40^{b}
	MF	4.01±0.03^{ab}	16.10±0.67^{b}	1.02±0.05^{b}	15.84±0.66^{a}	495.7±44.24^{b}	8.69±2.53^{b}	11.70±4.44^{a}	4.36±0.69^{a}
	BF	3.96±0.02^{b}	23.38±1.76^{a}	1.63±0.11^{a}	14.33±0.59^{a}	760.1±19.67^{a}	16.95±1.68^{a}	198.1±110^{a}	3.02±0.33^{ab}
26–40 cm	PF	4.16±0.04^{a}	6.83±0.30^{b}	0.70±0.04^{b}	10.03±0.96^{a}	323.0±20.37^{b}	1.53±0.67^{b}	91.24±74.54^{a}	1.66±0.37^{b}
	MF	4.15±0.03^{a}	8.49±0.49^{b}	0.72±0.06^{b}	12.06±0.72^{a}	456.4±40.12^{b}	1.85±0.39^{b}	30.19±18.04^{a}	4.31±1.15^{a}
	BF	4.03±0.01^{b}	12.57±1.07^{a}	1.05±0.09^{a}	12.06±0.73^{a}	531.3±21.94^{a}	4.89±0.69^{a}	112.6±60.89^{a}	2.76±0.23^{ab}
41–60 cm	PF	4.19±0.04^{a}	5.85±0.25^{b}	0.48±0.03^{b}	12.31±0.75^{a}	374.9±20.98^{a}	1.55±0.57^{a}	39.34±31.7^{a}	1.68±0.42^{a}
	MF	4.20±0.01^{a}	6.57±0.25^{ab}	0.60±0.03^{ab}	10.99±0.58^{a}	477.1±47.39^{a}	1.42±0.43^{a}	53.80±17.74^{a}	3.17±0.65^{a}
	BF	4.12±0.03^{a}	8.05±0.80^{a}	0.73±0.08^{a}	11.15±0.46^{a}	438.8±36.17^{a}	2.13±0.31^{a}	4.23±1.57^{a}	2.31±0.43^{a}

Values are means±standard errors (n=6). Within each depth, values in column followed by different superscript lowercase roman letters are significantly different at P<0.05

PF coniferous Masson pine forest, *MF* coniferous and broad-leaved mixed forest, *BF* monsoon evergreen broad-leaved forest, *SOC* soil organic C, *TN* total nitrogen

depth, total P concentration was higher in MF than that in PF and was intermediate in the BF (Fig. 1a). Although not significantly different between MF and BF at 0–10 cm soil depth and between MF and PF at 41–60 cm depth, occluded P concentrations were always higher in MF than those in the other stages for all soil layers (Fig. 1e). Organic P and intermediately available P_i concentrations were significantly affected by forest successional stage only for 0–10 cm and 11–25 cm soil depth (Fig. 1c, d). At 0–10 cm and 11–25 cm, organic P was significantly higher in BF than that in the other two stages (Fig. 1d), and intermediately available P_i concentrations were significantly higher in BF than those in the other two stages at 0–10 cm soil depth (Fig. 1c). Labile P_i and apatite P were not significantly affected by forest successional stage at any soil depth (Fig. 1b, f).

Percentages of total P present as soil P fractions

Organic P and occluded P were the dominant P fractions in all three forests at any soil depth; for example, at the 0–10 cm soil

Table 2 Soil microbial properties of the three successional forests at four depths in the Dinghushan Biosphere Reserve (DBR), Southern China

Soil depth	Forest	TB (nmol g^{-1})	G^+ (nmol g^{-1})	G^- (nmol g^{-1})	A (nmol g^{-1})	F (nmol g^{-1})	AM (nmol g^{-1})	F:B	G^+:G^-
0–10 cm	PF	21.66±0.80^{b}	4.92±0.35^{b}	4.46±0.16^{b}	0.38±0.01^{b}	2.18±0.25^{a}	0.33±0.01^{b}	0.23±0.02^{a}	1.12±0.11^{a}
	MF	25.09±1.60^{ab}	6.32±0.34^{ab}	4.93±0.39^{ab}	0.51±0.03^{b}	2.27±0.20^{a}	0.50±0.04^{a}	0.20±0.01^{a}	1.30±0.05^{a}
	BF	29.97±2.92^{a}	7.55±0.80^{a}	6.32±0.66^{a}	0.74±0.09^{a}	2.53±0.28^{a}	0.64±0.06^{a}	0.18±0.01^{a}	1.20±0.07^{a}
11–25 cm	PF	5.94±0.93^{b}	1.31±0.28^{b}	0.91±0.15^{b}	0.22±0.03^{b}	0.39±0.04^{b}	0.06±0.01^{b}	0.19±0.02^{a}	1.39±0.15^{a}
	MF	9.74±0.55^{ab}	2.40±0.18^{ab}	1.70±0.14^{a}	0.30±0.02^{ab}	0.74±0.07^{a}	0.16±0.01^{a}	0.18±0.02^{a}	1.43±0.11^{a}
	BF	14.03±2.44^{a}	3.58±0.74^{a}	2.51±0.41^{a}	0.51±0.09^{a}	0.98±0.18^{a}	0.21±0.05^{a}	0.16±0.01^{a}	1.42±0.14^{a}
26–40 cm	PF	5.84±1.32^{a}	1.27±0.37^{a}	0.87±0.21^{a}	0.19±0.05^{a}	0.36±0.07^{a}	0.05±0.02^{a}	0.20±0.03^{a}	1.37±0.10^{b}
	MF	5.52±0.99^{a}	1.30±0.28^{a}	0.66±0.15^{a}	0.17±0.04^{a}	0.33±0.06^{a}	0.07±0.02^{a}	0.17±0.01^{a}	1.99±0.07^{a}
	BF	9.95±2.03^{a}	2.38±0.67^{a}	1.40±0.43^{a}	0.29±0.09^{a}	0.60±0.12^{a}	0.13±0.04^{a}	0.18±0.02^{a}	1.77±0.07^{a}
41–60 cm	PF	3.58±0.63^{b}	0.72±0.17^{b}	0.35±0.05^{b}	0.09±0.03^{b}	0.21±0.02^{b}	0.01±0.01^{b}	0.23±0.04^{a}	1.92±0.25^{a}
	MF	4.89±0.70^{ab}	1.02±0.21^{ab}	0.51±0.10^{b}	0.13±0.04^{ab}	0.27±0.04^{b}	0.07±0.02^{ab}	0.19±0.02^{a}	1.98±0.10^{a}
	BF	8.21±1.28^{a}	2.00±0.39^{a}	1.10±0.24^{a}	0.25±0.05^{a}	0.47±0.06^{a}	0.11±0.02^{a}	0.17±0.02^{a}	1.90±0.12^{a}

Values are means±standard errors (n=6). Within each depth, values in column followed by different superscript lowercase roman letters are significantly different at P<0.05

PF coniferous Masson pine forest (pioneer successional stage), *MF* coniferous and broad-leaved mixed forest (transitional middle successional stage), *BF* monsoon evergreen broad-leaved forest (late successional stage), *TB* total microbial biomass, G^+ Gram-positive bacteria, G^- Gram-negative bacteria, *A* actinomycetes, *F* saprotrophic fungi, *AM* arbuscular mycorrhizal fungi, *F:B* saprotrophic fungi/bacteria (Gram-negative+Gram-positive) biomass ratio, G^+:G^- gram-positive bacteria/gram-negative bacteria biomass ratio

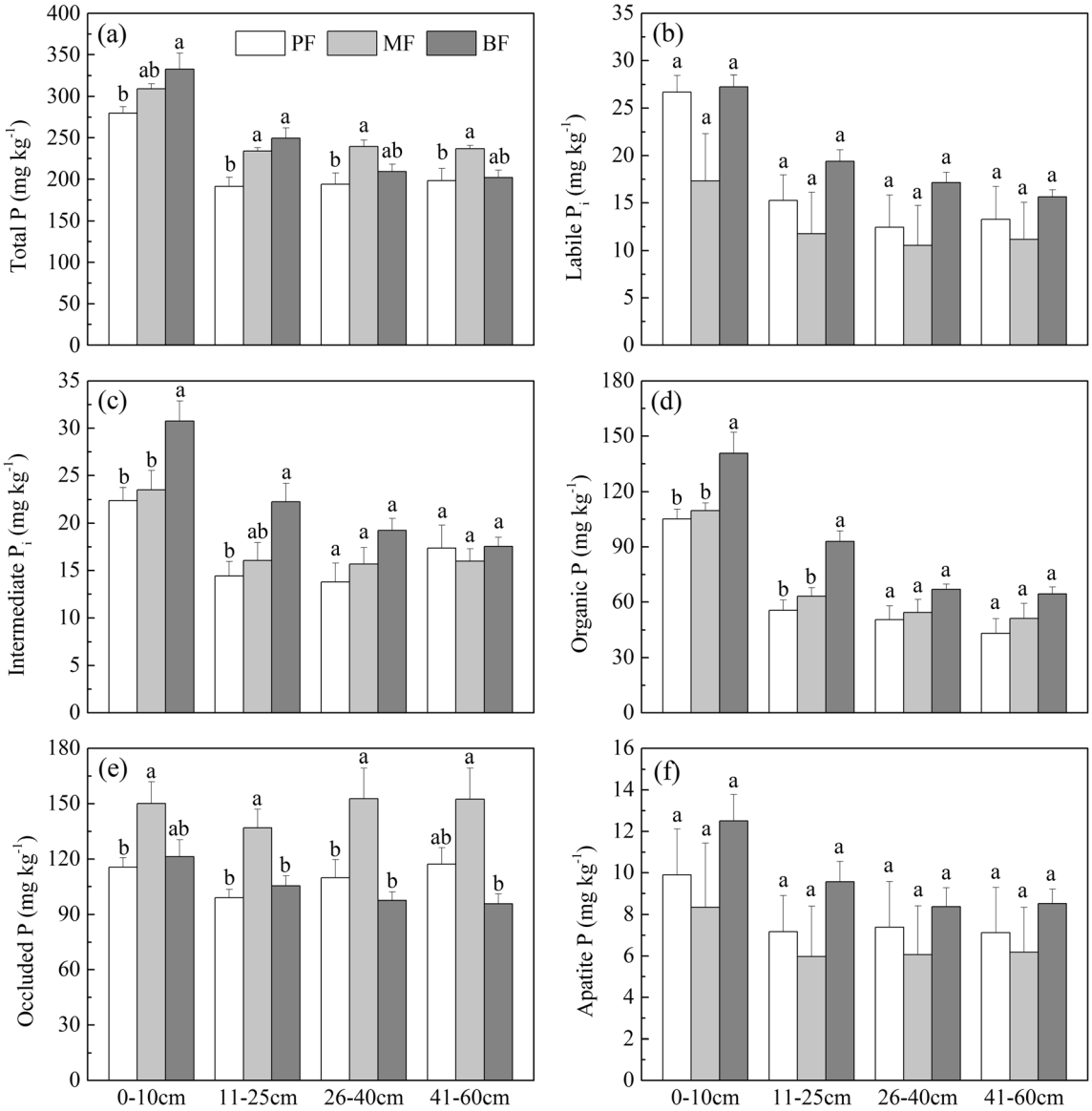

Fig. 1 Concentrations of soil total P (**a**), labile P_i (**b**), intermediately available P_i (**c**), organic P (**d**), occluded P (**e**), and apatite P (**f**) in the three successional forests at four soil depths at the Dinghushan Biosphere Reserve (DBR), Southern China. Values are means+standard errors (n=6). At each soil depth, means with *different letters* are significantly different at $P<0.05$. *PF* coniferous Masson pine forest, *MF* coniferous and broad-leaved mixed forest, *BF* monsoon evergreen broad-leaved forest

depth, these two fractions together accounted for 78.9, 84.2, and 78.6 % of the total P in the PF, MF, and BF, respectively (Fig. 2). The percentage of total P present as organic P was significantly affected by forest successional stage for all soil depths (Fig. 2). It was significantly higher in BF than that in MF and with no significant difference between MF and PF (Fig. 2). For example, 42.1 % of total P was present as organic P at 0–10 cm in the BF compared with 35.5 % in MF and 37.6 % in PF. The percentage of total P present as occluded P was significantly affected by forest successional stage only for the first two soil layers (Fig. 2). It was significantly lower in BF than that in MF and with no significant difference with PF at that two soil layers (Fig. 2). Percentages of total P present as labile P_i, intermediately available P_i (except for 26–40 cm), and apatite P were not significantly affected by forest successional stage (Fig. 2).

Soil acid phosphomonoesterase activity

Soil acid phosphomonoesterase activity was significantly affected by forest successional stage only at the 0–10 cm depth (Fig. 3; $P=0.03$). At that depth, acid phosphomonoesterase activity was significantly higher in BF and MF than that in PF (Fig. 3). Soil acid phosphomonoesterase activity was positively correlated with organic P ($n=72$, $r=0.49$, $P<0.01$), intermediately available P_i ($n=72$, $r=0.33$, $P<0.01$), and

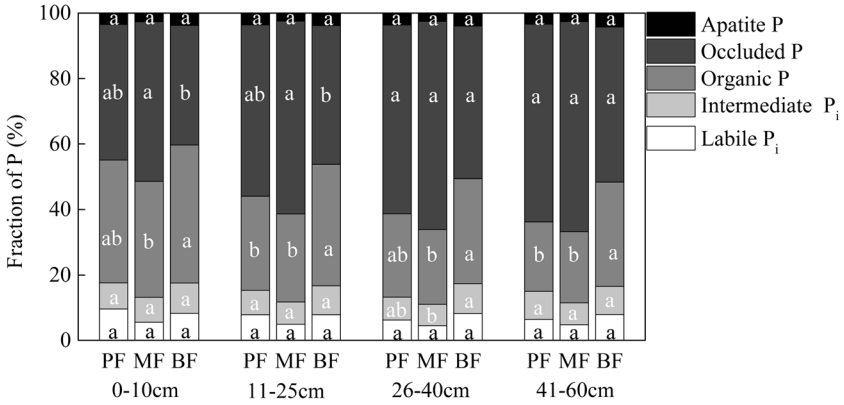

Fig. 2 The percentages of total P present as labile P_i, intermediately available P_i, organic P, occluded P, and apatite P relative to total P in the three successional forests at four soil depths at the Dinghushan Biosphere Reserve (DBR), Southern China. At each soil depth, means with *different letters* are significantly different at $P<0.05$. *PF* coniferous Masson pine forest, *MF* coniferous and broad-leaved mixed forest, *BF* monsoon evergreen broad-leaved forest

occluded P ($n=72$, $r=0.24$, $P<0.05$). Correlations were not significant between soil acid phosphomonoesterase activity and labile P_i or apatite P ($P>0.05$).

The relationships between P fractions and soil chemical and microbial properties

Redundancy analysis indicated that the environmental data (all soil chemical and microbial properties) explained 68.7 % of the variance of soil P fractions across the three successional forests for all soil layers, with axis 1 explaining 48.0 % of the variance and axis 2 explaining another 20.7 % (Fig. 4). For the surface soil (0–10 cm), the ordination biplot from RDA clearly distinguished the three successional forests (Fig. 4). The first axis of the RDA separated the soil P fractions of BF at 0–10 cm from those of the other two forests according to the higher contents of soil organic P and intermediately available P_i (Figs. 1 and 4). The high contents of these two soil P fractions in BF appeared to be associated with high soil microbial biomass, soil SOC, and TN, as well as with high concentrations of exchangeable Al and Fe (Fig. 4). These relationships in the RDA were consistent with the positive correlations between these properties and soil organic P and intermediately available P_i (Table 3). In contrast, soil P fractions in MF were segregated along the second axis, which was associated with

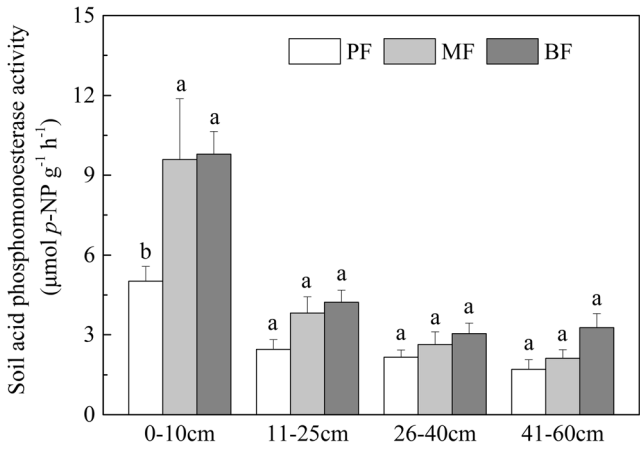

Fig. 3 Soil acid phosphomonoesterase activity in the three successional forests at four soil depths at the Dinghushan Biosphere Reserve (DBR), Southern China. Values are means+standard errors ($n=6$). At each soil depth, means with *different letters* are significantly different at $P<0.05$. *PF* coniferous Masson pine forest, *MF* coniferous and broad-leaved mixed forest, *BF* monsoon evergreen broad-leaved forest

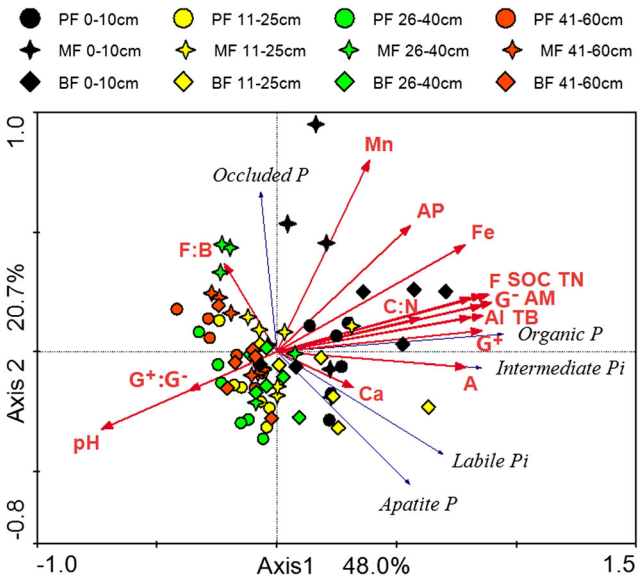

Fig. 4 Redundancy analysis of soil P fractions in the three successional forests at four soil depths at the Dinghushan Biosphere Reserve (DBR), Southern China. *PF* coniferous Masson pine forest, *MF* coniferous and broad-leaved mixed forest, *BF* monsoon evergreen broad-leaved forest, *AP* acid phosphomonoesterase activity, *TB* total microbial biomass, G^+ Gram-positive bacteria, G^- Gram-negative bacteria, *A* actinomycetes, *F* saprotrophic fungi, *AM* arbuscular mycorrhizal fungi, *F*:*B* saprotrophic fungi/bacteria (Gram-negative+Gram-positive) biomass ratio; G^+:G^-, Gram-positive bacteria/Gram-negative bacteria biomass ratio, *SOC* soil organic C, *TN* total nitrogen

Table 3 Spearman rank correlation coefficients between soil P fractions, acid phosphomonoesterase activity (AP), and soil microbial and chemical properties across the three successional forests and four soil depths in the Dinghushan Biosphere Reserve (DBR), Southern China

Soil property	Labile P_i	Intermediate P_i	Organic P	Occluded P	Apatite P
Chemical properties					
pH	−0.33**	−0.44**	−0.64**	−0.19	−0.01
SOC	0.50**	0.60**	0.77**	0.10	0.22
TN	0.44**	0.59**	0.75**	0.14	0.16
C:N	0.46**	0.43**	0.61**	0.10	0.22
Al	0.50**	0.67**	0.72**	0.09	0.31**
Fe	0.30*	0.45**	0.65**	0.25*	−0.02
Ca	0.62**	0.50**	0.50**	−0.07	0.49**
Mn	−0.02	0.18	0.39**	0.55**	−0.27*
Microbial properties					
AP	0.16	0.33**	0.49**	0.24*	−0.07
TB	0.63**	0.70**	0.81**	−0.06	0.45**
G^+	0.65**	0.73**	0.81**	−0.07	0.51**
G^-	0.61**	0.66**	0.81**	−0.04	0.41**
A	0.64**	0.72**	0.78**	−0.11	0.50**
F	0.58**	0.65**	0.79**	−0.04	0.36**
AM	0.62**	0.71**	0.82**	0.01	0.45**
F:B	−0.36**	−0.31**	−0.15	0.36**	−0.56**
G^+:G^-	−0.10	−0.09	−0.34**	−0.21	0.21

* and ** indicates significant correlation at $P<0.05$ and $P<0.01$, respectively

TB total microbial biomass, G^+ Gram-positive bacteria, G^- Gram-negative bacteria, *A* actinomycetes, *F* saprotrophic fungi, *AM* arbuscular mycorrhizal fungi, *F*:*B* saprotrophic fungi/bacteria (Gram-negative+Gram-positive) biomass ratio, G^+:G^- Gram-positive bacteria/Gram-negative bacteria biomass ratio, *SOC* soil organic C, *TN* total nitrogen

the high content of occluded P and the high concentration of exchangeable Mn in MF (Fig. 4, Table 1). Other correlations between soil P fractions and soil chemical and microbial properties are shown in Table 3.

Discussion

Our study revealed that total soil P and its fractions significantly changed with secondary forest succession at the DBR in Southern China (Fig. 1a, c, d, and e). In contrast to well-established conceptual model on long soil chronosequences which predicts that total soil P declines with succession (Walker and Syers 1976; Turner et al. 2013), the total soil P increases significantly in late successional forest compared with early successional PF in the present study (Fig. 1a). The different result is mainly attributed to the time scales of soil development we considered. In the long soil chronosequence studies, P declines with succession because of mineral weathering and P loss through runoff (Turner et al. 2013), whereas in the present study, all the three successional forests are developed on soils with similar weathering stages in the same tropical region. The mechanisms underlying the low total soil P in early successional PF could be the vegetation uptake: the PF would take up more P from soil than BF due to relatively higher net primary production (Tang et al. 2011). In addition, the P concentrations in leaves of *P. massoniana*, a dominant tree species in PF, are highest compared with dominant trees in other two forests (Huang et al. 2013).

The changes of soil P fractions along this secondary forest succession also differ with long-term ecosystem development (Turner et al. 2013). Unlike the occluded P fraction greatly increased and intermediate P_i decreased in late soil development stage (Walker and Syers 1976; Yang and Post 2011), we found that occluded P fraction was only significantly increased in middle successional forest (Fig. 1c) and organic P and intermediate Pi were significantly increased in late successional forest (Fig. 1c, d). The finding was similar with a previous study conducted at a Puerto Rico tropical forest where non-occluded P and organic P increased but occluded P decreased with forest development (Frizano et al. 2002).

Along with the secondary forest succession, organic P could increase in late successional forest mainly due the accumulation of soil organic C and N (Zhou et al. 2006; Tang et al. 2011). Previous studies in the same site showed that more soil organic C accumulates in this late successional forest (Zhou et al. 2006; Tang et al. 2011) because of high annual litterfall production (Zhou et al. 2007). Soil organic matter could contain substantial quantities of P (McGill and Cole 1981),

resulting in organic P accumulation. This was supported by the positive relationship between soil organic P and organic C and C:N ratio in this study (Table 3) and previous studies (e.g., Garcia-Monteil et al. 2000). In addition, increase in soil N may also increase organic P in the late succession through indirectly stimulating plant production and then higher C input into soil. In addition, a higher microbial biomass and biomasses of all individuals were found in late successional forest, together with a strong positive correlation between microbial biomass (total and all individual groups) and organic P (Table 3). Microbial biomass constitutes a large pool of soil P, and microorganisms mediate several key processes in the biogeochemical P cycle (Oberson and Joner 2005; Bunemann et al. 2011). More importantly, soil microorganisms can efficiently compete for inorganic P in the soil solution with plants (Olander and Vitousek 2004) and can transform inorganic P into organic forms. The third possible mechanism underlying the increase in soil organic P with succession may be the increase in exchangeable Fe and Al with succession in these soils (Table 1), because previous study suggested that most of the organic P is associated with Fe and Al oxides (Giesler et al. 2002; Giaveno et al. 2008). Consistent with this mechanism, our study indeed showed strong positive correlations between organic P and exchangeable Fe and Al contents (Table 3). In addition, we also found a weakly positive relationship between organic P and exchangeable Ca and Mn. This is mainly because both of the two cations may react with organic P in soil (Sims and Pierzynski 2005).

However, the pH decline may have induced the release of more exchangeable Fe and Al (Sanchez 1976). This could have caused the increase in intermediately available P_i associated with aluminum/iron minerals in the late successional forest soil. A study in lowland Amazonian forest ecosystem found that a substantial proportion of added P was transformed into intermediately available P_i due to the ability of exchangeable Fe and Al to quickly adsorb inorganic P dissolved in the soil solution and transform it into intermediately available P_i (McGroddy et al. 2008). The increase in intermediately available P_i in late successional forest could be also due to the increase in the biomass of all microbial groups and acid phosphomonoesterase activity which may enhance the potential of inorganic P release through mineralization (Olander and Vitousek 2004; Jones and Oburger 2011).

Occluded P is considered the most recalcitrant P fraction and is unavailable to plants and soil microorganisms at least over short time scales (Tiessen and Moir 2007). The present study found that the content of occluded P in soil changed with forest succession at the DBR, where the content was usually higher in the middle successional stage than that in the early and late successional stages (Fig. 1e). The accumulation of occluded P in the middle successional forest may be explained by a coupled microbial and chemical mechanism. Higher soil acid phosphomonoesterase activity in this successional stage may accelerate the release of P_i into soil solutions (Nannipieri et al. 2011; Marklein and Houlton 2012), which could be quickly precipitated by the high concentrations of exchangeable Mn (Table 2) and subsequently transformed into solid forms and then into occluded forms (Sanyal and De Datta 1991; Sims and Pierzynski 2005). This explanation is indirectly supported by the relatively low labile P_i content in middle successional forest (Fig. 1b) and by the weakly positive correlation between the occluded P fraction and acid phosphomonoesterase activity. In addition, the composition of microbial community (F:B) and exchangeable Fe also positively related to occluded P fraction (Table 3). This is because the potential of mineralization would be enhanced by a soil microbial community dominated by fungi (Jones and Oburger 2011), and the exchangeable Fe is an efficient agent to occlude inorganic P (Sims and Pierzynski 2005). In addition, P biocycling may be enhanced by low amount of soil P as observed in previous studies (Celi et al. 2013), so enhanced P_i release from microbial mineralization in P-deficient and Fe/Al-rich tropical soils may also contribute to accumulation of occluded P.

Conclusions

This study revealed that soil P fractions change significantly with forest succession at the DBR. In particular, fractions of potential sources of labile P_i (i.e., organic P and intermediately available P_i) were greater in the late successional stage than those in earlier successional stages. Across the three successional forests, soil labile P_i, organic P, and intermediately available P_i were closely related to microbial and chemical properties. The positive relationships suggested that both microbial and chemical processes help maintain the high levels of available or potentially available P fractions during this forest succession. Our results also suggest that P cycling will remain active and efficient in the late successional forest because of relative higher soil microbial biomass and because of larger pool of potential sources of available P fractions in this late successional stage than in the two earlier stages. Overall, our study indicates that unlike soil development and primary succession, secondary succession in tropical forest ecosystems may not always decrease P availability.

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