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Physiological linkage in co-variation of foliar nitrogen and phosphorus in tropical tree species along a gradient of soil phosphorus availability

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Abstract: In order to understand the stoichiometric balance between foliar nitrogen (N) and phosphorus (P) on P-poor soils, we investigated how foliar N and P attributes (i.e. N and P concentrations in green and senesced leaves, N and P resorption efficiencies) of 30 tropical tree species co-vary along a gradient of soil P availability across three forests on Mount Kinabalu, Borneo. We found strong and positive correlations between foliar N and P in the concentrations and resorption efficiencies within each forest and across the three forests. Slopes of standardized major axis between foliar N and P concentrations for both green and senesced leaves were not different among the three forests, although the values of the scaling exponent in the relationships of foliar N to P across the three forests were significantly lower than 1. We suggest that down-regulation of N concentrations in green leaves on P-poor soils is one of several possible mechanisms explaining why N concentrations decrease with decreasing P concentrations in both green and senesced leaves toward a lower P availability in soils. On the other hand, the physiological and ecological reasons why N and P resorption efficiencies are positively correlated with each other across tree species remain unclear.

Key Words: leaf traits, litter, nitrogen, nutrient limitation, phosphorus, resorption, stoichiometry, tropical montane rain forest

INTRODUCTION

Nitrogen (N) and phosphorus (P) are essential nutrients in plant metabolism (Marschner 1995), and their availabilities in soils frequently regulate plant growth and productivity in terrestrial ecosystems (Elser *et al*. 2007, Harpole *et al*. 2011). N and P concentrations in green leaves are important leaf traits of plant species, because they relate functionally to photosynthetic assimilation rates and growth rates (Aerts & Chapin 2000, Ågren 2008, Wright *et al*. 2004). In addition, foliar N and P are the important components controlling carbon and nutrient cycles and food webs in terrestrial ecosystems (Chapin *et al*. 2011). Therefore, knowledge of foliar N and P attributes in relation to nutrient availabilities provides important insight into understanding plant nutritional strategies and terrestrial ecosystem functions. In tropical regions, tree growth and productivity are frequently limited by low P availability in soils (Cleveland *et al*. 2011, Vitousek 1984). Therefore, foliar P attributes and P-use strategies of tropical tree species have been investigated in relation to soil P availability (Cordell *et al*. 2001, Hidaka & Kitayama 2009, 2011). On the other hand, how tropical tree species control foliar N dynamics in relation to efficient foliar P-use under P limitation remains unclear, although it is suggested that soil P availability may influence N cycle in tropical rain forests (Hall *et al*. 2004, Kitayama *et al*. 1998, Quesada *et al*. 2010).

The stoichiometric balance between N and P in green leaves has been a particular focus in understanding life-history strategies of plant species (e.g. photosynthesis and growth rates) (Ågren 2008, Niklas *et al*. 2005, Reich *et al*. 2009). Earlier studies showed that N and P concentrations in green leaves are positively correlated with each other within and among various plant species (Kerkhoff *et al*. 2006, Niklas *et al*. 2005, Reich *et al*. 2010, Wright *et al*. 2004). It was suggested that a general 2/3- or 3/4-power law exists in the scaling relationship of N to P concentrations in green leaves among plant species across biomes (Niklas *et al*. 2005, Reich *et al*. 2010). This finding suggests that foliar N:P ratio increases with decreasing foliar P concentration among plant species, although

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foliar N concentration decreases with decreasing foliar P concentration. Because foliar P concentration generally decreases with decreasing soil P availability (Cleveland *et al*. 2011, Hidaka & Kitayama 2009), plant species will control the stoichiometric balance between N and P in green leaves and consequently disproportionately increase foliar N:P ratio in response to a lower P availability in soils. On the other hand, how the positive correlation between foliar N and P varies in response to a lower P availability in soils is poorly understood.

Nutrient resorption prior to leaf abscission is one of the important foliar strategies to conserve growth-limiting nutrients, because the retranslocated nutrients are used for new leaves and other organs for plant productivity and growth (Aerts 1996, Killingbeck 1996). Species-level N and P resorption efficiencies (i.e. the proportion of reduction in N and P concentration from green to senesced leaves) (NRE and PRE, respectively) have been frequently investigated in various vegetations (Kobe *et al*. 2005, Vergutz *et al*. 2012, Yuan & Chen 2009). Reed *et al*. (2012) suggested that the ratio of NRE per PRE (i.e. N:P resorption ratio) reflects soil P availability in tropical rain forests, and that tropical tree species growing on P-poor soils translocate more P than N prior to leaf abscission. On the other hand, the importance of N resorption of tropical tree species is not well characterized with soil P availability. Although several experiments showed that P fertilization decreases or does not change NRE of tropical tree species (Cordell *et al*. 2001, Mayor *et al*. 2014, Treseder & Vitousek 2001), how tropical tree species control N resorption on P-poor soils remains unclear.

Our aim in this study is to test the following hypotheses for understanding how foliar N and P attributes co-vary with each other along a gradient of soil P availability. First, the magnitude of reduction of foliar N concentration with decreasing P availability is smaller than that of foliar P concentration, because soil P availability more strongly and directly influences foliar P than foliar N. Secondly, tree species translocate more P than N prior to leaf abscission and N:P resorption ratio decreases with decreasing P availability. We investigated N and P concentrations and N:P ratios in green and senesced leaves, NRE, PRE and N:P resorption ratio of 30 tropical tree species across three forests with differing soil P availability on Mount Kinabalu, Borneo.

METHODS

Study sites

This study was conducted in three tropical montane rain forests on the southern slopes of Mount Kinabalu (6°05′N, 116°33′E, 4095 m asl), Sabah, Malaysian Borneo. These forests are the same as those in Hidaka & Kitayama (2011) and are called lower slope sites in Takyu *et al*. (2002). The three study sites were nearly the same in altitude (1560–1860 m asl) and had a comparable climate (mean annual air temperature was 18°C and mean annual precipitation was 2700 mm) (Kitayama 1992). On the other hand, the three sites were different in soil P availability due to the differences in geological substrates (Quaternary and Tertiary sedimentary rocks and ultrabasic rock) (Takyu *et al*. 2002). Hereafter, we name the three sites as Site 1 (ultrabasic), Site 2 (Tertiary sediment) and Site 3 (Quaternary sediment) in the increasing order of soil P availability (0.02, 0.12 and 0.19 g m^{-2} soluble P, respectively) (Table 1). Inorganic-N pool (i.e. NO_3 + NH_4) in soils and rates of net soil N mineralization decreased, and soil C:N ratio increased with decreasing soil P availability across the three forests (Table 1) (Hall *et al*. 2004, Kitayama *et al*. 2004, Takyu *et al*. 2002). It was suggested that soil N availability was down-regulated by soil P availability via activity of microbes (i.e. ammonifier and nitrifying bacteria) (Hall *et al*. 2004, Kitayama *et al*. 1998, 2004). Although soil N availability decreased with decreasing soil P availability across the three sites, the magnitude of soil P variation was greater than that of soil N variation, and soil P availability rather than soil N availability more strongly influenced plant nutritional strategies and ecosystem processes (Kitayama & Aiba 2002, Kitayama *et al*. 2004, Takyu *et al*. 2003).

Table 1. Soil properties (top 15 cm depth) of nitrogen (N) and phosphorus (P) in three tropical montane rain forests on Mount Kinabalu. Data are from Takyu *et al*. (2002, 2003).

	Site 1	Site 2	Site 3
C:N ratio	20.3	17.4	13.0
Inorganic N			
NH_4-N (g m^{-2})	1.27 ± 0.34	1.54 ± 0.37	0.66 ± 0.19
NO_3-N (g m^{-2})	0.02 ± 0.01	0.01 ± 0.02	1.11 ± 0.24
NH_4+ NO_3-N (g m^{-2})	1.29	1.56	1.77
Soluble P (g m^{-2})	0.02 ± 0.16	0.12 ± 0.03	0.19 ± 0.09

Sampling and chemical analysis

In each forest, we selected 10 dominant tree species (Table 2), of which seven species were previously studied for understanding foliar P-use strategies (Hidaka & Kitayama 2011) and three species were additionally selected in this study. We collected sun leaves of three to five trees per species from crown tops using a catapult, and collected fresh fallen leaf litter at 2-d intervals using litter traps on the ground within each permanent plot (2000 m^2 in Site 1, 10 000 m^2 in Site 2 and Site 3). After drying these samples at 60°–70°C for 72 h to a constant weight, each sample including veins after removing petiole was ground for the measurement of nutrients. N concentration was measured using a CN

Table 2. Nitrogen (N) and phosphorus (P) concentrations (mg g^{-1}) and N:P ratios (mass basis) in green and senesced leaves, N and P resorption efficiencies corrected with calcium concentrations (NRE and PRE, respectively) (%), and N:P resorption ratio (i.e. ratio of NRE per PRE) of 30 tree species in three tropical montane rain forests on Mount Kinabalu. A part of the data is from Hidaka & Kitayama (2011).

		Green leaves			Senesced leaves			Resorption		
Species	Family	N	P	N:P	N	P	N:P	NRE	PRE	N:P
Site 1										
Agathis kinabaluensis de Laub.	Araucariaceae	11.1	0.37	29.8	6.5	0.10	62.1	51.6	76.9	0.67
Dacrydium pectinatum de Laub.	Podocarpaceae	9.9	0.42	23.5	6.3	0.11	55.5	47.5	77.7	0.61
Garcinia sp.	Clusiaceae	10.0	0.31	32.4	7.8	0.14	54.2	23.0	53.8	0.43
Gynotroches axillaris Blume	Rhizophoraceae	13.2	0.47	28.3	9.2	0.19	47.8	37.9	63.4	0.60
Lithocarpus rigidus Soepadmo	Fagaceae	10.0	0.25	39.5	5.4	0.08	68.4	53.0	73.0	0.73
Podocarpus gibbsiae N.E.Gray	Podocarpaceae	9.7	0.27	36.0	6.9	0.12	59.6	59.6	75.5	0.79
Quercus lowii King	Fagaceae	11.6	0.33	35.5	7.1	0.09	76.6	58.5	80.8	0.72
Tristaniopsis cf. *elliptica* (Stapf) Peter G.Wilson & J.T.Waterh.	Myrtaceae	10.4	0.28	37.9	4.5	0.04	110.3	48.2	82.2	0.59
Weinmannia cf. *blumei* A.Gray	Cunoniaceae	9.7	0.24	41.0	3.8	0.05	71.3	64.6	79.7	0.81
Xanthophyllum tenue Chodat	Polygalaceae	20.8	0.49	42.4	13.5	0.22	61.3	54.8	68.7	0.80
Site 2										
Aglaia squamulosa King	Meliaceae	25.5	1.06	24.2	13.4	0.29	46.2	67.0	82.8	0.81
Dacrycarpus imbricatus (Blume) de Laub.	Podocarpaceae	15.1	0.74	20.5	9.3	0.26	35.6	57.8	75.8	0.76
Dacrydium gracile de Laub.	Podocarpaceae	14.0	0.54	25.7	7.5	0.20	37.6	50.6	66.2	0.76
Lithocarpus clementianus (King ex Hook.f.) A.Camus	Fagaceae	13.7	0.44	30.9	8.9	0.15	57.6	67.8	82.8	0.82
Payena microphylla (de Vriese) Burck	Sapotaceae	14.3	0.53	26.9	9.9	0.25	39.0	52.3	67.1	0.78
Syzygium kunstleri (King) Bahadur & R.C.Gaur	Myrtaceae	13.0	0.75	17.4	6.6	0.17	39.1	68.8	86.1	0.80
Syzygium napiforme (Koord. & Valeton) Merr. & L.M.Perry	Myrtaceae	12.4	0.43	29.0	5.9	0.15	38.6	66.9	75.1	0.89
Syzygium pachysepalum Merr. & L.M.Perry	Myrtaceae	11.0	0.41	26.5	5.3	0.14	37.8	60.7	72.4	0.84
Tristaniopsis clementis (Merr.) Peter G.Wilson & J.T.Waterh.	Myrtaceae	10.2	0.42	24.1	4.1	0.08	54.3	48.1	76.9	0.63
Tristaniopsis sp.	Myrtaceae	14.9	0.49	30.7	7.7	0.20	39.4	66.0	73.6	0.90
Site 3										
Cinnamomum subcuneatum Miq.	Lauraceae	16.2	0.66	24.6	15.7	0.63	25.2	8.0	9.8	0.82
Lithocarpus confertus Soepadmo	Fagaceae	18.7	0.66	28.2	17.6	0.63	28.0	11.8	11.2	1.05
Lithocarpus lampadarius (Gamble) A.Camus	Fagaceae	16.5	0.62	26.5	13.2	0.49	27.1	22.1	24.2	0.91
Litsea ochracea (Blume) Boerl.	Lauraceae	17.6	0.83	21.3	11.3	0.28	40.1	37.2	66.7	0.56
Madhuca endertii H.J.Lam	Sapotaceae	18.1	0.70	26.1	14.9	0.65	23.0	19.0	8.6	2.21
Magnolia carsonii Dandy ex Noot.	Magnoliaceae	19.8	0.75	26.2	15.9	0.55	29.0	29.8	36.4	0.82
Syzygium castaneum (Merr.) Merr. & L.M.Perry	Myrtaceae	17.3	0.68	25.4	11.1	0.51	21.8	41.1	31.5	1.30
Syzygium napiforme (Koord. & Valeton) Merr. & L.M.Perry	Myrtaceae	12.3	0.56	22.0	5.6	0.21	26.6	70.2	75.4	0.93
Syzygium pachysepalum Merr. & L.M.Perry	Myrtaceae	13.9	0.46	29.9	7.9	0.34	23.4	50.8	36.9	1.38
Ternstroemia magnifica Stapf ex Ridl.	Theaceae	11.6	0.44	26.7	7.2	0.23	31.4	44.7	53.1	0.84

analyser (JM 1000CN, J-Science Lab Co., Kyoto, Japan). P and calcium (Ca) concentrations were measured using an inductively coupled plasma atomic emission spectrometer (ICPS-7510; Shimadzu Co., Kyoto, Japan) after digesting samples with H_2SO_4 and H_2O_2. After calculating mean concentrations of N, P and Ca in green and senesced leaves in each species, mean NRE and PRE per species was respectively calculated as the following equation for correcting a loss of leaf mass by using Ca concentrations: NRE (%) or PRE (%) = 100 − (N or P concentration in green leaves/N or P concentration in senesced leaves) × (Ca concentration in senesced leaves/Ca concentration in green leaves) × 100.

Statistical analysis

We tested the differences in mean values of foliar N and P attributes among the three forests by ANOVA with a post hoc Tukey HSD test. The relationships of

Table 3. Mean values ± SE (CV) of nitrogen (N) and phosphorus (P) concentrations (mg g^{-1}) and N:P ratios (mass basis) in green and senesced leaves, N and P resorption efficiencies corrected with calcium concentrations (NRE and PRE, respectively) (%), and N:P resorption ratio (NRE:PRE) of tree species on three tropical montane rain forests with differing soil P availability on Mount Kinabalu, Borneo. P-values are shown for testing differences in the mean values among the three sites. Pairwise significant differences at $P < 0.05$ among sites are shown in different letters.

	All (n = 30 spp.)	Site 1 (n = 10 spp.)	Site 2 (n = 10 spp.)	Site 3 (n = 10 spp.)	P-values
Green leaves					
N (mg g^{-1})	14.1 ± 0.7 (27.6)	11.6^{b} ± 1.1 (29.2)	14.4^{ab} ± 1.3 (29.2)	16.2^{a} ± 0.9 (16.9)	0.02
P (mg g^{-1})	0.52 ± 0.04 (37.2)	0.34^{b} ± 0.03 (26.5)	0.58^{a} ± 0.07 (36.0)	0.64^{a} ± 0.04 (19.1)	<0.001
N:P ratio	28.6 ± 1.9 (36.7)	34.6^{a} ± 1.9 (36.7)	25.6^{b} ± 1.4 (16.8)	25.7^{b} ± 0.8 (10.1)	<0.001
Senesced leaves					
N (mg g^{-1})	9.0 ± 0.7 (42.6)	7.1^{b} ± 0.9 (38.6)	7.9^{b} ± 0.8 (34.0)	12.0^{a} ± 1.3 (34.2)	0.004
P (mg g^{-1})	0.25 ± 0.03 (72.4)	0.11^{b} ± 0.02 (50.0)	0.19^{b} ± 0.02 (33.9)	0.45^{a} ± 0.05 (38.1)	<0.001
N:P ratio	45.6 ± 3.6 (43.4)	66.7^{a} ± 5.5 (26.3)	42.5^{b} ± 2.4 (17.9)	27.6^{c} ± 1.7 (19.2)	<0.001
Resorption					
NRE (%)	48.0 ± 3.2 (36.5)	49.9^{a} ± 3.8 (24.1)	60.6^{a} ± 2.5 (13.0)	33.5^{b} ± 6.1 (57.4)	<0.001
PRE (%)	61.5 ± 4.3 (38.6)	73.2^{a} ± 2.8 (12.2)	75.9^{a} ± 2.1 (8.7)	35.4^{b} ± 7.4 (66.6)	<0.001
NRE:PRE	0.85 ± 0.06 (37.6)	0.68^{b} ± 0.04 (17.8)	0.80^{ab} ± 0.02 (9.6)	1.08^{a} ± 0.15 (42.8)	0.01

nutrient concentrations between green and senesced leaves, and between N and P concentrations in green and senesced leaves were analysed after base-10 log-transformation, because earlier studies have shown that these are best expressed as scaling relationships (Kobe *et al.* 2005, Reich *et al.* 2010). We also tested the difference in their relationships in standardized major axis (SMA) among the three forests by using *SMATR* (Warton *et al.* 2012). According to our hypothesis, slopes of SMA between foliar N and P concentrations for both green and senesced leaves will become flatter with decreasing P availability, although foliar N concentration decreases with decreasing P availability. Statistical analyses were performed using R 2.14.1 (http://www.R-project.org).

RESULTS

Foliar N concentrations and N resorption efficiency

N concentration in green leaves (N_G) varied 2.6-fold from 9.7 mg g^{-1} in *Podocarpus gibbsiae* and *Weinmannia* cf. *blumei* at Site 1 to 25.5 mg g^{-1} in *Aglaia squamulosa* at Site 2, and N concentration in senesced leaves (N_S) varied 4.6-fold from 3.8 mg g^{-1} in *Weinmannia* cf. *blumei* at Site 1 to 17.6 mg g^{-1} in *Lithocarpus confertus* at Site 3 (Table 2). Both mean N_G and N_S significantly increased from Site 1 to Site 3 with increasing soil P availability (Table 3). Across the three sites, N_G and N_S were positively correlated with each other, and the value of the scaling exponent in the relationship was significantly higher than 1 (1.40 ± 0.15) (Figure 1a), and the SMA slope of N_S to N_G was significantly higher than 1 (Table 4). N_G and N_S were also significantly and positively correlated with each other within each site, and the SMA slopes were higher than 1 at Site 3, but not different from 1 at Site 1 and Site 2, and were not different among the three sites (Figure 1c, Table 4). N resorption efficiency varied 8.6-fold from 8.0% in *Cinnamomum subcuneatum* at Site 3 to 68.8% in *Syzygium kunstleri* at Site 2 (Table 2), and the mean was significantly higher at Site 1 and Site 2 than at Site 3 (Table 3).

Foliar P concentrations and P resorption efficiency

P concentration in green leaves (P_G) varied 4.4-fold from 0.24 mg g^{-1} in *Weinmannia* cf. *blumei* at Site 1 to 1.06 mg g^{-1} in *Aglaia squamulosa* at Site 2, and P concentration in senesced leaves (P_S) varied 16-fold from 0.04 mg g^{-1} in *Tristaniopsis* cf. *elliptica* at Site 1 to 0.65 mg g^{-1} in *Madhuca endertii* at Site 3 (Table 2). Both mean P_G and P_S significantly increased from Site 1 to Site 3 with increasing soil P availability (Table 3). Across the three sites, P_G and P_S were positively correlated with each other, and the value of the scaling exponent in the relationship was significantly higher than 1 (1.55 ± 0.22) (Figure 1b), and the SMA slope of P_S to P_G was significantly higher than 1 (Table 4). P_G and P_S were also positively correlated with each other at Site 1 and Site 2 but not at Site 3, and the SMA slopes were higher than 1 at Site 1, but not different from 1 at Site 2 (Figure 1d, Table 4). P resorption efficiency varied 10-fold from 8.6% in *Madhuca endertii* at Site 3 to 86.1% in *Syzygium kunstleri* at Site 2 (Table 2), and the mean was significantly higher at Site 1 and Site 2 than at Site 3 (Table 3).

Relationships between foliar N and P in the concentrations and resorption efficiencies

N and P concentrations were positively correlated with each other in both green and senesced leaves across the three sites, and the values of the scaling exponent in

Table 4. Standardized major axis (SMA) relationships of N and P concentrations in green and senesced leaves (N_G, P_G, N_S and P_S, respectively) and of N and P resorption efficiencies (NRE and PRE, respectively) of tree species on three tropical montane rain forests with differing soil P availability on Mount Kinabalu, Borneo. Slopes (lower and upper 95% confidence intervals) for SMA regressions in the upper line and R^2 and P-values in the lower line. P-values are shown for testing differences in slopes among the three sites.

Y	X	All (n = 30 spp.)	Site 1 (n = 10 spp.)	Site 2 (n = 10 spp.)	Site 3 (n = 10 spp.)	P-values
N_S	N_G	1.62 (1.33, 1.96) 0.75, <0.001	1.52 (0.95, 2.42) 0.65, 0.005	1.38 (0.97, 1.96) 0.81, <0.001	2.14 (1.50, 3.05) 0.80, <0.001	0.17
P_S	P_G	1.96 (1.55, 2.48) 0.63, <0.001	2.07 (1.24, 3.45) 0.57, 0.01	1.19 (0.69, 2.07) 0.50, 0.02	2.15 (1.11, 4.15) 0.25, 0.14	NA
N_G	P_G	0.69 (0.56, 0.85) 0.72, <0.001	0.91 (0.53, 1.56) 0.53, 0.02	0.78 (0.50, 1.23) 0.67, 0.004	0.89 (0.59, 1.35) 0.73, 0.002	0.87
N_S	P_S	0.57 (0.47, 0.68) 0.78, <0.001	0.67 (0.50, 0.89) 0.87, <0.001	0.90 (0.64, 1.28) 0.81, <0.001	0.89 (0.64, 1.24) 0.83, <0.001	0.25
NRE	PRE	0.74 (0.61, 0.90) 0.74, <0.001	1.35 (0.85, 2.14) 0.66, 0.004	1.20 (0.68, 2.12) 0.46, 0.03	0.81 (0.54, 1.23) 0.73, 0.002	0.22

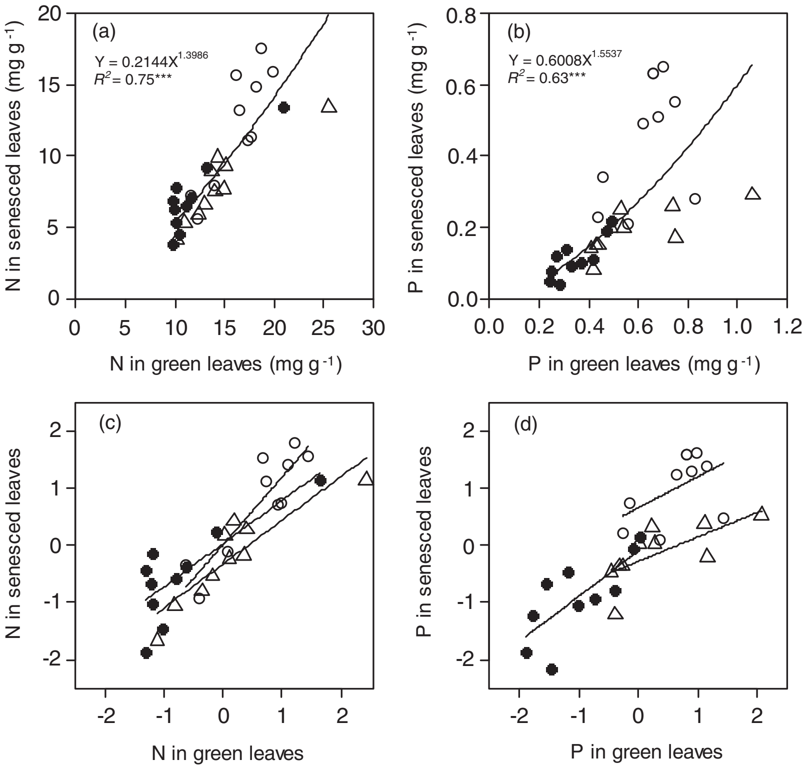

Figure 1. The relationships of N (a) and P (b) concentrations in green and senesced leaves, and their relationships in standardized major axis (SMA) after base-10 log-transformation (c, d) of 30 tropical tree species on Mount Kinabalu. Symbols: Site 1 (solid circles), Site 2 (triangles) and Site 3 (open circles). Detailed SMA slopes are shown in Table 3.

the relationships were both significantly lower than 1 (0.58 ± 0.07 in green leaves, 0.50 ± 0.05 in senesced leaves) (Figure 2a, b), and the SMA slopes of N_G to P_G and of N_S to P_S across three forests were significantly lower than 1 (Table 4). N and P concentrations were also positively correlated with each other in both green and senesced leaves within each site (all $P < 0.05$) (Figure 2d, e, Table 4), and the SMA slopes of N_G to P_G and of N_S to P_S within each site were not different among sites (Table 4).

N:P ratio in green leaves varied 2.4-fold from 17.4 in *Syzygium kunstleri* at Site 2 to 42.4 in *Xanthophyllum tenue* at Site 1 (Table 2), and the mean was significantly higher at Site 1 than at Site 2 and Site 3 (Table 3). N:P ratio in senesced leaves varied 5.1-fold from 21.8 in *Syzygium castaneum* at Site 3 to 110 in *Tristaniopsis* cf. *elliptica* at Site 1 (Table 2), and the mean value increased significantly with decreasing soil P availability (Table 3).

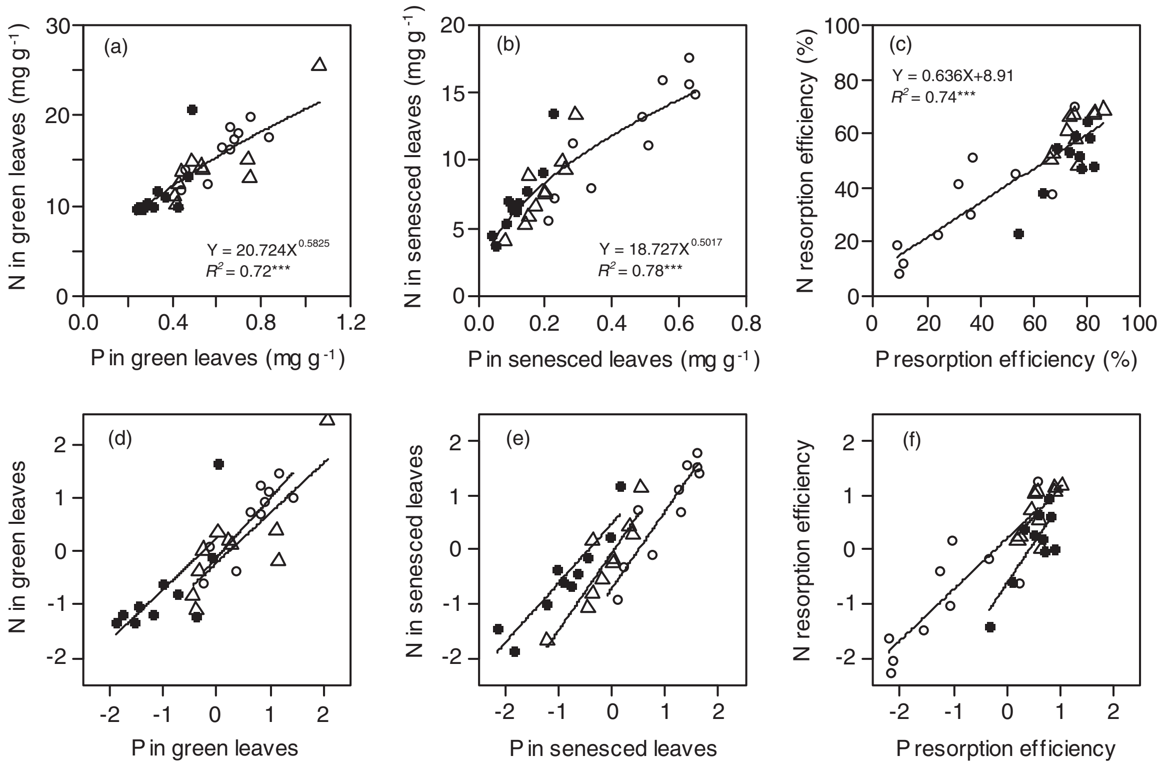

Figure 2. The relationships between N and P concentrations in green (a) and senesced (b) leaves, and between N- and P-resorption efficiencies (c), and their relationships in standardized major axis (SMA) after base-10 log-transformation (d, e, f) of 30 tropical tree species on Mount Kinabalu. Symbols are the same as in Figure 1. Detailed SMA slopes are shown in Table 3.

NRE and PRE were positively correlated with each other across the three sites ($P < 0.001$) (Figure 2c) and within each site (all $P < 0.05$) (Figure 2f). N:P resorption ratio was significantly higher at Site 3 than at Site 1, and was not different between Site 1 and Site 2 and between Site 2 and Site 3 (Table 3).

DISCUSSION

In this study, we found strong and positive correlations between foliar N and P in the concentrations and resorption efficiencies within each forest and across the three forests with differing soil P availability. Such positive correlations across the forests may be partly influenced by the difference of soil N availability among the forests; it was suggested that soil N availability was down-regulated by soil P availability in our study sites (Hall *et al.* 2004, Kitayama *et al.* 1998, 2004). On the other hand, the positive correlations within each forest cannot be explained by co-variation of soil N and P availability alone, because the small-scale spatial heterogeneity of soil N does not always correspond to soil P. Rather, observed patterns within each forest can be explained by plant nutritional strategies; foliar N-use strategy is influenced by foliar P-use strategy in each tree species in response to soil P availability.

Against our hypothesis, there was no difference in SMA slopes between foliar N and P concentration for both green and senesced leaves among the three forests. This result suggests that foliar N strongly decreases with decreasing foliar P within each forest and across the three forests, although the scaling relationship of foliar N to P (Figure 2a, b) suggests that soil P availability more strongly and directly influences the variation of foliar P than that of foliar N. We suggest that down-regulation of N concentrations in green leaves on P-poor soils is one of several possible mechanisms explaining why N concentrations decrease with decreasing P concentrations in both green and senesced leaves. When photosynthetic rates are limited by low P concentrations in green leaves, tree species will down-regulate the investment of N to the proteins of photosynthetic machinery (i.e. ribulose-1,5-bisphosphate carboxylase oxygenase). In addition, in order to prolong leaf lifespan as a strategy of conserving P on P-poor soils, tree species on P-poor soils tend to have tougher leaves and/or decrease N concentration of green leaves to reduce herbivory risks (Coley *et al.* 1985, Endara & Coley 2011). Such down-regulation of foliar N may give rise to the observed positive correlation between foliar N and P concentrations along a gradient of soil P availability. Furthermore, the down-regulation of foliar N coupled with low foliar P concentrations will decrease decomposition rates and consequently decrease soil N availability on P-poor soils, eventually causing a positive correlation between foliar N and P.

Stoichiometry of N and P in plant cells provides another explanation. Theoretical studies have applied a biochemical relationship in plant cells between N in proteins and P in ribosomes used for protein synthesis (Ågren 2008, Niklas *et al.* 2005), and suggest that the scaling exponent of N to P in green leaves becomes lower than 2/3 or 3/4 when plants have relatively slow growth rates under P limitation. In agreement with their suggestion, our species have a lower value (0.582) than 2/3 or 3/4 in the scaling exponent of N to P in green leaves (Figure 2a) under P limitation, which is suggested by the extremely high foliar N:P ratios of our species (Table 1) compared with those of other tropical regions (mean value 26.1 on relatively P-poor Oxisols and Ultisols, Townsend *et al.* 2007). An experimental observation suggests that relative growth rates partly explain a within-site variation of foliar N:P ratios among tropical tree species (Cernusak *et al.* 2010). On the other hand, some studies argue that such a stoichiometric view based on plant growth rates does not always explain the balance between foliar N and P, because various forms of P-containing biochemical compounds in addition to ribosomes are involved in determining the relationship between foliar N and P (Hidaka & Kitayama 2011, Matzek & Vitousek 2009). In addition, such a stoichiometric view based on plant growth rates does not consider that plants store excess P in vacuoles, but not N (Ostertag 2010, Veneklaas *et al.* 2012). Further physiological and ecological studies are necessary to explain why N concentration tightly and positively relates to P concentration in green leaves and how high foliar N:P ratios are regulated on P-poor soils in tropical rain forests.

Reed *et al.* (2012) suggested that the ratio of NRE per PRE (i.e. N:P resorption ratio) reflects soil P availability in tropical rain forests. Our result that N:P resorption ratio increased with decreasing soil P availability agrees with their suggestion. Tree species translocate more P than N prior to leaf abscission with decreasing soil P availability. On the other hand, the reason why NRE and PRE are positively correlated with each other across tree species within each forest (Table 4, Figure 2f) remains unclear. Such a positive correlation between NRE and PRE was found also in other tropical forests (Cai & Bongers 2007, Lal *et al.* 2001) and in wetlands (Güsewell 2005). In addition, Tully *et al.* (2013) found a positive correlation between NRE and PRE in a symbiotic N-fixing tree species irrespective of soil P availability in tropical rain forests in Costa Rica. Earlier studies suggested that interspecific variation in NRE and PRE within a tropical rain forest reflect small-scale edaphic differences (Reed *et al.* 2012) or a wide range of adaptive nutrient-use strategies and genetically constrained flexibility in nutrient resorption among tree species (Hättenschwiler *et al.* 2008, Mayor *et al.* 2014). As stated earlier, the co-variation of soil N and P availabilities within a forest is unlikely. Tree species at Site 1 (lower P availability) translocated P from their leaves by hydrolysing phospholipids and nucleic acids (Hidaka & Kitayama 2011), although PRE was not different between Site 1 and Site 2 (Table 3). Because nutrient resorption costs carbon and N in enzymes such as protease and phosphatase to hydrolyse high-molecular-weight compounds to simpler compounds (Wright & Westoby 2003), nutritional strategies and genetically constrained flexibility (Mayor *et al.* 2014, Treseder & Vitousek 2001) will be different among tree species in response to soil P availability. A high NRE on P-poor soils implies that tree species hydrolyse N-containing compounds with a cost of N while hydrolysing P-containing compounds. The reason why tree species on P-poor soils need to hydrolyse N-containing compounds, which is an additional cost of N, remains unclear.

We have demonstrated that plant nutritional strategies balance N and P and form a basis of ecosystem N and P stoichiometry. The positive correlations between foliar N and P in concentrations and resorption efficiency along a gradient of soil P availability will influence consequent soil processes and may feed back to plant nutrient uses. Further analyses are needed to elucidate the physiological background of the correlation.

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