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Foliar nutrient allocation patterns in *Banksia attenuata* and *Banksia sessilis* differing in growth rate and adaptation to low-phosphorus habitats

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• **Background and Aims** Phosphorus (P) and nitrogen (N) are essential nutrients that frequently limit primary productivity in terrestrial ecosystems. Efficient use of these nutrients is important for plants growing in nutrient-poor environments. Plants generally reduce foliar P concentration in response to low soil P availability. We aimed to assess ecophysiological mechanisms and adaptive strategies for efficient use of P in *Banksia attenuata* (Proteaceae), naturally occurring on deep sand, and *B. sessilis*, occurring on shallow sand over laterite or limestone, by comparing the allocation of P among foliar P fractions.
• **Methods** We carried out pot experiments with slow-growing *B. attenuata*, which resprouts after fire, and faster growing opportunistic *B. sessilis*, which is killed by fire, on substrates with different P availability using a randomized complete block design. We measured leaf P and N concentrations, photosynthesis, leaf mass per area, relative growth rate and P allocated to major biochemical fractions in *B. attenuata* and *B. sessilis*.
• **Key Results** The two species had similarly low foliar total P concentrations, but distinct patterns of P allocation to P-containing fractions. The foliar total N concentration of *B. sessilis* was greater than that of *B. attenuata* on all substrates. The foliar total P and N concentrations in both species decreased with decreasing P availability. The relative growth rate of both species was positively correlated with concentrations of both foliar nucleic acid P and total N, but there was no correlation with other P fractions. Faster growing *B. sessilis* allocated more P to nucleic acids than *B. attenuata* did, but other fractions were similar.
• **Conclusions** The nutrient allocation patterns in faster growing opportunistic *B. sessilis* and slower growing *B. attenuata* revealed different strategies in response to soil P availability which matched their contrasting growth strategy.

Key words: Leaf phosphorus fractions, phosphate, phospholipid, phosphorus allocation, photosynthetic P use efficiency, nucleic acid, relative growth rate.

INTRODUCTION

Understanding strategies of nutrient allocation and their underlying mechanisms in plants adapted to phosphorus- (P) impoverished soils is an important topic in plant physiological ecology (Lambers *et al.*, 2006; Veneklaas *et al.*, 2012). Phosphorus-impoverished soils limit the growth and yield of crops, pastures and forests throughout the world (Conroy *et al.*, 1990; Herbert and Fownes, 1995; Seneweera and Conroy, 1997; Fujita *et al.*, 2003; Thomas *et al.*, 2006). Moreover, a recent meta-analysis showed that P limitation of above-ground plant production is pervasive in natural terrestrial ecosystems (Hou *et al.*, 2020). Low soil P availability is widespread in Australia (Viscarra Rossel and Bui, 2016; Kooyman *et al.*, 2017), and plants generally respond to low soil P availability by having a low foliar P concentration (Epstein and Bloom, 2005).

Foliar P concentration is the sum of the concentrations of several major P fractions in leaf cells including inorganic P (Pi) and various P-containing organic compounds (i.e. nucleic acids, phospholipids and small phosphate esters; Veneklaas *et al.*, 2012). The allocation of P to foliar fractions is likely to be related to life history strategy, because these fractions are functionally related to growth, reproduction and stress tolerance (Hidaka and Kitayama, 2011). Shifting P allocation patterns in leaves is an important mechanism for plants to acclimate to low soil P availability (Hidaka and Kitayama, 2011; Yan *et al.*, 2019). If strong P limitation occurs, plants shift the allocation of P among foliar P fractions, and this might increase their fitness under the prevailing conditions (Hidaka and Kitayama, 2011).

Chapin and Kedrowski (1983) found no evidence in four Alaskan tree species for adaptations to nutrient stress through major changes in biochemical use of nitrogen (N) and P by investigating foliar P fractions; however, they found important changes in allocation of N and P to different leaf fractions, during the growing season (Chapin and Kedrowski, 1983). Hidaka and Kitayama (2009) found that plants growing on P-impoverished

tropical soils increased both leaf mass per area (LMA) and photosynthetic P use efficiency (PPUE) compared with plants on P-richer soil. These authors suggested that a greater proportion of cellular P may be allocated to metabolic P, rather than to structural P to maintain high PPUE. Yan *et al.* (2019) investigated foliar P fractions of three species along a 2 million year chronosequence with a strong gradient of available P in south-western Australia, and found that their P allocation pattern was associated with their distribution along the chronosequence, concluding that the differences are likely to be adaptive. How plants allocate P among foliar P fractions and exhibit adaptive strategies to use P efficiently in two species in the same genus with contrasting life history strategies in extremely P-impoverished ecosystems with a Mediterranean climate remains unclear.

Comparison of the relationship between growth rate and P investment showed that species with fast growth rates exhibit low N:P ratios (Elser and Hamilton, 2007). This pattern has been explained by the growth rate hypothesis (GRH), which proposes that fast growth rates require greater allocation of resources (mainly P) to P-rich ribosomal RNA (rRNA) to meet the protein synthesis demands needed to support the rapid growth rates (Elser *et al.*, 1996; Sterner and Elser, 2002; Elser and Hamilton, 2007; Reef *et al.*, 2010; Hidaka and Kitayama, 2011). Nucleic acids have an N:P stoichiometry of 4:1 (Reef *et al.*, 2010), and are a major fraction of organic P, with RNA by far the largest proportion (Geider and La Roche, 2002). Within the RNA pool, rRNA is the largest P fraction.

Tree species in ancient landscapes have experienced long-term low soil P status; thus, they probably possess adaptations to P limitation. Non-mycorrhizal Proteaceae are particularly successful on the most P-impoverished soils in south-western Australia (Pate *et al.*, 2001; Lambers *et al.*, 2013; Hayes *et al.*, 2014). These species with specialized cluster roots effectively mine soil P by releasing carboxylates to replace P sorbed to soil particles (Shane and Lambers, 2005) and exhibit relatively fast rates of area-based photosynthesis, despite having extremely low leaf P concentrations (Denton *et al.*, 2007; Lambers *et al.*, 2012; Sulpice *et al.*, 2014), while leaves of P-starved crop plants tend to have slow rates of photosynthesis per unit leaf area (Brooks *et al.*, 1988; Rao *et al.*, 1989; Fredeen *et al.*, 1990). Consequently, some of these Proteaceae exhibit a very high PPUE (Denton *et al.*, 2007; Lambers *et al.*, 2010; Sulpice *et al.*, 2014). This high PPUE in Proteaceae from severely P-impoverished habitats is brought about mainly by low foliar rRNA concentrations (Sulpice *et al.*, 2014) and extensive replacement of phospholipids by galactolipids and sulfolipids during leaf development (Lambers *et al.*, 2012).

The slow-growing resprouter *Banksia attenuata* and the faster growing seeder *B. sessilis* (Pate *et al.*, 1991) both inhabit low-P soils and produce compound cluster roots (Shane and Lambers, 2005), but have different life histories (Shi *et al.*, 2020); the soil total N concentrations in their natural habitats is 0.24–0.27 g kg^{-1} (Hayes *et al.*, 2014). *Banksia sessilis* is a short-lived obligate seeder that occurs on shallow sand over laterite or limestone (Pate and Bell, 1999; Hayes *et al.*, 2019) and allocates more biomass to cluster roots than *B. attenuata*, which invests more in deep roots (Shi *et al.*, 2020). This strategy enhances P mobilization from laterite or limestone by releasing more carboxylates and/or exuding these at a faster rate than *B. attenuata* (Shi *et al.*, 2020). In contrast to *B. sessilis*, *B. attenuata* is restricted to deep sand (FloraBase, http://florabase.dpaw.wa.gov.au/), grows slowly and is long lived (Pate *et al.*, 1990; Knox and Clarke, 2005; Bowen and Pate, 2017). McArthur and Wilson (1967) coined the terms *r* strategy and *K* strategy to describe selection for rapid population growth in uncrowded populations and selection for competitive ability in crowded populations, respectively. Over time, the meaning of these terms has broadened (Parry, 1981) and, according to the broader concept, *B. sessilis* is an *r* strategist, while *B. attenuata* is a *K* strategist. We do not know the physiological pattern of allocating P among foliar P fractions that allows species to exhibit a particular life history strategy and efficient use of P in contrasting low-P environments. Therefore, we aimed to compare P allocation patterns in these two *Banksia* species with contrasting life history. Thus, we measured leaf P and N concentrations, LMA, and concentrations and proportions of P in foliar P-containing fractions in *B. attenuata* and *B. sessilis* grown with different substrate P availability.

We hypothesized that: (1) with decreasing soil P availability, the foliar total P concentrations of both *B. attenuata* and *B. sessilis* would decrease; and (2) *B. sessilis*, which exhibits a more opportunistic *r* strategy than *B. attenuata*, would have a higher foliar N_{Total}:P_{Total} ratio and invest more P in nucleic acids than *B. attenuata* when grown on the same substrate.

MATERIALS AND METHODS

Sowing method

Seeds of *Banksia attenuata* R.Br. and *B. sessilis* (Knight) A.R. Mast & K.R. Thiele (purchased from Nindethana Seed Company, King River, Western Australia) were sown on filter paper. One seedling was transferred into each experimental pot on 22 May 2016. According to the supplier, the seeds of *B. sessilis* were collected from a coastal population, growing over limestone near Jurien Bay, Western Australia (30°18′S, 115°3′E). The provenance of the *B. attenuata* seed was unknown.

Experimental design

A pot experiment was carried out in a glasshouse at the University of Western Australia, Perth, Australia (31°59′S, 115°53′E) using a randomized complete block design. Glasshouse temperature fluctuated between 13 and 33 °C over a whole year, and transmission of radiation into the glasshouse was 60 % of natural light. The experiment was designed to explore why *B. sessilis* is able to grow across a wider range of P-impoverished soil types and maintain a greater relative growth rate (RGR) than *B. attenuata* by comparing the use and allocation of P among foliar P fractions in the two species. Three soil treatments were imposed, based on washed river sand: sand only, sand + laterite (SLAT) and sand + limestone (SLIM). The substrate total P availability

was sand > SLAT > SLIM (Supplementary data Fig. S1; Shi *et al.*, 2020). The pots (100 mm inner diameter × 400 mm tall PVC cylinders) were lined with plastic bags. For each soil treatment, 3.0 kg of substrate was added to the pots. For the SLAT and SLIM treatment, a 100 mm layer of laterite or limestone gravel, respectively, was added 50 mm below the soil surface, and other layers were filled with sand. There were ten replicates for each species in each treatment. Field capacity of soils in each treatment was calculated as [(wet mass – dry mass)/dry mass] × 100 %. The pots were watered to a constant weight of 80 % of field capacity three times a week. A 20 mL aliquot of basal liquid nutrient solution lacking P and containing (per kg of soil) 217.5 mg of KNO_3; 74 mg of $CaCl_2$; 140 mg of K_2SO_4; 80 mg of $MgSO_4{\cdot}7H_2O$; 28.9 mg of $MnSO_4{\cdot}H_2O$; 10 mg of $ZnSO_4{\cdot}7H_2O$; 5 mg of $CuSO_4{\cdot}5H_2O$; 0.7 mg of H_3BO_3; 0.5 mg of $CoSO_4{\cdot}7H_2O$; 0.4 mg of $Na_2MoO_4{\cdot}2H_2O$; and 20 mg of FeNaEDTA was applied to each pot once every second week.

Photosynthesis measurement

Prior to the final harvest, the net photosynthetic rate (P_n) of attached leaves was measured between 10.00 and 11.00 h on 7 and 9 March 2017 using a red/blue LED light source (LI-6400, LI-COR Inc., Lincoln, NE, USA). The plants were watered on the day before the photosynthesis measurement. One mature leaf of each plant was measured at a photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1} and a CO_2 concentration of 400 µmol mol^{-1}. The leaves used for photosynthesis measurements were sampled, and the projected leaf area measured at 200 dpi (Epson 1680, Long Beach, CA, USA) and calculated (ImageJ 1.4, NIH, Bethesda, MD, USA). Leaves were then dried at 70 °C for 72 h to measure dry mass (DM).

Harvest

After 50 weeks of growing in pots, a total of 20 fully expanded leaves with no visible damage or discolouration were harvested from each plant. The leaves were immediately scanned at 200 dpi to calculate leaf area (LA_1), then submerged in liquid nitrogen and stored at –80 °C. Frozen leaves were freeze-dried for 7 d (VirTis Benchtop 'K', New York, USA) and DM was determined (DM_1). The remaining leaves on each plant were harvested and scanned at 200 dpi to calculate the remaining leaf area (LA_2). Total LA = LA_1 + LA_2. The remaining leaves, stem and roots were separated and dried at 70 °C for 72 h. The DM was determined for the remaining leaves (DM_2) and for stems plus roots (DM_3). Total leaf DM = DM_1 + DM_2. Total plant dry mass M_2 = DM_1 + DM_2 + DM_3. The LMA was calculated as total leaf DM/total LA. Seed weight (W_1) was measured using four lots of ten (*B. attenuata*) or 30 (*B. sessilis*) seeds that were dried (70 °C, 48 h) and weighed before calculating the average seed weight. The RGR was calculated as $(\ln M_2 - \ln W_1)/(T_2 - T_1)$, where T_1 and T_2 are the dates of sowing and harvesting, respectively, expressed in weeks.

Leaf nutrient analyses

Freeze-dried leaves were ground to a fine powder (Geno/Grinder 2010, Spex SamplePrep, Metuchen, NJ, USA). A 50 mg sample was used to determine inorganic P (Pi; Yan *et al.*, 2019).

The P allocated to the nucleic acid, lipid, small metabolite (Pi + other metabolites) and residual fractions was determined in a 50 mg portion of powdered leaves using the differential solubility method (Hidaka and Kitayama, 2013), as modified by Yan *et al.* (2019). Metabolite P is defined here as small metabolite P – Pi.

Phosphorus concentrations in extracts and residues from the above procedures were measured as described by Matusiewicz and Golik (2004) using the molybdenum blue method (Ames, 1966). Total leaf P is the sum of Pi, nucleic acids, lipids, metabolites and the residual fraction. Total leaf P was confirmed by acid digestion of ground leaf material, followed by Pi assay. Total foliar N concentration was determined by combustion of approx. 30 mg of dried leaf sample (Vario Macro Combustion Analyser, Elementar Analysensysteme GmbH, Langenselbold, Germany).

Leaf area-based P concentration was calculated as total leaf P concentration × LMA; PPUE was calculated as the ratio of photosynthesis rate to area-based P concentration; leaf area-based N concentration was calculated as leaf N concentration × LMA; and photosynthetic N use efficiency (PNUE) was calculated as the ratio of photosynthesis rate to area-based N concentration.

Statistics

The differences in means between *B. attenuata* and *B. sessilis* on the same substrate were analysed by two-way analysis of variance (ANOVA) with 95 % confidence intervals, while the differences in means within each species across substrate types were analysed by Tukey's method. The relationships of foliar P fractions to total foliar P concentration, LMA and RGR to nucleic acid P, and foliar N and foliar N to nucleic acid P were determined by linear regression analysis; the correlation coefficients were analysed by Student's *t*-test. All statistical analyses were performed using the SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA), and graphed with OriginPro 9.5 (OriginLab Corporation, Northampton, MA, USA).

RESULTS

Leaf P and N concentrations

The effect of P availability on P and N relationships in leaves of *B. attenuata* and *B. sessilis* was tested by growing plants in sand, SLAT and SLIM. Foliar total P concentrations on a mass basis for both *B. attenuata* and *B. sessilis* were highest

when grown in sand, and lowest in SLIM (Fig. 1A). However, there were no significant differences between *B. attenuata* and *B. sessilis* in foliar total P concentrations for any substrate type (Fig. 1A). Mass-based foliar total P concentrations for *B. attenuata* differed on all three substrates ($P < 0.05$): sand > SLAT > SLIM. In contrast to foliar total P concentration, foliar total N concentration on a mass basis for *B. sessilis* was greater than that for *B. attenuata* in each substrate type (Fig. 1B). The leaf total N concentrations on a mass basis for both species were the same in sand and SLAT, and higher than when grown in SLIM ($P < 0.05$).

The differences in mass-based foliar total P concentration across substrates for both *B. attenuata* and *B. sessilis* were also apparent when P concentrations were expressed on an area basis ($P < 0.05$; Fig. 1C). However, on an area basis, the foliar P concentration for *B. attenuata* was higher than that for *B. sessilis* in all substrates. There were also substrate-dependent differences in area-based foliar total N concentration for *B. attenuata*, but not for *B. sessilis* (Fig. 1D). In contrast to higher mass-based foliar N concentrations in *B. sessilis* than in *B. attenuata* in all substrates, the area-based foliar N concentration was higher in *B. attenuata* in SLAT, or was not distinguishable (Fig. 1D).

The concentration ratio of foliar total N to foliar total P (N_{Total}:P_{Total}) had the same pattern for the two species across the three substrate types (Table 1). The N_{Total}:P_{Total} ratio for both species was lower for plants grown in sand than for those grown in SLAT or SLIM ($P < 0.05$). However, there was no significant

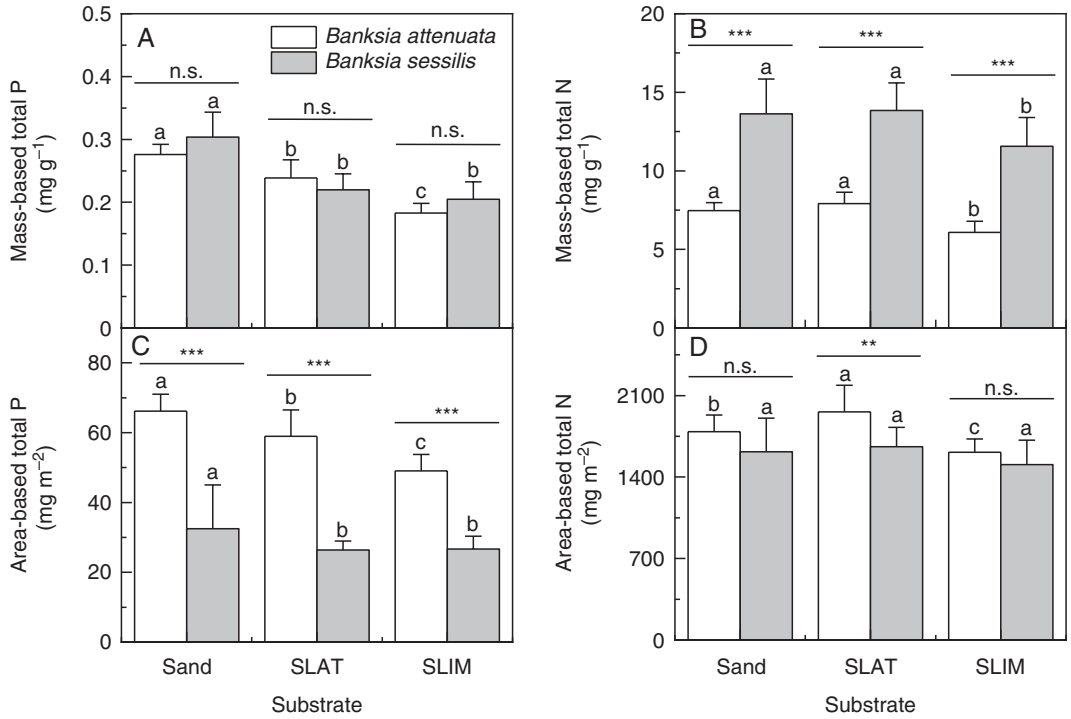

FIG. 1. Leaf mass-based total P (A), and N (B) concentrations and area-based P (C) and N (D) concentrations of two *Banksia* species, *Banksia attenuata* and *B. sessilis*, that were grown in different substrates. Values are means ± s.e. ($n = 10$); the asterisks denote significant differences between the species by *t*-test. n.s., not significant, $^{**}P < 0.01$, $^{***}P < 0.001$. Different letters indicate significant differences among substrates ($P < 0.05$). SLAT, sand plus laterite; SLIM, sand plus limestone.

TABLE 1. *Mass ratios for total foliar nitrogen (N) to total foliar phosphorus (P) and to P in each foliar P-containing fraction, and relative growth rate (RGR) for two* Banksia *species,* B. attenuata *and* B. sessilis*, grown in different substrates*

Items	*Banksia attenuata*			*Banksia sessilis*		
	Sand	SLAT	SLIM	Sand	SLAT	SLIM
Leaf N/total leaf P	27.1 ± 1.8^{b}	33.4 ± 2.5^{a}	33.1 ± 3.6^{a}	$45.9 \pm 8.9^{b***}$	$63.2 \pm 7.4^{a***}$	$56.9 \pm 7.72^{a***}$
Leaf N/Pi	100 ± 14^{b}	161 ± 46^{a}	176 ± 46^{a}	$253 \pm 86^{b***}$	$390 \pm 67^{a***}$	$356 \pm 80^{a***}$
Leaf N/lipid P	159 ± 25^{a}	168 ± 27^{a}	158 ± 23^{a}	$294 \pm 76^{b***}$	$436 \pm 127^{a***}$	$398 \pm 95^{ab***}$
Leaf N/metabolic P	119 ± 10^{b}	168 ± 36^{a}	177 ± 38^{a}	$189 \pm 29^{b***}$	$274 \pm 41^{a***}$	$239 \pm 54^{a***}$
Leaf N/nucleic acid P	105 ± 9^{a}	114 ± 11^{a}	108 ± 13^{a}	$136 \pm 29^{b**}$	$186 \pm 28^{a***}$	$159 \pm 25^{ab***}$
Leaf N/residual P	416 ± 94^{a}	447 ± 94^{a}	375 ± 66^{a}	$692 \pm 195^{a***}$	$607 \pm 98^{a**}$	$772 \pm 258^{a***}$
RGR (mg g^{-1} $week^{-1}$)	95 ± 2.6^{a}	93 ± 3.7^{a}	86 ± 4.6^{b}	$123 \pm 4^{a***}$	$115 \pm 7.6^{b***}$	$110 \pm 6.1^{b***}$

Values are means ± s.e. ($n = 10$); asterisks indicate significant differences between the species by *t*-test. $^{**}P < 0.01$, $^{***}P < 0.001$. Different letters indicate significant differences for the two species among the substrates ($P < 0.05$). SLAT, sand plus laterite; SLIM, sand plus limestone.

difference for either species when grown in SLAT or SLIM. The N_{Total}:P_{Total} ratio in *B. sessilis* was significantly higher than that in *B. attenuata* in every substrate.

Photosynthetic rates, PPUE and PNUE

Net photosynthetic rates (P_n) for *B. attenuata* grown in SLIM were lower than for plants grown in sand or SLAT, while there were no substrate-dependent differences for *B. sessilis* ($P > 0.05$; Table 2). There were no differences in photosynthesis rate between the two species in any substrate ($P > 0.05$). Moreover, there were no substrate-dependent differences in PPUE within either species (Table 2). However, the PPUE of *B. sessilis* was significantly higher than that of *B. attenuata* in all three substrates ($P < 0.05$; Table 2). The PNUE for *B. attenuata* grown in sand was higher than that of plants grown in SLIM ($P < 0.05$; Table 2), while PNUE for *B. sessilis* was the same in all substrate types (Table 2). Moreover, there were no significant differences in PNUE between *B. attenuata* and *B. sessilis* for any substrate type (Table 2).

Leaf P fractions

Banksia attenuata and *B. sessilis* had different patterns of allocating leaf P to lipid, metabolite, nucleic acid and residual fractions in all substrates (Fig. 2). The lipid P concentrations of *B. attenuata* grown in SLIM were lower than in plants grown in sand and SLAT (Fig. 2A), while the difference in metabolite P concentration in *B. attenuata* grown in the three substrate types was sand > SLAT > SLIM (Fig. 2B). The nucleic acid P concentrations of *B. attenuata* grown in SLIM were lower than in plants grown in sand and SLAT (Fig. 2C). The lipid P, metabolite P, nucleic acid P and Pi concentrations of *B. sessilis* grown in sand were greater than those in plants grown in SLAT and SLIM (Fig. 2A–C, E).

The lipid P concentration of *B. attenuata* was greater than that of *B. sessilis* in SLAT and SLIM (Fig. 2A). The metabolite P and nucleic acid P concentrations of *B. attenuata* were lower than those of *B. sessilis* in both sand and SLIM, but were the same in SLAT (Fig. 2B, C). The residual P concentration only differed between the species when grown in SLAT, with the concentration in *B. attenuata* being lower than that in *B. sessilis* (Fig. 2D). The Pi concentrations of *B. attenuata* were greater than those of *B. sessilis* in sand and SLAT, but not in SLIM (Fig. 2E).

The N_{Total}:$P_{Fraction}$ ratios for metabolite P and Pi for *B. attenuata* were significantly lower in sand than in SLAT or SLIM, while the ratios for the other fractions were indistinguishable among the substrates (Table 1). This result shows that metabolite P and Pi fractions were drivers for the observed difference in leaf N_{Total}:P_{Total} ratio for *B. attenuata* in the three substrates. For *B. sessilis*, all P factions except residual P contributed to the lower leaf N_{Total}:P_{Total} ratio in sand. The ratios of leaf N_{Total}:$P_{Fraction}$ in each P fraction were lower in *B. attenuata* than in *B. sessilis* for plants grown in any of the three substrates (Table 2).

The proportions of foliar P fractions to total foliar P

The proportions of lipid P and nucleic acid P in *B. attenuata* were significantly lower for plants grown in sand than for those grown in SLAT or SLIM ($P < 0.05$; Fig. 3A, C). Conversely, the proportion of total P in Pi for *B. attenuata* was greater for plants grown in sand than for plants grown in SLAT or SLIM ($P < 0.05$; Fig. 3E). There was no significant difference in the proportions of total P in lipids, metabolites, nucleic acids and Pi for *B. sessilis* grown in any of the three substrates (Fig. 3A).

The proportion of total P in lipid P in *B. attenuata* was greater than that of *B. sessilis* in SLAT and SLIM ($P < 0.01$; Fig. 3A). Conversely, the proportion of total P in nucleic acid P in *B. sessilis* was significantly greater than that in *B. attenuata* in all substrates (Fig. 3C); likewise, the proportion of metabolite P in *B. sessilis* was greater than that in *B. attenuata* in SLIM (Fig. 3B). The proportion of residual P was <10 % in all substrates, except for *B. sessilis* grown in SLAT (Fig. 3D). The proportion of total P in Pi in *B. attenuata* was greater than that of *B. sessilis* in sand and SLAT ($P < 0.01$; Fig. 3E).

The relationships of RGR and foliar nutrient concentrations, foliar P fractions and leaf mass per area

The RGR of *B. attenuata* was slower than for *B. sessilis* in all substrates (Table 1). In *B. attenuata*, the RGR was the same in sand and SLAT, but slower in SLIM, while in *B. sessilis*, RGR was also fastest in sand, but slower and equal in both SLAT and SLIM. The RGR of *B. attenuata* and *B. sessilis* was positively

Table 2. *Photosynthesis rates, photosynthetic P use efficiency (PPUE), photosynthetic N use efficiency (PNUE) and foliar mass per area (LMA) of two* Banksia *species grown in different substrates:* B. attenuata *and* B. sessilis

Items	*Banksia attenuata*			*Banksia sessilis*		
	Sand	SLAT	SLIM	Sand	SLAT	SLIM
Photosynthesis rates (μmol m^{-2} s^{-1})	15.3 ± 3^{a}	15.4 ± 1.3^{a}	10.8 ± 1.8^{b}	13.4 ± 2.2^{a}n.s.	13.2 ± 6.2^{a}n.s.	10.7 ± 2.3^{a}n.s.
PPUE (μmol g^{-1} P s^{-1})	234 ± 53^{a}	264 ± 38^{a}	223 ± 52^{a}	377 ± 86.4^{a}***	506 ± 269^{a}**	410 ± 116^{a}***
PNUE (μmol g^{-1} N s^{-1})	8.9 ± 2.2^{a}	7.9 ± 1.0^{ab}	6.7 ± 1.0^{b}	8.6 ± 2.4^{a}n.s.	7.9 ± 3.5^{a}n.s.	7.3 ± 1.9^{a}n.s.
LMA (g m^{-2})	240 ± 15^{b}	248 ± 21^{b}	267 ± 24^{a}	119 ± 7^{b}**	121 ± 9^{b}**	131 ± 6^{a}**

Values are means ± s.e. ($n = 10$); asterisks indicate significant differences between the species by *t*-test. n.s., not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Different letters indicate significant differences for the species among the substrates ($P < 0.05$). SLAT, sand plus laterite; SLIM, sand plus limestone

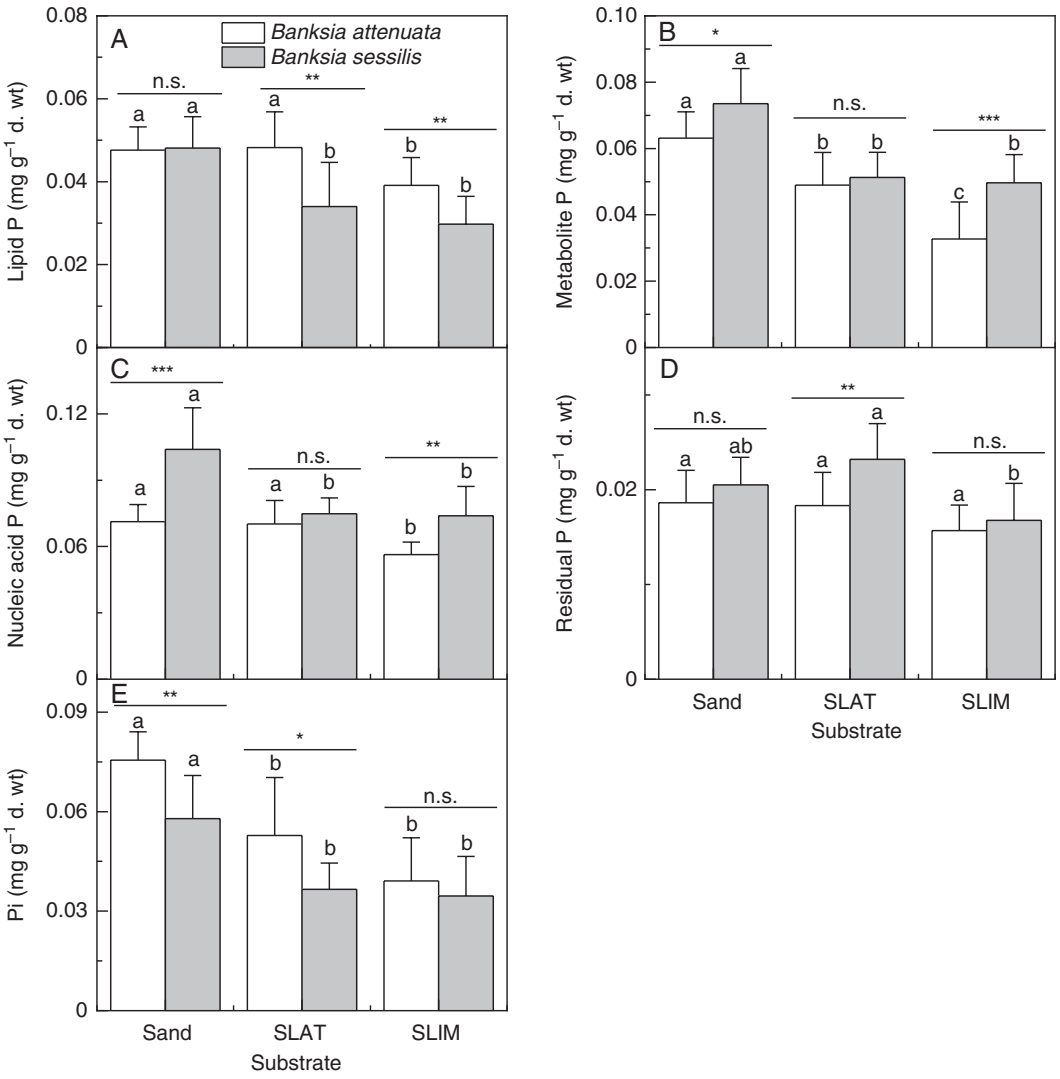

FIG. 2. The lipid phosphorus (P) (A), metabolite P (B), nucleic acid P (C), residual P (D) and inorganic P (Pi) concentrations (E) in leaves of two *Banksia* species, *B. attenuata* and *B. sessilis*, grown in different substrates. Values are means ± s.e. (n = 10); asterisks indicate significant differences between the two species by t-test. n.s., not significant, *P < 0.05, **P < 0.01, ***P < 0.001. Different letters indicate significant differences for the two species among substrates (P < 0.05). SLAT, sand plus laterite; SLIM, sand plus limestone.

correlated with foliar nucleic acid P concentration (Fig. 4A) and foliar N concentration (Fig. 4B). There was also a positive correlation between foliar N concentration and nucleic acid P concentration for both species (Fig. 4C). No other correlations were detected in any other pairwise comparison of P concentration with N concentration. In both species, the concentrations of P in lipid, metabolite, nucleic acid, residual and Pi fractions were all correlated positively with foliar total P concentration (Fig. 5).

The LMA varied from 240 to 267 g m^{-2} in *B. attenuata* and from 119 to 131 g m^{-2} in *B. sessilis* depending on the growth substrate (Fig. 5; Table 2). The concentrations of the P fractions and total P generally decreased with increasing LMA (Fig. 5). This relationship was not significant for lipid P and nucleic acid P in *B. sessilis*. The only exception to a negative correlation between leaf P fraction and LMA was the lack of a relationship between residual P and LMA in *B. attenuata*.

DISCUSSION

Foliar total P and N concentrations

Leaf N and P concentration can be expressed on a leaf area (N_{area}) or leaf mass basis (N_{mass}; Li *et al.*, 2016). Concentrations on an area basis are useful when comparing rates of photosynthesis expressed on an area basis. However, mass-based values are useful in terms of expenditures and returns per unit investment, and allow broader comparisons with the literature (Wright *et al.*, 2004). Our first hypothesis was that with decreasing soil P availability, the mass-based foliar total P concentrations of both *B. attenuata* and *B. sessilis* would decrease, and this was supported (Fig. 1). An important finding was that the area-based foliar P concentrations were higher in *B. attenuata* than in *B. sessilis* in all three substrates, but there was no significant difference between *B. attenuata* and *B. sessilis* in mass-based foliar P concentration for any substrate type. We found

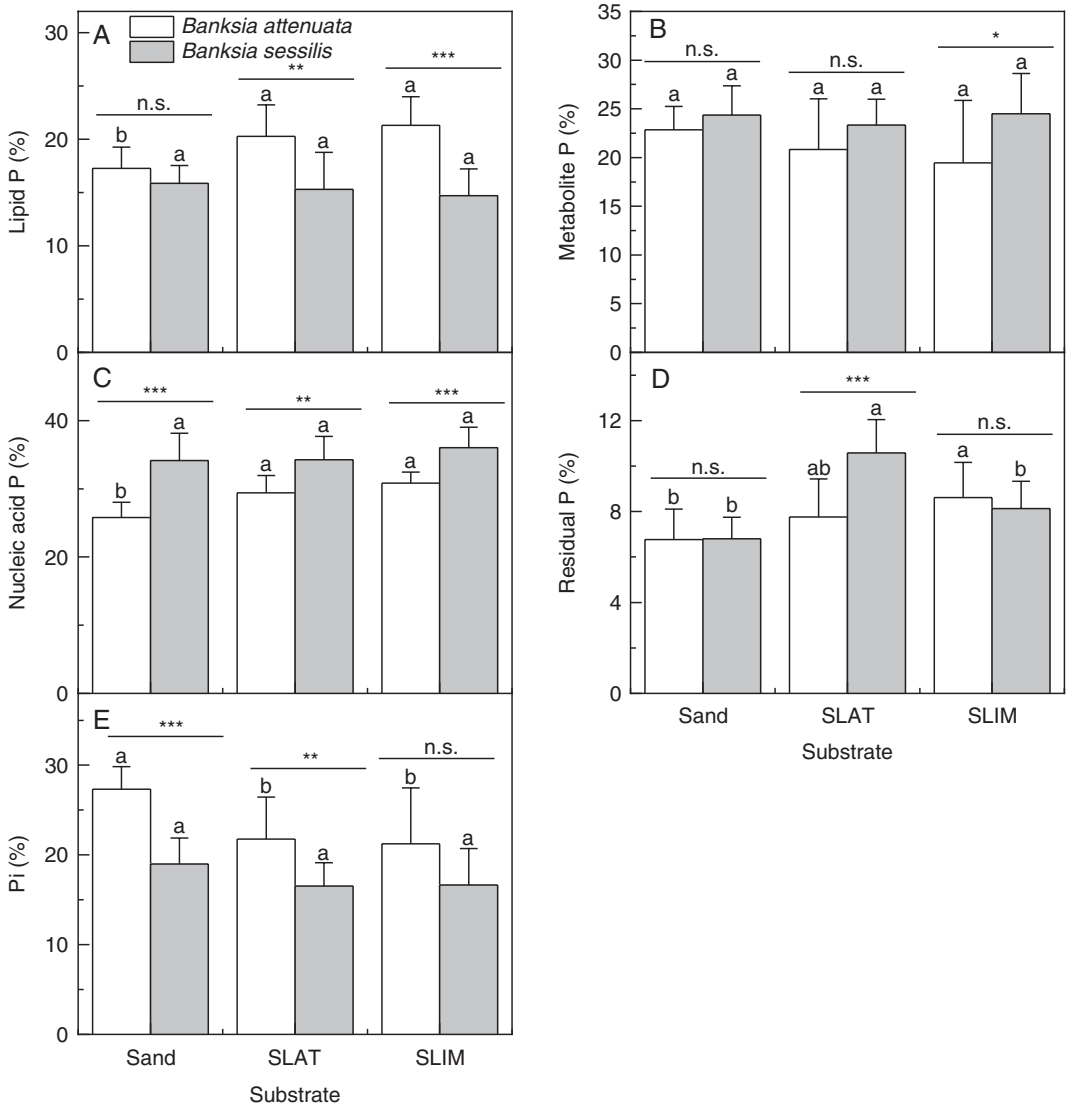

FIG. 3. The proportions of total foliar phosphorus (P) as lipid P (A), metabolite P (B), nucleic acid P (C), residual P (D) and inorganic P (Pi) (E) for two *Banksia* species, *B. attenuata* and *B. sessilis*, grown in different substrates. Values are means ± s.e. ($n = 10$); asterisks indicate significant differences between the two species by *t*-test. n.s., not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Different letters indicate significant differences for the two species among substrate types ($P < 0.05$). SLAT, sand plus laterite; SLIM, sand plus limestone.

the same patterns of mass-based foliar total P concentration for both *B. attenuata* and *B. sessilis* grown in all three substrates; the foliar total P concentrations of both species were greatest in sand, and about 35 % lower in SLIM. The greatest carboxylate-extractable P concentration was in sand, and the lowest was in limestone gravel (Supplementary data Fig. S1).

In contrast to the similar mass-based foliar total P concentrations in the two species in each substrate, the mass-based foliar total N concentration of *B. sessilis* was almost twice that of *B. attenuata* grown in the same substrate. Leaf N concentrations in *B. attenuata* and *B. sessilis* grown on SLIM were approx. 20 % lower than those in plants grown in sand and SLAT. Our result differs from results on *Hakea prostrata* (Proteaceae), showing that P availability did not influence leaf N concentration (Prodhan *et al.*, 2016). The foliar total N concentrations of both species were very low compared with those of plants from other environments (Reich *et al.*, 1991), which reflects the low foliar rRNA concentration in *B. attenuata* (Sulpice *et al.*, 2014) and, presumably, in *B. sessilis*, based on the similar size of their nucleic acid P pools. In our study, the relatively low foliar N concentrations in *B. attenuata* and *B. sessilis* indicate that protein concentrations were very low, which implies a low demand for rRNA and, thus, P.

Whilst the leaf N concentrations in both species were low compared with the global average (Reich *et al.*, 1991; Wright *et al.*, 2004), they were distinctly higher in *B. sessilis* than in *B. attenuata*. The higher N concentration correlated with greater allocation of P to the nucleic acid P fraction in *B. sessilis*. However, rates of photosynthesis and leaf N concentrations expressed on an area basis were similar for the two species, and hence so was the PNUE. Therefore, the 'extra'

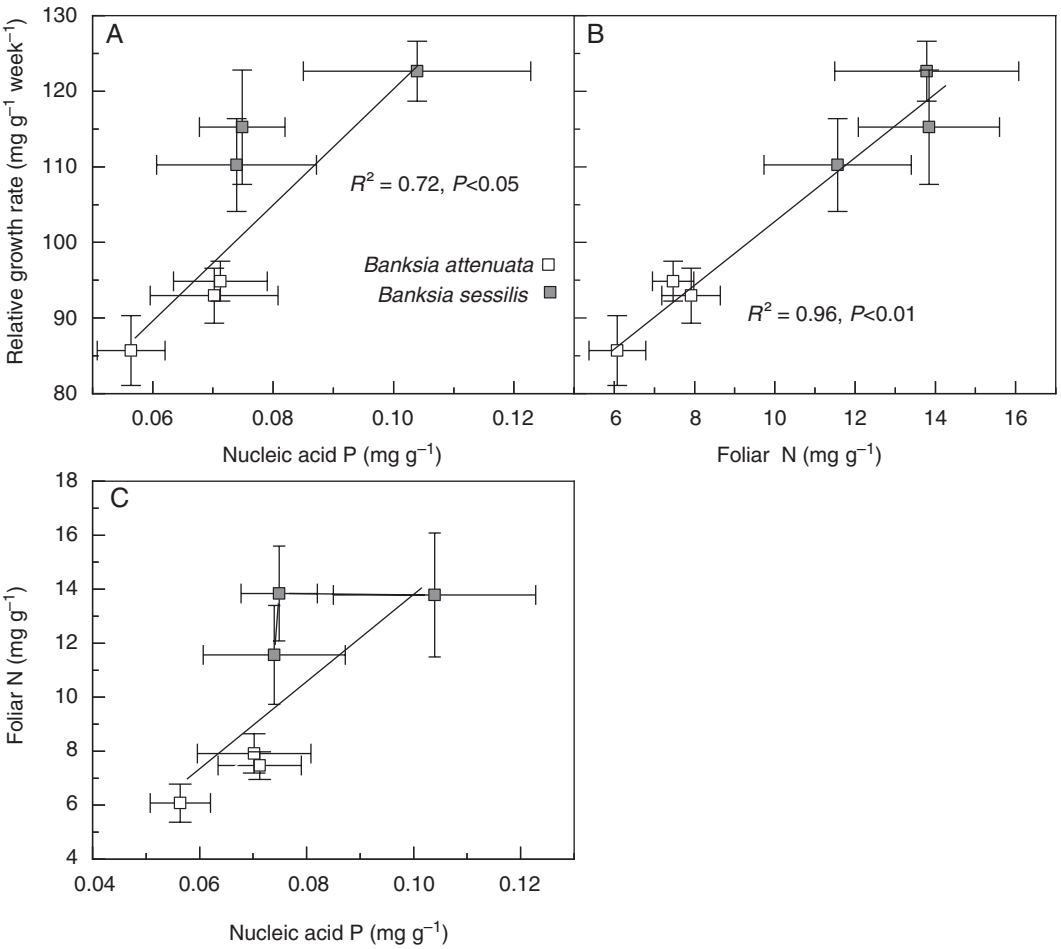

FIG. 4. Relationships of relative growth rate (RGR) to foliar nucleic acid phosphorus (P), foliar nitrogen (N), and foliar N to nucleic acid P for *Banksia attenuata* and *B. sessilis*. Values are means ± s.e. (n = 10). Individual data can be found in Figs 1 and 2, and Table 1.

N in *B. sessilis* on a mass basis was a reflection of a lower investment in sclerenchymatic tissue, as evidenced by its lower LMA. A low ribosome abundance can be expected to decrease the rate of protein synthesis, and hence the protein and N concentrations. Therefore, lower leaf N concentrations in *B. attenuata* compared with *B. sessilis* on the three substrates tested is consistent with lower rRNA concentrations and lower rates of protein synthesis. However, since we studied mature non-growing leaves, which do not rapidly change in protein concentration (Kuppusamy *et al.*, 2014), the faster rate of protein synthesis must have been balanced by a faster rate of protein breakdown, and hence protein turnover (Sulpice *et al.*, 2014).

According to the GRH, species with rapid growth rates would have low foliar N:P ratios, because of their high P-rich rRNA concentrations. In our analysis, we found that RGR was strongly correlated with leaf N and nucleic acid P concentrations in both species; this would appear to support the GRH within each species, but we should bear in mind that our results on N and P concentrations refer to fully expanded leaves that had stopped growing. Also, RGR, N concentration and foliar N:P in *B. sessilis* were significantly greater than those in *B. attenuata* when grown in the same substrate. This supports our second hypothesis that *B. sessilis*, which exhibits a more opportunistic growth strategy than *B. attenuata* (Shi *et al.*, 2020), has a higher foliar N_{Total}:P_{Total} ratio than *B. attenuata*, and invests more P in nucleic acid P to support a higher N concentration. This finding is in line with a study on *Pinus* species in a glasshouse experiments (Matzek and Vitousek, 2009). The higher leaf N concentration found here and the higher capacity to acquire P (Shi *et al.*, 2020) in *B. sessilis* than in *B. attenuata* when grown in the more P-limiting SLIM may explain the different distribution patterns of the two species. A higher capacity to acquire P presumably allows it to colonize and become established on different P-impoverished soils (sand over laterite or over limestone), compared with *B. attenuata*, which is restricted to deep sand (FloraBase, http://florabase.dpaw.wa.gov.au/).

Foliar traits and P fractions

The different foliar P allocation patterns combined with differences in LMA between the two species reflects differences in their life history strategies and resource requirements. Plants like *B. sessilis* with an *r* selection life history typically grow fast (Clarke *et al.*, 2013) and produce seeds before the next catastrophe, i.e. fire or drought (Pate *et al.*, 1990; Knox and Clarke, 2005; Bowen and Pate, 2017). This

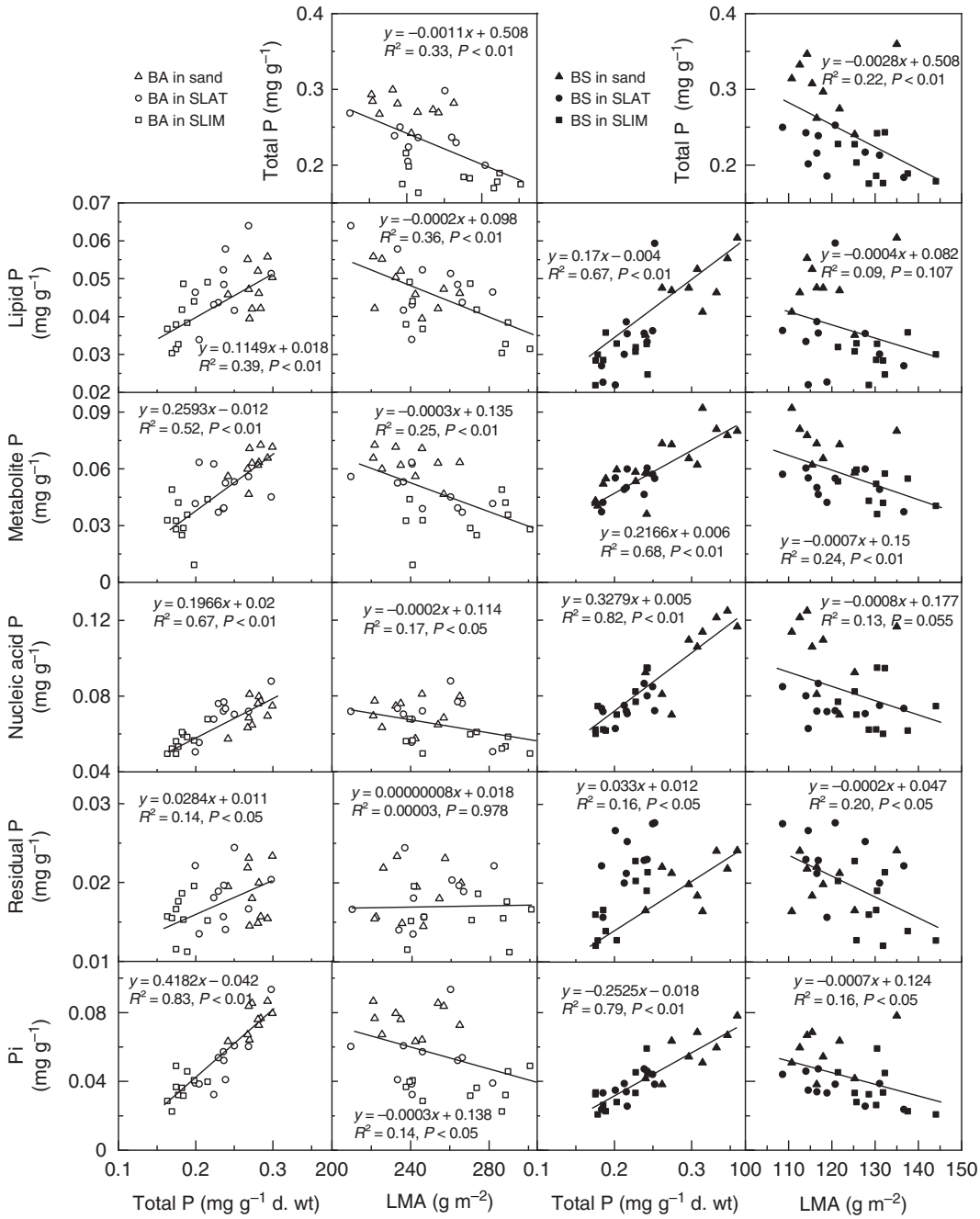

FIG. 5. Relationships between foliar phosphorus (P) fractions and total foliar P concentration (left), and leaf mass per area (LMA) (right) for *Banksia attenuata* and *B. sessilis* grown in three substrates (n = 30). Each symbol represents an individual plant. BA, *B. attenuata*; BS, *B. sessilis*; SLAT, sand plus laterite; SLIM, sand plus limestone.

strategy may require relatively greater investment in P-rich rRNA, and thus ribosomes, to support rapid protein synthesis and turnover, including replacement of damaged proteins (Raven, 2012). A high protein synthesis capacity may provide flexibility to acclimate to variable and changing environments (i.e. shallow sand over laterite or limestone, where water availability may fluctuate) and complete the life cycle quickly. Unlike *B. sessilis*, *B. attenuata* with larger seeds (Shi *et al.*, 2020) and higher LMA has the ability to resprout from epicormic buds or lignotubers (Pate *et al.*, 1991; Groom and Lamont, 2011), a strategy associated with a slower RGR (Pate *et al.*, 1990; Knox and Clarke, 2005; Bowen and Pate, 2017). Thus, selection in *B. attenuata* was based on a lower investment in nucleic acid P, as well as the ability to allocate more biomass to deep roots compared with *B. sessilis* (Shi *et al.*, 2020). Thus, it does not need to grow fast and complete its life cycle quickly (Pate *et al.*, 1990; Knox and Clarke, 2005; Bowen and Pate, 2017).

The Pi concentration in slow-growing *B. attenuata* was higher than that in the faster growing *B. sessilis* when grown in sand and SLAT, slightly higher than when grown in SLIM. Cell vacuoles serve as a reservoir for excess Pi in most plants which can then be drawn upon as P availability decreases (Mimura, 1995). Changes in total foliar P concentration with Pi supply generally reflect the accumulation of Pi in vacuoles which is typically greater in slow-growing species than in fast-growing species (Güsewell, 2004). Thus, fast-growing species convert Pi into growth-sustaining organic P, rather than accumulating Pi, as in slow-growing species.

The metabolite P concentrations and the proportions of total P in metabolites for *B. sessilis* were significantly greater than those for *B. attenuata* for plants grown in SLIM. Moreover, the PPUE of *B. sessilis* was greater than that of *B. attenuata* grown on all substrates. Hidaka and Kitayama (2009) suggested that high PPUE is sustained by the allocation of a greater proportion of P to metabolic P (metabolite P + Pi) than to structural P, as we have shown here. In addition, *B. sessilis* had a lower LMA than *B. attenuata* on all substrates tested; however, *B. attenuata* had higher lipid P concentrations when grown in SLAT and SLIM in response

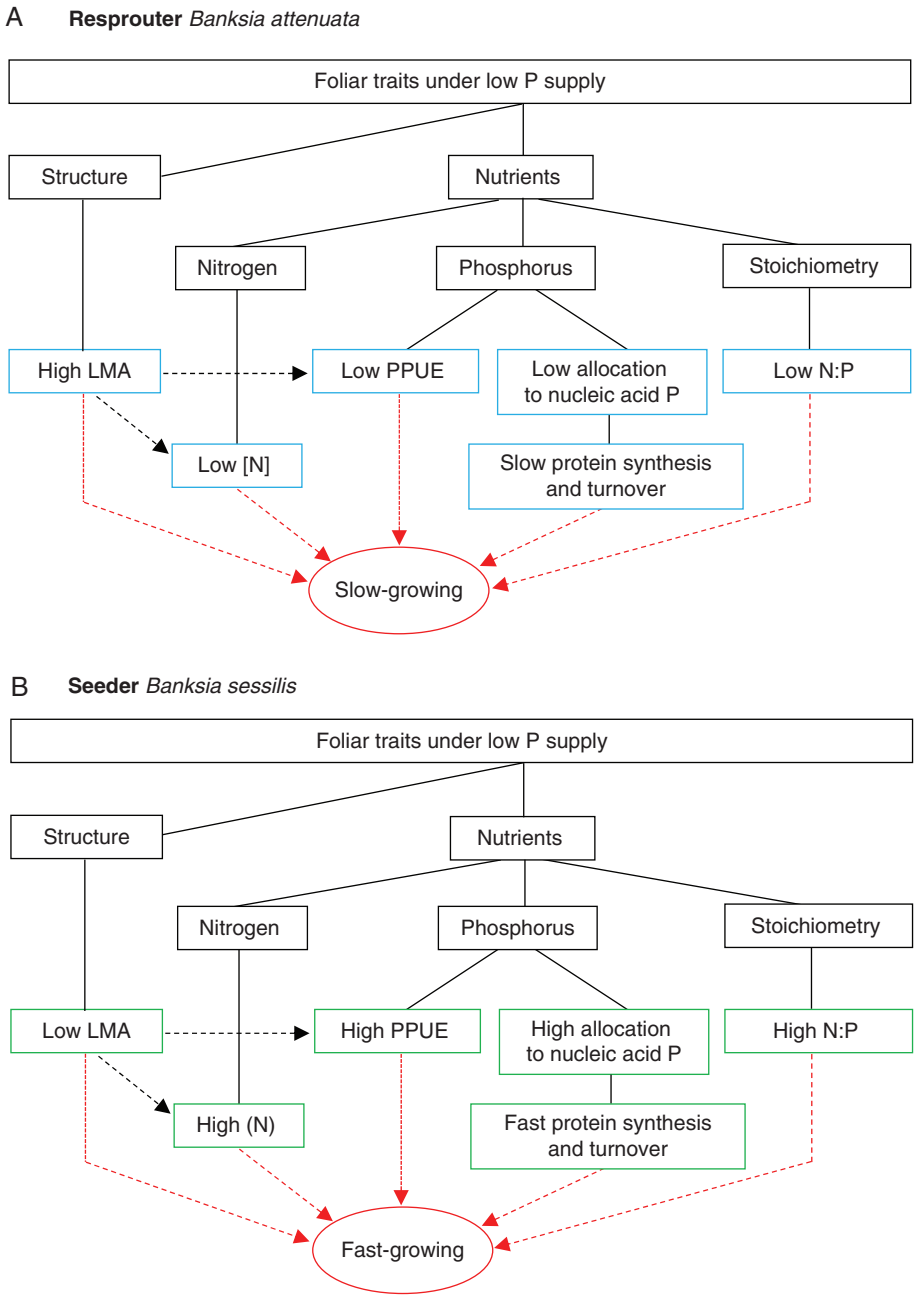

FIG. 6. Diagrams summarizing foliar traits affecting relative growth rates for the resprouter *Banksia attenuata* (A) and the seeder *Banksia sessilis* (B). Solid lines indicate connections between factors; dashed arrows indicate factors that affect another factor; the red dashed arrow indicates that the factor affects the relative growth rate. P, phosphorus; LMA, leaf mass per area; PPUE, photosynthetic P use efficiency.

to the lower P availability compared with sand alone. This finding was partially in line with a study that showed that the concentration of structural P is greater in slow-growing plants with high LMA than in fast-growing plants with low LMA (Villar *et al.*, 2006). In other words, a greater proportion of nucleic acid P, a lower proportion of lipid P and a lower LMA in *B. sessilis* than in *B. attenuata* are all traits associated with a faster RGR and shorter leaf life span (Veneklaas *et al.*, 2012). Overall, the unique foliar traits of the two species revealed different patterns of P allocation in response to soil P availability and associated with growth strategy that may define the ecological niches in which they are found (Fig. 6).

Conclusions

Both *B. attenuata* and *B. sessilis* exhibited a unique pattern of allocating P to different P fractions within the leaves under P limitation. Faster growth of *B. sessilis* was associated with greater allocation of P to nucleic acids than in the slower growing *B. attenuata* to support greater protein synthesis, which is likely to be needed for greater protein turnover associated with rapid growth rates. The observations that *B. sessilis* had a lower LMA and higher N concentration, PPUE, allocation of P to nucleic acids and N:P ratios than *B. attenuata* are possibly adaptive traits to growth in more severely P-impoverished soils, and may account for the different distributions of the two species. We surmise that P allocation patterns are probably the functional basis explaining why plants can reduce foliar P concentrations on P-impoverished soils. The foliar nutrient-allocation patterns and distinct foliar traits of the two *Banksia* species reveal different adaptive strategies in response to soil P availability and match their differences in growth strategies.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of Figure S1: the phosphorus concentration in soil solution extracted by citrate and *iso*-citrate for different soil substrates.

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