Leaf phosphorus allocation to chemical fractions and its seasonal variation in south-western Australia is a species-dependent trait

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HIGHLIGHTS

- Little convergence of foliar P allocation was found within family, season or habitat.
- Plants in south-western Australia exhibited species-dependent foliar P-allocation patterns.
- Species converged on high photosynthetic P-use efficiency, each with their specific way.

GRAPHICAL ABSTRACT
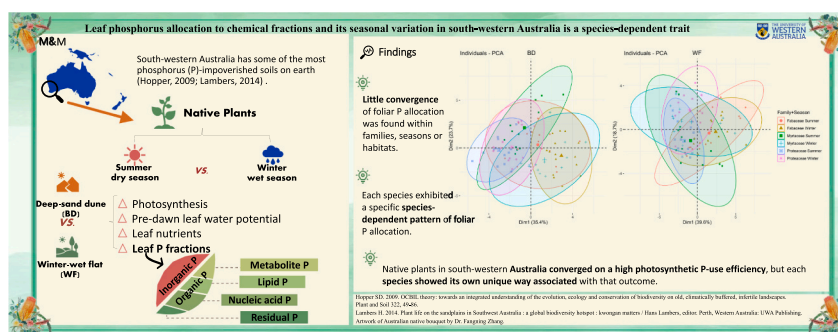
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ABSTRACT

South-western Australia is a global biodiversity hotspot and has some of the oldest and most phosphorus (P)-impoverished soils in the world. Proteaceae is one of the dominant P-efficient plant families there, but it is unknown how leaf P concentrations and foliar P allocation of Proteaceae and coexisting dominant plant families vary between seasons and habitats. To investigate this, we selected 18 species from Proteaceae, Myrtaceae and Fabaceae, six from each family, in two habitats from Alison Baird Reserve (32°1′19″S 15°58′52″E) in Western Australia. Total leaf P and nitrogen (N) concentrations, leaf mass per area, photosynthetic rate, pre-dawn leaf water potential and foliar P fractions were determined for each species both at the end of summer (March 2019 and early April 2020) and at the end of winter (September 2019). Soil P availability was also determined for each site. This is the very first study that focused on seasonal changes of foliar P fractions from different P-impoverished environments in three plant families. However, contrary to our expectation, we found little evidence for convergence of foliar P allocation within family, season or habitat. Each species exhibited a specific species-dependent pattern of foliar P allocation, and many species showed differences between seasons. Native plants in south-western Australia converged on a high photosynthetic P-use efficiency, but each species showed its own unique way associated with that outcome.

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1. Introduction

South-western Australia is a global biodiversity hotspot (Myers et al., 2000), and has some of the oldest and most phosphorus (P)-impoverished soils in the world (Hopper, 2009; Lambers, 2014). Phosphorus is an essential plant macronutrient that plays a key role in plant productivity in natural and managed ecosystems (Vitousek et al., 2010), including in south-western Australia (Hopper, 2009; Hopper and Gioia, 2004; Lambers et al., 2010). Leaf P concentrations of native plants in south-western Australia are well below the global average values, including those on plants in other P-impoverished areas like South Africa and south-eastern China (Lambers et al., 2010; Wright et al., 2004).

Foliar P concentrations are closely associated with leaf photosynthetic traits. Reich and Schoettle (1988) reported that the net photosynthetic capacity (A_{max}) of white pine (*Pinus strobus*) is strongly positively correlated with foliar P concentration. Wright et al. (2004) found that lower foliar P concentrations and lower mass-based photosynthetic rates are often correlated with higher leaf mass per area (LMA) in their compilation of leaf trait data for more than 2500 species over 175 sites. Moreover, a low foliar P concentration is associated with a higher photosynthetic P-use efficiency (PPUE) in 340 tropical species (Hidaka and Kitayama, 2009). In another study, 10 tropical tree species had both higher LMA and higher PPUE on low-P soils (Hidaka and Kitayama, 2013). Foliar P concentrations may differ with species and leaf developmental stage (Tsujii et al., 2017), temperature and precipitation, hence water availability (Guo et al., 2020; Reich and Oleksyn, 2004; Yuan and Chen, 2009) and other soil conditions, including soil nutrient availability (Hayes et al., 2014; Yan et al., 2019) and soil pH (Lambers et al., 2008).

Proteaceae is one of the dominant plant families in south-western Australia. Many pivotal traits have evolved in Proteaceae that allow them to survive in P-impoverished soils (Hayes et al., 2021; Lambers et al., 2015; Pate et al., 2001). For instance, they acquire P from severely P-impoverished soil very efficiently by producing specialised carboxylate-releasing cluster roots (Neumann and Martinoia, 2002; Shane and Lambers, 2005). Proteaceae also have efficient strategies of P utilisation, such as highly efficient remobilisation of P from senescing organs to growing organs (Hayes et al., 2021) and delayed greening in young leaves (Kuppusamy et al., 2014; Lambers et al., 2012). They also tightly control their nitrate uptake (Liu et al., 2022; Prodhan et al., 2019; Prodhan et al., 2016) and function at low protein concentrations (Lambers et al., 2015) which reduces their demand for P, including a low investment of P in rRNA for protein synthesis (Sulpice et al., 2014). Proteaceae from P-impoverished landscapes also preferentially allocate P to photosynthetically-active mesophyll cells, rather than epidermal cells (Hayes et al., 2018). These traits allow Proteaceae to thrive in south-western Australia, especially on the most severely P-impoverished soils (Lambers et al., 2010).

One adaptive P-efficiency trait that evolved in Proteaceae is the ability to form cluster roots that allow P acquisition when soil P concentrations are extremely low (Lambers, 2022). Plants that produce cluster roots exhibit significant seasonal variation in P acquisition (Jeschke and Pate, 1995). In regions with a Mediterranean climate, such as south-western Australia, precipitation is strongly seasonal (Gentilli, 1972). Rainfall in south-western Australia mostly occurs in winter (June-early September), when the greater water availability in soil would result in more P available for plant uptake. Native cluster-rooted plants in south-western Australia produce cluster roots only during the wet winter season, when they acquire P that is stored until it is used during spring and summer for leaf growth (Jeschke and Pate, 1995). For example, Australian Proteaceae like *Banksia menziesii*, *Ba attenuata* and *Ba hookeriana* develop cluster roots only for two to three months in winter-spring (Lamont, 1982).

We know much about the P nutrition of Proteaceae on severely P-impoverished soils, as shown above. Even though, Fabaceae and Myrtaceae are also dominant families in these habitats, we know relatively little about their P-efficiency traits. Like Proteaceae, some Myrtaceae and Fabaceae have high P-use efficiency traits. For example, two Myrtaceae species have nitrate-uptake restraint, which is related to their low demand for P (Liu et al., 2022), and some Fabaceae species produce cluster roots (Nge et al., 2020). However, how Myrtaceae and Fabaceae allocate P to different fractions in their leaves is unknown.

Phosphorus in plant cells can be chemically separated into five functional fractions: inorganic phosphate (Pi), and the organic fractions nucleic acids, phospholipids, metabolites, and a residual fraction of poorly-characterised composition (Chapin and Bieleski, 1982; Hidaka and Kitayama, 2013; Yan et al., 2019). Inorganic phosphate is a significant fraction of the total leaf P pool when P availability is high (Veneklaas et al., 2012). Nucleic acid P is typically the largest among the organic P fractions (Suriyagoda et al., 2023). Leaf P allocation patterns give an insight into P-use strategies. Native plant species in south-western Australia have low leaf P concentrations while exhibiting rapid rates of photosynthesis (Lambers et al., 2010; Lambers et al., 2015; Sulpice et al., 2014). Studying leaf P allocation of native species would provide valuable information into P-efficient strategies which is crucially important to ecosystem restoration and future P-efficient crop development. The allocation patterns of P to different fractions in leaves might depend on soil nutrient availability and soil age, which would impact species distribution (Veneklaas et al., 2012; Yan et al., 2019). However, it is unknown how leaf P concentrations and foliar P allocation vary between seasons, or how they respond to habitats that provide a different availability of both P and water.

The aim of this research was to discover how leaf P concentrations and foliar P fractions vary between summer and winter and between relatively wetter and dryer habitats. This is the very first study that focuses on seasonal changes of P fractions in three plant families. We determined foliar total P concentrations, allocation of P to chemical fractions and photosynthetic traits among Proteaceae, Myrtaceae and Fabaceae from two severely P-impoverished study sites in south-western Australia at the end of the dry summer (March/April) and at the end of the wet winter (September). We hypothesised that Pi would be the largest P pool among the five fractions and that native species would have higher leaf total P concentrations and a greater proportion of Pi in winter than in summer due to seasonal changes of precipitation in south-western Australia. We also hypothesised that species from the same family would show convergence of their leaf P-allocation pattern: nucleic acid P of Proteaceae and Myrtaceae species would not change significantly between seasons since Proteaceae and Myrtaceae species tightly control their nitrate uptake (Liu et al., 2022; Prodhan et al., 2016), which is necessary for protein synthesis. Fabaceae would generally have a greater proportion of nucleic acid P than Proteaceae and Myrtaceae, since most Fabaceae species symbiotically fix N_2 and generally have higher leaf N concentrations than co-occurring non-Fabaceae. Proteaceae would show more seasonal variation than other species since the development of cluster roots, and therefore P acquisition is restricted to the wet winter season (Jeschke and Pate, 1995).

Walker and Syers (1976) emphasised all terrestrial ecosystems begin with a limited amount of P in the parent material that then gets weathered; therefore, even a small loss of P might not be replenished again which makes it important to understand how P-efficient native species use P at both ecological and physiological levels. Moreover, the overuse of P fertiliser in agriculture has tripled the speed of depletion of P reserves (Smit et al., 2009) and has led to eutrophication of waterways and terrestrial systems (Lambers et al., 2015). This is the very first study that investigates seasonal changes of foliar P fractions in plants from different P-impoverished environments in three plant families. This study will provide a better understanding of ecosystem functioning in severely P-impoverished environments, and may be an important foundation for efforts to restore degraded ecosystems, as well as increase P-utilisation efficiency in crops (Calvo et al., 2014; Cong et al., 2020).

2. Materials and methods

2.1. Study site and species selection

Field work for this study was conducted at two sites in Alison Baird Reserve (32°1′19″S 15°58′52″E). The climate in the study area is Mediterranean, with warm dry summers (December to February) and cool wet winters (June to August) (Gentilli, 1972). Rainfall mostly occurs from late May to early September. The mean annual rainfall (1989–2020) is 804.3 mm according to Gosnells City weather station (32°3′S, 115.58′48″E) (Australian Government Bureau of Meteorology, 2022). The site on top of a Bassendean sand dune (BD) has soil that is moist in winter, but never waterlogged due to its elevation and well-drained sandy composition, and dry in summer, while the West flat (WF) site has a five-meters lower elevation where water accumulates and waterlogs plant in winter, but is dry in summer (Gao et al., 2020) (Fig. 1) Waterlogging at WF usually occurs intermittently from July to early September when rainfall is more abundant during the rainy season. The soils at both sites are characteristic of old climatically-buffered infertile landscapes (OCBILs) (Hopper, 2009; Hopper et al., 2016) which belong to the most P-impoverished soils globally (Leopold and Zhong, 2019). The mean annual maximum temperature is 25.6 °C, while the mean monthly highest and lowest temperature are 33 °C (January) and 18.7 °C (July), respectively (Australian Government Bureau of Meteorology, 2022).

The target species were Proteaceae, Fabaceae and Myrtaceae, which are three dominant native plant families in south-western Australia, including the reserve. Three species from each family were selected from each site (Table S1). In general, plants on the Bassendean dune have deeper roots than plants in the flat area, as shown by analysis of stable isotopes of soil water and xylem sap (Gao et al., 2020). All targeted species are evergreen; Proteaceae produce new leaves in spring, while Fabaceae and Myrtaceae produce new leaves all year round, providing water is adequate for leaf growth.

2.2. Plant and soil collection

Fieldwork was conducted at the end of winter (September 2019) before soil inundation ceased, and at the end of summer (early April 2019 and March 2020), before there were substantial autumn/winter rain events. Leaf sample collection was conducted between 9 am and 11.30 am on sunny days in both seasons. Mature most-recently fully-expanded leaves with no visible damage were sampled from five individuals of each species (Fig. 1). Targeted plants did not have significant shading within their canopies, and leaves were collected from around the sun-exposed canopy of the plants. Samples were snap-frozen in liquid nitrogen at harvest. At each WF and BD site, six replicates of soil samples evenly distributed around the study site were collected (Fig. 1). Each replicate comprised three pooled subsamples (20 cm deep) that were taken about one meter apart.

2.3. Pre-dawn water potential

The pre-dawn leaf water potential of individual plants was measured using a PMS Model 1000 Pressure Chamber (PMS Instrument Company, Corvallis, OR, USA). Leaves or leafy twigs were cut, immediately placed in plastic zip bags and transferred to the pressure chamber for measurement. All measurements were done on cloudless mornings before sunrise both at the end of winter (September 2019) and at the end of summer (early April 2019 and March 2020).
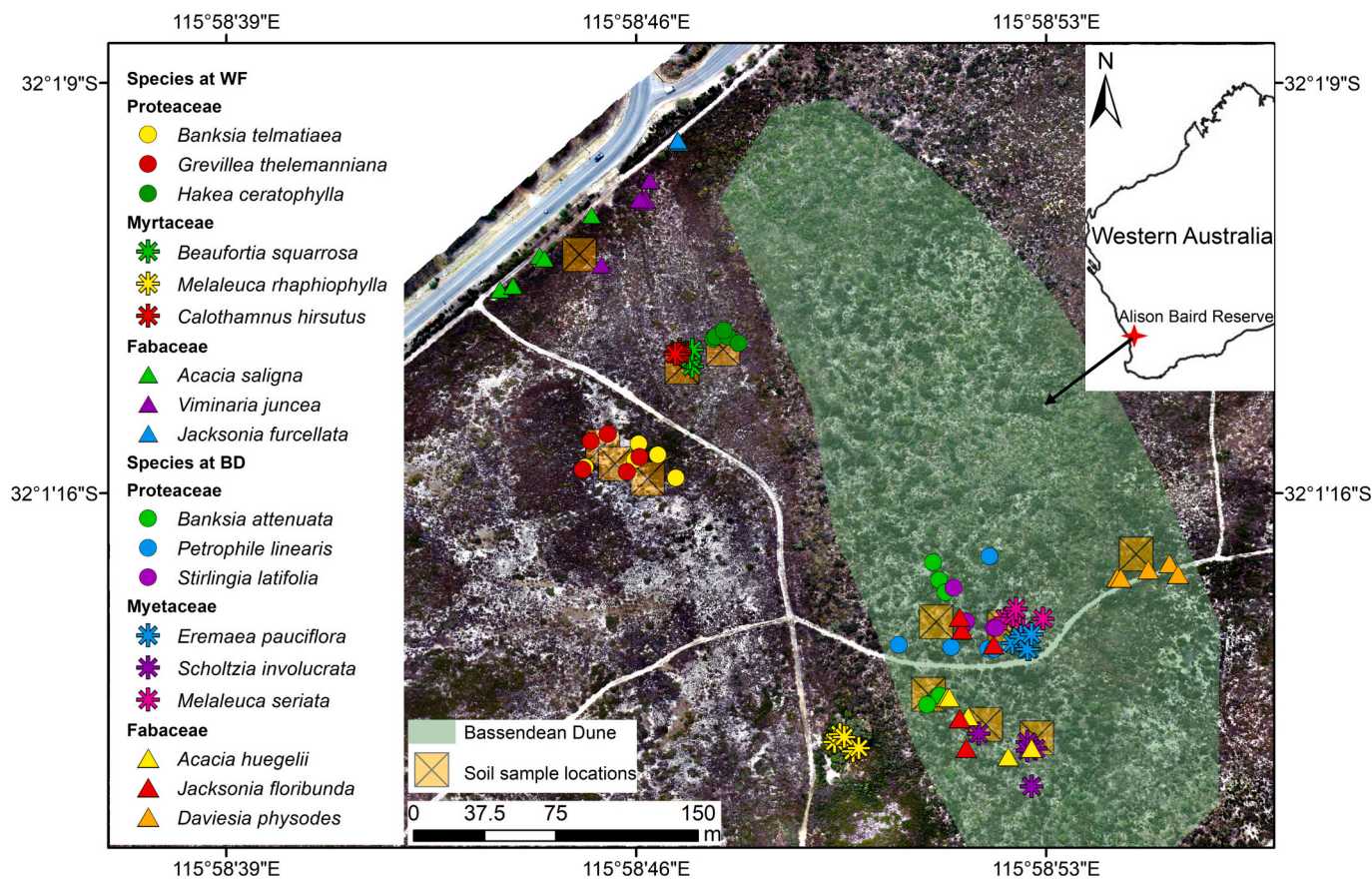

Fig. 1. Sampling sites for soil and 18 targeted plant species in Alison Baird Reserve, which is located about 20 km southeast of Perth. WF: West flat area of the reserve, BD: top of the Bassendean dune. The inset shows the location of Alison Baird Reserve in south-western Australia. The image has been falsely coloured to highlight the Bassendean dune (green). The map was generated using ArcMap 10.8.2 GIS software.

2.4. Photosynthetic traits and photosynthetic nutrient-use efficiency

Leaf photosynthesis was measured for each individual between 9 am and 11.30 am on sunny days both at the end of winter (September 2019) and at the end of summer (early April 2019 and March 2020) using a portable gas exchange system (Li-6400XT, LiCor, Lincoln NE, USA) with reference CO_2 level set at 400 μmol CO_2 mol^{-1}, chamber temperature at 25 °C and relative humidity controlled at 50 % to 70 %. Li-6400 measures the differences in CO_2 and H_2O in an air stream that is flowing through the leaf cuvette. Leaf area in the cuvette was measured to calculate the photosynthetic rate on a leaf area basis.

Photosynthetic P-use efficiency (PPUE) and photosynthetic N-use efficiency (PNUE), the photosynthetic rate per unit of leaf P and leaf N, respectively, were calculated by dividing maximum net photosynthetic rate A_{max} (mass basis) by leaf P concentrations or leaf N concentrations (mass basis). Photosynthetic rate on a mass basis was calculated by dividing photosynthetic rate on an area basis by leaf mass per area (LMA).

To measure LMA, fresh leaves were scanned (Epson Perfection V800 Photo Scanner, Epson, Los Alamitos, USA) to determine their leaf area (WinRHIZO, Regent Instruments, Québec City, Canada), then dried at 70–80 °C for 72 h until constant weight. Leaf mass per area was calculated by dividing oven-dried leaf mass by leaf area.

2.5. Leaf nutrient analyses

Leaf samples were freeze-dried for seven days (VirTis Bench Top freeze dryer, SP Scientific, New York, USA) then ground to a fine powder (Geno Grinder, Spex SamplePrep, Metuchen, NJ, USA).

Total leaf P concentrations were determined using a malachite green-based method (Motomizu et al., 1983) after acid digestion of samples. Aliquots of 100 mg of ground freeze-dried samples were acid digested with 3 mL of 70 % HNO_3 at 80 °C to 90 °C, followed by digestion with 0.5 mL of 70 % $HClO_4$ at 130 °C to 150 °C, then with 1 mL of 32 % HCl at 130 °C. Deionised water was added to adjust the final volume to 10 mL before determining P.

Inorganic phosphate (Pi) was extracted from 25 mg freeze-dried leaf samples by homogenising in ice-cold 1 % (v/v) acetic acid (Precellys 24 Homogeniser, Thermo Fisher Scientific, Waltham, USA) and special care was taken to keep the samples and solutions cold. The homogenate was clarified twice by centrifugation at 21,000 $\times g$ for 15 min at 4 °C (Yan et al., 2019). The extracts were treated with acid-washed activated charcoal to remove interfering substances (Dayrell et al., 2022). The P concentration in the final extracts was determined colorimetrically using a malachite green-based method (Motomizu et al., 1983).

Aliquots of 50 mg of freeze-dried ground leaf samples were used to determine P allocation to nucleic acids, phospholipids, small P-containing metabolites and residual P using the method of Yan et al. (2019), adapted from Hidaka and Kitayama (2013). Fractions were separated based on differential solubility in various solvents. Metabolic P, which contains Metabolite P and Pi, was highly water-soluble material. Lipid P was chloroform-soluble material. Nucleic acid P was insoluble in 5 % trichloroacetic acid but soluble in 2.5 % trichloroacetic acid at 95 °C. Residual P was material that was not soluble in either 5 % or 2.5 % trichloroacetic acid.

Total leaf N concentrations were determined by combustion using an elemental analyser (Elementar Australia Pty Ltd., Sydney, Australia).

2.6. Soil nutrient analyses

Total soil P concentrations were determined by perchloric acid digestion (Olsen and Dean, 1982) followed by a malachite green-based assay (Motomizu et al., 1983). Soil resin P, which is readily plant-available P, was extracted using an anion exchange membrane (VWR Chemical, Leuven, Belgium) from 1:10 soil:water solution by shaking for eight hours. The resin-absorbed P was eluted from the membranes by

transferring them to 0.5 M HCl and shaking for one hour (Bentley et al., 1999). The P concentration in the extracts was determined by a malachite green-based method (Motomizu et al., 1983).

2.7. Statistics

Statistical analyses were carried out in R studio v.3.6.0 (R Development Core Team, 2023). Differences in each parameter between seasons and between sites were tested using Student's t-test at $P < 0.05$ after testing the variances of each group. Differences in each parameter among species were analysed by one-way analysis of variance (ANOVA) using Fisher's least significant difference (LSD) test at $P < 0.05$ after testing assumptions of ANOVA.

Principal component analysis (PCA) of all parameters for each species, family and study site were conducted using the 'devtools' package in R studio v.3.6.0 (R Development Core Team, 2023).

3. Results

3.1. Soil P concentrations

Soil on top of the Bassendean dune had a lower average concentration of total P (9.0 mg kg^{-1}) and organic P (7.3 mg kg^{-1}) than the soil on the West flat area (14.3 mg kg^{-1} and 11.8 mg kg^{-1}, respectively), although they were not significantly different between the two sites ($P > 0.05$), because of a large variability at the West flat area (Fig. S1). Soil resin P concentration was the same for both sites, both around 0.6 mg kg^{-1} ($P > 0.05$).

3.2. Pre-dawn water potential

All targeted species had significantly higher (less negative) pre-dawn leaf water potentials in winter than in summer (Fig. 2). In winter, the pre-dawn water potentials varied from −0.50 MPa for *Jacksonia furcellata* to 0 MPa for *Stirlingia latifolia*. *Jacksonia furcellata* and *Acacia huegelii* had a relatively more negative pre-dawn leaf water potential of −0.50 MPa and − 0.48 MPa, respectively, than that of other species. There were no significant differences among species from the two study sites tested in winter ($P > 0.05$). In summer, species at the West flat area had more negative leaf water potentials than species on the Bassendean dune ($P < 0.01$). The pre-dawn water potential of species at the West flat area in summer varied from −3.20 MPa for *Grevillea thelemanniana* to −1.20 MPa for *A. saligna*, while the values for species on the Bassendean dune ranged from −2.20 MPa for *Scholtzia involucrata* to −0.96 MPa for *Ba attenuata*.

3.3. Leaf P concentrations and photosynthetic traits

For species on top of the Bassendean dune, *Petrophile linearis*, *Sc involucrata*, *Eremaea pauciflora*, *Daviesia physodes* all had significantly higher leaf P concentrations (both dry mass-based and leaf area-based) in winter than in summer. *Acacia huegelii* had a significantly higher dry mass-based P concentration in winter than in summer ($P < 0.05$), *J. floribunda* had a lower leaf area-based P concentration in winter than in summer ($P < 0.05$), while leaf P concentration in all other species could not be distinguished between seasons ($P > 0.05$) (Fig. 3a, b). For species in the West flat area, *Calothamnus hirsutus* had higher dry mass-based and leaf area-based leaf P concentrations in summer than in winter ($P < 0.05$). In contrast, *Viminaria juncea* had a higher dry mass-based leaf P concentration in winter than in summer ($P < 0.05$) (Fig. 3a, b).

Overall, Fabaceae had the highest leaf P concentrations, averaging 0.45 mg P g^{-1} DW, while Proteaceae had the lowest, averaging 0.26 mg P g^{-1} DW ($P < 0.05$) (Fig. S2). The species at the West flat area also had higher leaf P concentrations, averaging 0.40 mg P g^{-1} DW, than the species on top of the Bassendean dune, averaging 0.31 mg P g^{-1} DW (P
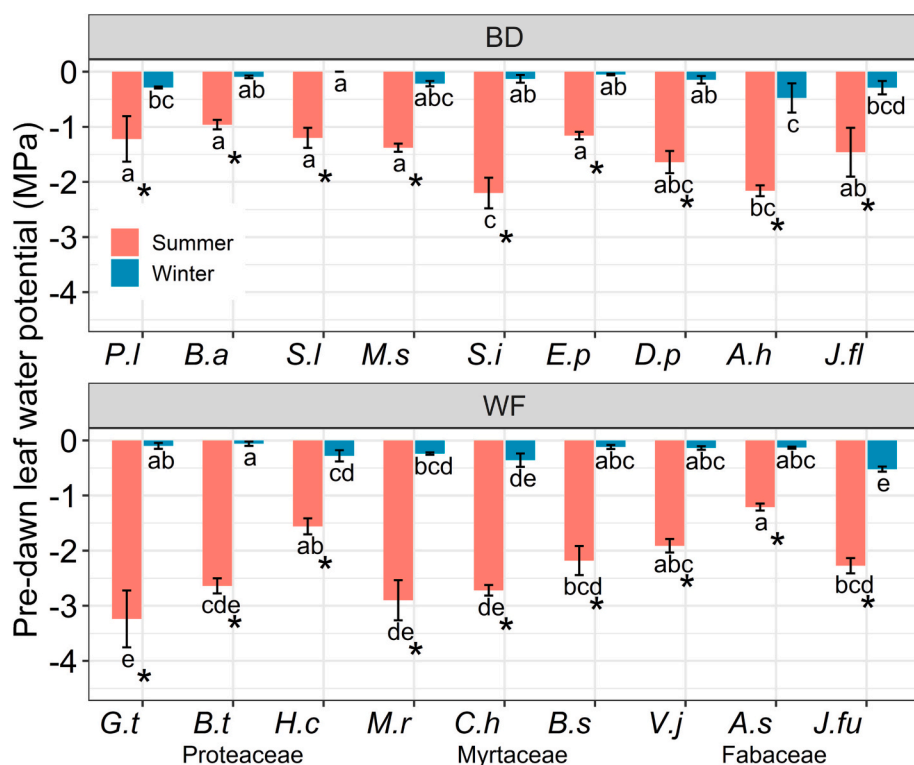
Fig. 2. Pre-dawn leaf water potential of 18 species in Alison Baird Reserve in both summer and winter. Means ± standard errors ($n = 5$) are given. For each species, significant differences between seasons were determined by Student's t-test ($P < 0.05$) and are indicated by *. For each season, significant differences among species at each study site were determined by ANOVA with Fisher's least significant difference (LSD) test ($P < 0.05$) and indicated by different letters. BD: top of the Bassendean dune, WF: West flat area of the reserve. *P.l, Petrophile linearis; B. a, Banksia attenuata; S.l, Stirlingia latifolia; M.s, Melaleuca seriata; S.i, Scholtzia involucrata; E.p, Eremaea pauciflora; D.p, Daviesia physodes; A.h, Acacia huegelii; J.fl, Jacksonia floribunda; G.t, Grevillea thelemanniana; B.t, Banksia telmatiaea; H.c, Hakea ceratophylla; B.s, Beaufortia squarrosa; C.h, Calothamnus hirsutus; M.r, Melaleuca rhaphiophylla; A.s, Acacia saligna; V.j, Viminaria juncea; J.fu, Jacksonia furcellata.*

< 0.05) (Fig. S3). Intriguingly, Proteaceae and Fabaceae on top of the Bassendean dune had significantly higher leaf P concentrations in winter than in summer ($P < 0.05$), while species from all three families at the West flat area had indistinguishable leaf P concentrations in summer and winter ($P > 0.05$) (Fig. S4a).

Leaf mass per area (LMA) ranged from 104.7 g m^{-2} to 584.0 g m^{-2} for all species and both seasons. *Melaleuca seriata, Sc involucrata, J. floribunda* on top of the Bassendean dune and *Ba telmatiaea* and *J. furcellata* at the West flat area all had significantly higher LMA in winter than in summer ($P < 0.05$), while all other species had a indistinguishable LMA in summer and winter ($P > 0.05$) (Fig. 3c).

No difference of leaf area-based photosynthetic rate was found between the sites ($P > 0.05$) (Fig. S5). Species at the West flat area had faster dry mass-based photosynthetic rates than those on the Bassendean dune ($P < 0.05$). These species also had both faster leaf area-based and dry mass-based photosynthetic rates in winter than in summer ($P < 0.05$), while species on the Bassendean dune did not (Figs. S5, S6). Among the three families, Fabaceae had the fastest average photosynthetic rates, both on a leaf area basis ($P < 0.05$) and a dry mass basis ($P < 0.05$), averaging 12.1 μmol CO$_2$ m^{-2} s^{-1} and 58.3 μmol CO$_2$ g^{-1} s^{-1}, respectively. Myrtaceae had the slowest photosynthetic rates on a leaf area basis, averaging 8.8 μmol CO$_2$ m^{-2} s^{-1}, lower than Proteaceae, 11.5 μmol CO$_2$ m^{-2} s^{-1} ($P < 0.05$), while Proteaceae had the slowest photosynthetic rate on a dry mass basis, averaging 37.7 μmol CO$_2$ g^{-1} s^{-1}, slower than Myrtaceae, 50.1 μmol CO$_2$ g^{-1} s^{-1} ($P < 0.05$). On top of the Bassendean dune, *P. linearis* and *Ba attenuata* had both a significantly faster photosynthetic rate and higher PPUE in winter than in summer ($P < 0.05$), while *M. seriata* and *E. pauciflora* had both a significantly faster photosynthetic rate and higher PPUE in summer than in winter ($P < 0.05$) (Fig. 3d-f). *Stirlingia latifolia* had a higher PPUE in summer than in winter ($P < 0.05$). At the West flat, *G. thelemanniana, Ba telmatiaea* and *J. furcellata* had both a significantly faster photosynthetic rate and higher PPUE in winter than in summer ($P < 0.05$), while *M. rhaphiophylla* had a faster photosynthetic rate in winter but did not show a difference in PPUE between seasons ($P > 0.05$). All other species at both sites did not show significant differences in photosynthetic rate

or PPUE between summer and winter ($P > 0.05$). We found no strong correlations between PPUE and any leaf P fraction ($R^2 < 0.25$) (Figs. S7, S8).

Stomatal conductance of targeted specie showed similar patterns between seasons and among species as photosynthesis rate in general (Fig. S9). However, more species at West flat showed differences in stomatal conductance than in photosynthesis rate between seasons. All species at West flat except for *C. hirsutus, A. saligna* and *J. furcellata*, had higher stomatal conductance in winter than in summer ($P < 0.05$). *Petrophile linearis, Ba attenuata* and *D. physodes* from Bassendean dune also showed higher stomatal conductance in winter than in summer ($P < 0.05$). Among species from Bassendean dune, *P. linearis, Ba attenuata* had relatively higher stomatal conductance in winter than other species ($P < 0.05$), while *Sc involucrata* had the highest stomatal conductance in summer ($P < 0.05$). Among species from West flat, *A. saligna* showed the highest stomatal conductance in both summer and winter ($P < 0.05$).

3.4. Foliar P fractions

Every targeted species allocated P to major fractions differently. Although not every fraction of the targeted species showed differences between seasons, those of many species differed between winter and summer (Figs. 4, 5). Irrespective of season and location, the most abundant P fraction was nucleic acid P, except for *P. linearis, D. physodes* and *M. rhaphiophylla*. The species on top of the Bassendean dune had a significantly higher nucleic acid P concentration in winter than in summer ($P < 0.05$), except *P. linearis* and *J. floribunda* (Fig. 4a). *Petrophile linearis* and *E. pauciflora* had significantly higher metabolite P concentrations in winter than in summer ($P < 0.05$), while *Ba attenuata* showed exactly the opposite. *Daviesia physodes* had a higher lipid P concentration in winter than in summer ($P < 0.05$). Some species including *St latifolia, M. seriata, E. pauciflora, D. physodes* and *A. huegelii* had a higher residual P concentration in winter than in summer ($P < 0.05$). Phosphorus concentrations for all other fractions in the other species were indistinguishable between summer and winter ($P > 0.05$) (Fig. 4a).
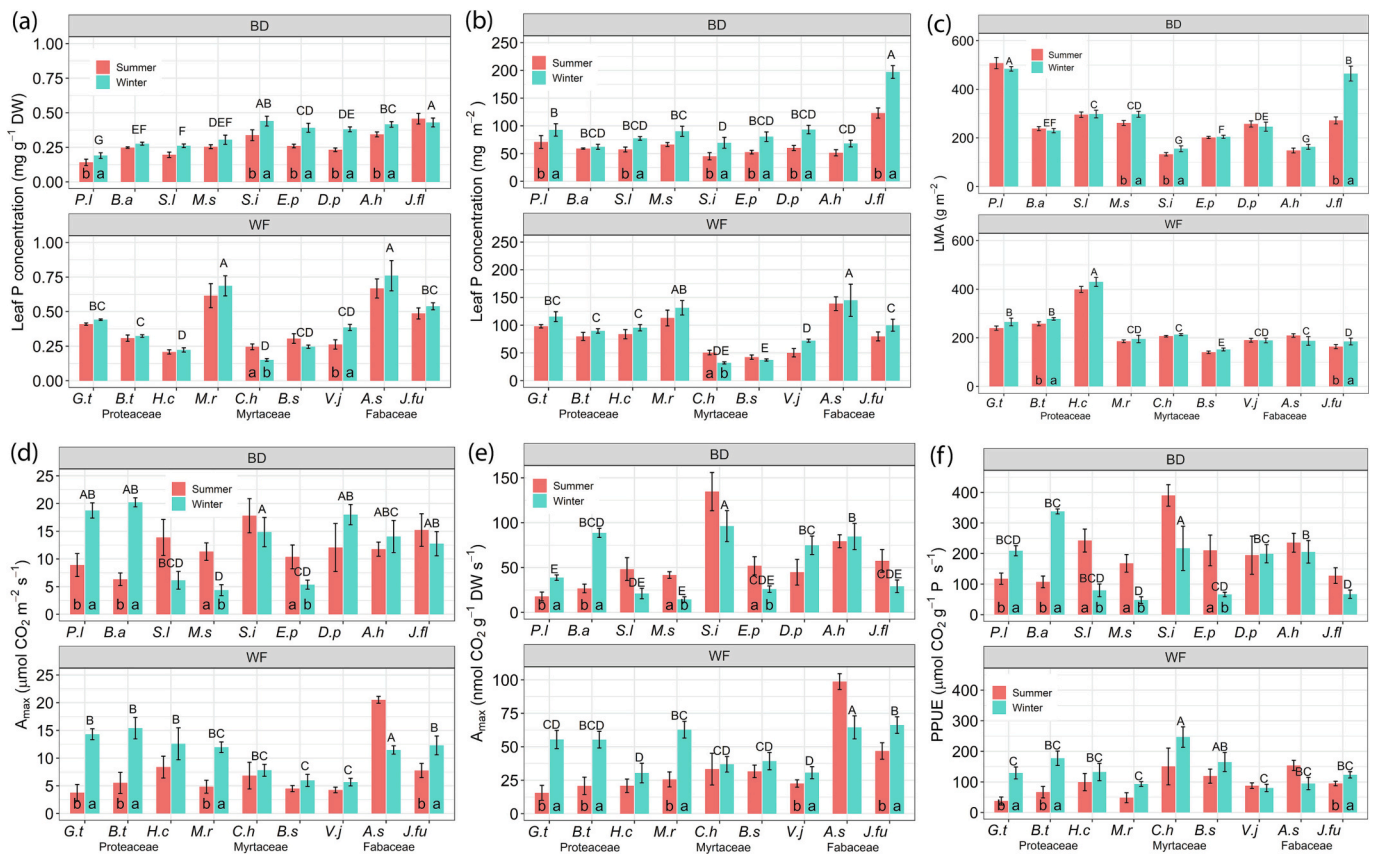
Fig. 3. Leaf phosphorus (P) concentrations and photosynthetic traits of targeted species in summer and winter in Alison Baird Reserve. (a) Leaf P concentrations per unit area, (b) Leaf P concentrations per unit dry weight, (c) Leaf mass per area (LMA), (d-e) Area-based and mass-based maximum net photosynthetic assimilation rate, respectively, and (f) Photosynthetic P-use efficiency (PPUE) of targeted species in both summer and winter. Means ± standard errors ($n = 5$) are given. For each species, significant differences between seasons were determined by Student's t-test ($P < 0.05$) and are indicated by lowercase letters. For each site, significant differences among species were determined by ANOVA with Fisher's least significant difference (LSD) test ($P < 0.05$) and indicated by uppercase letters. BD: top of the Bassendean dune, WF: West flat area of the reserve. *P.l*, Petrophile linearis; *B.a*, Banksia attenuata; *S.l*, Stirlingia latifolia; *M.s*, Melaleuca seriata; *S.i*, Scholtzia involucrata; *E.p*, Eremaea pauciflora; *D.p*, Daviesia physodes; *A.h*, Acacia huegelii; *J.fl*, Jacksonia floribunda; *G.t*, Grevillea thelemanniana; *B.t*, Banksia telmatiaea; *H.c*, Hakea ceratophylla; *B.s*, Beaufortia squarrosa; *C.h*, Calothamnus hirsutus; *M.r*, Melaleuca rhaphiophylla; *A.s*, Acacia saligna; *V.j*, Viminaria juncea; *J.fu*, Jacksonia furcellata. Leaf P concentrations of *M. seriata*, *St latifolia* and *D. physodes* in summer are from Shen (2023).

Unlike species on top of the Bassendean dune, *J. furcellata* was the only species on the West flat that showed a higher nucleic acid P concentration in winter than in summer ($P < 0.05$) (Fig. 4b). *Grevillea thelemanniana*, *Ba telmatiaea*, *A. saligna* and *J. furcellata* all had a significantly higher Pi concentration in winter than in summer ($P < 0.05$). *Grevillea thelemanniana* was the only species that had a significantly higher lipid P concentration in winter than in summer ($P < 0.05$). In contrast, *Be squarrosa* had a lower lipid P concentration in winter than in summer ($P < 0.05$), while all other species in the flat area did not differ significantly in lipid P concentration between seasons ($P > 0.05$). *Viminaria juncea* had higher metabolite P concentrations in winter than in summer ($P < 0.05$), *Ba telmatiaea* had a lower metabolite P concentration in winter ($P < 0.05$). A few species, including *H. ceratophylla*, *C. hirsutus* and *V. juncea* exhibited differences in residual P concentration between summer and winter ($P < 0.05$); other than that, all other P fractions of the other species were indistinguishable between seasons ($P > 0.05$) (Fig. 4b).

Intriguingly, the proportion of total P in each fraction had different patterns from the mass-based P concentrations in each fraction (Fig. 5). For species on top of the Bassendean dune, *P. linearis*, *St latifolia*, *Sc involucrata* and *E. pauciflora* had a significantly higher proportion of lipid P in winter than in summer ($P < 0.05$) (Fig. 5a). *Banksia attenuata*, *St latifolia* and *Sc involucrata* had a significantly higher proportion of nucleic acid P in winter than in summer ($P < 0.05$), while *P. linearis* had a higher proportion of nucleic acid P in summer than in winter ($P <$

0.05) (Fig. 5b). All other species on top of the dune had the same proportion of nucleic acid P in summer and winter ($P < 0.05$). *Petrophile linearis* and *A. huegelii* both had a significantly higher proportion of Pi to total P in winter than in summer ($P < 0.05$), *Ba attenuata* showed a lower proportion of Pi in winter ($P < 0.05$). *Petrophile linearis* showed a higher proportion of metabolite P in winter ($P < 0.05$), mirroring the pattern of the absolute P concentration in its metabolite P. In contrast, *Ba attenuata* had a lower proportion of metabolite P in winter ($P < 0.05$) (Fig. 5a). For species at the West flat, *V. juncea* was the only species that had a significantly different proportion of nucleic acid P between seasons, with a higher proportion of nucleic acid P in summer than in winter ($P < 0.05$). *Grevillea thelemanniana*, *Ba telmatiaea*, *M. rhaphiophylla* and *A. saligna* all had a higher proportion of Pi in winter than in summer ($P < 0.05$), while the proportion of the Pi pool of other species was indistinguishable between summer and winter ($P > 0.05$) (Fig. 5b).

3.5. Leaf N concentrations, N:P ratios and photosynthetic nitrogen-use efficiency (PNUE)

Most of the targeted species from both sites had a significantly higher mass-based leaf N concentration in winter than in summer ($P < 0.05$) (Fig. 6a). *Eremaea pauciflora*, *C. hirsutus*, *Be squarrosa* and *A. saligna* were the only species out of the 18 examined that had indistinguishable leaf N concentration in both seasons ($P > 0.05$). On average, Fabaceae had the highest leaf N concentration among all species, with an average of 16.8
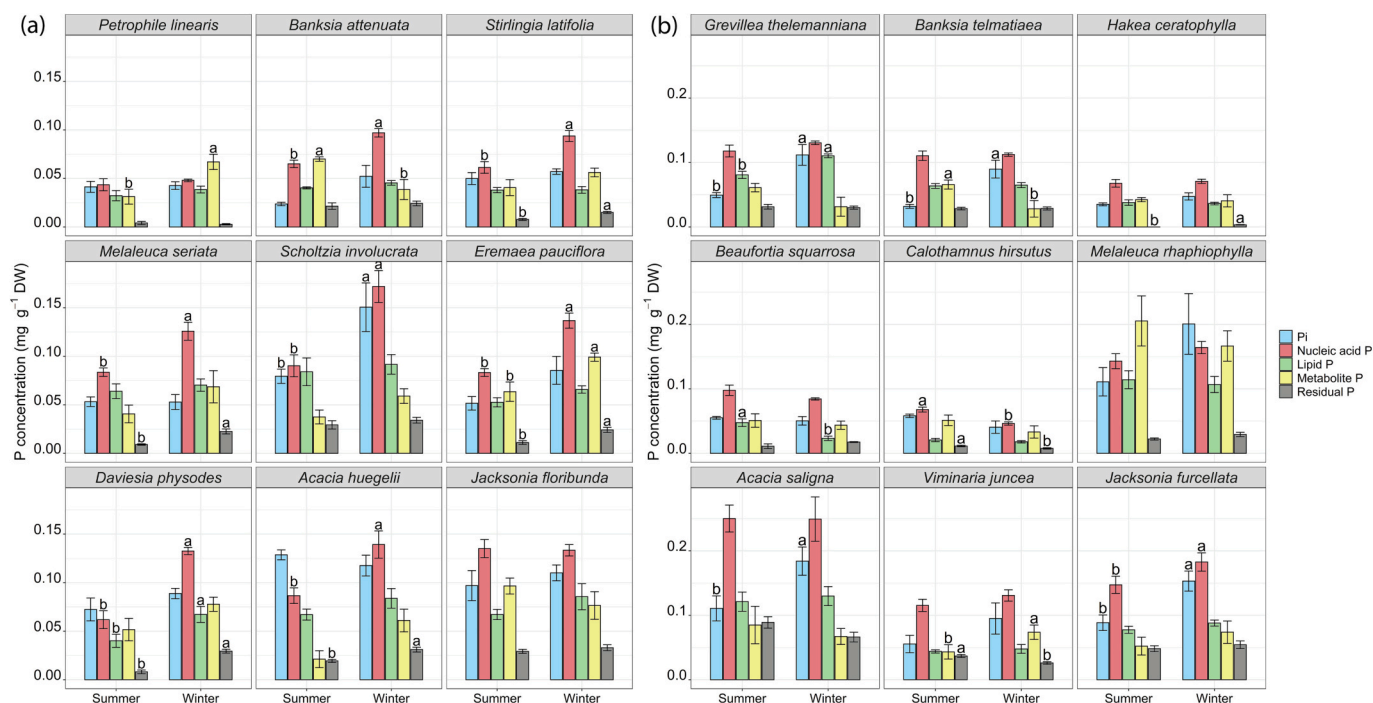
Fig. 4. Foliar phosphorus (P) fraction concentrations of targeted species in Alison Baird Reserve. Foliar P fraction concentrations of species (a) on the Bassendean dune and (b) at the West flat area in summer and winter. Means ± standard errors (*n* = 5) are given. For each species, significant differences between seasons were determined by Student's *t*-test (*P* < 0.05) and indicated by lowercase letters. Data of *M. seriata*, *St latifolia* and *D. physodes* in summer are from Shen (2023).
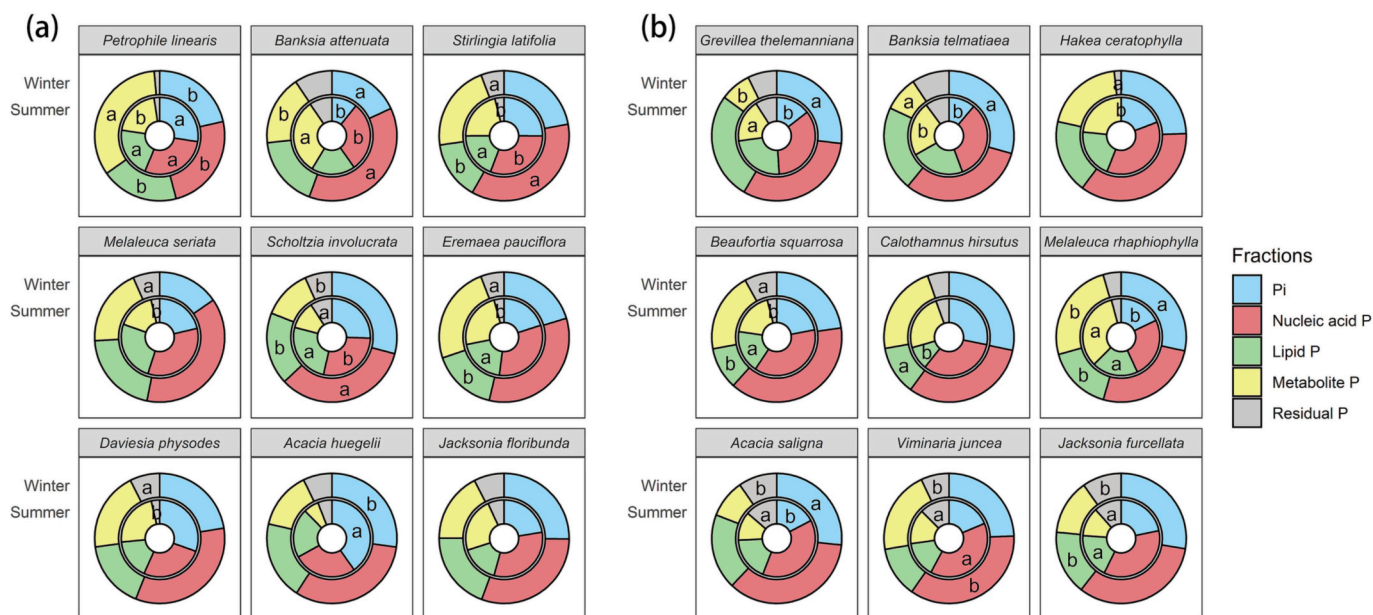

Fig. 5. Foliar phosphorus (P) fractions as a proportion of leaf total P in targeted species in Alison Baird Reserve. Proportions for species (a) on the Bassendean dune and (b) at the West flat area in summer (inner circle) and winter (outer circle). Means (*n* = 5) are given. For each P fraction of species, significant differences between seasons were determined by Student's *t*-test (*P* < 0.05) and indicated by lowercase letters. Data of *M. seriata*, *St latifolia* and *D. physodes* in summer are from Shen (2023).

mg N g^{-1} DW, followed by Myrtaceae (9.5 mg N g^{-1} DW) and Proteaceae (6.9 mg N g^{-1} DW) (*P* < 0.05) (Fig. S10). There was no difference in leaf N concentration between sites (Fig. S11). Leaf N concentration correlated with leaf P concentration most strongly in Myrtaceae (R^2 = 0.69) and least strongly in Proteaceae (R^2 = 0.44) (Fig. S12). Of the P fractions, leaf N concentration correlated most strongly with leaf nucleic acid P concentration. The correlation was strongest for Myrtaceae (R^2 = 0.73), followed by Fabaceae (R^2 = 0.69) and Proteaceae (R^2 = 0.49)

(Fig. S13). All families on top of the Bassendean dune had higher leaf N concentrations in winter than in summer, as did Proteaceae at the West flat (*P* < 0.05) (Fig. S4b).

Banksia attenuata, *G. thelemanniana*, *C. hirsutus* and *Be squarrosa* had a significantly higher leaf N:P ratio in winter than in summer (*P* < 0.05) (Fig. 6b). In contrast, *E. pauciflora* and *D. physodes* were the only two species that had a lower leaf N:P ratio in winter than in summer (*P* < 0.05), while all other species had indistinguishable leaf N:P ratio
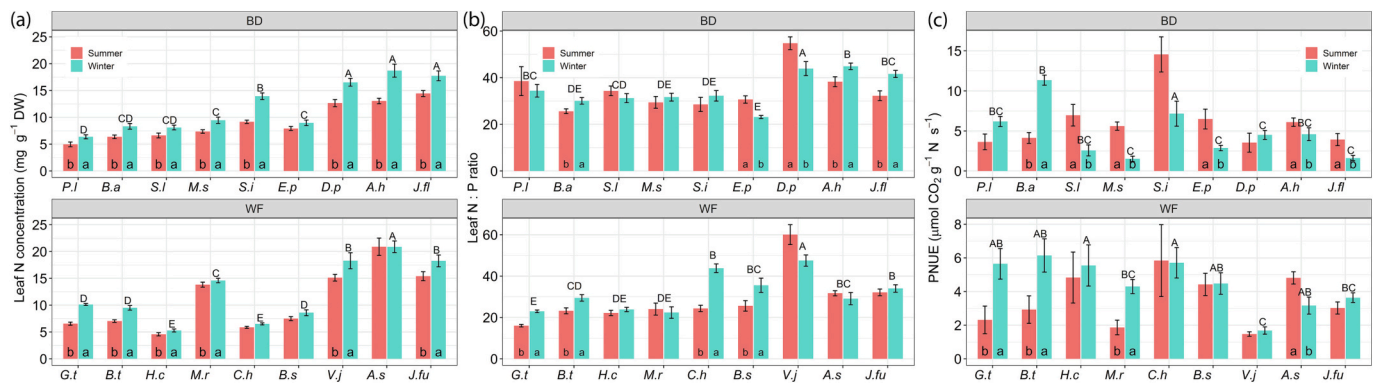
Fig. 6. Leaf nitrogen (N) concentrations (a), leaf N: phosphorus (P) ratios (b) and photosynthetic N-use efficiency (PNUE) (c) of targeted species in summer and winter in Alison Baird Reserve. Means ± standard errors ($n = 5$) are given. For each species, significant differences between seasons were determined by Student's t-test ($P < 0.05$) and are indicated by lowercase letters on the bars. Significant differences among species at each study site were determined by ANOVA with Fisher's least significant difference (LSD) test ($P < 0.05$) and are indicated by uppercase letters. BD: top of the Bassendean dune, WF: West flat area of the reserve. *P.l, Petrophile linearis*; *B.a, Banksia attenuata*; *S.l, Stirlingia latifolia*; *M.s, Melaleuca seriata*; *S.i, Scholtzia involucrata*; *E.p, Eremaea pauciflora*; *D.p, Daviesia physodes*; *A.h, Acacia huegelii*; *J.fl, Jacksonia floribunda*; *G.t, Grevillea thelemanniana*; *B.t, Banksia telmatiaea*; *H.c, Hakea ceratophylla*; *B.s, Beaufortia squarrosa*; *C.h, Calothamnus hirsutus*; *M.r, Melaleuca rhaphiophylla*; *A.s, Acacia saligna*; *V.j, Viminaria juncea*; *J.fu, Jacksonia furcellata*. Leaf P concentrations data of *M. seriata, St latifolia* and *D. physodes* and leaf N concentrations data of *M. seriata, St latifolia, E. pauciflora* and *D. physodes* in summer are from Shen (2023).

between seasons ($P > 0.05$).

On top of the dune, only *Ba attenuata* had a significantly higher photosynthetic N-use efficiency (PNUE) in winter than in summer ($P < 0.05$), whereas *Sc involucrata, M. seriata, E. pauciflora, A. huegelii* and *J. floribunda* had a significantly higher PNUE in summer than in winter ($P < 0.05$) (Fig. 6c). On the West flat, *G. thelemanniana, Ba telmatiaea* and *M. rhaphiophylla* had significantly higher PNUE in winter than in summer ($P < 0.05$), whereas only *A. saligna* had a significantly higher PNUE in summer than in winter ($P < 0.05$) (Fig. 6c). There were no strong correlations between PNUE and any of the leaf P fractions ($R^2 < 0.15$) (Figs. S8, S14).

3.6. Principal component analysis (PCA)

Based on principal component analysis (PCA), season, location and family each explained around 60 % of the variation among the measured traits (Fig. S15). Most of the data points were in overlapping parts of the circles, except for a small number that were outside the overlapping area. There was no clear pattern between seasons, locations or among families (Figs. S15, S16, S17). However, PCA was not representative of the species within the respective group at either ecosystem or family level, as each species expressed a different pattern (Figs. 7, 8). Species from the same family also showed different patterns (Fig. S18), although many species were separated by season (Figs. 7, 8).

4. Discussion

We explored seasonal differences in foliar P fractions and several leaf traits of species from three prominent families in south-western Australia growing at two nutrient-poor sites that differed in water availability. Contrary to our expectation, there was no trait common to all species in the same family at each season and each study site. The key message is that there was little convergence of foliar P fractions for family, season or location, and that each species showed a species-specific pattern of foliar P fractions. Native plants in south-western Australia converged on a high photosynthetic P-use efficiency, but each species showed its own unique way associated with that outcome.

4.1. Plant water relations

The differences in pre-dawn leaf water potentials between the two sites showed that they comprised habitats with different water availability for the targeted species. Differences in pre-dawn leaf water

potential as well as stomatal conductance between seasons also reflected the different water availability between seasons. The greater water availability at the wetter site will have made P available for root uptake for a longer period.

4.2. Differences between sites and seasons

The differences of total leaf P concentrations and photosynthetic traits of the targeted species were associated with different habitats. Species at the West flat had higher leaf P concentrations and lower PPUE than those on the Bassendean dune. Leaf N concentrations were not associated with the different habitats. This might be because native species in severely P-impoverished habitats in south-western Australia tend to restrain their nitrate uptake, regardless of nitrate supply (Liu et al., 2022; Prodhan et al., 2019; Prodhan et al., 2016).

The variation in total leaf P and N concentrations between seasons was impacted by habitat which might be related to different water availability. Precipitation mainly occurs in winter (Australian Government Bureau of Meteorology, 2022) and water accumulates and waterlogs plants at the West flat, while the soil on the Bassendean dune is moist. Since waterlogging is not continuous through the entire rainy season, P availability is unlikely to be affected by redox effects. In contrast, the extra and more persistent water in winter might make more P available for plant uptake. All three families on top of the Bassendean dune had higher leaf P concentration in winter, when plants take up and store most P (Jeschke and Pate, 1995; Lamont, 1982), while we found no seasonal difference at the West flat area; likewise, leaf N concentrations were the same between seasons, except for Proteaceae, which showed seasonal differences at both study sites. Plants exhibit different nutrient preferences and nutrient-utilisation strategies among habitats; differences in leaf P and N concentrations are related to temperature and precipitation, hence water availability (Guo et al., 2020; Reich and Oleksyn, 2004; Yuan and Chen, 2009).

4.3. Differences among families

Total leaf P and N concentrations showed clear differences among families, with Fabaceae having the highest total P and N concentrations and Proteaceae the lowest (Figs. S2, S8). Despite this, leaf P concentrations of Fabaceae were still well below global average values, while leaf N concentrations of Fabaceae were similar to global average values (Tian et al., 2018; Wright et al., 2004). The higher leaf N concentrations of Fabaceae might be associated with their symbiotic N-fixation. The
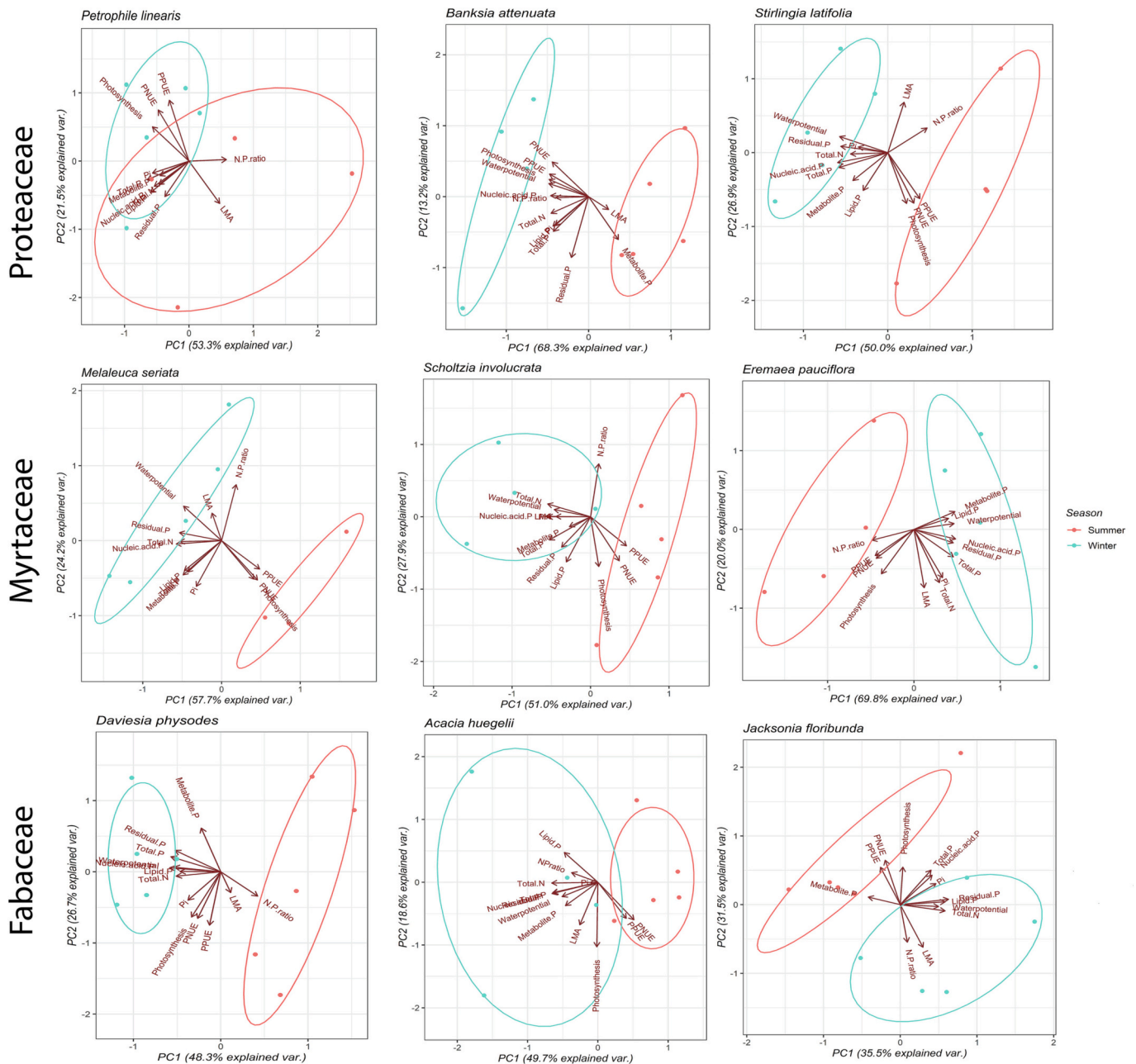
Fig. 7. Principal component analysis (PCA) of all parameters for nine species on top of the Bassendean dune of Alison Baird Reserve in summer (red ellipse) and winter (blue ellipse).

higher leaf P concentrations of Fabaceae species might be related to the distribution of targeted Fabaceae species in the reserve. A study on plant P across a soil chronosequence located near Jurien Bay in Western Australia showed that *A. rostellifera* (Fabaceae) tends to grow on P-richer soils and generally has higher leaf P and N concentrations than *H. prostrata* (Proteaceae) and *M. seriata* (Myrtaceae) in the same habitat (Yan et al., 2019). It is worth noting that the three targeted Fabaceae at the West flat in this study were only found close to a track where there is a higher P concentration due to human activities that disturb the soil (Shen, 2023). *Jacksonia floribunda* and *A. huegelii* on top of the Bassendean dune were often found next to *Ba attenuata,* which produces cluster roots that exude carboxylates into soil and mobilise P from poorly-soluble P complexes, thus creating a slightly P-richer environment for its neighbours (Shane and Lambers, 2005; Shen, 2023). It is quite common for cluster-rooted species such as *Banksia* to make P and manganese (Mn) more available for their neighbours in severely P-

impoverished soil (Abrahão et al., 2018; Lambers et al., 2018; Lambers et al., 2022; Muler et al., 2014; Shen, 2023). Likewise, facilitation of P uptake by carboxylate-releasing neighbours occurs in other natural systems as well as in managed intercropping systems (Li et al., 2014; Yu et al., 2021; Zhou et al., 2022).

Despite clear patterns of total P in terms of families and study sites, there was no leaf P allocation trait common to all species in the same family at each season. We found little convergence of foliar P allocation, either within families or within season, and not within habitat either, based on PCA results (Figs. S15, S17).

4.4. Species differences

Every species allocated P to leaf P fractions differently (Figs. 4, 5). Principal component analysis indicated that each species showed differences in allocation of P to different chemical fractions between
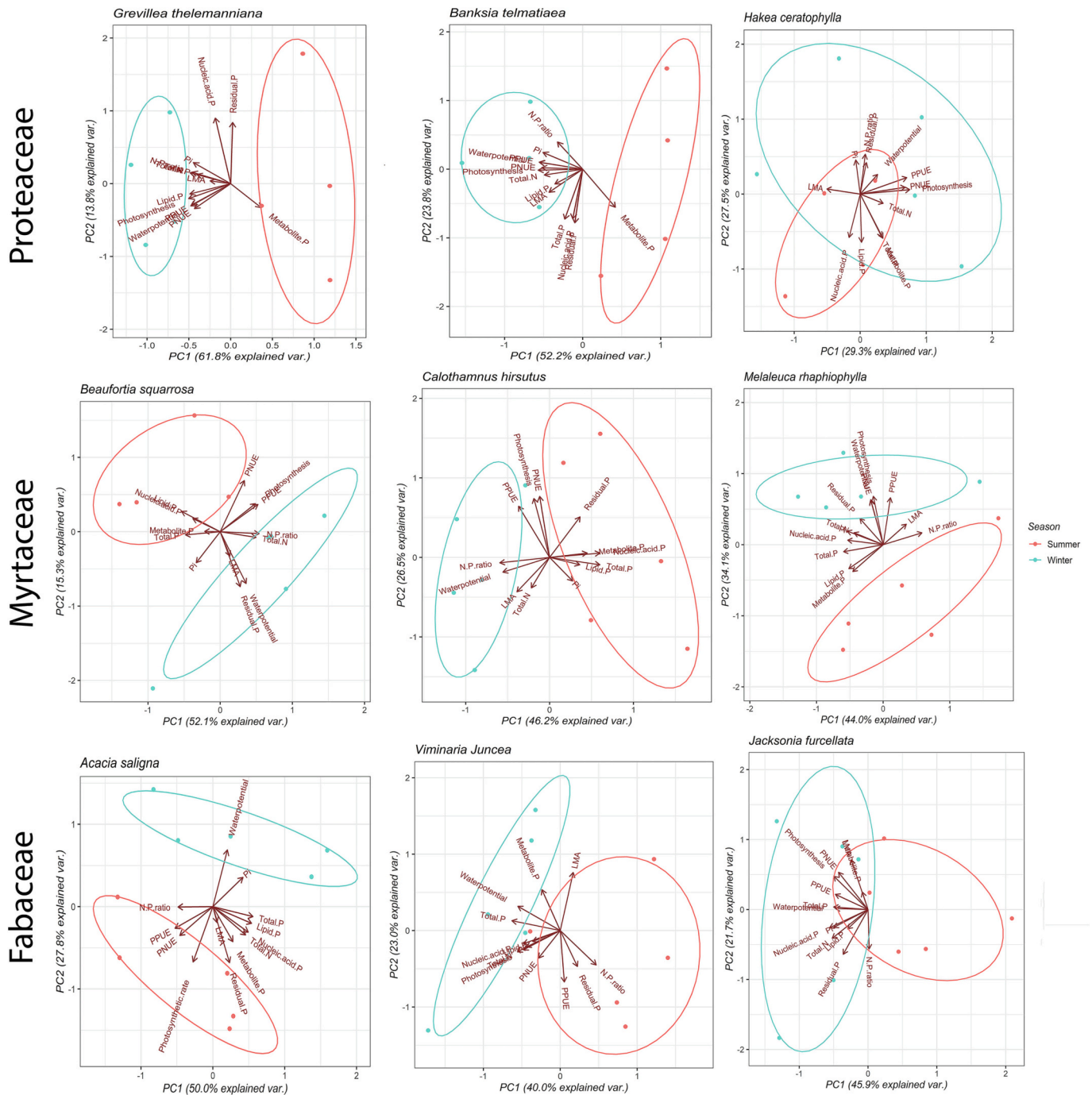
Fig. 8. Principal component analysis (PCA) of all parameters of nine targeted species at the West flat area of Alison Baird Reserve in summer (red ellipse) and winter (blue ellipse).

seasons, but these differences varied as well (Figs. 7, 8). Native plants in south-western Australia are typically slow-growing and converge on exhibiting high PPUE and being efficient at P remobilisation (Denton et al., 2007; Guilherme Pereira et al., 2018; Hayes et al., 2014), but each species appeared to have its own unique way of reaching that outcome.

A similar conclusion on allocation of P to different fraction as we found was reached by Suriyagoda et al. (2023), who reviewed results on P fractions in plant species across a wide range of studies. Therefore, this study provides direct evidence that different species from the same family in the same habitat function quite differently from each other in terms of leaf P-allocation patterns. The finding that such different responses occur among three dominant families in two habitats is a strong

argument that this conclusion will apply to other ecosystems, as shown by Tsujii et al. (2023).

Foliar P fractions have been studied in detail in various species in different ecosystems (Chapin and Bieleski, 1982; Hidaka and Kitayama, 2013; Suriyagoda et al., 2023; Yan et al., 2019). This study sheds new light on seasonal changes of foliar P fractions in plants from different P-impoverished environments in three plant families. However, there are still many issues to be explored further. For instance, what exactly are the molecules in the leaf metabolite P pool? This can be explored using enzymatic assays that failed to work with Proteaceae (Sulpice et al., 2014) or ^{31}P NMR (McQuillan et al., 2020). Also, whether the concentrations of these molecules change between seasons or habitats would

provide more detailed insights into how plants adjust their P utilisation towards a change in environment. Proteomics and genetics research on native plants in south-western Australian plants are especially worth conducting, as they would provide a deeper insight into how native plants express P-efficiency strategies.

CRediT authorship contribution statement

Shu Tong Liu: Designed the research, Investigation, Formal analysis, Visualization, Writing - original draft. **Clément E. Gille** and **Toby Bird**: Contributed to investigation and to the final manuscript. **Kosala Ranathunge** and **Patrick M. Finnegan**: Supervision, contributed to investigation, Writing-Review & Editing. **Hans Lambers**: Supervision, Writing-Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166395.

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