New Phytologist

Research

Life at the conservative end of the leaf economics spectrum: intergeneric variation in the allocation of phosphorus to biochemical fractions in species of *Banksia* (Proteaceae) and *Hakea* (Proteaceae)

Clément E. Gille[1], Patrick E. Hayes[1], Kosala Ranathunge[1], Shu Tong Liu[1], Robert P. G. Newman[1], Félix de Tombeur[1,2], Hans Lambers[1] and Patrick M. Finnegan[1]

[1]School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA, 6009, Australia; [2]CEFE, Université Montpellier, CNRS, IRD, EPHE, Montpellier, 34000, France

Author for correspondence:
Clément E. Gille
Email: clement.gille@uwa.edu.au

Received: *29 February 2024*
Accepted: *16 July 2024*

New Phytologist (2024)
doi: 10.1111/nph.20015

Key words: leaf economics spectrum, nitrogen, nucleic acids, nutrient-use efficiency, phospholipids, phosphorus fractions, Proteaceae, stoichiometry.

Summary

- In severely phosphorus (P)-impoverished environments, plants have evolved to use P very efficiently. Yet, it is unclear how P allocation in leaves contributes to their photosynthetic P-use efficiency (PPUE) and position along the leaf economics spectrum (LES). We address this question in 10 species of *Banksia* and *Hakea*, two highly P-efficient Proteaceae genera.
- We characterised traits in leaves of *Banksia* and *Hakea* associated with the LES: leaf mass per area, light-saturated photosynthetic rates, P and nitrogen concentrations, and PPUE. We also determined leaf P partitioning to five biochemical fractions (lipid, nucleic acid, metabolite, inorganic and residual P) and their possible association with the LES.
- For both genera, PPUE was negatively correlated with fractional allocation of P to lipids, but positively correlated with that to metabolites. For *Banksia* only, PPUE was negatively correlated with residual P, highlighting a strategy contrasting to that of *Hakea*. Phosphorus-allocation patterns significantly explained PPUE but were not linked to the resource acquisition vs resource conservation gradient defined by the LES.
- We conclude that distinct P-allocation patterns enable species from different genera to achieve high PPUE and discuss the implications of different P investments. We surmise that different LES axes representing different ecological strategies coexist in extremely P-impoverished environments.

Introduction

Phosphorus (P) is an essential nutrient for plant growth and is involved in many physiological processes. Thus, P fertilisation is pivotal to high productivity in agriculture. However, rock-derived P fertilisers are not renewable, and global reserves continue to be consumed, mainly in the agricultural sector, due to the dependence of current agricultural systems on P fertilisers (Fixen & Johnston, 2012). Moreover, P limitation in natural terrestrial ecosystems has been widely underestimated (Hou *et al.*, 2020) and is becoming more critical under global change (Wassen *et al.*, 2005; Zhou *et al.*, 2021; Tian *et al.*, 2022). Climate change is predicted to alter plant nutrient stoichiometry, affecting competitive interactions and species distribution and diversity (Elser *et al.*, 2010; Yuan & Chen, 2015). As such, it is essential to understand how plants use P efficiently to sustain growth under P-limiting conditions (Pang *et al.*, 2018).

The leaf economics spectrum (LES) defines a global trade-off that contrasts fast-growing species with acquisitive traits with slow-growing species with conservative traits (Wright *et al.*, 2004). Acquisitive traits are characterised by fast nutrient acquisition and photosynthetic rates, while conservative traits are characterised by long-lived leaves with high investment in leaf structure (Wright *et al.*, 2004). Plants growing on infertile soils, including P-impoverished sites, tend to exhibit more conservative growth strategies and retain scarce nutrients in the soil–plant system (Hayes *et al.*, 2014; Guilherme Pereira *et al.*, 2019). For instance, along a 2-million-year dune chronosequence in south-western Australia, plants growing on older severely P-impoverished dunes have higher leaf mass per area (LMA), leaf dry matter content (LDMC) and defence strategies based on silicon, a beneficial nutrient, but lower concentrations of essential nutrients (i.e. P and nitrogen (N)) than plants growing on younger P-richer dunes (Hayes *et al.*, 2014; Guilherme Pereira *et al.*, 2019; de Tombeur *et al.*, 2020b, 2021). While LMA increases with declining soil P availability along this natural nutrient gradient, photosynthetic rates do not decrease, when expressed either on an area or on a mass basis (Guilherme Pereira

et al., 2019). Furthermore, photosynthetic rates are faster for species of *Banksia* and *Hakea* grown in soil from the older, more P-impoverished sites along this chronosequence than in those in soil from younger, less P-impoverished sites (Hayes *et al.*, 2024). However, it is not clear how plants allocate nutrients within their leaves, particularly P, to achieve high photosynthetic P-use efficiency (PPUE) and how P allocation and leaf traits associated with the LES drive photosynthetic nutrient-use efficiency in severely P-impoverished environments.

One way for plants to adapt or acclimate to P limitation is by reducing the concentration of P in their leaves (Veneklaas *et al.*, 2012). Phosphorus in leaves comprises five operational biochemical pools: nucleic acids, phospholipids, metabolic P (comprising inorganic P (Pi) and small P-containing metabolites) and a residual fraction that likely contains phosphorylated proteins among other P-containing chemical compounds not captured in other fractions (Hidaka & Kitayama, 2011, 2013; Mo *et al.*, 2019; Yan *et al.*, 2019; Liu *et al.*, 2023). Along a 120-yr deglaciation chronosequence on the eastern Tibetan Plateau with varying soil P speciation and availability, evergreen species maintain their PPUE by decreasing the total amount of P in their leaves and by adjusting the allocation of P among fractions, with fast and slow economic strategies driving plant succession along the chronosequence (Lei *et al.*, 2021). Similar results were reported for a fast-growing *Banksia sessilis* (Proteaceae), allocating more P to nucleic acids than the slow-growing *Banksia attenuata* in severely P-impoverished soil (Han *et al.*, 2021). A recent survey demonstrated a partial association between variation in P allocation along the LES in 12 evergreen species co-occurring on P-impoverished soils in south-eastern Australia (Tsujii *et al.*, 2023). However, it is not clear how the allocation of P to different P fractions in highly P-efficient plant species under extremely P-limiting conditions might be linked to the gradient of resource acquisition vs conservation that is defined by the LES.

South-western Australia is one of the most nutrient-impoverished regions in the world (Lambers *et al.*, 2010; Viscarra Rossel & Bui, 2016; Kooyman *et al.*, 2017) and is recognised as a global biodiversity hotspot (Myers *et al.*, 2000; Williams *et al.*, 2011). The Proteaceae family is prominent, with most species in the family being endemic to the region (Beard *et al.*, 2000; Hopper, 2009). Adaptations have evolved in the Proteaceae that provide them with a high P-acquisition efficiency in extremely P-impoverished soils (Hayes *et al.*, 2021; Lambers, 2022). The high P-acquisition efficiency is complemented by numerous adaptations that also give Proteaceae a high internal P-use efficiency (Hayes *et al.*, 2021). Proteaceae function at a very low abundance of ribosomal RNA (rRNA) and low concentrations of protein in mature leaves (Lambers *et al.*, 2015a; Liu *et al.*, 2022). During leaf development, Proteaceae replace phospholipids with lipids that do not contain P, such as sulfolipids and galactolipids (Lambers *et al.*, 2012). The demand for P is further spread over time with the leaf growth being dissociated from the P-demanding development of the photosynthetic apparatus, a phenomenon known as 'delayed greening' (Lambers *et al.*, 2011; Kuppusamy *et al.*, 2014, 2021; Bird *et al.*, 2024). These adaptations have allowed Proteaceae to function at low foliar P and N concentrations ([P] and [N], respectively) without compromising photosynthetic performance, as is usually the case under extremely low P availability (Veneklaas *et al.*, 2012; Guilherme Pereira *et al.*, 2019; Hayes *et al.*, 2021). This results in a high leaf PPUE and photosynthetic N-use efficiency (PNUE) (Denton *et al.*, 2007; Lambers *et al.*, 2012; Sulpice *et al.*, 2014; Hayes *et al.*, 2018; Guilherme Pereira *et al.*, 2019). Liu *et al.* (2023) showed that P allocation patterns among a wide range of species from different families in south-western Australia are species-dependent. However, it remains unknown how the P investment at the biochemical level explains the relatively high PPUE of species from different genera within the Proteaceae.

In this study, we combined major leaf traits associated with the LES framework (i.e. LMA, mass-based photosynthetic rates, leaf [P] and [N]) with the allocation of P to the major biochemical fractions described above in a range of species of *Banksia* and *Hakea* that occur on extremely P-impoverished soils in Badgingarra National Park, Western Australia. *Banksia* and *Hakea* are emblematic and phylogenetically well-separated genera of the Proteaceae family, with long evolutionary histories and strong diversification (Hopper, 2009; Hayes *et al.*, 2021). We aimed to determine the dependence of physiological processes such as photosynthesis and PPUE on P allocation in leaves, and how P-allocation patterns are associated with traits related to the LES. For the first time, we investigated whether P-allocation patterns contribute to the distribution of species along a continuum of resource-use strategies (i.e. acquisitive vs conservative) in highly P-efficient genera of Proteaceae occurring in some of the most P-impoverished soils on earth. We hypothesised that (1) the allocation of P to lipids and small metabolites would be negatively and positively correlated with PPUE, respectively, as observed in other studies (Hidaka & Kitayama, 2013; Suriyagoda *et al.*, 2023); (2) in relation to their evolutionary history, *Banksia* and *Hakea* species would be positioned at the highly conservative end of the LES, and their leaf P allocation, particularly that to lipids, metabolites and nucleic acids would contribute to the position of these species along the LES.

Materials and Methods

Species selection and study area

Five *Banksia* species (Proteaceae) and five *Hakea* species (Proteaceae) were selected as the highly abundant species in the targeted extremely P-impoverished area of Badgingarra National Park (S30.412, E115.367), *c.* 200 km north of Perth, Western Australia (Fig. 1). The vegetation is kwongan heath, dominated by sclerophyllous shrubs of the family Proteaceae, followed by Myrtaceae and Fabaceae (Pate & Beard, 1984). The climate is Mediterranean with hot dry summers and cool wet winters, with a mean annual maximum temperature of 26°C and a mean annual rainfall of 440 mm (1999–2018, Badgingarra Research Station, Australian Bureau of Meteorology, http://www.bom.gov.au). Five individuals of each species were sampled at four sites along a *c.* 8 m elevation gradient with contrasting soils

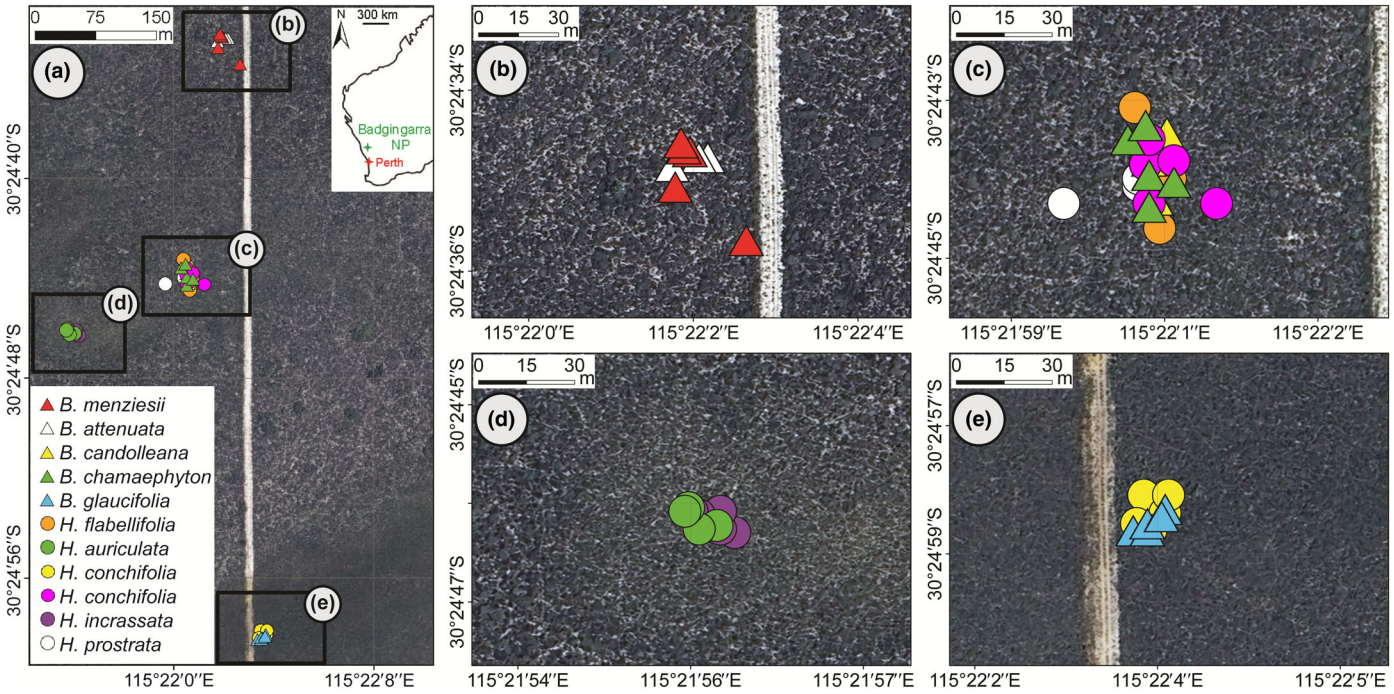

Fig. 1 Sampling location of five *Banksia* species (triangles) and five *Hakea* species (circles) naturally occurring at four extremely phosphorus-impoverished sites along a slight elevation gradient in Badgingarra National Park, Western Australia: (a) overall study area; the inset shows the location of Badgingarra National Park in south-western Australia, *c.* 200 km north of Perth; (b) 'bottom' site with silty sand; (c) 'slope' site with sand; (d) 'top' site with exposed laterite interspersed with sand; and (e) 'laterite' site with little sand. Elevation differs *c.* 8 m from (b) to (e). Elevation at (c) was similar to (d). The maps were edited using ArcMap 10.8.2 GIS software using Google Earth imagery (Google Inc., Mountain View, CA, USA).

(Fig. 1), with one species (*Hakea conchifolia*) found at two sites (Fig. 1c,e).

Leaf characteristics and nutrient analyses

Mature fully expanded undamaged and sun-exposed leaves were collected on 16 or 17 June 2020, scanned with an optical scanner (Epson Perfection V800 Photo; Epson, Los Alamitos, CA, USA) and the images analysed for projected leaf area (WinRHIZO Pro Software, Regent Instruments Inc., Québec, QC, Canada). Leaf thickness was measured using a portable digital calliper and calculated as the average thickness of three positions on the lamina along the axis from the base to the tip of the leaf. Leaves were oven-dried for one week at 70°C to a constant weight, and leaf mass per area (LMA; g m^{-2}) was calculated as the total dry weight of the sample divided by its total area. Leaf dry matter content (LDMC; %) was calculated as the total dry weight of the samples used for determining LMA divided by their total fresh weight. Leaf lifespan was not measured due to the high leaf longevity of Australian Proteaceae as reported in previous studies (Veneklaas & Poot, 2003; Denton *et al.*, 2007; Shane *et al.*, 2014); instead, other traits (i.e. LMA and LDMC) were used as proxies to estimate the LES (Wright *et al.*, 2004).

In parallel with leaf collection for LMA and leaf thickness, different mature fully expanded undamaged and sun-exposed leaves from the same individuals were harvested at the same time and immediately snap-frozen in liquid N and stored at −80°C before being freeze-dried for 7 d (VirTis BenchTop Pro 'K' Freeze Dryer, SP Scientific, Warminster, PA, USA). Freeze-dried material was finely ground using plastic vials and zirconium beads in a vertical ball-mill grinder (GenoGrinder; Spex SamplePrep, Metuchen, NJ, USA). Leaf total [N] was determined by combustion with a glutamic acid standard using a CN analyser (Elementar Vario Macro CNS Analyser, Langenselbold, Hesse, Germany). Leaf total [P] was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300DV; PerkinElmer, Waltham, MA, USA) following acid digestion with a mixture of concentrated nitric and perchloric acids (Zarcinas *et al.*, 1987).

Analysis of leaf P fractions

Leaf inorganic P (Pi) concentration was determined after extraction with acetic acid (Hurley *et al.*, 2010). In brief, freeze-dried and ground leaf material was mixed with cold 1% (v/v) acetic acid, shaken with zirconium beads in bursts of 5000 rpm at 4°C for 15 s with 5 min breaks between bursts (Precellys 24 Tissue Homogenizer; Bertin Instruments, Montigny-le-Bretonneux, France). After clarification by centrifugation at 14 000 ***g*** at 4°C for 15 min, the extract was purified with activated charcoal (Dayrell *et al.*, 2022), and the Pi concentration was determined colorimetrically using a malachite green-based method (Motomizu *et al.*, 1983).

Foliar P was separated into lipid P, metabolic P (comprising both metabolite P and Pi), nucleic acid P and a residual P fraction

by sequential extraction based on the differential solubility of each class of P-containing compound using a modification of the method described previously (Kedrowski, 1983; Hidaka & Kitayama, 2013; Hayes *et al.*, 2022). In brief, ground leaf material was extracted with 12 : 6 : 1 (v/v/v) chloroform : methanol : formic acid, then with 1 : 2 : 0.8 (v/v/v) chloroform : methanol : water. The extracts were combined and extracted with chloroform-saturated water into an organic phase and an aqueous phase, which contained the lipid P and metabolic P fractions, respectively. The pellet was resuspended in 85% (v/v) methanol and extracted with 5% (w/v) trichloroacetic acid (TCA). After centrifugation of the sample at 5000 ***g*** at 4°C for 15 min, the clear supernatant was added to the metabolic P fraction. The pellet was resuspended in 2.5% (w/v) TCA at 95°C to extract the nucleic acids. The pellet was then transferred to a digestion flask by suspending in 85% (v/v) methanol three times to make the residual P fraction. All fractions were dried at *c.* 50°C and digested with a mixture of concentrated nitric and perchloric acids to hydrolyse esterified P (Zarcinas *et al.*, 1987). The Pi concentration in each fraction was determined colorimetrically as described above (Motomizu *et al.*, 1983). Metabolite P was calculated by subtracting Pi from metabolic P, where Pi was determined as described above. The recovery rate (%) was calculated as the sum of all P fractions divided by total P measured directly from the ground leaves by ICP-OES and ranged from 86% to 94% for our P fractionation method (Supporting Information Table S1). Therefore, we present total P as the sum of the four P fractions (lipid P, metabolic P, nucleic acid P and residual P).

Gas exchange measurements

Leaf gas exchange was measured on attached leaves from the same individuals and at the same time as leaves were collected to measure other leaf traits. Care was taken during sampling to ensure that leaves used for gas exchange measurements were as similar as possible to those sampled for the other traits, that is similar size, age, intactness and position. Gas exchange measurements were made on clear sunny days between 8:30 h and 10:30 h on 16 or 17 June 2020 using a portable open-system infrared gas analyser (LI6400XT; LI-COR Biosciences, Lincoln, NE, USA) with 1500 $\mu mol^{-1}\ s^{-1}$ photosynthetic photon flux density, 400 $\mu mol\ mol^{-1}$ CO_2 with a chamber temperature of 23 ± 1°C and relative humidity of 55–70%. When the chamber area (6 cm^2) was not completely filled, the leaf area inside the chamber was measured by scanning as described above. Light-saturated net photosynthetic rates were expressed on a leaf area basis ($A_{sat,area}$; $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) and on a leaf dry mass basis, using LMA for conversion ($A_{sat,mass}$; $nmol\ CO_2\ g^{-1}\ s^{-1}$). Instantaneous PPUE and instantaneous PNUE were calculated as the rate of net photosynthetic CO_2 assimilation per unit P ($\mu mol\ CO_2\ g^{-1}\ P\ s^{-1}$) and N ($\mu mol\ CO_2\ g^{-1}\ N\ s^{-1}$), respectively.

Soil sampling and analyses

Three soil samples were collected within 300 mm around the base of three plants of each targeted species at each site on 16 June 2023. While soils were sampled 3 yr after the leaf traits were measured, the timing within the year was matched to the timing of the leaf sampling to minimise possible annual variation in soil characteristics. Soils sampled in May 2017 at the same site, but around different plants, were indistinguishable from the soils sampled in 2023 in all parameters tested (EC, pH, concentrations of total P and resin P; Table S2). The three subsamples of soil from each plant were collected using a hand trowel (depth = 100 mm) and mixed to form one soil sample for each plant. The soil samples were air-dried at room temperature (*c.* 20°C) for 2 wk and then sieved (< 2 mm) to remove gravel and large organic debris, including roots.

Soil pH and electrical conductivity (EC) were measured using pH and EC probes calibrated with pH 4 and 7 buffers or a 1314 $\mu S\ cm^{-1}$ solution, respectively (Orion Versa Star Pro; Thermo Fisher Scientific, Waltham, MA, USA). Soil EC was measured in 1 : 5 (w/v) soil : deionised water, and pH was measured in 0.01 M $CaCl_2$ (1 : 5 (w/v) soil : solution).

Soil total [P] was determined by ignition (Saunders & Williams, 1955). In brief, air-dried soil was heated at 550°C for 1 h and allowed to cool before extraction by shaking with 1 M HCl (1 : 30 (w/v) soil : solution) for 16 h. A second soil subsample was extracted with 1 M HCl (1 : 10 (w/v) soil : solution) for 16 h without prior ignition for the determination of inorganic P (Saunders & Williams, 1955). Both subsamples were filtered using Whatman No.42 filter papers, and the [P] was determined colorimetrically (Motomizu *et al.*, 1983). Organic P (Po) was calculated by subtracting the [P] in the nonignited sample from the [P] in the ignited sample.

Resin P, a measure of 'plant-available' soil P, was extracted using anion exchange membranes (AEM; Turner & Romero, 2009). Air-dried soil was shaken in deionised H_2O (1 : 6 (w/v) soil : water) with four anionic-form AEM strips (10 × 40 mm; manufactured by BDH, Poole, UK, and distributed by VWR International) for 16 h. After shaking, the AEM strips were rinsed free of soil particles with deionised H_2O, and the Pi was recovered by shaking the strips in 10 ml of 0.5 M HCl for 1 h. Resin [P] in the extract was determined colorimetrically (Motomizu *et al.*, 1983). Soil [P] was expressed on a dry weight basis ($mg\ P\ kg^{-1}$ soil DW).

Statistical analyses

Data were analysed using the R software platform (R Core Team, 2023). One-way ANOVAs were performed to test the significance of differences in all measured variables among all species or among species within both genera, and Tukey's HSD *post hoc* tests were run to determine significant differences. The differences between both genera were tested for some variables (i.e. PPUE, PNUE and percentage P allocated to residual P) using linear mixed-effects models that consider species as a random effect using the '*nlme*' package (Pinheiro & Bates, 2000). The homogeneity of variances was verified using the Levene's test, and the normality of the residuals was verified using the Shapiro–Wilk test ($P > 0.05$). Data on $A_{sat,area}$, $A_{sat,mass}$, leaf Pi (% and absolute concentration) and soil Pi concentration were

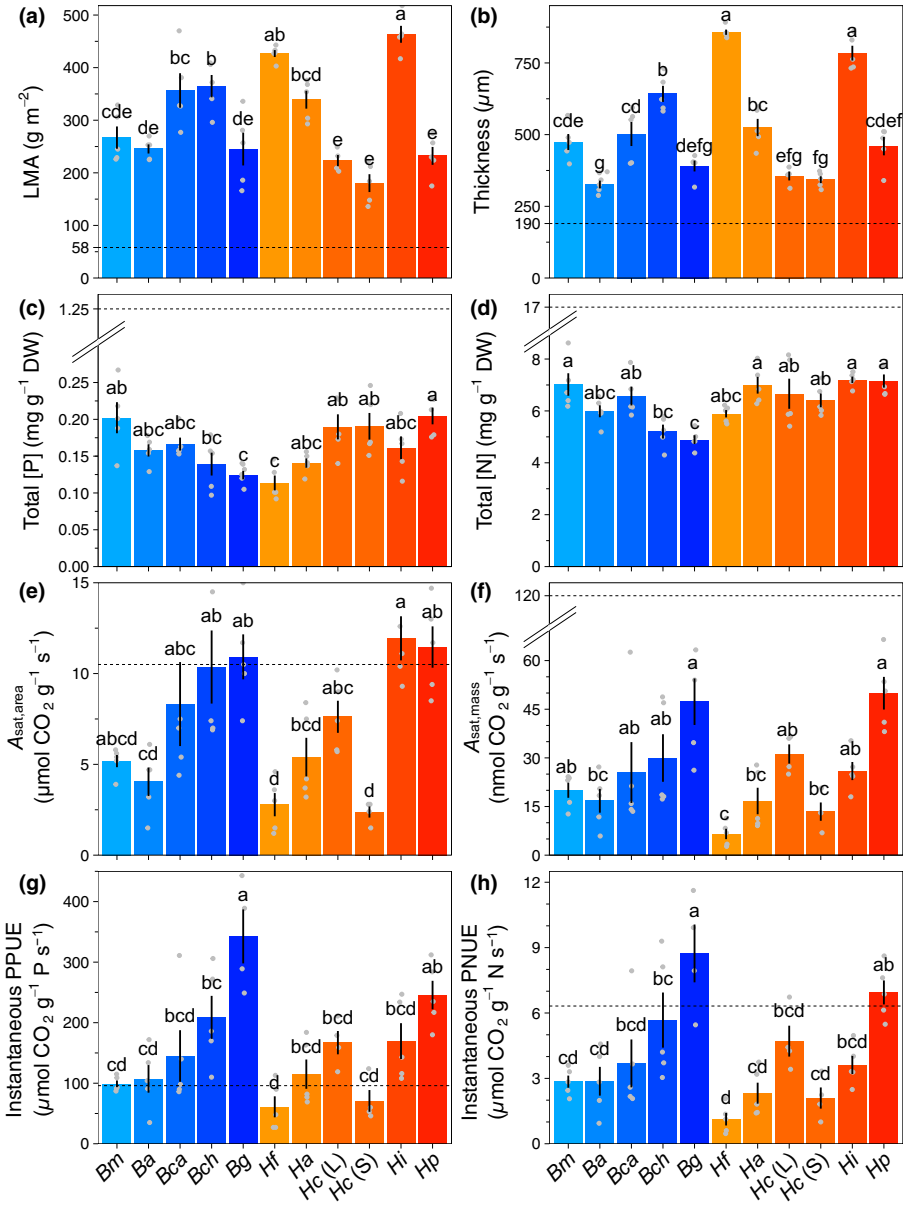

Fig. 2 Foliar traits of five *Banksia* and five *Hakea* species naturally occurring on extremely phosphorus (P)-impoverished soils: (a) leaf mass per unit area (LMA), (b) leaf thickness, (c) leaf phosphorus concentration ([P]), (d) leaf nitrogen concentration ([N]), (e) area-based light-saturated photosynthetic rate ($A_{sat,area}$), (f) mass-based light-saturated photosynthetic rate ($A_{sat,mass}$), (g) instantaneous photosynthetic P-use efficiency (PPUE) and (h) instantaneous photosynthetic N-use efficiency (PNUE). Values are means ± SE (n = 4 or 5). Different letters indicate significant differences among species (*post hoc* Tukey's HSD test, $P < 0.05$). The horizontal dashed lines represent global median values extracted from the TRY plant trait database; the associated variation is presented in Supporting Information Table S3 (Kattge *et al*., 2011). *Bm*, *Banksia menziesii*; *Ba*, *Banksia attenuata*; *Bca*, *Banksia candolleana*; *Bch*, *Banksia chamaephyton*; *Bg*, *Banksia glaucifolia*; *Hf*, *Hakea flabellifolia*; *Ha*, *Hakea auriculata*; *Hc*, *Hakea conchifolia* (Laterite and Slope); *Hi*, *Hakea incrassata*; *Hp*, *Hakea prostrata*.

log_{10}-transformed when either condition was not met. Due to different sampling times, which prevented us from matching soil and leaf samples, the linear regressions between leaf [P] and soil [P] were run on unmatched averaged data with variation shown for both variables. The principal component analyses (PCAs) characterising functional foliar traits defining LES and P-related traits were run using the '*FactoMineR*' package on log_{10}-transformed data (Lê *et al*., 2008). The optimal number of principal components (PCs) retained in the PCA was decided using the function '*estim_ncpPCA*' of the '*missMDA*' package, and missing values including null values were imputed with the regularised iterative PCA algorithm using the function '*imputePCA*' from the same package, with the fixed number of PCs estimated from the previous step (Josse & Husson, 2016). In the case of no missing data, PCs explaining ≥ 90% of the variation were retained. Pearson's correlation analysis was used to analyse the correlation between the allocation of P to the different fractions and the individual coordinates extracted from individual PCAs for each genus. Global median values for leaf traits presented in Fig. 2 were extracted with permission from the published TRY plant trait database with the variation shown in Table S3 (Kattge *et al*., 2011), and global data presented in Fig. S1 were extracted with permission from the GLOPNET data set from the leaf economics spectrum initiative (Wright *et al*., 2004).

Results

Banksia and *Hakea* species had a high LMA, averaging 306 g m^{-2}, compared with a global average of 60 g m^{-2} (Figs 2a, S1). However, there was a wide variation, reflecting differences in leaf structure among species. The LMA closely aligned

New Phytologist

Table 1 Soil chemical characteristics of the top layer (0–100 mm depth) under five *Banksia* and five *Hakea* species naturally occurring on extremely P-impoverished soils.

Species	Site	EC (μS cm^{-1})	pH ($CaCl_2$)	Total P (mg kg^{-1} DW)	Inorganic P (mg kg^{-1} DW)	Organic P (mg kg^{-1} DW)	Resin P (mg kg^{-1} DW)
B. menziesii	Bottom	17 ± 1 a	4.5 ± 0 b	4.5 ± 0.6 c	0.18 ± 0.01 ab	4.3 ± 0.5 c	0.23 ± 0.00 a
B. attenuata	Bottom	14 ± 1 a	4.5 ± 0.1 b	3.9 ± 0.5 c	0.19 ± 0.03 ab	3.7 ± 0.4 c	0.22 ± 0.04 a
B. candolleana	Slope	17 ± 1 a	4.6 ± 0.1 b	4.2 ± 0.8 c	0.16 ± 0.05 abc	4.0 ± 0.7 c	0.27 ± 0.01 a
B. chamaephyton	Slope	15 ± 5 a	4.6 ± 0 ab	3.4 ± 0.6 c	0.12 ± 0.01 abc	3.3 ± 0.5 c	0.24 ± 0.05 a
B. glaucifolia	Laterite	23 ± 4 a	4.6 ± 0.1 b	12.2 ± 2.6 ab	0.48 ± 0.12 a	11.7 ± 2.5 ab	0.16 ± 0.04 a
H. flabellifolia	Slope	27 ± 7 a	4.7 ± 0.1 ab	5.5 ± 1.1 c	0.07 ± 0.03 bc	5.4 ± 1.0 c	0.17 ± 0.02 a
H. auriculata	Top	21 ± 4 a	5.0 ± 0.1 a	8.3 ± 0.9 bc	0.12 ± 0.02 abc	8.2 ± 0.9 bc	0.10 ± 0.02 a
H. conchifolia	Laterite	27 ± 1 a	4.5 ± 0 b	16.7 ± 1.8 a	0.52 ± 0.13 a	16.2 ± 1.6 a	0.11 ± 0.02 a
H. conchifolia	Slope	21 ± 5 a	4.6 ± 0 b	4.2 ± 0.7 c	0.05 ± 0.02 c	4.2 ± 0.7 c	0.23 ± 0.07 a
H. incrassata	Top	22 ± 2 a	4.8 ± 0.1 ab	9.0 ± 1.1 bc	0.15 ± 0.03 abc	8.9 ± 1.1 bc	0.14 ± 0.03 a
H. prostrata	Slope	22 ± 6 a	4.5 ± 0.1 b	4.5 ± 1.5 c	0.08 ± 0.03 bc	4.4 ± 1.5 c	0.20 ± 0.08 a

DW, dry weight; EC, electrical conductivity.
Values are means ± SE (n = 3). Different letters indicate significant differences among species (*post hoc* Tukey's HSD test, $P < 0.05$).

with leaf thickness (Fig. 2b). Leaf dry matter content was relatively high and constant for all samples of the targeted species, averaging 56% ± 0.5% (n = 54) across our entire data set (data not shown). Leaf [P] were low compared with a global average with *Banksia* species ranging from 0.124 to 0.202 mg P g^{-1} DW for *B. glaucifolia* and *B. menziesii*, respectively, and *Hakea* species ranging from 0.114 to 0.205 mg P g^{-1} DW for *H. flabellifolia* and *H. prostrata*, respectively (Figs 2c, S1). Leaf [N] were also low compared with a global average, between 4.9 and 7.2 mg N g^{-1} DW, with only *B. chamaephyton* and *B. glaucifolia* having significantly lower [N] than some of the other species (Figs 2d, S1). Foliar [N] was relatively more conserved among all Proteaceae tested than foliar [P] (1.5 and 1.8-fold variation across all species, respectively). This conservation was particularly pronounced among *Hakea* species (1.2-fold variation), with an average of 6.7 mg N g^{-1} DW with no strictly significant differences within this genus ($P > 0.05$; Fig. 2d). The average N : P ratio for the 10 species was 41, which was notably high compared with a global average of 13 (Fig. S2).

Light-saturated photosynthetic rates were more variable than leaf [P] and [N] within each genus (Fig. 2e,f). Area-based photosynthetic rates ($A_{sat,area}$) were spanning the entire range of that measured in the LES (Fig. S1), although most species had slower rates than the global average. However, when expressed on a mass basis, photosynthetic rates ($A_{sat,mass}$) were no more than 6% (*H. flabellifolia*) to 43% (*H. prostrata*) of the global average rate, reflecting the high LMA of these species (Fig. 2a,f).

The instantaneous PPUE of the study group ranged from 61 μmol CO_2 g^{-1} P s^{-1} for *H. flabellifolia* to 375 and 246 μmol CO_2 g^{-1} P s^{-1} for *B. glaucifolia* and *H. prostrata*, respectively (Fig. 2g). Therefore, the values were at or above the global median values, except for *H. flabellifolia* and *H. conchifolia* growing on the slope. The instantaneous PNUE followed the same relative pattern across species as the PPUE, but the values were all less than half of the global average, except for *B. glaucifolia*, which was near the global average (Fig. 2h). Both PPUE and PNUE were indistinguishable between *Banksia* and *Hakea* ($P > 0.05$), but varied among species within each genus (Fig. 2g,h).

There were no significant differences in pH or EC in the soil under any of the species (Table 1). Soil total [P] was significantly higher under *B. glaucifolia* and *H. conchifolia*, the two species found on the lateritic site, consistent with higher soil organic [P] under these plants (Fig. S3a; Table 1). There were no significant differences in soil resin P under any of the species (Fig. S3b; Table 1). In contrast to the species-level comparison, combining the data from these same soils by site revealed differences in soil [P] (Table S4). The soil at the upper-most laterite site had the highest soil [P], while the bottom and slope sites had the lowest soil total [P], respectively. The soil at the top site was between these extremes. Soil total [P] at the site level were consistent with soil Pi and Po concentrations. Interestingly, the slope and top sites, which were intermediate in elevation, had the lowest and highest resin [P], respectively (Table S4). Despite these differences in soil total [P] and resin [P] among the four sites, there was no significant correlation between leaf [P] and either soil total [P] or resin [P] (Fig. S3).

There was a high level of consistency in the proportion of P each species allocated to nucleic acid P, with an overall average of 36.6% of total P allocated to this fraction (Fig. 3a). The proportional allocation of P to lipids was also relatively conserved across species, particularly among *Hakea* species, with the greatest variation of 1.4-fold between *H. conchifolia* (on the slope) and *H. incrassata* (Fig. 3b). The allocation of P to Pi was also conserved across all species with no differences among *Banksia* species ($P > 0.05$). The only statistically significant differences among all species were that *H. conchifolia* (on the slope) had a greater P allocation to Pi than *H. prostrata*, *B. menziesii* and *B. attenuata* (Fig. 3c).

The allocation of P to small metabolites and the residual fraction was much more variable, with a 2.5-fold and 14.9-fold variation among all species, respectively (Fig. 3d,e). Within the respective genera, *B. glaucifolia* had a higher proportion of P allocated to small metabolites than *B. attenuata* and *B. candolleana*, while *H. conchifolia* (on the slope) had a lower proportional allocation than the other *Hakea* species ($P < 0.05$; Fig. 3d). Residual P only represented a small proportion of total foliar P compared

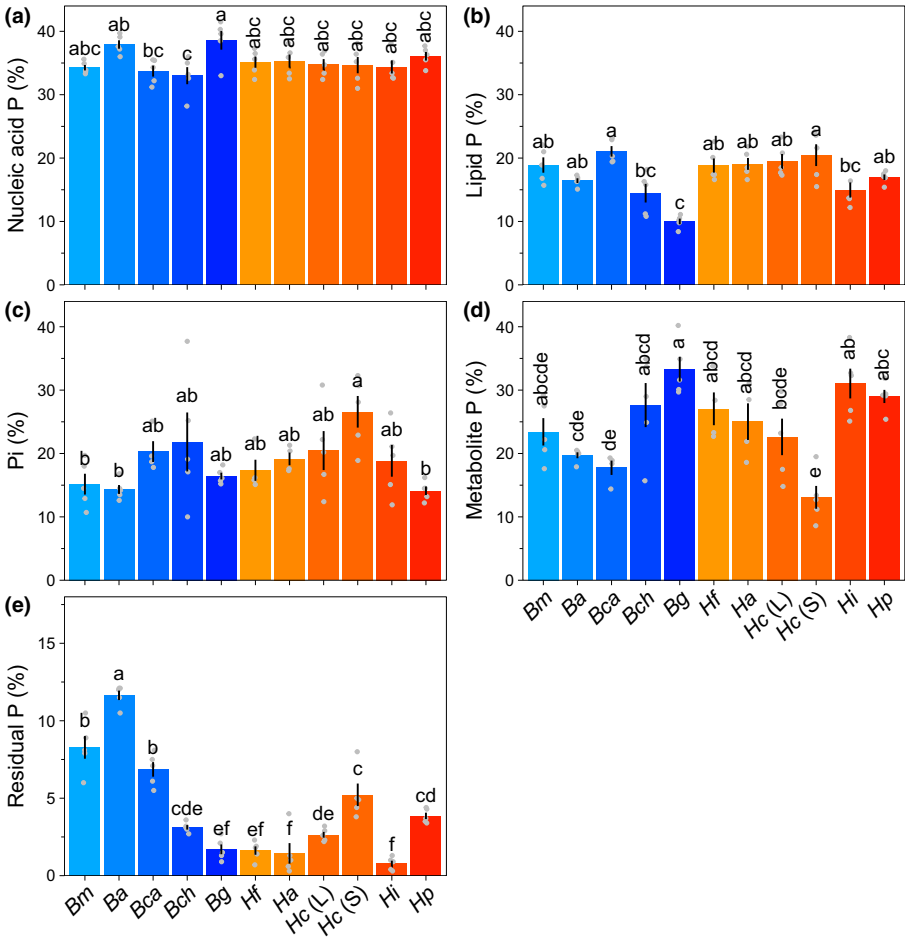

Fig. 3 Phosphorus (P) allocation to five biochemical fractions as a proportion of total foliar P for five *Banksia* and five *Hakea* species naturally occurring on extremely P-impoverished soils: (a) nucleic acid P, (b) lipid P, (c) inorganic P (Pi), (d) metabolite P (metabolic P–Pi) and (e) residual P. Values are means ± SE (n = 4 or 5). Different letters indicate significant differences among species (*post hoc* Tukey's HSD test, $P < 0.05$). Actual concentrations of P (mg P g^{-1} DW) in each fraction are given in Supporting Information Fig. S4. *Bm*, *Banksia menziesii*; *Ba*, *Banksia attenuata*; *Bca*, *Banksia candolleana*; *Bch*, *Banksia chamaephyton*; *Bg*, *Banksia glaucifolia*; *Hf*, *Hakea flabellifolia*; *Ha*, *Hakea auriculata*; *Hc*, *Hakea conchifolia* (Laterite and Slope); *Hi*, *Hakea incrassata*; *Hp*, *Hakea prostrata*.

with the other fractions. There was a larger variation in residual P allocation among the species of *Banksia* than among the species of *Hakea*. However, this P allocation was not significantly different ($P = 0.052$) at the genus level between *Banksia* and *Hakea* with averages of 6.5% and 2.6% of total P, respectively (Fig. 3e).

A correlation analysis across the two genera showed significant correlations between PPUE and most of the leaf traits, as well as P fractions expressed on a fractional basis (Fig. 4). The strongest correlations were between PPUE and $A_{sat,area}$ and $A_{sat,mass}$ which were supported by strong correlations for each genus examined individually. By contrast, there was a weak correlation between PPUE and LMA, which was not sustained by either genus individually. Interestingly, no correlation was found between PPUE and total foliar [P], despite a nearly twofold variation in leaf [P] within each genus (Fig. 2). Thus, the differences in PPUE were associated with differences in photosynthetic rates, rather than leaf [P]. Like PPUE, PNUE was strongly correlated with photosynthetic rates, rather than foliar [N] or LMA (Fig. S5). Therefore, PPUE and PNUE were strongly correlated ($R^2 = 0.97$ and $R^2 = 0.88$ for *Banksia* and *Hakea* species, respectively; $P < 0.001$; Fig. S5).

The only correlations between PPUE and the fractional allocation of P that was supported by both genera assessed individually were a negative correlation of PPUE with lipid P (Fig. 4f) and a positive correlation of PPUE with metabolite P (Fig. 4h). There was a weak positive correlation of PPUE with nucleic acid P, but this was not supported by either genus individually. At the level of all plants examined, there was a negative correlation between PPUE and residual P. This correlation was driven by the *Banksia* species, but not by the *Hakea* species. Conversely, there was a negative correlation between PPUE and Pi only for the *Hakea* species examined.

Correlations specific to each genus were also found when fractions were expressed as an absolute concentration of P, that is PPUE correlated with P concentration in lipids for *Banksia* species and P concentration in metabolites for *Hakea* species (Fig. S6). Also, there was a strong correlation for *Banksia* species between PPUE and the absolute P concentration in the residual fraction. Interestingly, P allocated to the lipid and residual fractions (both fractional and actual concentrations) were positively correlated with leaf [N] only for the *Banksia* species. Moreover, there was a strong correlation between leaf [N] and nucleic acid P concentrations supported by both genera assessed individually (Fig. S7).

In a PCA, the first two PCs explained 70% of the total variance for all individuals of *Banksia* and *Hakea* when describing functional foliar traits associated with the LES (Fig. 5a; Table S5). When P-related foliar traits were combined with the foliar functional traits, PC1 and PC2 encompassed 59% of the variation in all individuals (Fig. 5b; Table S5). There was no distinction between the two genera when all traits were considered

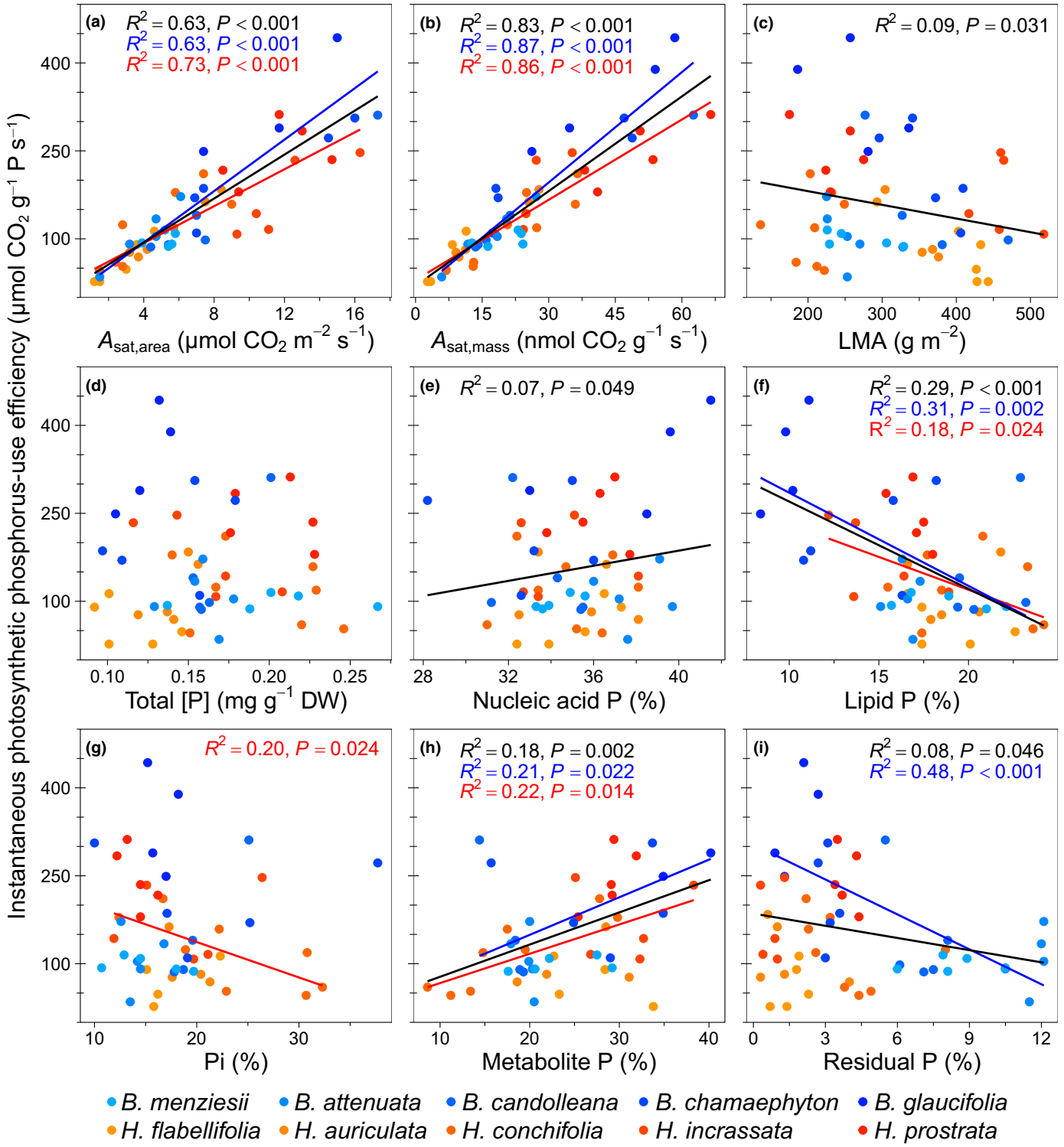

Fig. 4 Correlations between instantaneous photosynthetic phosphorus (P)-use efficiency (PPUE) and leaf traits for five *Banksia* and five *Hakea* species naturally occurring on extremely P-impoverished soils. Correlations between PPUE and major leaf traits (a) area-based light-saturated photosynthetic rate ($A_{sat,area}$), (b) mass-based light-saturated photosynthetic rate ($A_{sat,mass}$), (c) leaf mass per unit area (LMA), (d) leaf P concentration, and correlations between PPUE and percentages of P allocated to (e) nucleic acid P, (f) lipid P, (g) inorganic P (Pi), (h) metabolite P (metabolic P–Pi) and (i) residual P are shown. Solid lines indicate significant linear correlations (black: among all individuals, n = 52–55; blue: among *Banksia* species, n = 24 or 25; and red: among *Hakea* species, n = 28–30; $P < 0.05$).

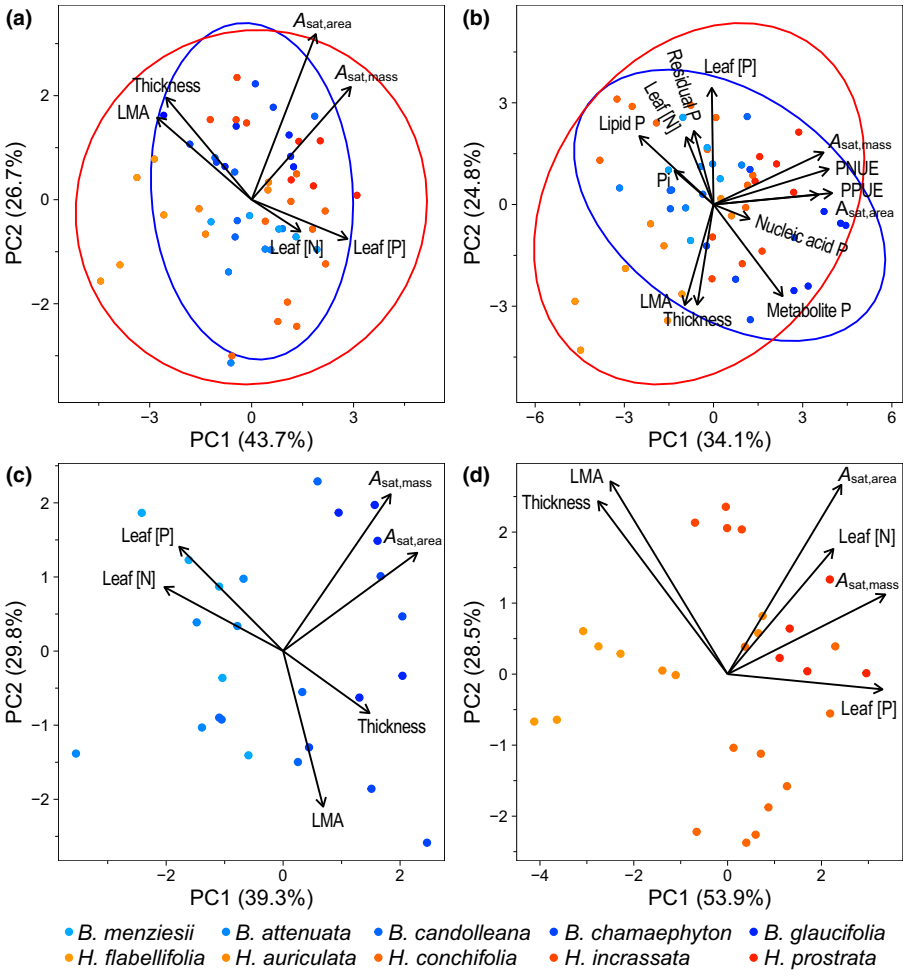

Fig. 5 Principal component analysis (PCA) of (a) functional leaf traits defining the leaf economics spectrum and (b) after adding in phosphorus (P)-related leaf traits of five *Banksia* (blue) and five *Hakea* species (red) naturally occurring on extremely P-impoverished soils; PCA of functional leaf traits of (c) *Banksia* species and (d) *Hakea* species. Detailed results are presented in Supporting Information Tables S5 and S6. Nucleic acid P, lipid P, inorganic P (Pi), metabolite P and residual P are expressed as a fraction of leaf total P concentration ([P]). $A_{sat,area}$, area-based light-saturated photosynthetic rate; $A_{sat,mass}$, mass-based light-saturated photosynthetic rate; LMA, leaf mass per unit area; PNUE, instantaneous photosynthetic nitrogen-use efficiency; PPUE, instantaneous photosynthetic P-use efficiency.

together. However, in accordance with the LES framework, structural traits (i.e. LMA and leaf thickness) were placed opposite to total leaf [P] and [N] in both PCAs (Fig. 5a,b; Table S5). Surprisingly, photosynthesis-related traits (i.e. $A_{sat,area}$, $A_{sat,mass}$, PPUE and PNUE) were dissociated from the contrasts between structural traits and nutrient concentrations and were placed along a different PC.

The fractional allocation of P to metabolites and lipids grouped with and opposite to photosynthesis-related traits, respectively, along PC1 in the PCA describing functional and P-related traits. The allocation to residual P was strongly associated with leaf [N] along PC2, but not with nucleic acid P that was placed on PC3 (Fig. 5b; Table S5).

The individual PCAs of functional leaf traits comprising the LES for *Banksia* (Fig. 5c) and *Hakea* species (Fig. 5d) showed similar patterns, with structural traits positioned orthogonally to photosynthesis-related traits. For *Banksia* species, structural traits were opposed to both nutrient concentrations and photosynthesis-related traits along PC2, which we define as the LES for this genus (Fig. 5c; Table S6). On the other hand, nutrient concentrations were opposed to both structural and photosynthesis-related traits in PC1; therefore, we also present correlations between the allocation of P to the different fractions for PC1 (Fig. 5c; Tables S6, S7). However, for *Hakea* species, photosynthesis-related traits and nutrient concentrations grouped together orthogonally to structural traits along PC1 (Fig. 5d; Table S6). As structural traits had a high loading on both PC1 and PC2 for *Hakea* species, but physiological traits had a high loading only on PC1, we define PC1 as the LES axis for this genus (Fig. 6; Tables S6, S7).

All five P fractions expressed as an absolute P concentration were significantly positively correlated with the distribution along PC1 for *Hakea* species, but we did not observe any significant correlations between the fractional P allocation and the distribution of species along PC1 (Fig. 6; Table S7). This highlights the tight link between an increase in P in those fractions and increase in total [P]. Similarly for *Banksia* species, the allocation of P to most P fractions except Pi and metabolite P was significantly correlated with the position of individual plants along PC1, which describes the resource-use gradient for this genus and was driven by total [P] (Fig. 6; Table S7). Only nucleic acid P concentration was significantly correlated with the distribution of species along PC2, defined as the LES, but this was not retained for the percentage of P allocated to nucleic acids (Fig. 6; Table S7). The percentage of P allocated to lipid P, metabolite P and residual P significantly correlated with the distribution along PC1, substantiating the correlations found between those P fractions and PPUE for *Banksia* species (Figs 4, 6).

New Phytologist

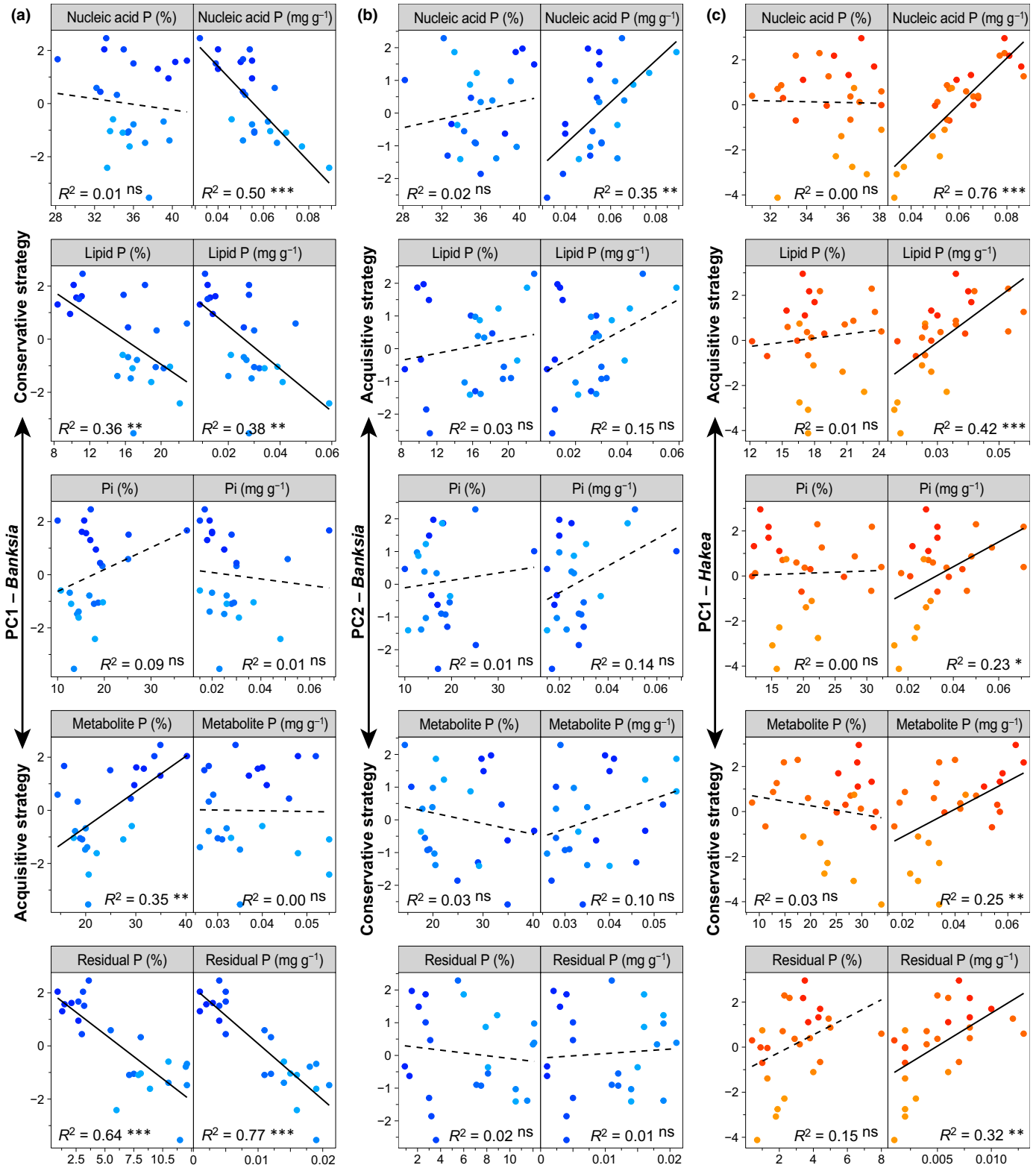

Fig. 6 Correlations between the distribution of individuals of *Banksia* (blue) and *Hakea* (red) along a continuum of resource-use strategies (i.e. acquisitive vs conservative) defined by each individual principal component (PC) analysis in Fig. 5 and phosphorus (P) allocated to five biochemical fractions, expressed as a percentage of total P and absolute concentration. (a) PC1 and (b) PC2 are shown for *Banksia*, but (c) only PC1 is shown for *Hakea*, as explained in the text. Solid and broken lines represent significant and nonsignificant linear correlations, respectively (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant, i.e. $P > 0.05$).

Discussion

Our results support the notion that Proteaceae naturally occurring in extremely nutrient-impoverished environments modulate the allocation of P in their leaves to achieve high PPUE. Of the five P fractions measured, the allocation of P to lipids and to small metabolites was the allocation most closely associated with variation in PPUE. The allocation of P to other fractions, especially to nucleic acids and Pi, was more conserved among species of both genera. These observations support our first hypothesis that the allocation of P to lipids and small metabolites would be negatively and positively correlated with PPUE, respectively. The high PPUE and PNUE values observed for all Proteaceae in this study are largely explained by their extremely low leaf [P] and [N]. However, the interspecific variation in PPUE and PNUE was largely explained by the variation in photosynthetic rates ($A_{sat,area}$ and $A_{sat,mass}$). While photosynthetic rates were generally slower than global averages, they were more variable than leaf nutrient concentrations among the species examined and spanned the range of global values (Wright *et al.*, 2004; Kattge *et al.*, 2011, 2020). All species had highly conservative leaf traits, such as extremely high LMA and low leaf nutrient concentrations, placing them on the far conservative end of the LES, supporting our second hypothesis. Our correlation analyses show that the P-allocation patterns of *Banksia* and *Hakea* species, specifically the allocation to small metabolites and residual P, contributed differently to their high PPUE. Within each genus, species were distributed along a genus-defined LES axis on which structural and physiological traits were located at opposite ends. However, the distribution of these species along these axes, which represent the contrast between relatively more acquisitive to more conservative resource-use strategies, was not correlated with their allocation of P to the different fractions. These findings do not support our second hypothesis. We suggest that, while species of Proteaceae modulate their leaf P allocation, this does not explain the variation in the resource-use strategy they exhibit to cope with an extremely P-impoverished environment.

On the far 'conservative' end of the leaf economics spectrum

Leaf [P] of *Banksia* and *Hakea* growing on extremely P-impoverished soils were extremely low with an average of 0.16 mg P g^{-1} DW, which is even lower than in plants found in typical kwongan vegetation (*c.* 0.3 mg P g^{-1} DW; Hayes *et al.*, 2018; Guilherme Pereira *et al.*, 2019). Leaf [P] in this study was among the lowest recorded world-wide (world-wide average: 1.23 mg P g^{-1} DW; Wright *et al.*, 2004; Kattge *et al.*, 2011; Kattge *et al.*, 2020). In alignment with low leaf total [P], all P fractions had significantly lower concentrations than the global averages for perennial species (Suriyagoda *et al.*, 2023). All Proteaceae in this study had similarly conservative leaf traits, for example, low $A_{sat,mass}$ and high LMA and LDMC associated with scleromorphy. This result highlights the dependence of mass-based traits (e.g. $A_{sat,mass}$ and leaf [P]) on LMA and LDMC (Poorter *et al.*, 2009). However, the variation in those traits cannot solely be explained by LMA and deserves further investigation. Moreover, it is likely that all targeted species have relatively long lifespans, since leaf lifespan for *B. menziesii*, *B. attenuata* and *H. prostrata* is 2 yr or more (Veneklaas & Poot, 2003; Denton *et al.*, 2007; Shane *et al.*, 2014). A longer leaf lifespan allows for a longer residence time of nutrients in leaves. The P-resorption efficiency (PRE) is high in leaves of some of the species, for example > 90% for *B. menziesii*, 82% for *B. chamaephyton* and 69% for *B. attenuata* (Denton *et al.*, 2007; de Campos *et al.*, 2013; Hayes *et al.*, 2014). High PRE is another indicator of the high P-use efficiency for Proteaceae naturally occurring in extremely P-impoverished environments.

Slight variation in soil total [P] and resin [P] was not associated with higher leaf [P]. On the lateritic site, soil was shallower than on the sandy nonlateritic sites. Yet, cluster-rooted species such as *Banksia* and *Hakea* are able to efficiently access sufficient nutrients from this lower soil volume and also directly from lateritic gravels (Han *et al.*, 2021). Moreover, at a very low P supply like that of our study site in Badgingarra National Park, which has some of the most P-impoverished soils in the world (Kooyman *et al.*, 2017), P that is taken up by plants is mainly used to support growth, rather than accumulating in leaves to a high [P] (De Groot *et al.*, 2003; Shane *et al.*, 2003; Gille *et al.*, 2024). Accumulating more biomass rather than increasing leaf [P] further emphasises the extreme P-conserving strategy of these species.

There was no appreciable difference in soil characteristics between our results from May 2017 and June 2023. Furthermore, both of these collections also differed little from those taken at a nearby site within Badgingarra National Park in October 2017 (Dayrell, 2020). Finally, in the studied environment, 3 yr is likely much too short for significant changes to emerge in soil physicochemical properties that are driven by long-term pedogenic processes (de Tombeur *et al.*, 2020a). Therefore, we are confident that the differences in timing between leaf and soil measurements did not impact the outcome of our results.

We observed some variation in structural (e.g. LMA) and physiological (e.g. A_{sat}, nutrient concentrations) traits among species of both genera. These two sets of traits comprising the LES were well-separated from each other in the PCA including both *Banksia* and *Hakea* species, as well as in PCAs defined by each genus. However, nutrient concentrations and photosynthesis-related traits were orthogonal to one another on different axes in the PCA of functional leaf traits. This arrangement was particularly true for *Banksia* species. The divergence between these two sets of traits was at odds with the principles of the LES, where these two sets of traits on the global scale are at opposite ends along a single axis (Wright *et al.*, 2004). Leaf physiology constrains the correlations between photosynthetic activity and nutrient concentrations or LMA. At a smaller scale, such as in extremely P-impoverished environments in south-western Australia, plants have adaptations that allow them to cope with extreme environmental conditions (e.g. low nutrient availability and high light intensity), likely without compromising leaf physiology and therefore disrupting the single axis theorised in the global LES. In our study, the measurements of leaf traits were performed on

different leaves. However, we ensured that leaves of similar condition (i.e. age, intactness and position in the canopy) were sampled at the same time and from the same individuals for the various analyses. Therefore, it is highly unlikely that the divergences observed between structural and physiological traits in our PCAs result from our sampling design. A similar methodology was used in previous studies (Guilherme Pereira *et al.*, 2019; Tsujii *et al.*, 2024).

In agreement with recent studies, we observed significant correlations between PPUE or A_{sat} and the allocation of P to different fractions (Tsujii *et al.*, 2023, 2024). Here, we show that the allocation of P to these fractions is correlated with PPUE, but not with the LES axes opposing contrasting resource-use strategies among highly P-efficient species from two Proteaceae genera. Interestingly, we did not find significant correlations between P-allocation patterns (i.e. percentage of P allocated to each fraction) and the distribution of individuals along the axes defining their genus-specific LES, despite a gradient from relatively more acquisitive to conservative strategies among species within each genus. We suggest that the extremely conservative strategies displayed by these species from a severely P-impoverished environment limit intrageneric variation along those axes. However, we highlight different strategies in the two genera to achieve high PPUE and discuss below the implications of P allocation on physiological processes.

Higher LMA is usually associated with slower growth rates, long leaf lifespans and lower leaf protein concentrations (Poorter *et al.*, 2009). Our high LMA leaves had low leaf [N] and nucleic acid [P], indicative of low protein and rRNA concentrations, respectively, suggesting a low capacity to produce and replace proteins. *Hakea prostrata* had a higher leaf nucleic acid P concentration, but similar leaf total [N], as the other *Hakea* species. This indicates that mature *H. prostrata* leaves function at a lower leaf protein: rRNA ratio, suggesting a faster protein turnover rate than that of other species of *Hakea* (Matzek & Vitousek, 2009; Lambers, 2022). In a glasshouse experiment conducted in acidic soil similar to that found at our study site in Badgingarra National Park, *H. prostrata* and *B. menziesii* had faster relative growth rates than *H. incrassata* (Hayes *et al.*, 2024). However, the potential correlation between P fractions, leaf [N] and protein turnover has yet to be explored. For this exploration, it will be crucial to consider expanding leaves of species with contrasting growth rates.

Interconnection of N and P efficiency

Leaf N and nucleic acid P proportions were remarkably conserved among all Proteaceae in our study. *Hakea prostrata*, *B. attenuata* and *B. thelemanniana* restrain their nitrate uptake even when provided with a large amount of nitrate (Prodhan *et al.*, 2016; Liu *et al.*, 2022). This nitrate-uptake restraint trait is also found in two Myrtaceae co-occurring with Proteaceae in a highly P-impoverished environment, suggesting that it is a convergent trait in species that evolved in these environments (Liu *et al.*, 2022). Limiting N uptake appears to be a strategy that allows plants to enhance P-use efficiency and is associated with low concentrations of rRNA and, therefore, proteins (Matzek & Vitousek, 2009; Prodhan *et al.*, 2019). The low leaf [N] and extremely high N: P ratios in leaves of all species in this study, as well as the lack of variation among species, suggest nitrate-restraint occurs in these species. Conversely, species growing in fertile environments that are not limited by P or when supplied with additional nitrate acquire and accumulate N to high levels (Greenwood & Hunt, 1986; Tschoep *et al.*, 2009; Prodhan *et al.*, 2019). This supports the view that nitrate-uptake restraint is an adaptation in Proteaceae and at least one other plant family to low-P environments (Liu *et al.*, 2022). Further studies need to be carried out to explore the prevalence of this trait among Proteaceae.

The lack of correlation between photosynthetic rates and [N] or nucleic acid P concentrations indicates that photosynthesis was limited by metabolic factors other than protein concentration (Sulpice *et al.*, 2014; Ellsworth *et al.*, 2022). Moreover, a recent study demonstrated that two Proteaceae species from southwestern Australia, *Grevillea thelemanniana* and *Hakea ceratophylla*, prioritise leaf N investment to photosynthesis-related proteins while sacrificing proteins related to abiotic stress tolerance to maintain rapid photosynthetic rates at low leaf [N], compared with *Arabidopsis thaliana* (Liu, 2024). However, we suggest that *Banksia* species might also function with higher enzyme concentrations in parallel with lower substrate abundance, considering the strong correlation for this genus only found between PPUE and residual P. The residual fraction is thought to contain a mixture of insoluble P compounds, likely to include phosphorylated proteins based on their solubility in the solvents used in the sequential P fractionation (Chapin & Kedrowski, 1983; Kedrowski, 1983). The strong correlation between leaf residual [P] and leaf [N], which is a proxy for leaf protein concentration, supports this hypothesis (Hidaka & Kitayama, 2011; Shen, 2023; Tsujii *et al.*, 2023, 2024). Interestingly, the correlation between leaf [N] and residual [P] was only significant for species of *Banksia* in our study, suggesting that species of *Hakea* function differently. The lack of correlation between PPUE and the P concentration in small metabolites in *Banksia* species supports the idea that there is a tight balance between substrate and enzyme abundance (Dourado *et al.*, 2021). The strong correlations between PPUE, PNUE or A_{sat} and residual [P] further suggest that these proteins are involved in photosynthesis (Tsujii *et al.*, 2023, 2024). The lack of correlation between residual P and either leaf [N] or PPUE for *Hakea* species highlights the different biochemical strategies in this genus to adapt to the extremely nutrient-impoverished environment compared with the *Banksia* species. *Hakea* species potentially function at higher substrate concentrations than *Banksia* species, which exhibited a positive correlation between PPUE and P concentration in small metabolites. Determining the content of the residual P fraction and its proportion of phosphorylated proteins is an essential challenge to the recently evolving field of P fractions in leaves (Liu *et al.*, 2023; Suriyagoda *et al.*, 2023; Tsujii *et al.*, 2024).

Implications of biochemical investment of P on PPUE

Foliar lipid P concentrations among the *Banksia* and *Hakea* species examined here were low and similar to those found in several

other species growing in severely P-impoverished environments (Hidaka & Kitayama, 2011, 2013; Lambers *et al.*, 2012; Yan *et al.*, 2019). It is likely that all Proteaceae in this study replaced phospholipids by other lipids that do not contain P, such as sulfolipids and galactolipids as previously determined for five of the present species (Lambers *et al.*, 2012). The endoplasmic reticulum (ER) contains > 60% phospholipid by mass in a variety of cells (Lagace & Ridgway, 2013). Therefore, the negative correlation between lipid P and PPUE may indicate that the replacement of phospholipids involves a trade-off between saving P and maintaining the function of cellular membranes, particularly that of the ER. The ER is involved in synthesising proteins destined for endomembranes or export (Sadowski *et al.*, 2008), so the trade-off may be associated with both low protein and rRNA concentrations. The link between phospholipids in the ER membranes and protein synthesis, as well as protein turnover, in relation to P limitation requires further attention.

Inorganic phosphate concentration is tightly linked with that of small metabolites via phosphorylation (Plaxton & Tran, 2011). The fact that PPUE was positively correlated with metabolite P but not with Pi indicates that plants growing under extremely low P availability function at the lower limit of Pi concentrations. Excess Pi is stored in the vacuole and remobilised for metabolism when needed (Yang *et al.*, 2017). Up to 95% of Pi can be stored in the vacuole of plants growing high-P conditions (Bieleski, 1973). This figure is likely not representative of plants such as the Proteaceae that naturally occur in severely P-limiting environments and function at very low leaf [P]. A typical P-starvation response in a wide range of species is the reduction in the concentration of Pi (Bieleski, 1968; Veneklaas *et al.*, 2012; Yan *et al.*, 2019). However, there is currently no information on the cellular compartmentation of P in P-efficient plants growing in extremely P-limiting environments. Using ^{31}P-nuclear magnetic resonance (^{31}P-NMR), Pratt *et al.* (2009) showed that a decrease in cytosolic Pi concentration is the first response following Pi starvation but that Pi efflux from the vacuole is not sufficient to fully compensate for the lack of Pi supply in *Acer pseudoplatanus* (Sapindaceae) and *A. thaliana* (Brassicaceae). Further investigation is required to identify the extent of the metabolic importance of both cytosolic and vacuolar Pi pools under very low P availability. These aspects can be measured using metabolomics approaches combined with ^{31}P-NMR (Pratt *et al.*, 2009; Gout *et al.*, 2011).

Positive correlations between PPUE and the fractional allocation of P to small metabolites are consistent with previous studies (Wen *et al.*, 2023), although we were able to distinguish low-molecular-weight esterified metabolites (metabolite P) from Pi which are sometimes pooled as metabolic P (Hidaka & Kitayama, 2013). A decrease in the concentration of phosphorylated metabolites involved in major metabolic pathways, for example, Benson–Bassham–Calvin cycle and glycolysis, decreases their activity unless compensated by increases in enzyme quantity that use these metabolites or changes in the catalytic properties of the enzymes (Lambers *et al.*, 2015b). Producing more enzyme is unlikely to occur under low P availability as it would require a greater investment of P in rRNA to support protein synthesis (Veneklaas *et al.*, 2012; Lambers, 2022). To achieve higher PPUE, *Banksia* and *Hakea* species might increase P allocation to small metabolites to support greater enzyme activity (i.e. higher substrate concentration) and maintain relatively fast photosynthetic rates. However, we observed a positive correlation between nucleic acid P and metabolite P (R^2 = 0.18, P = 0.002), consistent with previous studies (Hidaka & Kitayama, 2013; Yan *et al.*, 2021; Suriyagoda *et al.*, 2023), but contradictory to the idea that lower enzyme concentration is compensated for by higher small metabolite concentration. The weak correlations between PPUE and metabolite P (absolute and proportional) might be explained by the presence of phytate in the metabolite pool. However, phytate is synthesised in the leaves as a storage compound and to regulate cytoplasmic [Pi] (Strother, 1980; Raboy, 2003), but is not a metabolite feeding into photosynthesis. Some P metabolites are products of photosynthesis-related enzymes (e.g. glucose 1-P and fructose 6-P). Functioning at high concentrations of photosynthetic intermediates might allow plants to decrease the amount of N needed for protein, therefore decreasing the need for P in rRNA (Sulpice *et al.*, 2014; Lambers *et al.*, 2015b; Prodhan *et al.*, 2019). Understanding how the concentrations of phosphorylated metabolites are regulated and at which scale the composition of this pool is regulated is warranted.

Concluding remarks

This study highlights critical differences in P-allocation patterns between two genera of Proteaceae naturally occurring on extremely P-impoverished soils, *Banksia* and *Hakea*, demonstrating that these genera have contrasting strategies to achieve high PPUE. For the first time, our study established LES gradients within individual genera that range from species with relatively more acquisitive to ones with more conservative resource-use strategies, and that these gradients lacked obvious links with P-allocation patterns in leaves. This may be explained by the remarkably conservative nature of the studied species; each species possessing contrasting P-use strategies to cope with extremely P-limiting conditions, however independently from the acquisition-conservation continuum defined globally by the LES. Our results suggest that the LES alone cannot capture all available resource-use strategies, particularly in the highly P-limited environment studied here. Our results show that high PPUE was achieved by a proportionally greater P allocation to small metabolites, which may include photosynthetic intermediates (e.g. glucose 1-P and fructose 6-P), and a proportionally smaller P allocation to lipids for both *Banksia* and *Hakea* species. Interestingly, for the *Banksia* species only, the actual concentration and relative proportion of P allocated to the residual fraction, which likely contains phosphorylated proteins, was negatively correlated with PPUE and positively with leaf [N], highlighting that both genera exhibit different strategies to enhance photosynthetic activity. These findings have clear implications and further our understanding of the differences and similarities of plant functional traits related to biochemical, physiological

and structural parameters, as well as trade-offs, that impact the fitness, survival, growth and performance of Proteaceae. Further investigation is necessary to explore the nature of molecules comprising those P fractions, particularly the small P metabolites and the residual fraction.

Acknowledgements

CEG is supported by a Scholarship for International Research Fees from The University of Western Australia and a University Postgraduate Award cofunded by The University of Western Australia and Australian Research Council grant DP200101013 to HL and PMF. Project funding was provided by the Australian Research Council grants DP200101013 to HL and PMF and FT170100195 Future Fellowship to KR. FdT is supported by the EU Horizon 2020 Research and Innovation Program under Marie Skłodowska-Curie grant agreement 101021641. We are grateful to Toby Bird, Li Yan and Hirotsuna Yamada for assistance with fieldwork. We thank three anonymous reviewers for their constructive comments that helped improve our manuscript.

Competing interests

None declared.

Author contributions

CEG and PMF designed the study; CEG performed the experiment and collected the data with contributions from HL, STL, RPGN and PMF; CEG, PEH, FdT, PMF, KR and HL analysed the data; CEG wrote the manuscript. All authors contributed critically to improve the final version of the manuscript and gave approval for publication.

ORCID

Patrick M. Finnegan https://orcid.org/0000-0001-5021-1138
Clément E. Gille https://orcid.org/0000-0003-3890-0402
Patrick E. Hayes https://orcid.org/0000-0001-7554-4588
Hans Lambers https://orcid.org/0000-0002-4118-2272
Shu Tong Liu https://orcid.org/0000-0002-5346-1711
Robert P. G. Newman https://orcid.org/0009-0000-5901-0367
Kosala Ranathunge https://orcid.org/0000-0003-2826-9936
Félix de Tombeur https://orcid.org/0000-0002-6012-8458

Data availability

The data that support the findings of this study are available at doi: https://doi.org/10.26182/xqwv-y130/.

References

Beard JS, Chapman AR, Gioia P. 2000. Species richness and endemism in the Western Australian flora. *Journal of Biogeography* **27**: 1257–1268.

Bieleski RL. 1968. Effect of phosphorus deficiency on levels of phosphorus compounds in *Spirodela*. *Plant Physiology* **43**: 1309–1316.

Bieleski RL. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annual Review of Plant Physiology* **24**: 225–252.

Bird T, Nestor BJ, Bayer PE, Wang G, Ilyasova A, Gille CE, Soraru BEH, Ranathunge K, Severn-Ellis AA, Jost R *et al.* 2024. Delayed leaf greening involves a major shift in the expression of cytosolic and mitochondrial ribosomes to plastid ribosomes in the highly phosphorus-use-efficient *Hakea prostrata* (Proteaceae). *Plant and Soil* **496**: 7–30.

de Campos MCR, Pearse SJ, Oliveira RS, Lambers H. 2013. Downregulation of net phosphorus-uptake capacity is inversely related to leaf phosphorus-resorption proficiency in four species from a phosphorus-impoverished environment. *Annals of Botany* **111**: 445–454.

Chapin FS, Kedrowski RA. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous Taiga trees. *Ecology* **64**: 376–391.

Dayrell RLC. 2020. *Regeneration in old climatically-buffered infertile landscapes*. PhD thesis, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil, and University of Western Australia, Perth, WA, Australia.

Dayrell RLC, Cawthray GR, Lambers H, Ranathunge K. 2022. Using activated charcoal to remove substances interfering with the colorimetric assay of inorganic phosphate in plant extracts. *Plant and Soil* **476**: 755–764.

De Groot CC, Marcelis LFM, Van Den Boogaard R, Kaiser WM, Lambers H. 2003. Interaction of nitrogen and phosphorus nutrition in determining growth. *Plant and Soil* **248**: 257–268.

Denton MD, Veneklaas EJ, Freimoser FM, Lambers H. 2007. *Banksia* species (Proteaceae) from severely phosphorus-impoverished soils exhibit extreme efficiency in the use and re-mobilization of phosphorus. *Plant, Cell & Environment* **30**: 1557–1565.

Dourado H, Mori M, Hwa T, Lercher MJ. 2021. On the optimality of the enzyme–substrate relationship in bacteria. *PLoS Biology* **19**: e3001416.

Ellsworth DS, Crous KY, De Kauwe MG, Verryckt LT, Goll D, Zaehle S, Bloomfield KJ, Ciais P, Cernusak LA, Domingues TF *et al.* 2022. Convergence in phosphorus constraints to photosynthesis in forests around the world. *Nature Communications* **13**: 5005.

Elser JJ, Fagan WF, Kerkhoff AJ, Swenson NG, Enquist BJ. 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist* **186**: 593–608.

Fixen PE, Johnston AM. 2012. World fertilizer nutrient reserves: a view to the future. *Journal of the Science of Food and Agriculture* **92**: 1001–1005.

Gille CE, Finnegan PM, Hayes PE, Ranathunge K, Burgess TI, de Tombeur F, Migliorini D, Dallongeville P, Glauser G, Lambers H. 2024. Facilitative and competitive interactions between mycorrhizal and nonmycorrhizal plants in an extremely phosphorus-impoverished environment: role of ectomycorrhizal fungi and native oomycete pathogens in shaping species coexistence. *New Phytologist* **242**: 1630–1644.

Gout E, Bligny R, Douce R, Boisson AM, Rivasseau C. 2011. Early response of plant cell to carbon deprivation: *in vivo* ^{31}P-NMR spectroscopy shows a quasi-instantaneous disruption on cytosolic sugars, phosphorylated intermediates of energy metabolism, phosphate partitioning, and intracellular pHs. *New Phytologist* **189**: 135–147.

Greenwood DJ, Hunt J. 1986. Effect of nitrogen fertiliser on the nitrate contents of field vegetables grown in Britain. *Journal of the Science of Food and Agriculture* **37**: 373–383.

Guilherme Pereira C, Hayes PE, O'Sullivan OS, Weerasinghe LK, Clode PL, Atkin OK, Lambers H. 2019. Trait convergence in photosynthetic nutrient-use efficiency along a 2-million years dune chronosequence in a global biodiversity hotspot. *Journal of Ecology* **107**: 2006–2023.

Han Z, Shi J, Pang J, Yan L, Finnegan PM, Lambers H. 2021. Foliar nutrient allocation patterns in *Banksia attenuata* and *Banksia sessilis* differing in growth rate and adaptation to low-phosphorus habitats. *Annals of Botany* **128**: 419–430.

Hayes P, Turner BL, Lambers H, Laliberté E. 2014. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million year dune chronosequence. *Journal of Ecology* **102**: 396–410.

Hayes PE, Adem GD, Pariasca-Tanaka J, Wissuwa M. 2022. Leaf phosphorus fractionation in rice to understand internal phosphorus-use efficiency. *Annals of Botany* **129**: 287–302.

Hayes PE, Clode PL, Lambers H. 2024. Calcifuge and soil-indifferent Proteaceae from south-western Australia: novel strategies in a calcareous habitat. *Plant and Soil* **496**: 95–122.

Hayes PE, Clode PL, Oliveira RS, Lambers H. 2018. Proteaceae from phosphorus-impoverished habitats preferentially allocate phosphorus to photosynthetic cells: an adaptation improving phosphorus-use efficiency. *Plant, Cell & Environment* **41**: 605–619.

Hayes PE, Nge FJ, Cramer MD, Finnegan PM, Fu P, Hopper SD, Oliveira RS, Turner BL, Zemunik G, Zhong H *et al.* 2021. Traits related to efficient acquisition and use of phosphorus promote diversification in Proteaceae in phosphorus-impoverished landscapes. *Plant and Soil* **462**: 67–88.

Hidaka A, Kitayama K. 2011. Allocation of foliar phosphorus fractions and leaf traits of tropical tree species in response to decreased soil phosphorus availability on Mount Kinabalu, Borneo. *Journal of Ecology* **99**: 849–857.

Hidaka A, Kitayama K. 2013. Relationship between photosynthetic phosphorus-use efficiency and foliar phosphorus fractions in tropical tree species. *Ecology and Evolution* **3**: 4872–4880.

Hopper SD. 2009. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. *Plant and Soil* **322**: 49–86.

Hou E, Luo Y, Kuang Y, Chen C, Lu X, Jiang L, Luo X, Wen D. 2020. Global meta-analysis shows pervasive phosphorus limitation of aboveground plant production in natural terrestrial ecosystems. *Nature Communications* **11**: 637.

Hurley BA, Tran HT, Marty NJ, Park J, Snedden WA, Mullen RT, Plaxton WC. 2010. The dual-targeted purple acid phosphatase isozyme AtPAP26 is essential for efficient acclimation of *Arabidopsis* to nutritional phosphate deprivation. *Plant Physiology* **153**: 1112–1122.

Josse J, Husson F. 2016. missMDA: a package for handling missing values in multivariate data analysis. *Journal of Statistical Software* **70**: 1–31.

Kattge J, Bönisch G, Díaz S, Lavorel S, Prentice IC, Leadley P, Tautenhahn S, Werner GDA, Aakala T, Abedi M *et al.* 2020. TRY plant trait database – enhanced coverage and open access. *Global Change Biology* **26**: 119–188.

Kattge J, Díaz S, Lavorel S, Prentice IC, Leadley P, Bönisch G, Garnier E, Westoby M, Reich PB, Wright IJ *et al.* 2011. TRY – a global database of plant traits. *Global Change Biology* **17**: 2905–2935.

Kedrowski RA. 1983. Extraction and analysis of nitrogen, phosphorus and carbon fractions in plant material. *Journal of Plant Nutrition* **6**: 989–1011.

Kooyman RM, Laffan SW, Westoby M. 2017. The incidence of low phosphorus soils in Australia. *Plant and Soil* **412**: 143–150.

Kuppusamy T, Giavalisco P, Arvidsson S, Sulpice R, Stitt M, Finnegan PM, Scheible W-R, Lambers H, Jost R. 2014. Lipid biosynthesis and protein concentration respond uniquely to phosphate supply during leaf development in highly phosphorus-efficient *Hakea prostrata*. *Plant Physiology* **166**: 1891–1911.

Kuppusamy T, Hahne D, Ranathunge K, Lambers H, Finnegan PM. 2021. Delayed greening in phosphorus-efficient *Hakea prostrata* (Proteaceae) is a photoprotective and nutrient-saving strategy. *Functional Plant Biology* **48**: 218–230.

Lagace TA, Ridgway ND. 2013. The role of phospholipids in the biological activity and structure of the endoplasmic reticulum. *Biochimica et Biophysica Acta* **1833**: 2499–2510.

Lambers H. 2022. Phosphorus acquisition and utilization in plants. *Annual Review of Plant Biology* **73**: 17–42.

Lambers H, Brundrett MC, Raven JA, Hopper SD. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. *Plant and Soil* **334**: 11–31.

Lambers H, Cawthray GR, Giavalisco P, Kuo J, Laliberté E, Pearse SJ, Scheible W-R, Stitt M, Teste F, Turner BL. 2012. Proteaceae from severely phosphorus-impoverished soils extensively replace phospholipids with galactolipids and sulfolipids during leaf development to achieve a high photosynthetic phosphorus-use-efficiency. *New Phytologist* **196**: 1098–1108.

Lambers H, Clode PL, Hawkins HJ, Laliberté E, Oliveira RS, Reddell P, Shane MW, Stitt M, Weston P. 2015a. Metabolic adaptations of the non-mycotrophic Proteaceae to soils with low phosphorus availability. In: *Annual plant reviews, volume 48, phosphorus metabolism in plants*. Chicester, UK: John Wiley & Sons, 289–336.

Lambers H, Finnegan PM, Jost R, Plaxton WC, Shane MW, Stitt M. 2015b. Phosphorus nutrition in Proteaceae and beyond. *Nature Plants* **1**: 15109.

Lambers H, Finnegan PM, Laliberté E, Pearse SJ, Ryan MH, Shane MW, Veneklaas EJ. 2011. Phosphorus nutrition of Proteaceae in severely phosphorus-impoverished soils: are there lessons to be learned for future crops? *Plant Physiology* **156**: 1058–1066.

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software* **25**: 1–18.

Lei Y, Du L, Chen K, Plenković-Moraj A, Sun G. 2021. Optimizing foliar allocation of limiting nutrients and fast-slow economic strategies drive forest succession along a glacier retreating chronosequence in the eastern Tibetan Plateau. *Plant and Soil* **462**: 159–174.

Liu ST. 2024. *Phosphorus-use and nitrogen-use strategies of native plants in south-western Australia*. PhD thesis, University of Western Australia, Perth, WA, Australia.

Liu ST, Gille CE, Bird T, Ranathunge K, Finnegan PM, Lambers H. 2023. Leaf phosphorus allocation to chemical fractions and its seasonal variation in south-western Australia is a species-dependent trait. *Science of the Total Environment* **901**: 166395.

Liu ST, Ranathunge K, Lambers H, Finnegan PM. 2022. Nitrate-uptake restraint in *Banksia* spp. (Proteaceae) and *Melaleuca* spp. (Myrtaceae) from a severely phosphorus-impoverished environment. *Plant and Soil* **476**: 63–77.

Matzek V, Vitousek PM. 2009. N : P stoichiometry and protein : RNA ratios in vascular plants: an evaluation of the growth-rate hypothesis. *Ecology Letters* **12**: 765–771.

Mo Q, Za L, Sayer EJ, Lambers H, Li Y, Zou B, Tang J, Heskel M, Ding Y, Wang F. 2019. Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. *Functional Ecology* **33**: 1–11.

Motomizu S, Wakimoto T, Tôei K. 1983. Spectrophotometric determination of phosphate in river waters with molybdate and malachite green. *The Analyst* **108**: 361–367.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.

Pang J, Ryan MH, Lambers H, Siddique KH. 2018. Phosphorus acquisition and utilisation in crop legumes under global change. *Current Opinion in Plant Biology* **45**: 248–254.

Pate JS, Beard JS. 1984. *Kwongan, plant life of the sandplain*. Nedlands, WA, Australia: University of Western Australia Press.

Pinheiro J, Bates D. 2000. *Mixed-effects models in S and S-PLUS*. New York, NY, USA: Springer.

Plaxton WC, Tran HT. 2011. Metabolic adaptations of phosphate-starved plants. *Plant Physiology* **156**: 1006–1015.

Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**: 565–588.

Pratt J, Boisson AM, Gout E, Bligny R, Douce R, Aubert S. 2009. Phosphate (Pi) starvation effect on the cytosolic Pi concentration and Pi exchanges across the tonoplast in plant cells: an *in vivo* ^{31}P-nuclear magnetic resonance study using methylphosphonate as a Pi analog. *Plant Physiology* **151**: 1646–1657.

Prodhan MA, Finnegan PM, Lambers H. 2019. How does evolution in phosphorus-impoverished landscapes impact plant nitrogen and sulfur assimilation? *Trends in Plant Science* **24**: 69–82.

Prodhan MA, Jost R, Watanabe M, Hoefgen R, Lambers H, Finnegan PM. 2016. Tight control of nitrate acquisition in a plant species that evolved in an extremely phosphorus-impoverished environment. *Plant, Cell & Environment* **39**: 2754–2761.

R Core Team. 2023. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Raboy V. 2003. *myo*-Inositol-1,2,3,4,5,6-hexakisphosphate. *Phytochemistry* **64**: 1033–1043.

Sadowski PG, Groen AJ, Dupree P, Lilley KS. 2008. Sub-cellular localization of membrane proteins. *Proteomics* **8**: 3991–4011.

Saunders WMH, Williams EG. 1955. Observations on the determination of total organic phosphorus in soils. *Journal of Soil Science* **6**: 254–267.

Shane MW, De Vos M, De Roock S, Cawthray GR, Lambers H. 2003. Effects of external phosphorus supply on internal phosphorus concentration and the initiation, growth and exudation of cluster roots in *Hakea prostrata* R.Br. *Plant and Soil* **248**: 209–219.

Shane MW, Stigter K, Fedosejevs ET, Plaxton WC. 2014. Senescence-inducible cell wall and intracellular purple acid phosphatases: implications for phosphorus remobilization in *Hakea prostrata* (Proteaceae) and *Arabidopsis thaliana* (Brassicaceae). *Journal of Experimental Botany* **65**: 6097–6106.

Shen Q. 2023. *Phosphorus-acquisition and phosphorus-utilisation strategies of native plants in south-western Australia*. PhD thesis, University of Western Australia, Perth, WA, Australia.

Strother S. 1980. Homeostasis in germinating seeds. *Annals of Botany* **45**: 217–218.

Sulpice R, Ishihara H, Schlereth A, Cawthray GR, Encke B, Giavalisco P, Ivakov A, Arrivault S, Jost R, Krohn N *et al.* 2014. Low levels of ribosomal RNA partly account for the very high photosynthetic phosphorus-use efficiency of Proteaceae species. *Plant, Cell & Environment* **37**: 1276–1298.

Suriyagoda LDB, Ryan MH, Gille CE, Dayrell RLC, Finnegan PM, Ranathunge K, Nicol D, Lambers H. 2023. Phosphorus fractions in leaves. *New Phytologist* **237**: 1122–1135.

Tian Q, Lu P, Zhai X, Zhang R, Zheng Y, Wang H, Nie B, Bai W, Niu S, Shi P *et al.* 2022. An integrated belowground trait-based understanding of nitrogen-driven plant diversity loss. *Global Change Biology* **28**: 3651–3664.

de Tombeur F, Laliberté E, Lambers H, Faucon MP, Zemunik G, Turner BL, Cornelis JT, Mahy G. 2021. A shift from phenol to silica-based leaf defences during long-term soil and ecosystem development. *Ecology Letters* **24**: 984–995.

de Tombeur F, Turner BL, Laliberté E, Lambers H, Cornelis J-T. 2020a. Silicon dynamics during 2 Myr of soil development in a coastal dune chronosequence under a Mediterranean climate. *Ecosystems* **23**: 1614–1630.

de Tombeur F, Turner BL, Laliberté E, Lambers H, Mahy G, Faucon MP, Zemunik G, Cornelis JT. 2020b. Plants sustain the terrestrial silicon cycle during ecosystem retrogression. *Science* **3699**: 1245–1248.

Tschoep H, Gibon Y, Carillo P, Armengaud P, Szecowka M, Nunes-Nesi A, Fernie AR, Koehl K, Stitt M. 2009. Adjustment of growth and central metabolism to a mild but sustained nitrogen-limitation in *Arabidopsis*. *Plant, Cell & Environment* **32**: 300–318.

Tsujii Y, Atwell BJ, Lambers H, Wright IJ. 2024. Leaf phosphorus fractions vary with leaf economic traits among 35 Australian woody species. *New Phytologist* **241**: 1985–1997.

Tsujii Y, Fan B, Atwell BJ, Lambers H, Lei Z, Wright IJ. 2023. A survey of leaf phosphorus fractions and leaf economic traits among 12 co-occurring woody species on phosphorus-impoverished soils. *Plant and Soil* **489**: 107–124.

Turner BL, Romero TE. 2009. Short-term changes in extractable inorganic nutrients during storage of tropical rain forest soils. *Soil Science Society of America Journal* **73**: 1972–1979.

Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible W-R, Shane MW, White PJ *et al.* 2012. Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist* **195**: 306–320.

Veneklaas EJ, Poot P. 2003. Seasonal patterns in water use and leaf turnover of different plant functional types in a species-rich woodland, south-western Australia. *Plant and Soil* **257**: 295–304.

Viscarra Rossel RA, Bui EN. 2016. A new detailed map of total phosphorus stocks in Australian soil. *Science of the Total Environment* **542**: 1040–1049.

Wassen MJ, Venterink HO, Lapshina ED, Tanneberger F. 2005. Endangered plants persist under phosphorus limitation. *Nature* **437**: 547–550.

Wen Z, Pang J, Wang X, Gille CE, De Borda A, Hayes PE, Clode PL, Ryan MH, Siddique KHM, Shen J *et al.* 2023. Differences in foliar phosphorus fractions, rather than in cell-specific phosphorus allocation, underlie contrasting photosynthetic phosphorus use efficiency among chickpea genotypes. *Journal of Experimental Botany* **74**: 1974–1989.

Williams KJ, Ford A, Rosauer DF, De Silva N, Mittermeier R, Bruce C, Larsen FW, Margules C. 2011. Forests of east Australia: the 35th biodiversity hotspot. In: *Biodiversity Hotspots*. Berlin, Germany: Springer, 295–310.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M *et al.* 2004. The worldwide leaf economics spectrum. *Nature* **428**: 821–827.

Yan L, Sunoj VSJ, Short AW, Lambers H, Elsheery NI, Kajita T, Wee AKS, Cao KF. 2021. Correlations between allocation to foliar phosphorus fractions and maintenance of photosynthetic integrity in six mangrove populations as affected by chilling. *New Phytologist* **232**: 2267–2282.

Yan L, Zhang X, Han Z, Pang J, Lambers H, Finnegan PM. 2019. Responses of foliar phosphorus fractions to soil age are diverse along a 2 Myr dune chronosequence. *New Phytologist* **223**: 1621–1633.

Yang S-Y, Huang T-K, Kuo H-F, Chiou T-J. 2017. Role of vacuoles in phosphorus storage and remobilization. *Journal of Experimental Botany* **68**: 3045–3055.

Yuan ZY, Chen HYH. 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. *Nature Climate Change* **5**: 465–469.

Zarcinas BA, Cartwright B, Spouncer LR. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* **18**: 131–146.

Zhou J, Li XL, Peng F, Li C, Lai C, You Q, Xue X, Wu Y, Sun H, Chen Y *et al.* 2021. Mobilization of soil phosphate after 8 years of warming is linked to plant phosphorus-acquisition strategies in an alpine meadow on the Qinghai–Tibetan Plateau. *Global Change Biology* **27**: 6578–6591.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Distribution of five *Banksia* and five *Hakea* species in the space defined by the leaf economics spectrum.

Fig. S2 Leaf nitrogen to phosphorus ratio.

Fig. S3 Correlations between foliar total phosphorus (P) concentrations and soil total P and soil resin P concentrations.

Fig. S4 Phosphorus allocated to five major biochemical fractions in leaves of five Banksia and five Hakea species.

Fig. S5 Correlations between photosynthetic nitrogen-use efficiency and major leaf traits.

Fig. S6 Correlations between photosynthetic phosphorus (P)-use efficiency and P allocated to five major leaf biochemical fractions.

Fig. S7 Correlations between leaf nitrogen concentrations and phosphorus allocated to five major biochemical fractions.

Table S1 Recovery rate of the phosphorus-fractionation method.

Table S2 Comparison of soil characteristics in Badgingarra National Park at different sampling times.

Table S3 Variation associated with traits extracted from the TRY plant trait database.

Table S4 Soil chemical characteristics at four locations in Badgingarra National Park.

Table S5 Output of the principal component analyses of functional leaf traits defining the leaf economics spectrum and

combined with phosphorus-related traits of five Banksia and five Hakea species.

Table S6 Output of the principal component analyses of functional leaf traits of five Banksia and five Hakea species.

Table S7 Output of the correlations between the distribution of individuals of *Banksia* and *Hakea* along a gradient of resource-use strategies and allocation of phosphorus to leaf biochemical fractions.

Please note: Wiley is not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.