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RESEARCH ARTICLE

Leaf phosphorus allocation and photosynthesis in native Australian plants differs between soil parent material types

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Abstract

Background and aims There is considerable interest in how plants allocate phosphorus (P) and how this varies by environment. How leaf P fractions change with soil P supplies and soil type is essential to understand the roles of P in storage, structure and biochemistry including photosynthesis. Here, we contrasted the P allocation patterns for native woody plants in south-eastern Australia on soils derived from either P-poor sedimentary or P-rich igneous parent materials.

Methods We measured total leaf P and four leaf P fractions: inorganic phosphate (P_i), metabolite P, nucleic acid P, and lipid P for 33 native species. We also measured photosynthetic capacity (A_{sat}) for species across four sites with contrasting soil P and parent material types: high total soil P from basalt versus low total soil P in sands.

Results The leaf P_i fraction scaled consistently with total leaf P within and across the high-P versus low-P sites. Species growing on high P soils from basalt tended to have similar P fractions of total P to those of species on low P soils, except for the lipid P fraction which was greater for species at high P sites. There was a substantial reduction in P allocated to the lipid fraction for species on low P soils, especially in Proteaceae species. Leaf photosynthetic P-use efficiency was significantly higher with lower leaf P concentration.

Conclusion The adaptive strategy of reducing phospholipids in leaves occurs in multiple species in low-P environments, including non-Proteaceae. This resulted in species from more than one plant family maintaining lower leaf P concentrations on sedimentary-derived soils than on igneous soils, thus achieving more efficient P use in photosynthesis at low P.

Keywords Leaf phosphorus fractions · Leaf inorganic phosphate · Phospholipids · Photosynthesis · Phosphorus-use efficiency · Soil phosphorus

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Introduction

Low phosphorus (P) content is a distinctive aspect of Australian soils (Viscarra Rossel and Bui 2016) due to the ancient, highly weathered soils that occur over large parts of its landmass (Beadle 1962). This low soil P concentration ([P]) has shaped vegetation function in a variety of important ways, such as

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reducing plant growth and leaf turnover and selecting for P-conserving strategies (Aerts and Chapin 2000; Lambers et al. 2022). How plant species that are adapted to low-P soils can effectively acquire and use scarce P resources for maintaining growth and productivity has been a long-standing question for ecologists (Beadle 1966; Lambers et al. 2015a; Reed et al. 2015; Veneklaas et al. 2012).

To better understand how P is allocated on infertile sites, several analyses of leaf P fractions have emerged in recent years (McQuillan et al. 2020; Yu et al. 2022; Meng et al. 2025; Tsujii et al. 2024). Some analyses of leaf P fractions have utilised soil P gradients (Yan et al. 2019; Liu et al. 2023; Tsujii et al. 2024) or P addition experiments (Mo et al. 2019) to understand P allocation in leaves in relation to soil P supply. Analyses of leaf P fractions have separated storage P, cell ultrastructure P and P in biochemical compounds, and these can broadly be classed by the roles of P in metabolic processes versus structural materials. According to the chemical P fractionation scheme of Chapin and Kedrowski (1983), leaf P can be separated into fractions such as inorganic phosphate (P_i), P-containing small metabolites, organic phosphate esters in nucleic acids, and phospholipids based on differential solubility or hydrolysis (also see Kedrowski 1983; Veneklaas et al. 2012). A further P fraction is the residual of the sequential chemical extractions which contains chemically recalcitrant compounds, such as phosphorylated proteins and other organic P constituents. Although this leaf P chemical fractionation technique has been employed for > 40 years, there is still a relatively small set of plant families, species and regions in the global data (see Suriyagoda et al. 2023; Meng et al. 2025), especially for sites on native soils (i.e., unploughed and unfertilised).

Plants adjust to low soil [P] by reducing leaf P concentrations (Hidaka and Kitayama 2009; Lambers et al. 2010; Hayes et al. 2014), with trade-offs against photosynthetic carbon (C) assimilation (Hidaka and Kitayama 2009; Reich et al. 2009) and other P-demanding processes (Denton et al. 2007; Hidaka and Kitayama 2011, 2013; Lambers et al. 2015a; Wright et al. 2001). The ratio of photosynthetic C assimilation to the P deployed in leaves defines the instantaneous photosynthetic P-use efficiency (PPUE; see Reich and Schoettle 1988). Often, on low-P soils PPUE increases in leaves in spite of reduced photosynthetic C assimilation (Shen et al. 2024). Hence PPUE is an important plant trait which signifies effective utilisation of limited P resources for short-term C gain. The mechanism for how high PPUE is achieved is still not fully understood (Hidaka and Kitayama 2009, 2011; Lambers et al. 2011, 2012; Ellsworth et al. 2022), though overall reductions in leaf P concentrations and possible certain P constituents contribute to P-use efficiency. Thus, there is strong evidence that high PPUE is achieved in plants by i) maintaining leaf function in the face of low leaf P concentrations and ii) allocating P in leaves differently on P-scarce soils than on P-fertile soils (Lambers et al. 2012).

There are a number of ways that P can be deployed differently on low-P soils: Australian Proteaceae from severely P-depleted areas have shown a preferential allocation of P in photosynthetically active mesophyll cells (Guilherme Pereira et al. 2018; Hayes et al. 2018; Shane et al. 2004), a low investment of P in ribosomal-RNA (Sulpice et al. 2014), and some replacement of phospholipids with non-phospholipids (Lambers et al. 2012). These strategies allow plants from Proteaceae to retain photosynthetic function while maintaining lower leaf P concentrations than plants occurring on more fertile sites (Lambers et al. 2022). However, phospholipid replacement by non-phospholipids has only been demonstrated for a taxonomically-limited set of species from one part of Australia and is contradicted by some studies in east Asia (Hidaka and Kitayama 2013; Meng et al. 2025; Dong et al. 2025). This begs the central question of which mode for reassigning leaf P is a central strategy for plants on low-P soils, and how it can be generalised to other species groups outside of the Proteaceae (see Shen et al. 2024).

Plant P allocation among different chemical fractions can be optimised when P is scarce in soils to reduce overall leaf P concentrations, which allows survival on low P sites by reducing the cost of P acquisition from soils (Hidaka and Kitayama 2013; Tsujii et al. 2024). Understanding how these P fractions vary with P availability and soil types is crucial for distinguishing between the roles of P for storage versus biochemical function via its allocation to biochemically active compounds versus cell ultrastructure (Crous and Ellsworth 2020). One feature of P allocation that has been identified when soil [P] is high is the accumulation of available or labile P forms within leaves such as P_i which is thought to have a

storage role (Bieleski 1968; McQuillan et al. 2020; Mimura et al. 1996; Veneklaas et al. 2012), resulting in a proportionally higher fraction of total leaf P in such instances compared to when soil [P] is low. Tsujii et al. (2024) observed this pattern when contrasting species on a low-P sandstone soil with those on a higher-P, basaltic soil in southeastern Australia, though species variation in leaf P allocation to chemical fractions within these sites was large. Veneklaas et al. (2012) and McQuillan et al. (2020) contended that excess orthophosphate (P_i) pools stored in vacuoles allowed for finely-balanced P_i available in the cytosol at times when required for metabolic processes (Dietz and Foyer 1986). However, the range in soil P levels at which P_i is preferentially accumulated in plant leaves remains unclear.

To understand species P allocation patterns and if P_i can be a storage compound under high P availability in native species on basalt soils as observed by Tsujii et al. (2024), we established plots at multiple sites with contrasting soil [P] as expected based on contrasting soil parent materials in eastern Australia. These sites with highly contrasting parent materials would provide a test for how foliar P fractions are adjusted in native species in relation to soil P supply avoiding short-term accumulation of excess P as occur in cropping systems (Veneklaas et al. 2012), soil P gradient studies (Liu et al. 2023; Tsujii et al. 2024), and P addition experiments (such as Mo et al. 2019). Investigating the allocation of foliar P in plants on soils with contrasting P availability can reveal different mechanisms and strategies for plant P utilisation with different soil P supplies, thereby enhancing our understanding of nutrient storage and partitioning in ecological systems.

Low-nutrient, sandy soils often lacking in total and available P are common in Australia (Beadle 1966; Viscarra Rossel and Bui 2016). In contrast, volcanically-derived basalt soils are relatively rare on the continent, though where they occur they support different vegetation and much greater total soil P than the surrounding sandstone substrates (Porder and Ramachandran 2013). In this study, we aimed to elucidate how native plants in south-eastern Australia growing in natural environments with contrasting soil P levels have adapted their P allocation strategies to these soils. We measured four leaf P fractions: P_i, metabolite P, nucleic acid P and lipid P, as well as net photosynthetic capacity and PPUE for > 30 species across four sites in New South Wales, Australia. These sites comprise a natural contrast in total soil [P] due to their origin from either sedimentary or igneous parent material (Table 1). We hypothesised (i) that in soils with a high [P] derived from igneous parent material, a large proportion of total leaf P would be allocated to readily-available, labile forms within leaves due to uptake in excess of requirements and subsequent storage (see McQuillan et al. 2020). This should result in a more pronounced increase in P_i relative to organic P fractions with increasing total leaf P. We also hypothesised (ii) that the PPUE of plants would be higher on low-P soils, such as those derived from sedimentary parent material, than on soils with high soil P status. We further expected (iii)

Table 1 Description of site characteristics and mean values ± SE for soil and leaf characteristics of the study sites. Shown are the site descriptions and soil total P concentration, soil extractable P concentration (Bray P), and leaf P concentration as the bulk total averaged across all sampled species (Table S2) for the four study sites. When site differences were found (for $p < 0.001$), different letters in each row are used to indicate significant differences among sites ($p < 0.001$, Tukey's HSD post-hoc test). Soil extractable P concentration was not significantly different among sites

Characteristic	North Head	Davies Park	Sun Valley	Mount Banks
Location	33.81 °S, 151.30 °E	33.70 °S, 150.55 °E	33.72 °S, 150.59 °E	33.58 °S, 150.37 °E
Elevation a.s.l. (m)	95	395	210	1060
Parent material and type	Aeolian sand (sedimentary)	Sandstone (sedimentary)	Diatreme (igneous)	Basalt cap (igneous)
Soil total P (μg g^{-1})	58.1 ± 12.0 c	83.6 ± 14.0 c	212.7 ± 53.8 b	1367.3 ± 10.1 a
Soil extractable P (μg g^{-1})	0.81 ± 0.07 a	2.29 ± 1.00 a	2.06 ± 0.41 a	1.85 ± 0.25 a
Site mean leaf P concentration (mg g^{-1})	0.34 ± 0.04 c	0.37 ± 0.02 c	1.14 ± 0.05 b	1.32 ± 0.06 a
Number of sampled species	8	13	7	6*

* 4 species were sampled for photosynthesis, 6 for leaf P

that high PPUE would be associated with high P_i or metabolic P fractions (combined P_i and metabolite P), ensuring more efficient use of P to maintain photosynthetic rates for survival on low P soils.

Materials and methods

Sites and species

To investigate the leaf P allocation patterns among P-containing biochemical pools in species adapted to habitats with strongly contrasting soil P levels, four sites in New South Wales (NSW), Australia were selected comprising two each of each parent material type with sandy soils (Table 1). North Head and Davies Park are underlain by sedimentary parent materials (sandstone), resulting in relatively low soil total P pools. The aeolian sand at North Head is from the Tertiary period, while Davies Park is underlain by Triassic Hawkesbury sandstone (ca. 240 Mya; Ellsworth et al. 2015). In contrast, Sun Valley and Mount Banks are underlain by igneous parent materials (Keith and Benson 1988), leading to higher soil total P pools at these two sites (Table 1).

North Head is a coastal site on sand and surrounded by sea cliffs (Table 1), described in Rogers et al. (2024). The vegetation at this site is predominantly heathland, dominated by *Banksia aemula* R.Br. and *Gaudium laevigatum* (Gaertn.) Peter G. Wilson, with a diverse low-stature understory of sclerophyllous shrubs. Davies Park features a dry sclerophyll forest with a diverse shrub understory on a southeast-facing plateau, as described in Ellsworth et al. (2015) and Dhakal et al. (2025). This site is dominated by *Corymbia gummifera* (Gaertn.) K.D. Hill & L.A.S. Johnson, *Eucalyptus piperita* Sm., and *Syncarpia glomulifera* (Sm.) Nied., characteristic of the silica-rich Hawkesbury sandstone-derived soils found throughout the Greater Blue Mountains area (Table 1).

The two sites on igneous parent materials, Sun Valley and Mount Banks, are both basaltic but from different origins. Sun Valley is a gently sloping valley located on residual Jurassic volcanic soil (ca. 175 mya) derived from a volcanic diatreme. The Mount Banks site was near the summit of a basalt cap formed by a series of volcanic flows in Tertiary period (14–16 Mya; Keith and Benson 1988), overlaying Hawkesbury Sandstone. The vegetation at Sun Valley is considered wet sclerophyll forest and is dominated by *Eucalyptus amplifolia* Naudin, accompanied by a grassy understory. At Mount Banks the rich soil and relatively high elevation (over 1000 m) supports a wet sclerophyll forest dominated by *Eucalyptus cypellocarpa* L.A.S. Johnson, *Doryphora sassafras* Endl., and *Hedycarya angustifolia* A.Cunn. This site exhibits a stark contrast in vegetation height and species composition compared to the heathland community and dwarf plants found on the surrounding Hawkesbury sandstone-derived soils. The sites and soils are described in Table 1, and mean annual temperatures and precipitation for the sites are given in Table S1.

A total of 33 different species were sampled across all four sites for leaf P concentration and its chemical fractions (Table 1, Fig. 1, Table S2; *Lambertia* was at both of the sandstone sites), encompassing 16 different families. The leaves exhibited significant morphological diversity, ranging from simple to compound, needle-like to bipinnate, and from true leaves to phyllodes. Of these species, 31 species were measured for photosynthesis (Table 1, Table S2 for within-species replicates). Two species from the Mount Banks site could not be measured for photosynthesis due to the remoteness of the site. The genus *Acacia* was the only one represented at all sites (one unique species at each site). Species from the Proteaceae family were found at sites derived from sedimentary parent material, including North Head (3 species) and Davies Park (7 species) but were entirely absent from the igneous-derived sites.

Soil sampling and analyses

Soil samples were collected in May (North Head, Davies Park, and Sun Valley) and July (Mount Banks) of 2022 from 2 to 6 plots at each site. Soil plots were randomly selected within the area of the sampled plants. Each plot was comprised by a composite sample made from soils collected from five subplots within a 2 m radius of the plot centre. Soil was extracted from a depth of 10 cm using a hand-held auger after removing the surface organic matter layer. Samples were placed in zip-lock polyethylene bags and stored on ice until air-drying. Soils were then sieved through a 2 mm sieve to remove stones and

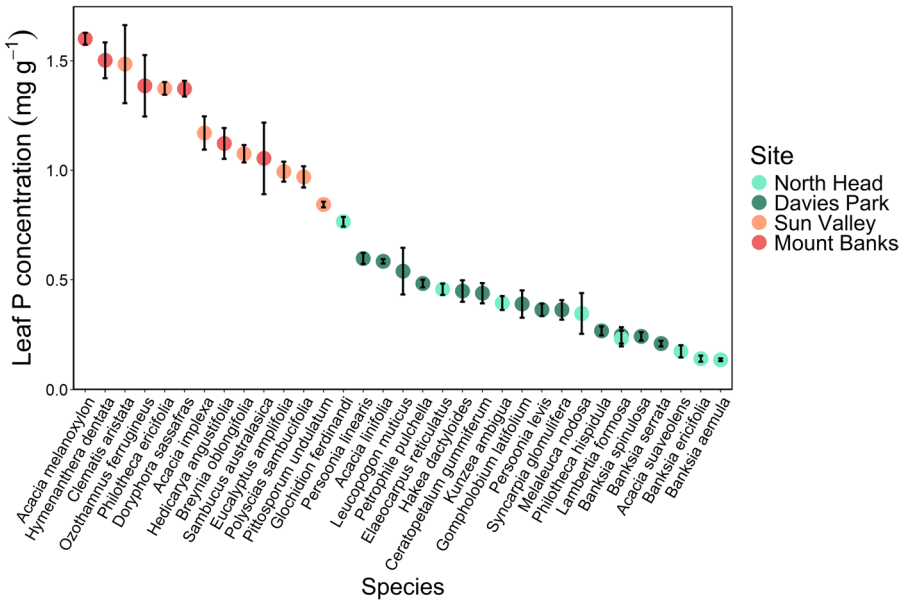

Fig. 1 Mean ± SE for total leaf P concentration for the study species at all four study sites ordered from high to low. Some SE values are obscured by the points. Values are grouped according to parent material separated by the vertical dashed line, with species on igneous parent material indicated with two shades of green points and species on sandstone parent material shown as two shades of orange points. Data for one species (*Lambertia*) are shown for the two sites on sedimentary soils on which it was measured (two means ± SE bars)

organic debris, ensuring a more homogeneous sample before chemical analyses.

Soil total P concentration for each composite sample from the plots was determined by digesting the oven-dried soil with a 1:3 mixture of nitric acid and hydrochloric acid using a microwave digester (Speedwave 4, Berghof Instruments, Eningen, Germany). Soil extractable P concentrations were measured by extracting the soil with a 0.03 M ammonium fluoride and 0.025 M hydrochloric acid in a 1:7 soil-to-extractant ratio, following the Bray I procedure (Bray and Kurtz 1945).

Measurement of photosynthesis and photosynthetic phosphorus use efficiency

A portable gas exchange system with infrared gas analysers (Li6400XT, Li-Cor, Lincoln, NE USA) was used to measure net photosynthesis on clear, sunny mornings at each site, using sunlit, attached, and fully expanded leaves. The measurement protocol followed Ellsworth et al. (2015) and Rogers et al. (2024), with measurements made at light saturation (photon flux density of 1800 µmol m^{-2} s^{-1}) with a LED bank of lights and using leaf temperatures regulated to 25 °C (24.9 ± 0.1 °C), and a controlled CO_2 supply at 401 ± 1 µmol mol^{-1} (mean ± s.d.). The chamber was humidified during measurements, achieving an average leaf-air vapour pressure difference of 1.41 ± 0.1 kPa (mean ± s.d.) across the measurements. Photosynthesis measured on sunny days in morning hours under these conditions approximate well the peak rates of net photosynthesis close to photosynthetic capacity (Ellsworth et al. 2015; Rogers et al. 2024). Measurements were made in the growing season at all sites: spring for North Head and Mount Banks, summer-autumn for Davies Park and autumn for Sun Valley, from April 2021 to November 2023. Light-saturated photosynthetic rates were expressed on a leaf area basis ($A_{sat,area}$; µmol CO_2 m^{-2} s^{-1}) and a per unit dry mass basis ($A_{sat,mass}$; nmol CO_2 g^{-1} leaf s^{-1}). When the chamber area (6 cm^2) was not completely filled, the leaf area inside the chamber was determined by scanning and rates were corrected for the appropriate leaf area inside the chamber. Photosynthetic rates were also expressed on a per unit leaf P basis using the leaf P concentration and leaf mass per unit area. We defined instantaneous photosynthetic P-use efficiency (PPUE) as the rate of light-saturated photosynthesis on a per unit leaf P basis (units: µmol CO_2 g^{-1} P s^{-1}).

Photosynthesis measurements were taken on 2 to 7 individuals per species at all sites (Table S2). To ensure adequate tissue for chemical analysis, the measured leaf, along with neighbouring leaves of the same age class, was harvested from the same or nearest branch or branchlet. The leaves were placed in labelled zip-lock polyethylene bags and stored on ice

until further processing and chemical analyses in the laboratory.

Analyses of total leaf P and leaf P fractions

After returning to the laboratory, leaf samples were freeze-dried for > 48 h and then ground to a fine powder using a mixer mill (Retsch Mixer Mills MM 400). Total P concentrations of leaves were determined after digesting the ground plant samples with concentrated (99.99%) sulfuric acid and hydrogen peroxide for 1 h at 30 bar pressure in a microwave digester (Crous et al. 2015). The digests were diluted with pure water (Milli-Q® Ultrapure, Merck, Vic., Australia) and checked to ensure pH was not different between samples and standards. Then total leaf P concentrations were colorimetrically analysed at 880 nm after a molybdate reaction in the SEAL AQ2 discrete flow autoanalyzer (SEAL model AQ270, Milwaukee, USA). Internal and internationally-accepted leaf standards for P (*Eucalyptus* leaves and NIST Standard Reference Material 1515, respectively) were used with each batch that was analysed.

We measured four different chemical fractions of total P in leaves: free orthophosphate (P_i), metabolite P comprising low molecular weight adenylates and related metabolites as well as phytate (myo-inositol hexakis phosphate), nucleic acid P including DNA and RNA, and lipid P were extracted. Concentrations of P_i in leaves were determined after extracting 20 mg of the ground plant samples with 2 ml 0.3 M trichloroacetic acid (TCA) for 1 h at 4 °C, with vortex every 10 min (Close and Beadle 2004; Crous et al. 2015). The extraction was followed by centrifuging at 15,000 rpm for 5 min at 4 °C. The P concentration of the supernatant was analysed in a similar manner as the assay for total P, after dilution with ultrapure water. Metabolic P (P_i + metabolite P) and nucleic acid P fractions were sequentially extracted from 400 mg of the ground plant samples following the methodology of Chapin and Kedrowski (1983), with modifications by Polglase et al. (1992) and further by Close and Beadle (2004). Ground plant samples were extracted with 50 ml of cold 0.3 M TCA at 4 °C for 1 h, with a vortex mixer every 10 min. The extracts were centrifuged at 4,000 rpm for 20 min at 4 °C. The supernatants were removed and refrigerated for later digestion in a block digester (SEAL Analytical, BD50 and BD28 Block Digestion System) to determine metabolic P concentrations. The remaining pellet after cold extraction was re-extracted with 50 ml of 1.5 M TCA at 90 °C for 1 h in a hot water bath. The subsequent extracts were cooled to room temperature, centrifuged, followed by removal and storage of the supernatant for later digestion (in block digester) for nucleic acid P determination.

It is possible that P_i was overestimated in our measurements since samples were stored on ice and not flash-frozen, so some RNA and lipid degradation could have released P_i. This could also mean that some organic P fractions were somewhat underestimated with our storage technique relative to studies that have flash-frozen samples in liquid N_2. We are unaware of any systematic comparisons of preservation effects on leaf P fractions involving samples flash-frozen in liquid N_2 compared to those stored on ice but such work should be undertaken given the different sample preservation techniques used in different studies.

Lipid P was extracted separately, rather than sequentially with metabolic and nucleic acid P, using a method adapted from Hidaka and Kitayama (2013) and modified by Yan et al. (2019). Ground plant samples (50 mg each) were mixed with 1 ml of 1:2:6 CMF (chloroform, methanol, and formic acid), vortex-mixed for 10 s, followed by centrifuging at 15,000 rpm for 10 min at 4 °C. The supernatant was transferred to a new tube (15 ml) and the procedure was repeated two more times. The remaining pellet was mixed with 1.26 ml of 1:2:0.8 CMW (chloroform, methanol, and water), vortexed, centrifuged and the supernatant was transferred to the same 15 ml tube. This procedure was also repeated two more times. The liquid extracts in 15 ml tubes were mixed with 1.9 ml chloroform-washed water, vortexed and centrifuged to separate the lipid-rich phase accumulated at the bottom from the aqueous upper phase. The lipid-containing bottom layer was transferred to the digestion vessel with a Pasteur pipette. All the extracts/supernatants from the three different P extraction processes were evaporated until dryness at maximum 130 °C (for metabolic and nucleic acid P extracts) or 70 °C (for lipid P extracts). They were then digested with concentrated sulfuric acid and hydrogen peroxide for 3 h in the digestion block for Kjeldahl digestion. The P concentration of the digests was analysed similarly as done for total P after dilution with ultrapure water.

The concentration of the metabolite P was determined by subtracting the P_i concentration from the metabolic P concentration. The residual P fraction of total P is typically small (see Suriyagoda et al. 2023) and is comprised by recalcitrant P forms such as phosphorylated proteins and complex phosphate esters as well as other P-containing organic compounds not extracted by the preceding methods. The residual P fraction was not measured in this study as it is not easily interpretable and we were not interested in making inferences about it.

Statistical analyses

All statistical analyses were conducted using R software (R Core Team 2024). One-way ANOVA was performed to compare the means of soil total and extractable P, total leaf P, A_{sat_mass} and PPUE among sites, as well as the concentrations and relative allocation of each P fraction within sites. Post-hoc Tukey's HSD test was used to determine pairwise differences between groups when $p < 0.05$. Linear regression models were fitted to examine relationships among P fraction concentrations, their relative allocations, and their associations with A_{sat_mass} and PPUE and Pearson's correlation analysis was also used for these relationships with $\log_{10}$ transformations. We used diagnostic plots, such as Q-Q plots, residual and scale-location plots to verify the assumptions of normality and homoscedasticity of residuals. Additionally, the Shapiro–Wilk test was performed to assess the normality of residuals. These checks confirmed that the assumptions of linear regression were met. Logarithmic transformations ($\log_{10}$) were applied where necessary to meet the assumptions of parametric tests.

Results

Total soil P concentration was significantly different amongst the sites ($p < 0.001$). This soil P measure was markedly higher, by at least twofold, for the igneous-derived soils than for the sedimentary-derived soils (Table 1). Hence the overall P pool that plants could potentially access from the soils varied strongly between contrasting parent material types, although the extractable P concentration was not significantly different between the two different parent material types or among sites (Table 1, $p > 0.05$). As further evidence, mean total leaf P concentration for these species was significantly higher for the igneous-derived soils than for both of the sedimentary-derived soils ($p < 0.001$, Fig. 1, Table 1) showing that parent material had a strong influence on leaf P even though soil extractable P was similar. In fact, the mean leaf P concentration was on average more than three-fold higher at the sites with igneous parent material (Sun Valley and Mount Banks) compared to the sites with sedimentary parent material (North Head and Davies Park; Table 1). For the only genus that co-occurred at all sites (*Acacia*), leaf P concentration also differed by 3.5-fold between sites with sedimentary parent material compared with sites with igneous parent material (Fig. 1), again reflecting the differences in P-containing minerals between these different substrates.

Leaf P fractions

Concentrations of leaf P fractions, including P_i, metabolite P, nucleic acid P, and lipid P, all increased linearly with increasing total leaf P concentration (Fig. 2a). Hence concentrations of each of these P fractions were also higher for igneous than sedimentary parent materials, but in proportion to total leaf P. We sought to establish if there was a steeper increase in P_i concentration with increasing total leaf P compared to organic P concentrations as would be suggested if this was a storage form of P. However, we did not observe the expected exponential increase between the concentrations of P fractions and total leaf P (Fig. 2a), nor did we observe this when total metabolic P was considered as the sum of P_i and metabolite P (not shown). Instead, all P fractions showed a linear increase with total P concentration, with each fraction exhibiting significant positive correlations with leaf total P ($R^2 = 0.87$, 0.61, 0.93 and 0.92 for P_i, metabolite P, nucleic acid P and lipid P respectively, all $p < 0.001$, Fig. 2a). Indeed, the total organic P (P_o) was found to linearly increase with increasing P_i without reaching a plateau, further confirming that P_i was not stored in excess of either the organic component or leaf total P at these sites (data not shown). Here we subtracted P_i from the total P pool to represent all components of P_o ($R^2 = 0.71$, $p < 0.001$, Fig. 2b). The mean ratio of P_o to P_i did not differ significantly between the two different parent material types

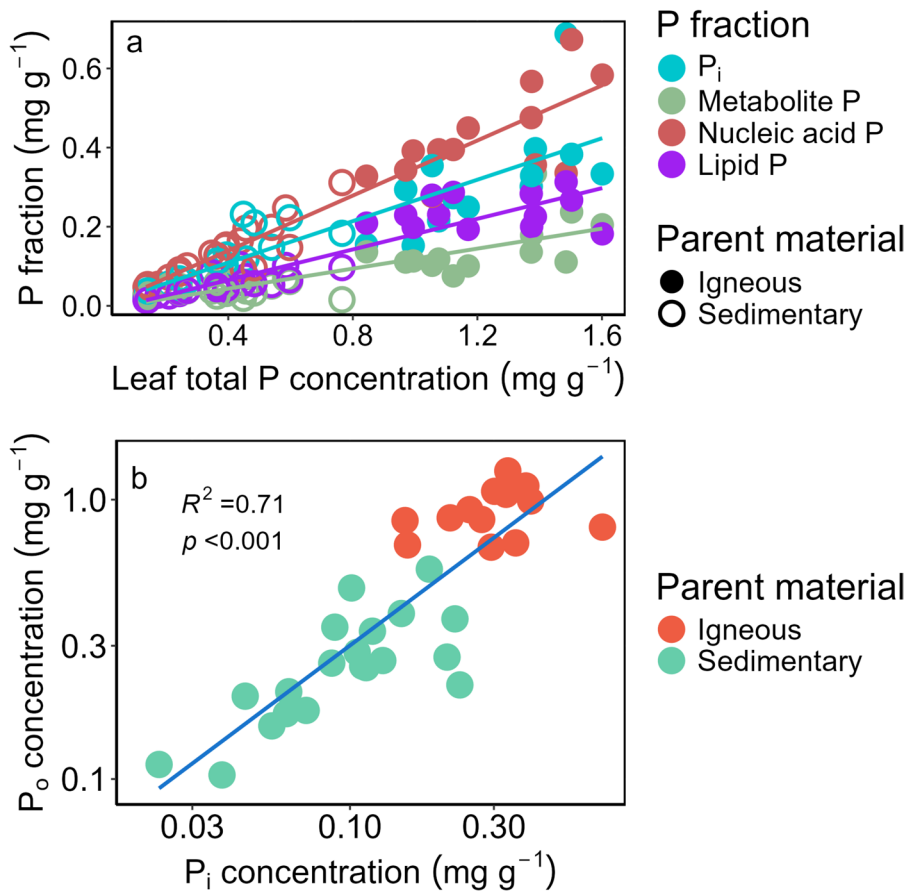

Fig. 2 (**a**) The relationships between concentrations of total leaf P and each of four P fractions. (**b**) The relationship between inorganic P (P_i) concentration and organic P (P_o, containing metabolic P, nucleic acid P and lipid P) concentration for study species (n = 33 across all four study sites). Both the x and y-axis in panel B are presented on a $\log_{10}$ scale

(0.46 ± 0.12 for sedimentary-derived soils versus 0.18 ± 0.12 for igneous-derived soils, $p > 0.05$ using the chi-square test).

Mean concentrations of leaf P fractions for P_i, metabolite P, nucleic acid P, and lipid P all exhibited marked and significant differences between sites with contrasting parent materials in our study (all $p < 0.001$). These concentrations were also not statistically different between sites within each parent material type, except for metabolite P concentrations within the igneous parent material type ($p < 0.001$, Tukey's HSD post-hoc test, Fig. 2a). Averaged within each parent material type, the concentrations of each of these P fractions showed between 3.2 to 4.6-fold differences from sedimentary to igneous-derived soils, commensurate with the 3.5-fold difference in total leaf P concentration between these parent materials (Table 1, Fig. 1). The largest difference in P fractions across parent materials was observed for the lipid P fraction, which showed a 4.6-fold increase in concentration from sedimentary to igneous-derived soils, compared to the other P fractions (Fig. 3a).

Whilst the concentrations of the four P fractions that we separated differed substantially between the two contrasting parent materials, we sought to understand if the relative proportion of different P containing compounds per unit total P differed among the four sites or with regard to parent material. The relative proportion of P both in the inorganic fraction of total P and in nucleic acids per total leaf P was not significantly different among the four sites nor across the two parent materials (Fig. 3b). In contrast, the lipid P fraction of total leaf P was significantly different between parent materials ($p < 0.001$, Tukey's HSD post-hoc test, Fig. 3b; see also Fig. S2). In fact, the lipid P fraction of total leaf P was lower by about a third at the two sites with sedimentary-derived soils compared to the two sites with igneous-derived soils (Fig. 3b). A consistent compensation of the reduction in lipid P fraction by an increase in another

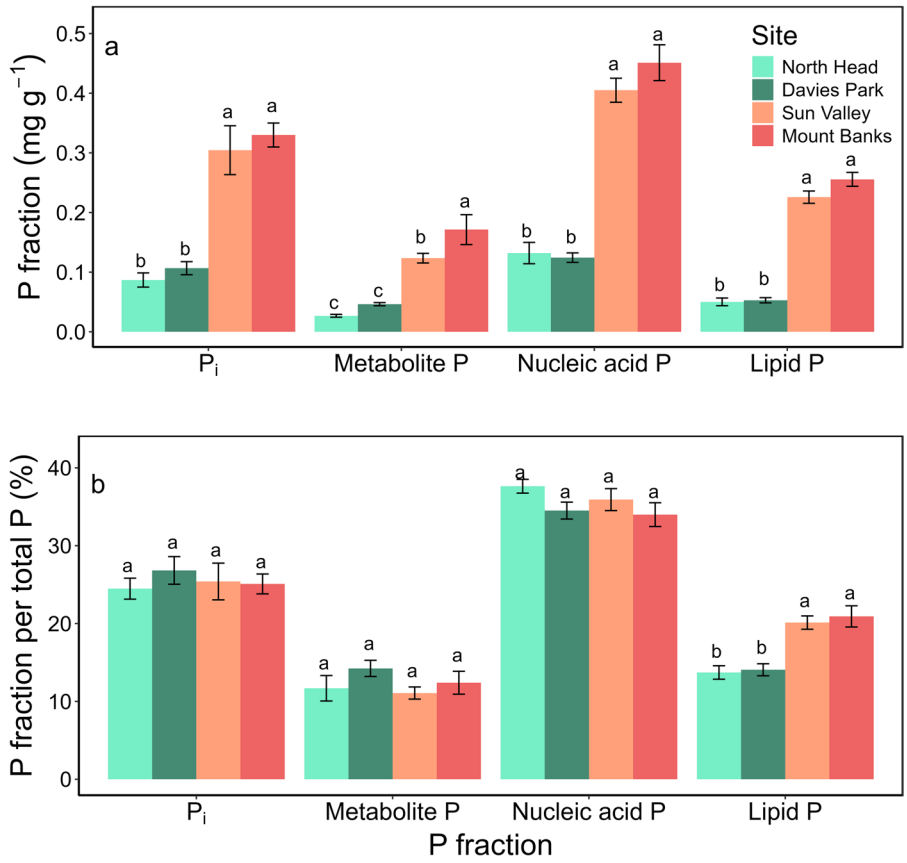

Fig. 3 Mean ± SE for leaf P concentrations (**a**) and proportions of leaf P fractions (**b**) for study species at four different study sites. Different letters in each fraction indicate significant differences among sites ($p < 0.05$, Tukey's HSD post-hoc test). As in Fig. 1, green colour tones denote sedimentary parent material and orange tones indicate igneous parent material

fraction was not apparent from the slightly (but not significantly) higher P_i and metabolite P fractions for Davies Park and the nucleic acid P fraction for North Head for sedimentary parent materials ($p > 0.05$, Fig. 3b). However, we observed a negative correlation between the lipid P fraction as an indicator of leaf structure and the sum of the biochemical compounds of the leaf using metabolic P (both P_i and metabolite P, mostly adenylates) and nucleic acid P ($R^2 = 0.14$, $p = 0.027$, Fig. S3). This weak negative correlation indicates some offsetting may have occurred for the structural versus biochemical fractions of total leaf P.

To understand the lower lipid P fractions of total P on the sedimentary-derived sites compared to the igneous-derived sites, we investigated how the species from highly P-efficient cluster rooted Proteaceae family (see Shane and Lambers 2005) allocate P to lipid fraction compared to the non-Proteaceae families at these sites (Fig. 4, Fig S2). Species from Proteaceae family exhibited 13–28%

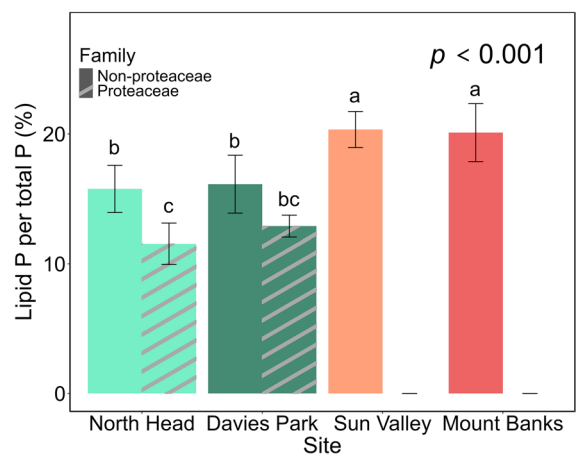

Fig. 4 Mean ± SE for lipid P proportion per total P in Proteaceae and non-Proteaceae species at the study sites. North Head and Davies Park contain 3 and 7 Proteaceae species respectively, whereas the other two sites lack Proteaceae. Different letters indicate significant differences among the six means ($p < 0.001$, pairwise t-test). Within-site differences are indicated in the text

lower lipid P fraction of total P compared to the species from non-Proteaceae families within the sedimentary sites, with a significant family difference apparent for North Head ($p = 0.029$, pairwise t-test) but not Davies Park ($p = 0.18$, Fig. 4). The cluster-rooted yet non-Proteaceae species *Gompholobium latifolium* (in the Fabaceae family; see Nge et al. 2020) had the lowest lipid P fraction of total P among all the species we measured (Fig. S1), as an exception to the low lipid P fraction of total P in Proteaceae. When *Gompholobium latifolium* was removed from analysis, a significant family difference was also apparent for Davies Park ($p = 0.036$, pairwise t-test, Fig. S2). In contrast to the general pattern of lower lipid P fraction of total P with respect to site parent material shown in Fig. 3, congeners in both *Acacia* (across all sites) and *Philotheca* (contrasting Davies Park with Sun Valley sites) were not significantly different for this parameter ($p > 0.05$, Fig. S1). The mean lipid P fraction of total P for *Acacia* at each of the four sites was $15.8 \pm 2.1\%$.

Photosynthesis and Photosynthetic P-use efficiency

Area-based photosynthetic capacity did not differ much among sites, and in all cases the site average A_{sat_area} ranged from 10–14 μmol m^{-2} s^{-1} (data not shown). However, A_{sat_mass} was significantly different among the sites ($p < 0.001$), with values that were 70% higher for species on igneous-derived soils compared to those on sandstone-derived soils (Fig. 5a). As a result, instantaneous PPUE of plants was significantly different among the sites ($p < 0.001$, Fig. 5b) and particularly between parent material types (also $p < 0.001$), with plants growing at each of the sedimentary-derived soils exhibiting higher PPUE compared to PPUE of the plants growing in the igneous-derived soils (Fig. 5b). The higher PPUE for species on the sandstone-derived sites was largely related to the lower leaf P concentration for these species (see Table 1 and Fig. 6b).

To investigate how leaf photosynthetic capacity and PPUE varied with leaf P among sites and species, we examined a set of correlations for PPUE with total leaf P concentration and the concentrations

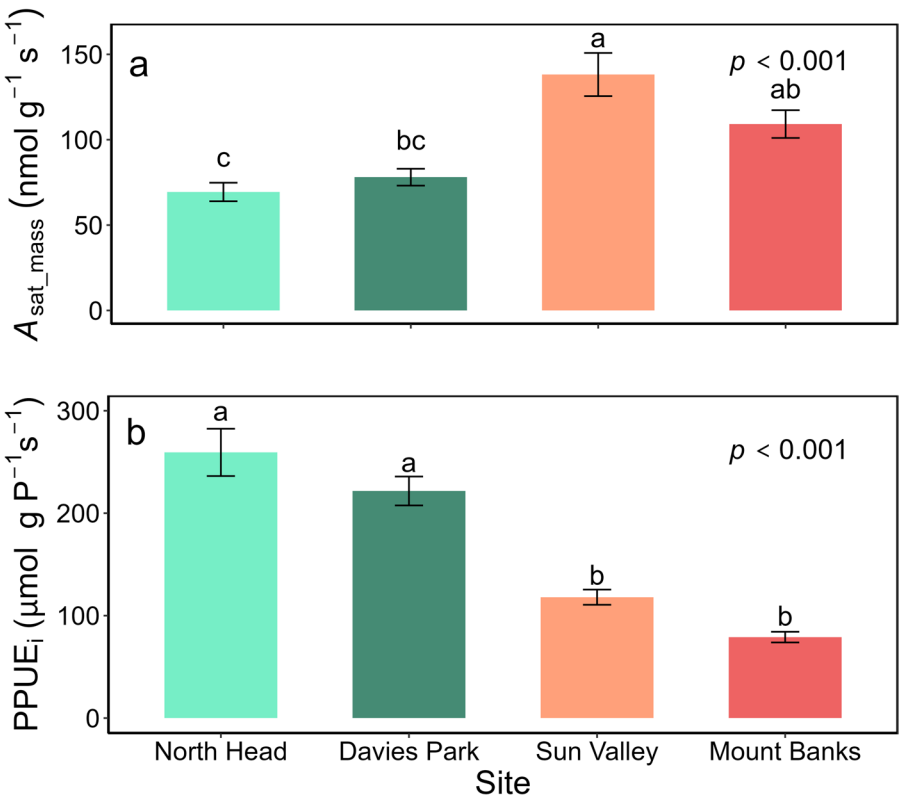

Fig. 5 Mean ± SE for (**a**) A_{sat_mass} and (**b**) PPUE for the study species at four different study sites. Different letters indicate significant differences among sites ($p < 0.001$, Tukey's HSD post-hoc test). Green tones denote sedimentary parent material and orange tones indicate igneous parent material

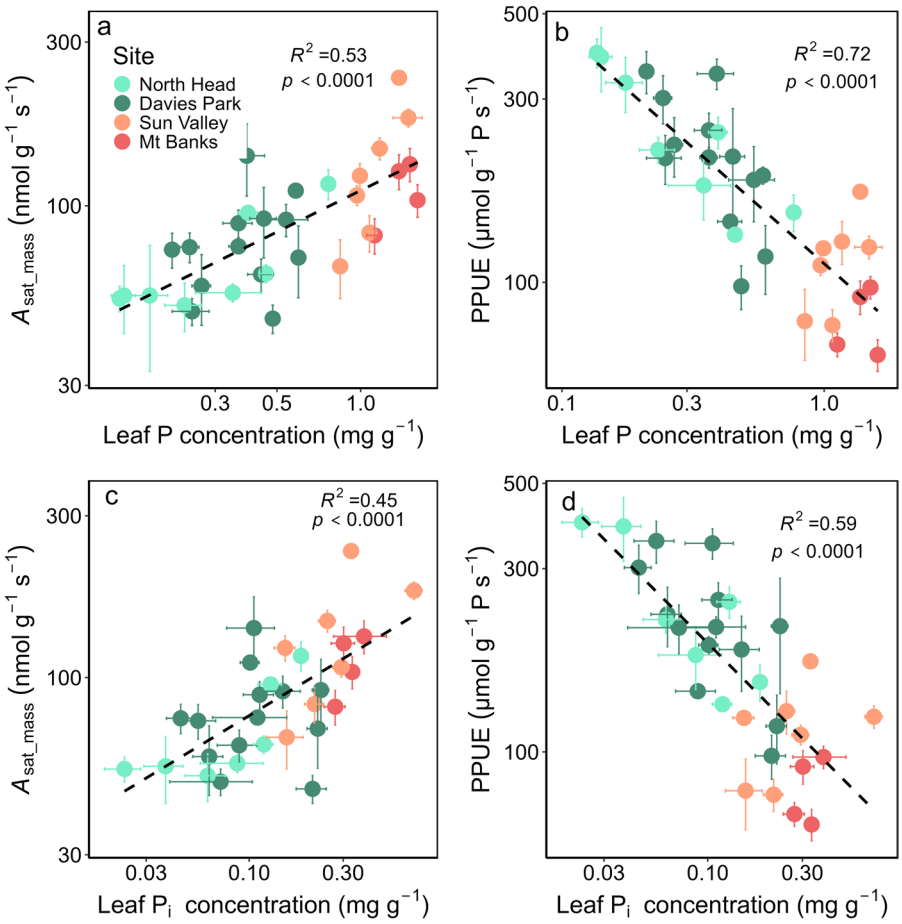

Fig. 6 The relationships between (**a**) A_{sat_mass} and total leaf P concentration for the study species (n = 31 species, n = 32 species-site combinations) across all four study sites, (**b**) instantaneous PPUE and total leaf P concentration, (**c**) A_{sat_mass} and leaf P_i concentration, and (**d**) instantaneous PPUE and leaf P_i concentration. Points are species means and bars indicate standard errors, with colours indicating the different sites. Data from sites are shown in green tones for species on sandstone parent material and red tones denote species on igneous-derived parent material. Lines show the best-fit relationship with R^2 for the fit shown in each panel. See Table S3 for all regression statistics. Both axes in each panel employ a $\log_{10}$ scale

of different P fractions in leaves. Among the species studied, A_{sat_mass} was strongly correlated with total leaf P concentration ($R^2 = 0.53$, $p < 0.00001$, Fig. 6a, Table S3) and was also correlated with total leaf P concentration among species on sandstone-derived soils only (n = 21 species, $R^2 = 0.31$, $p = 0.008$; Table S3). Instantaneous PPUE was negatively correlated with total leaf P concentration across all species ($R^2 = 0.72$, $p < 0.001$, Fig. 6b), as expected from how PPUE is mathematically computed.

In addition to total leaf P concentration, A_{sat_mass} across the full set of species was also correlated with leaf P_i concentration ($R^2 = 0.45$, $p < 0.001$, Fig. 6C). The lower goodness of fit for the A_{sat_mass}-leaf P_i correlation than for total leaf P concentration (Table S3) was due to greater variation in leaf P_i concentration than total leaf P (Fig. 6a, c). PPUE was negatively correlated with leaf P_i concentration ($R^2 = 0.59$, $p < 0.001$, Fig. 6d) and all other leaf P components (Fig. 7). PPUE also varied negatively with leaf P_i concentration across species within the sedimentary-derived sites ($R^2 = 0.59$, $p < 0.001$, Table S3) but this relationship was not significant within the sites from igneous parent material ($p > 0.05$, Fig. 6d; significant linear correlation only shown across all species, see Table S3). Similar to total leaf P concentration, an increase in each P fraction concentration was associated with a decrease in PPUE across all study species and sites ($R^2 = 0.39$—0.76, $p < 0.001$, Fig. 7), even when non-metabolic P fractions were considered.

Discussion

We demonstrated that differences in soil P concentration arising from different parent materials can lead to significant differences in total leaf P concentrations. Alongside this, there were differences in the fractional allocation of leaf P to the structural and biochemical pools within plant leaves of native species

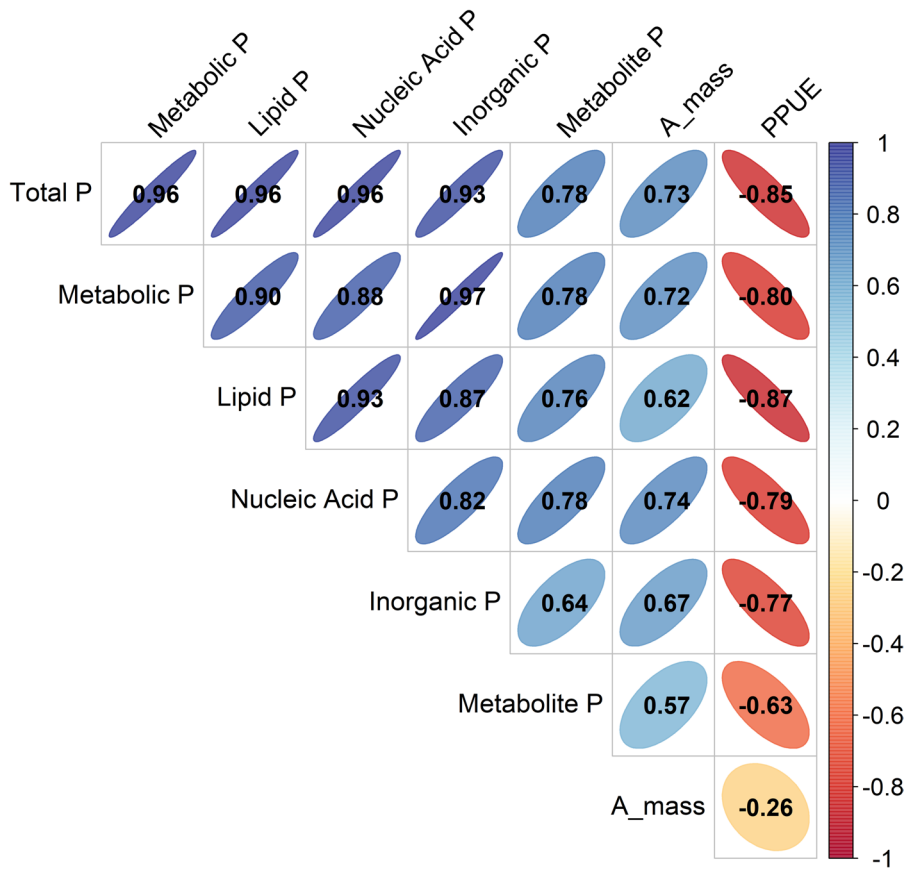

Fig. 7 Correlations among photosynthesis and leaf P fractions depicted as ellipses with widths and colour shades corresponding to the strength of the correlation (magnitude of Pearson's R values). The direction of the ellipse and its colour indicate the correlation direction (up = positive correlation) and strength, with positive correlations depicted in blue shades and negative correlations in orange shades. Correlation coefficients are indicated by the numbers in the ellipses. All variables are log-transformed

(Table 1, Figs. 3 and 4). This was in spite of the similarities of these sites in extractable P (Table 1) suggesting that the species on these sites, and particularly those on the igneous-derived soils, had greater access to P than is considered 'labile' according to the Bray P extraction that we used for the surface soils (see also Lambers et al. 2011). Understanding the mode of cellular P allocation in leaves in response to such differences in total soil P status is key to understanding plant P-use for physiological function in the field (see Fig. 6). The mode of P allocation to different chemical fractions shows how plants may reduce their requirements for P in leaves (Suriyagoda et al. 2023) to underpin survival in places where plant access to P supplies is very low.

Generally when P supply is high, it is expected that more P is allocated to available forms within leaves, with P_i in excess of current cytoplasmic needs stored in the vacuole and later remobilised to maintain P_i concentration in the cytoplasm when P supply is low (Foyer and Spencer 1986; Mimura et al. 1990). However, in our study we showed that in spite of significant differences in soil P levels across the sites, the relative proportion of P_i to total P within plants remained remarkably consistent (Fig. 2) across species and parent materials. In our study, P_i was not preferentially accumulated as a storage form in high P sites, in contrast to what has been suggested by McQuillan et al. (2020) and Suriyagoda et al. (2023). It is possible that the soil P concentrations among the soils studied here was in a lower range than in those previous studies, and it is also possible that cropping systems with added available P may stimulate excess P_i and storage. The lack of P_i accumulation in amounts in excess of the fraction expected based on total leaf P is in contrast with our first hypothesis, as we had expected a large amount of P in labile forms in leaves on the high-P soils like the basaltic, igneous-derived soils here. A difference in our results from what we expected from our first hypothesis may be explained by the low overall extractable P on all our sites, both basaltic as well as sandstone, which

possibly precluded an excess of P_i beyond a constant fraction of the size of the leaf total P pool. Even on high-P sites, the excess P in leaves was apparently not stored as readily-available P_i, at least not out of proportion to the organic or total leaf P pool (Fig. 2a, b). Accordingly, as opposed to our first hypothesis, we found that the concentration of P_i does not show a steeper increase with increasing total leaf P compared to other P fractions (Fig. 2a), suggesting that the excess P from high P environments such as those with igneous-derived soils is small. As a caveat, sample preservation techniques can affect P_i concentrations, so further research on this as well as broad agreement among the community for how to best collect and analyse P fractions is needed. With that caveat in mind, our results do suggest that high P_i allocation in leaves, relative to organic P fractions, may be more common in plants with much higher total leaf P concentrations than those of plants in this study, such as fertilised crop plants as were analysed in Suriyagoda et al. (2023) and Veneklaas et al. (2012).

We found not only 70% lower leaf P concentration for native species on sandstone-derived sites compared with igneous sites (Table 1), but also a significantly lower proportional allocation of lipid P per unit total leaf P between these sites (Fig. 3). Species on sedimentary soils had 32% lower proportional allocation of lipid P to total leaf P, a result broadly consistent with a comparison of sedimentary and ultrabasic-derived soils (Hidaka and Kitayama 2011). The substitution of phospholipids in membranes by other lipids which do not contain P, referred to as remodelling phospholipids with other lipids such as galactolipids and/or sulfolipids, has been recognised in a range of Proteaceae species growing at low soil P availability in Australia (Lambers et al. 2012; Liang et al. 2024). Lipid remodelling in this fashion has been observed in other species subjected to P-deficiency (Andersson et al. 2003; Härtel et al. 2000; Tjellström et al. 2008), though it is important to distinguish that low-P-adapted species could respond with lipid remodelling differently than those under P-starvation (Lambers et al. 2015b). A rapid increase in the transcription of genes involved in galactolipid and sulfolipid synthesis in leaves during P starvation has been found for some model plants (Hammond et al. 2003, 2011; Morcuende et al. 2007), indicating that reduced lipid P fractions can be an effective strategy for conserving P (Lambers et al. 2015a).

Reduced lipid P concentrations per unit total P in leaves have been demonstrated for Proteaceae (Lambers et al. 2012; Liang et al. 2024). For the Proteaceae in our study there was 11% and 13% of the fractional allocation of total leaf P to lipid P for the two sedimentary sites, which was 41% lower than the lipid P fraction in species on igneous-derived soils (Fig. 4). Although we did not measure P-free lipids, similar work at a similar but higher soil P sedimentary site nearby has done so (Liang et al. 2024). Hence we interpret our result in Fig. 4 as evidence of lipid P substitution, recognising that this may indicate a reduction in the mass of P-containing lipids in leaves (Liang et al. 2024). In part, this effect could pertain to the presence/prevalence of Proteaceae species on low-nutrient sandy soils (Lambers et al. 2012) such as the sedimentary sites we sampled, given that Proteaceae in our study had significantly lower P concentrations than co-occurring species (Lambers et al. 2015a; Rogers et al. 2024; Liang et al. 2024). Still, other taxa can also show reduced lipid P fractions in leaves (Suriyagoda et al. 2023; Tsujii et al. 2024). Indeed, in our study the non-Proteaceae species showed a 23% lower proportional allocation of total P to lipid P on low-nutrient sandstone sites compared to non-Proteaceae plants on igneous-derived soils (Fig. 4), indicating a reduction in lipid P fractions in these leaves, although species identity also plays a role in these differences. An important caveat to our study is that we cannot exclude the possibility of some lipid degradation during sample storage, although all our samples were treated consistently and thus our finding of lower lipid P fractions on sandstone versus igneous soils (Fig. 4, Fig. S1) can be considered robust.

The comparison of the fractional allocation of leaf P to lipid P across sites and parent material is not possible for the Proteaceae family in our study because they are either not present or extremely rare and not found on the igneous sites where we worked. In contrast, *Acacia* species occurred on all the sites we studied and these species all showed similar fractional allocation of leaf P to lipid P on sandstone-derived soils compared to igneous-derived soils. Yan et al. (2019) also found that the fractional allocation of leaf P to lipid P was invariant for an *Acacia* species growing along a soil P gradient, supporting our *Acacia* results. Similar studies on the fractional allocation of leaf P should involve more species or genera that occur across a range in soil P availability as done

by Yan et al. (2019) and Shen et al. (2024). Further work on the plasticity of lipid P fractions across soils would be needed to determine the extent to which low P allocation to the lipid pool in leaves is a taxon-specific strategy for adapting to low P environments, a mode that may only be possible for certain phylogenetic branches of plants that are common at low P, rather than being general for most plant species. The use of other techniques such as ultra-performance liquid chromatography and high-resolution mass spectrometry would be valuable for identifying and quantifying various lipid compounds in the cellular membranes of our study species (see Hummel et al. 2011).

Achieving higher instantaneous rates of photosynthesis per unit total leaf P in low P environments (e.g., high instantaneous PPUE) is a consequence of reduced total P concentration in leaves (Reich et al. 2009) both ecologically as well as mathematically, which may include reducing the allocation of P to structural, non-photosynthetic components such as lipids. When a higher fraction of leaf P can be allocated to metabolic processes in leaves of plants and less P is allocated to structures such as membranes, PPUE is high as has particularly been observed in *Banksia* species in south-western Australia (Lambers et al. 2011). The shift in P allocation to membranes presupposes that the functionality of photosynthetic membranes like the chloroplast envelope and thylakoid membranes are not affected by a reduced fractional allocation of P. This is expected since the thylakoid membrane of chloroplasts as sites for photosynthetic electron transport are largely composed by glycolipids (Härtel et al. 2000; Gasulla et al. 2019).

Significantly higher PPUE across species was observed in species from each of the sedimentary-derived sites in this study compared to the other two P-rich sites (Figs. 5 and 6) confirming our second hypothesis. An association between low P investment in phospholipids and high PPUE was also observed in tropical tree species on Mount Kinabalu, Borneo (Hidaka and Kitayama 2013) and in two highly P-efficient Proteaceae genera, *Banksia* and *Hakea* in south-western Australia (Gille et al. 2024) as well as other taxa in that region (Shen et al. 2024). We note that the expression of PPUE as the ratio of A_{sat_mass} per unit total leaf P is a convenience but potentially misleading if only some fractions of total leaf P contribute directly to photosynthetic capacity and if species differ greatly in these fractions of leaf P. Refining our definition of PPUE in the light of within-leaf P allocation to function versus structure is an area for further study.

Since concentrations of all four P fractions we measured were correlated with total P among species and sites, there were correlations of varying strength between A_{sat_mass} and each of the P fractions in our study (Fig. 7). However, among these we suggest that the P_i concentration in leaves is most closely functionally related to photosynthetic capacity among the species in our study, noting that phosphorylated protein concentrations were not measured. This relationship with P_i is supported by theory (see Ellsworth et al. 2015) and prior study (Thomas et al. 2006) as well as a strong goodness of fit here ($R^2 = 0.41$, $p < 0.0001$, Fig. 6c). Although RuBP pools are a part of the metabolites we measured and should also relate to photosynthetic capacity as a substrate for photosynthesis, the metabolite P correlation with A_{sat_mass} was lower than for P_i (Pearson's $R = 0.65$ versus $R = 0.5$, respectively; Fig. 7). It is unclear if this reflects a more peripheral relationship to photosynthesis for metabolite P concentrations or not, requiring detailed quantification of these components as well as phosphorylated proteins in future photosynthetic work. Metabolic P as the sum of the P_i and metabolite P components was also correlated with A_{sat_mass}, although P_i made up on average 70% of the metabolic P fraction of the leaves in our study (range among species between 44 to 98%). As a result, the evidence suggests that higher leaf P_i concentrations are best associated with higher A_{sat_mass} amongst the chemical fractions of P we measured.

The denominator in the calculation of instantaneous PPUE is leaf P concentration, so higher leaf P concentrations and higher P_i concentrations are characteristic of richer soils and are both associated with lower PPUE (Fig. 6b, d). The species on igneous-derived substrates had higher total leaf P concentrations (Table 1) and showed reduced PPUE by more than half compared with the sedimentary-derived soils (Fig. 5b). In comparison, the species on sedimentary soils had high PPUE which was associated with low leaf P concentrations and also with the lower lipid proportion of total leaf P (Fig. S1). The positive intercept of the A_{sat_mass}-P relationship in Fig. 6a would also tend to enhance PPUE at low leaf P concentrations. Taken together, the results indicate that

high instantaneous PPUE is achieved in the majority of the species we sampled on sedimentary-derived soils, but especially Proteaceae (Figure S2), by maintaining low leaf P concentrations by reducing all leaf P pools but in part facilitated by a greater-than-proportional reduction in the lipid fraction of leaf P (see also Shen et al. 2024).

Conclusions

Across four sites on different parent materials, the concentrations of biochemically-active metabolic P (both P_i and metabolite P) as well as more recalcitrant structural P (lipid P) in leaves differed for species growing in P-poor sedimentary-derived soils and relatively P-rich igneous-derived soils in south-eastern Australia. Maintaining low leaf P concentrations resulted in reduced A_{sat_mass} but higher phosphorus-use efficiency in photosynthesis (PPUE). Ultimately, the savings in leaf P achieved by P reallocation away from lipids in leaves lowers species requirements for P uptake, so along with higher PPUE this may enhance the survival of these species on soils with very low P stocks and availability.

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Author contributions DSE conceived the project and acquired the funding; KRM, DSE, and EIER designed the study; EIER and DSE carried out the field measurements and collections; KRM, and EIER conducted the laboratory measurements; KRM, DSE, and EIER analysed the data. KRM wrote the first draft with input from DSE; all authors contributed to the subsequent versions of the manuscript. All authors have read and approved the submitted version of the manuscript.

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Data availability The data supporting the results and findings are openly available at https://doi.org/10.26183/0rsm-yz41 and indexed at Research Data Australia upon publication.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose. The funding organisation had no role in the design or implementation of the study, or in the decision to publish the results.

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