Review

New Phytologist

Research review

Phosphorus fractions in leaves

Author for correspondence:
Lalith D. B. Suriyagoda
Email: lalith.suriyagoda@agri.pdn.ac.lk; lalith.suriyagoda@uwa.edu.au

Received: *12 July 2022*
Accepted: *25 October 2022*

Lalith D. B. Suriyagoda[1,2], Megan H. Ryan[3,4], Clément E. Gille[2], Roberta L. C. Dayrell[2], Patrick M. Finnegan[2], Kosala Ranathunge[2], Dion Nicol[3,4,5] and Hans Lambers[2,4]

[1]Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya 20400, Sri Lanka; [2]School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia; [3]UWA School of Agriculture and Environment, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia; [4]Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia; [5]Department of Primary Industries and Regional Development, Western Australia, Dryland Research Institute, Merredin, WA 6415, Australia

New Phytologist (2023) **237:** 1122–1135
doi: 10.1111/nph.18588

Key words: crop improvement, organic phosphorus, phospholipids, phosphorus fractions, phosphorus-use efficiency.

Summary

Leaf phosphorus (P) comprises four major fractions: inorganic phosphate (P_i), nucleic acids, phospholipids, P-containing metabolites and a residual fraction. In this review paper, we investigated whether allocation of P fractions varies among groups of terrestrial vascular plants, and is indicative of a species' strategy to use P efficiently. We found that as leaf total P concentration increases, the P_i fraction increases the most, without a plateau, while other fractions plateau. Variability of the concentrations of leaf P fractions is greatest among families > species(family) > regions > plant life forms. The percentage of total P allocated to nucleic acid-P (20–35%) and lipid-P (14–34%) varies less among families/species. High photosynthetic P-use efficiency is associated with low concentrations of all P fractions, and preferential allocation of P to metabolite-P and mesophyll cells. Sequential resorption of P from senescing leaves starts with P_i, followed by metabolite-P, and then other organic P fractions. Allocation of P to leaf P fractions varies with season. Leaf phytate concentrations vary considerably among species, associated with variation in photosynthesis and defence. Plasticity of P allocation to its fractions is important for acclimation to low soil P availability, and species-specific P allocation is needed for co-occurrence with other species.

Introduction

Phosphorus (P) frequently limits plant productivity, and its availability in soil determines species distribution in many terrestrial ecosystems (Veneklaas *et al.*, 2012; Zemunik *et al.*, 2015). Molecules that contain P are involved in many metabolic processes (Bieleski, 1973). Based on their chemical structure, leaf P compounds are broadly divided into four chemical fractions: inorganic phosphate (P_i) and three organic P (P_o) fractions (small metabolites, nucleic acids and phospholipids), as well as a residual fraction of uncharacterised composition (Bieleski, 1973; Chapin III & Bieleski, 1982; Veneklaas *et al.*, 2012; Hidaka & Kitayama, 2013). The metabolically active P_i fraction is located in the cytoplasm within a narrow range of concentrations (Bieleski, 1968; Mimura *et al.*, 1996), and excess P_i is stored in the cell vacuole as a buffer to regulate [P_i] in the cytoplasm (Bieleski, 1968; Tachibana, 1987; Lee & Ratcliffe, 1993), or in other membrane-surrounded structures and organelles presently poorly characterised (Ryan *et al.*, 2019). Small metabolites represent low-molecular-weight P-esters, such as sugar phosphates and nucleotides (e.g. ATP and NAD(P)H). Inorganic P and small metabolites are sometimes reported together as the 'metabolic' P pool, which is inappropriate as only a fraction of the P_i is metabolically active (Bieleski, 1968; Veneklaas *et al.*, 2012). Nucleic acids are the major P_o fraction in leaves, of which up to 85% is RNA and the remainder is DNA (Bieleski, 1968; Tachibana, 1987; Matzek & Vitousek, 2009). Phospholipids are components of cellular membranes including endoplasmic reticulum, plasmalemma, Golgi apparatus and tonoplast, as well as membranes of the nucleus, mitochondria and chloroplasts (Jouhet *et al.*, 2004; Andersson

et al., 2005). The endoplasmic reticulum accounts for > 60% of phospholipid mass in a variety of cell types (Lagace & Ridgway, 2013). The residual-P fraction comprises P compounds that do not fall in any of the other four categories (Kedrowski, 1983). It includes chemically recalcitrant compounds that are not co-extracted with the above-mentioned organic compounds and likely represent mainly phosphorylated proteins. Variation in total leaf [P] among plant families and species, functional types and environments reflects differences in both the absolute concentrations of each P fraction and their relative proportions (Chapin III & Bieleski, 1982; Hidaka & Kitayama, 2011). However, we lack a conceptual framework to predict the contribution of ecological and phylogenetic factors, as well as their interaction, to the variability of total leaf P and each P fraction.

Exploring the variation in leaf P fractions among habitats, families, species and plant life forms is important to appreciate the diversity among plants in approaches to use P efficiently. General trends of leaf P fractions have been synthesised by Veneklaas *et al.* (2012) with a focus on possibilities to improve P-use efficiency in crops. Since then, the number of publications on leaf P fractions in the 'Web of Science' database has increased to 84% (Supporting Information Fig. S1), and new research avenues have been explored. As leaf P fractionation data from different plant life forms (i.e. trees and herbaceous plants, perennials and annuals), habitats, families and species are now available, new analyses with a broader focus and application are possible. For instance, information derived from coexisting species in their natural habitat could be used to understand the combinations of leaf P allocation that have been evolutionarily viable. Whether and how plants can partition leaf P into the four fractions to adjust physiological processes in response to P supply may help us understand the competitive ability of species when coexisting in their natural habitats and devise strategies towards more sustainable crop genotypes. Moreover, the relationships between leaf P fractions and leaf traits such as net photosynthetic rate (A_n), leaf mass per area (LMA), P-resorption efficiency (PRE, i.e. the percentage reduction in leaf P concentration in mature green leaves during senescence; Killingbeck, 1996) and photosynthetic P-use efficiency (PPUE, i.e. the rate of photosynthesis per unit leaf P) are poorly understood across habitats and species. These are some of the key variables that plant breeders might consider when aiming to improve the PUE of crops for sustainable agriculture.

Our objectives were to analyse published data to (1) identify correlations among the concentration of leaf P fractions, (2) assess the importance of the environment (i.e. region and habitat), plant life form and phylogeny (i.e. families and species) in determining leaf P fraction allocation patterns (i.e. the proportion of total P allocated to each fraction) and (3) explore associations between the concentrations of leaf P fractions and other leaf traits (i.e. A_n, LMA, PRE and PPUE) of terrestrial vascular plants. These analyses will increase our understanding of how plants adjust their allocation of total P to leaf P fractions and whether this is associated with other leaf traits. Finally, we explore the potential for leaf P fraction allocation traits to be used to improve PPUE in crops.

Materials and Methods

We compiled the literature on the concentrations of leaf P fractions for a variety of terrestrial plant species (Table S1). We considered studies presenting the concentrations of P fractions in the form of P_o and P_i, or P_o fractions as P in metabolites, nucleic acids, lipids and a residual fraction. Data were tabulated on country or region, family, species, life form, types of leaves used to measure P (i.e. mature or senesced), soil P status (i.e. deficient or sufficient, based on leaf [P]) and types and concentrations (mg P g^{-1} DW) of leaf P fractions measured (Tables S1, S2). Leaf total [P] and its fractions were expressed on a leaf dry weight (DW) basis in all studies. Soil [P] was not included, despite a wide variation, as this was not measured consistently, and thus not comparable across studies; it was used to compare P treatments within a study. Leaves of annuals had been collected from plants grown either in the field or in pots, with or without supplementary P (Table S1). Annuals were mostly crop species. Leaves of perennials had been collected from field-grown plants, mostly in their natural habitat, either from one field site or from several sites with different soil [P] (Table S1). Complete data set can be accessed through the doi: 10.26182/26rb-mv37.

When studying relationships among the concentrations of leaf P fractions, species mean values from different studies for both annuals and perennials were used (data sources are given in Tables S1, S2). This approach allowed generation of generic relationships among the concentrations of leaf P fractions without restriction to growth forms, habitats, families or species. Out of the linear and nonlinear models (logarithmic, polynomial and non-rectangular hyperbola), the model with the highest coefficient of determination (r^2) and least error variance was selected as the best-fitting model to explain the relationship between each pair of P fractions. When comparing the concentrations of leaf P fractions between annuals and perennials, data across P treatments (for annuals) or sites (for perennials) were used (Table S2). Perennials represented herbaceous perennials, shrubs, vines and trees from East Asia, Australia and New Zealand, Europe and North America. In a few instances, P fraction data were collected from plants grown in both low-P (i.e. P-deficient) and high-P (i.e. P-sufficient) field conditions (Table S2). Those data were used to compare the variation in leaf P fractions as dependent on soil P availability. Except for the comparison of P fractions in mature and senesced leaves (Table S2), all analyses were based on P fractions in mature green leaves. A regression analysis identified the best fit correlations between the concentrations of leaf P fractions and the strength of those correlations was determined using the coefficient of determination (r^2) (Fig. 1). The effect of region, family, species within each family and plant life form on the concentration of leaf P fractions (mg P g^{-1} DW) and on the percentage of total leaf P (%) allocated to each P fraction was tested using four-way analysis of variance (ANOVA) and log-linear models, respectively. An ANOVA was used to compare the concentration of leaf P fractions among annual and perennial plant species (Fig. 2), perennial plant families (Figs 3, 4), co-occurring species within the same family (Fig. 5) and green and senescing leaves (Fig. 6). The variability of leaf P concentrations and percentages of leaf P fractions among

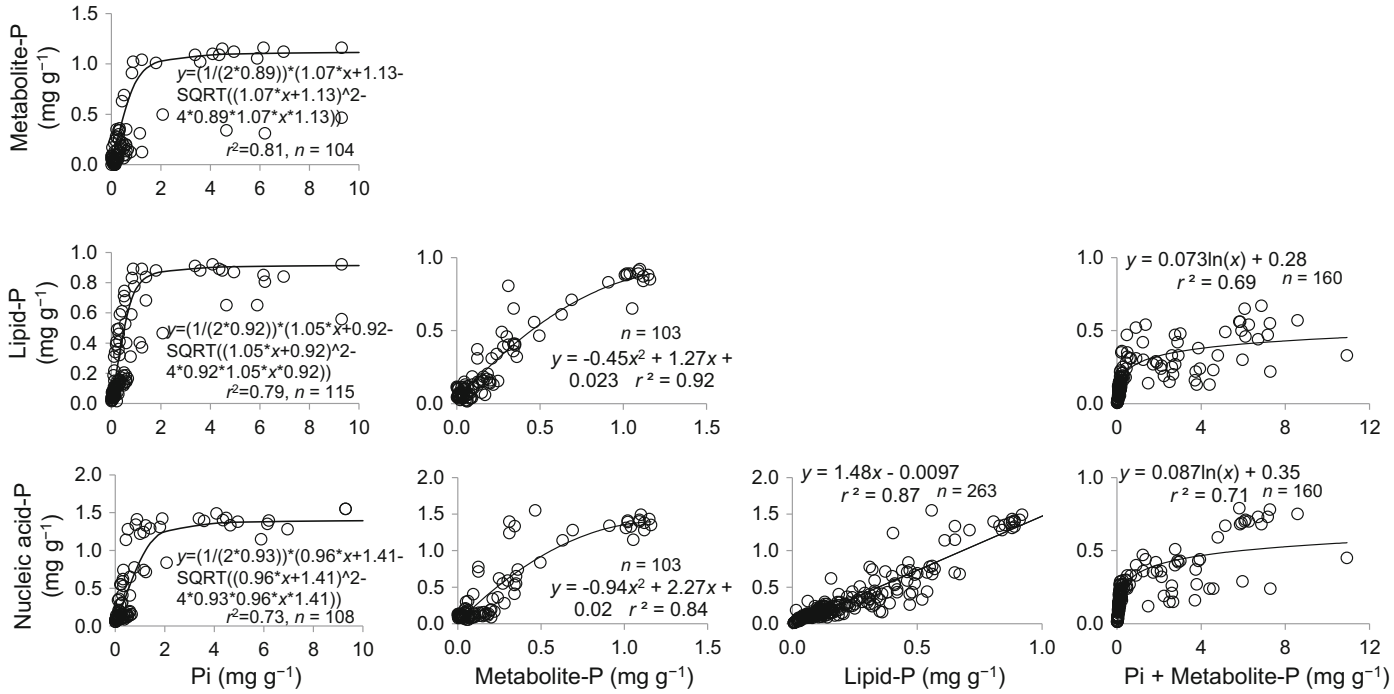

Fig. 1 Correlations between leaf inorganic phosphate (P_i), small metabolites containing phosphorus (P), phospholipids (lipid-P) and nucleic acid-P concentrations of species from numerous families. Data sources are given in Supporting Information Table S2. Each point represents a species mean value of a study. Pearson's correlation coefficient (r^2) and number of paired observations (n) are given. All the relationships were significant at $P < 0.05$ in the regression analysis. In some studies, P_i and metabolite-P concentrations were not separated, and these are presented in the panels at the far right. P-hyperaccumulating outlier species, *Ptilotus exaltatus* 'Joey' (Mulla Mulla) and *Kennedia prostrata* R.Br. from Ye *et al*. (2021), were not included. However, the four data points that deviate from the rest in the first column are *Hordeum leporinum* Link and *Hordeum vulgare* L. from Chapin III & Bieleski (1982).

plant families was determined using coefficients of variance (CV). All data were analysed using SAS software (SAS, 2003).

Relationships among leaf P fractions

The concentration of all leaf P fractions increases with increasing leaf total [P] (Fig. S2). While the sum of the P_o fractions reaches a plateau at < 3 mg P g^{-1} DW, $[P_i]$ continues to increase with increasing leaf total [P]. The maximum values of P_i, metabolite, lipid, nucleic acid and residual [P] are 16.5, 4.2, 2.05, 1.6 and 0.22 mg P g^{-1} DW, and the minimum values are 1×10^{-2}, 8×10^{-5}, 6×10^{-3}, 1.3×10^{-2} and 6.7×10^{-3} mg P g^{-1} DW, respectively. The correlations between lipid [P] and metabolite [P] (polynomial; $r^2 = 0.92$), lipid [P] and nucleic acid [P] (linear; $r^2 = 0.87$), and nucleic acid [P] and metabolite [P] (polynomial; $r^2 = 0.84$) are stronger than those between metabolite [P] and $[P_i]$ ($r^2 = 0.81$), lipid [P] and $[P_i]$ ($r^2 = 0.79$), and nucleic acid [P] and $[P_i]$ ($r^2 = 0.73$) (Fig. 1). Moreover, the relationships of metabolite [P], lipid [P] and nucleic acid [P] with $[P_i]$ were nonrectangular hyperbolic.

The correlations of P_i with metabolite-P, lipid-P and nucleic acid-P exhibit similar trends: with increasing P_i (or P_i + metabolite-P), there is a steep increase in the other fractions until these reach a plateau at which their concentration is mostly unaffected by $[P_i]$. This reflects the storage role of P_i when leaves are functioning at high total [P] with 'excess' P_i stored in vacuoles and only slowly released into the cytoplasm when required for cellular functions, such as photosynthesis and respiration (Bieleski, 1968, 1973; Mimura *et al*., 1990; Lee & Ratcliffe, 1993). This vacuolar P_i may be as much as two-thirds of leaf total [P] (Bieleski, 1973; Sinclair & Vadez, 2002). The $[P_i]$ in the cytosol of P-sufficient plants is commonly in the range of 5–10 mM (Bieleski, 1973). Therefore, during P deficiency, growth may be limited by the rate at which P is transported across the tonoplast into the cytoplasm and then to meristems.

The first-order polynomial correlations between metabolite [P] and lipid [P] and nucleic acid [P] show a rapid increase in lipid [P] and nucleic acid [P] initially which then stabilises as metabolite [P] increases further. Moreover, the rate of increase in nucleic acid [P] was greater than that in lipid [P]. This indicates that the allocation of P to nucleic acids was evolutionarily favoured over allocation to metabolite-P. There was also a linear correlation between nucleic acid [P] and lipid [P]. Therefore, overall results indicate that the nucleic acid [P] and lipid [P] fractions are the most conserved among the leaf P fractions.

The effect of region, plant life form, family and species on leaf P fractions

The four-way ANOVA showed that for the concentration of each leaf P fraction and total P, 'family' explains the largest proportion of the variability, followed by 'species within family', 'region' and 'plant life form', that is 30–66%, 6–15%, 1–20% and < 0.03%, respectively (Table 1). As most of the families studied were not present in all regions, an interaction between region × family or region × species (family) could not be examined.

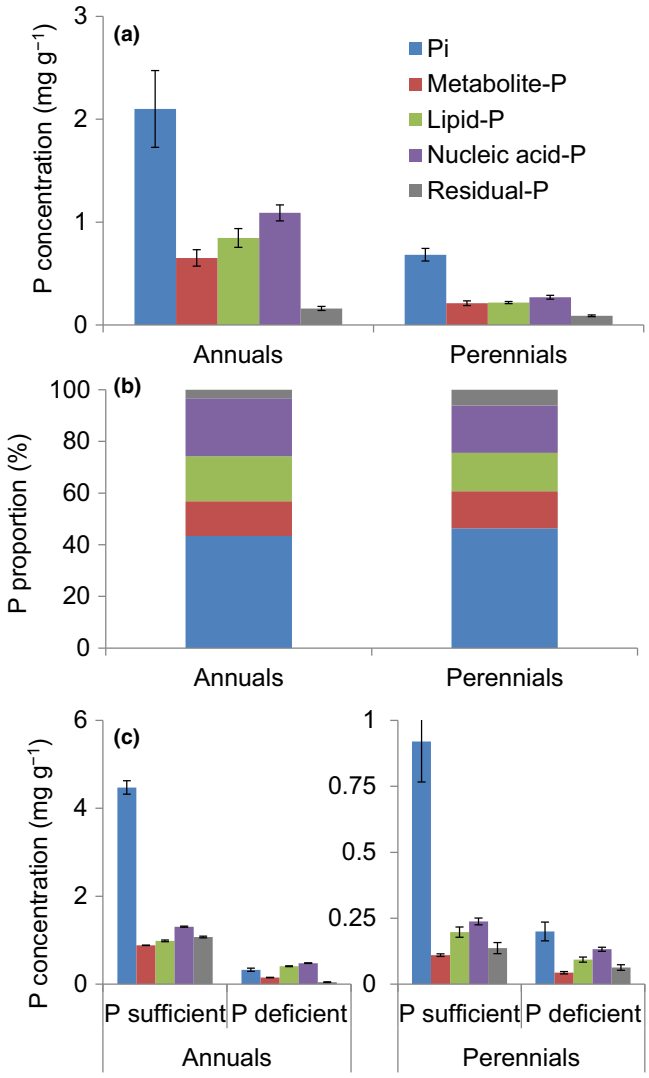

Fig. 2 Inorganic phosphate (P_i), small metabolites containing phosphorus (P) (metabolite-P), phospholipids (lipid-P), nucleic acid-P and residual-P in leaves expressed as concentrations (a, c) and as percentages of leaf total [P] (b), and compared between annual and perennial species (a–c) when growing in P-sufficient and P-deficient soils (c) (mean ± SE, $n > 34$). Source: studies summarised in Supporting Information Table S2.

Region

Looking at each of the factors included in ANOVA in turn, for 'region', we found that leaf total P, P_i and metabolite-P concentrations in plants from East Asia are higher than those in Australia and New Zealand, Europe and North America (Table S3). Region accounts for 19% of the variation of metabolite-P, but only 1% of the variation of lipid-P. Therefore, region is an important factor, but with contrasting explanation power for specific fractions.

Plant life form

For plant life form, we found that herbaceous perennials contain higher concentrations of leaf P fractions and leaf total P than other perennial plant life forms (i.e. shrubs, vines and trees) (Table S4). Moreover, it seems that the concentrations of leaf P fractions in herbaceous perennials are similar to those in annuals (Table S4). Both annuals and herbaceous perennials contain 46–47% of their leaf P as P_i, while shrubs and trees exhibit only 25–30% of leaf P as P_i (Table S4). In summary, leaf P allocation of herbaceous perennials resembles those of annuals, while shrubs and trees show a different pattern.

Additionally, we compared the P concentrations of annuals and perennials (ferns, herbaceous perennials, shrubs, trees and vines together). Annuals have higher leaf total [P] and P in their fractions than perennials (Fig. 2a), but the proportion of P allocated to each fraction (%) is similar; for example, nucleic acid-P represents the largest P_o fraction at 20 ± 3.2% of leaf total P and 33 ± 2.9% of leaf P_o, for both annuals and perennials (Fig. 2b). Despite growing under fertile soil conditions and at a fast growth rate, mature leaves of annuals maintain a similar proportional P allocation to that of perennials. It is important to note that the leaf dry matter content (DMC) of annuals is lower than that of perennials and in young expanding leaves than mature leaves (Lambers & Poorter, 1992). Therefore, the difference in P allocation between leaves of annuals and perennials as affected by leaf DMC needs to be explored further: DMC may explain why the differences disappear when expressed as percentages, rather than as absolute amounts (Fig. 2).

For annuals and perennials grown under P-deficient and P-sufficient conditions, annuals increase their [P_i] 14-fold and metabolite [P] sixfold under P-sufficient conditions, much more than the increase for perennials (fivefold and threefold, respectively; Fig. 2c). Similarly, under P-sufficient conditions, annuals increase lipid [P] and nucleic acid [P] 2.4- and 2.7-fold, while perennials increase 2.1- and 1.8-fold, respectively. Thus, annuals reach a higher [P_i] and [P_o], possibly to support faster metabolic activities than perennials and/or due to luxury consumption. Under P-deficient conditions, annuals reduce P in their fractions to a greater extent than perennials, possibly at the expense of maintaining metabolic activities (Tawaraya *et al.*, 2018). However, this conclusion has to be made in the context of the association of leaf [P] with other leaf traits (Lambers & Poorter, 1992). As Nicol & Ryan (2021) observed, annual and perennial pasture species exhibit higher leaf total [P] and [P_i] when grown under heavily P-fertilised conditions than the values reported for those species under low-P conditions (Fig. S3). In such comparisons, the high leaf total [P] and [P_i] in both annuals and short-lived perennials is likely due to the herbaceous nature and fast growth rate of both plant types. Organic P fractions occur at higher concentrations when plants are grown under P-sufficient conditions than P-deficient conditions (Fig. 2c); some P_o is hydrolysed as P becomes limiting for growth, in particular that contained in phospholipids (Tawaraya *et al.*, 2018).

There are few data on P fractions in annuals in comparison with perennials, and data include wild or crop species grown either in pots or in controlled environments (Tables S1, S2). Therefore, the rest of the discussion is based on data from perennials, and most of which were growing in their natural habitats without fertiliser application (Table S1).

Family

Leaf total [P] of perennial plant species representing different families shows large variation (Fig. 3). Plant family represents

New Phytologist

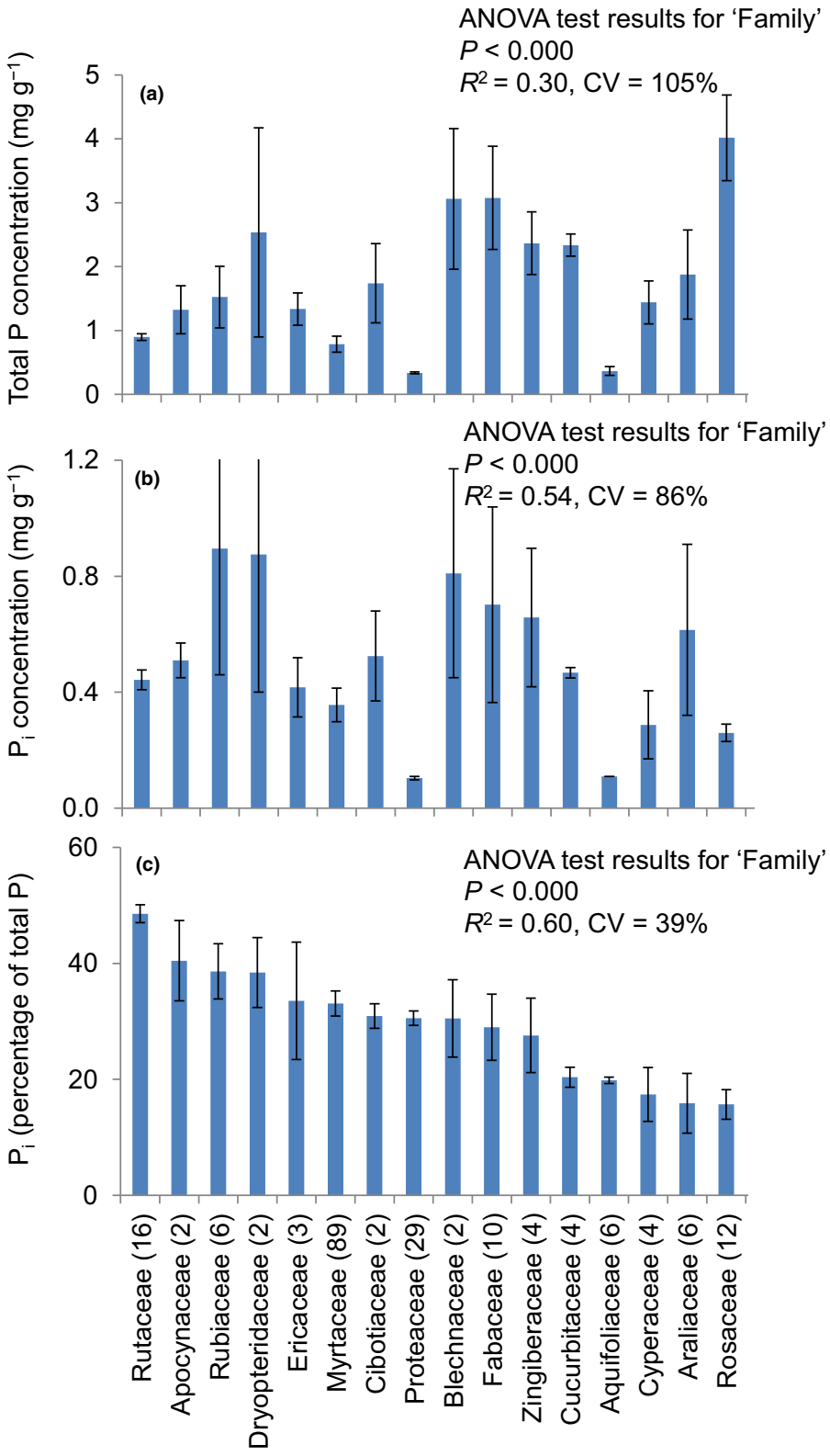

Fig. 3 Leaf total phosphorus (P) (a) and inorganic phosphate (P_i) (b) concentrations and the percentage of leaf P_i (c) for perennial plant species from 16 families (mean ± SE). Data across species and treatments were considered only when more than one value was found for a family. R^2, coefficient of determination; CV, coefficient of variance for the comparison among families in analysis of variance are also given. Values in parentheses following the family name are sample sizes. Source: studies summarised in Supporting Information Table S2.

only 30% of the variation in total [P] (R^2 = 0.30; coefficient of variance (CV) = 105%). Compared with total [P], [P_i] is more conserved within a family, which represents 54% of the variation (R^2 = 0.54; CV = 86%). Moreover, the percentage of [P_i] relative to leaf total [P] is even more conserved within a family than either factor alone (R^2 = 0.60; CV = 39%). Similar patterns appear in studies where both [P_i] and metabolite [P] were measured combined (Fig. S4).

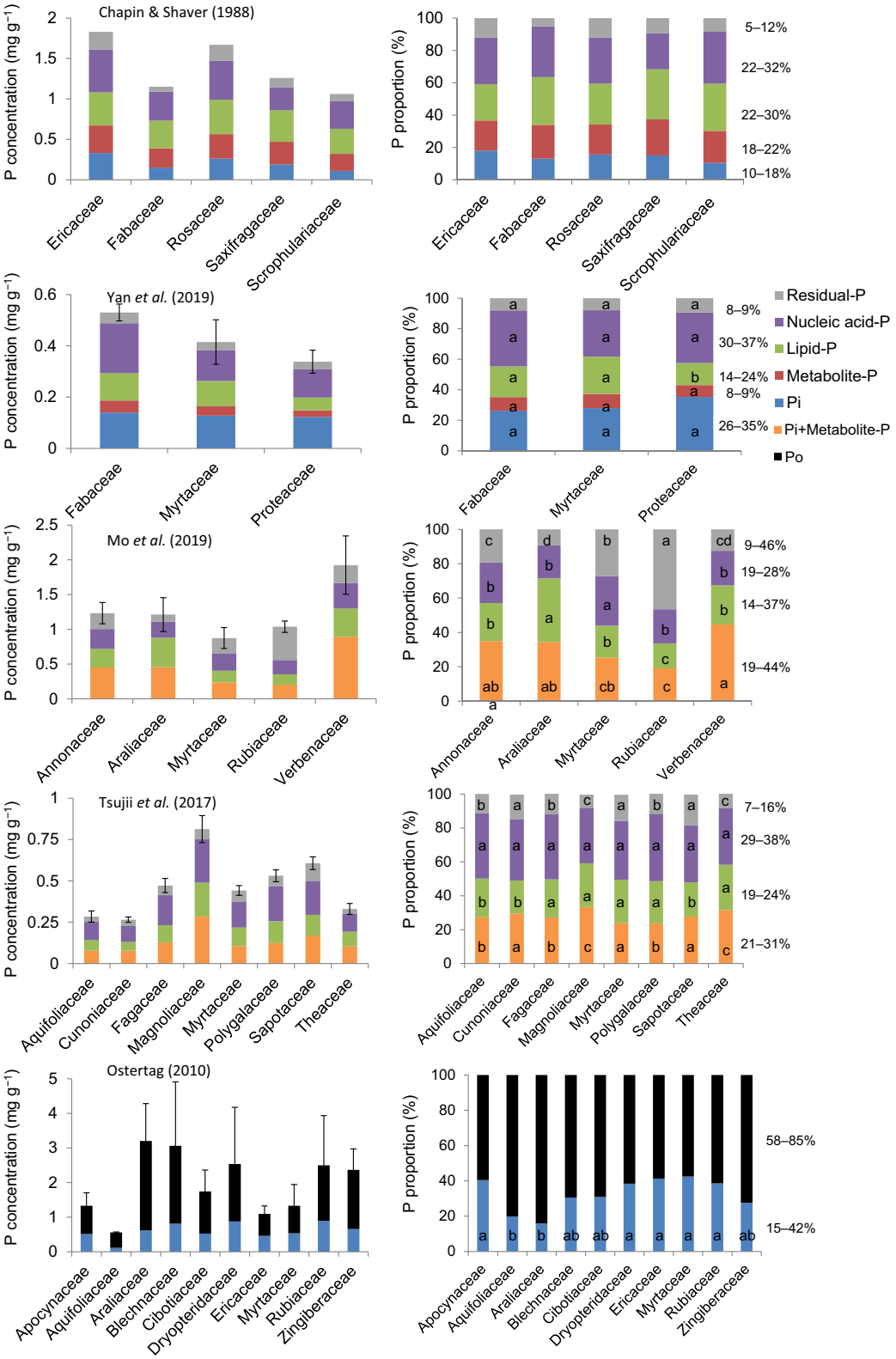

Fig. 4 Concentrations of metabolic (i.e. inorganic phosphate (P_i) + small metabolites containing phosphorus (metabolite-P)), phospholipids (lipid-P), nucleic acid-P and residual-P in leaves or P_i and organic P (P_o) concentrations (left column) and the percentage of each fraction relative to total leaf P (right column) of coexisting families as reported in five studies (mean ± SE). Different letters denote significant differences in proportion of leaf P in each fraction among families within a given study ($P < 0.05$) according to the chi-square test. Source: studies summarised in Supporting Information Table S2. Data collected from co-occurring species from multiple sites were averaged when producing bar charts using data from Yan *et al*. (2019), Mo *et al*. (2019), Tsujii *et al*. (2017) and Ostertag (2010).

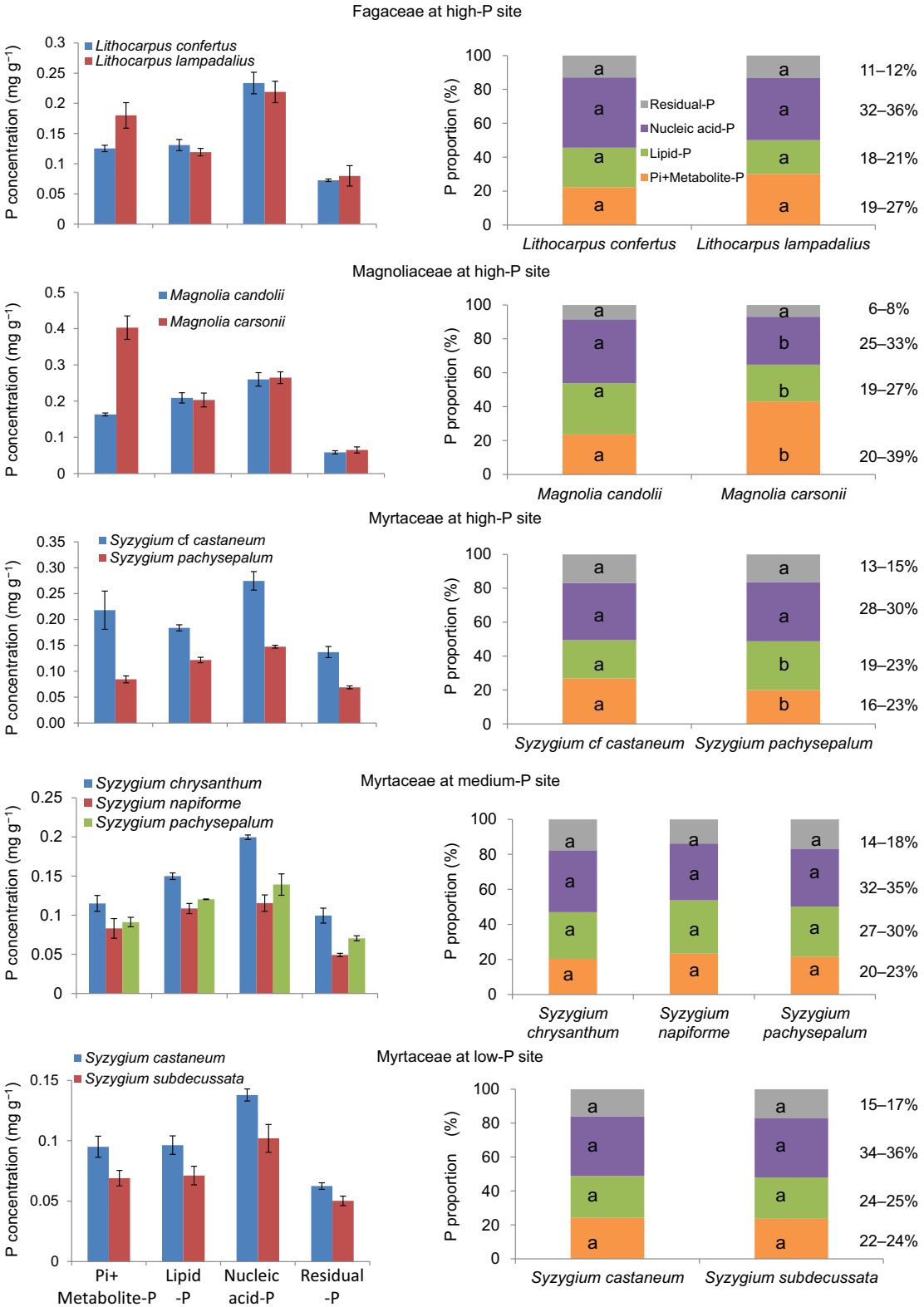

Fig. 5 Concentrations of metabolic (i.e. inorganic phosphate (P_i) + small phosphorus (P)-containing metabolites), phospholipid (lipid-P), nucleic acid and residual-P in leaves (left column) and the percentage of each fraction of total leaf P (right column) of co-occurring perennial plant species from three families as reported by Tsujii *et al*. (2017) (mean ± SE, $n = 3$). The soil soluble P concentrations, extracted with 0.03 M NH_4F/0.1 M HCl solution, are 0.02, 0.12 and 0.19 g m^{-2} in the topsoil for low-, medium- and high-P sites, respectively. According to Takyu *et al*. (2002), these soil P concentrations are equivalent to 0.44, 2.32 and 3.31 g P kg^{-1} dry soil, respectively. Different letters denote significant differences in proportion of leaf P in each fraction among species within a given study ($P < 0.05$) according to the chi-square test.

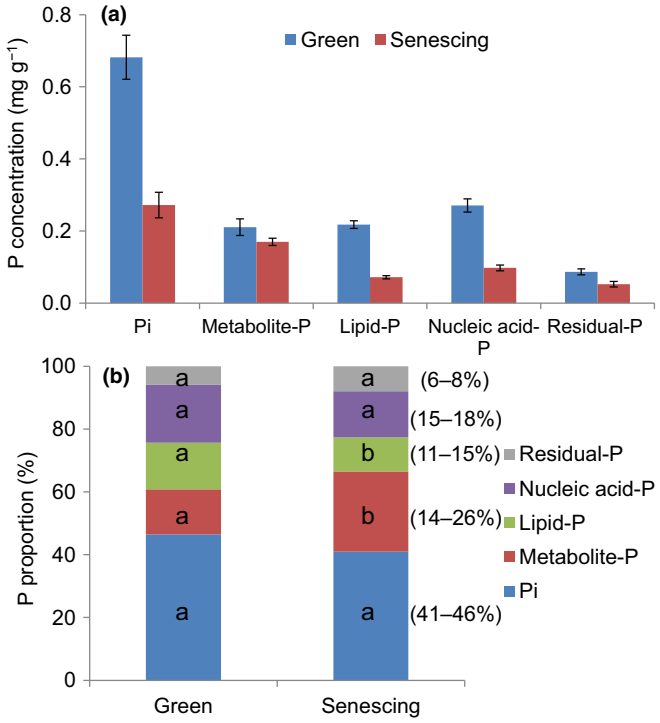

Fig. 6 Concentrations of inorganic phosphate (P_i), small metabolites containing P (metabolite-P), phospholipids (lipid-P), nucleic acid-P and residual-P (a) and the percentage of each fraction relative to total leaf P (b) in mature green leaves and senesced leaves of perennial plant species (mean ± SE, $n = 82$ and 35 for green and senesced leaves, respectively). Same letter within a leaf P fraction is statistically similar ($P > 0.05$) according to the chi-square test. Source: studies summarised in Supporting Information Table S2.

Among the families for which P_i data were available, Proteaceae from severely P-impoverished habitats in Australia, and Aquifoliaceae from nutrient-impoverished Hawaiian forests, show the lowest leaf total [P] (0.34–0.38 mg P g^{-1} DW) and [P_i] (0.10–0.12 mg P g^{-1} DW; Fig. 3). However, those families exhibit P_i values that are 20–31% of the total [P], which is in the range of that in other families (16–49%). Therefore, the variability of P among families included in this study decreases from leaf total [P] > [P_i] > percentage allocated to leaf P_i relative to total leaf P. It is important to note that for some families, only a limited number of samples (species) were available, and thus the relative position of those families in Fig. 3 may vary as more data become available.

Variation in leaf P allocation among coexisting taxa

To further our understanding of the role of region in influencing P allocation, we selected studies comparing families with only coexisting plant species and assessed whether the results on a global scale are consistent in studies from different regions.

The mean leaf total [P] across families varies widely, from 0.25 mg P g^{-1} DW for Cunoniaceae to 3.1 for Araliaceae (Fig. 4). The total [P] and [P] in the various leaf P fractions differs significantly among co-occurring families (Fig. 4; Table S5). For example, Ericaceae and Rosaceae exhibit a higher total [P] and [P] in each fraction than co-occurring species from other families in Alaskan tundra vegetation (Chapin III & Shaver, 1988). Fabaceae, Verbenaceae and Magnoliaceae show higher leaf total [P] and its fractions than co-occurring species in a Mediterranean biodiversity hotspot in south-western Australia (Yan *et al.*, 2019), a tropical coastal ecosystem in Guangdong province in China (Mo *et al.*, 2019) and tropical forests in Mount Kinabalu, Borneo (Tsujii *et al.*, 2017), respectively. Species in families with higher leaf total [P] tended to retain most P in P_i and metabolite-P (Fig. 4; Table S5). Despite the large variability in leaf total [P] and [P] in the various leaf P fractions, the percentage of P allocated to those fractions is less variable among the families tested. Myrtaceae sampled in four of five studies show that the percentage of P allocated to nucleic acids (29–32%) and lipids (19–24%) is a conserved trait in this family.

We calculated the variability of each P fraction among the families (Table S6). While the concentrations of P_i, metabolite-P and residual-P fractions are highly variable among families, the percentage of P allocated to nucleic acids and lipids is more conserved (CV = 18 and 25%, respectively; Table S6). The percentages of leaf P as nucleic acid-P and lipid-P across families vary by 20–35% and 14–34%, respectively. Moreover, the allocation of P to total P_o is 25–55% to nucleic acids, 20–44% to phospholipids and 6–35% to the residual-P fraction. Overall, these results reveal a large variability in leaf total [P] and its fractions among co-occurring families (Tables S5, S6), but the percentage of P allocated to the P-containing organic fractions, particularly nucleic acids and lipids, is less variable and hence more conserved.

So far, only one study tested whether co-occurring congeneric species within a family have similarities of P allocation in sites with different soil P availability; in this case, high, medium and low availability (Tsujii *et al.*, 2017). At the high-P site (Bray II topsoil

Table 1 Percentage of total variability of the concentration of leaf phosphorus (P) fractions and leaf total P explained by a four-way analysis of variance (ANOVA) with the factors of region (Australia and New Zealand, East Asia, Europe and North America), plant life form (annuals, ferns, herbaceous perennials, shrubs, trees and vines), family and species (family).

Source	df	P_i (%)	Metabolite P (%)	Lipid P (%)	Nucleic acid P (%)	Residual P	Total-P (%)
Region	3	3.85	19.41	1.36	6.93	8.61	7.18
Plant life form	5	0.03	0.00	0.00	0.00	0.00	0.02
Family	34	30.22	29.94	56.28	63.73	66.17	48.19
Species (Family)	28	13.40	9.69	15.00	9.54	5.88	10.19
Error	241	52.50	40.96	27.36	19.80	19.34	34.42
R^2		0.48	0.59	0.72	0.80	0.80	0.65

P = 0.19 g m^{-2}), two species from each of Fagaceae, Magnoliaceae and Myrtaceae were present (Fig. 5). *Lithocarpus lampadalius* (Gamble) A. Camus (Fagaceae) and *Magnolia carsonii* Dandy ex Noot. (Magnoliaceae) have a higher metabolic [P] (i.e. P_i + metabolite-P) than co-occurring species from the same family, despite having similar lipid [P], nucleic acid [P] and residual [P] (Fig. 5; Table S7). *Syzygium* cf. *castaneum* (Merr.) Merr. & L.M. Perry. (Myrtaceae) exhibits higher concentrations of all P fractions than its counterpart from the same family. Consistent with earlier comments, species with higher leaf total [P] contain more P in P_i and metabolite-P fractions (Fig. 5; Table S7). At both the medium- and low-P sites (Bray II topsoil P = 0.12 and 0.02 g m^{-2}, respectively), there are significant differences in leaf total [P] and its fractions among Myrtaceae (Fig. 5; Table S7). Based on the limited data, we suggest that the leaf P allocation pattern of co-occurring species within the same family is different and species-specific. However, as more data from diverse environments and plant functional types become available, generic conclusions should be possible in regard to the concentration of P fractions of co-occurring species of a family and species × region interactions. Moreover, as shown in Fig. 4 for co-occurring families, the percentage of P allocated to each of P fractions is less variable for congeneric species, despite a large variability in leaf total [P] and [P] in the various leaf P fractions. Understanding the mechanism and signification of this variation is important when screening varieties with the aim of improving PUE in crops.

Plant traits

Changes in A_n and PPUE

Mass-based A_n is positively correlated with leaf total [P] (Veneklaas *et al.*, 2012). However, the relationship between A_n and leaf P fractions has not been widely explored. Hidaka & Kitayama (2013) measured leaf A_n of 10 tree species from a tropical montane rainforest and found that A_n is positively correlated with leaf total [P] and metabolic [P] (i.e. P_i + metabolite-P) across species. Moreover, Hidaka & Kitayama (2011, 2013) noted a strong correlation between metabolic [P] and nucleic acid [P]. Our analysis across environments and families extends this relationship more generally ($r = 0.80$; $P < 0.001$) (Fig. 1). Overall, tree species on P-poor soils appear to function at low levels of both metabolic-P and nucleic acid-P.

Several mechanisms allow a high PPUE in plants: (1) preferential allocation of P among leaf P fractions, for example greater allocation to metabolite-P; (2) preferential allocation of P among leaf tissues, for example greater P allocation to photosynthetically active mesophyll cells; (3) the net effect of a lower concentration of each leaf P fraction; and (4) various combinations of the above possibilities. In all these scenarios, the relative reduction in A_n is smaller than the reduction in leaf [P] under low soil P availability (Lambers *et al.*, 2012; Sulpice *et al.*, 2014; Mo *et al.*, 2019). The net outcome, then, is a higher PPUE.

Supporting the hypothesis that a high PPUE can arise from a more efficient distribution of P among leaf P fractions, tree species from tropical montane rainforests with high PPUE had a relatively greater investment of P in P-containing metabolites and a relatively smaller investment in phospholipids (i.e. higher metabolic [P] to lipid [P] ratio) than those with lower PPUE (Hidaka & Kitayama, 2013). In another example, Proteaceae that grow on severely P-impoverished soils function at very low leaf rRNA levels (i.e. low nucleic acid-P), but maintain fast A_n (Sulpice *et al.*, 2014), which is a main reason for their exceptionally high PPUE (Denton *et al.*, 2007; Lambers *et al.*, 2012; Sulpice *et al.*, 2014). With this supporting evidence from a limited number of species, the hypothesis that greater allocation of P to P-containing metabolites helps maintain A_n now needs to be tested for a wider range of species or genotypes within a species.

Eudicots from severely P-impoverished environments in Australia, South Africa and Brazil preferentially allocate P to their photosynthetically active mesophyll cells (Lambers *et al.*, 2015; Hayes *et al.*, 2018; Guilherme Pereira *et al.*, 2019; Ye *et al.*, 2021). However, eudicots inhabiting other environments, such as P-rich soils, either preferentially allocate P to epidermal cells (Conn & Gilliham, 2010; Hayes *et al.*, 2018; Guilherme Pereira *et al.*, 2019) or exhibit a lack of preferential allocation, for example the P-hyperaccumulating *Ptilotus exaltatus* (Amaranthaceae) (Ye *et al.*, 2021). Preferential allocation of P to mesophyll cells allows plants to efficiently use P for photosynthesis, which occurs in mesophyll cells, but not in epidermal cells (with the exception of guard cells). Thus, preferential P allocation contributes to a high PPUE.

Species of *Banksia* and *Hakea* (Proteaceae) substitute phospholipids with lipids that do not contain P, such as galactolipids and sulfolipids, during leaf development, which contributes to their high PPUE even under low leaf total [P] (Lambers *et al.*, 2012; Kuppusamy *et al.*, 2014). Phosphorus substitution in lipids is indicated by gene expression profiling during grain filling of rice (*Oryza sativa* L.) at low-P availability (Jeong *et al.*, 2017; Hayes *et al.*, 2022). Therefore, plants that adjust and/or substitute leaf P fractions, and preferentially allocate P to photosynthetic tissues, exhibit a high PPUE in low-P soils.

Resorption of P fractions during leaf senescence

Plants resorb P from senescing leaves before abscission. The amount of P resorbed largely depends on soil P status and species. Phosphorus-resorption efficiency (PRE) is defined as the percentage of P resorbed from senescing leaves before abscission relative to the amount in mature green leaves (Killingbeck, 1996). Phosphorus-resorption efficiency increases with decreasing soil P availability (Hidaka & Kitayama, 2011; Reed *et al.*, 2012; Hayes *et al.*, 2014; Suriyagoda *et al.*, 2017; Guilherme Pereira *et al.*, 2019). The global average of PRE is *c.* 50–60% in evergreen angiosperms (Yuan & Chen, 2009; Vergutz *et al.*, 2012), while PRE can exceed 80% in some species native to very low-P habitats (Denton *et al.*, 2007; Hidaka & Kitayama, 2011; Lambers *et al.*, 2015; Suriyagoda *et al.*, 2017; Tsujii *et al.*, 2017; Hayes *et al.*, 2018). However, so far, little is known about the biochemical mechanisms underlying this greater PRE and the types of P fractions resorbed.

Leaf P fractions differ in the extent to which they can be remobilised, for example soluble P forms such as P_i can easily be

resorbed, before the degradation of P_o (Ostertag, 2010; Hidaka & Kitayama, 2011). Mao *et al.* (2015) found that PRE increases with an increasing ratio of P_i to P_o in mature leaves. Phosphorus-resorption efficiency also varies among tissues and families, for example *Templetonia retusa* (Vent.) R.Br. (Fabaceae) preferentially allocates P to its upper epidermis and shows a low PRE (Guilherme Pereira *et al.*, 2018), while Proteaceae that preferentially allocate P to mesophyll cells exhibit higher PRE (Hayes *et al.*, 2018). However, it cannot be generalised that PRE is high due to P allocation to mesophyll cells, as *Hordeum vulgare* L. (Poaceae) shows low PRE despite allocating P to its mesophyll (Dietz *et al.*, 1992). It is clear that resorption of P from different fractions and from different leaf tissues and families needs to be studied further.

Phosphorus-resorption efficiency often exceeds the proportion of soluble P, especially for plants grown in low-P soils (Denton *et al.*, 2007; Hidaka & Kitayama, 2011; Tsujii *et al.*, 2017). For example, Tsujii *et al.* (2017) tested the PRE for metabolic, lipid, nucleic acid and residual-P fractions of 14 species from eight families growing at two sites differing in soil P availability. At the higher-P site, average PRE for metabolic, lipid, nucleic acid and residual-P pools was 52%, 65%, 56% and 32%, respectively, while at the lower-P site, average PRE was 74%, 86%, 79% and 70%, respectively. Similarly, in *Eriophorum vaginatum* L. (Cyperaceae) in Alaskan tussock tundra, all P_o fractions in leaf blades decreased to a similar extent during senescence, showing that retranslocation of P from leaves was independent of the nature of different P fractions (Chapin III *et al.*, 1986). Therefore, the amount of P resorbed before leaf abscission in perennials exceeds that of P_i in mature leaves, confirming those species that achieve a high PRE do so by remobilising P from P_o fractions in addition to P_i. This conclusion is further supported by the observation that genes related to the remobilisation of P_i are upregulated before the breakdown of P_o fractions in rice leaves (Jeong *et al.*, 2017). These observations suggest that species inhabiting P-poor environments have higher PRE due to an ability to degrade a greater proportion of P_o and/or a greater capacity to transport P_i from the Po that is degraded.

Nucleic acid-P and lipid-P are converted to metabolite-P and then to P_i before being translocated from leaves and resorbed. Data from mature green and senesced leaves of over 25 perennial plant species from Alaska, Australia and Indonesia show that, on average, 74% of nucleic acid-P, 80% of lipid-P, 47% of residual-P and 44% of P_i are resorbed (Fig. 6; Table S2). Despite the P_i fraction being expected to be preferentially resorbed due to its higher mobility, its percentage resorption is lower than that of the nucleic acid and lipid-P fractions (Fig. 6). Moreover, the metabolite-P fraction in senesced leaves increased. This does not mean that P_i and metabolite-P are resorbed less from senescing tissues. The amount of P_i and metabolite-P can increase during the degradation of P_o in senescent tissues (Chapin III & Kedrowski, 1983; Tsujii *et al.*, 2017). Therefore, such transient degradation products likely result in lower net resorption of P_i and increased metabolite [P]. Trees on P-rich soils may allocate excess P to soluble P fractions (Fig. 2) and to epidermal cells, and this excess P may remain in senesced leaves (Guilherme Pereira *et al.*, 2019). In general, P resorption from the residual fraction is smaller than that from the nucleic acid and lipid fractions (Fig. 6). The residual-P fraction most likely contains phosphorylated proteins and is retained in senesced leaves more than any other P fraction. Nevertheless, species with greater PRE demonstrate a high percentage resorption from this fraction, similar in magnitude to that of the other fractions (Tsujii *et al.*, 2017). Therefore, the percentage P resorbed from residual-P is dependent on soil P availability.

According to Tsujii *et al.* (2017), variation in PRE in perennial tree species from Mount Kinabalu, Borneo is accounted for by both genus (i.e. phylogeny) and site (i.e. soil P availability). The variation in P resorption from each P fraction is in part due to unique contributions of genus (25–43%) and site (20–37%), and the interaction of genus and site (6–24%). Overall, available results indicate that plants selectively degrade organic compounds depending on soil P availability, making this a key mechanism underlying variation in PRE. However, more data across plant functional types with different PRE are needed to gain a better understanding of the degradation of leaf P fractions under different conditions of P availability as differential resorption of particular P fractions is not known. The variation of P resorption from different leaf tissues such as mesophyll cells and epidermal cells also needs to be further explored to broaden our understanding with more plant functional types, species and a broader range of soil P availability.

Seasonal variation and variation during leaf development

Chapin III & Kedrowski (1983) studied seasonal changes in P-containing chemical fractions in the leaves of deciduous (larch – *Larix laricina* (Du Roi) K. Koch, birch – *Betula papyrifera* Marshall and alder – *Alnus crispa* Ehrh. K.Koch) and evergreen (black spruce – *Picea mariana* Mill.) Alaskan tree species. They found no important differences in patterns of P distribution among the major chemical fractions in the leaves of deciduous trees. In deciduous species, concentrations of all P fractions are highest in mature green leaves and decline throughout the season, first as they are diluted by increasing leaf biomass, and later as Po fractions are hydrolysed and P_i retranslocated out of mature leaves. The quantities of nucleic acids and phospholipids hydrolysed in autumn are 40–47% and 26–38%, respectively, of the total P retranslocated from leaves of deciduous species before abscission. In buds and stems, P found during winter primarily comprises metabolite-P, phospholipid-P and nucleic acid-P, and this Po pool is converted into P_i in spring. In the evergreen species, P is present in the same types of compounds as in deciduous species, but the P in leaves involves no winter translocation to stems as in deciduous species. Similarly, Hellin & Alcaraz (1980) studied the changes in leaf total [P] and its fractions in lemon (*Citrus limon* L.; Rutaceae) leaves over the course of 1 yr and found that leaf total P, Pi, lipid-P and metabolite-P concentrations decline during spring, and then increase from late summer into autumn and winter. However, nucleic acid [P] does not show remarkable differences among seasons. Chapin III *et al.* (1986) observed similar results in *E. vaginatum* (Cyperaceae) in an Alaskan tussock tundra. The declined concentrations of leaf P fractions in spring would be due to remobilisation of P from existing leaves to drive new leaf

growth, and an increase in the lipid-P fraction from autumn to winter reflects its role in cold hardiness (Chapin III *et al.*, 1986).

Nucleic acid [P] is generally high in young actively growing leaves and stems, where rates of protein synthesis are fast (Brady, 1973). Phospholipids and metabolite-P (possibly phytate) are apparently the overwintering forms of P, as they increase in autumn and decline in spring. Similar changes in lipid-P occur in older stems and roots (Siminovitch *et al.*, 1968). Overall, perennials exhibit changing P forms and locations of storage depending on environment. This seasonal variation in P fractions must be considered when collecting leaf samples for P fraction studies and making comparisons among plant life forms (e.g. annuals vs perennials or deciduous vs evergreen).

Proteaceae species from severely P-impoverished habitats spread investment of P in rRNA during leaf development (Sulpice *et al.*, 2014). Compared with *Arabidopsis thaliana* L., immature leaves of Proteaceae contain low levels of rRNA, especially plastidic rRNA. Accordingly, Proteaceae show a delayed development of their photosynthetic apparatus with young leaves having low levels of Rubisco and chlorophyll ('delayed greening'). Sulpice *et al.* (2014) showed that low ribosome abundance contributes to the high PPUE of Proteaceae by investing less P in ribosomes and maintaining low abundance of plastidic ribosomes in young leaves and of cytosolic ribosomes in mature leaves, thus spreading investment of P in rRNA.

Apart from seasonal and leaf development-dependent variation in leaf P fractions, diurnal variation in leaf P fractions, particularly in photosynthetically active leaves, can also be expected, because A_n is positively correlated with metabolic [P] and nucleic acid [P] (Hidaka & Kitayama, 2011, 2013). Both leaf total [P] and [P_i] in cotton (*Gossypium hirsutum* L.) and white clover (*Trifolium repens* L.) increase from morning to evening and decline during the night (Phillis & Mason, 1942; Hart & Jessop, 1984). It is, however, not yet clear whether the reduction in [P_i] is due to the formation of other P-containing compounds or export of P_i from the leaves via the phloem, or both.

Phytate-P in leaves

One of the adaptations to regulate [Pi] in the cytoplasm is to synthesise *myo*-inositol hexakisphosphate, phytate (Strother, 1980). Phytate is a small P-rich compound that functions as the major storage compound (up to 90%) of P in seeds (Raboy, 2003). It accumulates during seed development and is enzymatically hydrolysed to release P_i during germination. Phytate also accumulates in other plant organs, such as pollen, roots, tubers, stems and leaves (Reddy *et al.*, 1982; Alkarawi & Zotz, 2014a,b; Takagi *et al.*, 2020) where it also serves to store P (Raboy, 2003).

Alkarawi & Zotz (2014a) reviewed the literature for phytate in green leaves. They found that phytate-P of 35 plant species accounts for 1–27% of total P (average, 8%; median, 5%). These values are much lower than those for seeds (Madsen & Brinch-Pedersen, 2020). At low concentrations, phytic acid is involved in cellular signal transduction in eukaryotic cells (Kumar *et al.*, 2021). However, the above concentrations are too high to fully account for a signalling function. Phytate occurs in leaves in many plant families (Table S8), and its concentration ranges from 0.2% of total P in Poaceae to 14.2% in Gnetaceae. Despite this high concentration of phytate in leaves of certain species, its role is largely unknown. Recently, Takagi *et al.* (2020) showed that an excessive P_i supply increases the cytosolic sugar phosphate concentration and activates phytate synthesis, decreasing leaf A_n and disrupting the reactive oxygen species defence system. More studies are required to assess the effect of phytate on photosynthesis in other species.

Although phytate concentrations increase with increasing leaf total [P], there is a negative correlation between the proportion of total P in the form of phytate and leaf total [P] in *Manihot esculenta* Crantz (Euphorbiaceae) and *Taraxacum officinale* (L.) Weber ex F.H.Wigg (Asteraceae) (Alkarawi & Zotz, 2014a,b). A positive correlation can be explained by the fact that phytate synthesis usually starts when the supply of P exceeds the requirement of basic plant metabolism when no other sinks for P exist (Bieleski, 1973). We do not know if the trend towards lower proportions of phytate-P with increasing leaf total [P] is a general phenomenon, and this deserves further studies.

Conclusions

While the concentrations of leaf total [P] and its fractions are highly variable among families and species, the proportion of P allocated to each fraction is less variable, especially for nucleic acid-P and lipid-P. Moreover, species-specific P allocation patterns are evident (e.g. P allocation to specific leaf tissues and changes during leaf development). Despite these variations, there are significant positive correlations in concentrations among the five leaf P fractions. The variability of concentration of leaf P fractions decreases in the order of families > species(family) > region > plant life form. When annual and perennial plant species are compared, the percentage of P allocated to the leaf P fractions is similar, despite the absolute concentrations being higher in annuals. All P fractions can be resorbed from senescing leaves, including residual-P. Temporal (i.e. seasonal and diurnal) fluctuations of leaf P fractions may allow leaves to respond to environmental changes.

When expressed on a DW basis, leaf [P] is affected by leaf DMC. Most studies do not report this trait, but it may have affected the comparisons made in this paper among species and families. This highlights the importance of presenting leaf [P] in relation to leaf DW, leaf area, leaf fresh weight (FW), leaf [N] or together with specific leaf area and leaf DMC (Sulpice *et al.*, 2014).

Whereas P fraction data are relatively abundant for perennial plant species, there is limited information on annuals and crop species. Therefore, comparisons lack the robustness required to offer a clear scheme of differences in P allocation among plant functional types. Moreover, changes in leaf P fractions during leaf development and seasonal and diurnal variation need to be further explored. As leaf morphological and physiological adaptations enhancing PRE, PUE and PPUE have been explored widely, genetic analyses are needed to identify genes responsible for such adaptations, both in annuals and in perennials. This would pave the way towards more P-efficient crops.

Acknowledgements

Funding received from the Endeavour Leadership Program to LDBS under grant number 7178-2019, Australian Research Council Discovery Grants, DP130100005 to HL, and DP200101013 to HL and PMF is greatly acknowledged. HL also acknowledges support from the Deputy Vice Chancellor Research at the University of Western Australia. The authors also thank anonymous reviewers for their constructive comments to improve an earlier version of the manuscript.

Competing interests

None declared.

Author contributions

LDBS, MHR and HL developed the structure of the manuscript. LDBS compiled the literature, analysed the data and developed the initial draft. MHR, CEG, RLCD, PMF, KR, DN and HL contributed to the writing and improvement of the manuscript.

ORCID

Roberta L. C. Dayrell https://orcid.org/0000-0002-4770-9100
Patrick M. Finnegan https://orcid.org/0000-0001-5021-1138
Clément E. Gille https://orcid.org/0000-0003-3890-0402
Hans Lambers https://orcid.org/0000-0002-4118-2272
Kosala Ranathunge https://orcid.org/0000-0003-2826-9936
Megan H. Ryan https://orcid.org/0000-0003-0749-0199
Lalith D. B. Suriyagoda https://orcid.org/0000-0002-5253-2717

Data availability

Complete data set is now available at doi: 10.26182/26rb-mv37.

References

Alkarawi HH, Zotz G. 2014a. Phytic acid in green leaves. *Plant Biology* **16**: 697–701.

Alkarawi HH, Zotz G. 2014b. Phytic acid in green leaves of herbaceous plants-temporal variation *in situ* and response to different nitrogen/phosphorus fertilizing regimes. *AoB Plants* **6**: plu048.

Andersson MX, Larsson KE, Tjellström H, Liljenberg C, Sandelius AS. 2005. Phosphate-limited oat: the plasma membrane and the tonoplast as major targets for phospholipid-to-glycolipid replacement and stimulation of phospholipases in the plasma membrane. *Journal of Biological Chemistry* **280**: 27578–27586.

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.

Brady CJ. 1973. Ch. 24, Changes accompanying growth and senescence and effect of physiological stress. In: Butler GW, Bailey RW, eds. *Chemistry and biochemistry of herbage, vol. 2.* London, UK: Academic Press, 317–351.

Chapin FS III, Bieleski RL. 1982. Mild phosphorus stress in barley and a related low-phosphorus-adapted barleygrass: phosphorus fractions and phosphate absorption in relation to growth. *Physiologia Plantarum* **54**: 309–317.

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

Chapin FS III, Shaver GR. 1988. Differences in carbon and nutrient fractions among arctic growth forms. *Oecologia* **77**: 506–514.

Chapin FS III, Shaver GR, Kedrowski RA. 1986. Environmental controls over carbon, nitrogen and phosphorus fractions in *Eriophorum vaginatum* in Alaskan tussock tundra. *Journal of Ecology* **74**: 167–195.

Conn S, Gilliham M. 2010. Comparative physiology of elemental distributions in plants. *Annals of Botany* **105**: 1081–1102.

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.

Dietz KJ, Schramm M, Betz M, Busch H, Dürr C, Martinoia E. 1992. Characterization of the epidermis from barley primary leaves. *Planta* **187**: 425–430.

Guilherme Pereira C, Clode PL, Oliveira RS, Lambers H. 2018. Eudicots from severely phosphorus-impoverished environments preferentially allocate phosphorus to their mesophyll. *New Phytologist* **218**: 959–973.

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

Hart AL, Jessop D. 1984. Diurnal variation in starch and inorganic phosphate concentrations in leaves of white clover and lotus. *New Zealand Journal of Agricultural Research* **27**: 1–3.

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, 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.

Hellin E, Alcaraz CF. 1980. Influence of manganese deficiency on phosphorus fractions in lemon leaves. *Journal of Plant Nutrition* **2**: 323–333.

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.

Jeong K, Julia CC, Waters DLE, Pantoja O, Wissuwa M, Sigrid H, Liu L, Rose TJ. 2017. Remobilisation of phosphorus fractions in rice flag leaves during grain filling: implications for photosynthesis and grain yields. *PLoS ONE* **12**: e0187521.

Jouhet J, Maréchal E, Baldan B, Bligny R, Joyard J, Block MA. 2004. Phosphate deprivation induces transfer of DGDG galactolipid from chloroplast to mitochondria. *Journal of Cell Biology* **167**: 863–874.

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

Killingbeck KT. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecology* **77**: 1716–1727.

Kumar A, Singh B, Raigond P, Sahu C, Mishra UN, Sharma S, Lal MK. 2021. Phytic acid: blessing in disguise, a prime compound required for both plant and human nutrition. *Food Research International* **142**: 110193.

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.

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

Lambers H, Cawthray GR, Giavalisco P, Kuo J, Laliberte E, Pearse SJ, Scheible WR, 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, Finnegan PM, Jost R, Plaxton WC, Shane MW, Stitt M. 2015. Phosphorus nutrition in Proteaceae and beyond. *Nature Plants* **1**: 15109.

Lambers H, Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* **34**: 187–261.

Lee RB, Ratcliffe RG. 1993. Subcellular distribution of inorganic phosphate, and levels of nucleoside triphosphate, in mature maize roots at low external phosphate concentrations: measurements with ^{31}P-NMR. *Journal of Experimental Botany* **44**: 587–598.

Madsen CK, Brinch-Pedersen H. 2020. Globoids and phytase: the mineral storage and release system in seeds. *International Journal of Molecular Sciences* **21**: 7519.

Mao R, Zeng D-H, Zhang X-H, Song C-C. 2015. Responses of plant nutrient resorption to phosphorus addition in freshwater marsh of North-east China. *Scientific Reports* **5**: 8097.

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.

Mimura T, Dietz KJ, Kaiser W, Schramm MJ, Kaiser G, Heber U. 1990. Phosphate transport across biomembranes and cytosolic phosphate homeostasis in barley leaves. *Planta* **180**: 139–146.

Mimura T, Sakano K, Shimmen T. 1996. Studies on the distribution, re-translocation and homeostasis of inorganic phosphate in barley leaves. *Plant, Cell & Environment* **19**: 311–320.

Mo Q, Li Z, 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**: 503–513.

Nicol D, Ryan H. 2021. *Dataset. Leaf phosphorus fractions at a field site in southern Western Australia.* The University of Western Australia. [WWW document] URL doi: 10.26182/4vwc-e382. https://research-repository.uwa.edu.au/en/persons/megan-ryan/datasets/ [accessed 14 January 2022]

Ostertag R. 2010. Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. *Plant and Soil* **334**: 85–98.

Phillis E, Mason TG. 1942. On diurnal variations in the mineral content of the leaf of the cotton plant. *Annals of Botany* **6**: 437–442.

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

Reddy NR, Sathe SK, Salunkhe DK. 1982. Phytates in legumes and cereals. In: Chichester CO, Mrak EM, Stewart GF, eds. *Advances in food research*. New York, NY, USA: Academic Press, 1–92.

Reed SC, Townsend AR, Davidson EA, Cleveland CC. 2012. Stoichiometric patterns in foliar nutrient resorption across multiple scales. *New Phytologist* **196**: 173–180.

Ryan MH, Kaur P, Nazeri NK, Clode PL, Keeble-Gagnère G, Doolette AL, Smernik RJ, Van Aken O, Nicol D, Maruyama H *et al.* 2019. Globular structures in roots accumulate phosphorus to extremely high concentrations following phosphorus addition. *Plant, Cell & Environment* **42**: 1987–2002.

SAS. 2003. *Statistical analysis system. SAS release 9.1 for windows.* Cary, NC, USA: SAS Institute.

Siminovitch D, Rheaume B, Pomeroy K, Lepage M. 1968. Phospholipid, protein, and nucleic acid increases in protoplasm and membrane structures associated with development of extreme freezing resistance in black locust tree cells. *Cryobiology* **5**: 202–225.

Sinclair TR, Vadez V. 2002. Physiological traits for crop yield improvement in low N and P environments. *Plant and Soil* **245**: 1–15.

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, Rajapaksha R, Pushpakumara G, Lambers H. 2017. Nutrient resorption from senescing leaves of epiphytes, hemiparasites and their hosts in tropical forests of Sri Lanka. *Journal of Plant Ecology* **11**: 815–826.

Tachibana S. 1987. Effect of root temperature on the concentration of various forms of phosphorus in cucumber and figleaf gourd plants. *Bulletin of the Faculty of Agriculture - Mie University* **74**: 1–8.

Takagi D, Miyagi A, Tazoe Y, Suganami M, Kawai-Yamada M, Ueda A, Suzuki Y, Noguchi K, Hirotsu N, Makino A. 2020. Phosphorus toxicity disrupts Rubisco activation and reactive oxygen species defence systems by phytic acid accumulation in leaves. *Plant, Cell & Environment* **43**: 2033–2053.

Takyu M, Aiba SI, Kitayama K. 2002. Effects of topography on tropical lower montane forests under different geological conditions on Mount Kinabalu, Borneo. *Plant Ecology* **159**: 35–49.

Tawaraya K, Honda S, Cheng W, Chuba M, Okazaki Y, Saito K, Oikawa A, Maruyama H, Wasaki J, Wagatsuma T. 2018. Ancient rice cultivar extensively replaces phospholipids with non-phosphorus glycolipid under phosphorus deficiency. *Physiologia Plantarum* **163**: 297–305.

Tsujii Y, Onoda Y, Kitayama K. 2017. Phosphorus and nitrogen resorption from different chemical fractions in senescing leaves of tropical tree species on Mount Kinabalu, Borneo. *Oecologia* **185**: 171–180.

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

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB. 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecological Monographs* **82**: 205–220.

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.

Ye D, Clode PL, Hammer TA, Pang J, Lambers H, Ryan MH. 2021. Accumulation of phosphorus and calcium in different cells protects the phosphorus-hyperaccumulator *Ptilotus exaltatus* from phosphorus toxicity in high-phosphorus soils. *Chemosphere* **264**: 128438.

Yuan Z, Chen HYH. 2009. Global trends in senesced-leaf nitrogen and phosphorus. *Global Ecology and Biogeography* **18**: 532–542.

Zemunik G, Turner BL, Lambers H, Laliberté E. 2015. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants* **1**: 15050.

Supporting Information

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

Fig. S1 Number of records for topic 'leaf phosphorus fractions' per publication year.

Fig. S2 Relationships between different leaf phosphorus fractions and leaf total phosphorus.

Fig. S3 Leaf total phosphorus and inorganic phosphate concentrations in the shoots of annual and perennial species.

Fig. S4 Leaf total phosphorus (P) and metabolic P concentrations in perennial plant species.

Table S1 Families and species in which phosphorus fractions were studied.

Table S2 Sources of data used to generate figures.

Table S3 Concentrations of leaf phosphorus fractions among regions.

Table S4 Concentrations of leaf phosphorus fractions among plant life forms.

Table S5 Statistical significance of leaf phosphorus fractions among co-occurring families.

Table S6 Coefficient of variation of leaf phosphorus fractions of perennial plant families.

Table S7 Statistical significance of leaf phosphorus fractions among coexisting species from a single family.

Table S8 Phytate concentration in leaves of species from numerous families.

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.