REGULAR ARTICLE

Contrasting phosphorus sensitivity of two Australian native monocots adapted to different habitats

Felipe E. Albornoz ⬤ **· Michael W. Shane · Hans Lambers**

Abstract

Aims Contrasting nutrient-acquisition strategies would explain why species differ in their distribution in relation to soil phosphorus (P) availability, promoting diversity. However, what drives the differential distribution of plant species with the same P-acquisition strategy remains poorly understood.

Methods We selected two Haemodoraceae species, *Anigozanthos flavidus* and *Macropidia fuliginosa*, to investigate physiological responses in non-mycorrhizal monocots adapted to different edaphic habitats that vary in P availability. Plants were grown in nutrient solution in large tanks at a range of P concentrations (0, 0.1, 1, 10 μM P). After seven months, we measured growth, photosynthetic rate, net P-uptake capacity, and leaf [P].

Results Fresh weights of *A. flavidus* plants were highest at 1 μM P and lowest at 0 μM P. Fresh weights of *M. fuliginosa* plants were lowest at 10 μM P compared with those at other P levels. Rates of P uptake by *A. flavidus* showed a steady decline with increasing P level during growth from 0 to 1 μM P, and then a sharp decline from 1 to 10 μM P. Rates of P uptake in

M. fuliginosa did not differ among growth P levels, except between 0 and 1 μM P. Both species showed a drastic increase in the concentration of both total P and inorganic P at 10 μM P.

Conclusions The results support our hypothesis that *A. flavidus* is efficient in down-regulating its P-uptake capacity, while *M. fuliginosa* is not. Thus, partly explaining the narrower and wider distribution of these species.

Keywords Endemism · Haemodoraceae · Phosphate uptake · Phosphorus sensitivity · Phosphorus toxicity

Michael W. Shane is deceased

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Introduction

Hyperdiverse terrestrial ecosystems tend to occur on some of the most nutrient-impoverished soils (Lambers 2014; Lambers et al. 2010; Oliveira et al. 2015). These ecosystems are characterised by high spatial heterogeneity in soils and species composition, with high species turnover across a soil nutrient availability gradient (Hulshof and Spasojevic 2020; Latimer et al. 2005; Zemunik et al. 2016; Lazarina et al. 2019; Leopold and Zhong 2019). Understanding the diversity, ecology, and distribution of plant species in such diverse ecosystems has been one the main questions ecologists seek to answer. In the last decade, several hypotheses have been proposed to explain hyperdiversity, such as nutrient-acquisition strategies (Lambers et al. 2008), and phosphorus (P) sensitivity (Hayes et al. 2019b). Lambers et al. (2008) proposed that species capable of releasing

carboxylates exhibit the most efficient P-acquisition strategy on severely P-impoverished soils, explaining their high diversity on the most P-improvised soils in the world. This would explain why species with contrasting nutrient-acquisition strategies differ in their distribution in relation to soil P availability. However, what drives the differential distribution of plant species with the same P-acquisition strategy remains poorly understood.

South-western Australia is a global biodiversity hotspot (Myers et al. 2000) on severely nutrient-impoverished soils (Lambers 2014), and harbours a diverse range of species and nutrient-acquisition strategies, such as mycorrhizas, cluster roots, dauciform roots, capillaroid roots and sand-binding roots (Zemunik et al. 2015). Many species in this ecosystem have evolved adaptations to extremely low P availability, and some even exhibit P-toxicity when exposed to slightly higher levels than in their ecological niche (Shane and Lambers 2006; de Campos et al. 2013; Hayes et al. 2019a,b). Symptoms of P-toxicity include leaf chlorosis, necrosis, and stunted growth (Shane and Lambers 2006). Phosphorus toxicity is relatively common in dicotyledonous species from severely P-impoverished habitats in Australia and South Africa, in particular in Proteaceae (Lambers et al. 2015; Hayes et al. 2019b); it is caused by accumulation of P to $\geq 10,000$ mg P kg^{-1} of leaf dry mass, as a consequence of limited down-regulation of the plant's P-uptake capacity (Lambers et al. 2015). This has been relatively well studied for dicotyledonous species in Australia (Shane et al. 2004), South Africa (Shane et al. 2008) and Brazil (Abrahão et al. 2014), but the role of soil P availability in determining species distribution has never been studied in monocotyledonous species from the same regions.

Kangaroo paws (Haemodoraceae) are a group of monocot species endemic to south-western Australia (Hopper and Campbell 1977). Kangaroo paw species are non-mycorrhizal (Brundrett and Abbott 1991), and commonly produce sand-binding roots (Smith et al. 2011). Sand-binding roots are perennial roots covered with a thick sheath of sand particles, root hairs, exudates, and microorganisms (Smith et al. 2011). One of their functions is assumed to be related to reduction of water loss (Smith et al. 2011), but another function involves P acquisition associated with carboxylate release (Hayes et al. 2014; Zemunik et al. 2016). Kangaroo paws inhabit a variety of both wet and dry habitats, but are invariably restricted to severely P-impoverished soils (https://florabase.dpaw.wa.gov.au). Hence, these

species pose a unique study subject given their restricted distribution within a biodiversity hotspot. Here, we selected two Haemodoraceae species, *Anigozanthos flavidus* and *Macropidia fuliginosa*, for an experiment designed to investigate physiological responses in monocot species adapted to different edaphic habitats (Fig. 1) that vary in P availability, as done before for dicot Proteaceae (Shane et al. 2004, 2008; Shane and Lambers 2006). These species are endemic to contrasting natural habitats in south-western Australia (Fig. 1). *Macropidia fuliginosa* (black kangaroo paw) occurs in a semi-arid to Mediterranean climate (Fig. 1), is not restricted to sandy soils, and is notable in its ability to occupy hilltops on lateritic gravel conglomerates (https://florabase.dpaw.wa.gov.au). *Anigozanthos flavidus* (tall kangaroo paw), on the other hand, occurs in a Mediterranean to temperate climate, at lower latitudes than *M. fuliginosa*. *Anigozanthos flavidus* grows in clay-loams as well as peaty sands, and even partly immersed in brackish estuarine water along the south coast (Hopper 1993). This latitudinal separation in the distribution of these two plant species could be related to differences in soil P availability and rainfall. Higher rainfall indirectly affects soil chemical properties, increasing the mobility of P (Lambers and Oliveira 2019; Pandey and Srivastava 2009). Hence, we hypothesised that *M. fuliginosa*, which is restricted to drier and more P-impoverished habitats, has a very low ability to down-regulate its P-uptake capacity at higher P availability, while *A. flavidus*, which grows in wetter habitats with higher P availability, is capable of down-regulating its P-uptake capacity, explaining its wider distribution. To test our hypothesis, plants were grown in nutrient solution in large tanks at a range of P concentrations, to study their response to P supply and their P sensitivity. Their P-uptake capacity was determined as dependent on the P supply during growth, to assess if any differences in P sensitivity was accounted for by the regulation of their P-uptake system.

Materials and methods

Growth conditions

Two-month-old seedlings, each with 2–4 leaves, of *M. fuliginosa* (Hook.) Druce and *A. flavidus* DC. were obtained from Lullfitz Nursery (Wanneroo). Soil was
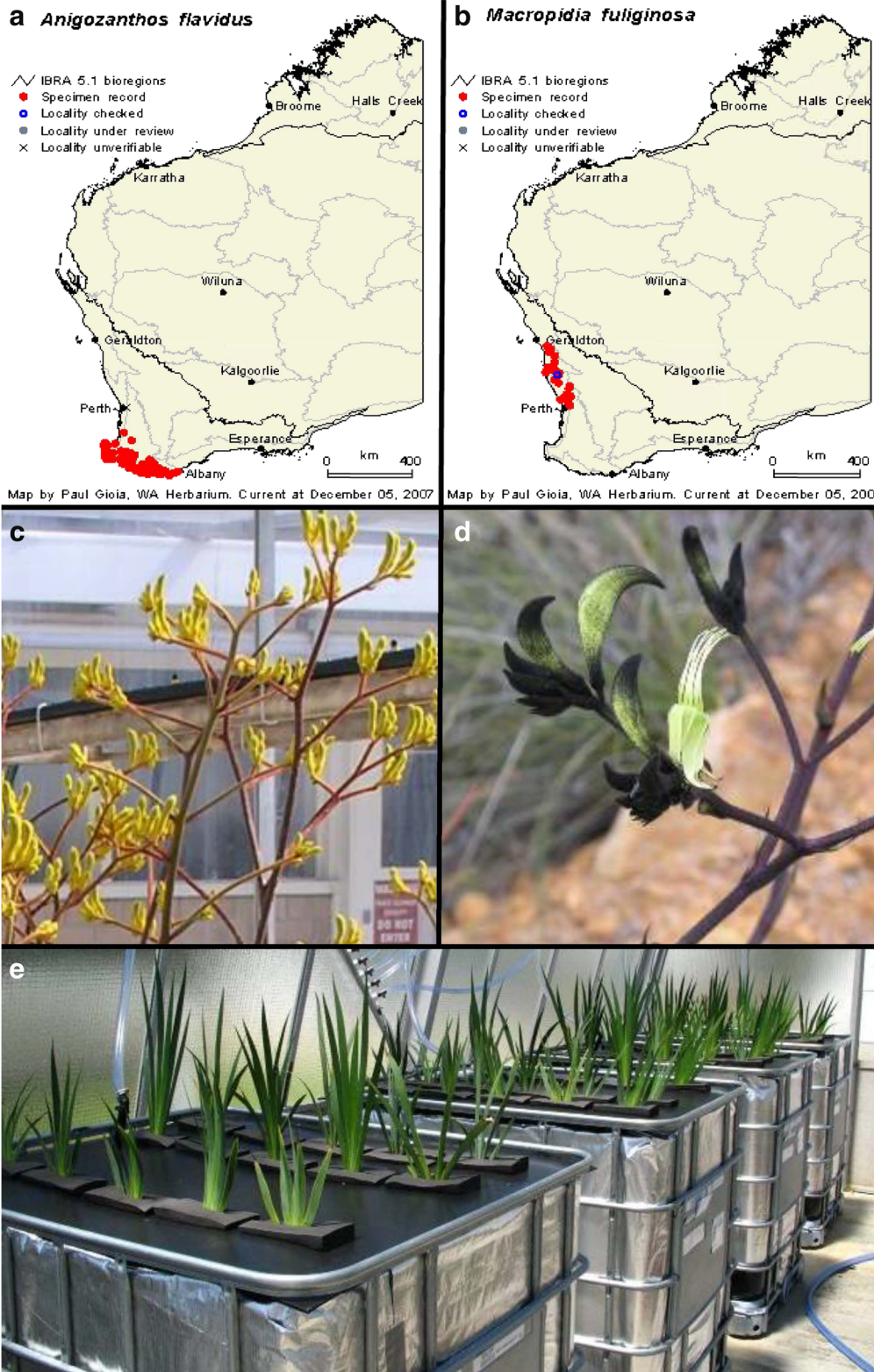
Fig. 1 (**a**) Geographic distribution of (**c**) tall kangaroo paw (*Anigozanthos flavidus*). Grows on wet road verges, river banks and swamps. (**b**) Geographic distribution of (**d**) black kangaroo paw (*Macropidia fuliginosa*). Grows on lateritic plateau terrain usually in low heath and low mallee. (**d**) Hydroponic tanks used for the experiment. Map by Paul Giogia, WA Herbarium

washed from root systems using tap water, and seven to eight seedlings of each species were transferred to large-volume hydroponics tanks (1000 L, Fig. 1) in a glasshouse at the beginning of November 2006. Tanks contained aerated nutrient solution (pH 5.8) composed of the following (in μM): 400 NO_3^-, 200 Ca^{2+}, 200 K^+, 154 SO_4^{2-}, 54 Mg^{2+}, 20 Cl^-, 2.0 Fe-EDTA, 0.24 Mn^{2+}, 0.10 Zn^{2+}, 0.02 Cu^{2+}, 2.4 H_3BO_3, 0.3 Mo^{4+} made up in deionised water. After an adjustment period of four weeks without P addition, seedlings were culled to five per species and hydroponic solutions were supplemented with KH_2PO_4 to give four P levels 0, 0.1, 1 and 10 μM P. The range of [P] used for this experiment was based on results from a previous large volume hydroponics experiment with South African native Proteaceae (Shane et al. 2008). The P treatment lasted eight months (harvested in July 2007). Day/night temperatures in the glasshouse ranged from 40 to 16°C in summer, and 30 to 16°C in winter, while that of the nutrient solution was 18 to 22°C. Relative humidity was 40 to 70%, and maximum photosynthetically active radiation in summer and winter was 2000 and 1000 μmol m^2 s^{-1}, respectively. The complete nutrient solution (1000 L) was replaced every 4th day, and [P] in solution was measured and adjusted daily to provide near-constant [P] in the treatments.

Measurements of growth, P uptake and gas exchange

Initial whole plant fresh weight (FW_i) was determined in the glasshouse for plants after seven months of P treatment, and final whole plant fresh weight (FW_f) was determined after another 30 days growth at the end of the experiment. The relative growth rate (RGR) was calculated as $((FW_f - FW_i) / FW_i) / 30) \times 1000 = $ mg FW g^{-1} FW day^{-1}.

Gas exchange measurements and subsequent measurements of P uptake were made in July 2007 between 11h00 and 14h00, prior to harvesting the plants. Gas exchange of youngest fully-expanded leaves of each plant was measured using a LI-6400 portable photosynthesis system (Licor, Lincoln, NE, USA) at 1500 μmol quanta m^{-2} s^{-1} supplied by the blue-red light-emitting diode bank of the Licor LI-6400-02B light source. Cuvette conditions were 400 μl CO_2 L^{-1}, air temperature 25 °C, leaf-air vapour pressure deficit was 0.9 to 1.5 kPa, and relative air humidity was 40 to 50%.

Net P-uptake rates were determined for whole root systems as described in Shane et al. (2004). Briefly, P depletion was measured by immersing roots in an external solution (6 L) of initial [P] of 5 μM for three hours. In preliminary experiments, 6 L of nutrient solution containing 5 μM gave a linear P-depletion rate over 3 h. During all P-uptake measurements, plants remained in the glasshouse in which they were grown. A small volume of concentrated KH_2PO_4 solution (less than 100 μL) was added to each pot containing fresh nutrient solution minus P (i.e. one plant per 6 L pot) to give a final concentration of 5 μM. After 120 s of mixing by vigorous aeration in each pot, a 1 mL sample (time zero) was taken from each pot, and subsequent samples were taken at 30-min intervals.

Post-harvest analyses

After measuring RGR, gas exchange, and net P uptake, plants were removed from nutrient solutions, and their fresh weight (FW) was recorded. Root diameter was determined for the main root axes of fresh material. Diameters were measured using digital callipers at 100 mm from the tips for each species (four roots per plant, $n = 20$).

For total leaf [P], fresh samples were digested in concentrated HNO_3 : $HClO_4$ (3:1) at 175˚C. Total [P] in leaf digests and in solutions used for P-depletion studies were determined using the malachite green colorimetric method (Motomizu et al. 1983). Inorganic P (Pi) concentrations of fresh leaf material (50 mg per sample; $n = 5$) was determined using a modified method from Irving and Bouma (1984) (according to Delhaize and Randall 1995). Digested samples were also analysed for magnesium (Mg), sulfur (S), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) by inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Optima 5300DV, PerkinElmer, Waltham, MA, USA). Quantification was carried out against a series of standard plant-reference material. A subsample was then used to calculate percentage of water content. Samples were weighed fresh (i.e. FW) and again after drying (i.e. dry weight; DW) for two days at 70°C. The percent water content was calculated as (FW – DW / FW)*100. Then, water content was used to express leaf nutrient concentration by dry weight.

Statistical analyses

We used generalised mixed-effect models (Pinheiro et al. 2018) to test for differences in plant responses between species and among P-addition levels. In all analyses, we selected the best model by first inspecting residuals to check model assumptions. When models did not meet assumptions, variance structures were specified in a second model, and we compared both models using the Akaike Information Criterion (AIC) and likelihood ratio tests (Zuur et al. 2009). When a main term (namely P treatment) or interaction between species and P treatment was significant, *post hoc* Tukey tests were performed. All analyses and figures were made in R (R Core Team 2016) using the *nlme* and *multcomp* packages (Hothorn et al. 2012; Pinheiro et al. 2018).

Results

Growth, photosynthesis and P uptake

There was a significant interaction between species and P treatment for FW ($F_{1,3} = 50.16$; $P < 0.0001$). Fresh weights of *A. flavidus* plants grown at 1 µM P were three times greater than those of plants grown at 0.1 and 10 µM P, and more than 10-fold greater than those of plants grown at 0 µM P (Fig. 2). Fresh weight of plants grown at 0.1 and 10 µM P were four times greater than of those grown at 0 µM P; there were no differences between them (Fig. 2). Fresh weights of *M. fuliginosa* plants, on the other hand, were lowest for plants grown at 10 µM P compared with all other P levels (Fig. 2); we found no differences among the other three P levels.

Anigozanthos flavidus plants grown with 0 to 1 µM P exhibited a gradual increase in root diameter (Fig. S1). Increasing the P supply had no further influence on root diameter (Fig. S1). In contrast, *Macropidia fuliginosa* supplied with 0 to 1 µM P during growth showed no differences in root diameter (Fig. S1), whereas this parameter decreased significantly for the corresponding plants supplied with 10 µM P (Fig. S1).

There was a significant interaction between species and P treatment for RGR ($F_{1,3} = 16.7$; $P < 0.0001$). The RGR of *A. flavidus* increased over 10-fold in plants growing at 0.1, 1 and 10 µM P, compared with plants grown at 0 µM P (Fig. 3); there were no differences among the three higher P levels. *Macropidia fuliginosa* plants grew twice as fast when grown at 0.1 µM P than

at the other three P levels, and no differences were found among them (Fig. 3).

Rates of photosynthesis of *A. flavidus* were slowest when plants were grown at 0 µM P, and fastest when grown at 0.1 µM P (Fig. 4); while there was a decrease in photosynthetic rate from 0.1 to 1 and 10 µM P, it remained almost three times faster than at 0.1 µM P (Fig. 4). *Macropidia fuliginosa*, on the other hand, showed a steady increase in photosynthetic rate with increasing P level, peaking at 1 µM P (Fig. 4). The photosynthetic rate of *M. fuliginosa* grown at 10 µM P was similar to that of plants grown at 0 µM P. Interestingly, at the highest P supply, the rate of photosynthesis was almost twice as fast in *A. flavidus* as in *M. fuliginosa*.

Rates of P uptake by *A. flavidus* measured at a standard P concentration, independent of that during growth, showed a steady decline with increasing P level during growth from 0 to 1 µM P, and then a sharp decline from 1 to 10 µM P (Fig. 5). Rates of P uptake in *M. fuliginosa*, on the other hand, did not statistically differ among P levels, except between 0 and 1 µM P (Fig. 5).

Leaf nutrient concentrations

Total and inorganic P concentrations were determined in the youngest fully-expanded leaves, showing an increase in total P and Pi concentration with increasing P supply (Fig. 6). Both species showed a drastic increase in both total P and Pi concentration at 10 µM P (Fig. 6). Leaves of *A. flavidus* accumulated less total P at 0 and 0.1 µM P compared with *M. fuliginosa*; but it was similar at 1 µM P (Fig. 6a). However, at the highest external P level (10 µM), *A. flavidus* accumulated more total P in its leaves than *M. fuliginosa* (Fig. 6a). Inorganic P accumulated in leaves was relatively similar among the two species at 0 µM P, but at higher external P level (i.e. 0.1, 1, and 10 µM), *A. flavidus* and *M. fuliginosa* accumulated similar amounts of Pi in their leaves (Fig. 6b).

Leaf concentrations of K gradually increased with increasing P supply, but only in *A. flavidus* (Table 1). The opposite trend was found for leaf Mg concentrations, which gradually decreased with increasing P supply, but only for *A. flavidus* (Table 1). Leaf calcium concentrations did not vary appreciably with increasing P supply for either species, but were always significantly greater in *A. flavidus* than in *M. fuliginosa* (Table 1). Leaf Mn concentrations in *A. flavidus* were lowest at the
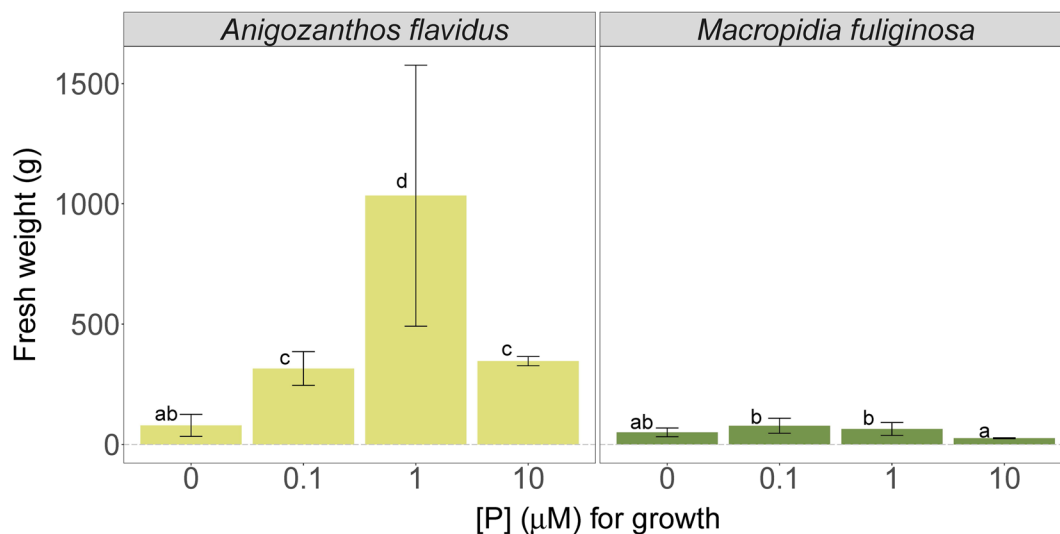
Fig. 2 Fresh weight of *Anigozanthos flavidus* and *Macropidia fuliginosa* accumulated after seven month growing in hydroponic tanks at different phosphorus concentrations ([P]) in nutrient solutions. Values shown are means ± 95% confidence intervals. Different letters indicate statistical differences among P treatments and species ($P < 0.05$)

lowest P level, while those of *M. fuliginosa* did not vary with P supply (Table 1). Interestingly, leaf Mn concentrations were more than an order of magnitude higher in *A. flavidus* than in *M. fuliginosa*. Leaf Fe and Zn concentrations were highest at intermediate P supply in leaves of *A. flavidus*, while they did not vary with P supply in *M. fuliginosa* and were consistently lower than those of *A. flavidus* (Table 1). Finally, leaf Cu and S

concentrations did not vary with P supply in either *A. flavidus* or *M. fuliginosa* (Table 1).

Discussion

Phosphorus toxicity has been reported for several Australian and South African species adapted to nutrient-
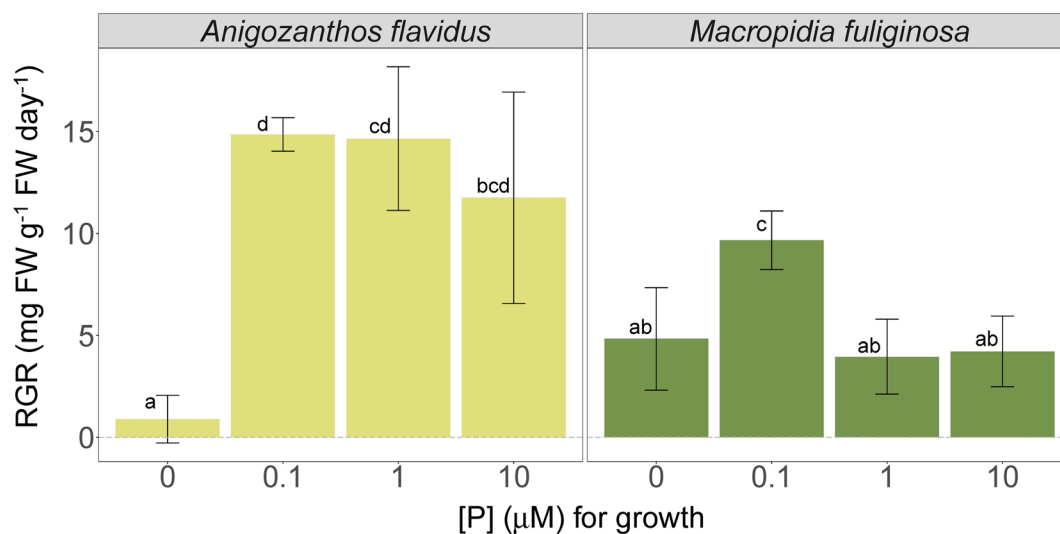

Fig. 3 Relative growth rate (RGR) of *Anigozanthos flavidus* and *Macropidia fuliginosa* after seven months growing in hydroponic tanks at different phosphorus concentrations ([P]) in nutrient solutions. Relative growth rate was measured as the difference in biomass between seven- and six-month-old plants. Values shown are means ± 95% confidence intervals. Different letters indicate statistical differences among P treatments and species ($P < 0.05$)
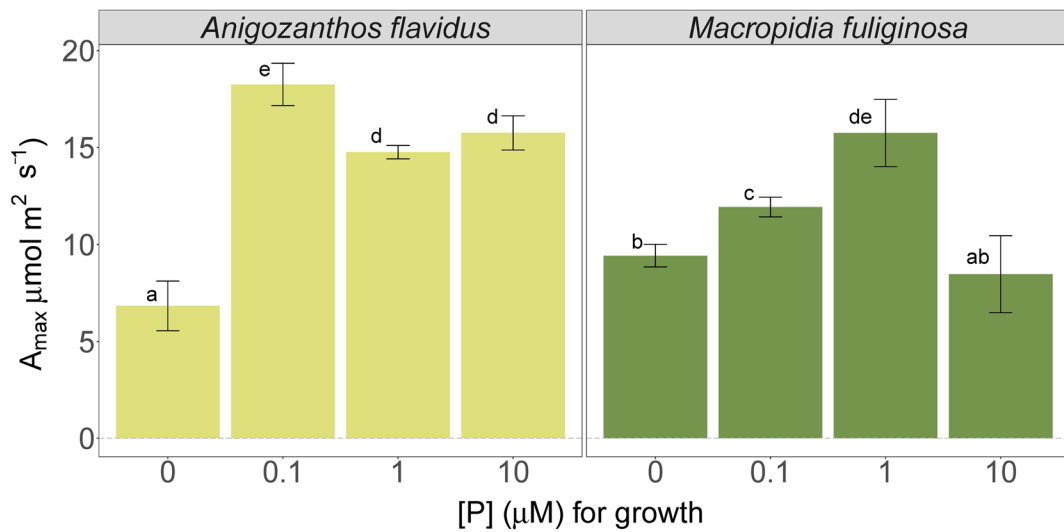
Fig. 4 Rates of photosynthesis (A_{max}) of youngest fully expanded mature leaves of *Anigozanthos flavidus* and *Macropidia fuliginosa* grown in hydroponic tanks at different phosphorus concentrations ([P]) in nutrient solutions. Values shown are means ± 95% confidence intervals. Different letters indicate statistical differences among P treatments and species ($P < 0.05$)

impoverished environments (Specht 1963; Grundon 1972; Groves and Keraitis 1976; Ozanne and Specht 1981; Handreck 1997a; Hawkins et al. 2008; Shane et al. 2008; de Campos et al. 2013). Down-regulation of the P-uptake capacity is the most important mechanism to avoid P toxicity (Shane and Lambers 2006; de Campos et al. 2013; Lambers and Plaxton 2015). Both Haemodoraceae showed symptoms of P toxicity at a high P supply. However, the symptoms of *A. flavidus* were much milder than those of *M. fuliginosa*. This was associated with a difference in their ability to down-regulate their P-uptake capacity. These results support our hypothesis that *A. flavidus* is much more efficient in down-regulating its P-uptake capacity than *M. fuliginosa*. Thus, these findings at least partly explain the narrower and wider distribution of these species.
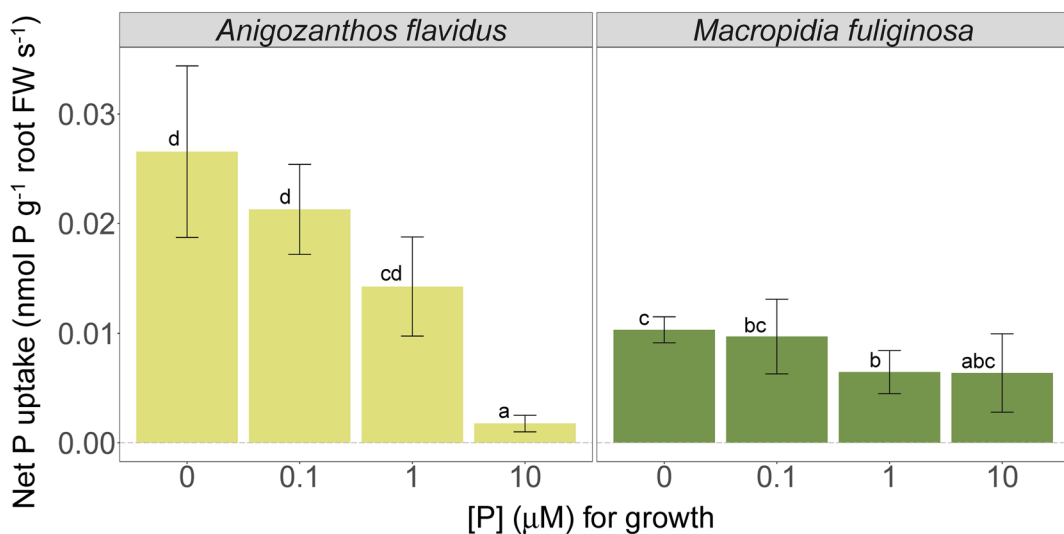

Fig. 5 Rates of net phosphorus (P) uptake determined at 5 µM P for intact whole root systems of *Anigozanthos flavidus* and *Macropidia fuliginosa* grown in hydroponic tanks at different [P] in nutrient solutions. Phosphorus uptake was calculated from P-depletion curves. Values shown are means ± 95% confidence intervals. Different letters indicate statistical differences among P treatments and species ($P < 0.05$). FW, fresh weight
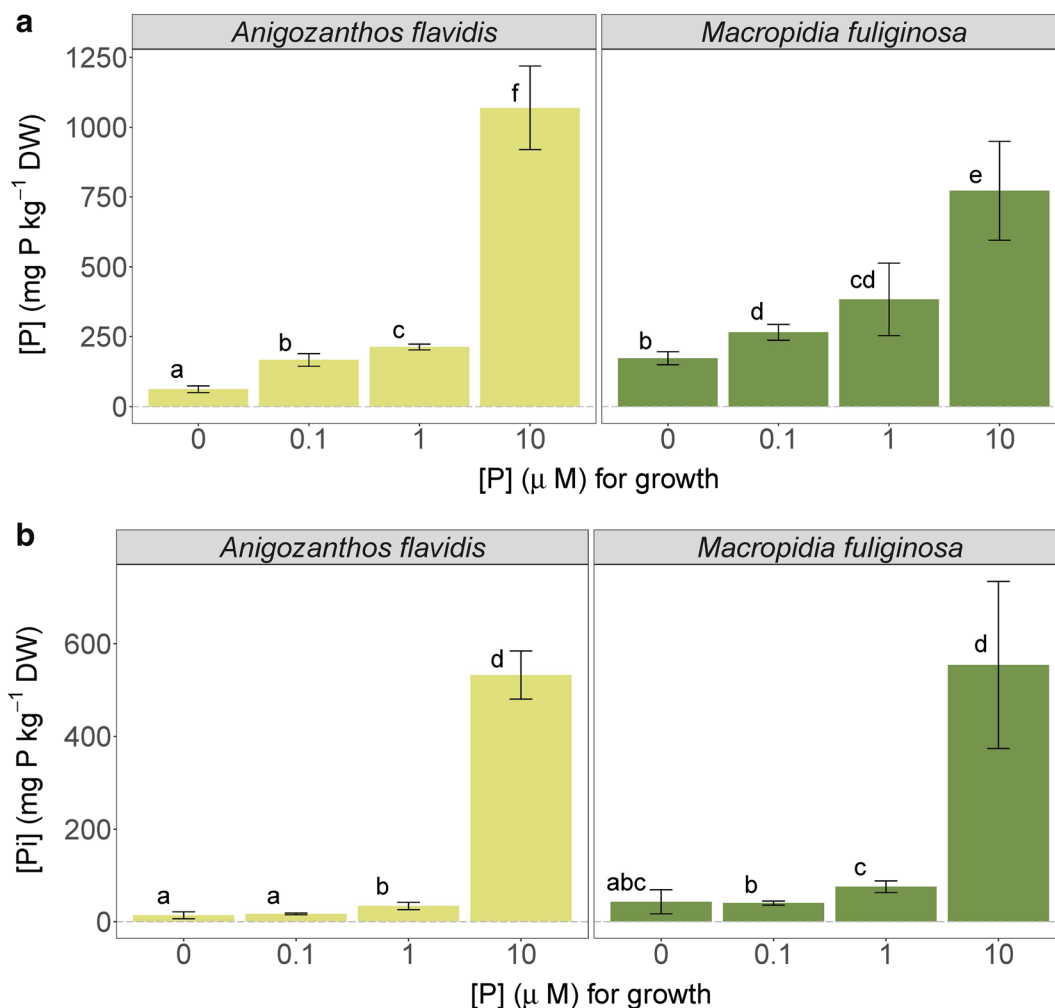
Fig. 6 Leaf phosphorus concentrations ([P]) of dry mature leaves of *Anigozanthos flavidus* and *Macropidia fuliginosa* grown in hydroponic tanks at different [P] in nutrient solutions. (a) Represents total leaf [P]; (b) represents inorganic leaf P [Pi]. Values shown are means ± 95% confidence intervals. Different letters indicate statistical differences among P treatments within each species ($P < 0.05$)

Previous studies have suggested nutrient requirements as a key factor in determining species distribution in hyperdiverse ecosystems (Mustart and Cowling 1993; Cowling et al. 1994; Willis et al. 1996; Gao et al. 2020; Zhong et al. 2020). When P is extremely limiting, there is no need for mechanisms that down-regulate and limit uptake of P. However, absence of these mechanisms in favour of rapid uptake, restricts the ability of the plant to colonise richer environments, due to P-sensitivity. In this study, we show that the magnitude of the response to high P supply differed strongly between the two species, as found before when comparing P-sensitive and P-insensitive Proteaceae (Shane et al. 2004, 2008; Shane and Lambers 2006).

We found little to no down-regulation of P-uptake capacity in *M. fuliginosa*, but a stronger down-regulation in *A. flavidus*. However, this down-regulation in *A. flavidus*, was still insufficient to completely avoid P toxicity at the highest P supply. Tolerance to high P have previously been found for other species within *Anigozanthos* (Handreck 1997b). This suggests that the same principle regarding P sensitivity proposed for dicotyledonous species in South Africa and Australia, applies to perennial monocotyledonous species.

Symptoms of P toxicity found here were decreased biomass, RGR, photosynthetic rate, and leaf necrosis (*not shown*). Plant species adapted to infertile soils tend to be slow-growing, while species adapted to fertile soils

Table 1 Nutrient concentrations in dry leaves of *Anigozanthos flavidus* and *Macropidia fuliginosa*, grown in nutrient solution containing 0, 0.1, 1 or 10 μM phosphate. Values are mean ± 95% confidence intervals. Nutrients are: magnesium (Mg), sulfur (S), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn). Different letters indicate statistical differences among P treatments within each species ($P < 0.05$). Sufficient levels of nutrients are based on Epstein and Bloom (2005)

	Anigozanthos flavidis				*Macropidia fuliginosa*				Sufficient crop levels
	0	0.1	1	10	0	0.1	1	10	
K (g kg^{-1})	5 ± 0.3ab	6.4 ± 0.4bc	7.8 ± 0.5 cd	8.5 ± 0.4d	7.5 ± 0.2a	8.5 ± 0.3a	7.6 ± 0.7a	8.8 ± 0.6a	10
Mg (g kg^{-1})	1.2 ± 0.1c	0.9 ± 0.1b	0.6 ± 0.1a	0.6 ± 0.1a	0.6 ± 0a	0.6 ± 0a	0.7 ± 0a	0.5 ± 0a	2
Ca (g kg^{-1})	7 ± 0.4a	7.6 ± 0.9a	5.4 ± 0.3a	7.4 ± 0.4a	1.6 ± 0.3a	2.1 ± 0.3a	2.3 ± 0.4a	1.6 ± 0.3a	5
Mn (mg kg^{-1})	116 ± 22a	293 ± 59b	286 ± 57b	315 ± 68b	22 ± 3a	21 ± 3a	46 ± 10a	26 ± 3a	50
Fe (mg kg^{-1})	36 ± 2a	60 ± 6b	29 ± 2a	38 ± 1a	19 ± 2a	17 ± 1a	26 ± 4a	14 ± 2a	100
Zn (mg kg^{-1})	12 ± 1a	31 ± 3c	33 ± 3c	21 ± 2b	17 ± 2a	15 ± 1a	19 ± 2a	13 ± 1a	20
S (g kg^{-1})	1.3 ± 0.2a	1.5 ± 0.2a	1.3 ± 0.2a	1.5 ± 0.2a	0.8 ± 0.2a	0.7 ± 0.1a	1.4 ± 0.3a	1.5 ± 0.2a	1
Cu (mg kg^{-1})	1.3 ± 0.1a	1.2 ± 0.1a	1.3 ± 0.1a	1.2 ± 0.1a	2.7 ± 0.3a	2.5 ± 0.2a	2.9 ± 0.3a	2.5 ± 0.2a	6

have relatively faster growth rates (Lambers and Poorter 1992). Slow growing plant species tend to exhibit a higher leaf dry matter content (DMC) than fast growing species (Lambers and Oliveira 2019). In this study, the biomass accumulation of *A. flavidus* was an order of magnitude greater than that of *M. fuliginosa*. Further, DMC of *A. flavidus* and *M. fuliginosa* were 20 and 38 mg g^{-1}, respectively. The significantly higher DMC and lower biomass and RGR of *M. fuliginosa* confirmed that it is an inherently slower-growing species than *A. flavidus*, potentially as an adaptation to infertile soils. Our results indicate P toxicity at 10 μM for both species. For *M. fuliginosa*, however, biomass was less than half at the highest P level compared with that at all other P levels, indicating stronger P toxicity when compared with *A. flavidus*.

Leaves of *A. flavidus* accumulated significantly less leaf P up to an external supply of 1 μM P than those of *M. fuliginosa* did which explains why *M. fuliginosa* showed P-toxicity symptoms at lower P concentrations in the nutrient solution. Excess of P in leaves can inhibit Rubisco, hampering photosynthesis (Takagi et al. 2020). *Anigozanthos flavidus* showed a maximum photosynthetic rate at 0.1 μM, while this was at 1 μM for *M. fuliginosa*. Although photosynthetic rate of *A. flavidus* was only mildly inhibited by increasing P supply, that of *M. fuliginosa* was almost half at 10 μM compared with that at 1 μM of P. This suggests that the reduced ability to down-regulate P-uptake results in leaf [P] above non-toxic levels, hindering photosynthesis.

Leaf [P] ranged between 62 and 1,069 mg kg^{-1} DW for *A. flavidus*, and between 172 and 772 mg kg^{-1} DW for *M.* fuliginosa, between the 0 and 10 μM P treatments. Excluding the 10 μM P treatment, leaf [P] did not exceed 212 and 383 mg kg^{-1} DW for *A. flavidus* and *M. fuliginosa*, respectively. This suggests that both *A. flavidus* and *M. fuliginosa* are more P-sensitive than the standard definition of 10,000 mg kg^{-1} (Marschner 2012). Most previous research on P accumulation and toxicity in native Australian plants has been conducted on dicot species (but see Handreck 1997b). Monocot plants can operate at lower leaf [P] than dicot species, partially due to them allocating less P to metabolically-inactive epidermal cells (Conn and Gilliham 2010). An additional explanation for low leaf [P] is the replacement of phospholipids by galactolipids and sulfolipids, and functioning at very low rRNA levels which has been shown for P-efficient Proteaceae species in this system (Lambers et al. 2012; Sulpice et al. 2014). However, this requires further investigation for Haemodoraceae.

Leaf concentrations of other macro- and micronutrients in *A. fuliginosa* were similar to what has been found for other plant species in south-western Australia (Hayes et al. 2014, 2019b; Table 1). Leaf nutrient concentrations in *M. fuliginosa*, on the other hand, were generally lower than those in *A. flavidus* (Table 1), native dicots (Hayes et al. 2019b), and another Haemodoraceae (Hayes et al. 2014). These lower leaf nutrient concentrations were persistent at all P levels.

Particularly, leaf Zn concentrations of *M. fuliginosa* were less than half of those of *A. flavidus*. Biological availability of Zn in cells is greatly reduced when excess P is accumulated in leaves, resulting in P toxicity (Cakmak and Marschner 1987) as seen in *M. fuliginosa*. In contrast, a high leaf Zn concentration would allow to tolerate higher leaf P concentrations, as is in the case of *A. flavidus*. This is in line with what has been found in P-sensitive Proteaceae species, where leaf Zn concentration in the most P-sensitive species was much lower than that in the less P-sensitive species (Hayes et al. 2019a, b).

It is worth noting, that rainfall might also contribute to the distribution of the two plant species studied here. *Anigozanthos flavidus* is restricted to drier environments, while *M. fuliginosa* naturally occurs in wetter habitats. In this study, plants were grown in hydroponics, and hence, water availability was not a variable. However, rainfall can increase soil moisture and the mobility of P in soil (Lambers and Oliveira 2019; Pandey and Srivastava 2009). Hence, we surmise that soil P availability can partly explain the distribution of these two plant species, acknowledging the potential role of other environmental variables, especially rainfall.

Conclusions

Soil nutrient spatial heterogeneity is likely a strong driver in plant species with contrasting P sensitivity, as previously concluded by Hayes et al. (2019a, b) for Proteaceae species. Having a greater tolerance to high P availability might allow *A. flavidus* to occupy a wider range of niches in regard to P availability, such as ruderal landscapes and valleys (https://florabase.dpaw. wa.gov.au). Here, we show that habitat specificity contributes to high species turnover and overall diversity of a biodiversity hotspot. Although P sensitivity of native Australian monocots have been tested before (Handreck 1997b), here we provide evidence, for the first time, of the role of down-regulation of P-uptake capacity in defining ecological distribution.

Author contributions MWS conducted the experiment and collected data. FEA analysed data and, together with HL, wrote the manuscript. Data availability Data will be archived in Dryad upon paper acceptance.

Compliance with ethical standards

Conflict of interest All authors, with the exception of Dr Shane, who passed away, state that they do not have any conflict of interest in the submission of this manuscript.

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