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RESEARCH ARTICLE

Facilitation of phosphorus acquisition by *Banksia attenuata* allows *Adenanthos cygnorum* (Proteaceae) to extend its range into severely phosphorus-impoverished habitats

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Abstract

Background and aims In extremely low-phosphorus (P) environments, most Proteaceae exude carboxylates from cluster roots. These carboxylates mobilise inorganic P which leads to a relatively high leaf manganese concentration ([Mn]). However, we found that *Adenanthos cygnorum* (Proteaceae) in a low-P habitat did not invariably have a high leaf [Mn] in south-western Australia. We aimed to explore how *A. cygnorum* acquires P in severely P-impoverished habitats.

Methods We determined soil P concentrations and leaf [Mn] of *A. cygnorum* growing within 1 m and more than 10 m away from other large Proteaceae. We also grew plants in a glasshouse to determine its root carboxylate exudation and rhizosheath phosphatase activity.

Results *Adenanthos cygnorum* did not produce functional cluster roots. It depended on carboxylates released by a P-mobilising neighbour, *Banksia attenuata* (Proteaceae), to acquire P when growing in severely P-impoverished soil (< 8 mg P kg^{-1} dry soil). In slightly less P-impoverished soil (> 11 mg P kg^{-1} dry soil), phosphatases released by *A. cygnorum* hydrolysed sufficient organic P that was relatively mobile.

Conclusion The reliance on facilitation of P acquisition in *A cygnorum* depended strongly on location. We demonstrated the exudation of phosphatases, which mobilise inorganic P; this P was adequate for growth when there was sufficient organic P in soil. Facilitation of P acquisition by *B. attenuata* allowed *A. cygnorum* to extend its range into severely P-impoverished habitats where it cannot exist without facilitation. This knowledge provides a better understanding of the diversity of P-acquisition strategies in severely P-impoverished environments.

Keywords *Adenanthos cygnorum* · Carboxylates · Cluster roots · P acquisition · Manganese · Phosphatases · Proteaceae

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Introduction

Phosphorus (P) is an essential plant nutrient, and frequently limits plant productivity, especially in biodiversity hotspots including south-western Australia (Kooyman et al. 2017). In response to low soil P availability, roots exhibit a variety of adaptive strategies. Many Fabaceae and Myrtaceae access

soil P by being mycorrhizal, provided the soil P concentration ([P]) is not extremely low (Abbott et al. 1984; Parfitt 1979; Treseder and Allen 2002). Most Proteaceae are non-mycorrhizal and develop cluster roots as an adaptation for nutrient acquisition when soil P availability is extremely low (Lamont 1982; Shane et al. 2004a). At the morphological level, each cluster root comprises hundreds to thousands of rootlets, which are covered in abundant root hairs, greatly increasing their surface area (Shane et al. 2004a). At the physiological level, cluster roots release exudates (e.g., carboxylates, protons, phenolics, phosphatases, phytases) into the rhizosphere that mobilise soil P (Dinkelaker et al. 1995; Shane and Lambers 2005).

The large amounts of carboxylates released into the soil by cluster roots mobilise both inorganic P (Pi) and organic P (Po) that are sorbed onto soil particles into the soil solution. However, roots only absorb Pi, and access organic forms of P following hydrolysis by phosphatases (Lambers et al. 2008; Lambers 2022), which releases Pi. Not all Proteaceae have functional cluster roots that release large amounts of carboxylates. For example, *Xylomelum occidentale* (Proteaceae) produces tiny cluster roots that only live for three days, and do not release carboxylates into the soil (Zhong et al. 2021).

Carboxylates mobilise a variety of Po molecules. They can mobilise strongly sorbed Po molecules, such as phytate, which can then be hydrolysed by phytases (Giles et al. 2017). Carboxylates are not needed to mobilise all Po molecules, since not all Po is as strongly sorbed as phytate to soil particles (Turner 2007; Giles et al. 2017). Strongly sorbed Po includes phytate and glucose-6-phosphate (Amadou et al. 2022), while phospholipids and nucleic acids (RNA and DNA) are less subject to strong sorption (Jarosch et al. 2019). Roots of *X. occidentale* that do not exude carboxylates acquire P from weakly sorbed soil phospholipids and RNA using exuded phosphatases, without involvement of carboxylates (Zhong et al. 2021).

Plants occurring next to P-mobilising plants may utilise Pi mobilised by those neighbours to facilitate their P uptake (Li et al. 2007). Facilitation of nutrient acquisition refers to a positive effect on nutrient uptake for at least one of the interacting species which may occur as a result of an increase in the availability of a limiting nutrient, such as iron (Fe), zinc (Zn) and manganese (Mn) (Li et al. 2014). For example, *Banksia attenuata* (Proteaceae) facilitates Mn acquisition of neighbouring *Scholtzia involucrata* (Myrtaceae), because carboxylates released by its cluster roots increase Mn availability in soil (Muler et al. 2014).

The uptake of Mn, including that mobilised by carboxylates, is poorly controlled (Lambers et al. 2015; Alejandro et al. 2020). Therefore, Proteaceae with carboxylate-releasing cluster roots (Lambers et al. 2021) take up more Mn from soil than mycorrhizal plants, because mycorrhizal hyphae intercept Mn (Kothari et al. 1991; Canton et al. 2016). The Mn is then exported from the roots to the shoot, giving rise to relatively high mature leaf Mn concentrations ([Mn]) in most Proteaceae. An exception is *X. occidentale*, due to its ineffective cluster roots (Zhong et al. 2021). Thus, leaf [Mn] is a proxy for carboxylate concentrations in the rhizosphere (Lambers et al. 2015; Pang et al. 2018), and, therefore, can also be used to assess facilitation of P acquisition. When using leaf [Mn] as a proxy for belowground carboxylate release, both positive and negative reference species are needed (Zhou et al. 2020). A positive reference is a species known to depend on carboxylate release for its P acquisition and has a high leaf [Mn], as found in *Banksia* species. A negative reference is a species such as *Xanthorrhoea preissii*, which is known to not depend on carboxylates for P acquisition and always has a low leaf [Mn]. Sometimes, when a negative reference cannot be found nearby, young leaves of the same test species can be used as the negative reference (Shane and Lambers 2005; Zhou et al. 2020; Lambers et al. 2021). When P is a severely limiting resource, plants will use all the absorbed Pi for growth so that leaf [P] does not increase (de Groot et al. 2003; Shane et al. 2003). In this case, leaf [P] cannot be used as a proxy for belowground interactions, while leaf [Mn] can be used.

Native plants in south-western Australia generally occur on extremely P-impoverished soils; they not only exhibit efficient P-acquisition strategies, but also use P very efficiently (Lambers et al. 2010). Photosynthetic rates are relatively fast in these scleromorphic plants (Lambers et al. 2012), in contrast to those of crop plants growing in P-impoverished soil which tend to have very slow photosynthetic rates per unit leaf area (Brooks et al. 1988). Many Proteaceae from this region have low leaf [P] compared with global values (Wright et al. 2004) and a high leaf mass per area (LMA) (Wright and Westoby 2003). Relatively fast photosynthetic rates combined with low leaf [P] typically provide

Proteaceae with very high photosynthetic P-use efficiencies (Denton et al. 2007).

Adenanthos Labill. (Proteaceae) is a genus of 33 species that are endemic to southern Australia, particularly in those parts with a Mediterranean-type climate (Nge et al. 2021). *Adenanthos cygnorum* Diels (common woolly bush) is endemic to south-western Australia (Fig. 1a; https://florabase.dpaw.wa.gov.au/browse/profile/1775). In Alison Baird Reserve (Fig. 1a and b and 32°01'10″S, 115°58'46″E), the main location of the present study, it has a low, but variable, leaf [Mn] compared with other Proteaceae (J. Wasaki et al. pers. obs.). We noticed during preliminary observations at this location that *A. cygnorum* only has tiny cluster roots compared with adjacent *B. attenuata* (Fig. S1). These observations suggested that cluster roots contribute little to its P acquisition, similar to what was found in *Xylomelum* species (Zhong et al. 2021), raising the question of how *A. cygnorum* survives on severely P-impoverished soils. We also noted that *A. cygnorum* only grows next to large *Banksia* species, *B. attenuata* and *B. menziesii*, on top of a two 2-million-year-old dune of highly weathered and severely nutrient-impoverished Bassendean sand (Turner et al. 2018) in the reserve. However, it can grow away from other Proteaceae at the bottom of the same dune. We hypothesise that soils at the bottom of the dune have a significantly higher [P] than those at the top of the dune, because P would have eroded down the slope towards the bottom of the dune.

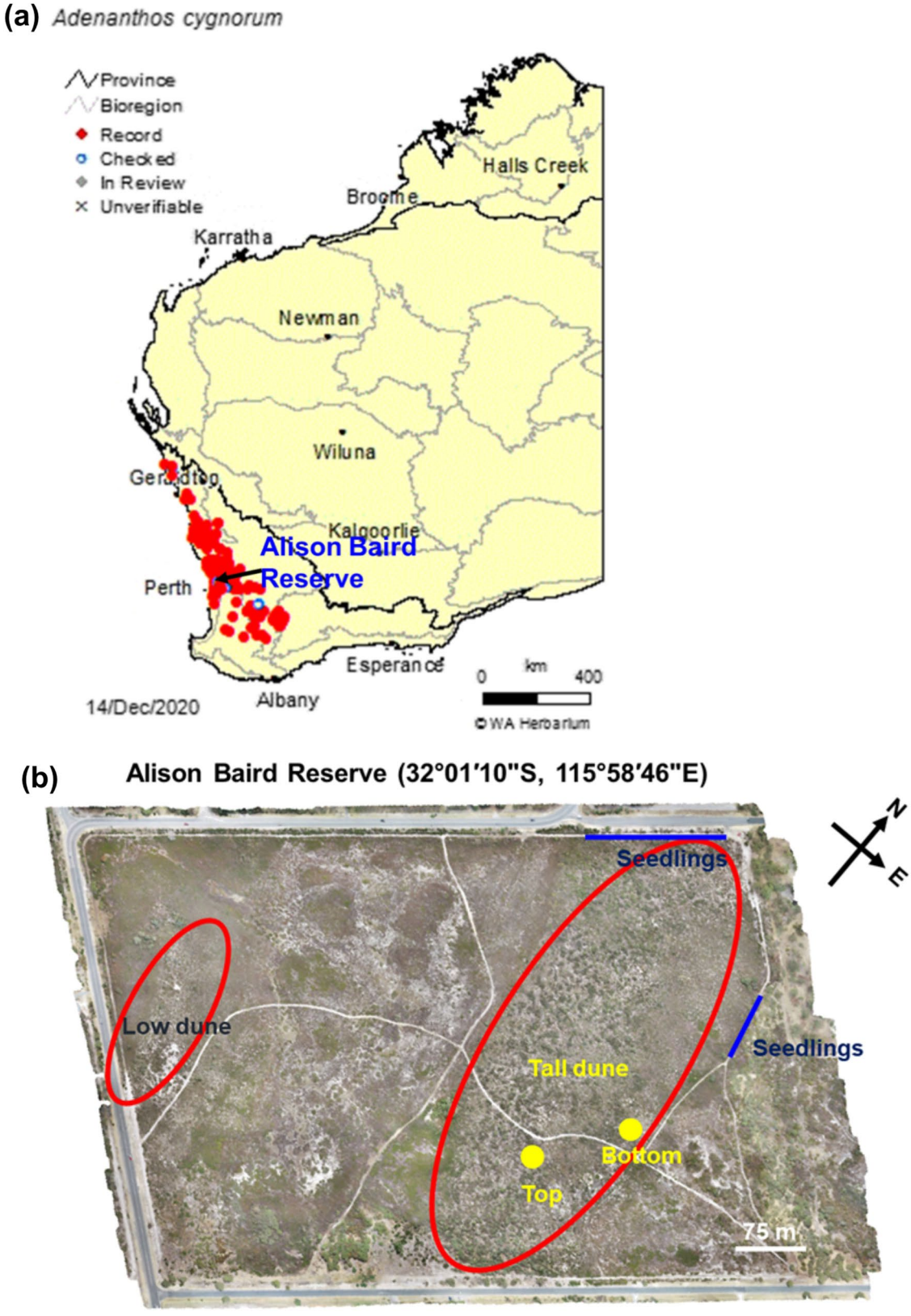
Based on our preliminary observations, we wondered how *A. cygnorum* acquired P in extremely P-impoverished habitats. We developed the following hypotheses: (1) Unlike most Proteaceae, *A. cygnorum* does not produce functional cluster roots that release large amounts of carboxylates. (2) Its roots release phosphatases into soil to hydrolyse those Po compounds that are not tightly sorbed onto soil particles, as is the case for *X. occidentale* (Zhong et al. 2021). (3) At locations where *Banksia attenuata* dominates and makes soil P available, such on top of a dune of Bassendean sand in Alison Baird Reserve, where we expect the availability of soil P to be very low (Hayes et al. 2014), P acquisition by *A. cygnorum* is facilitated by this P-mobilising neighbour. (4) At adjacent sites, where *A. cygnorum* does not grow next to tall Proteaceae, such as at the base of a dune in Alison Baird Reserve, the availability of soil P will be higher and *A. cygnorum* can acquire sufficient P without facilitation by P-mobilising neighbours. (5) *Adenanthos cygnorum* uses P efficiently in photosynthesis as most Proteaceae in south-western Australia do. To test these hypotheses, we measured soil [P] and [Mn] and leaf [P] and [Mn] in *A. cygnorum* both near and away from large *Banksia* plants, at three locations to test for facilitation of P acquisition. We also measured photosynthesis rates of *A. cygnorum* both near and away from *Banksia* plants in Alison Baird Reserve.

Materials and methods

Study area

The field experiment was conducted at three locations. The main location was in Alison Baird Reserve, a 34.6-ha flora reserve located 20 km southeast of Perth in south-western Australia (Fig. 1a, Supplementary Material 1). The other locations were near Jurien Bay (30°10'58.8"S 115°07'58.8"E), 200 km north of Perth (Hayes et al. 2014) and at Melaleuca Park (31°39′44″ S 115°55′29″ E), 70 km north of Perth (Veneklaas and Poot 2003). The climate of the study area is Mediterranean, with hot dry summers (Dec to Feb) and cool wet winters (Jun to Aug). The mean annual rainfall (1994–2022) in the Perth region is 736.8 mm, around 80% of which falls between May and September. The mean daily temperature range is 12.9–24.8 °C (1994–2022), with the highest temperature in February (18.4–31.6 °C), and the lowest in July (8.0-18.5 °C). In Jurien Bay, the mean annual rainfall is 545 mm (1968–2022), and the mean daily temperature range is 13.1–25.0 °C (1969–2022), with the highest temperature in February (18.0-30.8 °C) and the lowest in July (9.4–19.6 °C). Unless mentioned otherwise, the results shown pertain to the ecosystem in Alison Baird Reserve. The ecosystem represented by the three locations differs from most well-studied systems outside Australia (Supplementary Material 1). Each location is dominated by 2-million-year-old dunes of Bassendean sand (Turner et al. 2018). The sands of the dunes are podosols (Isabell and NCST 2016) of a marine origin.

In Alison Baird Reserve, we selected a habitat on top of a tall dune (Fig. 1b). At this site, all *A.*


(a) Adenanthos cygnorum
Province
Bioregion
Record
Checked
In Review
Unverifiable
Halls Creek
Broome
Karratha
Newman
Wiluna
Geraldton
Kalgoorlie
Alison Baird Reserve
Perth
Esperance
Albany
0 km 400
14/Dec/2020
© WA Herbarium
(b) Alison Baird Reserve (32°01′10"S, 115°58′46"E)
N
E
Seedlings
Low dune
Tall dune
Seedlings
Bottom
Top
75 m

◂**Fig. 1** **a** The distribution of *Adenanthos cygnorum* in south-western Australia. https://florabase.dpaw.wa.gov.au/browse/profile/1775. **b** Drone map of collection sites for leaf and soil samples of *Adenanthos cygnorum* in Alison Baird Reserve. On the top (yellow dot) of the taller dune (large red oval), *A cygnorum* grows next to *Banksia attenuata*. At the bottom of the taller dune (yellow dot) and on the low dune (small red oval), the sand profile is too shallow for *B. attenuata* to occur. At the bottom of the taller dune, *A. cygnorum* is able to grow in the absence of nearby Proteaceae, but it does not occur on the low dune. The locations for sampling *A. cygnorum* seedlings for phosphatase activity measurement are shown by blue lines. Bar = 75 m

cygnorum plants grow next to tall *Banksia* species such as *B. attenuata*. All *A. cygnorum* plants were within 5 m of a tall *Banksia* plant at this site, while roots of tall *Banksia* plants can extend over 10 m (Pate and Dixon 1996). We also selected a site at the bottom of the same tall dune, where tall Proteaceae other than *A. cygnorum* were absent and where *A. cygnorum* plants could be found away from all Proteaceae, except other individuals of *A. cygnorum*. All *A. cygnorum* plants in Alison Baird Reserve germinated after a fire in 2005, so plant size was similar. For comparison, we also selected a low dune (Fig. 1b) lacking both tall *Banksia* species and *A. cygnorum*. *Banksia attenuata* requires deep sand to access sufficient water during the Mediterranean summer (Canham et al. 2009); this habitat is available on top of the tall dune, but not on the low dune or at the bottom of the tall dune. Because the Bassendean dune is approximately two million years old, the seeds of both *B. attenuata* and *A. cygnorum* must have had sufficient time to arrive at the low dune from the tall dune which is less than 400 m away from the tall dune. Thus, absence of seeds of *A. cygnorum* on the low dune can be ruled out as an explanation of why it has not established there. At the Melaleuca Park and Jurien Bay locations, we also selected a habitat where all *A. cygnorum* grew next to tall *Banksia* species such as *B. attenuata* and another habitat where *A. cygnorum* grew away from all Proteaceae, except other individuals of *A. cygnorum*.

Leaf and soil sample collection

At each location, youngest fully-expanded undamaged mature leaves were collected from six individuals of *A. cygnorum* that were more than 10 m away from the nearest Proteaceae, except conspecifics, and from six individuals growing within 1 m of *B. attenuata*. For the sake of simplicity, the former plants will be referred to as *A. cygnorum* away from other Proteaceae. Leaves were collected in March, September and December 2019 in Alison Baird Reserve, in June 2020 near Jurien Bay and in June 2021 in Melaleuca Park. In Alison Baird Reserve, young and senesced leaves were also collected from the same *A. cygnorum* plants sampled for mature leaves. Senesced leaves were collected after spreading a white sheet under a bush and gently shaking the branches. In addition, at Alison Baird Reserve youngest fully-expanded undamaged mature leaves of *B. attenuata* were collected from randomly selected plants without considering their position relative to *A. cygnorum*. Leaf samples were oven-dried at 70 °C for two days, and finely ground into powder with zirconium beads (Geno/Grinder 2010, Spex SamplePrep, Metuchen, NJ, USA). Leaf samples were digested in hot concentrated HNO_3:$HClO_4$ (3:1) (Zarcinas et al. 1987) and total leaf [P] and [Mn] determined by inductively coupled plasma optical-emission spectrometry (ICP-OES, Model 5300DV, Perkin Elmer, Shelton, CT, USA).

Soil samples down to 10 cm depth and no more than 0.5 m from the base of all 12 *A. cygnorum* plants from each location were collected using a spade. We also randomly collected five soil samples from the low dune in Alison Baird Reserve down to 10 cm depth. Soil samples were air-dried at 40 °C for 72 h and sieved (< 2 mm) to remove roots and debris. Dried soil was finely ground for total element determination. Total [Mn] in sieved and ground soil was measured after an aqua regia (HNO_3/HCl) digestion by ICP-OES as previously described (Rayment and Higginson 1992) (Optima 7300DV; Perkin Elmer Inc, Wellesley, MA, USA). Total and organic [P] was measured by the ignition method and extracted from soil by 1 M HCl (Saunders and Williams 1955). For readily plant-available soil P (measured as resin [P], readily exchangeable Pi), soil samples were shaken for 16 h with 30 mL deionized (DI) water and Pi extracted using anion-exchange membrane (AEM) strips (Turner and Romero 2009). The AEM strips were then washed free of soil particles with DI water. After washing, the strips were transferred to 10 mL 0.5 M HCl and shaken for 1 h to extract the Pi.

Leaf gas exchange

Leaf gas exchange was measured on youngest fully-expanded undamaged green leaves between 9:00 and 11:00 am on plants in Alison Baird Reserve (LI6400XT portable photosynthesis system, Li-Cor Inc., Lincoln, NE, USA). During measurements, leaf temperature and photosynthetic photon flux density were maintained at 25.0 ± 0.5ºC and 1200 µmol photons m^{-2} s^{-1}, respectively. The reference CO_2 concentration, vapour pressure and relative humidity were 400 µmol mol^{-1}, 1.1 ± 0.05 kPa and 55–65%, respectively. Leaf area was measured by scanning at a resolution of 200 dpi (Epson Perfection V800, Seiko Epson, Nagano, Japan) and the images were analysed with WinRHIZO software (Regent Instruments, Quebec, Canada).

Glasshouse experiments

Glasshouse experiments were conducted at the University of Western Australia, Perth, Australia (31.57°S, 115.47°E) from September 2019 to June 2020 in a controlled glasshouse environment with min/max temperatures of 20/32˚C in summer and 13/23˚C in winter. Relative humidity was maintained at approximately 60% in the glasshouse. An overhead shade screen (~60% light transmission) automatically deployed if photosynthetically active radiation (PAR) exceeded 1650 µmol m^{-2} s^{-1}.

Hydroponic plant growth for root carboxylate measurement

Adenanthos cygnorum seedlings were collected from a fire break (Supplementary Material 1) on a Bassendean dune near Jurien Bay, south-western Australia, in September 2019 and transferred into 4.5 L pots containing 4 L continuously aerated nutrient solution with one plant per pot. Pots were placed in a root-cooling tank to maintain the root temperature at 18–20˚C for plant growth. Nutrient solution (pH 5.8) had the following composition (in µM): 0.5 P (applied as KH_2PO_4), 0.12 Mn^{2+}, 200 NO_3^-, 100 Ca^{2+}, 100 K^+, 77 SO_4^{2-}, 27 Mg^{2+}, 20 Cl^-, 1.0 Fe-EDTA, 0.05 Zn^{2+}, 0.02 Cu^{2+}, 1.2 H_3BO_3, 0.15 Mo^{4+}, and 50 SiO_3^{2-}. Nutrient solution was changed twice a week.

Plants were harvested 30 weeks after transfer to hydroponics. Carboxylate exudation was measured on whole root systems that included cluster roots and non-cluster roots, and on mature cluster roots separately. After excision, whole root systems or mature cluster roots were soaked in 15 ml or 1.5 ml, respectively, of basal nutrient solution without P and calcium (Ca) for 20 min to avoid effects from root detachment. Roots were then transferred to another tube containing the same nutrient solution, aerated gently with a stream of air, covered with aluminium foil, and incubated for 90 min. After incubation, the solution containing root exudates was filtered using a 0.2 µm syringe filter and fresh weight of the root was determined. The filtered solution (1.0 ml) was transferred to an HPLC vial to which 20 µl concentrated ortho-phosphoric acid was added and mixed well. The carboxylates exuded from the root were then determined (Cawthray 2003) (Waters HPLC, 600E dual head pump, 717 plus autosampler, and 996 photodiode-array detector; Milford, MA, USA).

Soil experiments for root phosphatase measurement

Soil for potting was collected near the bottom of the tall dune in Alison Baird Reserve more than 10 m from the nearest tall Proteaceae, dried at 40˚C for one week, and sieved using a 2-mm strainer to remove large plant debris. The *A. cygnorum* seedlings collected from the field were grown in hydroponics for 30 weeks and then eleven seedlings of uniform size were transferred to large free-draining PVC pots that contained 3.0 kg sieved soil. These pots were watered with deionised water to maintain adequate soil moisture.

After six months, *A. cygnorum* root systems were harvested. Rhizosheath soil, the soil compartment adhering to the root surface (Hinsinger et al. 2009), was collected by shaking plants gently to remove bulk soil, and then using a paint brush to remove the rhizosheath soil. Acid phosphatase activity was measured spectrophotometrically using *p*-nitrophenol as substrate (Tabatabai 1994). As a comparison, phosphatase activity was also determined for 11 *A. cygnorum* seedlings collected from two sites (Fig. 1b) in Alison Baird Reserve in October 2020 using the same method described above.

Calculations

Leaf mass per area (LMA) was calculated by dividing leaf dry weight (DW) by leaf area. Photosynthetic rate on a leaf DW basis was calculated by dividing photosynthetic

rate on a leaf area basis by LMA. Photosynthetic phosphorus-use efficiency (PPUE) was calculated by dividing photosynthetic rate on a leaf DW basis by leaf [P] on a leaf DW basis. Phosphorus-resorption was calculated by dividing senesced-leaf P content by mature-leaf P content. Weight loss was taken into consideration for senesced leaves to recalculate P content using mature leaf weight divided by senesced leaf weight.

Leaf anatomy

Cross-sections of fully expanded mature leaves of *A. cygnorum* were prepared using a microtome (Leica RM2125 RTS; Leica Biosystems Nussloch GmbH, Heidelberg, Germany). They were viewed under epifluorescence microscopy (Axioskop optical microscope, El-Einsatz; Carl Zeiss, Oberkochen, Germany), and photos were taken using an Axiocam digital camera (Carl Zeiss Microscopy, GmbH).

Statistics

Leaf and soil [Mn], leaf [P], leaf DW, photosynthetic rates, LMA, PPUE and phosphatase activity were analysed by unpaired Student's *t*-test (SAS 8.1, USA). Analysis of variance (ANOVA) for soil [P] was conducted using the one-way non-block experiment ANOVA model in SAS. Homogeneity of variance among the variables was distributed normally according to the SAS algorithm. Significant differences between or among means were determined by LSD at $P \leq 0.05$. All graphs were produced using SigmaPlot (SigmaPlot 10.0, USA).

Results

Leaf and soil manganese concentrations

Mature leaf [Mn] of *A. cygnorum* from both study sites on the tall dune at Alison Baird Reserve was around twice that of young leaves (Fig. 2a), while mature leaf [Mn] of *B. attenuata* was significantly higher than that of *A. cygnorum* growing at either site. In young *A. cygnorum* leaves on the tall dune, there was no significant difference in [Mn] next to *B. attenuata* or 10 m away from other Proteaceae. The mature leaf [Mn] at the other two locations, like that at Alison Baird Reserve, was two-fold greater in

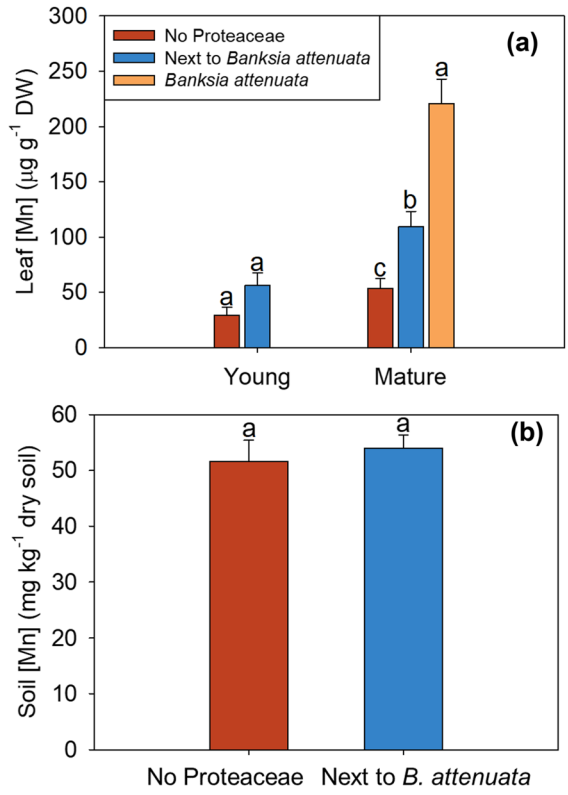


Fig. 2 **a** Manganese concentrations ([Mn]) of young and mature leaves of *Adenanthos cygnorum* growing more than 10 m away from the nearest large Proteaceae and within 1 m of *Banksia attenuata* and mature leaf [Mn] of *B. attenuata*. **b** Soil total [Mn] under *A. cygnorum* growing more than 10 m away from the nearest Proteaceae or within 1 m of *B. attenuata*. The bars represent means ± SE ($n = 6$). Different letters indicate significant differences at $p < 0.05$ within a leaf type (young / mature leaves) (**a**) or between sites (**b**)

plants next to *B. attenuata* than that in plants 10 m away from other Proteaceae (Figs. 2a, S2). However, soil [Mn] under *A. cygnorum* at these locations was similar next to *B. attenuata* and 10 m away from other tall Proteaceae (Figs. 2b, S3).

Leaf and soil phosphorus concentrations

In young leaves of *A. cygnorum* away from other Proteaceae at Alison Baird Reserve, [P] was 30% greater than that of plants next to *B. attenuata* (Fig. 3a). The [P] in young leaves was at least ten-fold higher than that in mature leaves at both sites (Fig. 3a). At each location, including Alison Baird Reserve, the [P] of mature leaves sampled next to *B. attenuata* and away from

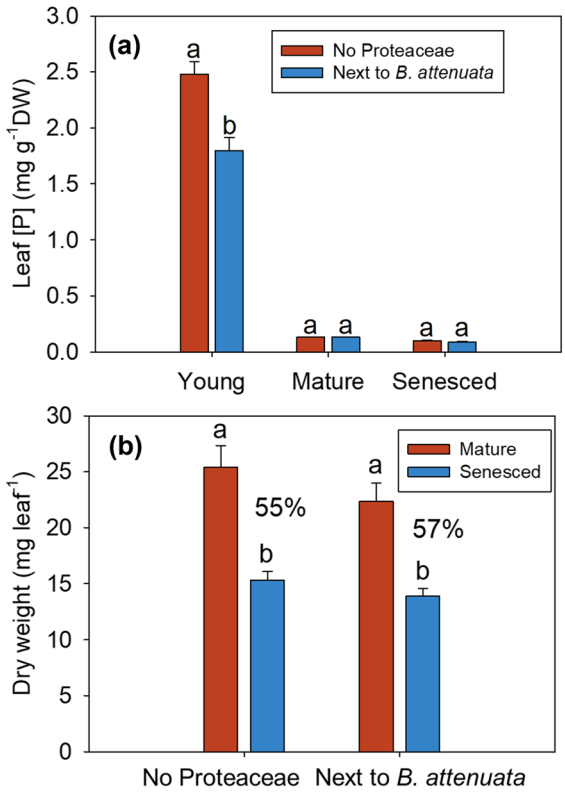


Fig. 3 **a** Phosphorus concentrations ([P]) of young, mature and senesced leaves, and **b** dry weight per mature or senesced leaf of *Adenanthos cygnorum* growing more than 10 m away from the nearest Proteaceae and within 1 m of *Banksia attenuata*. The percentage P resorption efficiency from senescing leaves is indicated above the bars for each location. The bars represent means ± SE ($n = 6$). Different letters indicate significant differences at $p < 0.05$ within a leaf type (**a**), and between sites (**b**)

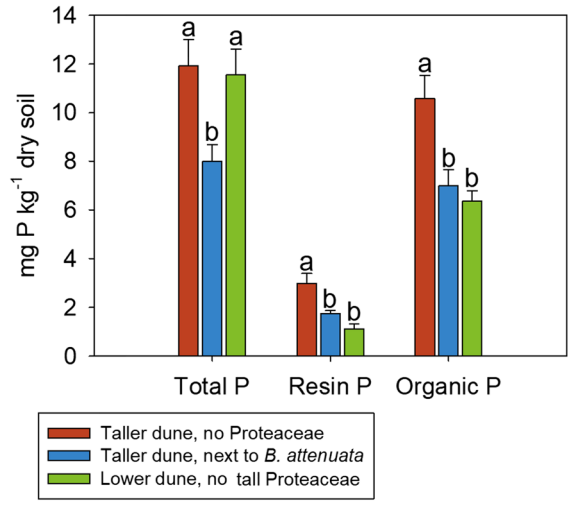


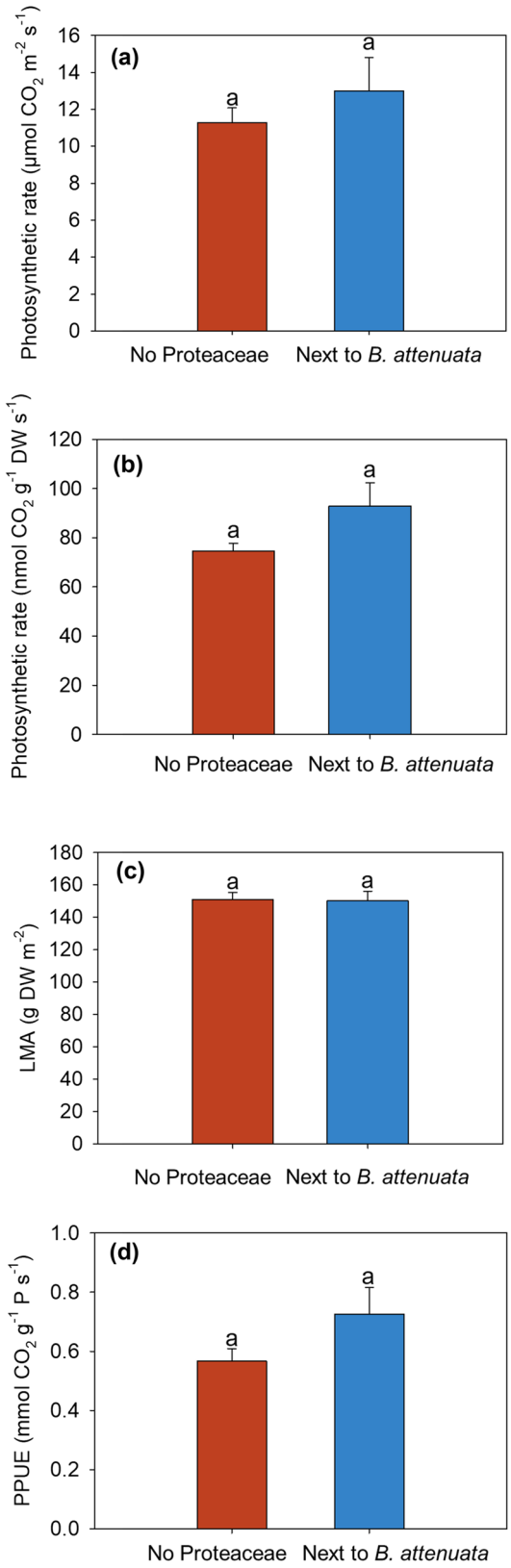
Fig. 4 Total phosphorus (P), resin P and organic P concentration of soils sampled under *Adenanthos cygnorum* growing more than 10 m away from the nearest Proteaceae and within 1 m of *Banksia attenuata*, and on the lower dune lacking both species. The bars represent means ± SE ($n = 5$). Different letters indicate significant differences at $p < 0.05$ among sites

other Proteaceae was very low and the same (Figs. 3a, S4). Similarly, there was no significant difference in [P] between senesced leaves at the contrasting sites in Alison Baird Reserve. On average, [P] of senesced leaves was only 30% lower than that of mature leaves (Fig. 3a). However, on average, leaf dry weight (DW) of mature leaves was about 60% greater than that of senesced leaves (Fig. 3b). Taking into account the loss of DW in senescing leaves, showed that P resorption was about 55% for leaves sampled either next to *B. attenuata* or away from other Proteaceae (Fig. 3b).

Soil organic P concentration ([Po]) was significantly greater than soil resin [P] at all three locations, irrespective of sampling site (Figs. 4, S5, S6, S7). At Alison Baird Reserve, soil resin [P] under *A. cygnorum* away from other Proteaceae, at 2.99 mg P kg^{-1} dry soil, was significantly higher than that of soils under *A. cygnorum* next to *B attenuata* and on the low dune, at 1.75 and 1.12 mg P kg^{-1} dry soil, respectively (Fig. 4). Similarly, soils under *A. cygnorum* growing away from other Proteaceae contained about 50% more Po than those under plants next to *B. attenuata* or soils sampled at the low dune (Fig. 4). Total [P] of soil under *A. cygnorum* growing away from other Proteaceae and of soil from the lower dune was about 50% greater than that under *A. cygnorum* next to *B. attenuata* (Fig. 4). At the other two sampling locations, soil total [P], resin [P] and organic [P] under *A. cygnorum* away from other Proteaceae was also significantly greater than that of soil under *A. cygnorum* next to *B. attenuata* (Figs. S5, S6, S7).

Statistical comparisons were only made between the two sites at each location but not among the sites at all three locations because the topography of the locations was not equivalent. At Alison Baird Reserve, there is a distinct dune with a top and a bottom deposited on a substrate with a different soil composition to the dune (Leopold and Zhong 2019). The locations sampled in Melaleuca Park and in Jurien Bay were deep Bassendean sand over a wide area where we were able to find some *A. cygnorum*

Fig. 5 **a** Net photosynthetic rates on a leaf area basis and **b** on a leaf mass basis, **c** leaf mass per unit area (LMA) and **d** photosynthetic phosphorus-use efficiency (PPUE) of youngest fully-expanded leaves of *Adenanthos cygnorum* growing more than 10 m away from the nearest Proteaceae and within 1 m of *Banksia attenuata*. The bars represent means ± SE ($n = 6$). Different letters indicate significant differences at $p < 0.05$



away from other large Proteaceae. Moreover, the substrate underlying the sand dunes at the three locations was not the same. The results we present are mainly based on the study location in Alison Baird Reserve, but, importantly, the results from the other two locations were consistent with what we found in Alison Baird Reserve (Figs. S5, S6, S7).

Photosynthetic rates and photosynthetic P-use efficiency (PPUE)

Photosynthetic rates on both a leaf area basis (Fig. 5a) and a leaf DW basis (Fig. 5b) for *A. cygnorum* plants growing next to *B. attenuata* were similar to those of plants growing > 10 m away from other large Proteaceae in Alison Baird Reserve. Leaf mass per area (LMA) was the same at both sites (Fig. 5c). Moreover, the photosynthetic P-use efficiency (PPUE) was similar for *A. cygnorum* at the two sites in Alison Baird Reserve (Fig. 5d). The average PPUE across the two sites was 0.65 mmol CO_2 g^{-1} P s^{-1} (average photosynthetic rate = 84 nmol CO_2 g^{-1} DW s^{-1}; average leaf [P] = 0.13 mg g^{-1} DW).

Leaf anatomy of *Adenanthos cygnorum*

There were no clear structural or anatomical differences in *A. cygnorum* leaves between sites at Alison Baird Reserve. Chloroplasts were mainly localised to two layers of palisade mesophyll cells beneath the upper epidermis (Fig. 6). There were abundant lignified sclereids with thick cell walls embedded in the palisade cell layers. A single layer of spongy mesophyll cells, which contained many calcium crystals, was present interior to the palisade mesophyll cells (Fig. 6). There were no stomatal crypts in the leaves.

Carboxylates and phosphatases released from *Adenanthos cygnorum* roots

Adenanthos cygnorum plants had tiny cluster roots that lived for no more than seven days in the

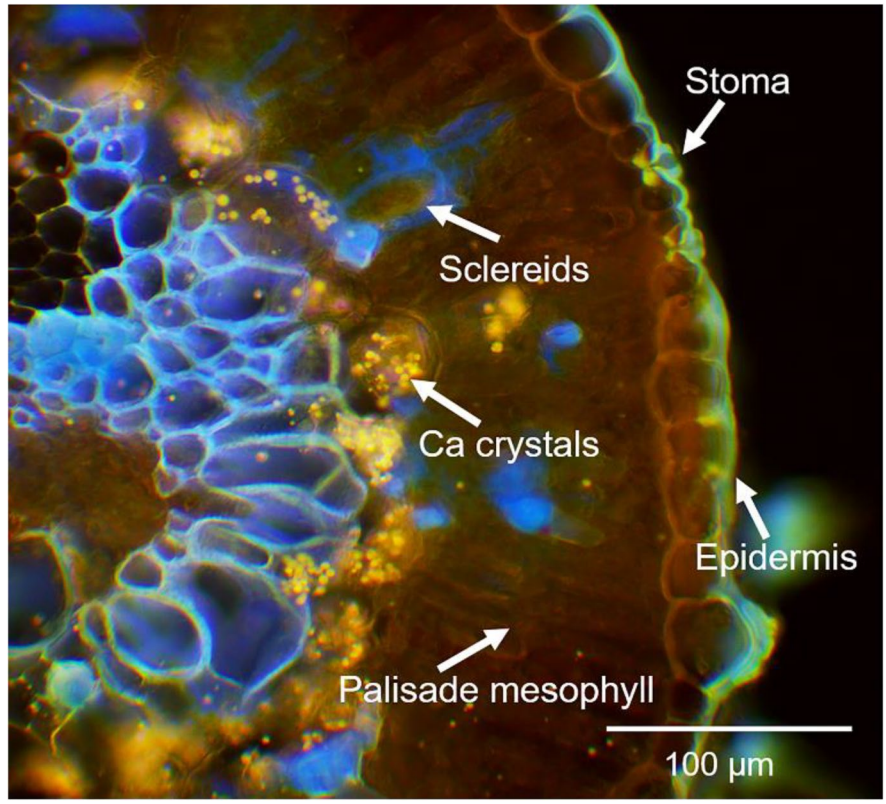


Fig. 6 Bright autofluorescence emitted from a cross-section of a mature leaf of *Adenanthos cygnorum* observed under epifluorescence microscopy with a UV-filter. Note the absense of stomatal crypts. Bar = 100 µm

present hydroponic experiment, in contrast with most Proteaceae species, including *Hakea prostrata* (Proteaceae), which produce larger cluster roots that live for up to three weeks (Fig. 7a). Citrate, malate, oxalate and *iso*-citrate were detected among the exudates released by whole root systems and cluster roots of *A. cygnorum* (Table 1). Total carboxylate exudation rates in whole root systems were significantly faster than those in cluster roots. The major exuded carboxylates collected from whole root systems were citrate and oxalate, which accounted for 45% and 41% of the total exuded carboxylates, respectively. For cluster roots, the major exuded carboxylate was oxalate, which accounted for 68% of the total exuded carboxylates, whereas citrate accounted for only 20%.

Carboxylate-exudation rates in both whole root systems and cluster roots of *A. cygnorum* were slow compared with those of other Proteaceae (Table 1). In *A. cygnorum*, the rate of citrate exudation from cluster roots was only 0.05 nmol g^{-1} root FW s^{-1}, which was much slower than that in other Proteaceae (Table 1).

Acid phosphatase activity in the rhizosheath of seedlings of *A. cygnorum* (Fig. 7b) from both the field and the glasshouse were similar at about 25 nkat g^{-1} soil, which was significantly higher than the activity in the surrounding bulk soil (Fig. 8). Acid phosphatase activity in the rhizosphere soil for seedlings from the field varied more widely than that of glasshouse-grown seedlings.

Discussion

The present results provide compelling evidence that in severely P-depleted soils, P acquisition by *A. cygnorum* was facilitated by the P-mobilising species *B. attenuata*. Based on an analysis of leaf [Mn], as outlined in Zhou et al. (2020), we conclude that P uptake in *A. cygnorum* was enhanced by P mobilisation by the root exudates released by *B. attenuata* (Shi et al. 2020). In contrast with the leaf [Mn] in *A. cygnorum*, the leaf [P] was not affected by facilitation. This is because when P is a severely limiting resource, plants use all the Pi that is taken up for growth, without increasing their leaf [P]

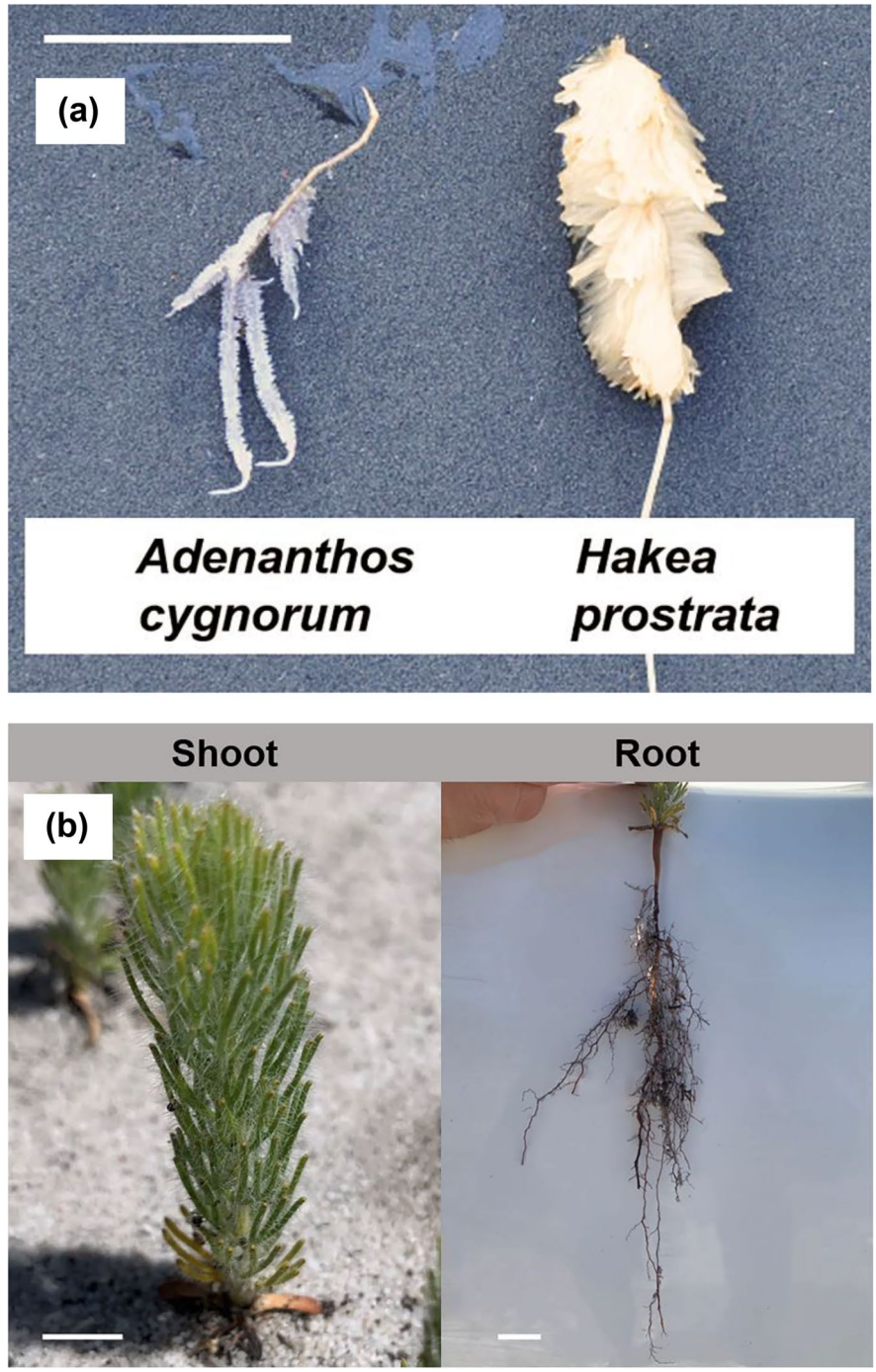


Fig. 7 **a** Cluster roots of *Adenanthos cygnorum* and *Hakea prostrata* (Proteaceae) plants grown in aerated hydroponics with low (1 µM) P supply. **b** Shoots and roots of *A. cygnorum* seedlings from Alison Baird Reserve. Photos: Qi Shen. Bars = 1 cm

(de Groot et al. 2003; Shane et al. 2003). *Adenanthos cygnorum* did not produce large and long-lived cluster roots that released significant amounts of carboxylates into soil as do most Proteaceae. However, this species did release phosphatases into soil which hydrolyse Po. Thus, we partly accept our first hypothesis that *A. cygnorum* does not produce functional cluster roots and accept our second hypothesis that *A. cygnorum* releases phosphatases that hydrolyse Po. The concentration of Po in old soils such as those in Alison Baird Reserve,

Table 1 Carboxylate-exudation rates of whole root systems or mature cluster roots of plants grown in aerated nutrient solution with low phosphorus (P) supply (mostly 1 µM P, with nutrient solutions replaced once a week)

Species	Organ	Exudation of carboxylates (nmol g^{-1} FW s^{-1})					Reference
		Citrate	Malate	Oxalate	*Iso*-citrate	Total carboxylates	
Adenanthos cygnorum	Whole root systems	0.29	0.02	0.26	0.07	0.64[a]	Present study
Adenanthos cygnorum	Cluster roots	0.05	0.02	0.17	0.01	0.25[b]	Present study
Banksia occidentalis	Cluster roots	0.52	0.21			1.4	Roelofs et al. (2001)
Banksia sessilis	Cluster roots	0.41	0.15			1.5	Roelofs et al. (2001)
Banksia prionotes	Cluster roots	0.23	0.12			1	Roelofs et al. (2001)
Hakea baxteri	Cluster roots	0.42	0.21			1.4	Roelofs et al. (2001)
Hakea prostrata	Cluster roots	0.77	0.22			2.2	Roelofs et al. (2001)
Hakea undulata	Cluster roots	0.5	0.25			2.5	Roelofs et al. (2001)
Hakea petiolaris	Cluster roots	0.25	0.2			1	Roelofs et al. (2001)
Hakea prostrata	Cluster roots					5	Lambers et al.(2003)
Hakea prostrata	Cluster roots	0.35	0.33			3.5	Shane et al. (2004b)
Embothrium coccineum	Cluster roots	0.85	0.32				Delgado et al. (2014)
Banksia attenuata	Cluster roots	0.01	0.35				Shi et al. (2019)
Banksia sessilis	Cluster roots	0.42	0.28				Shi et al. (2019)
Xylomelum occidentale	Whole root systems					ND	Zhong et al. (2021)
Lupinus albus	Cluster roots	0.22–0.66					Keerthisinghe et al. (1998)
Lupinus albus	Cluster roots	0.2–0.8					Kamh et al. (1999)
Lupinus albus	Cluster roots	0.2	0.02				Neumann et al. (1999)
Lupinus albus	Cluster roots	0.27	0.01				Hocking and Jeffery (2004)

Different letters indicate significant differences at $p < 0.05$ between root types for *Adenanthos cygnorum*

Hakea prostrata received 4 µM P every two weeks (Lambers et al. 2003). *Embothrium coccineum* received 1 µM P every day (Delgado et al. 2014). *Banksia attenuata* and *B. sessilis* received 1 µM P, replenished three times per week (Shi et al. 2020)

ND not detected

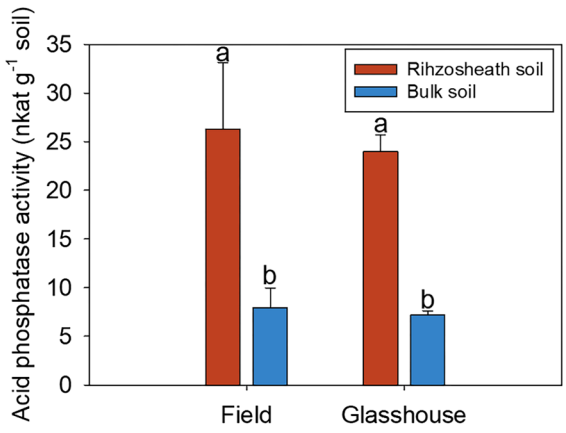


Fig. 8 Acid phosphatase activity of rhizosheath soil and bulk soil for *Adenanthos cygnorum* growing in the field and in pots of soil in the glasshouse. The bars represent means ± SE (n = 11). Different letters indicate significant differences at $p < 0.05$ between soil types

the main location for the present work, and the two other sampling locations (Fig. S6) was higher at the bottom of the dune, away from banksias than that on top of the dune, near banksias. The chemical nature of organic P in these old soils is such that it is not tightly bound (Zhong et al. 2021). Therefore, released phosphatases would provide access to Po, even without involvement of carboxylates which supports our third and fourth hypotheses. Also, *A. cygnorum* used P efficiently, as do most Proteaceae in P-impoverished habitats (Hayes et al. 2018) which is consistent with our fifth hypothesis.

Functional diversity of cluster roots and root exudation among species

Adenanthos cygnorum did not produce cluster roots that function like those in most Proteaceae (Shane and

Lambers 2005); its short-lived and tiny cluster roots did not release substantial amounts of carboxylates into the rhizosphere to mobilise P and Mn. Instead, acid phosphatase activity in its rhizosheath was significantly higher than that in bulk soil. When grown in a low-P nutrient solution, the total exudation rates of di- and tri-carboxylates by *A. cygnorum* in both whole root systems and cluster roots were slow compared with those of other Proteaceae (Table 1). Hundreds of lateral roots in a single cluster root release carboxylates in an exudative burst into soil, and thus solubilise large amounts of P sorbed onto soil particles (Shane and Lambers 2005). Since cluster roots of *A. cygnorum* are tiny and short-lived, their ability to solubilise sorbed P will be low, despite relatively fast citrate exudation in whole root systems. The importance of carboxylate release by cluster roots relative to release by the whole root system was shown in a field experiment with three high-carboxylate-exuding *Lupinus* (Fabaceae) species. The two species with cluster roots (*L. albus* and *L. luteus*) produced more biomass in P-impoverished soil than the one without cluster roots (*L. angustifolius*) (Bolland et al. 2000). No real-soil experiment was done to fill the gap between the field and hydroponic experiment in this study. However, the low leaf [Mn] in *A. cygnorum* growing without a P facilitator nearby confirmed that its exudates were ineffective. In summary, exuded carboxylates are more effective when released from cluster roots over a short time in a confined volume, rather than steadily from the entire root system into a larger volume.

Depending on species, the dominant carboxylates released by roots are malate or citrate, including in most Proteaceae (Table 1) (Gardner and Boundy 1983; Lambers et al. 2006). In *H. prostrata*, mature cluster roots released citrate and malate at 0.7 and 1.1 nmol g^{-1} root FW s^{-1}, respectively (Shane et al. 2004b; Roelofs et al. 2001) (Table 1). Many plants also release oxalate, especially under aluminium (Al) stress (Ryan et al. 1995). Tartrate is a dominant exudate in some species such as *Medicago sativa* (Fabaceae) (He et al. 2020). In contrast to most Proteaceae, oxalate was a dominant exudate from cluster roots of both *A. cygnorum*, and a phylogenetically distantly-related Proteaceae, *Euplassa cantareirea* (de Britto Costa et al. 2016).

Roots not only release carboxylates, but also phenolics, such as catechin (Weir et al. 2006), which may inhibit growth of neighbours because it triggers the production of toxic reactive oxygen species and may result in cell death in roots of neighbouring plants (Bais et al. 2003). However, oxalate reduces the negative effect of phenolics. For example, *Lupinus sericeus* and *Gaillardia grandiflora* (Asteraceae) release large amounts of oxalate in response to catechin exposure, and thus block the generation of reactive oxygen species and reduce oxidative damage caused by catechin (Weir et al. 2006). Cluster roots of *B. attenuata* also exude phenolics (Beeck 2017), which might lead to negative effects on *A. cygnorum*. Therefore, the release of oxalate by *A. cygnorum* may act as an antioxidant defence (Kayashima and Katayama 2002) to prevent oxidative damage caused by the phenolics released by *Banksia* cluster roots. This response would enhance the ability of *A. cygnorum* to be facilitated by *B. attenuata* to acquire P and provide a potential explanation of why oxalate was released from the entire root system, rather than predominantly from cluster roots.

Accessing phosphorus without carboxylates

In P-impoverished soil, it appears that acid phosphatases released by *A. cygnorum* can access P by hydrolysing forms of Po, such as phospholipids and nucleic acids, that are not tightly bound to soil particles, similar to the strategy used by *X. occidentale* to access soil Po (Zhong et al. 2021). However, different from *X. occidentale*, which does not show any evidence of facilitation because its leaf [Mn] is invariably low (Zhong et al. 2021), the habitat range of *A. cygnorum* was extended by being facilitated by neighbouring P-mobilising *Banksia* species to acquire P on soils with extremely low resin P and Po concentrations, which will be explained below. Like *A. cygnorum*, other plants exude acid phosphatases into soil to hydrolyse Po that is not tightly bound to soil particles. This is true for some Fabaceae in south-western Australia (Png et al. 2017). Generally, root phosphatase activity of legumes is higher than that of other species (Houlton et al. 2008; Olde Venterink 2011). *Nicotiana tabacum* (Solanaceae) also exudes enzymes that hydrolyse Po. In this case, the plants also exude citrate that helps desorb phytate from soil particles, making it more available for hydrolysis by released phytases, enhancing their effectiveness. The hydrolysis of phytate enhances shoot P accumulation

and soil Po depletion (Giles et al. 2017). This carboxylate-dependent strategy was not exhibited in *A. cygnorum*.

The mature leaf [Mn] of *Banksia* species varies from 126 to 439 µg Mn g^{-1} DW (Denton et al. 2007), much greater than the concentration considered adequate for crop growth (50 µg Mn g^{-1} DW) (Epstein and Bloom 2005). By comparison, in the present study, leaf [Mn] of *B. attenuata* was 220 µg Mn g^{-1} DW, and *A. cygnorum* growing next to it had a leaf [Mn] of 108 µg Mn g^{-1} DW, significantly higher than that of *A. cygnorum* growing away from other Proteaceae (50 µg Mn g^{-1} DW) (Fig. 2a). This difference in leaf [Mn] in *A. cygnorum* was despite there being no difference in soil [Mn] between the sites, indicating that differences in leaf [Mn] were due to differences other than soil [Mn]. The higher leaf [Mn] in *A. cygnorum* growing next to *B. attenuata* than in individuals growing > 10 m away from other large Proteaceae at all three sampling locations indicates facilitation of nutrient uptake, especially of P and Mn, promoted by carboxylates released by neighbouring *B. attenuata*. Plants cannot access sorbed P from soil directly, but both P and Mn are mobilised by carboxylates, which are released in large amounts by cluster roots (Lambers et al. 2015). In this study, soil resin [P] at the bottom of the dune at Alison Baird Reserve was significantly higher than that on top of the dune, whereas leaf [P] of plants growing on top of the dune was the same as that of plants growing at the bottom of the dune. Therefore, rather than accumulating P, the plants used all the absorbed P to support their growth, as has been shown before for other species when grown at very low P availability (de Groot et al. 2003; Shane et al. 2003). Low leaf [Mn] in *A. cygnorum* growing away from other large Proteaceae at all three of the locations we tested indicates that its roots did not release a large amount of carboxylates into the soil, or that the released carboxylates were rapidly metabolised by microbes in the rhizosheath soil (D'Angioli et al. 2017).

Nutrient-mobilisation-based facilitation by cluster roots involves the following processes. First, the release of nutrient-mobilising carboxylates or molecules with a similar effect into the rhizosphere (Lambers et al. 2018). Second, the ability of the roots of the facilitated plants or mycorrhizal hyphae to sense the activity of cluster roots and then intermingle with the cluster roots (Teste et al. 2020). As P is immobile in soil, root intermingling likely also happened between *A. cygnorum* and *B. attenuata* to allow facilitation of P uptake. Facilitation of Mn uptake by neighbouring plants is well documented (Gardner and Boundy 1983; Lambers et al. 2015). In steppe vegetation in Inner Mongolia, P-mobilising species also enhance leaf [Mn] in some of their non-P-mobilising neighbours (Yu et al. 2020). In a natural habitat on the Swan Coastal Plain of south-western Australia, leaf [Mn] in *Hibbertia hypericoides* (Dilleniaceae) is relatively high when growing next to *Banksia* species compared with the negative reference (Zhong et al. 2021), and the roots of *Hibbertia* species are found intermingled with cluster roots of adjacent Proteaceae (Lambers et al. 2022). In summary, P-mobilising species enhance Mn acquisition and leaf [Mn] in some of their neighbours, but leaf [P] may not be enhanced.

Leaf [P] of *Banksia* species in their natural P-impoverished habitats are extremely low, in the range of 0.14 to 0.32 mg P g^{-1} DW (Denton et al. 2007), compared with concentrations of typically 2 mg P g^{-1} DW that are required for crop species (Epstein and Bloom 2005). The average leaf [P] of 2548 species from 175 natural sites across the world is 0.99 mg P g^{-1} DW (Wright et al. 2004). Leaf [P] of plants sampled from low-P habitats in low-rainfall environments are also low (0.38 to 1.1 mg P g^{-1} DW) (Wright and Westoby 2003), but higher than those of *Banksia* species in south-western Australia (Denton et al. 2007). However, in the present study, irrespective of the location, leaf [P] in *A. cygnorum* mature leaves, at 0.13 mg P g^{-1} DW, was even lower than that in *Banksia* species. In contrast, leaf [P] in young expanding *A. cygnorum* leaves collected in the field was more than 10-fold higher than that in mature leaves of many Proteaceae species. This high [P] is associated with its strategy of delayed greening, which is a P-saving strategy in plants in severely P-impoverished habitats (Sulpice et al. 2014; Kuppusamy et al. 2021). Thus, *A. cygnorum* took up P that was available in the soil and accumulated it in young expanding leaves, to be used to support leaf maturation.

South-western Australia has some of the most P-impoverished soils in the world; 50% of the soils in south-western Australia have total [P] less than 100 mg P kg^{-1} dry soil, with 6.5% having no more

than 20 mg P kg^{-1} dry soil (Kooyman et al. 2017). In this study, soil total [P] was very low at all sampled sites, in the lowest or extremely P-poor category of no more than 20 mg P kg^{-1} dry soil. Plant-available [P] (approximated here as resin [P]) in soil under *A. cygnorum* was also very low. However, soil resin [P] under *A. cygnorum* > 10 m away from other Proteaceae species was significantly greater than that when *A. cygnorum* was < 1 m away from *B. attenuata* or on the low dune at Alison Baird Reserve, where *A. cygnorum* is absent. The low resin [P], combined with the low [Po], may explain why *A. cygnorum* does not grow on the low dune. With no facilitating tall *Banksia* trees on the low dune and very low plant-available [P] and Po in soil, the soil P availability is simply too low to sustain its growth.

Phosphorus-use efficiency in photosynthesis and growth

Adenanthos cygnorum used P very efficiently as an adaptation to extremely P-impoverished soils. Photosynthetic rates on a leaf area basis for *A. cygnorum* were around 12 µmol CO_2 m^{-2} s^{-1}, similar to that of *Hakea* species (Lambers et al. 2012). Photosynthesis rates for *Banksia* species are typically faster than those for *Hakea* species (20 µmol CO_2 m^{-2} s^{-1} and 13 µmol CO_2 m^{-2} s^{-1}, respectively) (Lambers et al. 2012). *Banksia* species with thick leaves have sunken stomata located in crypts (Lambers et al. 2014), epidermal depressions whose depth is tightly correlated with leaf thickness (Hassiotou et al. 2009). Deep stomatal crypts with numerous sunken stomata contribute to a shorter path for CO_2 diffusion from air to chloroplast which explains the faster photosynthesis rates in *Banksia* species compared with those of *Hakea* species with similar leaf thickness, but lacking crypts (Hassiotou et al. 2009). The pathway for water vapour diffusion is shortened to the same extent by stomatal crypts, and thus they have no significant effect on photosynthetic water-use efficiency (Roth-Nebelsick et al. 2009). We found that *A. cygnorum* leaves lacked stomatal crypts (Fig. 5a), similar to what has been found for *Hakea* species (Lambers et al. 2014). The lack of crypts likely contributed to the slow photosynthesis rate of *A. cygnorum* compared with that of *Banksia* species.

Photosynthetic P-use efficiency of *A. cygnorum* (0.65 mmol CO_2 g^{-1} P s^{-1}) was higher than that of *Banksia* species (0.19 to 0.46 mmol CO_2 g^{-1} P s^{-1}) (Denton et al. 2007), other Proteaceae (mean value 0.2 mmol CO_2 g^{-1} P s^{-1}) (Guilherme Pereira et al. 2019) or global average values determined under field conditions (0.10 mmol CO_2 g^{-1} P s^{-1}) (Wright et al. 2004). Photosynthetic P-use efficiency of *Pinus strobus* (Pinaceae) growing in soil with different nutrient availability is very low, at 0.02 to 0.08 mmol CO_2 g^{-1} P s^{-1} (Reich and Schoettle 1988). In a low-P tropical forests in Guyana, PPUE varies from 0.10 mmol CO_2 g^{-1} P s^{-1} to 0.26 mmol CO_2 g^{-1} P s^{-1} (Raaimakers et al. 1995), much lower than that of *A. cygnorum*. Despite its low photosynthetic rates, PPUE was very high in *A. cygnorum*, because of its extremely low leaf [P] of 0.13 mg P g^{-1}.

Plants can increase their P-use efficiency for growth by decreasing their demand for external P and efficiently resorbing and reallocating P from senescing leaves (Aerts 1996; Wright and Westoby 2003). Phosphorus re-mobilisation from senescing leaves is an important mechanism to maintain a high P-use efficiency for growth (Denton et al. 2007; Guilherme Pereira et al. 2019). Phosphorus resorption from senescing leaves is up to about 90% in Proteaceae growing on P-impoverished soils in south-western Australia (Wright et al. 2004; Denton et al. 2007). Phosphorus resorption from senescing leaves of *A. cygnorum* in this experiment was around 55%, taking into account the loss of leaf dry weight during senescence (Vergutz et al. 2012). Most Proteaceae from southern South America growing on P-richer soils than those in south-western Australia have much lower P-resorption efficiency from senescing leaves, i.e. about 30% (Diehl et al. 2003; Lambers et al. 2012), although high P-resorption efficiency was found in some species, such as *Embothrium coccineum* (Delgado et al. 2018). Evergreens are more proficient at resorbing P than deciduous species (Killingbeck 1996), though sustained, moderate drought can reduce the P proficiency in evergreens (Dallstream and Piper 2021). Leaf lifespan is also important for P-use efficiency, because nutrients can be used for a longer period when they have a longer residence time in leaves. The leaf life span of *A. cygnorum* is about 40 months (Veneklaas and Poot 2003), much longer than the average for 744 species growing in their native habitat (median 8.5 months; Wright et al. 2004) or *Banksia* species (24 to 38 months; Veneklaas and Poot 2003). So, P in *A.*

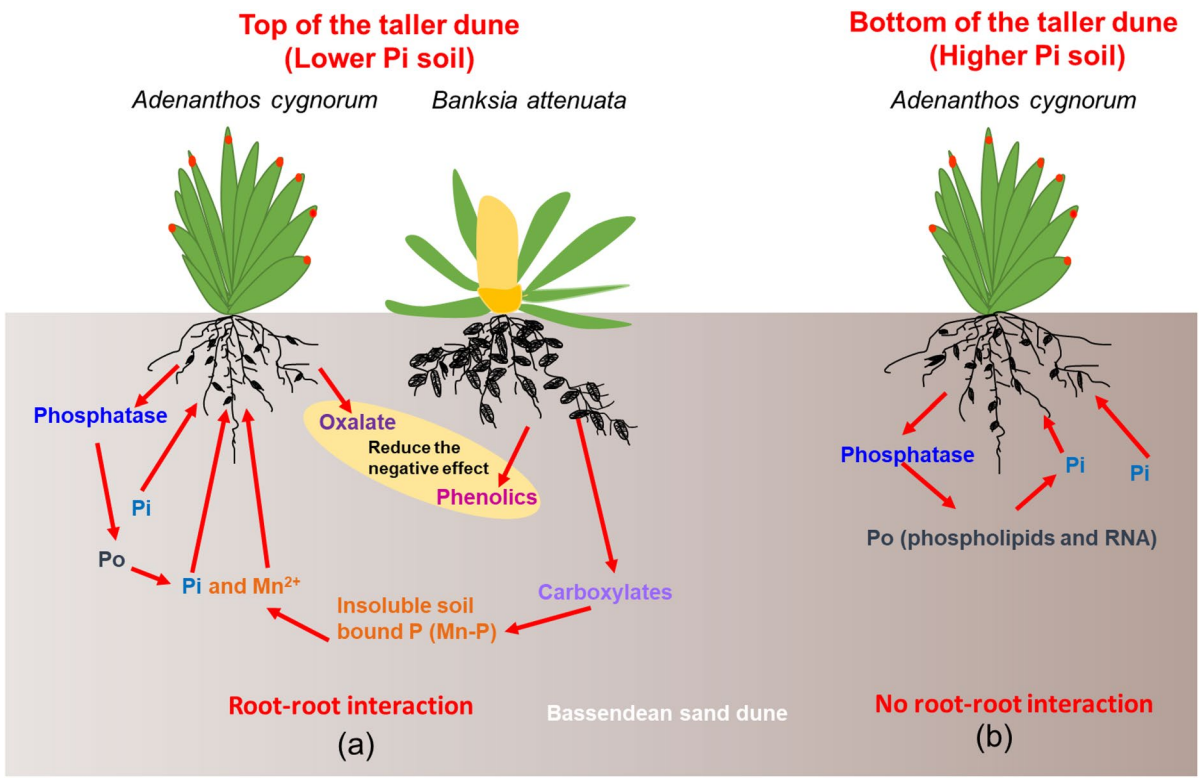


Fig. 9 Schematic diagram showing mechanisms of root phosphorus (P) acquisition in *Adenanthos cygnorum* at different locations. **a** *Adenanthos cynorum* neighbouring *Banksia attenuata* grew on top of the taller dune that has a severely low soil P concentration. Carboxylates released by *B. attenuata* can mobilise soil bound P into inorganic P (Pi) and other nutrient elements such as manganese. *Adenanthos cygnorum* can also release phosphatases into soil to hydrolyse orgainc P (Po) releasing Pi. *Banksia attenuata* can release phytotoxic phenolics, while *A. cygnorum* can release oxalate into soil that may reduce the phytotoxic effects of phenolics. **b** Soil at the bottom of the taller dune contains sufficient P for *A. cygnorum* to utilise, including Po, and it can absorb Pi directly. For the higher Po, *A. cygonrum* can release phosphatases into soil to hydrolyse Po that is not tightly bound, such as phospholipids and RNA (Zhong et al. 2021), to release Pi

cygnorum leaves has a longer residence time than that in *Banksia* species, contributing to efficient long-term P-utilisation efficiency.

Conclusion

Adenanthos cygnorum functions in a way that differs from what is known for most Proteaceae (Fig. 9). Rather than producing functional cluster roots, when growing in severely P-impoverished soils *A. cygnorum* depended on facilitation of its P acquisition by neighbouring *B. attenuata* to supplement its acquisition of P. When it grew in soil with higher [P], including higher [Po], *A. cygnorum* also occurred away from carboxylate-releasing neighbours. There, it could access sufficient Po that was relatively mobile by releasing acid phosphatases without substantial carboxylate exudation. Thus, by combining its ability to access P in Po by exuding phosphatases with its ability to be facilitated in its P acquisition by P-mobilising neighbours such as *B. attenuata*, *A. cygnorum* extended its range of habitats.

Compared with *Banksia* species that have thick leaves and sunken stomata in crypts, *A. cygnorum*, which lacked stomatal crypts, exhibited slower rates of photosynthesis, similar to those of *Hakea* species, which also lack stomatal crypts. However, because of its very low leaf [P], the PPUE of *A. cygnorum* was higher, despite slow photosynthetic rates, than that of *Banksia* species. The leaf lifespan of *A. cygnorum* is also longer than that of *Banksia* species (Veneklaas and Poot 2003), so the residence time of leaf P is

longer, contributing to a higher P-use efficiency for growth.

In severely P-impoverished landscapes such as in south-western Australia, where Proteaceae are common, different species within this family evolved different non-mycorrhizal P-acquisition strategies as adaptations to their severely P-impoverished environment. These new findings contribute to our understanding of ecosystem functioning in severely P-impoverished highly megadiverse systems.

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Author contributions QS, KR, PMF and HL designed the study. QS performed the experiment and collected the data with the contributions of KR and HZ. QS, KR, PMF and HL prepared the manuscript. KR, HZ, PMF and HL revised the manuscript. All authors read and approved the manuscript.

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