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RESEARCH ARTICLE

Carboxylate-releasing *Eucalyptus patens* in a low-diversity spot in a biodiversity hotspot in Southwest Australia is not a 'Darwinian demon'

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Abstract

Background and aims Plants in severely phosphorus (P)-impoverished environments in southwest Australia are highly P efficient. In a *Eucalyptus patens* forest on P-impoverished soils in this region, leaf P concentrations ([P]) are very low in common species, which release carboxylates to acquire P. We hypothesised that these species exhibit a high photosynthetic P-use efficiency (PPUE). Being mycorrhizal and releasing carboxylates, the eucalypts would be very strong competitors, also against N_2-fixing neighbours. Therefore, we hypothesised that the dominant species show relatively low leaf nitrogen concentrations ([N]) and a high photosynthetic N-use efficiency (PNUE). Conversely, we expected these species to have a low intrinsic water-use efficiency (iWUE). We also tested the hypothesis that *E. patens* would not show P-toxicity symptoms at high P availability, and its carboxylate release would not decline at high P supply.

Methods We selected common species such as *Eucalyptus patens*, *E. marginata*, *Corymbia calophylla*, *Banksia grandis* and *Bossiaea aquifolium*. We analysed their leaf [P], [N], photosynthesis rates, stomatal conductance, PPUE, PNUE, $\delta^{13}C$, WUE and iWUE. We grew *E. patens* seedlings in nutrient solution with increasing P supply and measured their carboxylate-exudation rates to test if it declined at high P supply.

Results *Eucalyptus patens* released large amounts of carboxylates without a decrease in carboxylate release with increasing P supply. *Eucalyptus patens* and co-occurring common species had similarly low leaf [P] and [N], high PPUE and PNUE, but low WUE and iWUE, when compared with literature values.

Conclusions The low leaf [N] of dominant species was compensated for by high stomatal conductance. This strategy would be viable only in habitats with abundant water. Therefore, P-efficient water-inefficient species would be restricted in their distribution to habitats with high water availability and not be 'Darwinian demons'.

Keywords *Banksia grandis* · Carboxylate exudation · *Eucalyptus patens* · Nitrogen · Phosphorus · Water-use efficiency

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Introduction

Southwest Australia is a global biodiversity hotspot (Myers et al. 2000), one of only 36 in the world (Habel et al. 2019), with some of the most phosphorus (P)-impoverished soils globally (Kooyman et al. 2017). Within the Southwest Australian Biodiversity Hotspot, tall eucalypt forests are characterised by a low diversity of vascular plants at the community level and limited diversity of growth forms (Hopper and Gioia 2004). For example, we collected 21 species at a *Eucalyptus patens* site for our previous paper (Zhou et al. 2022); this comprised most of the species at this site, except for some understorey orchids, sundews and sedges. The low diversity of this site is in strong contrast with numbers of 40–50 species per 100 m^2 in kwongan shrublands in the Southwest Biodiversity Hotspot (Zemunik et al. 2016). We therefore referred to the *E. patens* habitat as a 'cool spot in a biodiversity hotspot' (Zhou et al. 2022).

In southwest Australia, the greatest plant diversity is associated with some of the most P-impoverished soils in the world (Lambers et al. 2010). All *Eucalyptus* species in this region occur on P-impoverished soils, but their habitats differ in terms of rainfall (https://florabase.dpaw.wa.gov.au/browse/profile/21824). Compared with extremely low soil P availability, nitrogen (N) is relatively abundant in southwest Australia (Laliberté et al. 2012; Lambers et al. 2010; Turner and Laliberté 2014). Soil P availability declines during pedogenesis (Walker and Syers 1976), leading to a steadily increasing soil N:P ratio during long-term soil development which makes P the major macronutrient limiting plant productivity in southwest Australia (Guilherme Pereira et al. 2019; Lambers et al. 2010; Turner and Laliberté 2014). Many native plant species that evolved in this P-limiting environment are highly P-efficient, with specialised P-acquisition and P-utilisation strategies (Lambers 2022).

Proteaceae is one of the dominant plant families in southwest Australia; most of them are non-mycorrhizal (Brundrett 2017; Lambers et al. 2010). They efficiently acquire P from the soil through their specialised cluster roots that exude carboxylates into the soil and mobilise P from poorly-soluble P complexes (Shane and Lambers 2005). Proteaceae are prominent, with many species showing relatively fast rates of photosynthesis per unit leaf area (A_{area}) and a very high photosynthetic P-use efficiency (PPUE) in severely P-impoverished ecosystems in southwest Australia (Guilherme Pereira et al. 2019; Sulpice et al. 2014; Denton et al. 2007). *Eucalyptus* belongs to the family Myrtaceae, which plays an important role in southwest Australia; *Eucalyptus patens* (yarri or Swan River blackbutt) occurs in eucalypt forests in high-rainfall zones, where it dominates the forests (Zhou et al. 2022; French and Nicolle 2019.). Eucalypts do not have cluster roots, but some do exude carboxylates into the soil and mobilise P (Lambers et al. 2021; Zhou et al. 2022; de Andrade et al. 2022). Cluster roots in Proteaceae are ephemeral structures, and in *Hakea prostrata*, they live for about 20 days, from rootlet emergence to senescence (Shane et al. 2004). In *H. prostrata* (Shane et al. 2004) and *Lupinus albus* (Fabaceae) (Watt and Evans 1999), citrate and malate are released in an exudative burst at their mature stage. Also, in *L. albus*, acidification of the rhizosphere is associated with the exudation of protons just before the mature stage of cluster roots (Zhu et al. 2005). Cluster-root formation and carboxylate release in *L. albus* (Watt and Evans 1999) and *H. prostrata* (Shane et al. 2003) are suppressed at a high P availability, but how variation in P supply affects *Eucalyptus* root activity, especially the release of carboxylates, is unknown. Also, it is quite common in Proteaceae for excess P supply to cause P-toxicity symptoms (Shane et al. 2003). As a rare example among Myrtaceae, *E. marginata* also exhibits P-toxicity symptoms at elevated soil P availability when grown without mycorrhizal symbionts, but it does not when colonised by ectomycorrhizal and arbuscular mycorrhizal fungi (Kariman et al. 2014). We do not know whether *E. patens*, which co-occurs with *E. marginata* in high-rainfall areas (French and Nicolle 2019), is P sensitive, like many Proteaceae, or tolerant like most Myrtaceae (Lambers 2022; Liu et al. 2022).

Eucalyptus is a C_3 genus of woody species distributed across the Australian continent, with species occupying both mesic and xeric environments (Schulze et al. 2006; Cernusak 2020; Lamoureux et al. 2018; Nolan et al. 2017). Foliage $\delta^{13}C$ is considered a good indicator of a time-integrated water-use efficiency in C_3 plants, with more negative leaf $\delta^{13}C$ values indicating lower water-use efficiency (Farquhar and Richards 1984; Adams et al. 2020). *Eucalyptus* species native to mesic environments tend to have a

lower water-use efficiency than those native to xeric environments (Schulze et al. 2006). The photosynthetic activity of leaves is closely correlated with their N concentration ([N]) (Jia et al. 2021; Evans 1989; Field and Mooney 1986) because N is a component of photosynthetic enzymes such as Rubisco and proteins of the electron transport chain (Evans 1989). There is an inverse relationship between photosynthetic N-efficiency and intrinsic water-use efficiency (iWUE) (Hu et al. 2023; Querejeta et al. 2022; Fredeen et al. 1991; van den Boogaard et al. 1995). Under limited availability of N, stomatal conductance may be higher, increasing PNUE at the expense of iWUE (Renninger et al. 2015). Our study site of *E. patens* occurs in a region with an annual rainfall of 1224.5 mm (1934–2022; http://www.bom.gov.au/climate/data/); water is available throughout the year, even in the dry summer that is common in southwest Australia. To date, there is no information on the iWUE of *E. patens* and co-occurring species in *E. patens* forests.

We aimed to quantify the PPUE, PNUE, WUE and iWUE of common plant species from a low-diversity spot in a biodiversity hotspot. We hypothesised, first, that with a low leaf [P] and releasing carboxylates, the dominant species at the *E. patens* site would exhibit a very high PPUE, while leaf [N] would be low in the dominant species (except *B. aquifolium*; Fabaceae) and these species would exhibit a very high PNUE. Conversely, we hypothesised WUE would be low with a low leaf $\delta^{13}C$ value, and iWUE would be low, with a high stomatal conductance compensating for low leaf [N]. Second, we also tested the hypothesis that *E. patens* would not show P toxicity at high [P] in the root environment, in contrast with *E. marginata* where P toxicity is acute, because *E. patens* would down-regulate its P-uptake capacity at supraoptimal P supply. Third, we hypothesised that carboxylate release in *E. patens* would not decline by increasing [P] in the hydroponic solution. To test this hypothesis, we compared root exudation by measuring the carboxylate-release rate of the root system from plants that received increasing [P] in the hydroponic solution. Understanding how *Eucalyptus* species acquire and use P, N and water at physiological and ecological levels will increase our understanding of ecosystem functioning in a low biodiversity spot in a biodiversity hotspot.

Materials and methods

In situ measurements in a *Eucalyptus patens* forest

Site description

The study site was located at Cypress Form, Waroona (32°47′35”S, 116°0′46″E), ~100 km south of Perth in southwest Australia (Fig. 1a), a region characterised by a Mediterranean climate, with hot dry summers (December to February) and cool wet winters (June to August) with a mean annual rainfall of 1224.5 mm. The mean annual temperature is 22.0 °C and the mean monthly temperature maxima range from 16.7 °C in July to 32.5 °C in January (http://www.bom.gov.au/climate/data/; measured at Dwellingup, 1934–2022). Soils were acidic; pH ($CaCl_2$) was 4.8 ± 0.1 at the *E. patens* sampling site, and soil total [P] were very low (34 ± 2 mg P kg^{-1}). Readily-available soil [P], measured as resin-P concentrations, were very low (1.1 ± 0.1 mg kg^{-1}) across the site, and soil organic-P was the major fraction of soil P at all sites with a proportion of 88% (Zhou et al. 2022).

Selection of species

We used *Banksia grandis* Willd (Proteaceae), *Bossiaea aquifolium* Benth (Fabaceae), and three Myrtaceae, *Eucalyptus patens* Benth, *E. marginata* Sm. and *Corymbia calophylla* Hill & Johnson (Fig. 1b-e), including saplings (1–3 years old) and regrowth (around one year after coppicing) of *E. patens*, for gas-exchange measurements and leaf chemical analyses. These eucalypt species are the dominant species of the overstorey at the field site, and all have a high leaf [Mn] in their natural habitat and release carboxylates when grown in a nutrient solution with a low [P] (Lambers et al. 2021; Zhou et al. 2022). We used *B. prionotes* to show the sunken stomata of its thick leaves, but it was not sampled together with the other species as it does not occur in the same habitat.

Analyses of leaf samples

Leaf P concentrations The mature leaves of selected species were sampled to determine leaf [P], just after measuring the leaf gas exchange rates on 15 and 16 January 2021, and five to 10 plants

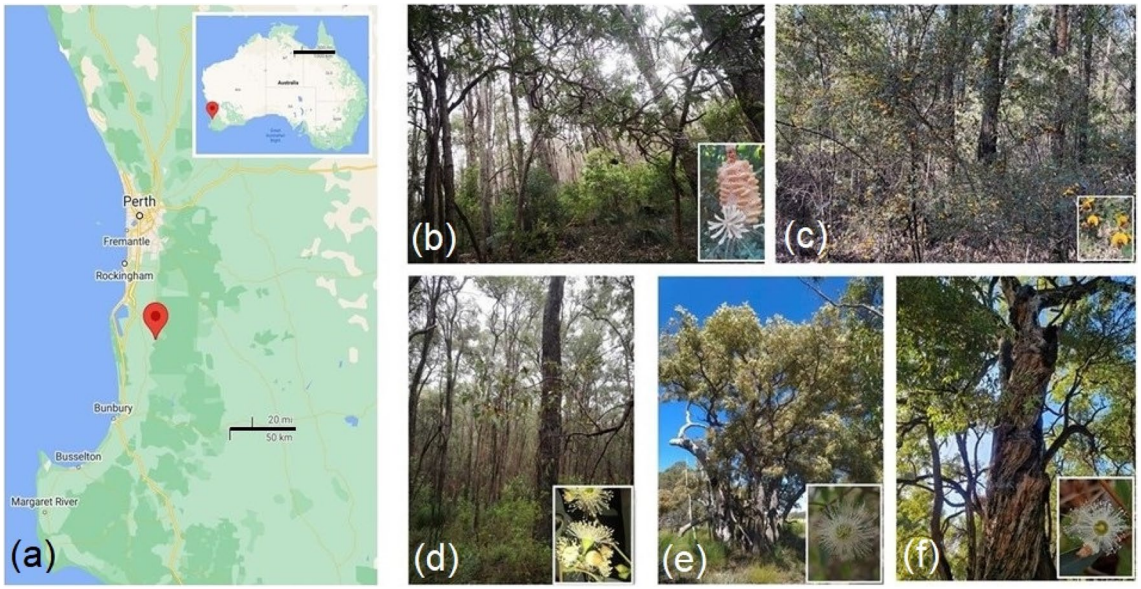

Fig. 1 Study site of the low-diversity 'cool' spot and studied species. **a** Study site of the *Eucalyptus patens* (yarri) forest at Cypress Form, Waroona (32°47′35"S, 116°0′46″E) in southwest Australia, located ~100 km south of Perth. Shades with different intensities of green represent the density of forest cover. The inset shows the map of Australia. Scale bars: on main map: 50 km; on inset: 1000 km. Source: https://www.google.com/maps/place/Waroona+WA. **b** *Banksia grandis,* **c** *Bossiaea aquifolium*, and (d) *Eucalyptus patens* at the study site; **e** *E. marginata* and (**f**) *Corymbia calophylla* photographed elsewhere. Insets of b-f are the flowers of each species. Photos: **b**, **c**, **d** Xue Meng Zhou; **e**, **f** Sophie Xiang

were sampled per species. After drying the leaves at 65 °C for three days, samples were ground in a ball-mill grinder (2010 Geno/Grinder, Metuchen, USA), using plastic vials and yttrium-stabilised zirconium ceramic beads. Samples were digested with hot concentrated HNO_3:$HClO_4$ (3:1) and analysed for [P] using ICP-OES (Model 5300DV, Perkin Elmer, Shelton, CT, USA) (Zarcinas et al. 1987).

Leaf N concentrations and carbon-isotopic composition The oven-dried finely-ground leaf samples of selected species were analysed for leaf [N] and carbon-isotopic composition ($\delta^{13}C$). Total [N] was measured by the combustion method using a Leco analyser (FP628, St. Joseph, MI, USA). Stable isotopes ($\delta^{13}C$) were measured using a Flash 1112 Elemental Analyser coupled via a continuous flow interface to Delta V Plus (Thermo-Finnigan, Bremen, Germany) isotope ratio mass spectrometer (Schulze et al. 2006). The natural stable isotope ratios were expressed as parts per thousand (‰), according to the following equation:

$$\delta X(\%_0) = \left(R_{sam}/R_{sta} - 1\right) \times 1000$$

where X is ^{13}C and R_{sam} and R_{sta} are the corresponding ratios of ^{13}C /^{12}C in sample and standard, respectively. We referenced our samples against Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}C$ (Skrzypek 2013). Standards were tested repeatedly after the completion of each batch of 10 measurements. Within a run, precision was ±0.10‰ for $\delta^{13}C$ (Coplen et al. 2006). Water-use efficiency (WUE) was calculated from $\delta^{13}C$ values (Du et al. 2021), as described by Farquhar and Richards (1984).

$$\Delta^{13}C = \left(\delta^{13}C_a - \delta^{13}C_p\right)/\left(1 + \delta^{13}C_p/1000\right)$$

in which $\delta^{13}C_a$ and $\delta^{13}C_p$ represent the $\delta^{13}C$ value of air and plant samples, respectively. The $\delta^{13}C_a$ was assumed to be −8‰, following Farquhar et al. (1989).

$$WUE = \left(b - \Delta^{13}C\right)/1.6(b - a)$$

where a is the diffusive fractionation of ^{13}C in air through the stomata (4.4‰), and b represents the net

fractionation caused by carboxylation (27‰) (Du et al. 2021; Farquhar et al. 1989).

Leaf anatomy

We collected intact mature leaves of the selected species for anatomical study. We used carrots as the supporting material to hold the leaves and cross-sections were prepared using a microtome (Leica RM2125 RTS; Leica Biosystems Nussloch GmbH, Heidelberg, Germany). Unstained cross-sections were viewed under an epifluorescence microscope in the darkfield with a UV filter, and photos were taken using an Axiocam digital camera (Carl Zeiss Microscopy GmbH, Oberkochen, Germany).

Leaf gas-exchange measurements

We measured the leaf gas exchange of selected species with an infrared gas analyser (LI-COR 6800XT; LI-COR Inc., Lincoln NE, USA) between 9 am and 12 am on 15 and 16 January 2021. Light-saturated photosynthesis (A_{area}) rates were measured in situ at a CO_2 concentration of 400 µmol CO_2 mol^{-1}, a chamber temperature of 25 ± 0.2 °C, and a vapour pressure deficit of 0.5–1.0 kPa. The photosynthetic photon flux density was 1500 µmol m^{-2} s^{-1}, with a relative humidity of 50–70%. Measurements were taken using undamaged, youngest fully-expanded and sun-exposed mature leaves, For the measurement, we cut a large branch from the tree and then measured the gas exchange rate immediately to minimise any errors on the reading as previously described (Grant 1992; Samuelson 1998). Stomatal conductance did not change after cutting the branch and during the gas-exchange measurements. We sampled five to 10 individuals per species.

After finishing each gas-exchange measurement, we collected the leaf material enclosed within the chamber of the LI-COR 6800XT. The area of those leaves was determined by scanning them using an Epson 1680 scanner (Epson America, Long Beach, CA, USA), and the images were analysed using the WINRHIZO v.4.1c scanner program (Regent Instructions, Quebec, Canada) to obtain leaf area data. Those leaves were then dried in an oven at 70 °C for three days to measure the dry mass. We calculated leaf mass per unit area (LMA) from the area and dry mass.

Experiments in a controlled-environment glasshouse

In vivo determination of changes in rhizosphere pH

We determined pH changes in the rhizosphere as described previously (Marschner et al. 1982). Agar (0.75% w/v; Sigma Chemical Company, St Louis, MO, USA) was heated to a clear liquid at ca. 80 °C, and 0.06% (w/v) bromocresol purple (pH indicator in the range of pH 5–8; Aldrich Chemical Company, Milwaukee, WI, USA) was added to the melted agar. The pH of the solution was adjusted to 6.5. The agar solution with pH indicator was divided into four equal parts and the pH of each part was adjusted to 5, 6, 7 and 8, respectively, by adding either 0.1 M HCl or 0.1 M NaOH. These solutions were poured into small Petri dishes and cooled to slightly below room temperature to observe the pH-dependent colour change of the agar solutions.

To observe the colour change of the agar solution with bromocresol purple as affected by root exudates, the pH of the agar medium was adjusted to 6.5 by adding 0.1 M NaOH (purple). The agar solution was poured into large Petri dishes and cooled to slightly below room temperature. To determine which parts of the root system changed the rhizosphere pH the most, we cut branched first-order lateral roots from the main root of *E. patens* seedling in hydroponics and separated mature, intermediate and younger zones, which contained second-order fine lateral roots. We also harvested younger tip regions of unbranched first-order lateral roots. We washed excised roots using double-distilled water and gently blotted them dry with paper towels and placed them in the agar medium separately. Root-induced changes in the rhizosphere pH by exudates, which were indicated by the colour change of the agar medium, were observed approximately 10–20 min after placing roots on the agar medium (Marschner et al. 1982; Shane et al. 2006; Tomasi et al. 2013).

Determination of root carboxylate-exudation rate in nutrient solution

We grew 10 seedlings of *E. patens* in a glasshouse at the University of Western Australia, under the controlled environment with an average daytime temperature of 25 °C from March to May 2022. Seedlings (around nine-months old) were purchased from Natural Area Holdings Pty Ltd. (Whiteman, WA 6068, Australia). All seedlings were transferred to aerated hydroponics in cooling tanks that maintained roots at 18 °C to 20 °C to start the pre-treatment period (Shane et al. 2004). Roots of the seedlings were washed and disinfected with 1% (v/v) sodium hypochlorite for 20 s before transferring them to 5-L plastic pots containing 4 L nutrient solution, which is commonly used for growing southwest Australian native plants (the nutrient solution contained, μM, 2.5 PO_4^{3-}, 500 NO_3^-, 200 Ca^{2+}, 500 K^+, 36 SO_4^{2+}, 36 Mg^{2+}, 10 Fe-EDTA, 0.24 Mn^{2+}, 0.10 Zn^{2+}, 0.018 Cu^{2+}, 2.4 H_3BO_3, 50 SiO_3^{2-}, and 0.3 Mo^{4+}. pH = 5.8 (Liu et al. 2022). Nutrient solutions were changed twice a week. Six weeks later, we excised one branched first-order lateral root from the main root and separated it into different parts, i.e. (1) younger zone of the branched lateral root with fine roots (YZBL), (2) mature zone of the branched lateral root with fine roots (MZBL), and (3) younger zone of unbranched lateral roots (YZUL) for the collection of root exudates and carboxylate analysis (Fig. S1). Root exudates were collected from these different parts of the roots in 3–10 mL of the same nutrient solution that was used to grow plants in hydroponics, but without P and Ca, for 1.5 h (Shane et al. 2006). Root exudates were then filtered using a 0.2 μm filter and stored at −20 °C until the samples were analysed. Root carboxylates were analysed using reverse phase liquid chromatography on an Alltima C-18 reverse phase column (250 × 4.6 mm, Alltech, Deerfield, IL, USA). The standards of malic, malonic, lactic, acetic, maleic, citric, succinic, *cis*-aconitic and *trans*-aconitic acid (ICN Biomedicals Inc., Aurora, OH) were used to identify carboxylates. The detection limit of the 10 acids ranged from 0.05 to 24 μM, as previously described by Cawthray (2003).

After the first harvest of roots to measure carboxylate-exudation rates, we left plants in hydroponics for two weeks to recover before harvesting roots again for the next carboxylate-exudation measurements. During this time, we changed the nutrient solution twice a week. After that, we gradually increased the [P] in the hydroponic medium from 1 μM to 20 μM, then to 40 μM, and finally to 80 μM. At each concentration, the seedlings were grown for two weeks before increasing [P] to the next level. The nutrient solution was changed twice a week. At each stage with different [P] in the medium, we collected exudates from one healthy branched lateral root from each plant, and analysed carboxylate exudation as described above for plants grown at 1 μM P. We also collected one or two mature fully-expanded mature leaves per plant grown at different P concentrations to analyse leaf [P]. We quantified P-toxicity symptoms (including chlorotic and necrotic areas on leaves) by ranking plants into six classes from 0 to 5, where the absence of any toxicity symptoms corresponded to 0, traces to 20% of symptomatic leaf tissue area (SLTA) as 1, 20 to 40% SLTA as 2, 40 to 60% of SLTA as 3, 60 to 80% of SLTA as 4 and more than 80% of SLTA as 5 (Kariman et al. 2014).

Statistical analyses

Individual trees were considered as the unit of replication. Differences among species, seedlings and regrowth for *E. patens* were analysed using one-way ANOVA. For each model, the normality of the residuals was assessed using the Shapiro-Wilk test, and heteroskedasticity was assessed by preparing a residual plot and visual inspection. Where appropriate (leaf [P], N:P ratio, PPUE and root exudation rates), data were $\log_{10}$ transformed before ANOVA. We used repeated-measures ANOVA with the R package rstatix to examine the difference among P treatments for the same individuals (Fig. 7b, c). We performed Tukey-Kramer HSD tests for a post-hoc mean comparison and differences were considered to be significant at $P \leq 0.05$. All analyses were conducted in R 4.2.1 (R_Development_Core_Team 2022) using base R packages.

Results

Leaf P and N concentrations

The dry mass (DM)-based leaf [P] and [N] across all species in this study ranged from 0.23–0.60 mg g^{-1}

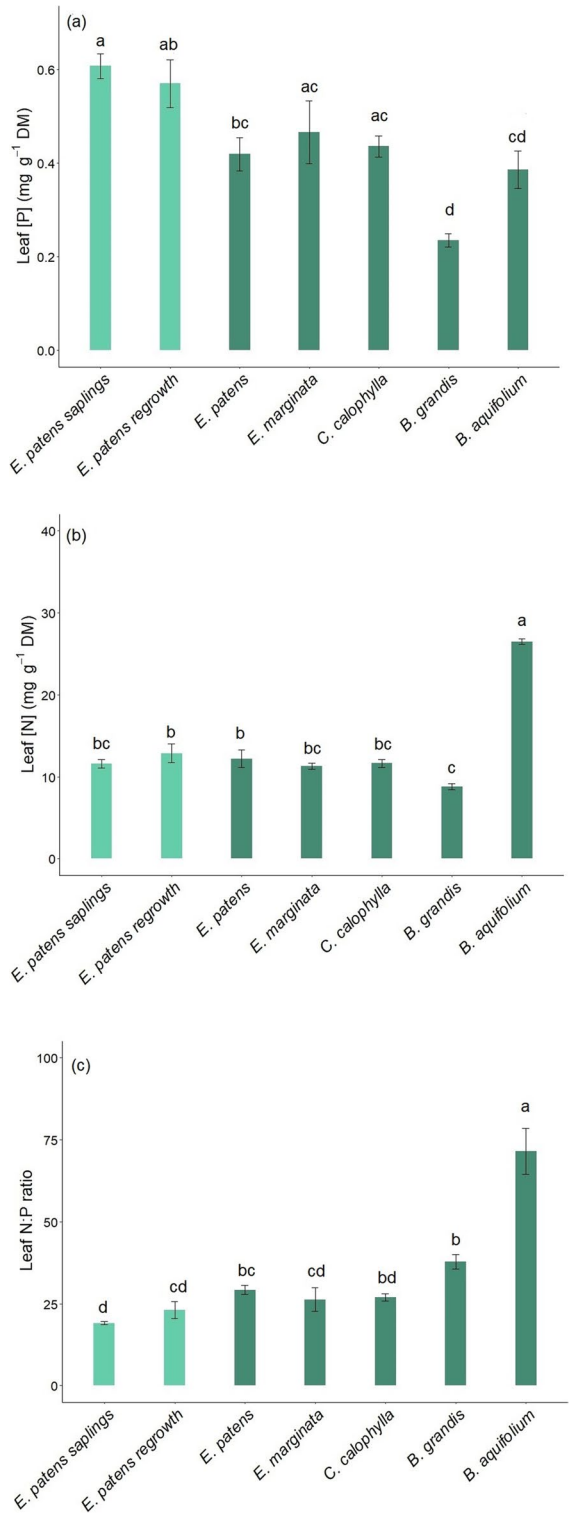

Fig. 2 **a** Leaf phosphorus concentrations ([P]), **b** leaf nitrogen concentration ([N]) and (**c**) leaf N:P ratio, on a dry mass (DM) basis of *Eucalyptus patens*, yarri (Myrtaceae) and co-occurring species at Cypress Form, Waroona. Data are means ± SE ($n = 5$). Different lowercase letters above the bars indicate significant differences among means, determined by analysis of variance (ANOVA) with Tukey's HSD test at $P \leq 0.05$ level

and 8.8–26.5 mg g^{-1}, respectively (Fig. 2a, b). *Bossiaea aquifolium* (Fabaceae) had the greatest leaf [N], 26.5 mg g^{-1}, and N:P ratio, 71, among all species studied (Fig. 2c). In contrast, *B. grandis* (Proteaceae) had the lowest leaf [P] (0.23 mg g^{-1}), and leaf [N] (8.8 mg g^{-1}). However, its leaf N:P ratio was intermediate (38). Mature leaves of *E. patens* saplings had significantly greater leaf [P] (0.60 mg g^{-1}) than those of adult trees (0.42 mg g^{-1}), whereas, it was intermediate for *E. patens* regrowth, following coppicing (0.55 mg g^{-1} for P). The mature leaf [P] of *E. marginata* and *C. calophylla* (0.47 and 0.44 mg g^{-1}, respectively) were the same as those of adult *E. patens*, and they were indistinguishable from each other. Leaf [N] of all Myrtaceae were similarly low, approximately 12 mg g^{-1}. The mature leaf N:P ratio of *E. patens* regrowth (23) was similar to that of adult *E. patens*, *E. marginata* and *C. calophylla*, while the N:P ratio of *E. patens* saplings (18) was significantly lower than that of adult *E. patens*.

Leaf gas exchange and carbon-isotope composition

Across all studied adult species, the mean values of area-based light-saturated photosynthetic rates of mature leaves (A_{area}) ranged from 7.2 (*B. aquifolium*) to 12.6 µmol CO_2 m^{-2} s^{-1} (*E. patens*) (Fig. 3a). *Eucalyptus patens* regrowth had the fastest A_{area} (22.2 µmol CO_2 m^{-2} s^{-1}) followed by *E. patens* saplings (17.3 µmol CO_2 m^{-2} s^{-1}). The former had significantly faster A_{area} than the adult leaves of all co-occurring species. The leaf stomatal conductance (g_{sw}) was similar for all studied species (approximately 0.2 mol H_2O m^{-2} s^{-1}), except for *B. aquifolium,* which had the lowest g_{sw} (0.08 mol H_2O m^{-2} s^{-1}) (Fig. 3b). The LMA varied among the co-occurring species, ranging from 56.5 g m^{-2} (*B. aquifolium*) to 177.8 g m^{-2} (*B. grandis*) (Fig. 3c). Adult plants of *E. patens, E. patens* saplings, *E. patens* regrowth, *E. marginata* and *C. calophylla* had a

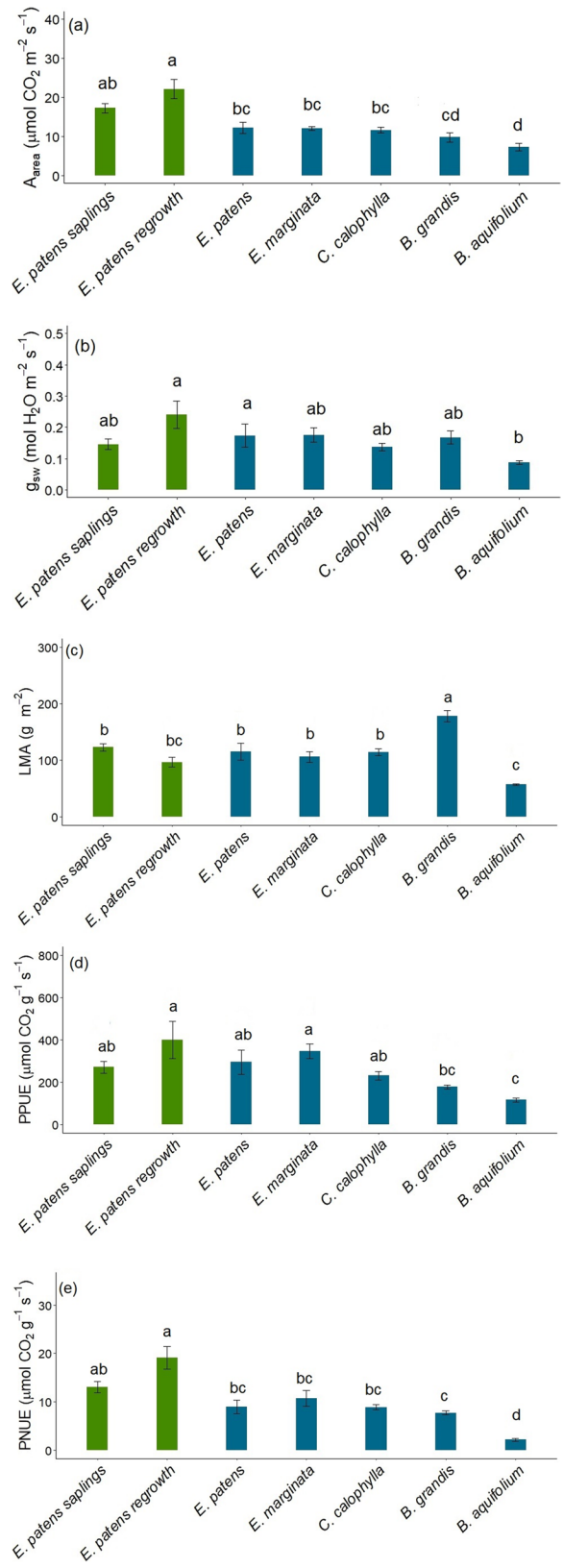

Fig. 3 Leaf gas exchange, leaf mass per area, and photosynthetic phosphorus (P)- and nitrogen (N)-use efficiencies of *Eucalyptus patens*, yarri (Myrtaceae) and co-occurring species at Cypress Form, Waroona measured in January 2021. **a** Area-based maximum photosynthetic rate (A_{area}); **b** leaf stomatal conductance (g_{sw}); **c** leaf mass per area (LMA); **d** photosynthetic P-use efficiency (PPUE); and (**e**) photosynthetic N-use efficiency (PNUE). Data are means ± SE ($n = 5$–10). Different lowercase letters above the bars indicate significant differences among means, determined by analysis of variance (ANOVA) with Tukey's HSD test at $P \leq 0.05$ level

similar LMA, approximately 100 g m^{-2}. Rates of photosynthesis per unit P (PPUE) (Fig. 3d) and N (PNUE) (Fig. 3e) of Myrtaceae were significantly greater than that of *B. aquifolium*, whereas it was intermediate for *B. grandis* (Proteaceae).

Banksia grandis had the most negative $\delta^{13}C$ value of −33.3‰ (Fig. 4a), and hence showed the lowest WUE calculated from $\delta^{13}C$ values (11 μmol mol^{-1}) (Fig. 4b); it also showed the lowest iWUE, calculated as A_{area} /g_{sw} (57 μmol mol^{-1}) (Fig. 4c). The $\delta^{13}C$ value of *B. aquifolium* was slightly less negative than that of *B. grandis* (−32.3‰), and hence *B. aquifolium* had a slightly higher WUE and iWUE (26 and 81 μmol mol^{-1}, respectively). *Eucalyptus patens* saplings and regrowth plants had the least negative $\delta^{13}C$ values of −29.9 and −30.5‰, respectively, like that of *C. calophylla* (−30.6‰). The Myrtaceae had the highest WUE and iWUE (62 and 120 for *E. patens* saplings, 54 and 88 for *E. patens regrowth* and 52 and 82 μmol mol^{-1} for *C. calophylla*, respectively. The $\delta^{13}C$ values of adult plants of *E. patens* (−31.1‰) and *E. marginata* (−31.3‰) were intermediate, and both had an intermediate WUE and iWUE (45 and 62; 41 and 64 μmol mol^{-1}, respectively), among the species. For all species, WUE calculated from $\delta^{13}C$ values was lower than iWUE calculated from A_{area}/g_{sw}. Importantly, all $\delta^{13}C$ values, WUE and iWUE values were low when compared with global values (O'Leary 1993) or values for other eucalypts (Schulze et al. 2006), indicating a very low efficiency of water used of all studied plants in the present habitat.

Leaf anatomy

Eucalyptus patens, *E. marginata*, *C. calophylla*, and *B. aquifolium* showed dorsiventral leaf anatomy with a single layer of palisade mesophyll and abundant spongy mesophyll cells (Fig. 5a-d). They also had

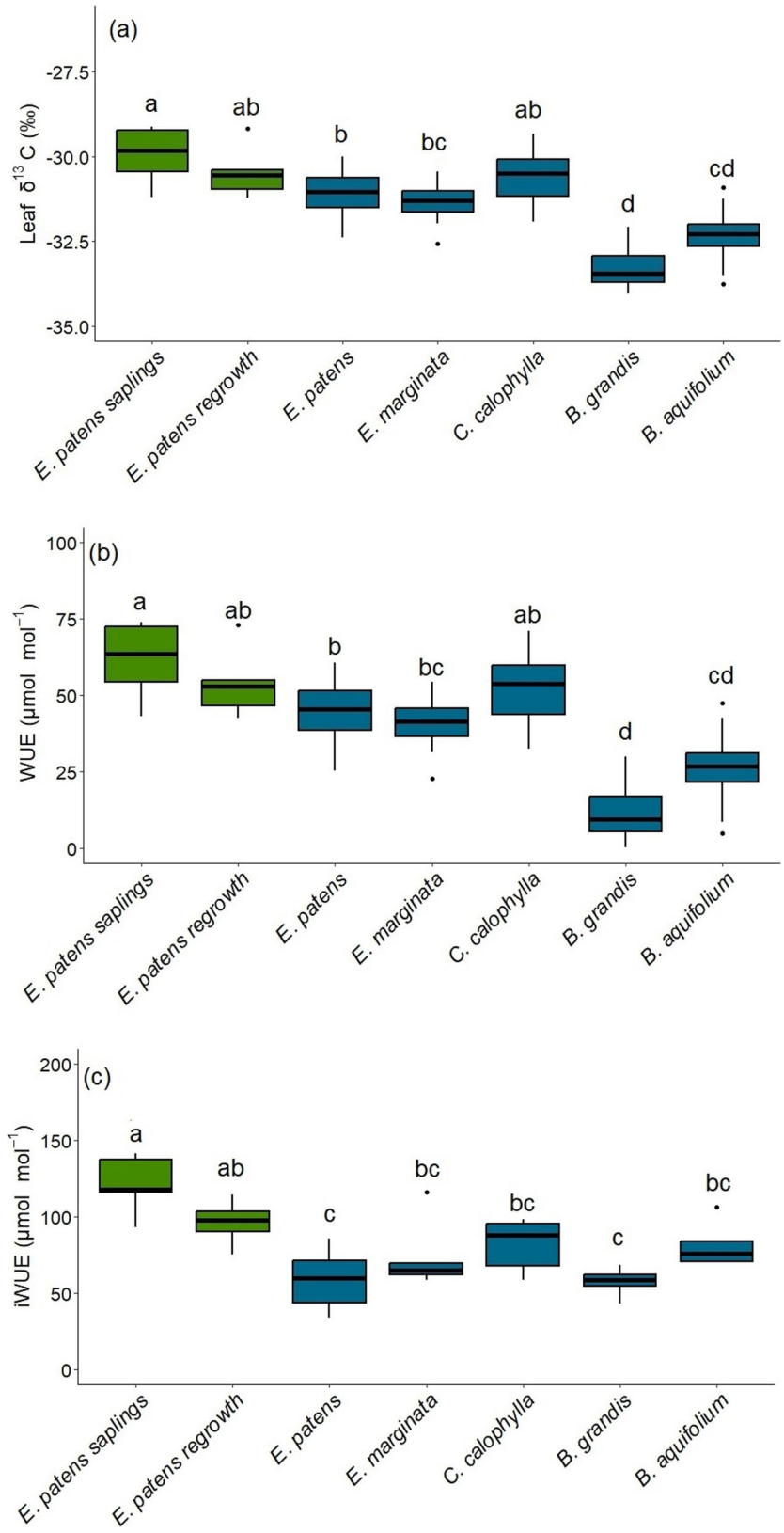

Fig. 4 Leaf carbon-isotope composition and water-use efficiency of *Eucalyptus patens*, yarri (Myrtaceae) and co-occurring species at Cypress Form, Waroona measured in January 2021. **a** Leaf carbon-isotope composition ($\delta^{13}C$); **b** water-use efficiency (WUE) calculated from $\delta^{13}C$ values.; **c** intrinsic water-use efficiency (iWUE) calculated as A_{area}/g_{sw}. Boxplots show medians, and 25th and 75th percentiles. Whiskers extend to 1.5 times the interquartile range. Data presented beyond whiskers represent outliers. ($n = 5$–10). Different letters indicate statistically-significant differences among means, analysed with one-way ANOVA and Tukey's HSD test at $P \leq 0.05$ level

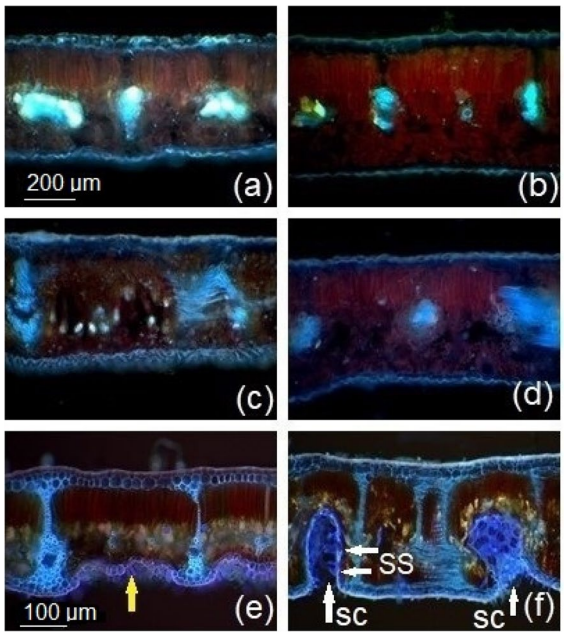

Fig. 5 Cross-sections of mature scleromorphic leaves of a range of species co-occurring with *Eucalyptus patens* (except *Banksia prionotes*, which was collected elsewhere and used to demonstrate the deep stomatal crypts and sunken stomata that are typical for thick-leaved *Banksia* species), viewed in dark-field with UV light using a UV filter. **a** *Eucalyptus patens*, **b** *E. marginata*, **c** *Corymbia calophylla*, **d** *Bossiaea aquifolium,* **e** *Banksia grandis,* **f** *B. prionotes*. UV-induced autofluorescence is visible in all micrographs, and different molecules and tissues exhibit different colours of autofluorescence. **a**-**f** Chlorophyll in the palisade and mesophyll parenchyma autofluoresced red, whereas lignified xylem vessels autofluoresced blue. **a**-**d**, **f** Blue/green and (**e**) purple/blue autofluorescence in the outer tangential walls of the upper and lower epidermis indicate the presence of thick cuticles. **e**, **f** Blue autofluorescence in the upper and lower epidermis of both *Banksia* species indicates lignified epidermal cell walls. Yellow arrow shows a shallow stomatal crypt in *B. grandis*. White arrows show deep stomal crypts (SC), and sunken stomata (SS) in *B. prionotes*. Scale bars: 200 µm (a-d) and 100 µm (**e**-**f**)

a thick cuticle on both the upper and lower epidermis. Like other studied species, *Banksia grandis* also showed dorsiventral leaf anatomy (Fig. 5e). Its lignified and thick-walled fibrous bundles of sclerenchyma cells (blue autofluorescence) divided the mesophyll tissue into small compartments. Despite their thickness, leaves of *B. grandis* did not exhibit deep stomatal crypts with sunken stomata and abundant long hairs originating from its lower epidermis, which are common in other thick-leaved *Banksia* species such as *B. prionotes* (Fig. 5f). Instead, *B. grandis* only had shallow stomatal crypts without sunken stomata (Fig. 5e).

Changes in rhizosphere pH and carboxylate release of plants grown at 1 µM P in nutrient solution

The addition of 0.1 M NaOH to the agar medium that contained a pH indicator, bromocresol purple, resulted in a notable colour change from yellow/orange (pH 5) to dark purple (pH 8) (Fig. 6a, b). To test the proton release from roots to the medium, the pH of the agar was adjusted to 6.5 (Fig. 6c, d). The strong colour changes from dark purple to bright yellow around the cluster roots (rhizosphere) of *B. grandis* indicated a strong acidification of the medium by cluster roots (Fig. 6c), which release carboxylates (Lambers et al. 2002). This colour change was mild for very young cluster roots (1–4 days old), whereas it was strong for mature cluster roots (13–15 days old). *Eucalyptus patens* roots also acidified the agar around them as indicated by the colour change (Fig. 6d). The mature zones of the branched lateral roots (MZBL) showed the strongest acidification of the medium, evidenced by the strongest colour change, followed by those around the young zone of the unbranched lateral roots (YZUL), and intermediate and younger zones of branched lateral roots (YZBL) with fine roots.

Different from the agar experiment, the younger zone of the branched lateral (YZBL) with fine roots of *E. patens* had the fastest rate of carboxylate release (0.13 nmol g^{-1} FM s^{-1}), followed by the young zone of the unbranched lateral roots (YZUL; 0.08 nmol g^{-1} FM s^{-1}) and mature zones of the branched lateral roots (MZBL; 0.04 nmol g^{-1} FM s^{-1}) (Fig. 7a). Remarkably, the root carboxylate-exudation rate of *E. patens* was independent of the [P] in the nutrient solution, in which even 80 µM P treatment failed to reduce the exudation rate significantly from that at 1 µM P ($F(3.12) = 0.96$, $p = 0.44$), even though there was a slight reduction of carboxylate exudation with 80 µM Pi treatment (Fig. 7b).

Changes in total leaf P concentration with different P treatments

There was no P left in the hydroponic medium three days after replacing the nutrient solution of the 1 µM P treatment, whereas more than 0.5 µM P was left

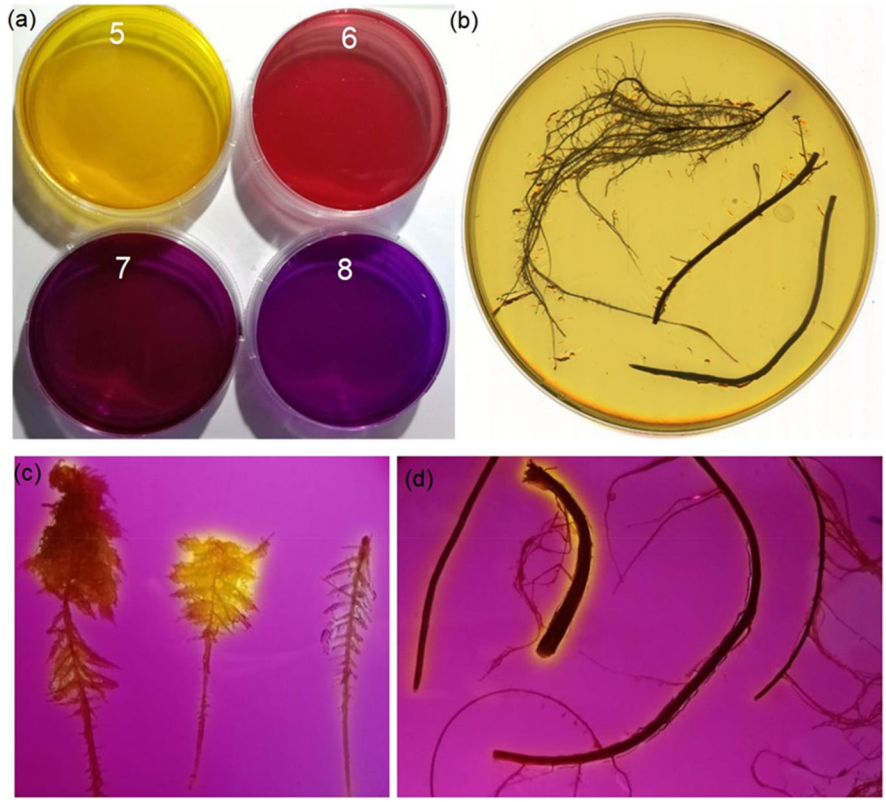

Fig. 6 Root-induced changes in rhizosphere pH after ca. 10–20 min on agar with a pH indicator (bromocresol purple). **a** The reference pH values and associated colours in the agar medium. **b** Roots of *Eucalyptus patens* when the initial pH of the agar medium with bromocresol purple was 5.0, but it was changed to 6.5 by adding 0.1 M NaOH (c and d). **c** Roots of *Banksia grandis*, from left to right: a senesced cluster root (16–19 days old), a mature cluster root (13–15 days old), and a young cluster root (1–4 days old). **d** Roots of *E. patens*, from left to right: younger zone of an unbranched lateral root, mature basal zone of a branched lateral root, intermediate zone of a branched lateral root and the younger zone of a branched lateral root with numerous second-order laterals (fine roots). The colour changes from purple to yellow around the roots on the agar medium reflects acidification of the medium from the initial pH of 6.5 to less than 5.0. The intensity of the yellow colour around the roots reflects the level of acidification of the medium (strong acidification results in intense bright yellow)

in the medium three days after replacing the nutrient solution with 20 µM P. In the 40 and 80 µM P treatments, even more P was left in the hydroponic medium. Total leaf [P] increased with increasing [P] in the nutrient solution ($F(3.12)=9.27$, $p=0.002$) (Fig. 7c). In the 1 µM P treatment, total leaf [P] was approximately 0.9 mg g^{-1} DM, increasing to 2.8 mg g^{-1} DM in the 80 µM P treatment, a 3.1-fold change in leaf [P] and an 80-fold change for the medium [P]. We did not find any P-toxicity symptoms (such as chlorotic and necrotic areas on leaves) of *E. patens*; this absence of any toxicity symptoms corresponded to toxicity class 0 (Kariman et al. 2014). All the plants had green and healthy leaves, without typical P-toxicity symptoms as found in previous studies (Kariman et al. 2014; de Campos et al. 2013; Shane et al. 2003), and maintained normal growth, even with higher P levels (Supplementary Fig. S1).

Discussion

The present study builds on recent findings that dominant mycorrhizal overstorey and understorey species in a tall eucalypt forest in southwest Australia depend on carboxylate release to acquire P (Lambers et al. 2021; Zhou et al. 2022). However, unlike cluster-root-producing Fabaceae (Keerthisinghe et al. 1998) and Proteaceae (Shane et al. 2003), *E. patens* did not show a decrease in carboxylate release from its roots with

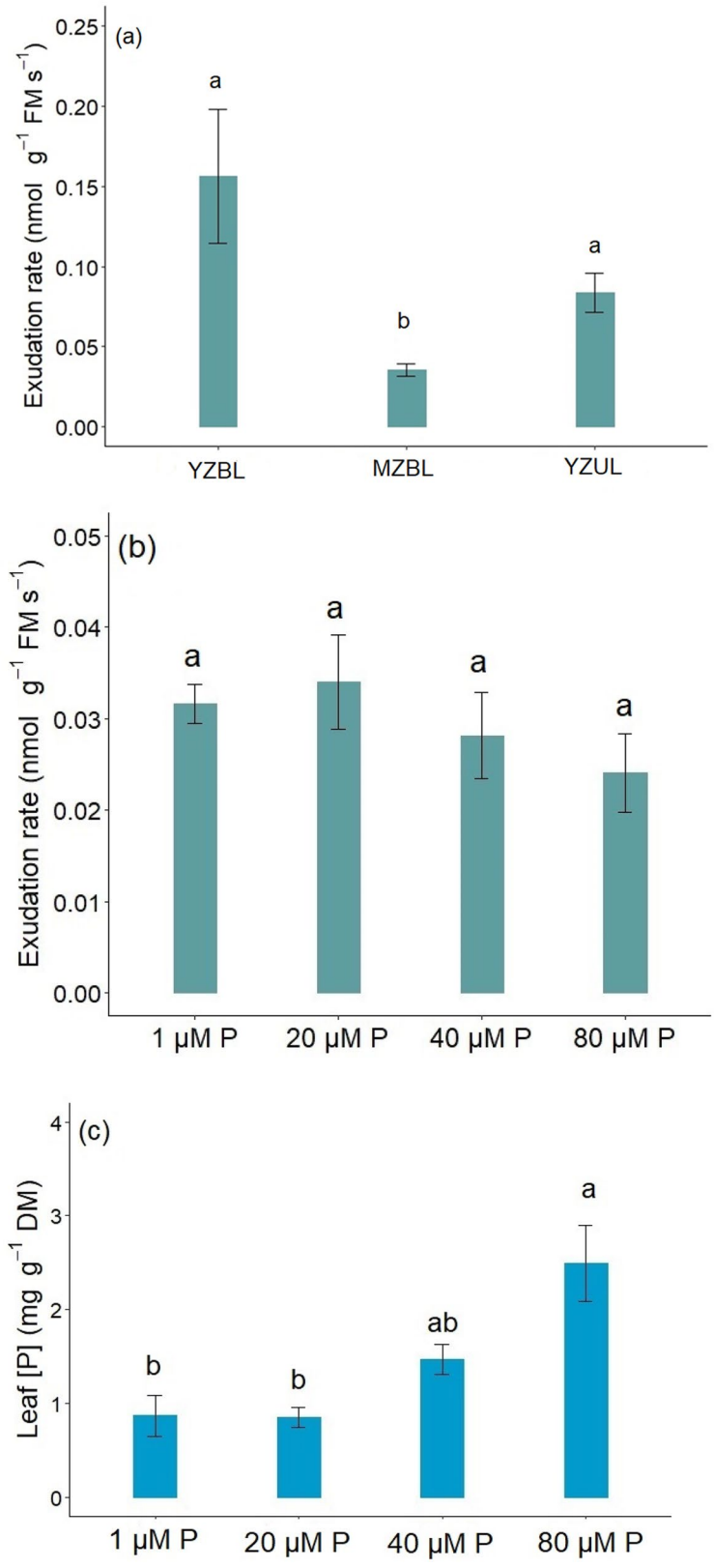

Fig. 7 Carboxylate-exudation rates of roots, and leaf phosphorus (P) concentrations ([P]) of *Eucalyptus patens* seedlings subjected to a gradual increase in [P] in the hydroponic medium. At each concentration, the seedlings were grown for two weeks, before measuring carboxylate release and leaf [P], followed by increasing [P] to the next level. **a** Carboxylate-exudation rates of different parts of the root system grown in nutrient solution with 1 µM P. YZBL: younger zone of branched lateral roots; MZBL: mature zone of branched lateral roots; YZUL: younger zone of unbranched lateral roots. **b** Carboxylate-exudation rates of branched lateral roots with fine roots, grown in different P treatments. **c** Phosphorus concentration in the mature leaves of *E. patens* seedlings subjected to a gradual increase in [P] in the nutrient solution. Data are means ± SE ($n = 5$). Different letters indicate significant differences among means, analysed with one-way ANOVA (**a**), repeated measures of ANOVA (**b**) and (**c**), Tukey's HSD test (**a**, **b** and **c**) at $P \leq 0.05$

increasing P supply and leaf [P] (Fig. 7b, c), indicating that carboxylate release was not dependent on the [P] in the medium or leaves. Being mycorrhizal, *E. patens* would be protected against pathogens (Albornoz et al. 2017; Cordier et al. 1998; Marx 1972; Pozo and Azcón-Aguilar 2007; Pozo et al. 2002), and it combines this with an effective carboxylate-releasing P-mobilising P-acquisition strategy. This implies that its P acquisition does not need to be facilitated by carboxylate-releasing P-mobilising neighbours (Lambers et al. 2018). Even if plants grow next to a carboxylate-releasing neighbour, this does not guarantee that their P uptake is facilitated by that neighbour (Yu et al. 2021; Yu et al. 2023). It takes two to tango (Yu et al. 2021). It requires the facilitated species to sense where P is mobilised and proliferate its roots accordingly (de Britto et al. 2021). With their effective P-acquisition strategy in a severely P-impoverished habitat, carboxylate release must give the P-mobilising species a competitive advantage, as has been shown for Proteaceae (Lambers 2022). The key difference, however, is that mycorrhizal Myrtaceae are protected against oomycete pathogens, whereas non-mycorrhizal Proteaceae are highly susceptible to these native pathogens (Albornoz et al. 2017). Therefore, these P-mobilising Myrtaceae combine the best of both worlds and are expected to be strong competitors, also against N_2-fixing species that lack a carboxylate-releasing P-acquisition strategy, such as *Acacia drummondii* and *Mirbelia dilatata* (Zhou et al. 2022). Below, we explore if this trait might make *E. patens* and other carboxylate-releasing eucalypts 'Darwinian demons' (sensu Law 1979).

Carboxylate and proton exudation

To assess the effect of external P supply and carboxylate release of *E. patens*, we gradually increased the P supply in the nutrient solution and measured the root carboxylate-exudation rate after each increase. Interestingly, *E. patens* did not respond as is known for Fabaceae (Keerthisinghe et al. 1998) and Proteaceae (Shane et al. 2003), because it did not show a significant decrease in carboxylate release with increasing P supply and increasing leaf [P] (from 0.9–2.8 mg g^{-1}), similar to some other *Eucalyptus* species studied by de Andrade et al. (2022). The absence of an effect of leaf [P] on the rate of carboxylate release is inconsistent with that in species producing cluster roots (Shane and Lambers 2005) or dauciform roots (Shane et al. 2006). However, this absence of a response to leaf [P] has also been noted for other species without specialised root structures (He et al. 2021; Huang et al. 2017; Wouterlood et al. 2004). This suggests that carboxylate release in *E. patens* may function to dispose of surplus carbon (Prescott 2022; Prescott et al. 2020), rather than releasing carboxylates in response to P limitation (Fig. 7c). However, independent of their control, the root exudates will mobilise sorbed P. The proton exudation of *E. patens* in agar suggests that the mature zones of branched lateral roots (MZBL) released more protons than the younger zone of branched laterals (YZBL) with fine roots (Fig. 6d), even though, the mature roots did not exhibit the fastest carboxylate-exudation rate in nutrient solution; that rate was faster in the younger roots (Fig. 7a). Further research is needed to test if exudate release from mature main roots of *E. patens* facilitates the P uptake of branched laterals with fine roots for the entire root system to acquire P efficiently, as shown for *Oryza sativa* (rice) (Kuppe et al. 2022).

Leaf P concentrations, photosynthesis, and photosynthetic P-use efficiency

The leaf [P] of *B. grandis* (Fig. 2a) was very low in this low-P habitat (Zhou et al. 2022), as recorded before (Barrick 2003), and other populations of this species (Denton et al. 2007; Sulpice et al. 2014; Guilherme Pereira et al. 2019). Leaf [N] of *B. grandis* (Fig. 2b) was also similar to that recorded for other *B. grandis* populations (Barrick 2003), and other Proteaceae in southwest Australia (Sulpice et al. 2014; Guilherme Pereira et al. 2019). In accordance, the leaf N:P ratio of *B. grandis* (45.5) was similar to that of three other *Banksia* species (Sulpice et al. 2014). *Banksia grandis* in the 'cool spot' showed relatively slow photosynthetic rates (A_{area}) compared with other *Banksia* species that have stomatal crypts in similar low-P habitats (Guilherme Pereira et al. 2019; Sulpice et al. 2014; Denton et al. 2007). Even though *B. grandis* has thick leaves, it did not have deep stomatal crypts and sunken stomata as found for *B. prionotes* (Fig. 5e vs. f) and other *Banksia* species with thick leaves (Hassiotou et al. 2009; Sulpice et al. 2014). The absence of deep stomatal crypts likely accounts for its relatively slow rates of photosynthesis, as observed in similarly thick-leaved

Hakea species without stomatal crypts (Lambers et al. 2012). Thick leaves without deep stomatal crypts imply a longer path for CO_2 diffusion from the air to the chloroplasts, thus restricting photosynthesis (Roth-Nebelsick et al. 2009).

The leaf PPUE of *B. grandis* was similar to that reported before for three other *Banksia* species (Denton et al. 2007). The leaf [P] and [N] of *E. patens, C. calophylla* and *E. marginata* were also similar to or somewhat lower than published data for these species (Grigg et al. 2009; Qiu et al. 2013). In accordance, the present N:P ratios for *C. calophylla* and *E. marginata* were similar to published data (Grigg et al. 2009); they were all >20, which indicates that their productivity was limited by P, rather than N (Güsewell 2004). There is no published record of the A_{area} of *E. patens*, but the present results of A_{area} of *C. calophylla* and *E. marginata* (Fig. 3a) were similar to or somewhat higher than published values (Grigg et al. 2009). The A_{area} of saplings and regrowth of *E. patens* were greater than that of adult *E. patens* (Fig. 3a). We did not find data on leaf [N] and [P] in the literature for *B. aquifolium*, but the present values were similar to published values for *B. laidlawiana* (renamed to *B. aquifolium* subsp. *laidlawiana*) in karri forest of southwest Australia (Grove 1990). The A_{area} of *B. aquifolium* was lower than that of the other four species at our study site, associated with a lower PPUE (Fig. 3d). The generally low leaf [N] and N:P values for the present species that do not fix N_2 agree with the low N availability at the *E. patens* site. This is possibly due to strong competition for P by P-mobilising eucalypts with N_2-fixing species in the study system, that are far less common and do not release carboxylates, unlike *B. aquifolium*, which is far more common and releases carboxylates (Zhou et al. 2022). In addition, there may be substantial leaching and/or denitrification associated with the high rainfall at this site which is typical for *E. patens* habitats, whereas *C. calophylla* and *E. marginata* also occur in low-rainfall habitats.

Leaf N concentrations and the trade-off between photosynthetic water-use efficiency and photosynthetic nitrogen-use efficiency

A low leaf [N] implies a low photosynthetic activity (Evans 1983; Field et al. 1983); this low activity can be partially compensated by a higher stomatal conductance (van den Boogaard et al. 1995; Wright et al. 2003). Plants tend to reduce stomatal conductance under water stress so that iWUE is maximised at the expense of PNUE (Limousin et al. 2015). Conversely, under limited availability of N, stomatal conductance may increase, increasing PNUE at the expense of iWUE (Renninger et al. 2015). This implies a trade-off between efficient use of either water or N (Fredeen et al. 1991; Limousin et al. 2015; van den Boogaard et al. 1995; Wright and Westoby 2002).

In the 'cool spot' ecosystem where P is severely limiting (Lambers et al. 2021; Zhou et al. 2022), we found that species belonging to three families exhibited different strategies for resource-use efficiency. *Banksia grandis* (Proteaceae), *E. patens, C. calophylla* and *E. marginata* (Myrtaceae) had similarly low leaf [N], restricting their photosynthetic activity, but the relatively high availability of water allowed them to partially compensate for their low photosynthetic activity by a high stomatal conductance. This suggests resource substitution in these species with limited access to N, maximising PNUE at the expense of iWUE, in agreement with previous studies (Fredeen et al. 1991; Nolan et al. 2017; Renninger et al. 2015; van den Boogaard et al. 1995). Conversely, *B. aquifolium* (Fabaceae) strongly depends on symbiotic N_2 fixation (Zhou et al. 2022) and functioned at a much higher leaf [N] (Fig. 2b). Despite this, it exhibited a low photosynthetic activity (Fig. 3a) and surprisingly low iWUE (Fig. 4c). This may account for the restriction of this species to high-rainfall regions (Hansen et al. 1991).

Can we consider *E. patens* to be a 'Darwinian demon'?

Law (1979) used the term 'Darwinian demons' for species that can maximise all aspects of fitness simultaneously but acknowledged there are costs associated with specific strategies. These are generally referred to as trade-offs, preventing the evolution of Darwinian demons (Pfautsch et al. 2018; Rees 1993). In biodiverse kwongan vegetation in the Southwest Biodiversity Hotspot, efficient acquisition of P in non-mycorrhizal Proteaceae is traded off against susceptibility to oomycete pathogens (Albornoz et al. 2017; Lambers et al. 2018). Some of the tall eucalypts we studied appear to combine the best of both worlds, because they exhibit a carboxylate-releasing

P-mining strategy (Lambers et al. 2021; Zhou et al. 2022), whilst their association with mycorrhizal fungi would provide protection against native oomycete pathogens (Albornoz et al. 2017; Cordier et al. 1998; Marx 1972; Pozo and Azcón-Aguilar 2007; Pozo et al. 2002). We surmise that this accounts for the low plant diversity in 'cool spots', but does it make these tall eucalypts Darwinian demons?

So far, we have only provided evidence for a combination of efficient P acquisition and pathogen defence in eucalypts from high-rainfall areas and found that this combination does likely not exist in *E. todtiana*, which grows in low-P habitats with less rainfall and exhibits a low leaf [Mn] (K. Ranathunge & H. Lambers, unpubl.). We use leaf [Mn] as a proxy for carboxylate-releasing P-acquisition strategies, based on the correlation between leaf [Mn] in the field and root carboxylate release in glasshouse studies (Lambers et al. 2021). We surmise that *Eucalyptus* species with high leaf [Mn] that release carboxylates are very strong competitors, leading to low-diversity habitats (Zhou et al. 2022), unlike the habitats where Myrtaceae depend on facilitation by carboxylate-releasing neighbours (Muler et al. 2014; Lambers et al. 2019). The low leaf [N] of the non-N_2-fixing species in the studied low-diversity environment was associated with a high stomatal conductance, as evidenced by low leaf $\delta^{13}C$ values (Fig. 4a). For *B. grandis*, the $\delta^{13}C$ values were slightly more negative (−33.2‰) than those for expanding leaves of *B. prionotes* during the wet growing season (−29‰ to −31‰), and considerably more negative than those of the same leaves later in the season, when they gradually increase to −26‰ while rainfall declines (Pate et al. 1998). This may account for *B. grandis* being restricted to habitats with greater availability of water than that of *B. prionotes* (https://florabase.dpaw.wa.gov.au/browse/profile/21316). For the eucalypts (*C. calophylla*, *E. marginata*, *E. patens*) the $\delta^{13}C$ values ranged from −30.7‰ to −31.3‰ (Fig. 4a). For 65 *Eucalyptus* species growing along rainfall gradients in southwest and central Western Australia, species-specific leaf $\delta^{13}C$ values decrease with increasing annual rainfall from −27.0‰ at 300 mm to −28.2‰ at 1000 mm (Schulze et al. 2006). For *E. marginata* at Wungong Catchment, in the same region as Waroona, leaf $\delta^{13}C$ values vary between −29‰ and −31‰ (Qiu et al. 2013). These comparisons show similar or more negative $\delta^{13}C$ values, suggesting greater stomatal conductance in the present tree species. The high stomatal conductance of the study species (Szota et al. 2011), allowed the leaves to function at a high internal CO_2 concentration (around 280 µmol mol^{-1}) to compensate for a low [N] (van den Boogaard et al. 1997; Warren and Adams 2006; Wright et al. 2001). In accordance, for *E. marginata*, a positive correlation between leaf [N] and leaf $\delta^{13}C$ was found at Wungong Catchment (Qiu et al. 2013). This shows a trade-off between efficient use of either water or N in photosynthesis.

In summary, the combination of traits involving pathogen defence, efficient P acquisition and efficient N use in plants was likely traded off against the water-use efficiency of the studied species. This nutrient-efficient strategy comes at the expense of profligate water use, restricting the nutrient-use efficient but water-use inefficient species to habitats with an abundant water availability but low nutrient availability throughout the year. Therefore, none of the eucalypts we have studied can be considered Darwinian demons, and they may be disadvantaged in a drying climate.

Concluding remarks

The present results provide further insights into a low-diversity 'cool spot' in a biodiversity hotspot. They indicate that *E. patens* in southwest Australia, and possibly other eucalypts elsewhere in Australia where they occur on P-impoverished sites and exhibit high leaf [Mn] (Lambers et al. 2021), function differently from what we might believe, based on mycorrhizal surveys (Albornoz et al. 2021; Brundrett and Abbott 1991). Surprisingly, carboxylate exudation in *E. patens* was not suppressed with increasing [P], and hence might function to dispose of surplus carbon (Prescott 2022; Prescott et al. 2020). Yet, the exuded carboxylates would mobilise sorbed P.

The combination of being mycorrhizal and releasing carboxylates would make *E. patens* a very strong competitor, also against N_2-fixing neighbours that do not release carboxylates. This likely accounts for the low diversity at the study site (Zhou et al. 2022), and also for the relatively low leaf [N] of *E. patens*. This low leaf

[N] implies a low photosynthetic activity, but this was compensated for by a high stomatal conductance, and thus low iWUE, but high PNUE (Wright et al. 2003; Wright and Westoby 2002). This trade-off would only be feasible in a high-rainfall region or in discharge zones in the landscape, and not outside these areas. Hence, *E. patens* is not a Darwinian demon that combines every single trait to be competitive everywhere.

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Author contribution Xue Meng Zhou was responsible for most of the field and glasshouse work and chemical and statistical analyses and contributed to writing the first draft of the manuscript; Kosala Ranathunge was responsible for the anatomical study, some of the glasshouse work and statistical analyses and contributed to the writing; Hans Lambers conceived the study and contributed to fieldwork and writing.

Declarations

Competing interests The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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